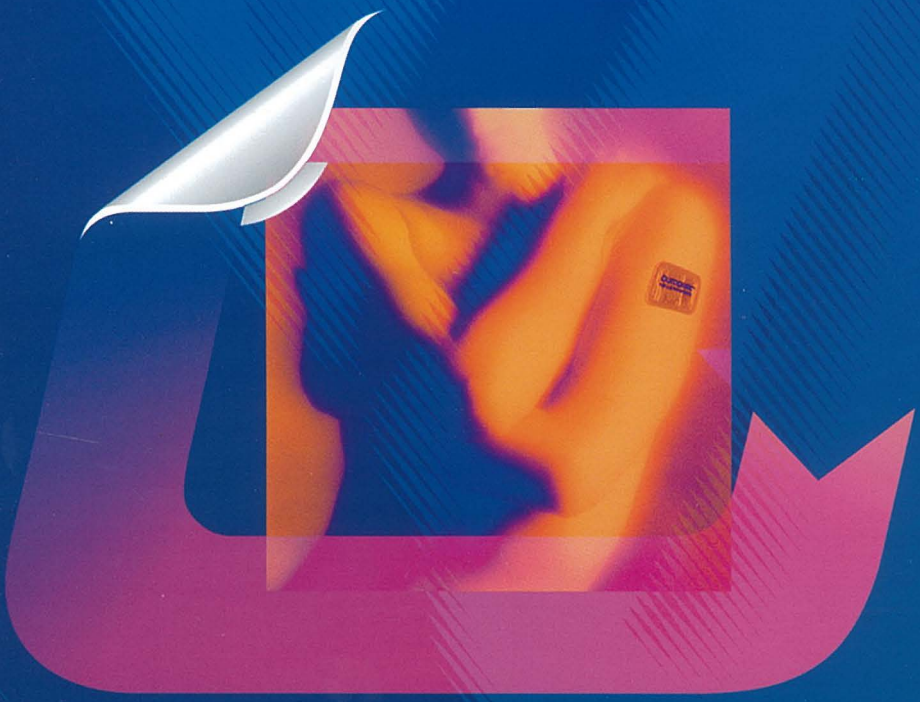
RADIOLOGY AND ONCOLOGY

March 2002
Vol. 36 No. 1
Ljubljana

ISSN 1318-2099

Durogesic®

TRANSDERMALNI FENTANIL



Nekaj cm^2 za 3 dni svobode

Dodatne informacije o zdravilu lahko dobite pri imetniku dovoljenja za promet:

JANSSEN-CILAG

JOHNSON & JOHNSON S. E., Podružnica Ljubljana, Šmartinska cesta 140, 1000 Ljubljana

E-mail: jac_slo@jnjsi.jnj.com

DUR-SLO-A-005-020801

RADIOLOGY AND ONCOLOGY

Editorial office
Radiology and Oncology
Institute of Oncology
Zaloška 2
SI-1000 Ljubljana
Slovenia
Phone: +386 1 4320 068
Phone/Fax: +386 1 4337 410
E-mail: gsersa@onko-i.si

March 2002
Vol. 36 No. 1
Pages 1-85
ISSN 1318-2099
UDC 616-006
CODEN: RONCEM

Aims and scope

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

Publisher
Association of Radiology and Oncology

Affiliated with
Slovenian Medical Association - Slovenian Association of Radiology, Nuclear Medicine Society, Slovenian Society for Radiotherapy and Oncology, and Slovenian Cancer Society
Croatian Medical Association - Croatian Society of Radiology
Societas Radiologorum Hungarorum
Friuli-Venezia Giulia regional groups of S.I.R.M. (Italian Society of Medical Radiology)

Reader for English
Mojca Čakš

Key words
Eva Klemenčič

Secretaries
Milica Harisch
Mira Klemenčič

Design
Monika Fink-Serša

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

Published quarterly in 700 copies

Bank account number 02010-0090006751
Foreign currency account number
010-7100-900067/4
NLB d.d., Podružnica Ljubljana Center, Ljubljana
S.W.I.F.T. Code LJBASI2X

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

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

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

This journal is printed on acid- free paper

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

RADIOLOGY AND ONCOLOGY

Ljubljana, Slovenia
March 2002
Vol. 36 No. 1

ISSN 1318-2099
UDC 616-006
CODEN: RONCEM

CONTENTS

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

Radiol Oncol 2002; 36(1): 1-4.

Primary non-Hodgkin lymphoma of the cecum: A case report

Mateja Kropivnik, Breda Jamar, Bojana Černelč

Clinical Institute of Radiology, University Medical Centre, Ljubljana, Slovenia

Background. *Primary lymphoma of the colon is rare, constituting 0.4 % of primary colonic malignancies and usually involves cecum or rectum. The aim of this paper is to present the role and the importance of double contrast barium enema (DCBE) in the diagnostic process.*

Case report. *A 77 years old male was admitted because of suspected inflammation in the area of total endoprosthesis of the left hip, inserted ten years before. Listeria monocytogenes was isolated from the aspirate and the patient treated with antibiotics. Twenty years ago the patient underwent nephrectomy because of hypernephroma of left kidney. At the time of admission he had sideropenic anaemia and he was febrile.*

Conclusion. *The patient underwent many diagnostic procedures: ultrasound (US), computed tomography (CT), double contrast barium enema, which showed a tumour in the cecum, small bowel follow-through and scintigraphy. The diagnosis of primary non-Hodgkin lymphoma was established by histology after biopsy at colonoscopy.*

Key words: cecal neoplasms – radiography; contrast media; barium; lymphoma, non-hodgkin

Introduction

Malignant lymphomas involve the gastrointestinal tract either as primary neoplasms or as part of disseminated disease. Primary lymphoma of the colon is rare, constituting 0.4 % of all colonic malignancies. Non-Hodgkin lymphoma accounts for almost all colonic lymphomas.[1-3] The signs and symptoms of colonic lymphoma are non-specific. The lack of specific symptoms can be the reason for delayed diagnosis.[4] The most common presentation is abdominal pain, with weight loss and changing bowel habits in 60-90 % of patients.[1,5]

A palpable abdominal mass can sometimes be noted on the initial physical examination.[6]

Double-contrast barium enema (DCBE) shows the changes in the large bowel, caused by lymphoma, but there is a broad spectrum of radiological variations: lymphoma is a great mimicker.[7]

Ultrasound (US) is often the first imaging modality used in patients with vague abdomi-

Received 28 February 2002
Accepted 6 March 2002

Correspondence to: Prim. Breda Jamar, M.D., Clinical Institute of Radiology, University Medical Centre, Ljubljana, Slovenia; Phone: +386 1 522-34-14; e-mail: breda.jamar@kclj.si

nal symptoms and can detect several patterns of involvement in cases of lymphoma.[8]

Computerised tomography (CT) is invaluable for the staging of the disease and is an essential complementary study to barium examination. It establishes the extent and shape of a lymphoma, demonstrates nodal involvement and possible infiltration of the liver or spleen.[9]

In our case, however, the location, the size and the appearance were determined by DCBE.

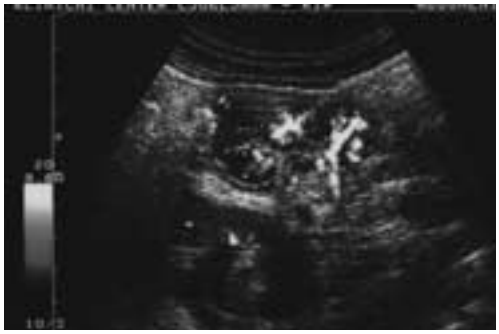


Figure 1. Ultrasound: a solid, hyperemic tumour in right lower quadrant of abdomen.

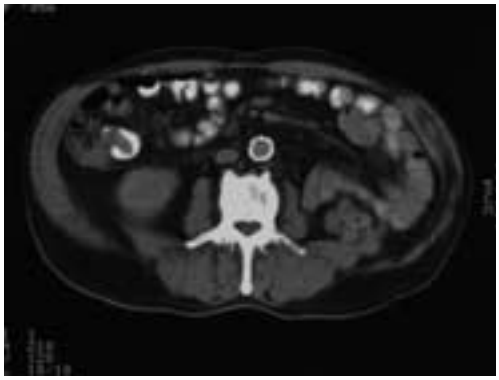


Figure 2. Computerised tomography: a slight mural thickening in the area of ileocecal valve.

Case report

A 77 years old male was admitted to the hospital at the beginning of August 2001, because of pain in the left hip and high body temperature. Ten years ago he had a total endoprosthesis of the left hip inserted and septic coxitis was suspected. He had had a hypernephroma of the left kidney, treated by nephrectomy, 20 year prior to admission. He was also complaining of vague lower abdominal pain, weakness and a short episode of diarrhoea.

On the admission his body temperature was 38.3 °C, he had sideropenic anaemia (E 3.25, Hb 189, and Fe 4.7) and his SR was 131. There was no palpable peripheral lymph nodes enlargement, abdominal mass or enlargement of liver or spleen.

The patient was treated by antibiotics, a needle aspiration of left hip was done and an orthopaedic surgeon was consulted. Scintigraphy showed a slightly higher activity in the area of left hip and in the right lower quadrant of the abdomen.

Listeria monocytogenes was isolated from the aspirate, antibiotics therapy was changed and an underlying disease was suspected.

US showed enlarged mesenterial and retroperitoneal lymph nodes and a bizarre, hyperaemic formation at the lower pole of the right kidney (Figure 1). CT showed normal liver parenchyma, slightly enlarged spleen, enlarged mesenterial and retroperitoneal lymphatic nodes, enlarged right kidney with parapelvic cysts, but no mass in the abdomen was described (Figure 2). Repeated US on the same day suggested that the tumour was in the area of distal segments of ileum.

At the beginning of September a double contrast barium enema was performed, which showed an approximately 4 × 4 cm large, lobulated, well defined tumour in the area of ileocecal valve (Figure 3). The tumour did not have characteristics of colon carcinoma. A small bowel follow- through was normal.

At colonoscopy an ulcerated, cauliflower mass was found in the cecum and biopsy was performed.

Figure 3. Double contrast barium enema: a well defined lobulated tumour in the cecum.

The histopathologic diagnosis was non-Hodgkin, B-cell lymphoma.

Discussion

Colon is a rare site of gastrointestinal non-Hodgkin lymphoma. Radiologic examination of large bowel and colonoscopy with biopsy are sufficient for definitive diagnosis, but US and/or CT are invaluable for the staging of the disease.[10]

Colonic lymphoma has a variety of different presentations, which are best seen by the use of double contrast barium enema technique.[11] The radiologic changes can be divided into five groups: mucosal nodularity, endo-exoenteric mass, intraluminal mass, mural infiltration and mesenteric invasion.[3] Intraluminal mass is the predominant feature, often lobulated and ranging in size up to 20 cm, mostly situated in the cecum, causing irregular enlargement of the ileocecal valve.

In our case the tumour, as seen on double contrast barium enema, met the criteria of colonic lymphoma as described in literature and it clearly did not have radiological characteristics of colonic carcinoma. At US, enlargement of lymph nodes was found and the tumour was seen, but its location and nature were not defined.

CT is invaluable for the staging in cases of primary colonic lymphomas, for definition of tumour invasion and spread. CT findings of intestinal tumour can throw a suspicion of lymphoma. Cecal tumours which are fairly demarcated from the surrounding pericolonic fat and show no evidence of invasion or obstruction of neighbouring viscera are suggestive of lymphoma.[12] In our case lymphoma of cecum was not obvious on CT examination. Because of US findings, which suggested pathology of the right kidney, the colon was not cleansed and there were a lot of faecal residua in the lumen, which made the proper evaluation of colonic disease difficult. Also, the tumour was small, only 4 cm in diameter. Nevertheless, on retrograde evaluation, the area of ileocecal valve showed mural thickening.

Conclusion

In the era of many imaging modalities, double contrast barium enema remains one of very sensitive, if not specific, diagnostic tools. The exact location and size can be determined and differential diagnoses suggested, even before the definitive diagnosis with biopsy is established.

References

1. Rubesin SE, Furth EE. Other tumors. In: Gore M E, Levine M S, Laufer I, editors. *Textbook of gastrointestinal radiology.* Philadelphia etc.: Saunders, 1994. p. 1200-7.

2. Cho MJ, Ha CS, Allen PK, Fuller LM, Cabanillas F, Cox JD. Primary non-Hodgkin lymphoma of the large bowel. *Radiology* 1997; **205:** 535-9.

3. O'Connell DJ, Thompson AJ. Lymphoma of the colon: the spectrum of radiologic changes. *Gastrointest Radiol* 1978; **2:** 377-85.

4. Doolabh N, Anthony T, Simmang C, Bieligk S, Lee E, Huber P, et al. Primary colonic lymphoma. *J Surg Oncol* 2000; **4:** 257-62.

5. Zinzani PL, Magagnoli M, Pagliani G, Bendandi M, Gherlinzoni F, Merla E, et al. Primary intestinal lymphoma: clinical and therapeutic features in 32 patients. *Haematologica* 1997; **3:** 305-8.

6. Zighelboim J, Larson MV. Primary colonic lymphoma. Clinical presentation, histopathologic features, and outcome with combination chemotherapy. *J Clin Gastroenterol* 1994; **4:** 291-7.

7. Mendelson RM. The gastrointestinal tract. In: Pettersson H, editor. *A global textbook of radiology*. Lund: The Nicer Institute; 1995. p. 891-1025.

8. Goerg C, Schwerk WB, Goerg K. Gastrointestinal lymphoma: sonographic findings in 54 patients. *Am J Roentgenol* 1990; **4:** 795-8.

9. Herlinger H, Maglinte DDT. Tumors of the small intestine. In: Herlinger H, Maglinte DDT, editors. *Clinical radiology of the small intestine*. Philadelphia etc.: Saunders; 1989. p. 399-451.

10. Montini F, Mascio DE, Fossaceca R, Frino F, Angelucci D, Errichi BM. Primary non-Hodgkin lymphomas of the colon: apropos of a case with double localisation. *Chir Ital* 1994; **46:** 59-65.

11. Torres WT, Gedgaudas-McClees RK. Lymphoma. In: Gore RM, Levine MS, Laufer I, editors. *Textbook of gastrointestinal radiology*. Philadelphia etc.: Saunders; 1994. p. 2570-82.

12. Wyatt SH, Fishman EK, Hruban RH, Siegelman SS. CT of primary colonic lymphoma. *Clin Imaging* 1994; **18:** 131-41.

Radiol Oncol 2002; 36(1): 5-12.

Carotid angioplasty with cerebral protection

Zoran Miloševič[1], Bojana Žvan[2], Marjan Zaletel[2], Miloš Šurlan[1]

[1]*Clinical Radiology Institute, University Medical Center, Ljubljana, Slovenia*
[2]*Neurology Clinic, University Medical Center, Ljubljana, Slovenia*

Background. *Carotid endarterectomy (CEA) is widely used in the management of high-grade carotid stenosis. It is a surgical procedure requiring general anaesthesia and is suitable only for lesions located at or close to the carotid bifurcation. It may develop complications, such as stroke, death, cranial nerve palsies, wound haematoma and cardiac complications. The risk of complications is increased in patients with recurrent carotid artery stenosis following CEA, in subjects undergoing radiotherapy to the neck, and in patients with cardiopulmonary disease. The drawbacks of CEA have led physicians to search for alternative treatment options. Carotid angioplasty and stenting (CAS) is less invasive than CEA. The method is particularly suitable for the treatment of recurrent stenosis after previous CEA and distal internal artery stenosis, which is inaccessible for CEA. CAS does not cause cranial nerve palsies. Moreover, it does not require general anaesthesia and causes lower morbidity and mortality in patients with severe cardiopulmonary disease. The complications of CAS include stroke due to distal immobilisation of a plaque or thrombus dislodged during the procedure, abrupt vessel occlusion due to thrombosis, dissection or vasospasm, and restenosis due to intimal hyperplasia. CAS is a relatively new procedure; therefore, it is essential to establish its efficacy and safety before it is introduced widely into clinical practice.*

Patients and methods. *In Slovenia, we have also started with carotid angioplasty by the study: Slovenian Carotid Angioplasty Study (SCAS). We performed CAS in 17 patients (12 males and 5 females) aged from 69 to 82 years. All patients were symptomatic with stenosis greater than 70 %. 10 patients suffered transient ischemic attacks, 4 patients minor strokes and 3 patients amaurosis fugax.*

Results. Technical success (< 30 % residual stenosis) was achieved in all cases. In 14 patients, no residual stenosis was found, in 2 patients a 15 % residual stenosis persisted and in 1 patient, a 30 % residual stenosis was detected. In 15 patients, CAS was performed without complications, in one patient the hyperperfusion syndrome occurred and in one periprocedural stroke occurred.

Conclusions. *According to our initial experience on 17 patients CAS could gain more importance in stroke prevention with proper selection of patients with brain ischemia and improved cerebral protection during procedure.*

Key words: carotid stroke; angioplasty; stents; cerebral infraction – prevention and control

Received 25 January 2002
Accepted 11 February 2002

Correspondence to: Zoran Miloševič, M.D., Clinical Radiology Institute, University Medical Centre Ljubljana, Zaloška 2, SI-1525 Ljubljana, Slovenia; Phone: +386 1 52 23 421; Fax: +386 1 43 31 044; E-mail: zoran.milosevic@guest.arnes.si

Introduction

Stroke is an important public health problem and the third most common cause of death, after heart diseases and cancer.[1] In Slovenia, stroke incidence, measured as a first ever stroke per 100000 population, is 190.5 and mortality rate is 19.3 %.[2] The proportion of ischemic stroke increases with age (33 % before 45 and 80 % after 50). Of all cases, 20 to 30 % are supposed to be due to carotid stenosis.[3] The most common cause of carotid stenosis is atherosclerosis. The mechanism of brain ischemia was thought to be either direct hemodynamic impact on the cerebral blood circulation or indirect as a source of thromboembolic material.[4] Three possible treatment modality are available to prevent stroke caused by carotid stenos is. The first is medical treatment, second surgical treatment, and third the newest approach, endovascular treatment by carotid angioplasty and stenting (CAS).

Platelet antiaggregants such as acetilsalicil acid or ticlopidine, reduce the risk of stroke.[5,6] Recently, preventive treatment with clopidogrel in combination with acetilsalicil acid are recommended.[7] Reducing the risk factors such as smoking, obesity, dyslipidemia, hypertension, diabetes is necessary.

In surgical approach, the atheromatous plaque is extirpated, removed and the artery is sutured. The first operation on carotid artery – carotid endarterectomy (CEA) was performed by DeBakey in 1953.[8] The number of the procedures increased in the following years. In 1984, 120000 CEA operations were performed.[9] After this year the number of CEA began to decrease because of its uncertain effectivness.[10] In 1991, randomised prospective surgical trials, North American Symptomatic Carotid Endarterectomy Trial (NASCET)[11] and European Carotid Surgery Trial (ECST)[12] showed a significant stroke risk reduction by CEA compared with medical treatment in symptomatic patients with carotid stenosis greater than 70 %. The reassessment of the results by the American Heart Association (AHA) Stroke Council indicated that CEA was three times as effective as medical treatment in reducing the frequency of stroke.[13] However, CEA carried a risk of cancer complications.[1] The benefit of CEA was dependent on maintaining a low complication rate. Most important complications during the procedure were perioperative stroke and death. Combined stroke and death rates exceeding 3 % for patients with asymptomatic stenosis and 6 % for patients with symptomatic stenosis would eliminate the benefit in stroke reduction.[14] Post-CEA restenosis should also be mentioned, since they are not rare. The rate is estimated between 1.2 and 23.9 %, depending on the operative technique.[15] The risk of complications by a reoperation was high.[16] Injuries of cranial nerves was seen due to the neck incision in 7.6 to 27%.[17]

CEA is the "gold standard" so far, but it is not without risks and limits as regards high-risk patients (elderly patients, patients suffering from coronary diseases, respiratory insufficiency...), supra-aortic lesions located in the upper section, and carotid lesions associated with severe intracranial lesions. Therefore, less invasive CAS seems to have its place in the treatment of carotid stenoses.

CAS has a history more than 20 years. After experiments on animal model Mathias in 1977 proposed the treatment of carotid stenosis for the first time using angioplasty.[18] The first carotid angioplasty was performed in 1980 by Kerber.[19] Carotid angioplasty with or without stenting has been investigated during last two decades. This procedure has not received wide acceptance because of the embolic stroke risk during the procedure. Till 1997 the perioperative stroke rate following CAS without cerebral protection ranged from 5.3 % – 8.2 %.[20,21] Initial results were criticised because of high neurological complications rate.[22] The main cause of perioperative complications are thought to be embolic particles

released from the carotid plaque during angioplasty.[23] In 1990, Theron, the father of cerebral protection, developed and advocated the use of cerebral protection device during CAS.[24] The risk of embolisation and the need for cerebral protection during CAS was confirmed later.[25]

Comparing the safety and efficacy of CAS with cerebral protection versus CEA, a prospective randomised trial was being organised: Carotid Revascularization Endarterectomy versus Stent Trial (CREST), which was started in the beginning of the year 2001.[26]

In Slovenia, we have also started with CAS by setting up the study "Slovenian Carotid Angioplasty Study (SCAS)" in order to evaluate the safety and efficacy of the method.

Patients and methods

Study protocol

The study has taken the form of a prospective clinical trial conducted over a period of 2 years on 60 patients enrolled according to well-defined inclusion and exclusion criteria. The patients were evaluated independently by a neurologist prior to and during the procedure and follow-up examination performed at 1, 6, 12 and 24 months. Evaluation of cerebral protection devices was incorporated into the study.

The safety of CAS was assessed on the basis of acute procedural success and occurrence of major clinical events during or within 30 days after the procedure. The efficacy of CAS was determined with respect to minor ipsilateral neurological events, major stroke and death occurring during or within 30 days of the procedure and recurrent stenosis established within 24 months of CAS.

Oral and written information on the study was provided to all patients, and a written, witnessed informed consent was obtained from each of them. The study was been approved by the National Medical Ethics Committee.

Patients

We performed CAS in 17 patients (12 of them were males and 5 females) aged from 69 to 82 years. All patients were symptomatic with stenosis greater than 70 %. 10 patients suffered transient ischemic attacks, 4 patients minor stroke and 3 patients amaurosis fugax. Seven patients had stenosis on right internal carotid artery, 8 on left internal carotid artery and 2 on right common carotid artery. Two patients had occlusion of the contralateral carotid artery. In the first 6 patients, we did not use cerebral protection devices. In other 11 patients, cerebral protection filter devices were used.

Procedure

All patients were give aspirin, 325 mg/d and clopidogrel (75 mg/d) starting the 7th day before the procedure. Heparin, given as an intra-arterial bolus, was titrated to maintain the activated clotting time between 200 and 250 seconds. The procedures were done with local anaesthesia. Neurologic status was monitored. Atropine (0.5-1 mg) was given as required during balloon inflation. Heart rate and blood pressure were monitored throughout the intervention.

Percutaneous access was gained through the femoral artery. Selective catheterization of carotid arteries was performed with standard techniques. The diagnostic angiography visualised the origins of the brachiocephalic arteries from the aortic arch, both carotid bifurcations, both vertebral arteries, intracranial parts of both carotid arteries and the dominant vertebral artery. Once the diagnostic angiography was completed and the stenotic internal carotid artery was identified, the 5F catheter was advanced using the 0.035-inch glide wire (Terumo Radiofocus Guide Wire, Terumo, Inc.) into the ipsilateral external carotid artery. The glide wire was withdrawn and replaced with an extra stiff 0.035-inch exchange wire (Extra Stiff Amplatz Wire, 260

cm; Cook, Inc.). The 5F catheter was withdrawn, and the 8F 90-cm guiding sheath (Carotid Vista Brite Tip; Cordis, Inc.) was advanced into the common carotid artery over the exchange Amplatz wire, which was anchored in the external carotid artery. Carotid angiography was again performed to measure the vessel diameter to facilitate the sizing of balloons, stents and cerebral protection filter devices. In the patients without cerebral protection, stenoses were then crossed with flexible coronary guidewires (V-18 Control Wire; Boston Scientific Corp, Watertown, Mass). Eleven patients underwent CAS with cerebral protection filter device Angioguard (Cordis, Inc): a low-profile guidewire-based, filter-type device (4F) that was placed in the distal ICA after crossing the stenotic lesion. It captured embolic debris while maintaining distal perfusion. After that we started with intervention on stenosis. The size of the initial angioplasty balloon was dictated by the severity of the stenosis. Very severe lesions were predilated with low-profile coronary balloons (Bypass Speedy Monorail Catheter, Boston Scientific Corp); in the case of less severe lesions, the initial dilatation may be performed with a definitive balloon sized to the distal normal artery. A Carotid Wallstent Monorail (Boston Scientific Corp) was deployed across the lesion. The stent was dilated at high pressure (14 to 16 atm) to firmly embed it into the vessel wall. After that filter with trapped emboli was removed and the procedure was finished. Completion angiography was performed on the ipsilateral intracranial vessels. The patients were transferred to the intensive care unit. The sheaths were removed. The patients were discharged on either the first or second day after the procedure. Clopidogrel was continued for 3 weeks, whereas aspirin was continued permanently.

Results

Procedural results are summarised in Table 1. Technical success (<30 % residual stenosis) was achieved in all cases. In 14 patients, no residual stenosis was found, in 2 patients a 15 % residual stenosis persisted and in 1 patient, a 30 % residual stenosis was detected.

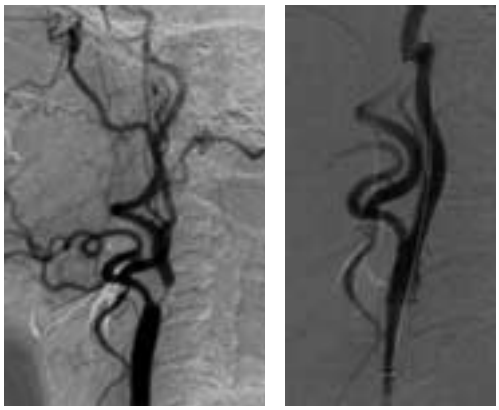


Figure 1. Digital subtraction angiography. Lateral views of the left carotid artery bifurcation. A. High grade circumferential, atherosclerotic stenosis of the internal carotid artery origin before CAS. B. No residual stenosis after CAS.

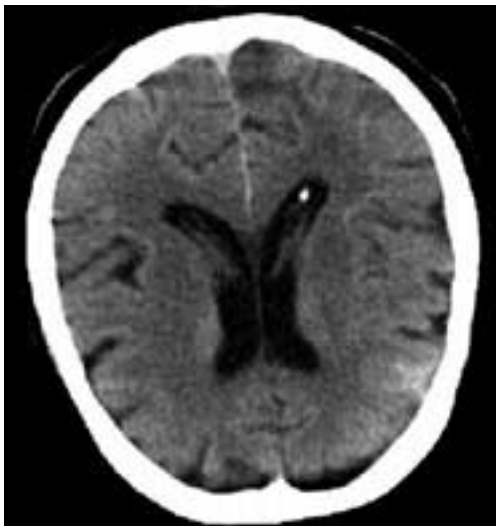


Figure 2. CT of the brain demonstrates small haemorrhage in the left frontal region.

Table 1. Procedural results in 17 patients

Pt	Vessel	Symptoms	Age in years	CLO	Stenosis % Pre	Post	Procedural Stroke	Severe CAD	Comments
1	R ICA	TIA	74		90	0	No	Yes	
2	L ICA	Stroke	72	Yes	99	0	No	No	
3	R ICA	TIA	63		70	0	No	No	
4	R CCA	TIA	68		80	0	No	Yes	
5	L ICA	Amaurosis fugax	72		95	0	No	No	After five days, an episode of seizure and transitory Tod's hemiparesis occurred. CT of the bran demonstrates small hemorrhage on the left frontal side. After a week, she recovered completely.
6	L ICA	Stroke	67	Yes	87	0	Yes	Yes	Occlusion of right ICA. Cerebral embolism occurred during the filter removal. He became aphasic and had right hemiplegy. We dissolved the embolus on the bifurcation of MCA with intraarterial thrombolysis using rTPA, but some hemiparesis persisted.
7	R CCA	TIA	66		80	0	No	No	The right iliac stenting followed same procedure.
8	L ICA	Amaurosis fugax	70	Yes	75	No	No	Yes	
9	R ICA	Stroke	64		90	15	No	No	
10	L ICA	TIA	68		76	0	No	No	
11	RICA	TIA	82		80	0	No	Yes	
12	R ICA	TIA	68		71	0	No	No	
13	L ICA	TIA	82		75	0	No	Yes	
14	R ICA	TIA	61		73	0	No	No	
15	L ICA	Stroke	76		99	30	No	Yes	
16	R ICA	Amaurosis fugax	57		85	0	No	No	
17	L ICA	TIA	53		75	15	No	No	

CAD = coronary artery disease; CCA = common carotid artery; CLO = contralateral carotid occlusion; ICA = internal carotid artery; L = left; MCA = middle cerebral artery; Pt = patient; R = right; TIA = transient ischemic attacks.

From patient 6 (Table 1), cerebral protection filter device was used.

In one patient (Patient 5, Table 1) hyperperfusion syndrome occurred. It occurred in 72-year-old female with carotid stenosis more than 90 %, who suffered from earlier amaurosis fugax. The stenting was performed successfully without residual stenosis and immediate complications (Figure 1). The 5th day after CAS, a generalized seizure with Tod's hemiparesis on the right side occurred. After admission, we performed brain CT that showed a small haemorrhage on the left front side (Figure 2). She recovered completely after a week.

Periprocedural stroke occurred in one patient (Patient 6, Table 1). This was 67 years old male with a previous minor stroke and 90 % stenosis of the left internal carotid artery due

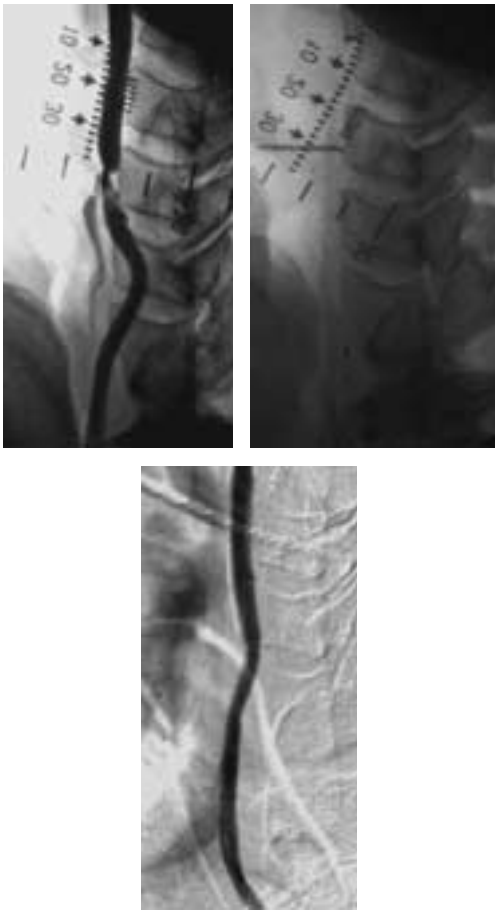


Figure 3. Digital subtraction angiography. Lateral views of the left carotid artery bifurcation. A. 90 % stenosis of left internal carotid artery before CAS. B. Cerebral protection filter device during CAS. C. No residual stenosis after CAS.

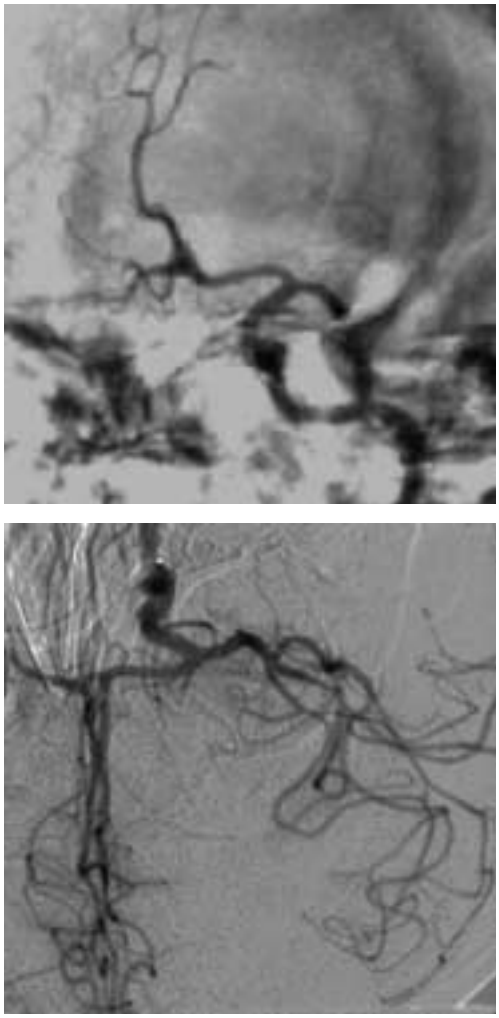


Figure 4. Digital subtraction angiography. Anteroposterior views of the left intracranial internal carotid artery with branches. A. An acute oclusion of left middle cerebral artery at the bifurcation. B. Recanalisation of the occlusion after intra-arterial thrombolysis.

to a lipid-laden plaque and the occluded right carotid artery. In this case we used a cerebral protective filter. CAS was successfully done (Figure 3). Cerebral embolism occurred during the filter removal. He became aphasic and had hemiplegy. We dissolved embolus on bifurcation of middle cerebral artery with intra-arterial thrombolisis using rTPA (Figure 4).

In 15 patients, CAS was performed without complications. The follow-up in all patients (average follow-up period of 3 months) revealed no transient ischemic attacks or new strokes. All patients remained at their neurologic baseline. Long-term clinical or imaging follow-up is not yet available.

Discussion

In the last years, angioplasty has been successfully used in coronary and peripheral disor-

ders and has been also applied at the carotid level. Throughout the world, several teams are actively engaged in research in order to determine the indications, the suitable techniques, the adjunct treatments, and the follow-up conditions. The final aim of carotid angioplasty is to prevent cerebral vascular neurological events and not to overshadow surgery. It could be an alternative or a complement to surgery if the results were comparable or better. Indications must be defined through randomised multi-centred studies and are currently much debated. Some would like them to be limited to high risk patients, restenosis, radiation-induced lesions, or lesions located in the upper internal carotid artery near the skull, while others would like them to be more extensive, including lesions of the carotid bifurcation.[27] In later time cerebral protection devices have the potential to enhance the safety of CAS.[28] First report of larger series by Wholey[29] shows that the perioperative complication rate after CAS with the cerebral protection is 1.6 % which is significantly lower than with CEA and CAS without cerebral protection.

We treat now all patients using cerebral protection filter device. In all filters we found embolic material. In two cases, filters were occluded due to a massive amount of embolic material. We suppose that, in such cases where a high risk of complications exists, it is very important to know the type of plaque, which can dislodge a large amount of embolic material. For the evaluation of plaque composition we performed ultrasound. We did not performed CAS in patients with echolucent plaques (Tip 1) due to high embolic risk.[30] Fibrous plaques seem to carry a very low risk of rupture and embolisation. We expect to learn more about plaque composition using MRI. MRI additionally shows the thickness of fibrous cap and pre-existent ruptures of the plaque.[31] By demonstrating thick or thin fibrous cap of the plaque and correlating data with the amount of emboli, trapped in the filter, we could be able to analyse the risk of periprocedural complications. This information would enable a better selection of patients for CAS. According to our initial experience on 17 patients CAS could gain more importance in stroke prevention with proper selection of patients and improved cerebral protection during procedure.

References

1. Hurst RW. Carotid angioplasty. *Radiology* 1996; **201:** 613-16.
2. Žvan B. Epidemiology of stroke in Republic of Slovenia. *Acta Clin Croat* 1998; **37 (Suppl 1):** 95-7.
3. De Bakey M. Carotid endarterectomy revisited. *J Endovasc Surg* 1996; **3:** 1-4
4. Riles TS, Lieberman A, Kopelman, I, Imparato AM. Symptoms, stenosis, and bruit: interrelationships in carotid artery disease. *Arch Surg* 1981; **116:** 218-20.
5. Sze PC, Reitman D, Pincus MM, Sacks HS, Chalmers TC. Antiplatelet agents in the secondary prevention of stroke: Meta-analysis of the randomized control trials. *Stroke* 1988; **19:** 436-42.
6. Hass WK, Easton JD, Adams HP and the Ticlopidine Aspirin Stroke Study Group. A randomized trial comparing ticlopidine hydrochloride with aspirin for the prevention of stroke in high-risk patients. *N Engl J Med* 1989; **321:** 501-7.
7. CAPRIE Steering Committee. A randomised, blinded trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). *Lancet* 1996; **348:** 1329-39.
8. Eastcott HHG, Pickering GW, Rob CG. Reconstruction of internal carotid artery in a patient with intermittent attacks of hemiplegia. *Lancet* 1954; **2:** 994-6.
9. Pokras R, Dyken ML. Dramatic changes in the performance of endarterectomy for diseases of the extracranial arteries of the head. *Stroke* 1988; **19:** 1289-90.
10. Barnett HJM, Plus F, Walton JN. Carotid endarterectomy: an expression of concern. *Stroke* 1984; **15:** 941-3.
11. North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med* 1991; **325:** 445-53.

12. European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: Interim results for symptomatic patients with severe (70-99 %) or with mild (0-29 %) carotid stenosis. *Lancet* 1991; **337:** 1235-43.

13. Albers GW, Hart RG, Lutsep HL, Newell DW, Sacco RL. Supplement to the guidelines for the management of transient ischemic attacks: from the Ad Hoc Committee on Guidelines for the Management of Transient Ischemic Attacks of the Stroke Council of American Heart Association. AHA medical/scientific statement. *Stroke* 1999; **30:** 2502-11.

14. Moore WS, Barnett HJ, Beebe HG, Bernstein EF, Brener BJ, Brott T, et al. Guidelines for carotid endarterectomy: a multidisciplinary consensus statement from the Ad Hoc Committee, American Heart Association. *Stroke* 1995; **26:** 188-201.

15. Raithel D. Recurrent carotid disease: optimum technique for redo surgery. *J Endovasc Surg* 1996; **3:** 69-75.

16. Bergeron P, Chambran P, Benichou H, Alessandri C. Recurrent carotid disease: will stents be an alternative to surgery? *J Endovasc Surg* 1996; **3:** 76-9.

17. Brown MM. Balloon angioplasty for extracranial carotid disease. *Advances in Vascular Surgery* 1996; **4:** 53-69.

18. Mathias K. Ein neues Katherersystem zur perkutanen transluminal Angioplastie von Karotidstenosen. *Fortschr Med* 1977; **95:** 1007-11.

19. Kerber CW, Hornwell LD, Loehden OL. Catheter dilatation of proximal carotid stenosis during distal bifurcation endarterectomy. *AJNR* 1980; **1:** 348-9.

20. Wholey MH, Eles G, Jarmolowski CR, Lim MC, Vozzi C, Londero H, et al. Percutaneous transluminal angioplasty and stents in the treatment of extra-cranial circulation. *J Invasive Cardiol* 1996; **9:** 225-31.

21. Henry M, Amor M, Masson I, Henry I, Tzvetanov K, Chati Z, et al. Endovascular treatment of atherosclerotic internal carotid artery stenosis. *J Endovasc Surg* 1997; **4(Suppl.1):** 1-14.

22. Naylor AR, Bolia A, Abbott RJ, Pye IF, Smilth J, Lennard N, et al. Randomized study of carotid angioplasty and stenting versus carotid endarterectomy: a stopped trial. *J Vasc Surg* 1998; **28(2):** 326-34.

23. DeMonte F, Peerless SJ, Rankin RN. Carotid transluminal angioplasty with evidence of distal embolisation. *J Neurosurg* 1989; **70:** 138-41.

24. Theron J, Courtheoux P, Alachkar F, Bouvard G, Maiza D. New triple coaxial catheter system for carotid angioplasty with cerebral protection. *AJNR* 1990; **11:** 869-74.

25. Ohki T, Marin M, Lyon R, Berdejo GL, Soundararajan K, Ohki M, et al. Ex vivo human carotid artery bifurcation stenting: correlation of lesion characteristics with embolic potential. *J Vasc Surg* 1998; **27:** 463-71.

26. Roubin GS, Hobson RW 2nd, White R, Diethrich EB, Fogarty TJ, Wholey M, et al. CREST and CARESS to evaluate carotid stenting: time to get to work! *J Endovasc Ther* 2001; **8(2):** 107-9.

27. Veith FJ, Amor M, Ohki T, Beebe HG, Bell PRF, Bolia A, et al. Current status of carotid bifurcation angioplasty and stenting based on a consensus of opinion leaders. *J Vasc Surg* 2001; **33(2 Suppl):** S111-6.

28. Jiri J., Vitek A, Gary SR, Al-Mubareka N, Gishel N, Sriram SI. Carotid artery stenting: technical considerations. *AJNR* 2000; **21:** 1736-43.

29. Wholey MH. Will protection devices set free carotid angioplasty? Carotid Angioplasty Conference 2001. CV Channel Internet.

30. de Bray JM, Baud JM, Delanoy P, Camuzat JP, Dehans V, Descamp-Le Chevoir J, et al. Reproducibility in ultrasonic characterization of carotid plaques. *Cerebrovasc Dis* 1998; **8(5):** 273-7.

31. Hatsukami TS, Ross R, Polissar NL, Chun Yuan C. Visualization of fibrous cap thickness and rupture in human atherosclerotic carotid plaque in vivo with high-resolution magnetic resonance imaging. *Circulation* 2000; **102:** 959-64.

Radiol Oncol 2002; 36(1): 13-22.

Endosonographic appearance of the anal sphincters in patients following colostomy

Iwona Sudoł-Szopińska[1], Marek Szczepkowski[2], Anna Panorska[3], Wiesław Jakubowski[1]

[1]*Department of Diagnostic Imaging, Second Faculty of Medicine, Warsaw, Poland*
[2]*Second Surgical Department, Bielany Hospital, Warsaw, Poland*
[3]*Desert Research Institute-DHS, Reno, NV, USA*

Background. *The aim of the study was to visualize, by anal ultrasound (AUS), the suspected defects of the anal sphincters in the patients after colostomy and to analyze possible factors that could have led to such defects.*

Patients and methods. *AUS, using a 7.0 MHz endorectal probe, was performed in a group of 25 patients with colostomy. The internal anal sphincter (IAS), external anal sphincter (EAS) and puborectalis muscle (PR) were visualized and the defects within them were qualified and quantified. For statistical analysis, the analysis of variance (ANOVA) was used.*

Results. *The IAS was thin in all but three patients (22 patients; 88 %) with the mean thickness of 1.62 mm. A circular reduction of the thickness along the entire length of the IAS was seen in 20 patients (90.9 %). The echogenicity of the IAS was increased in 15 patients (60 %), and in 10 of them (66.6 %), this defect embraced the whole length and circumference of the IAS. The margins of the IAS were not well-defined in 10 patients (40 %). A significant correlation was found between the length of the patient's life with the stoma and the IAS echogenicity defect (p-value = 0.0001). No significant correlation was found between the dynamic examination, the IAS thickness and the IAS borders definition.*

Conclusion. *The reduced thickness, increased echogenicity and borders definition defect of the IAS are seen in the patients after colostomy. The only significant correlation was confirmed between the length of the patient's life with the stoma and the IAS echogenicity defect.*

Key words: colostomy; anus – ultrasonography

Received 11 November 2001
Accepted 26 November 2001

Correspondence to: Iwona Sudoł-Szopińska, MD, PhD, Zakład Diagnostyki Ultrasonograficznej, Wojewódzki Szpital Bródnowski, ul. Kondratowicza 8, 03-285 Warszawa; Phone/Fax +48 22 811 95 91; Mobile 0 501 716 407; E-mail: mdyvonne@wp.pl

Introduction

Anal ultrasonography (AUS) is well established as a method of the visualization of normal and damaged sphincters. The defects of the internal anal sphincter (IAS) and external anal sphincter (EAS) are well represented. The structural abnormalities of either muscle as well as more subtle abnormalities of the internal sphincter smooth-muscle texture and composition can be identified.[1] AUS has now replaced electromyographic mapping in the demonstration of the defects of the sphincters.[2] This examination is safe, easy to perform and causes little patient discomfort.

Endosonographic assessment of the anal sphincters is very important before deciding whether or not to perform decolostomy or close a loop colostomy.

In order to recognize the suspected defects of the anal sphincters in patients after colostomy, we evaluated a group of patients with colostomy, assessed the IAS, EAS and puborectal muscle (PR), and analyzed possible factors that could have led to such defects.

Patients and methods

Patients

Twenty-five consecutive patients with colostomy were investigated by AUS between March 3, 2000 and June 30, 2000. The group comprised 17 women and 8 men with a median age of 71 (47 to 82 years old). No perianal operation was performed before and after colostomy. Two women had forceps deliveries (one gave birth to four children, with the largest baby weighing 3000 g and the second delivered twice with the babies weighing 3500 g each), two others had the second-degree tear of the peritoneum and four gave birth to babies with the birth weight exceeding 4000 g. Additional six with no complications at the delivery gave birth to one or two children with the birth weights below 4000 g and the last three women had not given birth.

The patients were inquired about defecation problems before operation: constipation and anal incontinence for gas and feces. Six patients (all women) complained about constipation present longer than ten years. All but one patient incontinent for gas were subjectively satisfied with the level of continence.

In our analyzed group of patients with colostomies, there were six patients with loop-colostomies; the remaining nineteen had end-colostomies in the course of Hartmann's operation. The most frequent indication for performing colostomy was adenocarcinoma of the sigma (fifteen patients). Others were as follows: rectal carcinoma (one patient), adenocarcinoma of the colon descendens (one), adenocarcinoma of the rectosigmoid (one), diverticulitis (two), complicated diverticulitis (one), colon ischemia (one), dehiscence anastomosis (two), tumor inflammatorious sigmae and urinary bladder (one).

The length of life with stoma ranged from eight weeks to sixteen years (median 38.17 months).

Methods

AUS was performed with the ultrasound scanner Bruel & Kjaer type 1846, Naerum, Denmark using a rotating 7.0 MHz transducer (type 1850). The transducer was covered by a plastic cone with an external diameter of seventeen mm and filled with degassed water. All the examinations were performed and read by one independent radiologist who was blind to the length of the patients life with the stoma and to clinical information. No bowel preparation was indicated. The examinations were performed in the left lateral position. Serial images were assessed along the length of the anal canal.

Image analysis

The thickness of the IAS was measured from the screen of the machine using electronic calipers in the distal and proximal part of the anal canal in each quadrant of the IAS. The echogenicity and margins of the IAS were assessed as increased or normal and not well-defined or well-defined, respectively. The EAS and the PR echogenicity were assessed as normal or not-homogenous/decreased. The dynamic activity of the EAS and PR was observed during rest and maximal contraction of these muscles and diagnosed subjectively by examining physician as: normal, poor or lack of contraction.

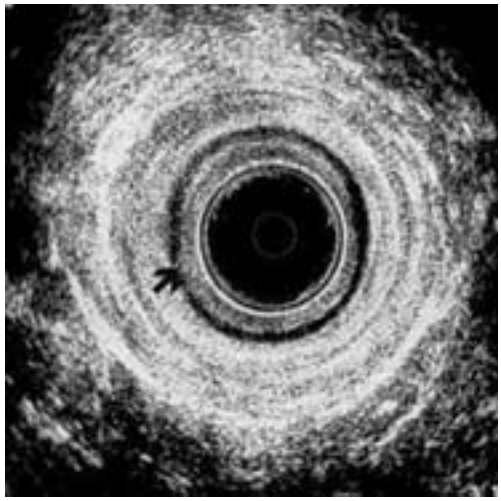


Figure 1. Endosonographic transverse image of the anal sphincters thinning in patient following colostomy: the thickness of the IAS in the distal part of the anal canal is less than 1 mm (arrow). Note that the echogenicity is normal (hypoechoic) and borders of the sphincter are well defined.

Results

In all but 3 patients (88 %), the IAS was thin with the mean thickness of 1.62 mm. Twenty patients had a circular reduction of the thickness (90.90 %) and remaining 2 (9.09 %) partial, i.e. restricted to one or two walls of the anal canal. In 12 patients, the entire length of the IAS was equally thin over the complete circumference, in 5, a circular reduction was seen only in the proximal part, and in 3 patients, only in the distal part of the anal canal (Figure 1). In one patient, the thinning of the IAS was not circular, but occurred on the posterior wall in a deep part, and in one patient, only on the posterior wall in a proximal part and on the lateral walls in the distal part of the anal canal. Three patients (12 %) had normal thickness of the IAS – at least 3.0 mm. Their length of life with stoma was 1.5, 14 and 18 months (mean 11.17 months). For the remaining patients with thin IAS, the thickness of the IAS was the smallest (less than 1 mm in nine cases) in patients with the colostomy from 8 weeks to 10 years (mean 24.94 months); the thickness of at least one but below 2 mm was seen in 7 patients living with the stoma from 3 months to 16 years (mean 43.57 months). The thickest IAS (at least 2 but less than 3 mm, six cases) was observed in the patients who had the stoma from 5 months to 10 years (mean 42.67 months).

Echogenicity of the IAS was increased in 15 patients (60 %) who lived with the stoma between 1.2 and 16 years (mean 7.71 years). Six of them (40 %) had colostomy for less than one year and remaining 9 (60 %) for a longer time. Ten patients out of 15 (66.6 %) had a circular increase of echogenicity seen within the entire length of the IAS (71.42 %) and their length of life with the stoma was from 6 months to 10 years, (mean 34.35 months). In 4 cases, it was seen only in the proximal part and the length of life with the stoma ranged from 1.5 months to 10 years (mean 56 months). One patient with the increased echogenicity had colostomy for 16 years in the distal part of the IAS.

The borders of the IAS were not well defined in 10 patients (40 %), including 7 who had the stoma from 1.5 to 11 months (mean 5.28 months) and 3 with the length of life with the

stoma of 4, 5 and 10 years (mean 6.33 years; i.e. 75.96 months). In 6 of these 10 patients, the margins of the IAS were not well defined only within a proximal part of the anal canal. All but one had stoma for less than one year. In 4 cases, the increased echogenicity was observed on the whole sphincter. These patients mostly had colostomy for a longer time.

The dynamic assessment of the EAS and PR in AUS was good in 18 patients (72 %), poor in 3 (12 %) and no contraction was seen in 4 patients (16 %). The length of life with the stoma in the patients with good contraction ranged from 1.5 months to 16 years (mean 40.72 months), in the patients with poor contraction from 5 to 18 months (mean 8.17 months), and in the patients without contraction of the muscles from 3 months to 3.5 years (mean 15.38 months).

Scars inside the EAS were seen in 4 patients and in one patient in both, the EAS and the PR.

We have performed standard statistical analysis to assess if any significant correlation exists between the length of the patient's life with the stoma and the IAS thickness, echogenicity, border and the results of the dynamic exam.

Detailed results are given below. All significance tests were performed on 0.05 significance level and the results were reported accordingly, unless otherwise noted.[3]

Statistical analysis of results

The length of the patient's life with the stoma versus IAS thickness

We have started by computing the Pearson correlation coefficient between the length of the patient's life with the stoma and the IAS thickness. It amounted to 0.025, which is not statistically significant, considering the 0.05 levels. The next step was to divide the observations into the following 4 categories with respect to IAS thickness: IAS thickness less than 1 mm, IAS thickness at least 1 but less than 2 mm (between 1 and 2), IAS thickness at least 2 but less than 3 mm (between 2 and 3), and IAS thickness at least 3 mm. The statistics for the length of the patient's life with the stoma for each IAS thickness category are summarized in Table 1.

With IAS thickness as a categorical variable we performed analysis of variance (ANOVA) to detect any significant relation between the IAS thickness and the length of the patient's life with the stoma. ANOVA yielded no significant results. Therefore we believe that we do not have enough evidence to draw any significant conclusion regarding the length of the patient's life with the stoma and IAS thickness.

The length of the patient's life with the stoma versus IAS echogenicity

The IAS echogenicity was recorded as a categorical variable with three categories: ″0″ –

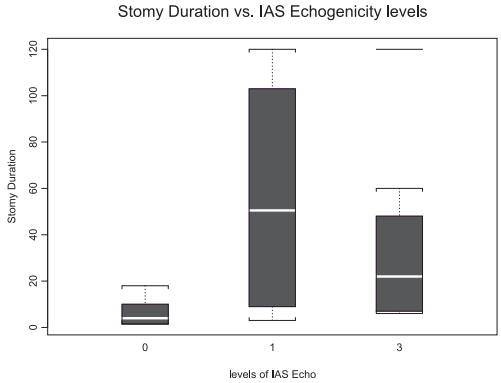
Table 1. Summary statistics for the length of the patient's life with the stoma versus IAS thickness category

IAS thickness (in mm)	Mean length of life with stoma (in months)	95 % Confidence Interval* for mean length of life with stoma (in months)	Number of patients
Below 1	24.94	(0, 55.6)	9
Between 1 and 2	43.57	(0, 110.3)	7
Between 2 and 3	42.67	(0, 86.17)	6
At least 3	11.17	NA (only 3 observations)	3
Overall	32.76	(12.8, 52.7)	25

*The confidence intervals were truncated from below at zero

normal, not changed, "1" – increased echogenicity in the deep part of the anal canal, "2" – increased echogenicity in the superficial part of the anal canal, "3" increased echogenicity of the entire muscle. ANOVA confirmed a significant effect (p value = 0.0001) of the length of the patient's life with the stoma on echogenicity. That means that not all the mean lengths in different echogenicity categories were the same. Further analysis (pairwise comparisons) showed that the mean length of the patients' life with the stoma in normal ("0") echogenicity category was significantly shorter (p value = 0.01) than that of the patients with an increased echogenicty (categories "1", "2" and "3" combined). Moreover, the mean length of the patients life with the stoma in the "0" echogenicity category was significantly shorter (p value = 0.0059) than that of the patients in the "1" echogenicity category. All other pairwise comparisons of the mean lengths of the patients' life with the stoma in different IAS echogenicity categories were not significant. The statistics for the length of the patient's life with the stoma for each IAS echogenicity category are summarized in Table 2.

The "box-and-whiskers" Figure 2 shows different distributions of the lengths of the patient's life with the stoma (in months) for the three main echogenicity categories. The solid horizontal line in each box is located at the median of the length of the patient's life with the stoma. The ends of the box show the lower and upper quartiles and "whiskers" extend to the maximum and minimum values of the length of the patient's life with the stoma for each IAS echogenicity category. Note the visible increase of the average length of life with the stoma of the patients with increased echogenicity.



Levels of IAS echogenicity: 0-normal IAS echogenicity; 1-increased echogenicity of the IAS in a deep part of the anal canal; 3-increased echogenicity of the whole sphincter.

Figure 2. The length of life with the stoma versus IAS echogenicity levels.

The length of the patient's life with the stoma versus IAS borders definition

The IAS border/margin definition was recorded as a categorical variable with three categories: "0" – normal, not changed, "1" – decreased border definition in the deep part of the anal canal only, and "2" – decreased bor-

Table 2. Summary statistics for the length of the patient's life with the stoma versus IAS echogenicity

IAS echogenicitys category	Mean length of life with stoma (in months)	95 % Confidence Interval* for length of life with stoma (in months)	Number of patients
0	5.95	(2.02, 9.88)	10
1	56	(0, 145.48)	4
2	NA (only one observation)	NA	1
3	34.35	(8.68, 60.02)	10

*The confidence intervals were truncated below at zero

Table 3. Summary statistics for stoma duration versus IAS border definition category

IAS border definition	Mean length of life with stoma (in months)	95 % Confidence Interval* for mean length of life with stoma (in months)	Number of patients
0	31.93	(2.41, 61.45)	15
1	17.67	(0, 52.86)	6
2	58.5	(0, 133.42)	4

*The confidence intervals were truncated below at zero

Table 4. Summary statistics for the mean length of life with stoma versus the results of the dynamic exam

Dynamic exam result	Mean length of life with stoma (in months)	95 % Confidence Interval* for mean length of life with stoma (in months)	Number of patients
1	15.38	(0, 43.98)	4
2	8.17	(0, 29.76)	3
3	40.72	(13.54, 67.91)	18

*The confidence intervals were truncated below at zero

der definition in the entire muscle. ANOVA detected no significant effect of the border definition on the mean length of the patient's life with the stoma. The statistics of the length of the patient's life with the stoma for each IAS border definition category are summarized in Table 3.

The length of the patient's life with the stoma versus the results of dynamic examination

Dynamic exam results were recorded as a categorical variable with three categories: "1" – low, "2" – poor, and "3" – good contraction capabilities. ANOVA showed no significant effect of the mean length of the patient's life with the stoma on the dynamic exam results. The statistics for the length of the patient's life with the stoma for each exam result category are summarized in Table 4.

Discussion

Endosonographic assessment of the anal sphincters is very important before deciding for decolostomy after Hartmann's operations or closing a loop colostomy.

We did not find any information on the anatomy of the anal sphincters after colostomy with defunctioning ileostomy in the literature. There is very little information about the anatomy of these muscles after IPAA where the thickness of the IAS is significantly smaller compared with normal subjects.[4,5] This finding was also the most striking abnormality we found in the majority of the patients after colostomy. Twenty two patients (88 %) had a thin IAS with the mean thickness 1.62 mm. Twenty of them (90.90 %) had a circular reduction of the IAS thickness and the remaining two (9.09 %) partial, i.e. restricted to one or two walls. Within the group with a circular thinning of the IAS, twelve (60 %) had the entire muscle equally thin, in the remaining eight (40 %), the thinning was seen only within the posterior or lateral walls and in the distal or proximal part of the anal canal.

The IAS thickness and proportion of fibrous tissue increases with age.[1] Several hypothetical reasons for the thinning of IAS are being proposed, such as denervation, ische-

mia or changes caused by direct trauma to the IAS as in patients after the ileal pouch anal anastomosis (IPAA) due to transanal mucosectomy.[4-6] This thinning, however, was visible in the patients with mucosectomy as well as after handsewn anastomosis. During the IPAA, the brunt of the injury is due to the dissection and mobilization of the anorectum, which may damage the extrinsic autonomic nerve supply that is crucial for the IAS function.[7] The transection of the rectal wall at the level of the levator ani muscles may cut through the layer of specialized circular muscle that forms the IAS. The damage so inflicted involves the intramural nerve plexus, blood supply and the muscle itself. Such direct damage to the IAS at this level is presumably inevitable.[6] The transection at a higher level, as in the case of our patients, is unlikely to produce such defect. In a study which used anal manometry and endosonography, a strong correlation between the endosonographic thickness of the IAS and the resting pressure was found.[1,8] There are, of course, several reasons leading to abnormal image of the IAS in AUS, including the thinning of this muscle. Passive fecal incontinence is related to the degeneration of the IAS smooth muscle, where the muscle atrophies and is replaced by fibrous tissue.[9] AUS reveals thin, hyperechoic sphincter with poor defined margins, without the usual increase in the thickness of the IAS with age. This condition affects predominantly older men and women. Mean age of our population with colostomy was 76 years and we cannot exclude this fact as accountable for the abnormalities in the IAS detected by the anal ultrasound, especially since very few patients had evidence of structural defects of IAS or EAS and of an EAS weakness. Likewise, in neurogenic, idiopathic incontinence, the denervation of the EAS and pelvic floor muscles is associated with the damage to the IAS.[10] The muscle damage is probably the result of autonomic denervation. In our material, the scars of the EAS were seen in 4 patients: in 3 women, of whom 2 were without risk factors for the sphincter damage and one having a typical episiotomy scar, and a man who was incontinent for gas. He was the only patient suffering from fecal incontinence before colostomy was performed. He was 76 years old with a colostomy for 16 years. Anal ultrasound detected a thinning only in the proximal part of the IAS, the increased echogenicity only in the distal part of the anal canal, and the scars in the EAS not affecting its contraction in the dynamic examination.

Thirty-eight per cent of women with forceps deliveries experience symptoms of incontinence and up to 2 % of women delivering vaginally have a third -degree obstetric tear involving one or both of the anal sphincters.[1] Although women constitute the main group suffering from fecal incontinence, none of our patients did have it. However, there were a few with the risk factors, including two with obstetric tears, two others after forceps deliveries, and four having delivered babies with the birth weight exceeding 4000 g. In AUS, the thinning of the IAS was seen in all of them, the increased echogenicity of the whole muscle in six; and in the proximal part of the anal canal in the remaining two. In one case, a scar after episiotomy was seen. The dynamic examination was normal. In this group, two women who had babies with the birth weight exceeding 4000 g and two with the second-degree tears of the peritoneum suffered from constipation for more than 10 years.

AUS may be considered to be used also in the patients with obstructed defecation in order to identify those patients with internal sphincter hypertrophy or its degeneration after permanent constipation with straining. In our group, 6 patients suffered from constipation for more than 10 years before colostomy was done.[11] AUS revealed thinning, increased echogenicity and not well-defined margins of the IAS in 4 of them; in another 2 only the thinning of the IAS was seen.

We regarded the length of the patient's life with the stoma as the main factor accountable for an abnormal image of the sphincters in AUS. Three patients had the normal thickness of the IAS (at least 3 mm) and their mean time of life with the colostomy was 11.17 months. Nine patients with the IAS thickness below 1 mm (which was the smallest) had the colostomy for mean 24.94 months. Seven patients with the IAS thickness at least one but below 2 mm had colostomy for 40.57 months on average. The thickest but still thin (at least 2 but below 3 mm) IAS was seen in 6 patients with colostomy for a mean time of 42.67 months. It is interesting that patients with the shortest life with the stoma (mean 11.17 months) had normal thickness of the IAS, whereas in the rest of the patients with longer life with the stoma the IAS was thinned. The results of the statistical analysis however didn't show any significant difference between the IAS thickness and the length of life with stoma. The IAS was the thinnest in the patients with the average life with the stoma of 24.94 months and it was the thickest in the patients with a longer period of colostomy.

The analyses of the IAS echogenicity showed that it was increased in 15 of the patients (60 %). Nine patients (60 %) had the colostomy for more than a year (mean 7.71 years) and 6 (40 %) less than a year. The length of time with the stoma appeared to correlate well with the echogenicity disturbances within the IAS. The most typical was the circular increase of echogenicity seen within the whole length of the IAS (10 patients – 66.62 %); however, there was no correlation, if not reverse, between the length of the patient's life with the stoma and the range of abnormal echogenicity. A circular defect was seen in the patients with the mean colostomy of 34.35 months. A partially increased IAS was seen in the deep part of the anal canal after an average of 56 months of life with colostomy and in the distal part of the anal canal in the patient with the longest life with the stoma (16 years). Our statistical analysis showed that the mean life with the stoma stoma in the patients with normal echogenicity of the IAS was significantly shorter than that in the patients with increased echogenicity ($p = 0.01$). However we could not find any significant correlation between the subgroups of patients with the abnormal echogenicity of the IAS and their length of life with colostomy.

The margins of the IAS were not well defined in 10 patients (40 %) and in most of them (seven cases – 70%) already after a mean colostomy of 5.28 months. Although this abnormality was seen in less than half of the patients, it appeared to have inflicted the IAS very early. Also, interestingly, 6 out of these 10 patients did not have well defined margins of the IAS only inside a deep part of the anal canal and all but one of them had the stomas for less than one year (mean 17.67 months). In 4 remaining cases, the whole sphincter had abnormal margins and these were all cases except one with a long life with the stoma (mean 58.5 months). ANOVA however, showed no significant correlation between the length of the patient's life with the stoma and the border definition defects.

We have not performed manometry or electromyography (EMG); so, the only way we could assess the function of the striated muscle was the anal ultrasound during contraction of the anal sphincter. This imaging technique applied during contraction of the anal sphincter is a useful adjunct to the standard study at rest. It gives better definition of the EAS and PR and is helpful in defining whether or not a defect is present.[2] The ultrasound image of a defect correlates well with absent electromyographic activity, and the former is more accurate at picking up lesions.[1] Eventually, anal endosonography reduced the need for EMG to identify EAS defects. In our group of patients with no contraction (4 cases), AUS depicted scars inside the EAS in one case only. There was no

history of obstetric trauma, operations or any factors that could lead to such a defect. Poor contraction was seen in 3 patients, 2 of them women (one delivered a large baby with the birth weight exceeding 4000 g, the second had forceps delivery) and one man with no risk factors. The impaired contraction of the EAS and PR was also not related to the length of life with the colostomy. The mean length of life with the stoma in the majority of the patients with good contraction (18 patients- 72%) was 40.66 months. In the patients with poor contraction (3 patients- 12%), that mean length was 8.16 months and in patients without contraction (4 patients; 16%), the mean life with the stoma was 15.37 months. No significant effect of the length of the patient's life with the stoma on the dynamic exam result was found.

This study confirmed the suspicion that the patients after colostomy have anal sphincters, especially IAS defects. In an attempt to identify other factors predictive of the anal sphincter complications, we took into account the age, gender, obstetric history, history of constipations, fecal incontinency and the length of the patient's life with the stoma. It appeared that only a few of analyzed factors had an effect on the sphincters defect. These findings were unexpected to us because we thought that constipation, obstetric trauma, as well as the length of the patient's life with the stoma would have a significant effect on the sphincter outcome. In our study, we found a correlation only between the length of the patient's life with the stoma and the changes in echogenicity of the IAS. However, there was no correlation between the length of life with colostomy and the range of those defects. The thickness of the IAS was preserved in patients with the short life with the stoma, but there was no correlation between the patient's length of life with the stoma and thinning of the IAS. Although our findings could implicate the dysfunction of the IAS, we would like to emphasize that their value is limited because manometry, electromyography or pudental nerve terminal motor latency (PNTML) were not performed in order to obtain a better assessment of the function deterioration of the IAS. The obstetric history, tears, forceps and birth weight exceeding 4000 g showed surprisingly little correlation with the IAS defects. All 8 women with the above risk factors had thin IAS, but only in 2 of them, the whole muscle was thin. In majority of cases, the increased echogenicity of the IAS was noted; in most of them, it involved the whole muscle, but 4 of them also experienced coexisting constipations. We have a few explanations for these findings. First, our population of patients was older (mean age 76 years) and presumably many other comorbid illnesses had influenced the image of the IAS, including degeneration mentioned above. Second, constipations lasting for a long time with accompanying straining can led to the degeneration manifested in AUS as thinning, increased echogenicity and poorly defined borders of the IAS. Six our patients with colostomy suffered from constipation and all of them had thin IAS, whereas increased echogenicity and not poorly defined margins were seen in four of them. Third, presumably the most crucial defect of the presented group of patients is the degeneration of the anal sphincters as the result of a lack of the intestinal passage in the rectum and of abnormal activity of the sphincter muscles during the defecation.

Endosonographic assessment of the anal sphincters is very important before deciding for decolostomy after Hartmann's operations or closing a loop colostomy. A reduction of thickness of the IAS and as circular reduction inside the whole muscle is the most typical disorder for this condition due to the stoma. No correlations were found between the extent of the thinning and the length of the patient's life with the stoma, although the thickness was well preserved through the first year after colostomy was done. A discrepancy

was also noticed among the length of life with the stoma and the range of increased echogenicity of the IAS. This defect occurred late after colostomy. Although echogenicity was normal in patients with the shortest colostomy in comparison to those with a longer ones, between those with a longer colostomy with different range of the echogenicity defects no correlation was found. Our statistical analysis showed that the only characteristic of the IAS and EAS significantly associated with the length of the patient's life with the stoma was the IAS echogenicity and that this variable was preserved only in the patients with the shortest colostomy.

The margins defect occurred early after the colostomy had been performed and, with time, the range of this defect increased. However, the results of the statistical analysis showed no significant correlation between those two: the length of life with colostomy and the borders defect.

References

1. Kamm MA. Obstetric damage and faecal incontinence. *Lancet* 1994; **10:** 730-3.

2. Rieger NA, Downey PR, Wattchow DA. Short communication: endoanal ultrasound during contraction of the anal sphincter-improved definition and diagnostic accuracy. *Br J Radiol* 1996; **69:** 665-7.

3. Johnson RA, Witchen DW. *Applied multivariate statistical analysis*. London: Prentice Hall; 1992.

4. Silvis R, Eekelen JW, Delemarre JBVM, Gooszen HG. Endosonography of the anal sphincter after ileal pouch-anal anastomosis. Dis Colon Rectum 1995; **38:** 383-8.

5. Tuckson W, Lavery I, Oakley J, Church J, Milsom J. Manometric and functional comparison of ileal pouch anal anastomosis with and without anal manipulation. *Am J Surg* 1991; **161:** 90-6.

6. Lavery IC, Tuckson WB, Easley KA. Internal anal sphincter function after total abdominal colectomy and stapled ileal pouch-anal anastomosis without mucosal proctectomy. *Dis Colon Rectum* 1989; **32:** 950-3.

7. Williams NS, Marzouk DEMM, Hallan RI, Waldron DJ. Function after ileal pouch and stapled pouch-anal anastomosis for ulcerative colitis. *Br J Surg* 1989; **76:** 1168-71.

8. Speakman CTM, Kamm MA. The internal anal sphincter-new insights into faecal incontinence. *Gut* 1991; **32:** 345-6.

9. Vaizey CJ, Kamm MA, Bartram CI. Primary degeneration of the internal anal sphincter as a cause of passive faecal incontinence. *Lancet* 1997; **349:** 612-5.

10. Lubowski DZ, Nicholls RJ, Burleigh DE, Swash M. Internal anal sphincter in neurogenic fecal incontinence. *Gastroenterology* 1988; **95:** 997-1002.

11. Nielsen MB, Rasmussen OO, Pedersen JF, Christiansen J. Anal endosonographic findings in patients with obstructed defecation. *Acta Radiol* 1993; **34:** 35-8.

Radiol Oncol 2002; 36(1): 23-31.

Endosonography of the puborectalis muscle- interobserver comparison of the anal and vaginal ultrasonography

Iwona Sudoł-Szopińska[1], Marek Szczepkowski[2], Anna Panorska[3], Wiesław Jakubowski[1], Dariusz Sarti[4]

[1]Department of Diagnostic Imaging, Second Faculty of Medicine, Warsaw, Poland,
[2]Second Surgical Department, Bielany Hospital, Warsaw, Poland,
[3]Desert Research Institute-DHS, Reno, NV, USA,
[4]Department of Proctology, Warsaw County Hospital, Poland

Background. *The aim of this study was to compare the anal ultrasonography (AUS) and transvaginal ultrasonography (TVUS) and also the interobserver variability assessment in the above comparison of visualization and dynamic activity assessment of the puborectalis muscle (PR).*

Patients and methods. *AUS and TVUS were performed in 25 women aged 20-72 years (median age 42). All examinations were performed by Bruel and Kjaer system, using a 7.0-MHz rotating endoprobe covered with a water-filled hard cone. All women were examined by two operators and AUS and TVUS were performed in each case.*

Results. *In 15 out of 25 women (60 %), a better definition of the PR was achieved in TVUS than in AUS. Both observers agreed with these findings. In the assessment of the PR function a discrepancy between the two methods and the two observers was found: in 4 women by both observers (16%) and in an additional 3 women by observer 1 (28 %). In all these cases, the PR function appeared to be better in TVUS than in AUS.*

Conclusions. *AUS and TVUS enable assessment of the morphology and dynamic activity of the PR. In the majority of cases (60 %), the PR was better visualized by means of TVUS than in AUS. In the assessment of the PR function, both methods were inconsistent in 7 cases (28 %) by the operator 1 and in 4 cases (16 %) by the operator 2. In all these cases, TVUS showed a better PR function than AUS. In 3 cases (12 %), we found the interobserver disagreement in the PR function assessment.*

Key words: anal ultrasonography; vagina – ultrasonography; puborectal muscle

Correspondence to: Iwona Sudoł-Szopińska, MD, PhD, Zaklad Diagnostyki Ultrasonograficznej, Wojewódzki Szpital Bródnowski, ul. Kondratowicza 8, 03 285 Warszawa: Phone/Fax +48 22 811 95 91, Mobile 0 501 716 407; E-mail: mdyvonne@wp.pl or uro@waw.pl

Received: 11 November 2001
Accepted: 4 January 2002

Introduction

Anal ultrasound (AUS) enables accurate assessment of the anal sphincters and is a useful method in the diagnostics of patients with different pathologies of the anal canal, including fecal incontinence. In addition to the visualization of the internal anal sphincter, the anal ultrasound allows the assessment of the morphology and contraction activity of the striated muscles of the anal canal, including the puborectalis muscle (PR).[1,2] AUS also supplements other traditional tests for the assessment of muscle contractions such as: electromyography (EMG), manometry and pudendal nerve terminal motor latency (PNTML).[3-5] The visualization of the anal canal is also possible after introduction of the endoanal probe into the lumen of the vagina.[6-8] The assessment of the deep part of the anal canal, representing the PR location, is then possible. The PR muscle loops around the posterior wall of the anal canal and, during contraction, creates a sharp angle between the rectum and the anal canal. It is this angle, together with the annulus anorectalis, that is considered to be among the most important factors responsible for gas and feces continence.[9]

The aim of this study was twofold:

1. a comparison of the transvaginal ultrasound (TVUS) and AUS with the use of the same anal endoprobe, and
2. an assessment of the interobserver variability in the above comparison of visualization and dynamic activity assessment of the puborectalis muscle.

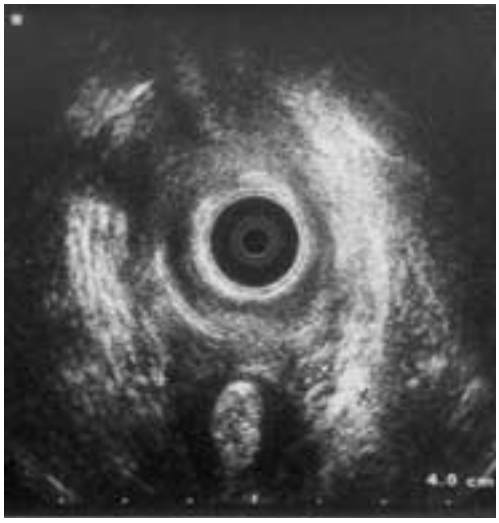


Figure 1a. Transvaginal endosonography with the use of axial endoprobe: resting image showing the loop of puborectalis (between crosses).

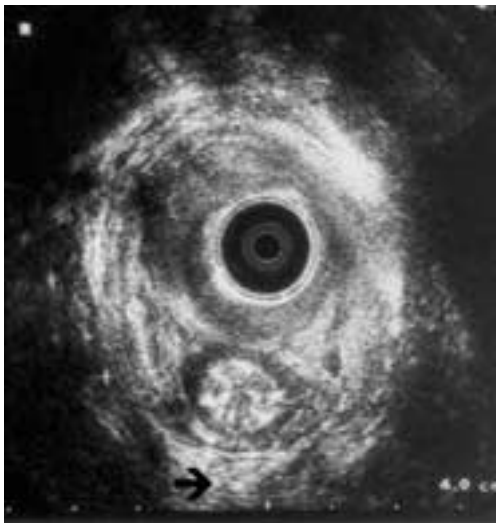


Figure 1b. Transvaginal endosonography with the use of axial endoprobe: image during contraction showing distinctly contracted puborectalis (between crosses), with well visible posterior aspect of the muscle (arrow).

Material and method

Ultrasonography was performed on a group of 25 women aged 20-72 years (median age 42). Eighteen women were multiparous and 7 nulliparous. Four women had Crohn's Disease (CD), 3 suffered from ulcerative colitis (UC), 2 had ileostomies because of UC, 1 had ileostomy because of CD and fecal incontinence after the delivery complicated by a third degree tear and recto-vaginal fistula, 1

had colostomy because of rectal adenocarcinoma, 2 suffered from constipations, 1 had a third degree tear after delivering a 4000 mg baby. The 11 remaining women did not suffer from any disturbances nor had a history of obstetric trauma or any surgery.

Eight of the 25 women were incontinent: 1 nulliparous with UC for gas and 7 for gas and feces, including both women with the history of the perineal tear, 2 with UC and 3 other who were multiparous.

To analyze the interobserver variability in the assessment of the PR contraction in TVUS and AUS, a study was designed with two doctors (observers). Each patient was examined by both of them. Before the study, they agreed on the examination procedure. All examinations were performed in the left lateral position. No bowel preparation was made. A Bruel and Kjaer ultrasound scanner type 1846 (Naerum, Denmark) was used. It was equipped with the 7.0 MHz rotating endoprobe with the focal range of 2-5cm and covered with a hard sonolucent plastic cone (external diameter 1.7 cm) and filled with degassed water.

First, the probe was introduced into the lumen of the vagina. Second, AUS was performed. Each time, the PR echotexture, outlines and dynamic activity were assessed.

The defect within the PR was defined on the basis of visualization of the hypoechoic area within the normal striated PR architecture and comparison with the opposite branch of this muscle. The muscle's outlines were assessed as distinct or not well visible. The dynamic activity of the muscle was assessed according to a subjective scale as lack of, poor or good (well visible) contraction. It was assessed on the basis of the comparison between the images of the PR taken at rest and during the maximal contraction.

The results of the TVUS and AUS performed by the two doctors were analyzed and compared retrospectively. The degree of agreement between the radiologists separately for

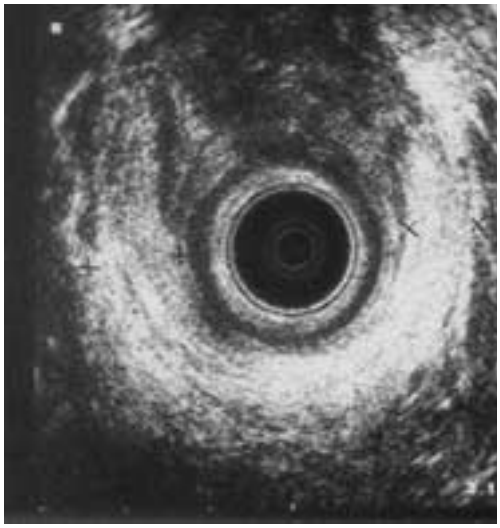


Figure 2a. Anal ultrasonography with the use of axial endoprobe: resting image showing the loop of puborectalis (between crosses).

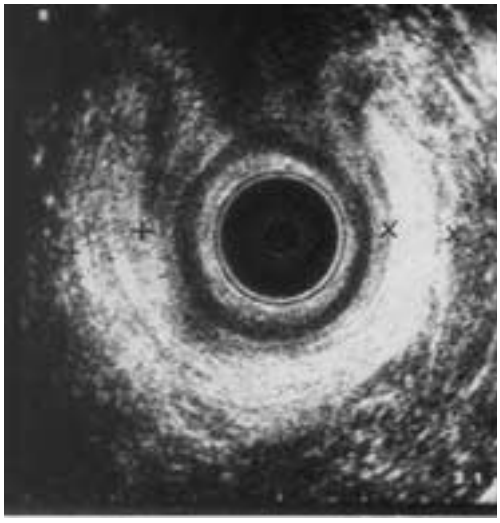


Figure 2b. Anal ultrasonography with the use of axial endoprobe: image during contraction without noticeable reaction of the muscle (the same patient).

AUS and TVUS was quantified. The percentage of the patients in the study on whose results the radiologists were in agreement were presented within the 95 % confidence intervals. The results of the two examination methods by the two radiologists were also compared.

Results

In 15 out of 25 women (60 %), a better definition of the PR outlines together with its posterior loop was obtained by TVUS than by AUS. Both observers agreed with this assessment.

In 2 women (8 %), a hypoechoic scar was visible in the right branch of PR. It was seen in both TVUS and AUS by both observers. Both women had episiotomies.

Table 1. Results of the study by observer and diagnostic method

Number of patients	TVUS 1	AUS 1	TVUS 2	AUS 2
1	0	0	0	0
2	1	1	1	1
3	1	1	1	1
4	2	1	2	1
5	2	0	2	0
6	2	2	2	2
7	2	2	2	2
8	2	2	2	2
9	1	1	1	1
10	2	2	2	2
11	2	1	2	1
12	1	0	1	0
13	0	0	0	0
14	1	1	1	1
15	2	2	2	2
16	2	2	2	2
17	1	0	1	1
18	1	1	1	1
19	2	1	1	1
20	0	0	0	0
21	2	2	2	2
22	2	1	1	1
23	1	1	1	1
24	2	2	2	2
25	0	0	0	0

TVUS1 = transvaginal ultrasonography made by radiologist 1; AUS1 = anal ultrasonography made by radiologist 1; TVUS2 = transvaginal ultrasonography made by radiologist 2; AUS2 = anal ultrasonography made by radiologist 2

0 = lack of contraction of the puborectalis muscle; 1 = poor contraction; 2 = good contraction of the puborectalis muscle

In the PR function assessment, a discrepancy between two methods and two observers was noted (Table 1). Discrepancies between TVUS and AUS results were found by both observers in 4 women (16 %) and in an additional 3 only by observer 1 (7 women, 28 %). These discrepancies between the diagnoses of both radiologists in the 4 women were as follows:

PR good contraction in TVUS and poor in AUS – 2 cases;

PR poor contraction in TVUS and lack of it in AUS – 1 case;

PR good contraction in TVUS and lack of it in AUS – 1 case.

Additionally, only observer 1 found the following differences in diagnoses for 3 more women:

a) PR good contraction in TVUS and poor in AUS – 2 cases;
b) PR poor contraction in TVUS and lack of it in AUS – 1 case.

In each of these 3 cases, the observer 2 consistently diagnosed poor PR contraction using both techniques. In the remaining 18 cases (72 %), the diagnoses of both observers for both methods were unanimous as follows:

a) PR good contraction – 8 cases;
b) PR poor contraction – 6 cases;
c) PR lack of contraction – 4 cases.

Discussion

Anal ultrasound is a reliable method for the visualization of the layered structure of the anal canal and it is a useful method for the diagnostics of the anal canal diseases and consequences of injuries to the anorectal area. The visualization of the anal canal is also possible using the transvaginal approach which has several advantages.[7] First, the anus is not disturbed, not compressed by the insertion of the probe into the lumen of the anal canal, so

the inner diameter of the IAS, its thickness and anal cushions may be measured in their true resting state.[7] Although this characteristics are of little diagnostic value with regard to the anal incontinence, this approach may allow the assessment of the anal canal if pain or stenosis are present. In the study by Poen et al.[8], TVUS added important information to that obtained by AUS in 25 % of the patients with fecal incontinence and perianal sepsis. In spite of a limited visualization range being considered as drawback of TVUS, the same study[8] concluded that it was not possible to image the anal sphincters in only 10 % of the patients. In the remaining 90 % the IAS, EAS and PR were visible and the defects such as sepsis possible to diagnose. The PR passes directly backward from the back of the pubis with its inner surface in contact with the lateral walls of the vagina or prostate and the anorectal junction.[9] Two legs of the PR meet at the posterior of the anorectal junction to form a sling with the angle to the anorectal junction of 92 ° during rest and 137 ° during straining.[9] The main function of the PR is to contribute to the maintenance of the anorectal angle, thereby producing a flap valve effect when the intra-abdominal pressure rises.[9] This effect can not be visualized by defecography and only EMG is available to determine the contribution of the EAS and the PR to the continence and defecatory mechanisms.[9] The denervation of the PR in cases of idiopathic fecal incontinence can also be demonstrated using transrectal PNTML measurements.[9] Although the results of manometry correlate well, but not always with EMG potentials for the EAS,[9] the assessment of only the PR is not possible with the use of manometry.

The loop of the PR is well visible in AUS and appoint the deep part of the anal canal. Transvaginal PR assessment is a useful adjunct technique to the standard anal ultrasonography. In spite of being limited to the deep canal level, TVUS, in many cases, allows a better visualization of the PR than AUS. In our study, such a situation was observed in 15 women (60 %) where both observers noted better visualization of the PR by TVUS than by AUS. We felt that an effect of perspective created by the distance between the vagina and the anal canal was the most probable reason for this finding. This perspective provided a better visualization of the whole loop of the PR and its external outlines as well as the muscle contraction. We therefore believe that the difficulties in the visualization of the posterior side of the EAS using TVUS, mentioned by Poen et al.[8] referred to the subcutaneous and superficial parts of the anal canal not its deep part and the level of PR, which is very well visible by TVUS.[7] Another finding of Poen et al.[8] was a poor correlation between TVUS and AUS in diagnosing the defects of the EAS which were seen in AUS but could not be reproduced by TVUS. The authors suggested that AUS might have overestimated the EAS defects and suspected that the artifacts from the air in vagina or fibres from the deep transverse perineal muscle or stretching the anal canal by a probe might have been responsible for that result. Again, this study referred only to the EAS and not the PR and we could not find any data in the literature concerning solely the PR and diagnosing its defects. In 8 % of our patients (two women), the hypoechoic defects were seen in the right branches of the PR. Both women had a history of episiotomies and both defects were seen by the two observers in TVUS and AUS. We do not have any reason to suspect any overaging of one method over another in defining the defects of the PR. Generally, because the definition of this muscle in TVUS as well as in AUS is very good and much less, if not at all, controversial to the EAS image, we believe that our diagnoses were correct.

Apart from the morphology of the anal canal muscles, endosonography enables the assessment of the contraction activity of the striated muscles of the anal canal, including

PR.[1] The imaging technique during the contraction of the anal sphincter is already known as a useful adjunct to the standard study at rest.[1] Given a better definition of the EAS, it is helpful in defining the defects in 62 % of the patients.

Because continence depends on the function of these muscles, their accurate diagnosis requires, first of all, an assessment of the neuromuscular axis of the anorectum. The evaluation usually begins with the palpation followed by manometric measurement of pressures within the anal canal at rest and during squeezing or by means of EMG. PNTML is also assessed.[6] Although manometry is the most widely used examination of the anal sphincter function, the assessment of the PR only cannot be achieved by this method. Additionally, in the patients with a deep defect of the EAS, the manometry does not always correlate with AUS findings and usually shows a normal function of the EAS. The reason can be a pull-through technique of manometry which may show a short anal canal or suggest a proximal defect, whereas a sleeve technique will not give this information.[10] Similarly, EMG, although correlating highly with AUS in mapping of the EAS defects, is suitable only for the assessment of a superficial and subcutaneous parts of the EAS.[11] The deep parts of the EAS and PR are beyond the reach of the standard 3 cm concentric needles.[10,11] In short, it is difficult to assess the function of the PR. First, apart from the limitations of the most common methods presented above, the majority of these methods cannot differentiate between the striated muscles, the EAS and PR. Second, interpretation of the findings of these tests frequently differs from one radiologist to another. Finally, they do not provide the surgeon with the anatomic information needed to plan an anatomic repair.[2,6] AUS enables a clear imaging of the IAS, the EAS and the PR. The accuracy of clinical examination in diagnosing these muscles' defects is 50 %, whereas that of EMG and of anal manometry is 75 % each.[2] AUS is more accurate than clinical and conventional physiological methods.[2] We did not find any data in the literature referring to the possibilities of endosonography in the assessment of the function of either the PR or EAS. This is not surprising since this examination is focused on the imaging of the morphology rather than physiology of the anal muscles. In this study, we found such a possibility for both AUS and TVUS which, especially in the light of the above difficulties in assessing the function of the PR, could have a considerable diagnostic value.

In the majority of women (18 women; 72 %), the assessment of the PR contraction (i.e. lack/poor/good) was identical in TVUS and AUS. The 95 % confidence interval for the overall percentage of identical diagnoses using TVUS and AUS was then 50.4 % and 87.1 %, respectively. We also analyzed the percentage of identical diagnoses for each radiologist. The 95 % confidence intervals for these individual percentages were 50.4 % and 87.1 % for radiologist 1 and 63.1 %, 94.7 % for radiologist. In order to find out whether the two diagnostic methods yielded significantly different results, we needed to decide on a threshold percentage of identical diagnoses. The threshold would be used as an evaluation tool. If the confidence interval for the percentage of identical diagnoses reaches the threshold, we may infer that the two diagnostic methods do not differ significantly. We decided to use a subjective, yet reasonable threshold of 85 %. Since all above confidence intervals reach the threshold of 85 %, we may conclude that with reference to 95 % confidence level the two diagnostic methods do not differ significantly. The details about the differences in diagnoses using TVUS and AUS are presented below.

Discrepancies were noted in 7 women by operator 1 and, in 4 of them, also by operator 2. In all these cases, the result of TVUS was better than that of AUS, i.e. the lack of or po-

or PR contraction in AUS appeared poor or good in TVUS. In one case, confirmed by the two operators, the discrepancy was most pronounced. A good contraction of the PR, detected by TVUS could not be confirmed by AUS, which showed a lack of contraction. None of these seven women with inconsistent diagnoses of the PR function suffered from fecal incontinence. All women with the symptoms of fecal incontinence were in the group of 18 women for whom both operators on the basis of two approaches consistently diagnosed the lack of contraction of the PR (4 women) and poor contraction (3 women). The eighth woman incontinent only for gas had good contraction of the PR. It is known that the damage to the PR may be inflicted during parturition.[10] All (10) women with impaired (poor or lack of) PR contraction diagnosed by both radiologists by TVUS and AUS were multiparous. Two of them had additional history of the third degree perineal tear and two suffered from UC. Two women suffering from constipations had normal function of the PR seen by the two operators by both methods, TVUS and AUS. It is known that, in idiopathic anorectal incontinence and rectal prolapse, with or without incontinence, often associated with a long history of excessive straining during defecation in the constipated patients, the PR is not at all or only sligthly damaged.[10,12] The innervation of the PR is most probably responsible for its preserved function in constipated patients. Likewise the anterior part of the EAS, the PR and levator ani muscle receive innervation from the perineal nerve.[13] The main nerve supply to PR arises from the direct branches of S3 and S4 reaching the muscle from above the pelvic floor.[10]

We assume that two reasons could be responsible for the discrepancies in the diagnosis of the PR contraction by TVUS and AUS (7 cases by operator 1 and 4 cases by operator 2). First, similar to a better visualization of the PR in TVUS, a better assessment of its activity by TVUS could be due to a different perspective that we achieved from the lumen of the vagina. Second, the discrepancies could be due to a kind of mental sensation in some patients who were unable to contract PR naturally having the probe introduced into the anal canal. Thus, the PR function visualized by TVUS was better. In our study, we did not observe any reverse reaction, namely worse (i.e. lack or poor) PR contraction by TVUS and better (i.e. good or poor) by AUS. This finding is in accordance with the second suggestion above. Moreover, it was supported by clinical data. Except multiple deliveries in 6 out of 7 women with worse diagnosis of the PR contraction by AUS than by TVUS, all had a history of peritoneal tear or constipation, therefore, we did not have any reasons to suspect that TVUS was incorrect.

In the assessment of the PR morphology in all 25 examined cases, the diagnoses of the two observers agreed. Knowing the endosonographic appearance of the PR, it is not difficult to recognize its defects. Such finding seems to be objective. The interpretation of the dynamic activity of the PR varied between the two operators and caused some diagnostic differences in our study. In 3 cases (12 %), we found the interobserver variability in the estimation of the PR contraction. This variability could have been due to a subjective scale we have used to assess the PR function. An objective scale, like the flow measurements, B-mode and Doppler ultrasound measurements, does not eliminate all the interobserver variability.[14] In an ideal situation, the data variance due to observers should be non-significant and the effect of the operator can be eliminated entirely by cooperative training and surely by using the same operator in both examinations.[14]

In this study, we chose 85 % as a threshold of agreement between the radiologists as adequate to define insignificant interobserver variability at a 95 % confidence level.

We quantified the degree of agreement between the radiologists separately for TVUS

and AUS. For TVUS, a 95 % confidence interval for the percentage of agreement was 72.5 % and 98.6 %, respectively. For AUS, the corresponding confidence interval was 77.7 % and 99.8%, respectively. The overall percentage of agreement between the two radiologists and for both methods was 72 % (18 patients out of 25) with a 95 % confidence interval (50.4 %, 87.1 %). Since our threshold of 85% was attained by all confidence intervals, we may conclude that the interobserver variability, although present, was not significant.

Conclusions

AUS is currently a method of choice for obtaining detailed images of the IAS, EAS and PR. It is unable to detect denervation of the EAS and PR, although the identification of the sphincters with no scars, a thickened IAS and a decreased anal sonography index (which means thin EAS and thick IAS) seem to be the indicators of a denervation pathogenesis.[15] Because it is painless, available, inexpensive and rapid, AUS is recommended as the examination suitable for screening.[3,4] Our study showed that AUS and TVUS of the anal canal can be useful adjuncts to physiologic studies of anorectal function. Of course, as imaging techniques they are not the methods of choice for the assessment of the anorectal functions. For this purpose, anal manometry, EMG and PNTML are suggested because they offer full and objective estimation of the functional disorders of the anal sphincters. The anal ultrasound supported by transvaginal ultrasonography seem to be valuable methods in assessing the PR morphology. They are also very promising in initial diagnosis of the PR function indicating an eventual necessity to perform more precise, more objective but less available method as e.g. manometry or EMG.

References

1. Rieger NA, Downey PR, Watchow DA. Short communication: endoanal ultrasound during contraction of the anal sphincter – improved definition and diagnostic accuracy. *Br J Radiol* 1996; **69:** 665-7.

2. Sultan AH, Kamm MA, Talbot IC, Nicholls RJ, Bartram CI. Anal endosonography for identification external sphincter defects confirmed histologically. *Br J Surg* 1994; **81:** 463-5.

3. Law PJ, Kamm MA, Bartram CI. A comparison between electromyography and anal endosonography in mapping external anal sphincter defects. *Dis Colon Rectum* 1990; **33(5):** 370-3.

4. Tjandra JJ, Milsom JW, Schroeder T, Fazio VW. Endoluminal ultrasound is preferable to electromyography in mapping anal sphincteric defects. *Dis Colon Rectum* 1993; **36(7):** 689-92.

5. Hill MC, Rifkin MD, Tessler FN. Ultrasound evaluation of the anal sphincter in fecal incontinence. *Ultrasound Quarterly* 1998; **14(4):** 209-17.

6. Alexander AA, Liu JB, Merton DA, Nagle DA. Fecal incontinence: transvaginal US evaluation of anatomic causes. *Radiology* 1996; **199:** 529-32.

7. Frudinger A, Bartram CI, Kamm MA. Transvaginal versus anal endosonography for detecting damage to the anal sphincter. *AJR* 1997; **168:** 1435-8.

8. Poen AC, Felt-Bersma RJ, Cuesta MA, Meuwissen GM. Vaginal endosonography of the anal sphincter complex is important in the assessment of faecal incontinence and perianal sepsis. *Br J Surg* 1998; **85:** 359-63.

9. Rasmussen O. Anorectal function. *Dis Colon Rectum* 1994; **37:** 386-403.

10. Bartolo DC, Jarratt JA, Read MG, Donnelly TC, Read NW. The role of partial denervation of the puborectalis in idiopathic faecal incontinence. *Br J Surg* 1983; **70:** 664-7.

11. Rieger NA, Sweeney JL, Hoffmann DC, Young JF, Hunter A. Investigation of fecal incontinence with endoanal ultrasound. *Dis Colon Rectum* 1996; **39(8):** 860-4.

12. Burnett SJ, Speakman CT, Kamm MA, Bartram CI. Confirmation of endosonographic detection of external anal sphincter defects by simultaneous electromyographic mapping. *Br J Surg* 1991; **78:** 448-50.

13. Parks AG, Swash M, Urich H. Sphincter denervation in anorectal incontinence and rectal prolapse. *Gut* 1997; **18:** 656-65.

14. Paivansalo MJ, Suramo I, Merikanto J, Lindholm EL. Interobserver, interequipement and intersubject variability of echo-Doppler examination of the common carotid and vertebral arteries. *Eur J Ultrasound* 1998; **7:** 145-51.

15. Emblem R, Dhaenens G, Stien R, Morkrid L, Aasen AO, Bergan A. The importance of anal endosonography in the evaluation of idiopathic fecal incontinence. *Dis Colon Rectum* 1994; **37(1):** 42-8.

Radiol Oncol 2002; 36(1): 33-40.

Modulation of radiotherapy- and chemotherapy-induced normal tissue response as prophylaxis of their side effects

Pavlína Plevová

Department of Radiotherapy, University Hospital, Ostrava, Czech Republic

Background. *Ionising radiation and cytostatic agents used in cancer therapy induce an immune response in normal tissues mediated by cytokines and adhesion molecules. Strategies modulating this response may downregulate cancer therapy side effects. The data published on the given topic have been reviewed.*
Conclusions. *The strategies influencing the tissue immune response with the aim to reduce the side effects of chemotherapy and radiotherapy are conflicting. Some of them inhibit this response supposing that an exaggerated reaction may have a damaging effect (e.g. corticosteroids, nonsteroidal anti-inflammatory drugs (NSAID), lisofylline, anti-cytokine antibodies, anti-sense oligonucleotides, sialyl Lewis X analogues), others promote this reaction by inducing endogenous production of cytokines (AS101) or use recombinant forms of appropriate cytokines involved in this response in order to intensify the physiologic tissue response. In clinical practice, corticosteroids and NSAID are widely used to modulate this response, while other agents are still experimental.*

Key words: radiotherapy – adverse effects; antineoplastic agents; antineoplastic agents – adverse effects; adjuvant, immunologic

Introduction

Ionising radiation and cytostatic agents used in cancer therapy exert damaging effects on normal tissues and induce there a complex response at the cellular and molecular levels. Cytokines and adhesion molecules are released during this response and mediate intercellular interactions among the effectors of immune and other systems.[1,2] Medical strategies that modulate this response in order to reduce chemotherapy- and radiotherapy-induced side effects are contradictory. Some of them inhibit this reaction, and their use is based on the hypothesis that exaggerated or persisting inflammatory response enhances the tissue damage; others stimulate this response in order to enhance physiological protective processes.

Received 25 February 2002
Accepted 11 March 2002

Correspondence to: Pavlína Plevová, M.D., Fr. Lyska 8, Ostrava-Belsky les, 700 30 Czech Republic, Phone: +420 69 6717841 or +420 69 6984370; Fax: +420 69 6919010; E-mail: pavlina.plevova@volny.cz

Acknowledgements: The author is indebted to Pavel Vodvářka for his helpful comments and Lenka Zivčáková for technical assistance.

a) Inhibition of the tissue response

Glucocorticoids exert strong anti-inflammatory effects including inhibition of pro-inflammatory cytokine production.[1-5] The molecular mechanism of their effects is not completely understood, but they inhibit the activity of some transcription factors.[3] In clinical practice, corticosteroids are used to prevent or treat chemotherapy-induced nausea and vomitus[6] and to prevent radiation- and chemotherapy-induced pneumonitis and fibrosis.[7,8] Although corticosteroids suppressed radiation pneumonitis in an experimental model they were not able to reduce pulmonary fibrosis development.[9] In another study, short-term use of dexamethasone suppressed temporarily radiation-induced pro-inflammatory cytokine gene expression in the mouse lung, but a rebound was observed after the drug withdrawal and the drug did little to change the essence and course of the pneumonitic process.[10] Dexamethasone is widely used in the prophylaxis of radiation-induced brain oedema and inflammation; this effect was demonstrated on an experimental model.[7,11] Dexamethasone significantly reduces the incidence of the somnolence syndrome after prophylactic cranial irradiation in children with leukemia.[12] Betamethasone was beneficial in radiation-induced oral mucositis in a few patients.[13] Dexamethasone delays the development of experimental radiation nephropathy; it does not stop the progression of injury.[14,15] Captopril, an angiotensin convertase enzyme inhibitor, enhanced the beneficial effect of dexamethasone in radiation nephropathy.[15] Corticosteroids suppress cytokine secretion in irradiated animal skin.[16] They reduce hematotoxic effects of 5-fluorouracil and methotrexate, but not of other cytostatic agents in an experimental model.[17]

Nonsteroidal anti-inflammatory drugs (NSAID) inhibit the prostaglandin synthesis through cyclooxygenase blockade,[18] activation of the transcription factor of nuclear factor κB (NF-κB)[19] and adhesion of neutrophils as a result of a decreased expression of L-selectine.[18] In clinical practice, they are used in the treatment of fever, pain, and fatigue associated with chemotherapy and radiotherapy. Mesalazine has been studied in the prevention of oral mucositis;[20] however, the result of this non-randomised study lacks clinical relevance. Indomethacine did not influence the survival of lethally irradiated mice.[21]

Lisofylline is a xanthine derivative able to inhibit the release of various cytokines, such as TNF-α, TGF-β, MIP-1α, IFN-γ, Il-1β, IL-6, IL-10.[22,23] Its mechanism of action is thought to involve inhibition of acyl-substituted unsaturated phosphatidic acid, a second messenger lipid implicated in pro-inflammatory cytokine cellular activation.[24,25] It also decreases white cell adhesiveness.[26] Lisofylline inhibited 5-fluorouracil-induced release of TGF-β and maybe also other hematopoiesis inhibiting cytokines and thus enhanced trilineage hematopoietic recovery after 5-fluorouracil treatment in mice.[27]

Pentoxifylline is a xanthine derivative with profound immunomodulatory properties in vitro, including inhibition of TNF-α, Il-1β and IL-10 release.[23,28] Elevated levels of TNF-α have been shown to correlate with both the development and severity of transplantation-related complications.[29] Although pentoxifylline reduced these complications in a study,[30] these results were not reproduced in others including the one focused on 5-fluorouracil-induced oral mucositis.[31-33]

The results of a study with a TNF-α neutralising monoclonal antibody in transplant patients lack clinical significance.[34]

Intravenous immunoglobulin, especially in high doses, has profound immunomodulatory effects, including the inhibition of anti-inflammatory cytokine release;[35,36] in addition, high TGF-β concentrations have been detected in intravenous immunoglobulin preparations.[37] Intravenous or intramuscular immunoglobulin has been studied sporadically in the prophylaxis or therapy of irradiation or

chemotherapy-induced oral mucositis and radiation pneumonitis. It is not possible to make a definite conclusion of its effects from these results.[38-41]

As TGF-β plays an important role in the pathogenesis of fibrosis development, its inhibition might reduce the risk of this complication. Neutralising antibodies to both TGF-β_1 and TGF-β_2 significantly reduced the bleomycin-induced increase in the accumulation of lung collagen in an experimental model;[42] However, fibrosis was ameliorated only partially.[43] TGF-β antisense oligonucleotides, short synthetic deoxyribonucleotide oligomers complementary to DNA, prevent protein production.[44] They have been investigated in the prevention of experimental peritoneal fibrous adhesions.[45] There are no reports on their use in association with chemotherapy or radiotherapy.

IL-4 has been shown to be able to downregulate radiation-induced production of mediators of inflammation, including IL1β in the lung, suggesting its anti-inflammatory potential in regulating the radiation-induced response.[46]

Interferon γ, taurine, and niacin reduced bleomycin-induced pulmonary fibrosis in an animal model via TGF-β inhibition and subsequent procollagen expression downregulation.[47-49]

The endothelial selectins (E-selectin and P-selectin) bind to sialylated tetrasaccharide sialyl Lewis X and A counter receptors on neutrophils, monocytes and lymphocytes, mediating their emigration into the tissue.[50,51] The analogues of sialyl Lewis X such as glycyrrhizin and carminic acid bind to E-selectin on irradiated endothelial cells and thereby inhibit adhesion of leukocytes and inflammatory response in vitro.[52]

b) Stimulation of the tissue response

It has been known for more than forty years that immunomodulators stimulating the cells of the reticulo-endothelial system can protect against deleterious effects of radiation.[53]

AS 101 (ammonium trichloro (dioxyethylene-0,0′) tellurate) stimulates some subpopulations of white cells and increases the release of various cytokines, including IL-1, IL-2, IL-6, TNF-α, GM-CSF, stem cell factor (SCF), and IFN-γ.[54-57] AS 101 reduces hematotoxic effects of cyclophosphamide, 5-fluorouracil, doxorubicine, lomustine, carboplatin and etoposide,[56-59] and alopecia after carboplatin and etoposide.[57] It also has been shown to exert radioprotective effects.[60]

The physiological role of cytokines in the immune response and tissue regeneration has led to experiments studying the effectiveness of recombinant forms of cytokines in the protection of normal tissues from damaging effects of chemotherapy and radiotherapy. The results of these experimental studies were successful, depending on the schedule and the dose of the cytokine used.[61,62]

Recombinant IL-1α, TNF-α, INF-γ administered before treatment reduced hematotoxic effects of both irradiation and chemotherapy with various agents.[21,62-70] G-CSF, GM-CSF, SCF act as radioprotectors both in vitro and in vivo;[71-75] on the contrary, their concomitant administration with chemotherapy increases the sensitivity of hematopoietic cells to its cytotoxic effects.[76] MIP-1α exerts chemoprotective effects on bone marrow cells.[77] IL-1α also reduced small gut and lung toxicity of radio- or chemotherapy.[78-80]

The combination of IL-1 and TNF-α had synergistic effects.[66] Both cytokines are relatively toxic due to their physiological roles in inflammation, especially after systemic application.[81] G-CSF and GM-CSF are well tolerated and potentiate radioprotective effects of IL-1.[66]

Local application of TGF-β3 on oral mucosa significantly reduced the 5-fluorouracil-induced oral mucositis in hamsters.[82,83] IL-1, EGF, FGF have been shown to protect mice against ARA-C-induced alopecia.[84,85]

The mechanism of the protective effects of cytokines might be explained by the following hypothesis: 1) Exogenous cytokines activate the physiological pathways of immune response through their receptors, thus activating and amplifying the defence of the organism. The induction of enzymes with antioxidant effects[86,87] could be a part of this response. 2) Some cytokines, such as IL-1 or SCF, may directly or indirectly, through release of other cytokines, stimulate hematopoietic progenitor cells.[61,62,74] 3) Cytokines might inhibit the cell proliferation, thus reducing the sensitivity to proliferation-inhibiting agents or inducing the cell-cycling so that the cells enter into the relatively radio- or chemoresistent phases of the cell cycle, the S and G1 ones.[61,77] 4) Certain cytokines, such as IL-6, IFN-γ, GM-CSF, inhibit cell apoptosis including its cytotoxic agents- and irradiation-induced activation.[88-91]

Conclusions

The modulation of the tissue response to the damaging effects of radiotherapy and chemotherapy may reduce toxic effects of these treatment modalities. Only corticosteroids and NSAID are used in clinical practice to reduce acute toxicity of cancer therapy. The agents that could affect late sequels are studied experimentally; AS101 is being tested at the clinical level. The response-modifying use of recombinant cytokines to reduce toxicity of radiotherapy or chemotherapy did not progress into clinical usage. The local use of TGF-β in association with chemotherapy-induced oral mucositis is promising.

The suppression of the inflammatory response must be used with caution in the clinical practice, however. Although corticosteroids are beneficial in the modulation of acute side effects, this effect results from inhibition of the protective response that is of pivotal importance in the maintenance of organism integrity and whose suppression might have detrimental end-effects as has been demonstrated by reduced survival of mice that were administered dexamethasone after irradiation.[92,93]

References

1. Thalmeier K, Meissner P, Reisbach G, Hültner L, Mortensen BT, Brechtel A, et al. Constitutive and modulated cytokine expression in two permanent human bone marrow stromal cell lines. *Exp Hematol* 1996; **24:** 1-10.

2. Koj A. Initiation of acute phase response and synthesis of cytokines. *Biochim Biophys Acta* 1996; **1317:** 84-94.

3. Barnes PJ, Karin M. Nuclear factor-κB – a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336:** 1066-71.

4. Kerner B, Teichmann B, Welte K. Dexamethasone inhibits tumor necrosis factor-induced granulocyte colony-stimulating factor production in human endothelial cells. *Exp Hematol* 1992; **20:** 334-8.

5. Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF. Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6 but not of M-CSF in human fibroblasts. *Blood* 1992; **79:** 45-51.

6. The Antiemetic Subcommittee of the Multinational Association of Supportive Care in Cancer (MASCC). Prevention of chemotherapy- and radiotherapy-induced emesis: results of Perugia Consensus Conference. *Ann Oncol* 1998; **9:** 811-9.

7. Rubin P, Constine LS, Williams JP. Late effects of cancer treatment: radiation and drug toxicity. In: Perez CA, Brady LW, editors. Principles and practice of radiation oncology. 3rd ed. Philadelphia: Lippincott-Raven Publishers; 1998. p. 155-212.

8. Khan A, McNally D, Tutschka PJ, Bilgrami S. Paclitaxel-induced acute bilateral pneumonitis. *Ann Pharmacother* 1997; **31:** 1471-4.

9. Ward HE, Kemsley L, Davies L, Holecek M, Berend N. The effect of steroids on radiation-induced lung disease in the rat. *Radiat Res* 1993; **136:** 22-8.

10. Hong JH, Chiang CS, Tsao CY, Lin PY, Wu CJ, McBride WH. Can short-term administration of dexamethasone abrogate radiation-induced acute cytokine gene response in lung and modify subsequent molecular responses? *Int J Radiat Oncol Biol Phys* 2001; **51:** 296-303.

11. Tada E, Matsumoto K, Kinoshita K, Furuta T, Ohmoto T. The protective effect of dexamethasone against radiation damage induced by interstitial irradiation in normal monkey brain. *Neurosurgery* 1997; **41:** 209-19.

12. Uzal D, Ozyar E, Hayran M, Zorlu F, Atahan L, Yetkin S. Reduced incidence of the somnolence syndrome after prohyplactic cranial irradiation in children with acute lymphoblastic leukemia. *Radiother Oncol* 1998; **48:** 29-32.

13. Abdelaal AS, Barker DS, Fergusson MM. Treatment for irradiation-induced mucositis [letter]. *Lancet* 1989; **1:** 97.

14. Robbins ME, Bonsib SM. Radiation nephropathy: a review. *Scanning Microsc* 1995; **9:** 535-60.

15. Geraci JP, Sun MC, Mariano MS. Amelioration of radiation nephropathy in rats by postirradiation treatment with dexamethasone and/or captopril. *Radiat Res* 1995; **143:** 58-68.

16. Beetz A, Messer G, Oppel T, van Beuningen D, Peter RU, Kind P. Induction of interleukin 6 by ionizing radiation in a human epithelial cell line: control by corticosteroids. *Int J Radiat Biol* 1997; **72:** 33-43.

17. Kriegler AB, Bernardo D, Verschoor SM. Protection of murine bone marrow by dexamethasone during cytotoxic chemotherapy. *Blood* 1994; **83:** 65-71.

18. Díaz-González F, Sánchez-Madrid F. Inhibition of leukocyte adhesion: an alternative mechanism of action for anti-inflammatory drugs. *Immunol Today* 1998; **19:** 169-72.

19. Pierce JW, Read MA, Ding H, Luscinskas FW, Collins T. Salicylates inhibit I kappa B-α phosphorylation, endothelial-leukocyte adhesion molecule expression and neutrophil transmigration. *J Immunol* 1996; **156:** 3961-9.

20. Rymes N, Glick L, Holmes JA. Topical mesalazine in the treatment of chemotherapy and radiotherapy-induced oral mucositis [letter]. *Bone Marrow Transplant* 1996; **18:** 484.

21. Neta R. Role of cytokines in radioprotection. *Pharmacol Ther* 1988; **39:** 261-6.

22. Rice GC, Rosen J, Weeks R, Michnick J, Bursten S, Bianco JA, et al. CT-1501R selectively inhibits induced inflammatory monokines in human whole blood ex vivo. *Shock* 1994; **1:** 254-66.

23. van Furth AM, Verhard-Seijmonsbergen EM, van Furth R, Langermans JA. Effect of lisofylline and pentoxyphylline on the bacterial-stimulated production of TNF-α, Il-1β, IL-10 by human leucocytes. *Immunology* 1997; **91:** 193-6.

24. Bursten S, Weeks R, West J, Le T, Wilson T, Porubek D, et al. A potential role for phosphatidic acid in mediating the inflammatory responses of TNF and IL-1. *Circ Shoc* 1994; **44:** 14-29.

25. Schwaighofer H, Kernan NA, O'Reilly RJ, Brankova J, Nachbaur D, Herold M, et al. Serum levels of cytokines and secondary messages after T-cell-depleted and non-T-cell-depleted bone marrow transplantation: influence of conditioning and hematopoietic reconstitution. *Transplantation* 1996; **62:** 947-53.

26. Waxman K, Daughters K, Aswani S, Rice G. Lisofylline decreases white cell adhesiveness and improves survival after experimental hemorrhagic shock. *Crit Care Med* 1996; **24:** 1724-8.

27. Clarke E, Rice GC, Weeks RS, Jenkins N, Nelson R, Bianco JA, et al. Lisofylline inhibits transforming growth factor β release and enhances trilineage hematopoietic recovery after 5-fluorouracil treatment in mice. *Cancer Res* 1996; **56:** 105-12.

28. Tilg H, Eibl B, Pichl M, Gachter A, Herold M, Brankova J, et al. Immune response modulation by pentoxifylline in vitro. *Transplantation* 1993; **56:** 196-201.

29. Holler E, Kolb HJ, Möller A, Kempeni J, Liesenfeld S, Pechumer H, et al. Increased serum levels of tumor necrosis factor α precede major complications of bone marrow transplantation. *Blood* 1990; **75:** 1011-6.

30. Bianco JA, Appelbaum FR, Nemunaitis J, Almgren J, Andrews F, Kettner P, et al. Phase I-II trial of pentoxifylline for the prevention of transplant-related toxicities following bone marrow transplantation. *Blood* 1991; **78:** 1205-11.

31. Clift RA, Bianco JA, Appelbaum FR, Buckner CD, Singer JW, Bakke L, et al. A randomized controlled trial of pentoxifylline for the prevention of regimen-related toxicities in patients undergoing allogeneic marrow transplantation. *Blood* 1993; **82:** 2025-30.

32. Attal M, Huguet F, Rubie H, Charlet JP, Schlaifer D, Huynh A, et al. Prevention of regimen-related toxicities after bone marrow transplantation by pentoxifylline: A prospective, randomized trial. Blood 1993; **82:** 732-6.

33. van der Jagt RHC, Pari G, McDiarmid SA, Boisvert DM, Huebsch LB. Effect of pentoxifyline on regimen related toxicity in patients undergoing allogeneic or autologous bone marrow transplantation. *Bone Marrow Transplant* 1994; **13:** 203-7.

34. Holler E, Kolb HJ, Mittermüller J, Kaul M, Ledderose G, Duell T, et al. Modulation of acute graft-versus-host disease after allogeneic bone marrow

transplantation by tumor necrosis factor α (TNFα) release in the course of pretransplant conditioning: Role of conditioning regimens and prophylactic application of a monoclonal antibody neutralizing human TNFα (MAK 195F). *Blood* 1995; **86:** 890-9.

35. Nydegger U. [Old and new views on intravenous immunoglobulin therapy.] *Schweiz Med Wochenschr* 1994; **124:** 5-25 (German).

36. Wolf HM, Eibl MM. Immunomodulatory effect of immunoglobulins. *Clin Exp Rheumatol* 1996; **14 (Suppl 15):** S17-25.

37. Kekow J, Reinhold D, Pap T, Ansorge S. Intravenous immunoglobulins and transforming growth factor β. *Lancet* 1998; **351:** 184-5.

38. Proske H, Pfab R. [Immunoglobulin preparations as antiinflammatory drugs in radiotherapy]. *Med Welt* 1992; **43:** 1025-26 (German).

39. Schedler MGJ, Bost P, Federspil P, Pautler M, Schatzle W. Treatment of radiogenic mucositis in patients with head and neck tumors with polyvalent intramuscular immunoglobulin. *Tumor Diagn Ther* 1994; **15:**184-91 (German).

40. Plevová P, Blažek B. Intravenous immunoglobulin as prophylaxis of chemotherapy-induced oral mucositis. *J Natl Cancer Inst* 1997; **89:** 326-7.

41. Seibel RM, Wendt BK. Immunoglobulin as prophylaxis of ionizing-radiation induced pneumonitis following high-volume irradiation for lung cancer. *Onkologie* 1986; **9:** 43-7 (German).

42. Giri SN, Hyde DM, Hollinger MA. Effect of antibody to transforming growth factor β on bleomycin induced accumulation of lung collagen in mice. *Thorax* 1993; **48:** 959-66.

43. Laurent GJ, Coker RK, McAnulty RJ. TGF-beta antibodies: a novel treatment for pulmonary fibrosis? *Thorax* 1993; **48:** 953-4.

44. Khanna A, Li B, Li P, Suthanthiran M. Regulation of transforming growth factor-beta 1 (TGF-β1) expression with a novel TGF-β1 complementary DNA. *Biochem Biophys Res Commun* 1994; **204:** 1061-6.

45. Chegini N. The role of growth factors in peritoneal healing: transforming growth factor β (TGF-β). *Eur J Surg* 1997; **577(Suppl):** 17-23.

46. Van der Meeren A, Monti P, Lebaron-Jacobs L, Marquette C, Gourmelon P. Characterization of the acute inflammatory response after irradiation in mice and its regulation by interleukin 4 (Il4). *Radiat Res* 2001; **155:** 858-65.

47. Gurujeyalakshmi G, Giri SN. Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-β and procollagen I and III gene expresssion. *Exp Lung Res 1995*; **21**: 791-808.

48. Gurujeyalakshmi G, Hollinger MA, Giri SN. Regulation of transforming growth factor-β1 mRNA epression by taurine and niacin in the bleomycin hamster model of lung fibrosis. *Am J Respir Cell Mol Biol* 1998; **18:** 334-42.

49. Gurujeyalakshmi G, Iyer SN, Hollinger MA, Giri SN. Procollagen gene expression is down-regulated by taurine and niacin at the transcriptional level in the bleomycin hamster model of lung fibrosis. *J Pharmacol Exp Ther* 1996; **277:** 1152-7.

50. Rosen SD. Cell surface lectins in the immune system. *Semin Immunol* 1993; **65:** 237-47.

51. Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. *Science* 1992; **258:** 964-9.

52. Hallahan DE, Kuchibhotla J, Wyble C. Sialyl Lewis X mimetics attenuate E-selectin-mediated adhesion of leukocytes to irradiated human endothelial cells. *Radiat Res* 1997; **147:** 41-7.

53. Mefferd RB, Henkel DT, Loeffer JB. Effect of piromen on survival of irradiated mice. *Proc Soc Exp Biol Med* 1953; **83:** 54-6.

54. Sredni B, Caspi RR, Lustig S, Klein A, Kalechman Y, Danzinger Y, et al. The biological activity and immunotherapeutic properties of AS-101, a synthetic organotellurium compound. *Nat Immun Cell Growth Regul* 1988; **7:** 163-8.

55. Shani A, Gurwith M, Tichler T, Catane R, Rozenszajan LA, Gezin A, et al. The immunologic effects of AS 101 in the treatment of cancer patients. *Nat Immun Cell Growth Regul* 1990; **9:** 182-90.

56. Kalechman Y, Zuloff A, Albeck M, Strassmann G, Sredni B. Role of endogenous cytokines secretion in radioprotection conferred by the immunomodulator ammonium trichloro(dioxyethylene-0-0′)tellurate. *Blood* 1995; 85: 1555-61.

57. Sredni B, Albeck M, Tichler T, Shani A, Shapira J, Bruderman I, et al. Bone marrow-sparing and prevention of alopecia by AS 101 in non-small-cell lung cancer patients treated with carboplatin and etoposide. *J Clin Oncol* 1995; **13:** 2342-53.

58. Kalechman Y, Sotnik-Barkai I, Albeck M, Sredni B. Protection of bone marrow stromal cells from the toxic effects of cyclophosphamide in vivo and of ASTA-Z 7557 and etoposide in vitro by ammonium trichloro(dioxyethylene-0-0′) tellurate (AS 101). *Cancer Res* 1993; **53:** 1838-44.

59. Kalechman Y, Shani A, Sotnik Barkai I, Albeck M, Sredni B. The protective role of ammonium trichloro(dioxyethylene-0,0)tellurate in combination with several cytotoxic drugs acting by different mechanisms of action. *Cancer Res* 1993; **53:** 5962-9.

60. Kalechman Y, Albeck M, Oron M, Sobelman D, Gurwith M, Sehgal SN, et al. Radioprotective effects of the immunomodulator AS 101. *J Immunol* 1990; **145:** 1512-7.

61. Dalmau SR, Freitas CS, Tabak DG. Interleukin -1 and tumor necrosis factor-alpha as radio- and chemoprotectors of bone marrow. *Bone Marrow Transplant* 1993; **12:** 551-63.

62. Wu SG, Tuboi A, Miyamoto T. Radioprotection of C3H mice by recombinant human interleukin-1 alpha. *Int J Radiat Biol* 1989; **56:** 485-92.

63. Neta R, Keller JR, Ali N, Blanchette F, Dubois CM. Contrasting mechanisms of the myeloprotective effects of interleukin-1 against ionizing radiation and cytotoxic 5-fluorouracil. *Radiat Res* 1996; **145:** 624-31.

64. Neta R, Sztein MB, Oppenheim JJ, Gillis S, Douches SD. The in vivo effects of interleukin 1: I. Bone marrow cells are induced to cycle after administration of interleukin 1. *J Immunol* 1987; **139:** 1861-6.

65. Castelli MP, Black PL, Schneider M, Pennington R, Abe F, Talmadge JE. Protective, restorative and therapeutic properties of recombinant human IL-1 in rodent models. *J Immunol* 1988; **140:** 3830-7.

66. Neta R, Oppenheim JJ, Douches SD. Interdependence of the radioprotective effects of human recombinant interleukin 1, tumor necrosis factor, granulocyte colony-stimulating factor, and murine recombinant granulocyte-macrophage colony-stimulating factor. *J Immunol* 1988; **140:** 108-11.

67. Futami H, Jansen R, MacPhee MJ, Keller JR, McCormick K, Longo DL, et al. Chemoprotective effects of recombinant human IL-1α in normal and tumor-bearing mice. Protection from acute toxicity, hematologic effects, development of late mortality and enhanced therapeutic efficacy. *J Immunol* 1990; **145:** 4121-30.

68. Slordal L, Warren DJ, Moore MAS. Effect of recombinant murine tumor necrosis factor on hemopoietic reconstitution in sublethally irradiated mice. *J Immunol* 1989; **142:** 833-5.

69. Slordal L, Warren DJ, Moore MAS. Protective effects of tumor necrosis factor on murine hematopoiesis during cycle-specific cytotoxic chemotherapy. *Cancer Res* 1990; **50:** 4216-20.

70. Gardner RV. Interferon-gamma (IFN-γ) as a potential radio- and chemoprotectant. *Am J Hematol* 1998; **58:** 218-23.

71. Uckun FM, Gillis S, Souza L, Song CW. Effects of recombinant growth factors on radiation survival of human bone marrow progenitor cells. *Int J Radiat Oncol Biol Phys* 1989; **16:** 415-35.

72. Uckun FM, Souza L, Waddick KG, Wick M, Song CW. In vivo radioprotective effects of recombinant human granulocyte colony-stimulating factor in lethally irradiated mice. *Blood* 1990; **75:** 638-45.

73. Waddick KG, Song CW, Souza L, Uckun FM. Comparative analysis of the in vivo radioprotective effects of recombinant granulocyte colony-stimulating factor (G-CSF), recombinant granulocyte-macrophage CSF, and their combination. *Blood* 1991; **77:** 2364-71.

74. Zsebo KM, Smith KA, Hartley CA, Greenblatt M, Cooke K, Rich W, et al. Radioprotection of mice by recombinant rat stem cell factor. *Proc Natl Acad Sci USA* 1992; **89:** 9464-8.

75. Liebmann J, DeLuca AM, Epstein A, Steinberg SM, Morstyn G, Mitchtell JB. Protection from lethal irradiation by the combination of stem cell factor and tempol. *Radiat Res* 1994; **137:** 400-4.

76. Meropol NJ, Miller LL, Korn EL, Braitman LE, MacDermott ML, Schuchter LM. Severe myelosuppression resulting from concurrent administration of granulocyte colony-stimulating factor and cytotoxic chemotherapy. *J Natl Cancer Inst* 1992; **84:** 1201-3.

77. Dalmau SR, Freitas CS, Savino W. Radio- and chemoprotection of bone marrow cells by opposite cell cycle-acting cytokines. *Leuk Res* 1997; **21:** 93-9.

78. Neta R, Douches S, Oppenheim JJ. Interleukin 1 is a radioprotector. *J Immunol 1986*; **136:** 2483-5.

79. Dorie MJ. Protection by interleukin 1 against lung toxicity caused by cyclophosphamide and irradiation. *Radiat Res* 1991; **128:** 316-9.

80. Damia G, Komschlies KL, Futami H, Back T, Gruys ME, Longo DL, et al. Prevention of acute chemotherapy-induced death in mice by recombinant human interleukin 1: protection from hematological and nonhematological toxicities. *Cancer Res* 1992; **52:** 4082-9.

81. Maisin JR. Bacq and Alexander Award Lecture. Chemical radioprotection: past, present and future prospects. *Int J Radiat Biol* 1998; **73:** 443-50.

82. Sonis ST, Lindquist L, van Vugt V, Stewart AA, Stam K, Qu GY, et al: Prevention of chemothe-

rapy-induced ulcerative mucositis by transforming growth factor β3. *Cancer Res* 1994; **54:** 1135-8.

83. Sonis ST, van Vugt AG, Brien JP, Muska AD, Bruskin AM, Rose A, et al. Transforming growth factor-β3 mediated modulation of cell cycling and attenuation of 5-fluorouracil induced oral mucositis. *Oral Oncol* 1997; **33:** 47-54.

84. Jimenez JJ, Wong GH, Yunis AA. Interleukin 1 protects from cytosine arabinoside-induced alopecia in the rat model. *FASEB J* 1991; **5:** 2456-8.

85. Jimenez JJ, Yunis AA. Protection from 1-beta-D-arabinofuranosylcytosine-induced alopecia by epidermal growth factor and fibroblast growth factor in the rat model. *Cancer Res* 1992; **52:** 413-5.

86. Masuda A, Longo DL, Kobayashi Y, Appella E, Oppenheim JJ, Matsushima K. Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB J* 1988; **2:** 3087-91.

87. Wong GHW, Goeddel DV. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* 1988; **242:** 941-4.

88. Lotem J, Sachs L. Differential suppression by protease inhibitors and cytokines of apoptosis induced by wild-type p53 and cytotoxic agents. *Proc Natl Acad Sci USA* 1996; **93:** 12507-12.

89. Lotem J, Sachs L. Control of apoptosis in hematopoiesis and leukemia by cytokines, tumor suppressor and oncogenes. *Leukemia* 1996; **10:** 925-31.

90. Lotem J, Sachs L. Cytokine suppression of protease activation in wild-type p53-dependent and p53-independent apoptosis. *Proc Natl Acad Sci USA* 1997; **94:** 9349-53.

91. Mor F, Cohen IR. IL-2 rescues antigen-specific T cells from radiation or dexamethasone-induced apoptosis. Correlation with induction of Bcl-2. *J Immunol* 1996; **156:** 515-22.

92. Nam SY, Cho CK, Kim SG. Correlation of increased mortality with the suppression of radiation-inducible microsomal epoxide hydrolase and glutathione D-transferase gene expression by dexamethasone: effects on vitamin C and E-induced radioprotection. *Biochem Pharmacol* 1998; **56:** 1295-34.

93. Rudat V, Kupper JH, Weber KJ. Trans-dominant inhibition of poly(ADP-ribosyl)ation leads to decreased recovery from ionizing radiation-induced cell killing. *Int J Radiat Biol* 1998; **73:** 325-30.

Radiol Oncol 2002; 36(1): 41-6.

review

Lymphangioleiomyomatosis

Franc Anderluh

Department of Radiotherapy, Institute of Oncology, Ljubljana, Slovenia

Background. *Lymphangioleiomyomatosis is a rare disease of unknown origin, which affects women in their reproductive period. It is characterised by non-neoplastic proliferation of atypical smooth muscle cells in the lung parenchyma, lymphatic vessels and mediastinal and abdominal lymph nodes. The most common presenting symptoms are spontaneous pneumothorax, dyspnea, hemoptysis and chylothorax.*
Conclusions. *High-resolution computed tomography (HRCT) and open lung biopsy followed by the immunohistologic studies are two diagnostic procedures with which diagnosis can be confirmed. Various treatment modalities are applied, particularly hormonal therapy, though their efficacy remain unknown. The prognosis of patients is bad.*

Key words: lymphangiomyomatosis – diagnosis – therapy

Introduction

Lymphangioleiomyomatosis (LAM) is a rare disease of unknown origin which occurs exclusively in women. It is characterised by non-neoplastic proliferation of atypical smooth muscle cells in the lung parenchyma and thoracic and abdominal lymph nodes and lymphatic vessels. This leads to progressive loss of lung function and, ultimately, death. The disease was first mentioned in medical literature in 1937, and until today, not much has been known about its aetiology and treatment efficacy. The incidence varies from country to country and it seems to be increasing in recent years, which is probably due to a more extensive use of, and easier accessibility to the high-resolution computed tomography (HRCT) that is essential in LAM diagnosing.[1] According to some estimates, there are around 300 cases of LAM in the USA, whereas exact data for Slovenia are not available. In the last 20 years, 2 cases have been diagnosed at the Clinical Department of Pulmonary Diseases and Allergology at Golnik, Slovenia.[2]

The first study in which the data on a greater number of patients was gathered, was the study by Cornog and Enterline which was published in 1966. They examined 20 patients with the disease that is today known as LAM and was earlier referred to as lymphangioma, lymphangiomyoma, lymphangiopericytoma, leiomyomatosys, lymphangious malformation and intrathoracic angiomatous hyperplasia. In these first reports arguments were made in favour of the malignant charac-

Received: 22 January 2002
Accepted 5 February 2002

Correspondence to: Franc Anderluh, MD, Department of Radiotherapy, Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia; Fax +386 1 43 14 180.

ter of the disease because of the diffuse and extensive infiltration of smooth muscle cells into the soft tissue, lymphatic vessels and lymph nodes in the thorax and abdomen. Cornog and Enterline supported the view that the disease could not be regarded as malignant because of a well organised structure of lung lesions and absence of mitotic overactivity, cell atypia and distant metastases.[3]

Several authors also suggest that LAM may be a form of tuberous sclerosis complex (TSC). TSC is an autosomally inherited form of congenital hamartomatosis, which usually affects the skin and central nervous system. It is characterised by mental retardation, epileptic attacks and angiofibromas on the face. The incidence of the disease is similar in both sexes. In contrast to epileptic attacks, the TSC pulmonary lesions develop only in adults and are detected in only 0,1-2-3 % of all patients, of whom 84 % are women. Histologically, TSC pulmonary lesions are similar to that of LAM lesions.[4] Nevertheless, the relation between TSC and LAM has not been definitely confirmed.[5]

Clinical features

LAM usually affects women aged 30 to 40 years although several patients have been diagnosed as having LAM after menopause. The initial and typical signs and symptoms are advancing dyspnea on exertion, cough, hemoptysis and recurrent pneumothorax. Other signs and symptoms that develop later in the course of the disease are persistent dry cough, chest pain, chylous pleural effusion and chylous ascites. The interrupted lymphatic flow may result in the chylous ascites, chyloptysis, chyluria, chylous pericardial effusion and oedema of the lower extremities. The physical examination may reveal crackles and wheezing, clubbing and signs and symptoms of pneumothorax, pleural effusion and ascites.[6] The rate of the disease progress varies from case to case and the survival usually ranges from 10 to 20 years from the diagnosis.[2]

LAM may develop or progress considerably during pregnancy. Yet, so far, it has not been made clear whether the symptoms and signs of LAM are only detected earlier due to hemodynamic and ventilatory changes associated with pregnancy. The application of exogenous estrogens is another cause that can induce the disease or worsen the clinical picture.[7]

Renal angiomyolipoma is a rare hamartomatous tumour, composed of smooth muscle cells, blood vessels and adipose tissue. It rarely occurs autonomously, but may develop in relation to TSC and is also frequent in the patients with LAM. Usually these tumours are asymptomatic, though they may be characterised by palpable mass and pain in the lumbar region or hematuria.[8]

Histopathology

Histopathologic characteristics of LAM are diffuse cystic changes in the lung related to the proliferation of the atypical smooth muscle cells, also termed as LAM cells. The proliferation can occur in all structures of the lung. The proliferation of LAM cells in the wall of bronchioles may result in the obstruction of small airways and formation of the air-filled parenchymal cysts. The rupture of the cysts located on the surface of the lung can lead to pneumothorax. Involvement of the venules and arterioles may result in partial or total occlusion of these vessels and subsequent pulmonary venous hypertension. Hemoptysis may be due to ruptures of the small blood vessels and minor bloodshedings into alveolar spaces. Obstruction of lymphatic channels may disrupt normal lymph flow and cause chylous pleural effusions or chyloptysis. Sometimes pleura is also involved.[9]

Pulmonary emphysema additionally impairs the pulmonary function. So far, it has not

been clarified how the emphysema develops. Some authors believe that it is a result of the respiratory tract obstruction whereas others support the theory that it may be due to disintegration of the elastic tissue caused by degranulation of the elastase- filled granules which were found in the cytoplasm of LAM cells.[10]

The thoracic duct may be considerably enlarged and divided into multiple microscopic channels with poor passage by the network of smooth muscle fibbers. Pathologic changes may be observed in the intra- and extrathoracic lymph nodes. Involved nodes appear grossly spongy and resilient. Microscopic examination shows progressive replacement of the lymphatic tissue with atypical LAM cells.[11]

Other associated abnormalities include renal angiomyolipomas and abdominal or pelvic masses, which may be cystical and histologically appear as cords of smooth muscle cells and a network of lymphatic channels.[12]

Many authors have reported the presence of oestrogen and progesterone receptors in the cytosol of LAM cells. In healthy subjects these receptors were found in the myometrium, but not in the smooth muscle cells in the colon, bladder and lung. As the normal lung tissue does not display any of these receptors, most probably the hormones affect LAM tissue. However, the mechanism of this process remains unclear.[13]

Monoclonal antibody HMB-45, that recognises antigens in the cytoplasm of melanoma cell lines, specifically binds to the LAM cells, too. As in the normal lung parenchyma or in the case of interstitial lung disease this type of binding does not occur, immunohistochemical staining with HMB-45 may be a very effective diagnostic method, particularly when only a transbronchial biopsy specimen is available. HMB-45 reactivity has also been documented in retroperitoneal LAM and in renal angiomyolipomas.[14,15]

Multifocal micronodular pneumocyte hyperplasia, clear-cell tumours of the lung and non-caseating granulomas have also been noted in patients with LAM.[16]

Diagnosis

Due to the rarity of LAM and because of the frequent non-specific nature of the clinical symptoms and signs and findings on the chest radiograph, many cases of LAM are initially misdiagnosed as more common diseases, such as asthma or chronic obstructive pulmonary disease. In most cases, patients are seeing the doctor and complaining about non-specific chest pain or dyspnea on exertion as a complication of spontaneous pneumothorax. Such clinical picture is present in number of other lung diseases, so it is not a surprise that the first diagnosis is usually incorrect in almost all cases. LAM should be included in differential diagnosis, when a young female patient presents with dyspnea, recurrent pneumothorax or chylothorax.[17]

Diagnostic imaging methods

In the initial stages of the disease, the changes in the lung are so discrete that they are seen only on CT image. At this stage chest X-ray (CXR) is usually misleading. In later stages of the disease, the CXR shows a generalised, symmetric reticular or reticulonodular interstitial infiltrate in the lung parenchyma as a consequence of the smooth muscle cell proliferation in the walls of lymphatic and small blood vessels. At the beginning the infiltration is limited to the base of the lung, in later stages it is found throughout the lung parenchyma. However, these findings are non-specific and may be detected in patients with other interstitial lung diseases, as well. The lung volume in LAM is usually not changed, but due to the pulmonary emphysema, the CXR may also show evidence of hyperinflation.[18-20]

The obstruction of lymphatic vessels is seen as septal or Kerley's B lines and a rough re-

ticular pattern. In the terminal stage of the disease honeycombing, pneumothorax or pleural effusion may be seen.[20]

HRCT is a highly sensitive method for the detection of the disease in its early stages as the changes in the lung parenchyma are present long before they are seen on CXR. HRCT scans reveal the diffuse cystic changes that account for the reticulation seen on CXR. The cysts are air-filled, have thin walls and are scattered all over the lung parenchyma. Their size ranges from 2 to 60 mm and are usually round, but may be polygonal or bizarrely shaped. The surrounding parenchyma is usually normal. With the progress of the disease, the cysts are growing in size and number. Though cysts are typical for LAM, they are not pathognomic, since they may be detected also in histiocytosis X.[18-20]

In order to detect or confirm renal angiomyolipomas and enlarged retroperitoneal, paraaortic and pelvic lymph nodes it is advisable to perform ultrasonography or computed tomography of the entire abdomen.[12] Lymphangiography may detect abnormal filling and cystic changes in the involved lymph nodes.[21] Abnormal pulmonary blood flow may be detected on nuclear perfusion scan.[22]

Lung function tests

The pulmonary physiologic features of patients with LAM are variable and depend on the severity of the disease. They include obstructive, restrictive or mixed patterns. The diffusing capacity for carbon monoxide (DLCO) is reduced in most patients, resulting in some degree of blood hypoxia. Total lung capacity and residual volume are usually increased.[6]

Lung biopsy

A definitive diagnosis of LAM usually requires an open lung biopsy and the judgement of the experienced pathologist. Occasionally, transbronchial lung biopsy or even cytological analysis of pleural fluid is sufficient. In case of the extrapulmonary tissues involvement, many authors recommend biopsy of the involved structures. Whenever lung biopsy cannot be performed, the diagnosis can be established with a fair degree of certainty when characteristic HRCT findings are seen along with renal angiomyolipoma.[23,24]

Therapy

Because of the rarity of the disease and the variable clinical course little data is available on different therapeutic strategies and their success. Given the occurrence of the disease in women in child-bearing years, reports of worsening of the clinical picture following administration of exogenous estrogens, and the presence of oestrogen and progesterone receptors in the proliferating LAM cells, hormonal manipulation would seem to be appropriate for therapy. Several techniques of hormonal blockage have been proposed: ovariectomy, progesterone, tamoxifen or other antiestrogen agents, luteinizing hormone releasing hormone (LHRH) agonists or radioablation of the ovaries. In some cases various combinations of these treatments have been attempted. Responses to such treatments have been variable, and no deffinitive conclusions should be drawn from these reports.[25-28]

In addition to hormonal medications intended to interrupt the proliferation of LAM cells, various interventions aimed at preventing or treating the complications of LAM, such as pneumotorax and pleural or peritoneal fluid collections, have been advised, too. The repetitive drainage of chylous pleural fluid collections may be hazardous due to the loss of proteins. Chemical or surgical pleurodesis has been performed with variable success in preventing recurrent pneumothorax or pleural effusion. The ligation or irradiation of the thoracic duct may prevent the depositi-

on of chylous pleural effusion. Recently, the option of the lung transplantation is considered in the patients with heavily impaired lung functions in the end-stage lung disease related to LAM.[29,30] A few cases of recurrence were reported in the patients who have undergone the lung transplantation. Interestingly, in some of these cases, the donor lungs were also from males, which raises the possibility of some kind of so far unknown circulating mitogen in the pathogenesis of the disease.[31]

Conclusion

Because LAM is the disease with low incidence and because little is known about its aetiology, it should be included in the differential diagnosis in women in child-bearing years presenting with clinical signs and symptoms of dyspnea, hemoptysis and recurrent spontaneous pneumothorax. HRCT and open lung biopsy followed by the immunohistologic studies are two diagnostic procedures with which diagnosis can be confirmed. More studies have to be performed, to understand the mechanisms of the disease better, for up to date, the treatment of the disease has been rather ineffective and its prognosis is bad.

References

1. Baum GL, Crapo JD, Celli BR. *Textbook of pulmonary diseases*. 6th ed. Vol. 1. London: Little Brown; 1998. p. 467-71.

2. Mušič E, Vencelj B, Bajrovič N, Kern I, Debeljak A, Gabrijelčič J. Limfangioleiomiomatoza kot vzrok spontanih pnevmotoraksov. *Zdrav Vestn* 2000; **69:** 15-8.

3. Cornog JL, Enterline HT. Lymphangiomyoma, a benign lesion of chyliferous lymphatics synonymous with lymphangiopericytoma. *Cancer* 1966; **19:** 1909-30.

4. Uzzo RG, Libby DM, Vaughan ED Jr, Levey SH. Coexisting lymphangioleiomyomatosis and bilateral angiomyolipomas in a patient with tuberous sclerosis. *J Urol* 1994; **151:** 1612-5.

5. Bonetti F, Chiodera P. Lymphangioleiomyomatosis and tuberous sclerosis: where is the border? *Eur Respir J* 1996; **9:** 399-401.

6. Kitaichi M, Nishimura K, Itoh H, Izumi T. Pulmonary lymphangioleiomyomatosis: a report of 46 patients including a clinicopathologic study of prognostic factors. *Am J Respir Crit Care Med* 1995; **151:** 527-33.

7. Shen A, Iseman MD, Waldron JA, King TE. Exacerbation of pulmonary lymphangioleiomyomatosis by exogenous estrogens. *Chest* 1987; **91:** 782-5.

8. Kerr LA, Blute ML, Ryu JH, Swensen SJ, Malek RS. Renal angiomyolipoma in association with pulmonary lymphangioleiomyomatosis; forme fruste of tuberous sclerosis? *Urology* 1993; **41:** 440-4.

9. Carrington CB, Cugell DW, Gaensler EA, Marks A, Redding RA, Schaaf JT, et al. Lymphangioleiomyomatosis: physiologic pathologic radiologic correlations. *Am Rev Resp Dis* 1977; **116:** 977-95.

10. Hayashi T, Fleming MV, Stetler-Stevenson WG, Liotta LA, Moss J, Ferrans VJ, et al. Immunohistochemical study of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in pulmonary lymphangioleiomyomatosis (LAM). *Hum Pathol* 1997; **28:** 1071-8.

11. Graham ML, Spelsberg TC, Dines DE, Payne WS, Bjornsson J, Lie JT. Pulmonary lymphangioleiomyomatosis with particular reference to steroid-receptor assay studies and pathologic correlation. *Mayo Clin Proc* 1984; **59:** 3-11.

12. Bernstein SM, Newell JD Jr, Adamczyk D, Mortenson RL, King TE Jr, Lynch DA. How common are renal angiomyolipomas in patients with pulmonary lymphangioleiomyomatosis. *Am J Respir Crit Care Med* 1995; **152:** 2138-43.

13. Berger U, Khaghani A, Pomerance A. Pulmonary lymphangioleiomyomatosis and steroid receptors: an imunnocytochemical study. *Am J Clin Pathol* 1990; **93:** 609-14.

14. Chan JKC, Tsang WY, Pau MY, Tang MC, Pang SW, Fletcher CD. Lymphangioleiomyomatosis and angiomyolipoma: closely related entities characterized by hamartomatous proliferation of HMB-45-positive smooth muscle. *Histopathology* 1993; **22(5):** 445-55.

15. Chan JK, Tsang WY, Pau MY, Tang MC, Pang SW, Fletcher CD. Lymphangioleiomyomatosis and angiomyolipoma: closely related entities characterized by hamartomatous proliferation of HMB-45 positive smooth muscle. *Histopathology* 1993; **22:** 445-55.

16. Flieder DB, Travis WD. Clear cell "sugar tumor" of the lung: association with lymphangioleiomyomatosis and multifocal micronodular pneumocyte hiperplasia in a patient with tuberous sclerosis. *Am J Surg Pathol* 1997; **21:** 1242-47.

17. Sullivan EJ. Lymphangioleiomyomatosis. *Chest* 1998; **114:** 1689-703.

18. Schiaffino E, Tavani E, Dellafiore L, Schmid C. Pulmonary lymphangioleiomyomatosis: report of a case with immunohistochemical and ultrastructural findings. *Appl Pathol* 1989; **7:** 265-72.

19. Lenoir S, Grenier P, Brauner MW, Frija J, Remy-Jardin M, Revel D, et al. Pulmonary lymphangioleiomyomatosis and tuberous sclerosis: comparison of radiographic and thin-section CT findings. *Radiology* 1990; **175:** 329-34.

20. Templeton PA, McLoud TC, Muller NL, Shepard JA, Moore EH. Pulmonary lymphangioleiomyomatosis: CT and pathologic findings. *J Comput Assist Tomogr* 1989; **13:** 54-7.

21. Sherrier RH, Chiles C, Roggli V. Pulmonary lymphangioleiomyomatosis: CT findings. *AJR Am J Roentgenol* 1989; **153:** 937-40.

22. Kawahara Y, Taniguchi T, Kadou T, Fujitani K, Yokoyama M, Fukuzaki H, et al. Elevated pulmonary arterial pressure in pulmonary lymphangioleiomyomatosis. *Jpn J Med* 1989; **28:** 520-2.

23. Guinee DG Jr, Feuerstein I, Koss MN, Travis WD. Pulmonary lymphangioleiomyomatosis: diagnosis based on results of transbronchial biopsy and immunohistochemical studies and correlation with high-resolution computed tomography findings. *Arch Pathol Lab Med* 1994; **118:** 846-9.

24. Itami M, Teshima S, Asakuma Y, Chino H, Aoyama K, Fukushima N. Pulmonary lymphangioleiomyomatosis diagnosed by effusion cytology: a case report. *Acta Cytol* 1997; **41:** 522-8.

25. de la Fuente J, Paramo C, Roman F, Perez R, Masa C, de Letona JM. Lymphangioleiomyomatosis: unsuccessful treatment with luteinizing-hormone-releasing hormone analogues. *Eur J Med* 1993; **2:** 377-8.

26. Klein M, Krieger O, Ruckser R, Rosen A, Waldner R, Preis P, et al. Treatment of lymphangioleiomyomatosis by ovariectomy, interferon alpha 2b and tamoxifen – a case report. *Arch Gynecol Obstet* 1992; **252:** 99-102.

27. Eysvogel MMM, Page PS. Lymphangioleiomyomatosis . *Chest* 1990; **98:** 1045-6.

28. Desurmont S, Bauters C, Copin MC, Dewailly D, Tonnel AB, Wallaert B. [Treatment of pulmonary lymphangioleiomyomatosis using a GnRH agonist]. [French]. *Rev Mal Respir* 1996; **13:** 300-4.

29. Fuleihan GE. Tissue-specific estrogens – the promise for the future. *N Engl J Med* 1997; 337: 1641-7.

30. Boehler A, Speich R, Russi EW, Weder W. Lung transplantation for lymphangioleiomyomatosis. *N Engl J Med* 1996; **335:** 1275-80.

31. Nine JS, Yousem SA, Paradis IL, Keenan R, Griffith BP. Lymphangioleiomyomatosis: recurrence after lung transplantation. *J Heart Lung Transplant* 1994; **13:** 714-9.

Radiol Oncol 2002; 36(1): 47-51.

Infantile myofibromathosis of the maxilla. A case report

Nataša Ihan Hren

Clinical Department of Maxillofacial and Oral Surgery, University Medical Centre, Ljubljana, Slovenia

Background. *Infantile myofibromatosis is a rare benign tumour in children. Its characteristic symptoms are firm masses in soft tissues, bones and visceral organs, and its common locations are head and neck. Three forms are well known: solitary, multicentric and visceral myofibromatosis. All have excellent prognosis, except the last one that may be lethal. Spontaneous regression can occur.*
Case report. *We present an unusual case of infantile myofibromatosis of the maxilla in an adolescent.*
Conclusions. *The infantile myofibromatosis should be managed with special caution because of the differential-diagnostic similarity with fibrosarcoma, leomyosarcoma, and histiocytosis.*

Key words: myofibromatosis; maxillary neoplasms; infant

Introduction

Infantile myofibromatosis (IM) is a rare benign tumor in children. It was first described as congenital fibrosarcoma. Later, sporadic cases were discussed, and in 1981, 61 cases were examined and named as IM. Three different forms were described: solitary, multicentric and visceral IM.[1]

Three quarters of soft tissue tumours in children and adolescents are benign, 95 % of them are fibromatosis, 80 % of fibromatosis are IM and 19 % aggressive desmoid fibromatosis.[2] The most frequent type is solitary IM. One third of these occur are in the head and neck.

Its aetiology is not known. First, it was believed that original cells are fibroblasts; but with the presence of desmins receptors in IM, this belief proved to be false. In fact, the original cells of IM growth are smooth muscular cells.[3] Genetic predisposition is likely since solitary form is twice as frequent in males than in females.

Clinically, IM is expressed by slowly growing painless firm solitary or multicentric nodes in the soft tissue, bones or visceral organs. Half of the cases develop in the dermis or subdermally.[4] Their size is few millimetres to few centimetres. Half of the cases are congenital, 90 % of all cases develop in first

Received 14 January 2002
Accepted 21 January 2002

Correspondence to: Assist. Prof. Nataša Ihan Hren, MD, PhD, Clinical Department of Maxillofacial and Oral Surgery, University Medical Centre, Zaloška 2, 1000 Ljubljana, Slovenia.

two years of life.[1] The symptoms are rare except when IM obstructs the visceral organs or nerves. Spontaneous ulcerations can also occur.

Pathohistological characteristics of IM are bundles of pinal cells – myofibroblasts, round and less differentiated cells may be found. In the centre, necrotic processes and calcinations are also seen. Mitotic activity is most diversified.

On the X-ray of the skeleton, the IM is seen as well differentiated osteolitic lesions with sclerotic margins. The IM may well be seen also by other diagnostic imaging techniques, such as CT and MR. Before final diagnosis, pathohistology of bioptic material should be performed. Differential diagnosis discovered some resemblances with fibrosarcoma, histiocytosis, and leiomyosarcoma; therefore, the misinterpretations are possible.

The visceral type is lethal in 75 % of cases in neonatal period because of an acute cardiopulmonary failure, haemorrhage or gastrointestinal obstruction,[1] whereas nonvisceral forms have excellent prognosis.

The conservative tumour excision is curative. Spontaneous regression may occur in the third of cases[4] 1 to 2 years;[5] therefore, the observation is a method of choice if IM is definitely pathohistologically conformed. The reported recurrence rates ranged from 7-10 % (1) to 31 % (4).[1,4]

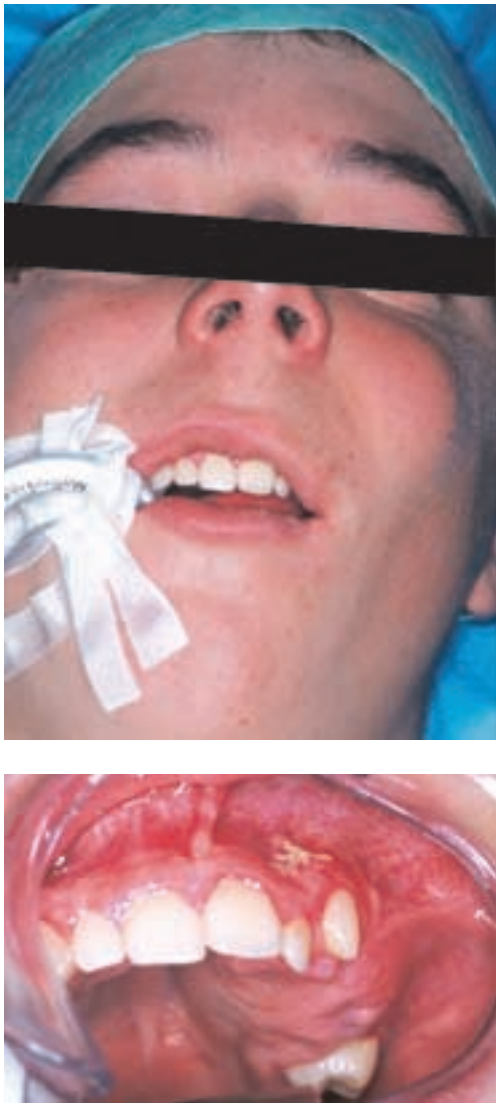


Figure 1a, 1b. The patient before surgery.

Case report

A fifteen-year-old boy came to our outpatients department because of a massive, firm oedema on the left cheek, which had been slowly progressing for 1 year. (Figures 1a, 1b) He as well as his parents connected it with the impacted upper canine tooth on the left that was operatively released by an oral surgeon one year ago. The X-ray of the teeth made at that period showed a tumour with a diameter of 2 cm in the alveoli of the maxilla. Medical history detected no genetic predisposition.

Clinical investigations confirmed a large tumour mass, which vaulted over the hard palate, alveolar and buccal tissues. The boy's face was strongly disfigured. The dermal and mucosal coating was intact. There were no other pathological changes in the oral cavity

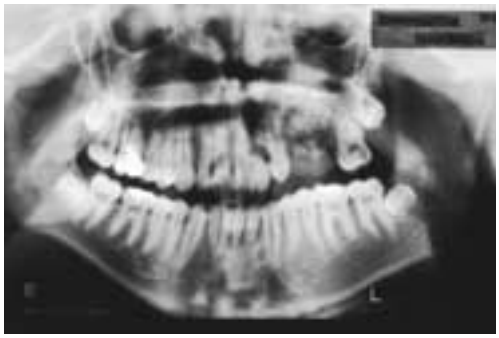
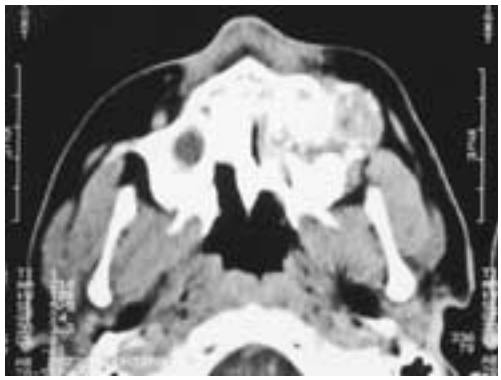


Figure 2. Panoramic X-ray.



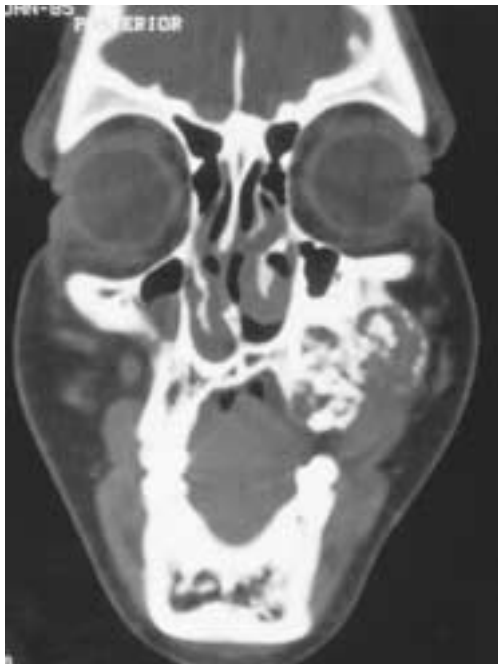


Figure 3a, 3b. CT scan of infantile myofibromatosis lesion in left maxilla before surgery.

and head, except the lacking permanent premolar and first permanent molar teeth in the left maxilla. From the medical history it is evident that they never existed.

X-ray showed significant changes in the bone of the maxilla, partly osteolytic partly calcinated. They spread over the alveoli from the canine tooth to maxillary tuber and invade the maxillary sinus too orbital floor (Figure 2). CT scan showed no invasion into the orbit, nasal cavity and pterigoids (Figures 3a, 3b). The serum alkaline phosphatase value was very high, the serum phosphate rate was also increased, whereas the values of the serum calcium and hormones were normal.

From the clinical viewpoint, it could be possible that the boy had a sarcoma or a rare odontogenic tumour because of the lacking teeth. We took the biotic material samples from three different parts. Pathohistologic results confirmed IM without mitotic activity and necrosis. The boy was operated on; the tumour was extirpated by conservative surgical approach. It size was minimally $7 \times 4 \times 4$ centimetres. After partial maxillectomy, the reconstruction was performed with local tissues. The final pathohistological findings were the same as those of previous biopsy. Moreover, the examination of IM tissue confirmed the presence of the ortotopic bone tissue.

No postoperative complications occurred. The disfigurement was restored, whereas the lacking the lacking alveolar bone and the teeth were replaced by obturator prosthesis.

A year and a half after surgery, the boy is without the local recurrence or recurrence elsewhere in the body (Figure 4a, 4b). The bone defect became smaller after the reparation of the marginal bone. After the boy's growth is completed, a reconstruction by autologous bone and dental implants is planned.

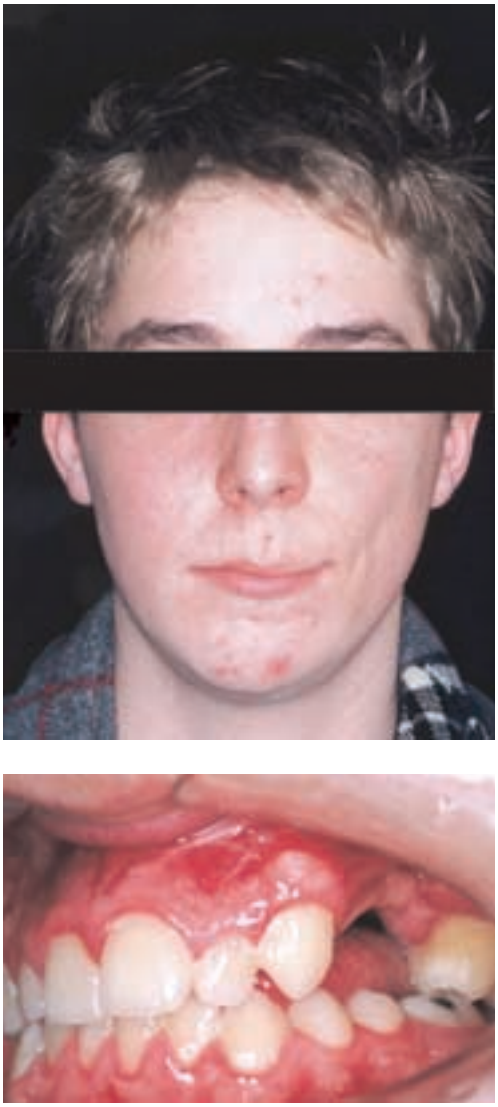


Figure 4a, 4b. The patient after surgery.

Discussion

IM is a rare, but important tumour in children and, because of its common localisation, it should be known to all the surgeons of the head and neck.

The spontaneous regression of IM is induced by programmed cell death – apoptosis.[6] This is probably the most significant example of this massively enhanced physiological mechanism. However, it does not occur in all cases, or it may be so slow that, in most cases, the conservative surgery is necessary. If its results are very mutilating, adjuvant chemotherapy may be applied.[4]

From the revised references, the maxilla, as in our case, is a rare localisation. The most frequent localisations are the dermis or subcutis.[4] Among the head bones, the calvarian bone is most frequently affected, whereas the temporal bone,[7] the orbita with the zygomatic bone8 and the nasal cavity with the inferior turbinate are less frequent localisations.[9]

What is also unusual in our case is the boy's age, which was relatively high for developing IM in spite of late diagnostic treatment. The teeth germs destruction is also most unclear. If the obstruction and necrosis had been the causes, it should have developed at the age of 4 to 6 years, well before the mineralization of the teeth germs occurred. In such circumstances it is unlikely that the tumour would grow so slow until the boy's age of 14 years and speeded-up so much in the last year. In our case, the differential diagnosis suspected of rare odontogenic tumours, which was exceptionality also in our case.

As with other fibrous tumours, IM is frequently misdiagnosed as malignancy, most commonly as fibrosarcoma.[10] It is also possible to mistake it for with leiomyosarcoma.[11] In case of the malignant fibrous tumour, surgery should be very radical and, if it was but a misdiagnosed IM, the morbidity unnecessary.

The recurrence and new growth of IM is possible also on localizations other than primary;[5] therefore, a long-term follow-up of these patients is recommended.

References

1. Chung EB, Enzinger FM. Infantile myofibromatosis. *Cancer* 1981; **48:** 1807-18.

2. Coffin CM, Dehner LP. Fibroblastic-myofibroblastic tumors in children and adolescent: a clinicopathologic study of 108 examples in 103 patients. *Pediatr Pathol* 1991; **11:** 559-88.

3. Fletcher CDM, Acho P, VanNoorden S, McKee PH. Infantile myofibromatosis: a light microscopic, histological and ammunohistochemical study suggesting true smooth muscle differentiation. *Histopathology* 1987; **11:** 245-58.

4. Beck JC, Devaney KO, Weatherly RA, Koopmann CF, Lesperance MM. Pediatric myofibromatosis of the head and neck. *Arch Otolaryngol Head Neck Surg* 1999; **125:** 39-44.

5. Schrodt BJ, Callen JP. A case of congenital multiple myofibromatosis developing in an infant. *Pediatrics* 1999; **104:** 113-5.

6. Fukasawa Y, Ishikura H, Takada A, Yokoyama S, Imamura M, Yoshiki T, et al. Massive apoptosis in infantile myofibromatosis. A putative mechanism of tumor regression. *Am J Pathol* 1994; **144:** 480-5.

7. Behar PM, Albritton FD, Muller S, Todd NW. Multicentric infantile myofibromatosis. *Int J Pediatr Otorhinolaryngol* 1998; **45:** 249-54.

8. Duffy MT, Harris M, Hornblass A. Infantile miofibromatosis of orbital bone. A case report with computered tomography, magnetic resonance imaging, and histologic findings. *Ophthalmology* 1997; **104:** 1471-4.

9. Walsh RM, Leen EJ, Gleeson MJ. Solitary infantile and adult myofibromatosis of the nasal cavity: a report of two cases. *J Laryngol Otology* 1996; **110:** 574-7.

10. Hartig G, Koopman C, Esclamado R. Infantile myofibromatosis: A commonly misdiagnosed entity. *Otolaryngol Head Neck Surg* 1993; **109(4):** 753-7.

11. De Saint Aubain Somerhausen N, Fletcher CDM. Leiomyosarcoma of soft tissue in children. Clinicopathologic analysis of 20 cases. *Am J Surg Pathol* 1999; **23(7):** 755-63.

Radiol Oncol 2002; 36(1): 53-62.

review

A brief overview of the tumor vaccines through the last decade

Srdjan Novaković[1], Barbara Jezeršek Novaković[2]

[1]Dept. of Tumor Biology, [2]Dept. of Internal Medicine, Institute of Oncology Ljubljana, Slovenia

How to destroy cancer cells without damaging the normal cells? How to make conventional methods of systemic cancer treatment that predominantly comprise cytotoxic drugs more selective and prevent the development of drug resistance? There is an abundance of such questions that do not have simple answers. If, a few years ago, unselective cytotoxic drugs were the method of choice for the treatment of cancer, in the last 25 years we are witnessing the rapid transition of immunotherapy from the laboratories to the clinics. Among the most attractive and promising immunotherapies for cancer, a special place is reserved for tumor vaccines. Exploiting the latest knowledge in immunology, tumor physiology, as well as in molecular biology, many outstanding approaches for the creation of tumor vaccines have been developed. With no intention to be comprehensive, in the present article some of those approaches are reviewed.

Key words: neoplasms; gene therapy; cancer vaccines

Introduction

In the last few years, we have witnessed a great progress in the fields of immunology, tumor physiology and molecular biology. Namely, the basic facts about the recognition of various structures by the immune system through the cooperation of MHC have been explained. The structures of MHC class I, and MHC class II have been studied and their function analyzed quite thoroughly. The complex mechanisms of antigen presentation and the role of presenting cells have been investigated in details.[1,2] Various cell receptors (especially T cell receptors) have been discovered, and the methods of signal transduction and the activation of T lymphocytes (the major performers of the cellular immunity) have been elucidated.[3,4] The production of monoclonal antibodies towards different well-defined structures has become a routine procedure, which facilitates the transition of such antibodies into clinical praxis.[5] Today, we are also familiar with the structure of and are equipped to produce various immunomodulatory cytokines, which in turn, assists their application in the treatment of some malignant diseases (hairy-cell leukemia, malignant melanoma, renal cell carcinoma).[6-8] On the other hand, also the methods for precise determination of different genes and their transduction into mammalian cells have been

Received 14 January 2002
Accepted 6 March 2002

Correspondence to: Assoc. Prof. Srdjan Novaković, Department of Tumor Biology, Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia. Tel. + 386 1 522 5118; Fax: +386 1 433 74 10; E-mail: snovakovic@onko-i.si

extensively studied. Thus, the researchers created various vector systems that can be divided into two large groups: (1) viral vectors (retroviral, adenoviral, adeno-associated viral vectors, herpesviral vectors)[9,10] and (2) nonviral vectors (calcium-phosphate precipitation, liposomes, microinjections, electroporation, poly-lysine conjugates, receptor-mediated endocytosis, gene gun).[9,11-16] These discoveries have become the groundwork for the renewed and new biological approaches towards the treatment of malignant diseases.

Tumor vaccines

The first tumor vaccines were created on the principles of classical immunology and comprised irradiated tumor cells and nonspecific immunomodulators. Further approaches towards the creation of tumor vaccines base on the principles of molecular immunology and quite often it is hard to distinguish them from the classical gene therapy approaches. The newer vaccines also include autologous or allogeneic cells, with the difference that various genes coding for proteins involved in the stimulation of immune response (e.g. genes coding for growth factors and cytokines, as well as genes coding for co-stimulatory molecules) are introduced into these cells.[17,18] Instead of whole cells, also certain specific structures that are responsible for the antitumor immune response can be used.[19,20]

Classical tumor vaccines

It was not until recently that we gained some information about the specific antigens that are present on the surface of the tumor cells, and about the co-stimulatory molecules that are necessary for the activation of the immune system, so the first true tumor vaccines were composed of (1) autologous or allogeneic irradiated tumor cells, (2) tumor cell lysates with viral antigens or (3) tumor cells with nonspecific immunomodulators (*Corynebacterium parvum, Bacillus Calmette-Guerin*).[21-27] These first-generation vaccines that base on the principles of classical immunology were termed classical tumor vaccines.

The classical tumor vaccines were applied predominantly for the treatment of malignant melanoma and in the following text this tumor type will be used as an example of the advances in the last years. The most extensively studied vaccine was the CancerVax that was prepared using three viable allogeneic malignant melanoma cell lines (MHC haplotype matches with 95 % of melanoma patients) chosen for their high immunogenicity.[28] As the non-specific immunostimulatory agent the BCG was given with the first two doses. When patients with malignant melanoma stage IV were treated with CancerVax in a clinical study, the five-years survival was as high as 25 %, which can be regarded as quite successful because the surviving fraction in the control group was only 6 %.[29]

In a different clinical trial, the melanoma patients received a classical tumor vaccine composed of autologous tumor cells and BCG. This therapy resulted in a complete response in four out of 40 malignant melanoma patients, and in a partial response in one patient.[30,31]

Worth mentioning are also the studies of Mitchell *et al*. In the first study the authors applied the tumor vaccine composed of the allogeneic cell lysate and Detox (i.e. the detoxified endotoxin from *Salmonella minesota*, cell wall skeletons of *Mycobacterium phlei*, squalane oil and emulsifier), while in the second they applied the Melacin (i.e. the lyophilized version of melanoma lysate vaccine and Detox). The first trial resulted in a partial or complete remission in 20 out of 106 melanoma patients, with the median duration of response of 21 months. The survival of the melanoma patients treated with Melacin in the second study equaled the survival of patients

treated with chemotherapy. As expected, the response rate was lower in the vaccinated group but the toxic side effects were much more pronounced in the chemotherapy group.[32,33]

Additionally, the results of our pre-clinical study with a syngeneic melanoma vaccine proved the high efficacy of the prepared vaccine. In the case of the aggressive intraperitoneal malignant melanoma tumor model we successfully protected more than 40 % animals from tumor development with just a single application of sublethally irradiated tumor cells admixed with MVE-2 (nonspecific immunomodulator). When such a vaccine was applied twice, the number of the protected animals rose to as much as 90 %. Concomitantly, we confirmed that our vaccine induced the long lasting protective immunity, since over 60 % of the former survivors that were rechallenged with the tumor cells survived again without any further treatment.[34,35]

Genetically modified and recombinant tumor vaccines

The group of second-generation vaccines that base on the principles of molecular immunology comprises *genetically modified* and *recombinant tumor vaccines*.

Just like the first-generation vaccines also the second-generation vaccines can be divided into the ones that utilize whole autologous or allogeneic cells, and ones that utilize only certain specific structures. Yet, unlike the first-generation vaccines that are prepared strictly with autologous or allogeneic tumor cells, the second-generation vaccines employ either tumor cells or non-tumor cells (mostly autologous) as fibroblasts and dendritic cells. Regarding the approach towards a better recognition of the tumor cells by the immune system, the second-generation vaccines can be subdivided into the (1) vaccines prepared with genetically modified tumor cells, (2) vaccines prepared with genetically modified non-tumor cells (most frequently dendritic cells and fibroblasts), and (3) recombinant vaccines.

The approach towards the creation of tumor vaccines on the base of genetically modified tumor cells includes different modes of preparation. The more promising modes of preparation are the transfection of tumor cells with genes coding for antigens that are being presented through MHC class I and II, with genes coding for co-stimulatory molecules, and with genes coding for various cytokines.

With the transfer of genes coding for the structures that are being presented through MHC class I into the tumor cells we expect to accomplish an enhancement of the presentation of specific antigens for the activation of CTL, while the transfer of genes coding for the structures that are being presented through MHC class II should augment the activation of helper T cells. Such studies were encouraged by the discovery of different specific tumor antigens that are quite often underexpressed in tumor cells. The transfer of genes coding for the above mentioned structures in different studies resulted in an augmented activation of autologous CTL.[36,37] Similarly to the transfer of genes into the antigen presenting cells (described below), the genes coding for tumor specific antigens from the group of MAGE, MART, MUC-1 and CEA were transferred in these studies.[38]

The transfection of tumor cells with genes coding for B7 ligand should enable a direct activation of CTL, thus bypassing the role of antigen presenting cells. Namely, sole tumor antigens on the surface of tumor cells are insufficient for the activation of the effector cells and can even trigger the development of a complete immune tolerance. Therefore, an additional signal is needed for the activation of the cytotoxic T cells and is mediated through the co-stimulatory molecules (B7.1 – CD80 and B7.2 – CD86) that bind to CD28 and CTLA-4. In humans, the B7 is expressed

only on antigen presenting cells – it is expressed constitutively on dendritic cells but can be induced also on activated B, T and NK cells, as well as on macrophages. The studies that applied the transfer of gene for B7 into tumor cells demonstrated the transition of non-immunogenic tumor cells into highly immunogenic tumor cells which resulted in tumor rejection *in vivo*.[39,40] Quite similar was the effect of the transfection of tumor cells with genes coding for other co-stimulatory molecules as ICAM-1 and LFA-3. For that reason, it can be concluded that the presence of co-stimulatory molecules is obligatory for the activation of T lymphocytes while these molecules are not needed for the function of already activated CTL, since the achieved immunity against the transfected cells was preserved also in the case of non-transfected cells.

The cytokines are expected to induce such a vigorous antitumor response that bystander native tumor cells would also succumb to it. Therefore, genes for various cytokines are being transferred into tumor cells in order to achieve a higher level of production of the cytokines that are involved in the complex immune reactions including stimulation of CTL activation, acceleration of the multiplication of activated cells (the cytokines act as growth factors), triggering of the expression of various cell receptors, cytokine cascades and antibody production and in some cases attaining of a direct cytostatic/cytotoxic effect on tumor cells. In contrast to the activities of exogenous cytokines, the cytokines produced in genetically changed tumor cells mimic the activities of natural endogenous cytokines (underlie to some extent control mechanisms of the organism), which on one hand improves their effectiveness and on the other hand minimizes their toxic side effects. When preparing tumor vaccines, different researchers introduced genes for numerous cytokines or growth factors (IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IFN-γ, TNF-α, GM-CSF and G-CSF), respectively, into tumor cells.[41-45] Preclinical results in various tumor models *in vivo* primarily confirmed the expectations concerning the action of this kind of vaccines on the immune system. It was shown clearly that vaccines containing an imunomodulatory cytokine are indeed capable of CD4+ and CD8+ T lymphocyte activation[46-49], activation of macrophages and neutrophils[50,51], as well as stimulation of differentiation of precursor blood cells and dendritic cells (important antigen-presenting cells for T lymphocytes).[52,53] Considering all mentioned facts, a slightly different effect was achieved on tumors than was expected. Even though the cytokine vaccines showed the potential to protect the animals from challenge with wild type tumor cells, none of these vaccines was efficient enough to cure the established tumors in a convincing proportion of experimental animals. The best protection, as well as the most pronounced antitumor activity against formed tumors, has been ascribed to vaccines created of tumor cells bearing gene for GM-CSF, while the most effective combination of genes for preparation of tumor vaccines comprised genes for IL-2 and GM-CSF.[52,53] The results of clinical trials are variable: there are reports about complete or partial tumor remissions after the treatment with cytokine vaccines, but also about an inadequate or missing clinical response.[20,27] Regarding the mechanisms of action of these vaccines in humans it is likely that they are pretty similar to those in animal tumor model: the vaccines should attack the tumor cells by activating the CD8 T cells, NK cell response, dendritic cells and macrophages. The most impressive antitumor (antimelanoma) immune reaction was displayed by the patient who received the GM-CSF vaccine.[54]

The purpose of the vaccination with genetically modified non-tumor cells is also the transfer and the mediation of immune active structures/substances to the effector cells in the organism. This mode of preparation beca-

me especially attractive after the discovery of specific tumor antigens and after the elucidation of the role of antigen presenting cells for the activation of naive T cells. The dendritic cells – DC (i.e. the most potent antigen presenting cells in the organism) that express the co-stimulatory and adhesion molecules (e.g. CD58, CD54, CD50, CD80 – B7.1, CD86 – B7.2) and MHC class I and II are employed in these reactions. The surface structures of DC bind to adequate structures on T cells (e.g. CD28) and through them mediate the signals for triggering of the primary immune response. This approach is thus opposite to the ones where the tumor cells were transfected with the genes encoding costimulatory molecules: if, with the transfection of tumor cells with B7 ligand the intention was to bypass the function of the antigen presenting cells, with this very approach it was intended to achieve the activation of specific CTL in the absence of tumor cells.

The first approaches using DC were based on *in vitro* activation of these cells with specific proteins as for example the OVA peptide, gp100, and MelaA/MART.[55,56] The DC prepared in this way were capable of triggering the CTL response leading to lysis of target cells that contained the corresponding antigen structures. Later on, the researchers in order to achieve the presentation of these structures by the DC, rather transfected the DC with the genes for tumor (more or less) specific antigens (instead of growing the DC *in vitro* together with certain antigen structures). Using various transfection techniques they succeeded to transfect the DC with MART-1, MUC-1, 2-galactosidaze gene[57-60], and demonstrated that the transfection of DC with genes coding for certain structures is a method that is superior in achieving the expression of these structures on the surface of antigen presenting cells to the *in vitro* activation of these cells with the very structures.[60] The latest approach in the field of DC application is the preparation of hybridomas, that is the fusion of DC with tumor cells. The resulting cells include all of the tumor antigens as well as all of the co-stimulatory molecules. In one study, where the researchers fused MC38 tumor cell line with DC from the bone marrow of the experimental animals, the vaccination with such vaccine prevented the development of lung metastases in 90 % of the treated animals.[61] In another research, 17 patients with renal carcinoma were treated with hybridomas created of autologous tumor cells and allogeneic DC. Thirteen months after the vaccination four patients were in complete remission, one in partial remission, and in two patients there was a less than 50 % reduction of the tumor burden.[62]

The group of recombinant vaccines comprises predominantly the peptides, fusion proteins and immunoglobulins. In this context, a question arises, if it is more rational to transfer the genes coding for tumor associated antigens into the antigen presenting cells and then apply these cells as tumor vaccines, or to apply such tumor antigens directly in the form of proteins and peptides as tumor vaccines. The immune response can be triggered by two main groups of peptides. Peptides with 8 to 11 amino acid residues that are bound in the MHC class I of the antigen presenting cells trigger through binding to the cell receptors of T lymphocytes (CD8+) the activation of CTL (cells responsible for the antitumor immunity).[63,64] The second group of peptides with 11 to 15 amino acid residues is expressed through MHC class II of the antigen presenting cells. These peptides are responsible for the activation of CD4+ lymphocytes that, in turn, produce cytokines involved in the activation of CD8+ cells.[65] The effectiveness of such vaccines depends upon if the treated organism carries the same MHC allele that will code for the recognizable paratopes on lymphocyte receptors. Also oligopeptides that include both epitopes (the MHC class I epitope and the MHC class II epitope) can be used as tumor vaccines. Most

of the specific oligopeptides represent malignant melanoma specific antigens (MAGE-1, MAGE-3, MART-1, gp100, tyrosinase, gp75), colorectal carcinoma specific antigens (CEA), breast cancer specific antigens (MUC-1). Since there are many different epitopes on these oligopeptides, some studies demonstrated that these antigens trigger the immune response (activate CTL) regardless of the type of the MHC present (MHC-unrestricted manner).[66,67] So far, only some of the above mentioned antigens were tested in clinical studies. In one of these studies, Rosenberg *et al*. achieved the development of immunity in 90 % of the patients vaccinated with the vaccine that included one of the peptides derived from gp100 (i.e. one of the epitopes). In 13 out of 31 patients with metastatic melanoma, that received IL-2 beside the vaccine, they observed an objective clinical response to treatment.[68] The preparation of fusion proteins and immunoglobulins is based on the use of monoclonal antibodies. Through the binding of the prepared monoclonal antibodies onto specific cell receptors the researchers aim to selectively influence the CTL and the antigen presenting cells. An example of such application is the preparation of monoclonal antibodies to CTLA-4 in order to reduce the weakened activation of CTL. Another example, already employed in the clinical practice, is the production of antibodies (Herceptin) to HER-2 (receptor for the growth factor in various types of carcinoma). These antibodies block the binding sites for the growth factor needed by the tumor cells and thus prevent the growth of the tumor.[69] The second mode of antibody application is the production of fusion proteins with cytokines. The most promising model is the employment of monoclonal antibodies bound to the IL-2 molecule. In both preclinical and clinical studies this fusion protein successfully activated the tumor infiltrating lymphocytes.[69]

Prospectus and conclusion

I intend to start the conclusion like I began the abstract with one of the general questions related to the cancer: Do we know enough about the tumor cells and their relations to the host? The answer is not simply YES or NO. Yes, we do know a lot about the tumor cells: their physiology, morphology, signal transduction, about gene susceptibility....We also do know a lot about the relations to the other – normal cells in the organism. Yet, I fell like we have all parts for the simple clock and some additional for an extremely sophisticated one. All the time we are discovering more and more additional parts belonging to the sophisticated clock, but unfortunately, there are still some missing parts that unable us to complete the clock. So instead of putting together the parts of the simple clock and constructing the usable device, we are trying to construct a complicated apparatus with many functions that are less important for daily determination of time.

Something similar is happening to us when we are trying to develop a systemic drug or therapy against cancer: we are targeting a single ultra-specific process offering the tumor cells plenty of time to rebuild the damaged functions. The researchers are discovering more and more details concerning the tumor cell but no one is open-minded enough to orchestrate all these pieces of information into a global prospect of surviving and proliferation of tumor cells in the host organism. Carcinogenesis as a process occurs in the living organisms much more often that could be concluded on the basis of real tumor incidence. Fortunately, only few of these malignantly transformed cells succeed to develop into tumor cell colonies and tumors. Majority of them are being recognized by the immune system and are destroyed before the tumor mass becomes clinically detectable. Considering the diverse origin of the transformed tumor cells (tumors of epithelial, me-

senchymal and other origins) that are recognized by the one immune system in the organism, it is clear that the immune system has the potency to distinguish between different types of tumor cells and control their proliferation. Once when we recognize the tiny borderline in the relation between the host immune system and the tumors the balance would be tilted in favor of the host. So speaking about the novel systemic cancer treatments, I am convinced that, rather than approaches where the therapeutics are acting directly against tumor cells, the approaches that propose the mobilization of protective mechanisms in the host will turn out to be more effective. That implies the development of the tumor vaccines that would be capable of triggering an antitumor immune response and preparing the host for a long lasting control of tumor growth and metastasis. At the moment, it is difficult to predict what kind of vaccine is going to be the most successful. It seams that the most effective one is going to be the vaccine that includes the tumor presenting cells armed with some genes encoding tumor antigens and immunostimulatory cytokines. On the other hand, the advantage will be given to the vaccine that comprises the elements of adjuvant and standard therapy allowing the application as a single adjuvant therapy after the surgical removal of the tumor, or in combination with the therapies that aggressively act directly against the tumor cells.

In conclusion, I should emphasize that classical tumor vaccines (first generation vaccines) are many times more potent than modern tumor vaccines (genetically modified second generation vaccines). The reason probably lies in the concepts used for the preparation of genetically modified vaccines (they are too specific in their activity). For that very reason the future of modern tumor vaccines is the preparation of vaccines that would be constituted of different major structures necessary for the triggering of the immune system or in combining of the currently available vaccines.

References

1. Townsend AR, Rothbard J, Gotch FM, Bahadur G, Wraith D, McMichael AJ. The epitopes of influenza nucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 1986; **44:** 959-68.
2. Lechler R, Aichinger G, Lightstone L. The endogenous pathway of MHC class II antigen presentation. *Immunol Rev* 1996; **151:** 51-79.
3. Viola A, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 1996; **273:** 104-6.
4. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* 1998; **393:** 480-3.
5. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; **256:** 495-7.
6. Quesada JR, Reuben J, Manning JT, Hersh EM, Gutterman JU. Alpha interferon for induction of remission in hairy-cell leukemia. *N Engl J Med* 1984; **310:** 15-18.
7. Nethersell A, Sikora K. Interferons and malignant disease. In: Taylor-Papadimitriou J, ed. *Interferons: their impact in biology and medicine*. Oxford: Oxford University Press, 1985: 127-44.
8. Neidhart JA, Anderson SA, Harris JE, Rinehart JJ, Laszlo J, Dexeus FH, *et al*. Vinblastine fails to improve response of renal cancer to interferon alfa-n1.: high response rate in patients with pulmonary metastases. *J Clin Oncol* 1991; **9:** 832-6.
9. Afione AS, Conrad KC, Flotte RT. Gene therapy vectors as drug delivery systems. *Clin Pharmacokinet* 1995; **28:** 181-9.
10. Jolly D. Viral vector systems for gene therapy. In: Sobol ER, Scanlon JK, eds. *The internet book of gene therapy/Cancer therapeutics*. Stamford: Appleton & Lange, 1995: 3-16.
11. Kubiniec RT, Liang H, Hui SW. Effects of pulse length and pulse strength on transfection by electroporation. *Biotechniques* 1990; **8:** 16-20.
12. Hug P, Sleight RG. Liposomes for the transformation of eukaryotic cells. *Biochim Biophys Acta* 1991; **1097:** 1-17.

13. Wagner E, Curiel D, Cotten M. Delivery of drugs, proteins and genes into cells using transferrin as a ligand for receptor-mediated endocytosis. *Adv Drug Del* 1994; **14:** 113-35.

14. Klein TM, Wolf ED, Wu R, Sanford JC. High-velocity microprojectiles for delivering nucleic acids into living cells. *Nature* 1987; **327:** 70-3.

15. Williams RS, Johnston SA, Riedy M, DeVit MJ, McElligott SG, Sanford JC. Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. *Proc Natl Acad Sci USA* 1991; **88:** 2726-30.

16. Wolff AJ, Budker V. Cationic lipid-mediated gene transfer. In: Sobol ER, Scanlon JK, eds. *The internet book of gene therapy/Cancer therapeutics*. Stamford: Appleton & Lange, 1995: 65-73.

17. Novaković S. Current approaches to gene therapy in oncology: Construction of tumor vaccines. *Radiol Oncol* 1996; **30:** 260-7.

18. Zöller M, Matzku S. Cancer therapy: New concepts on active immunization. *Immunobiology* 1999; **201:** 1-21.

19. Stevenson KF. DNA vaccines against cancer: From genes to therapy. *Ann Oncol* 1999; **10:** 1413-8.

20. Nawrocki S, Mackiewicz A. Genetically modified tumour vaccines—where we are today. *Cancer Treat Rev* 1999; **25:** 29-46.

21. McCune CS, O'Donnell RW, Marquis DM, Sahasrabudhe DM. Renal cell carcinoma treated by vaccines for acive specific immunotherapy: correlation of survival with skin testing by autologous tumor cells. *Cancer Immunol Immunother* 1990; **32:** 62-6.

22. Bystryn JC, Oratz R, Roses D, Harris M, Henn M, Lew R. Relationship between immune response to melanoma vaccine immunization and clinical outcome in stage II malignant melanoma. *Cancer* 1992; **69:**1157-64.

23. Hanna MG Jr., Brandhorst JS, Peters LC. Active specific immunotherapy of residual micrometastasis: an evaluation of sources, doses and ratios of BCG to tumor cells. *Cancer Immunol Immunother* 1979; **7:** 65-73.

24. Hoover HC Jr, Surdyke M, Dangel RB, Peters LC, Hanna MG Jr. Delayed cutaneous hypersensitivity to autologous tumor cells in colorectal cancer patients immunized with an autologous tumor cell: Bacillus Calmette-Guerin vaccine. *Cancer Res* 1984; **44:** 1671-6.

25. Schirrmacher V, Hoegan P, Schlag P, Liebrich W. Active specific immunotherapy with autologous tumor cell vaccines modified by Newcastle disease virus: Experimental and clinical studies. In: Schirrmacher V, Schwartz-Abiez R, eds. *Cancer Metastasis*. Berlin: Springer-Verlag, 1989: 157-70.

26. Hersey P, Edwards A, Coates A, Shaw H, McCarthy W, Milton G. Evidence that treatment with vaccinia melanoma cell lysates (VMCL) may improve survival of patients with stage II melanoma. Treatment of stage II melanoma with viral lysates. *Cancer Immunol Immunother* 1987; **25:** 257-65.

27. Sinkovics JG: Viral oncolysates as human tumor vaccines. *Int Rev Immunol* 1991; **7:** 259-87.

28. Conforti AM, Ollila DW, Kelley MC, Gammon G, Morton DL. Update on active specific immunotherapy with melanoma vaccines. *J Surg Oncol* 1997; **66:** 55-64.

29. Chan AD, Morton DL. Active immunotherapy with allogeneic tumor cell vaccines: present status. *Semin Oncol* 1998; **25:** 611-622.

30. Berd D, Maguire HC, Jr., Mastrangelo MJ. Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. *Cancer Res* 1986; **46:** 2572-77.

31. Berd D, Maguire HC, Jr., McCue P, Mastrangelo MJ. Treatment of metastatic melanoma with an autologous tumor-cell vaccine: clinical and immunologic results in 64 patients. *J Clin Oncol* 1990; **8:** 1858-67.

32. Mitchell MS, Harel W, Kempf RA, Hu E, Kan-Mitchell J, Boswell WD, *et al*. Active-specific immunotherapy for melanoma. *J Clin Oncol* 1990; **8:** 856-69.

33. Mitchell MS, Harel W, Kan-Mitchell J, LeMay LG, Goedegebuure P, Huang XQ, *et al*. Active specific immunotherapy of melanoma with allogeneic cell lysates. Rationale, results, and possible mechanisms of action. *Ann N Y Acad Sci* 1993; **690:** 153-66.

34. Novaković S, Ihan A, Wraber B, Jezeršek B. An effective tumor vaccine against malignant melanoma: irradiated autologous tumor cells admixed with MVE-2. *Int J Mol Med* 1999; **3:** 95-102.

35. Novaković S, Ihan A, Jezeršek B. Effectiveness of a simply designed tumor vaccine in prevention of malignant melanoma development. *Jpn J Cancer Res* 1999; **90:** 1130-8.

36. Boon T. Toward a genetic analysis of tumor rejection antigens. *Adv Cancer Res* 1992; 58: 177-210.

37. Deres K, Beck W, Faath S, Jung G, Rammensee HG MHC/peptide binding studies indicate hierarchy of anchor residues. *Cell Immunol* 1993; **151:** 158-67

38. Sobol ER, Shawler D, Dorigo O, Gold D, Royston I, Fakhrai H. Immunogene therapy of cancer. In: Sobol ER, Scanlon JK, eds. *The internet book of gene therapy/Cancer therapeutics*. Stamford: Appleton & Lange, 1995: 175-180.

39. Townsend SE, Allison JP. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. *Science* 1993; **259:** 368-70.

40. Baskar S, Ostrand-Rosenberg S, Nabavi N, Nadler LM, Freeman GJ, Glimcher LH. Constitutive expression of B7 restore immunogenicity of tumor cells expressing truncated major histocompatibility complex class II molecules. *Proc Natl Acad Sci USA* 1993; **90:** 5687-90.

41. Colombo MP, Ferrari G, Stoppacciaro A, Parenza M, Rodolfo M, Mavilio F, Parmiani G. Granulocyte colony- stimulating factor gene transfer suppresses tumorigenicity of a murine adenocarcinoma in vivo. *J Exp Med* 1991; **173:** 889-97.

42. Colombo MP, Lombardi L, Stoppacciaro A, Melani C, Parenza M, Bottazzi B, *et al*. Granulocyte-colony stimulating factor (G-CSF) gene transduction in murine adenocarcinoma drives neutrophil-mediated tumor inhibition in vivo. *J Immunol* 1992; **149:** 113-9.

43. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, *et al*. Vaccination with irradiated tumor cells engineered to secrete murine GM-CSF stimulates potent, specific and long lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 1993; **90:** 3539-43.

44. Asher AL, Mule JJ, Kasid A, Restifo NP, Salo JC, Reichert CM, *et al*. Murine cells transduced with the gene for tumor necrosis factor α. Evidence for paracrine immune effects of tumor necrosis factor against tumors. *J Immunol* 1991; **146:** 3227-34.

45. Hock H, Dorsch M, Kunzendorf Uquin Z, Diamanstein T, Blankenstein T. Mechanisms of rejection induced by tumor cell-targeted gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or interferon 3. *Proc Natl Acad Sci USA* 1993; **90:** 2774-8.

46. Fearon ER, Pardoll DM, Itaya T, Golumbek P, Levitsky HI, Simons JW, *et al*. Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. *Cell* 1990; **60:** 397-403.

47. Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, and Gilboa E. Interleukin-2 gene transferinto tumor cells abrogates tumorigenicity and induces protective immunity. *J Exp Med* 1990; **172:** 1217-24.

48. Gansbacher B, Zier K, Cronin K, Hantzopoulos PA, Bouchard B, Houghton A, Gilboa E, *et al*. Retroviral gene transfer induced constitutive expression of interleukin-2 or interferon gamma in irradiated human melanoma cells. *Blood* 1992; **80:** 2817-25.

49. Russell SJ, Eccles SA, Flemming CL, Johnson CA, Collins MK. Decreased tumorigenicity of a transpantable rat sarcoma following transfer and expression of an IL-2 cDNA. *Int J Cancer* 1991; **47:** 244-51.

50. Cavallo F, Giovarelli M, Gulino A, Vacca A, Stoppacciaro A, Modesti A, Forni G. Role of neutrophils and CD4+ T lymphocytes in the primary and memory response to nonimmunogenic murine mammary adenocarcinoma made imunogenic by IL-2 gene. *J Immunol* 1992; **149:** 3627-35.

51. Blankenstein T. Observations with tumor necrosis factor gene-transfected tumours. *Folia Biol – Prague* 1994; **40:** 19-28.

52. Mulligan R. The basic science of gene therapy. *Science* 1993; **260:** 926-32.

53. Golumbek PT, Azhari R, Jaffee EM, Levitsky HI, Lazenby A, Leong K, *et al*. Controlled release, biodegradable cytokine depots: a new approach in cancer vaccine design. *Cancer Res* 1993; **53:** 5841-4.

54. Ellem KAO, O'Rourke MGE, Johnson GR, Parry G, Misko IS, Schmidt CW, *et al*: Acase report: immune responses and clinical course of the first human use of granulocyte/mactophage-colony-stimulating factor-transduced autologous melanoma cells for immunotherapy. *Cancer Immunol Immunother* 1997; **44:** 10 20.

55. Celluzzi CM, Mayordomo JI, Storkus WJ, Lotze MT, Falo LD, Jr. Peptide-pulsed dendritic cells induce antigen-specific CTL-mediated protective tumor immunity. *J Exp Med* 1996; **183:** 283-7.

56. Bakker AB, Marland G, de Boer AJ, Huijbens RJ, Danen EH, Adema GJ, *et al*. Generation of antimelanoma cytotoxic T lymphocytes from healthy donors after presentation of melanoma-associated antigen-derived epitopes by dendritic cells in vitro. *Cancer Res* 1995; **55:** 5330-4.

57. Ribas A, Butterfield LH, McBride WH, Jilani SM, Bui LA, Vollmer CM, *et al*. Genetic immunization

for the melanoma antigen MART-1/Melan-A using recombinant adenovirus-transduced murine dendritic cells. *Cancer Res* 1997; **57:** 2865-9.

58. Song W, Kong HL, Carpenter H, Torii H, Granstein R, Rafii S, *et al*. Dendritic cells genetically modified with an adenovirus vector encoding the cDNA for a model antigen induce protective and therapeutic antitumor immunity. *J Exp Med* 1997; **186:** 1247-56.

59. Henderson RA, Nimgaonkar MT, Watkins SC, Robbins PD, Ball ED, Finn OJ. Human dendritic cells genetically engineered to express high levels of the human epithelial tumor antigen mucin (MUC-1). *Cancer Res* 1996; **56:** 3763-70.

60. Specht JM, Wang G, Do MT, Lam JS, Royal RE, Reeves ME, *et al*. Dendritic cells retrovirally transduced with a model antigen gene are therapeutically effective against established pulmonary metastases. *J Exp Med* 1997; **186:** 1213-21.

61. Gong J, Chen L, Chen D, Kashiwaba M, Manome Y, Tanaka T, *et al*. Induction of antigen specific antitumor immunity with adenoviral transduced dendritic cells. *Gene Therapy* 1997; **4**: 1023.

62. Kugler A, Stuhler G, Walden P, Zoller G, Zobywalski A, Brossart P, *et al*. Regression of human metastatic renal cell carcinoma after vaccination with tumorcell-dendritic cell hybrids. *Nat Med* 2000; **6:** 332-6.

63. Henkart PA. Lymphocyte-mediated cytotoxicity: two pathways and multiple effector molecules. *Immunity* 1994; **1:** 343-6.

64. Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, *et al*. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. *Science* 1994; **265:** 528-30.

65. Stuhler G, Schlossman SF. Antigen organization regulates cluster formation and induction of cytotoxic T lymphocytes by helper T cell subsets. *Proc Natl Acad Sci USA* 1997; **94:** 622-7.

66. Finn OJ, Jerome KR, Henderson RA, Pecher G, Domenech N, Magarian-Blander J, *et al*. MUC-1 epithelial tumor mucin-based immunity and cancer vaccines. *Immunol Rev* 1995; **145:** 61-89.

67. Kirii Y, Magarian-Blander J, Alter MD, Kotera Y, Finn OJ. Functional and molecular analysis of T cell receptors used by pancreatic- and breast-(mucin-) specific cytotoxic T cells. *J Immunother* 1998; **21:** 188-97.

68. Rosenberg S, Yang J, Schwartzentruber D. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treament of patients with metastatic melanoma. *Nat Med* 1998; **4:** 321-7.

69. Schlom J, Abrams IS. Tumor immunology. In: Bast CR, Kufe WD, Pollock ER, Weichselbaum RR, Holland FJ, Frei E, eds. *Cancer medicine*. London: B.C. Decker Inc. 2000: 153-67.

Radiol Oncol 2002; 36(1): 63-72.

review

Environment and breast cancer – the role of xenooestrogens in breast cancer carcinogenesis

Andrej Plesničar[1], Branko Družina[2], Viljem Kovač[3], Božo Kralj[1]

[1]University College of Health Care, [2]Institute of Public Health, [3]Institute of Oncology, Ljubljana, Slovenia

Background. *The survival rate of breast cancer patients has not changed much in the last few decades in developed countries. In order to improve the efficacy of breast cancer prevention and treatment, the role of xenooestrogens in the mechanisms of its development has been evaluated. These industrial chemicals bear little structural resemblance to each other and bind to the oestrogen receptors of exposed cells and/or trigger oestrogenic responses in laboratory test systems. Exposure to xenooestrogens has been regarded as a risk factor for carcinogenesis and a preventable cause of breast carcinoma. Several epidemiological and experimental studies in in vivo and in in vitro conditions of the influence of xenooestrogens on the occurrence of breast cancer have been conducted in the last decades and have shown ambiguous results.*

Conclusions. *No increase in breast carcinoma incidence could be found in women who were exposed to relatively high concentrations of xenooestrogens for extended periods and small quantities of these compounds that are present in the environment probably cannot act as etiological agents for the occurrence of this disease. A multi step approach is suggested regarding the sequence of studies and measures that should be taken to further assess the importance of xenooestrogens on breast cancer carcinogenesis.*

Key words: breast cancer; xenooestrogens; carcinogenesis

Introduction

The word cancer is used to describe several diseases that are caused by multiple genetic changes in the cells of different tissues. These changes may only manifest themselves after several years, causing the proliferation and immortalisation of affected cells. The clinical consequences of these changes are specific clinical states which lead to the premature death of 25-30 % of the population.[1] The improvement of survival in cancer, particularly breast cancer, the survival rate of which

Received 16 November 2001
Accepted 4 December 2001

Correspondence to: Andrej Plesničar, MD, MSc, University College of Health Care, Poljanska c. 26a, SI 1000 Ljubljana, Slovenia; Phone: +386 1 300 11 67; E-mail: andrej.plesnicar@vsz.uni-lj.si

has not changed significantly in the last few decades in the developed countries, is thus among the declared goals that were set to improve the health of the whole population by the year 2000.[2] The relative five-year survival rate of breast cancer patients in the United States (US) was practically constant at slightly less than 80 % for the 1974-1987 period.[1] The relative five-year survival rate for the patients with this disease who were diagnosed in the 1977-1985 period in Southern Australia was 72.8 %; however, patients who had breast cancer diagnosed there in the consequent 1986-1994 period had only slightly improved survival rate of 78 %.[3] In order to further improve the efficacy of breast cancer prevention and treatment, conditions and mechanisms that lead to its development must be known. An especially critical assessment of the significance of and role played by environmental risk factors is therefore necessary. According to some sources, xenooestrogens, industrial chemicals that bear little structural resemblance to each other and that bind to the oestrogen receptors (ER) of exposed cells and/or trigger oestrogenic responses in laboratory test systems, are among these factors.[4]

Xenooestrogens and breast cancer

Breast cancer: some characteristics

By definition, breast cancer is a disease in the course of which malignant proliferation of the epithelial cells in lobules and ducts of the breast tissue takes place.[5] However, this definition could be regarded as too limited since breast cancer is a field researched by experts that are not necessarily specialised in cell and tissue pathology. Breast cancer is the second most frequent form of cancer affecting women, non-melanocytic skin cancer being the most frequent.[6] It is also the most common cause of death for female cancer patients,[7] the incidence of which is up to five times higher in first-world countries than in some less developed Asian and African countries.[8] Moreover, the incidence of breast cancer in first-world countries still seems to be rising slowly.[1,9]

Histologically, breast cancer is usually a carcinoma that develops in the terminal ductal-lobular units. In most cases, a breast carcinoma develops from ductal cells and less frequently from lobular cells, whereas tumour formations that can be histologically classified as primary sarcoma, lymphoma or unclassified tumours are relatively rare, as are metastatic breast tumours that originate from other organs.[5,10-12] The occurrence and rapid development of breast carcinoma and the associated incidence of this disease in women is connected to the presence of certain risk factors. They include a relatively low age at first menstruation and a relatively high age at menopause, a high age at first birth, obesity and a fat-rich diet, excessive alcohol consumption, and benign breast disease and breast carcinoma in the family's medical history. The importance of oral contraception and postmenopausal hormone therapy for the occurrence and development of this disease is not yet completely clear.[1,5,11] Unfortunately, al-

Table 1. Characteristics of patients with symptomatic breast carcinoma at the time of diagnosis

1. The most common symptom among diagnosed breast carcinoma patients is a lump (present in 90 % of cases that exhibit symptoms at time of diagnosis), discovered by the patient during self examination, showering or bathing.
2. Pain is present in 10 % of breast carcinoma patients.
3. Skin-colour change on the skin of the affected breast, nodules or oedema (peau d'orange) are present in 5-10 % of breast carcinoma patients.
4. Changes on nipples are apparent in 5 % of patients, half of which have also exhibited discharge from the breast; with the other half, retraction or eczematoid changes can be observed.

most 80% of breast carcinoma patients only visit a doctor for the necessary diagnostic procedures after the symptoms and signs of the disease become apparent (Table 1).[5,9,11]

Xenooestrogens and breast carcinoma carcinogenesis

According to several experts in this field, the increase in breast cancer incidence in recent decades appears to be caused by prolonged exposure to oestrogens. It is a well-known fact that the incidence of breast carcinoma in developed countries has risen the most in the population of post-menopausal women.[4,13,14] Moreover, prolonged exposure to environmental oestrogens is probably a significant risk factor that contributes to the higher incidence of breast cancer in the US compared with the countries of East Asia. The importance of prolonged exposure to oestrogens in connection with cancer can be shown by the fact that, in the US, first menstruation occurs at the average age of 12.8 years whereas in China it occurs at the average age of 17 years.[4] Also noteworthy is the fact that the incidence of breast cancer in immigrants from East Asia and their descendants is practically the same as with women who were born in the US along with several generations of their ancestors.[15,16] Davis et al. set up a hypothesis in 1993 according to which xenooestrogens could be a risk factor for the carcinogenesis of breast carcinoma in women and that prolonged exposure to these compounds could be considered one of the preventable causes of this disease.[4,17] In the last few years, several structurally different xenooestrogens have been discovered which can, due to their tertiary structure and conformation, bind to oestrogenic receptors (ER) or induce oestrogenic responses in laboratory test systems, or can function using both mechanisms at the same time.[18-20] Among xenooestrogens are the halogenated organic compounds that were used until recently as pesticides. Some better known examples are kepone, toxaphene, endosulfan, dieldrin, *o,p'*-DDT, *p,p'*-DDE, some polychlorinated biphenyl (PCB) mixtures, hydroxy-PCB, nonlyphenol and some phthalates.[4]

Epidemiological studies of importance of xenooestrogens for breast cancer carcinogenesis

Epidemiological research into the importance of xenooestrogens for breast cancer carcinogenesis represents only a part of research on the environmental risk factors that may influence the occurrence and development of this disease, and this is despite a rising awareness of their importance.[21] The studies conducted have mostly been case-control studies and investigated the presence of organic halogenides, i.e. xenooestrogens in breast carcinoma patients compared to healthy control subjects.

The hypothesis that claims xenooestrogens are a risk factor for breast cancer formation and development has been confirmed by some studies but disproved by others.[22-28] According to some earlier studies, no difference was observed in PCB and DDE concentrations in the breast tissues of patients and control subjects. However, PCB concentrations in necropsy samples of breast carcinoma patients were higher than in the necropsy samples of control subjects.[22,23] A study published in 1992 by Falck et al. showed that PCB and DDE concentrations in breast carcinoma patients' breast tissue were higher than concentrations in healthy control subjects.[24] A similar study published later by Dewailly et al. only found higher DDE concentrations in breast tissue. Some groups of breast carcinoma patients had increased serum concentrations of DDE and PCB compared to healthy control subjects,[25] whereas an extensive study published in 1994 by Krieger et al. failed to confirm these differences.[27] In one of the extensive studies that followed, Henderson et al. found a large increase in the serum concentrations of polybrominated biphenyls (PBB)

in breast carcinoma patients compared to the control subjects.[28]

These epidemiological studies were unable to define the importance of xenooestrogens for the carcinogenesis of breast carcinoma, as higher serum concentrations of chlorinated organic compounds may have been, in some studies, caused by specific eating habits, since increased concentrations of these compounds were found in high-fat food as well as fish.[4] It should be noted, however, that the results of these and similar studies have in many cases led the laboratory scientists to seek to elucidate the role of xenooestrogens in the mechanisms of the breast cancer carcinogenesis.

Studying the importance of xenooestrogens for breast cancer carcinogenesis in experimental in vitro conditions

Several xenooestrogens that are able to bind to ER (Table 2) have been identified in the last few years using cell cultures in experimental *in vitro* conditions.[4,18-20] By binding to ER, the xenooestrogens could act as sex hormones and accelerate breast carcinoma cell proliferation. In this mitogenic effect, ER play the role of genetic transcription factors that release the block in the G1 phase of the cell cycle.[1]

Other mechanisms of the effects xenooestrogens have on breast carcinoma cells have also been researched with the help of *in vitro* conditions. The results of some studies indicate that the xenooestrogens reduce the enzymatic activity of 17β-estradiol 2-hydroxylase, and increase the enzymatic activity of E_2 16α-hydroxylase, which leads to a change in the ratio of the two respective metabolites in the cell. After exposure to oestrogens, the concentration of 16α-hydroxyoestrone in the cells is increased and is higher than the concentration of 2-hydroxyestrone, which is why the ratio of their concentrations is increased.[17,29] Previous studies also indicate the importance of the change of the ratio which showed that E_2 16α-hydroxylase acts as a strong oestrogen whereas 17β-estradiol 2-hydroxylase is a partial antagonist of the ER receptors.[30,31] In the MCF-7 breast carcinoma cell line, many xenooestrogens (Table 3) have the aforementioned effect on the two hydroxylases,[32] whereas the indole-3-carbinole (I3C), an ingredient of several vegetables, inhibits the development and growth of breast carcinoma in *in vitro* as well as *in vivo* models.[33,34] However, later studies have shown that a change in activity of E_2 2-hydroxylase in MCF-7 cells is not caused by inducing the P 450 system but is instead a result of this system's direct interactions with various chemical substrates. Moreover, E_2 2-hydroxylase activity was reduced after adding pure antioestrogen ICI 164,384. These results show that

Table 2. Xenooestrogens that bind to oestrogenic receptors (ER) (*in vitro* cell cultures)

Toxaphene
Endosulphan
Dieldrin
o,p'-DDT
p,p'-DDE
PCB (mixtures and PCB-like compounds)
Hydroxy-PCB
Bisphenol-A
Nonylphenol
Phthalates

Table 3. Xenooestrogens that reduce the enzymatic activity of 17-estradiole 2-hydroxylase and increase the enzymatic activity of 16-hydroxylase (*in vitro* cell cultures: MCF-7 cells)

Endosulphan
Kepone
p,p'-DDE
o,p'-DDE
o,p'-DDT
Atrazine
2,2',4,4',5 pentachlorobiphenyl
7,12-DMBA

modulation of E_2 2-hydroxylase activity in MCF-7 cells cannot serve as a reliable test for carcinogenic effects of oestrogenic pesticides and other compounds that are thought to promote the occurrence and development of breast carcinoma.[4]

Studying the importance of halogenated organic compounds in the development of breast carcinoma in experimental in vivo conditions

Some studies of the importance of halogenated organic compounds and related xenooestrogens in the occurrence and development of breast carcinoma were conducted using *in vivo* experimental models with animals (Table 4). Adding atrazine to Sprague-Dawley laboratory rats decreased the latent period of breast cancer development;[29] a similar study with Fisher laboratory rats showed no changes.[35] The results of the study on the Fisher laboratory rats also indicated that atrazine and related compounds influence the oestrus and regulation of synthesis of the luteinising hormone via non-oestrogenic pathways.[35] Additional studies showed that atrazine and simazine have no oestrogenic effect,[36] while a study done previously found that DDT caused an increase in breast cancer incidence in male Sprague-Dawley rats that were given acetamidophenantrene at the same time.[37] However, a much earlier study showed that DDT reduces the incidence of breast tumours induced by dimethylbenzanthracene (DMBA).[38] A review of these studies and examinations cannot prove the validity of the hypothesis of the important role of xenooestrogens in breast carcinoma carcinogenesis, as some halogenated organic compounds and xenooestrogens can both inhibit and accelerate it in *in vivo* conditions.

Halogenated organic compounds with antioestrogenic activity

Several of the numerous studies on the importance of xenooestrogens for carcinogenesis and the development of breast carcinoma found that there were some halogenated organic pollutants whose effects were directly opposite to those of oestrogens. One such antioestrogenic compound is 2,3,7,8-tetrachlorodibenzo-p-dioxine (TCDD), which according to some studies slows the occurrence of spontaneous breast and uterus tumours in female Sprague-Dawley rats and the occurrence of breast tumours in immunosupressed B6D2F1 mice with xenografts of the MCF-7 cells.[39-41] Additional studies confirmed the antioestrogen and antitumor effect of TCDD and some halogenated aromatic hydrocarbons (HAH) in human breast carcinoma cell lines in *in vivo* as well as *in vitro* conditions (Table 5).[42]

Table 4. Halogenated organic compounds that play a role in breast cancer development (animal experimental models – *in vivo*)

Compound	Animal model	Result	Reference
Atrazine	Rats (female) (Sprague-Dawley)	A decrease in the latent period of breast cancer development	29
Atrazine	Rats (female) (Fischer)	No change in the latent period of breast cancer development	35
ACTDP and DDT	Rats (male) (Sprague-Dawley)	Increased breast tumour incidence	37
DDT	Rats (female) (Fisher)	A decrease in DMBA-induced breast tumour incidence	28

ACTDP = acetamidophenantrene; DMBA = dimethylbenzanthracene

Table 5. Halogenated organic compounds that act as antioestrogens (in vitro cell cultures and in vivo animal experimental models)

Compound	Animal model	Result	Reference
TCDD	Rats (female) (Sprague-Dawley)	A decrease in the occurrence of spontaneous breast and uterine tumours	39
TCDD	Rats (female) (Sprague-Dawley)	Inhibition of breast tumour carcinogenesis	40
TCDD	Mice (immunosupressed with MCF-7 xenografts)	A decrease in the occurrence of spontaneous tumours	41
TCDD and HAH	Human breast cancer cell lines	Lower activity of peroxydases and binding of EGRF receptors, smaller amounts of m-RNA	42

TCDD = 2,3,7,8-tetrachlorodibenzo-p-dioxine; HAH = halogenated aromatic hydrocarbons

TCDD acts similarly to the "pure" antioestrogen ICI 164,384, which binds competitively to ER and is an ER antagonist.[43] Both compounds inhibit some of the oestrogenic responses and reduce concentrations of oestrogen-associated proteins in MCF-7 cells. However, TCDD does not bind to ER or receptors for other steroid hormones. Like the various classes of HAH and polychlorinated aromatic hydrocarbons (PAH), it binds to the aryl-carbohydrate receptors that trigger signal pathways similar to those triggered by the other ligand-induced transcription factors.[4, 42] Aryl-carbohydrate receptor agonists, which in addition to TCDD and similar pollutants from the class of halogenated hydrocarbons also include I3C and related hetero-PAH found in vegetables and PAH released with the cooking of fish and meat,[4] inhibit the growth-factor-induced growth of human breast carcinoma cells.[44-45]

Various oestrogenic and antioestrogenic compounds in the diet

An assessment of the influence of the dietary intake of xenooestrogens on breast carcinoma incidence should also include other compounds with oestrogenic and antioestrogenic effects that may be present in the diet. The characteristics of exposure to halogenated organic compounds with a weak oestrogenic effect are not known. However, there are estimates for average daily exposure to oestrogenic compounds in the diet according to regular measurements of their concentrations in various kinds of food. As reported by Safe and McDougal, the average daily intake of DDT, DDE, toxaphene and dieldrin is about 2.5 micrograms per day;[4] the average daily intake for other xenooestrogens is not known.[46] Bioflavonoids are an important dietary source of compounds with oestrogenic activity and can be found in most fruits, vegetables, walnuts and hazelnuts. The estimated dietary intake of oestrogenic bioflavonoids may reach up to 1000 milligrams per day. However, the concentrations of oestrogenic lignans in various kinds of foods, which have been found in human serum, are as yet unknown.[46-48] Besides these xenooestrogens, the usual diet contains compounds with antioestrogenic effects, as well as compounds that reduce the probability of breast carcinoma occurrence with other mechanisms. Some of them include various cell antioxidants and terpenoids.[4,46] Estimates have been made of the dietary concentrations of other halogenated organic compounds with antioestrogenic effects and other compounds that act as aryl-carbohydrate receptor antagonists, such as TCDD, HAH, PAH, and I3C.[49-51]

An assessment of the importance of xenooestrogens for breast carcinoma carcinogenesis

Studies of the occurrence and development of breast carcinoma in the last three decades have implicated exposure to natural or synthetic oestrogens as a risk factor for the occurrence of breast carcinoma in women.[1,4] Since 1993, when Davis et al. set up a detailed hypothesis according to which prolonged exposure to xenooestrogens could be considered a preventable cause for the occurrence of this disease in women, several epidemiological and experimental studies in *in vivo* as well as *in vitro* conditions have been conducted to ascertain the validity of this hypothesis.[17] It is impossible to either prove or deny it even with the collected results of a large number of studies.[4,17]

No increase in breast carcinoma incidence could be found in women who were exposed to relatively high concentrations of PCB and DDT for extended periods.[52] The small quantities of these compounds that are present in the environment probably cannot act as etiological agents for the occurrence of this disease. It should also be noted when studying the prolonged exposure of women to xenooestrogens that an ordinary diet contains several compounds that have been shown to prevent the occurrence and development of breast carcinoma in *in vivo* and *in vitro* conditions. Other xenooestrogen-like pollutants, in addition to various cell antioxidants and terpenoids, have been discovered in the diet, such as TCDD and HAH; these have been shown to act as antioestrogens, unlike other pesticides from the class of halogenated hydrocarbons.[4,49,53]

Also noteworthy is the fact that the intake of weakly oestrogenic pesticides from the class of halogenated hydrocarbons, whether in the diet or in some other way, represents only a minor part of daily exposure to oestrogens. Several women receive relatively high quantities of potent oestrogenic drugs either through hormone contraception or hormone replacement therapy, yet their risk of breast carcinoma is minimal.[16]

Xenooestrogens and breast carcinoma – a public health perspective

The environment we live in and depend on can influence our health for better or worse.[21] An increasing incidence of breast carcinoma has been observed in most industrially and agriculturally developed countries in the last three decades,[2,5-9] the causes of which are subject to a large number of epidemiological and clinical studies, as well as studies in experimental *in vivo* and *in vitro* conditions. Since pesticides have achieved widespread use in this period due to intensive agricultural production, a study of these industrially made compounds as risk factors is part of these ongoing research projects. Some of these compounds are called xenooestrogens due to the fact that they are a part of the environment and because of their weakly oestrogenic effects.[4,5,17,52-54]

The number of breast carcinoma casualties and their relative five-year survival rate after the diagnosis of the disease have not changed much despite advances in treating the symptomatic disease.[1-3] Although screening programmes using mammography are relatively expensive, several randomised studies have shown that a well-considered application of these programmes reduces the mortality rate of breast carcinoma patients.[1, 2, 10, 11] However, since these programmes cannot lead to an immediate reduction in the mortality rate,[55] it is important that the possibilities of improving the overall situation by applying primary prevention methods are taken into consideration. The fact that several cancer research and treatment institutions are conducting a number of studies on the efficacy of various pharmacological approaches to breast

carcinoma prevention is also important. The publication of results of studies on the chemoprevention of this carcinoma with Tamoxiphen has aroused considerable professional and public interest.[56]

A large part of the well-informed public shows concern about the possible influence environmental risk factors may have on the occurrence of various diseases. Part of this public is particularly concerned about problems relating to the occurrence of breast carcinoma and other cancerous diseases in relation to xenooestrogens.[4] Unfortunately, the results of studies of the influence of xenooestrogens on breast cancer occurrence are ambiguous, which makes educating the general public through clear and simple data rather complicated. In spite of this, doctors and other medical professionals in the primary health network should help breast carcinoma patients and everyone else to interpret the danger posed by the presence of xenooestrogens in their environment. They should also consider the possibility, when conducting clinical examinations and examining a medical history, that this disease forms due to a possible prolonged exposure to these chemicals.[1, 2]

Suggestions have been made regarding the sequence of studies and measures that should be taken to assess the importance of environmental risk factors. It is necessary to identify the compound – in this case, one or more xenooestrogens which, due to their presence in the environment, may represent a risk factor. Next, the time of exposure to one or more xenooestrogens, the number of such episodes, and their duration and intensity must be determined. The third step towards such an estimate is to identify the characteristics of acute or chronic exposure to xenooestrogens with regard to breast carcinoma carcinogenesis. Especially important is experimental work in laboratories. The characteristics and importance of the link between exposure to xenooestrogens and the predicted results of exposure must be determined. Finally, the risk of breast carcinoma carcinogenesis in every woman that has been exposed to environmental xenooestrogens must be defined, as must be the probable consequences of such exposure for a larger population over a longer time period and a calculation of population attributive risk, population attributive risk in percentages and odds ratios. By studying xenooestrogens' role in breast carcinoma carcinogenesis, such an estimate would be difficult to make, since the possible effects of exposure to relatively small concentrations of these compounds in the environment and in the routes of entry should be taken into account. Despite these potential problems, it is necessary to obtain such estimates so they can be used when promoting healthy lifestyles and also in order to introduce and justify all public and individual measures that must be taken to remove or at least reduce the presence of xenooestrogens in the environment. A further reduction in breast carcinoma incidence, and consequentially mortality due to breast carcinoma, could thus potentially be achieved.[1-5,11,46-50,52-54,57]

References

1. King RJB. *Cancer biology*. London: Addison Wesley Longman Limited; 1996.

2. Jekel JF, Elmore JG, Katz DL. *Epidemiology, biostatistics and preventive medicine.* Philadelphia (PA): W. B. Saunders Company; 1996.

3. South Australian Cancer Registry. *Epidemiology of cancer in South Australia. Incidence, mortality and survival 1977 to 1995 analysed by type and geographical location – nineteen years of data*. Adelaide: South Australian Health Commission; 1996.

4. Safe SH, McDougall A. Environmental factors and breast cancer. *Endocr-relat cancer* 1997; **4:** 113-123.

5. Lipmann SM, Lee JJ, Sabichi AL. Cancer chemoprevention: progress and promise. *J Natl Cancer Inst* 1998; **90:** 1514-28.

6. Parkin DM, Pisani P, Ferlay J. Estimates of worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 1993; **54:** 594-606.

7. Pisani P, Parkin DM, Ferlay J. Estimates of the worldwide mortality from eighteen major cancers in 1985. *Int J Cancer* 1993; **55:** 891-903.

8. Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. *Cancer incidence in five continents*. Lyon: International Agency for Research in Cancer; 1997.

9. Kricker A, Jeffs P. *Breast cancer in Australian women*. Canberra: Australian Institute of Health and Welfare; 1996.

10. Gillet D. Early breast cancer: surgical treatment. In: Bishop JF, editor. *Cancer facts. A concise oncology text.* Amsterdam: Harwood Academic Publisher; 1999. p. 133-41.

11. Williams CJ. Gynaecological cancers. In: Williams CJ, editor. *Cancer biology and management: An introduction*. Chichester (England): John Wiley & Sons Ltd; 1990. p. 285-320.

12. Plesničar A, Kovač V. Breast metastases from cutaneous melanoma. A report of three cases. *Tumori* 2000; **86:** 170-73.

13. Sondik EJ. Breast cancer trends. Incidence, mortality, and survival. *Cancer* 1994; **74:** 995-9.

14. Ries LAG. Stat bite: top 5 cancers for females and males in the US. *J Natl Cancer Inst* 1995; **87:** 867.

15. Pike MC, Bernstein L, Spicer DV. Exogenous hormones in breast cancer. In: Niederhuber JE, editor. *Current therapy in oncology*. St Louis: CV Mosby; 1993. p. 292-303.

16. Hulka BS, Liu ET, Lininger RA. Steroid hormones and risk of breast cancer. *Cancer* 1994; **74:** 1111-24.

17. Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer, *Environ Health Persp* 1993; **101:** 147-50.

18. Krishnan AV, Stathis P, Permuth SF, Tokes L, Feldman D. Bisphenol A. An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology* 1993; **132:** 2279-86.

19. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolyc compounds are estrogenic. *Endocrinology* 1993; **135:** 175-82.

20. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phtalate plasticizers, are weakly estrogenic. *Environ Health Persp* 1995; **103:** 582-7.

21. Selye H. The evolution of stress concept. *Am Sci* 1973; **61:** 692-9.

22. Unger M, Kiaer H, Blicher-Toft M, Olsen J, Clausen J. Organochlorine compounds in human breast fat from deceased with and without breast cancer and in biopsy material from newly diagnosed patients undergoing breast surgery. *Environ Res* 1984; **34:** 24-8.

23. Mussalo-Rauhamaa S, Häsänen E, Pyysalo H, Antervo K, Kauppila R, Pantzar P. Occurence of (-hexachlorocyclohexane in breast cancer patients. *Cancer* 1990; **66:** 2124-8.

24. Falck F, Ricci A, Wolff MS, Godbold J, Deckers P. Pesticides and polychlorinated byphenil residues in human breast lipids and their relation to breast cancer. *Arch Environ Health* 1992; **47:** 143-6.

25. Wolff MS, Toniolo PG, Leel EW, Rivera M, Dubin N. Blood levels of organochlorine residues and risk of breast cancer. *J Natl Cancer Inst* 1993; **85:** 648-52.

26. Dewailly E, Dodin S, Verreault R, Ayotte P, Sauvé L, Morin J, et al. High organochlorine body burden in women with estrogen receptor-positive breast cancer. *J Natl Cancer Inst* 1994; **86:** 232-4.

27. Krieger N, Wolff MS, Hiatt RA, Rivera M, Vogelman J, Orentreich N. Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women. *J Natl Cancer Inst* 1994; **86:** 589-99.

28. Henderson AK, Rosen D, Miller GL, Figgs LW, Zahm SH, Sieber SM, et al. Breast cancer among women exposed to polybrominated byphenils. *Epidemiology* 1995; **6:** 544-6.

29. Davis DL, Bradlow HL. Can environmental estrogens cause breast cancer? *Sci Am* 1995; **273:** 166-71.

30. Schneider J, Huh MM, Bradlow HL, Fishman J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. *J Biol Chem* 1984; **259:** 4840-5.

31. Swaneck GE, Fishman J. Covalent binding of the endogenous estrogen 16α-hydroxyestrone to estradiol receptor in human breast cancer cells: characterization and intranuclear localization. *Proc Natl Acad Sci* 1988; **85:** 7831-5.

32. Bradlow HL, Davis DL, Lin G, Sepkovic D, Tiwari RK. The ratio of 16α/2-hydroxyestrone as a biologic marker of breast cancer risk. *Environ Health Persp* 1995; **103:** 147-50.

33. Bradlow HL, Michnowicz JJ, Telang NT, Osborne MP. Effects of dietary indole-3-carbinol on estradiol metabolism snd spontaneous mammary tumors in mice. *Carcinogenesis* 1991; **12:** 1571-4.

34. Tiwari RK, Guo L, Bradlow HL, Telang NT, Osborne MP. Selective responsiveness of breast cancer cells to indole-3-carbinol, a chemopreventative agent. *J Natl Cancer Inst* 1994; **86:** 126-31.

35. Wetzel LT, Luempert LG, Breckenridge CB, Tisdel MO, Stevens JT, Thakur AJ, et al. Chronic effects of atrazine on estrus and mammary tumor formation in female Sprague Dawley and Fisher 344 rats. *J Toxicol Env Health* 1994; **43:** 169-82.

36. Connor K, Howell J, Chen I, Liu H, Berhane K, Sciaretta C, et al. Failure of chloro-s-triazine-derived compounds to induce estrogen receptor-mediated responses in vivo and in vitro. *Fund Appl Toxicol* 1996; **30:** 93-101.

37. Scribner JD, Mottet NK. DDt acceleration of mammary gland tumors induced in the male Sprague-Dawley rat by 2-acetamidophenantrene. *Carcinogenesis* 1981; **2:** 1235-9.

38. Silinskas KC, Okey AB. Protection by 1,1,1-trichloro-2,2bis(p-chlorophenyl)ethane (DDT) against mammary tumors and leukemia during prolonged feeding of 7,12-dimethylbenz(a)anthracene to female rats. *J Natl Cancer Inst* 1975; **55:** 653-7.

39. Kociba RJ, Keyes DG, Beger JE, Carreon RM, Wade CE, Dittenber DA, et al. Results of 2-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. *Toxicol Appl Pharmacol* 1978; **46:** 279-303.

40. Gierthy JF, Bennet JA, Bradley LM, Cutler DS. Correlation of *in vitro* and *in vivo* growth suspension of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Cancer Res* 1993; **53:** 3149-53.

41. Holcomb M, Safe S. Inhibition of 7,12-dimethybenzanthracene-induced rat mammary tumor growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Cancer Lett* 1994; **82:** 43-7.

42. Safe S. modulation of gene expression and endocrine response path ways by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. *Pharmacol Therapeut* 1995; **67:** 247-81.

43. Wakeling AE. Use of pure antiestrogens to elucidate the mode of action of oestrogens. *Biochem Pharmacol* 1995; **49:** 1545-9.

44. Fernandez P, Safe S. Growth inhibitory and antimitogenic activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in T47D human breast cancer cells. *Toxicol Lett* 1992; **61:** 185-97.

45. Liu H, Biegel L, Narasimhan TR, Rowlands C, Safe S. Inhibition of insulin-like growth factor-I responses in MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. *Mol Cell Endocrinol* 1992: **87:** 19-28.

46. Winter CK. Dietary pesticide risk assessment. *Rev Environ Contam Toxicol* 1992; 127: 23-67.

47. Verdeal K, Ryan DS. Naturally-occuring estrogens in plant foodstuffs – a review. *J Food Protect* 1979; **42:** 577-83.

48. Aldercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phytooestrogens in Japanese men. *Nature* 1993; **342:** 1209-10.

49. US Environmental Protection Agency. H*ealth assessment for 2,3,7,8-TCDD and related compounds*. External review draft 1994; EPA/600/BP-92/001 a-c.

50. Vaessen HAMG, Jekel AA, Wilbers AAMM. Dietary intake of polycyclic aromatic hydrocarbons. *Toxicol Environ Chem* 1988; **16:** 281-94.

51. Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, Bradfield CA. Aromatic hydrocarbon responsiveness – receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo* – comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. P*roc Natl Acad Sci* 1991; **88:** 9543-7.

52. Åhlborg UG, Lipworth L, Titusernstoff L, Hsieh CC, Hanberg A, Baron J, et al. Organochlorine compounds in relation to breast cancer, endometrial cancer, and endometriosis: an assessment of the biological and epidemiological evidence. *Crit Rev Toxicol* 1995; **25:** 463-531.

53. Ames BN, Gold LS, Willet WC. The causes and prevention of cancer. *Proc Natl Acad Sci* 1995; **92:** 5258-65.

54. Ma L, Selim HM. Atrazine retention and transport in soils. *Rev Environ Contam Toxicol* 1996; **145:** 129-73.

55. Burton CR, Giles GG. Cancer prevention, screening and early detection. In: Bishop JF, editor. *Cancer facts. A concise oncology text*. Amsterdam: Harwood Academic Publisher; 1999. p. 28-32.

56. Decensi A, Costa A. Recent advances in cancer chemoprevention, with emphasis on breast and colorectal cancer. *Eur J Cancer* 2000; **36:** 694-709.

57. Plesničar S, Plesničar A. Cancer, a reality in the emerging world. *Semin Oncol* 2001; **28:** 210-16.

Radiol Oncol 2002; 36(1): 1-4.

Primarni ne-Hodgkinov limfom v cekumu: prikaz primera

Kropivnik M, Jamar B, Černelč B

Izhodišča. Primarni limfom širokega črevesa je redek, predstavlja le 0,4 % primarnih malignomov v širokem črevesu, običajno pa zajame cekum ali rektum.
V članku želimo prikazati pomen rentgenske preiskave širokega črevesa v diagnostičnem postopku.
Prikaz primera. 77-letni bolnik je bil sprejet zaradi bolečin in suma na septično vnetje v levem kolku, kjer je imel deset let prej vstavljeno totalno endoprotezo. Iz punktata so izolirali Listerio monocytogenes. Dvajset let prej so mu odstranili levo ledvico zaradi hipernefroma. Ob sprejemu je bil febrilen in anemičen.
Zaključki. Pri bolniku so opravili več preiskav: ultrazvočno in računalniško tomografsko preiskao trebuha, scintigrafijo in jejunoileografijo Z dvojno kontrastno rentgensko preiskavo širokega črevesa smo prikazali tumor v predelu ileocekalne valvule. Diagnozo ne-Hodgkinovega limfoma so postavili histološko po biopsiji ob koloskopiji.

Radiol Oncol 2002; 36(1): 5-12.

Karotidna angioplastika z uporabo sredstev za zaščito možganov

Miloševič Z, Žvan B, Zaletel M, Šurlan M

Izhodišča. Karotidna endaterektomija (CEA) je kirurško zdravljenje zožitev notranje karotidne arterije, ki se v svetu in pri nas veliko uporablja. Pri CEA je v večini primerov potrebna splošna anestezija bolnika. Zapleti pri zdravljenju s CEA so možganska kap, paraliza obraznih živcev, hematom v pooperativnem področju in zapleti pri delovanju srca. Tveganje je večje pri bolnikih s ponovnimi zožitvami po CEA, pri bolnikih po radioterapevtskem obsevanju v področju vratu in pri srčnih bolnikih. Karotidna angioplastika s postavitvijo žilne opornice (CAS) je novejša in manj invazivna metoda zdravljenja zožitev notranje karotidne arterije. Metoda je zlasti primerna pri ponovnih zožitvah po CEA in pri distalnih zožitvah notranje karotidne arterije, ki so manj dostopne za CEA. Pri CAS ni potrebna splošna anestezija bolnika, zato je primernejša za težje srčne bolnike. Zapleti CAS so možganska kap zaradi distalne embolije plaka ali krvnega strdka med posegom, akutna zapora, disekcija ali vazospazem zdravljene arterije in ponovna zožitev zaradi hiperplazije intime arterijske stene. Ker je CAS relativno nova metoda, je potrebno opredeliti učinkovitost in varnost metode, preden se široko uvede v klinično prakso.
Bolniki in metode. V Sloveniji smo uvedli CAS v okviru raziskave Slovenian Carotid Angioplasty Study (SCAS). CAS smo izvedli pri 17 bolnikih (12 moških in 5 žensk), ki so bili stari od 69 do 82 let. Vsi bolniki so imeli simptome zožitve arterije karotis, stenoza pa je bila večja od 70 %.
Rezultati. CAS je bila tehnično uspešna pri vseh 17 bolnikih, saj smo pri vseh zmanjšali stenozo karotidne arterije in je bila po posegu manjša od 30 %. Pri 14 bolnikih stenoze nismo več ugotovili, pri 2 je bila 15 % in pri enem 30 %. Pri 15 bolnikih po CAS nismo opazili zapletov, ena bolnica je imela znake hiperperfuzijskega sindroma, ena pa znake crebrovaskularnega inzulta.
Zaključki. Glede na naše začetne izkušnje bo CAS lahko imela pomembno vlogo v okviru preprečevanja možganske kapi, predvsem s pravilnim izborom bolnikov in z uporabo sredstev zaščite možganov med posegom.

Radiol Oncol 2002; 36(1): 13-22.

Ultrazvočni prikaz analnega sfinktra po kolostomiji

Sudoł-Szopińska I, Szczepkowski M, Panorska A, Jakubowski W

Izhodišča. Namen raziskave je bil z analnim ultrazvokom (AUS) prikazati spremembe na analnem sfinktru pri bolnikih po kolostomiji. Želeli smo tudi najti in razčleniti vzroke ugotovljenih sprememb.

Bolniki in metode. AUS smo izvedli po kolostomiji pri 25 bolnikih in uporabili endorektalno sondo z 7.0 MHz. Okvare notranjega analnega sfinktra smo ovrednotili in za statistično analizo uporabili metodo variance (ANOVA).

Rezultati. Notranji analni sfinkter je bil stanjšan pri 22 bolnikih (88 %), srednja debelina pa je bila 1.62 mm. Krožno stanjšanje celotne dolžine je imelo 20 bolnikov (90.9 %). Povečano ehogenost notranjega analnega sfinktra smo ugotovili pri 15 bolnikih (60 %) in pri 10 od njih (66.6 %) je obsegala celotno dolžino in obod sfinktra. Robove notranjega analnega sfinktra ni bilo mogoče dobro določiti pri 10 bolnikih (40 %). Statistično značilno povezavo smo našli med časom trajanja stomije in okvaro ehogenosti notranjega analnega sfinkterja (p = 0.0001). Pri ostalih spremembah pa ni bilo najti statistično značilne povezave.

Zaključki. Pri bolnikih po kolostomiji smo ugotovili zmanjšano debelino in povečano ehogenost notranjega analnega sfinktra. Pri nekaterih bolnikih ni bilo mogoče prikazati robov sfinktra. Statistično značilno povezavo smo našli le med časom trajanja stomije in okvaro ehogenosti notranjega analnega sfinktra.

Radiol Oncol 2002; 36(1): 23-31.

Endoskopska ultrazvočna preiskava puborektalne mišice - primerjava izsledkov analne in vaginalne ultrazvočne preiskave dveh preiskovalcev

Sudoł-Szopińska I, Szczepkowski M, Panorska A, Sarti D, Jakubowski W

Izhodišča. Z raziskavo smo želeli primerjati rezultate analne in transvaginalne ultrazvočne preiskave. Primerjali smo tudi ocene različnih izvajalcev pri obeh preiskavah. Zanimala nas je različna vizualizacija kot tudi ocena dinamike puborektalne mišice.

Bolniki in metode. Analno in transvaginalno ultrazvočno preiskavo smo opravili pri 25 bolnicah, starih od 20 do 72 let (povprečna starost 42 let). Pri vseh preiskavah smo uporabili naprave Bruel &Kjaer s 7,0 MHz vrtljivo sondo s trdo konico, napolnjeno z vodo. Vse bolnice sta pregledala dva preiskovalca z analno in transvaginalno preiskavo.

Rezultati. Pri 15 (60 %) od 25 bolnic je bila puborektalna mišica natančneje določena s transvaginalno preiskavo kot z analno. Oba preiskovalca sta se strinjala v tej ugotovitvi. Njuni zaključki pa so se razlikovali pri oceni funkcionalnosti puborektalne mišice pri obeh metodah raziskave: pri 4 bolnicah (16 %) sta oba preiskovalca ugotovila razlike med metodama (16 %), pri 3 pa le prvi preiskovalec (28 %). V vseh primerih sta funkcijo puborektalne mišice ocenila boljše s transvaginalno ultrazvočno preiskavo kot z analno.

Zaključki. Z analno in transvaginalno ultrazvočno preiskavo ocenjujemo morfologijo in dinamično funkcionalnost puborektalne mišice. V večini primerov (60 %) je bila vizualizacija puborektalne mišice boljša pri transvaginalni metodi kot pri analni. Pri oceni funkcionalnosti puborektalne mišice so bili rezultati prvega preiskovalca pri obeh metodah protislovni v 7 primerih (28 %), rezultati drugega preiskovalca pa v 4 primerih (16 %). V vseh primerih pa je transvaginalna preiskava natančneje prikazala funkcionalnost puborektalne mišice kot analna metoda. V 3 primerih (12 %) smo med prvim in drugim preiskovalcem ugotovili razlike v njunih ocenah funkcionalnosti puborektalne mišice.

Radiol Oncol 2002; 36(1): 33-40.

Uravnavanje odgovora normalnega tkiva na radioterapijo in kemoterapijo zaradi preprečevanja stranskih učinkov

Plevová P

Izhodišča. Ionizirajoče sevanje in citostatske učinkovine, ki jih uporabljamo pri zdravljenju raka, povzročajo imunski odgovor normalnega tkiva. Ta imunski odgovor je uravnavan s citokini in adhezijskimi molekulami in če vplivamo na njih, lahko zmanjšamo stranske učinke zdravljenja. V članku podajamo pregled objavljenih podatkov o tej tematiki.
Zaključki. Načini, s katerimi lahko vplivamo na imunski odgovor tkiva, da bi omejili stranske učinke kemoterapije in radioterpije, so si nasprotni. Nekateri zavirajo odgovor na zdravljenje, saj bi pretirana odzivnost zdravega tkiva lahko imela škodljive posledice. To so na primer kortikosteroidi, nesteroidni antirevmatiki, lizofilin, protitelesa proti citokinom, oligonukletidi, analogi sialila Lewis X. Druge učinkovine pa spodbujajo odzivnost s pospeševanjem endogenega proizvajanja citokinov (AS101) ali pa uporabljajo rekombinantne oblike ustreznih citokinov, ki že sodelujejo v odgovoru na zdravljenje in zato še bolj stopnjujejo fiziološki odgovor tkiva na zdravljenje. V klinični praksi je uporaba kortikosteroidov in nestreroidnih antirevmatikov že močno uveljavljena, medtem ko je uporaba ostalih učinkovin še vedno v preizkusni fazi.

Radiol Oncol 2002; 36(1): 41-6.

Limfangioleiomiomatoza

Anderluh F

Izhodišča. Limfangioleiomiomatoza je redka bolezen neznanega vzroka, za katero zbolevajo ženske v rodnem obdobju. Za bolezen je značilna neneoplastična proliferacija atipičnih gladkomišičnih celic v pljučnem parenhimu, limfnih žilah ter mediastinalnih in abdominalnih bezgavkah. V klinični sliki se pojavljajo spontani pnevmotoraksi, dispneja, hemoptize in hilotoraks.
Zaključki. Računalniška tomografija visoke ločljivosti in imunohistološke metode v bioptičnih vzorcih pljučnega tkiva nam omogočajo pravilno postavitev diagnoze. Načini zdravljenja so različni, običajno hormonski, vendar je zaenkrat njihov uspeh še vprašljiv, prognoza bolnikov pa slaba.

Radiol Oncol 2002; 36(1): 47-51.

Infantilna miofibromatoza maksile. Prikaz primera

Ihan Hren N

Izhodišča. Infantilna miofibromatoza je redek benigni tumor otrok. Značilne so čvrste mase v mehkih tkivih, kosteh ali notranjih organih. Pogosto se pojavlja na glavi in vratu. Poznane so tri oblike: solitarna, multicentrična in visceralna miofibromatoza. Zadnja oblika je lahko smrtna, ostale imajo odlično prognozo. Znana je spontana regresija.
Prikaz primera. Opisan je manj značilen primer najstnika z obsežno solitarno miofibromatozo zgornje čeljustnice.
Zaključki. Pri diagnosticiranju infantilne miofibromatoze je potrebna posebna pozornost zaradi diferencialno diagnostične podobnosti s fibrosarkomom, leomiosarkomom, histiocitozo.

Radiol Oncol 2002; 36(1): 53-62.

Kratek pregled priprave tumorskih vakcin v zadnjem desetletju

Novaković S, Jezeršek Novaković B

Kako bi lahko uničili tumorske celice, ne da bi ob tem prizadeli tudi normalne celice v organizmu? Kako bi lahko spremenili sedanje pristope sistemskega zdravljenja, ki temelji predvsem na uporabi citostatikov, da bi le-to delovalo samo na tumorske celice in ne bi prišlo do pojava neobčutljivih celičnih klonov? Takšnih in podobnih vprašanj je pri zdravljenju raka veliko in predstavljajo osnovo za nove predklinične in klinične raziskave s skupnim ciljem odkriti učinkovito zdravilo ali način zdravljenja rakastih bolezni. Čeprav so še pred nekaj leti nesporno prevladovali citostatiki kot najučinkovitejše orožje v boju proti raku, se v zadnjih 25 letih na tem področju vse bolj uveljavlja biološka terapija. Posebej obetavna med biološkimi terapijami je priprava in uporaba tumorskih vakcin. Na osnovi novejših spoznanj s področij imunologije, tumorske fiziologije in molekularne biologije so raziskovalci oblikovali različne nadvse zanimive pristope k oblikovanju tumorskih vakcin. Pričujoči članek predstavlja kratek pregled nekaterih od navedenih pristopov.

Radiol Oncol 2002; 36(1): 73-9.

Radiol Oncol 2002; 36(1): 63-72.

Ksenoestrogeni in njihova vloga pri nastanku in razvoju karcinoma dojke

Plesničar A, Družina B, Kovač V, Kralj B

Izhodišča. Petletno preživetje bolnic s karcinomom dojke se v razvitih državah v zadnjih desetletjih ni bistveno izboljšalo. Za čim večjo uspešnost pri preprečevanju in zdravljenju karcinoma dojke je zato nujno temeljiteje poznati pogoje in mehanizme nastanka in razvoja te bolezni. Še posebej je potrebno oceniti vlogo najrazličnejših dejavnikov tveganja iz okolja, med njimi tudi ksenoestrogene. Ksenoestrogeni so strukturno raznoliki in se vežejo na estrogenske receptorje izpostavljenih celic v tkivih dojke in/ali sprožajo estrogenične odgovore na laboratorijskih testnih sistemih. Zato je bila že pred nekaj leti postavljena hipoteza, da je lahko dogotrajna izpostavljenost žensk ksenoestrogenom dejavnik tveganja pri razvoju raka dojke oz., da so ksenoestrogeni eden od možnih vzrokov nastanka te bolezni. Vendar z doslej zbranimi rezultati epidemioloških in laboratorijskih raziskav vloge in pomena ksenoestrogenov pri nastanku karcinoma dojke ni bilo mogoče natančno opredeliti. Tako posamezni polutanti med kloriranimi ogljikovodiki, ki so jih uporabljali kot pesticide, na primer TCDD in indol-3-karbinol, delujejo celo v nasprotju z odgovori, ki jih v celicah sprožajo estrogeni in učinkujejo kot antiestrogeni.
Zaključki. Ksenoestrogeni imajo šibke učinke, v vsakdanjem življenju jih zaužijemo ali prejmemo z drugačnimi aplikacijami le v zelo majhnih količinah. Njihova vloga pri nastanku raka dojke je nejasna. Celo pri ženskah, ki več let prejemajo sorazmerno velike količine estrogenih zdravil s kontracepcijo in hormonsko nadomestno terapijo, je ugotovljeno tveganje nastanka raka dojke minimalno. Tako je seveda tudi edukacija bolnic otežkočena in je potrebno previdno interpretirati morebitno nevarnost izpostavljenosti ksenoestrogenom v njihovem okolju. Kljub tem problemom priporočajo ukrepe, ki bi zmanjšali prisotnost ksenoestrogenov v okolju, kakor tudi nadaljnje večstopenjske raziskave o njihovem vplivu na karcinogenezo.

Notices

Notices submitted for publication should contain a mailing address, phone and/or fax number and/or e-mail of a ***Contact*** *person or department.*

Lung cancer

March 14-15, 2002

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

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

Gastroenterology and hepatology

March 14-16, 2002

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

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

Oncology

March 15-16, 2002

The course "Recent Advances in Cancer Treatment" will take place in Nicosia, Cyprus.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Breast cancer

March 19-23, 2002

The 3rd European Breast Cancer Conference will be offered in Barcelona, Portugal.

See http://www.fecs.be/conferences/ebcc3

Brachytherapy

March 24-26, 2002

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

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

Breast cancer

April 5-6, 2002

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

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

Head and neck

April 8-13, 2002

The master course about cancer in oral cavity and oropharynx will take place at European Institute of Oncology in Milan, Italy.

Call P. Lonati, +39 02 5748 9490; or fax +39 02 5748 9491; or e-mail head&neck@ieo.it

Thoracic surgery

April 11-12, 2002

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

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

Oncology

April 11-13, 2002

The 3rd European Oncology Nursing Society (EONS) Spring Convention will be offered in Venice, Italy.

See http://www.fecs.be@conferences/eons3

Radiation physics

April 14-18, 2002

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

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

Oncology

April 16-18, 2002

The ESO 20th anniversary special course "Advances in Clinical Oncology" will take place in Moscow, Russia.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Radiation oncology

April 21-25, 2002

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

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

Radiotherapy

April 21-25, 2002

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

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

Radiotherapy

May 9-11, 2002

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

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

Oncology

May 9-11, 2002

The ESO course "Oncologic and Reconstructive Surgery" will take place in Düsseldorf, Germany.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Nuclear medicine

May 12-15, 2002.

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

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

Radiation therapy

May 15-19, 2002

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

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

Oncology

May 30-31, 2002

The ESO course "Future Directions in Clinical Oncology" will take place in Belfast, North Ireland.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Radiotherapy

June 2-6, 2002

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

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

Breast cancer

June 5-7, 2002

The "4th Milan Breast Cancer Conference" will take place in Milan, Italy.

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

Radiology

June 9-11, 2002

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

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

Bronchology and bronchoesophagology

June 16-19, 2002

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

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

Brachytherapy

June 16-20, 2002

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

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

Clinical oncology

June 21-22, 2002

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

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

Radiotherapy

June 23-27, 2002

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

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

Radiotherapy

June 23-27, 2002

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

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

Oncology

June 30 – July 5, 2002

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

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

Radiology

July 1-5, 2002

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

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

Oncology

July 3-5, 2002

The ESO course "Cancer Economics and Evidence-Based Medicine" will take place in Sapporo, Japan.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Clinical Oncology

August 4-9, 2002

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

Contact Dr. Wolfgang Gatzermeier, ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 0258317850; or fax +39 02 433 59640; or e-mail es-oweb@tin.it

Radiation physics

August 25-29, 2002

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

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

Radiobiology

August 25-29, 2002

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

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

Oncohaematology

August 29-30, 2002

The ESO course will take place in Buenos Aires, Argentina.

Contact G. Farante, ESO Headquarters, ESO Latin America Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 58317318; or fax +39 02 58321266; or e-mail esolatin@tin.it; or see http://www.cancerworld.org; or Argentina Office, A. Rancati, Florida 833 (1o), 1005 Buenos Aires; Phone +54 11 45118078; Fax +54 11 45118079.

Oncohaematology

August 31 – September 1, 2002

The ESO course will take place in Bahia, Brazil.

Contact G. Farante, ESO Headquarters, ESO Latin America Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 58317318; or fax +39 02 58321266; or e-mail esolatin@tin.it; or see http://www.cancerworld.org; or e-mail afrasson@hotmail.com

Prostate cancer

September 1-3, 2002

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

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

Lung cancer

September 1-4, 2002

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

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

Lung cancer

September 8-12, 2002

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

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

Medical physics

September 9-13, 2002

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

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

Radiation therapy

September 17-21, 2002

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

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

Oncology

September 19-21, 2002

The ESO course "The Challenge of Cancer. A Central Role for General Practice" will take place in Dublin, Ireland.

Contact ESO Headquarters, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Oncology

September 29 – October 3, 2002

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

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

Radiation therapy

October 6-9, 2002

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

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

Salivary glands

October 7-12, 2002

The master course about cancer in salivary glands will take place at European Institute of Oncology in Milan, Italy.

Call P. Lonati, +39 02 5748 9490; or fax +39 02 5748 9491; or e-mail head&neck@ieo.it

Colorectal cancer

October 24-25, 2002

The "2nd Colorectal Cancer Conference" will take place in Rome, Italy.

Contact ESO Office, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Radiation oncology

November 10-16, 2002

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

Contact ESTRO office, Av. E. Mounier, 83/4, B-1200 Brussels, Belgium; or call +32 7759340; or fax +32 2 779 5494; or e-mail info@estro.be; or see http://www.estro.be

Breast cancer

November 12-13, 2002

The ESO course will take place in New York, USA.

Contact ESO Headquarters, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org; or R. Boschi-Belgin, ESO US Office, American-Italian Cancer Foundation, 112 East 71st Street – 2B, New York – NY 10021, USA; Phone +1 212 6289090; Fax +1 212 5176089; e-mail aicf@aicfonline.org; http://www.aicfonline.org

Breast cancer

November21-23, 2002

The ESO course "Current Breast Cancer Management" will take place in Johannesburg, South Africa.

Contact ESO Headquarters, Viale Beatrice d'Este 37, 20122 Milan, Italy; or call +39 02 43359611; or fax +39 02 43359640; or e-mail esomi@tin.it; or see http://www.cancerworld.org

Radiation oncology

March 15-19, 2003

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

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

Allergology and clinical immunology

June 7-11, 2003

The "22nd Congress of the European Academy of Allergology and Clinical Immunology" take place in Paris, France.

Contact Congrex Sweden AB, Attn: EAACI 2003, Linnegatan 89A, P.O. Box 5619, SE-114 86 Stockholm, Sweden, or call +46 8 459 66 00; or fax +46 8 661 91 25; or e-mail eaaci2003@congrex.se; or see http://www.eaaci.org

Lung cancer

August 10-14, 2003

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

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

Radiation therapy

September 21-25, 2003

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

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

Radiation therapy

October 19-23, 2003

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

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

Radiation therapy

September 12-16, 2004

The 23rd Annual ESTRO Meeting will be held.

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

Radiation therapy

October 3-7, 2004

ASTRO Annual meeting will be held in Atlanta, USA.

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

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

Fondacija "Docent dr. J. Cholewa"
je neprofitno, neinstitucionalno in nestrankarsko
združenje posameznikov, ustanov in organizacij, ki želijo
materialno spodbujati in poglabljati raziskovalno
dejavnost v onkologiji.

Mesesnelova 9
1000 Ljubljana
tel 01 519 12 77
faks 01 251 81 13

ŽR: 50100-620-133-05-1033115-214779

Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education - A Report for the First Quarter of 2002

The "Dr. J Cholewa" Foundation for cancer research and education is concerned as the change in the donors' attitude towards the Foundation in the last months of the year 2001 calls for the new ways to adapt to the given circumstances. These new circumstances and the difficulties and problems associated with them were taken into consideration and seriously discussed on all levels of the Foundation. It is hoped that some of the new approaches considered in contacts and communications with the donors will produce some tangible results in the near future.

The activity of the Foundation is by now well established as a result of a relatively long and substantial experience, and through the hard work of its members it offers a certain guarantee that the means from the possible donations will be spent in an efficient and impartial way. This attitude of the Foundation guarantees that the grants and supports are associated with excellent research work underway in Slovenia. The decision was also taken to increase the amount of the "Dr. J Cholewa" Foundation for cancer research and education annual prize in order to give further incentive to young researchers in all parts of Slovenia. It is a long-time held position of the Foundation that high quality research work in oncology and related scientific fields is taking place and should be further encouraged in all parts of Slovenia where the interest to promote such research exists.

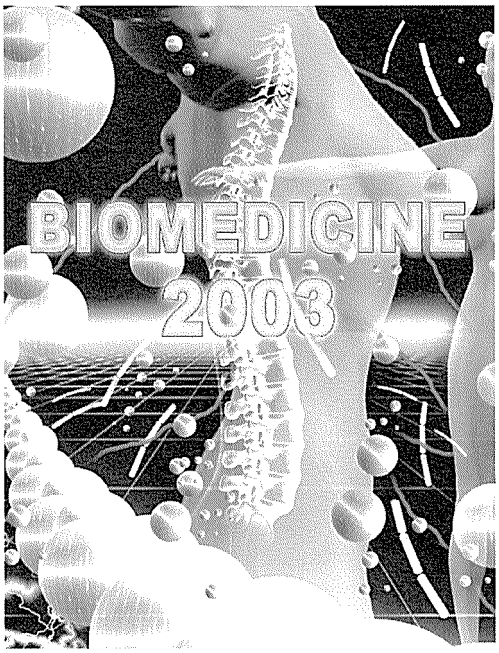
The Foundation continues to support the regular publication of "Radiology and Oncology" international scientific journal that is edited, published and printed in Ljubljana, Slovenia.

The Foundation has also received a number of grant applications and it has considered the merits of the proposed research work thoroughly. A number of grants was awarded to experts from various parts of Slovenia in order to attend various conferences and meetings in the field of oncology in Slovenia and around the world.

The Foundation invited new members to its Executive council in order to better represent and understand the present currents and advances in the continuos development of cancer research and education in Slovenia. The new members are Igor Bartenjev, MD, PhD, Janez Žagar, MD, PhD, and Rado Janša, MD, MSc.

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

Conference Announcement
Call For Papers



BIOMEDICINE 2003

Fifth International Conference on Simulations in Biomedicine

2 - 4 April 2003, Ljubljana, Slovenia

Organised by:
Wessex Institute of Technology, UK
University Medical Centre Ljubljana, Slovenia
Faculty of Computer and Information Science, Ljubljana, Slovenia

klinični center ljubljana

Abstracts can be submitted at:
www.wessex.ac.uk/conferences/2003/biomed03/

BIOMED 2003 provides an internationally recognised forum for disseminating the latest bioengineering research and its applications. The conference topics will cover a broad spectrum including the application of computers to simulate biomedical phenomena.

The conference will be held at the University Medical Centre, the most important medical institution in Slovenia and one of the leading modern medical hospitals in Central Europe. The meeting aims to bring together medical, physical scientists and engineers who are interested in the latest developments on simulations in medicine. It will also be relevant to professionals working in medical enterprises, which are actively involved in this field.

CALL FOR PAPERS

Papers are invited on the topics outlined opposite and others falling within the scope of the meeting. Abstracts of no more than 300 words should be submitted as soon as possible.

Abstracts should clearly state the purpose, results and conclusions of the work to be described in the final paper. Final acceptance will be based on the full-length paper, which if accepted, must be presented at the conference. Each submitted paper is subject to a separate registration. We strongly encourage the submission of abstracts electronically. The language of the conference will be English.

CONFERENCE SECRETARIAT

Gabriella Gossutta, Conference Secretariat, BIOMEDICINE 2003, WESSEX INSTITUTE OF TECHNOLOGY, Ashurst Lodge, Ashurst, Southampton, SO40 7AA. Telephone: 44 (0) 238 029 3223
Fax: 44 (0) 238 029 2853 Email: gcossutta@wessex.ac.uk

TOPICS

Simulation of Physiological Processes
Computational Fluid Dynamics in Biomedicine
Orthopaedics and Bone Mechanics
Simulations in Surgery Data Acquisition and Analysis
Image Processing Design and Simulation of Artificial Organs
Non-Conventional Therapy
Computers in Medicine
Expert Systems in Medicine
Advanced Technology in Dentistry
Gait and Motion Analysis
Computer Technology for Disabled
Cardiovascular System
Virtual Reality in Medicine
Electro-Magnetic Simulation
Biomechanics

VISIT THE CONFERENCE WEBSITE AT: www.wessex.ac.uk/conferences/2003/biomed03/

22nd INTERNATIONAL CONGRESS OF RADIOLOGY
ORGANIZED BY
INTERNATIONAL SOCIETY OF RADIOLOGY ISR
MEXICAN FEDERATION OF RADIOLOGY AND IMAGING FMRI

www.icr2002.org.mx

1 to 5 July 2002
CANCUN - MEXICO

CATEGORICAL AND REFRESHER COURSES

SPECIAL FOCUS CONFERENCES

SYMPOSIA

SCIENTIFIC EXHIBITS

WORKSHOPS

RADIOLOGISTS FROM THE FIVE CONTINENTS, WELCOME TO MEXICO

INFORMATION

B.P. Servimed, S.A. de C.V. Insurgentes Sur 1188-507, 03210 México, D.F.
TEL. (525) 575-99-31 FAX. (525) 559-94-97
Web http//www.icr2002.org.mx E-mail fmricr@servimed.com.mx

ENROLLMENT

1) Register to:
Stefaan Marcelis, Tielt Belgium
E-mail: medipoint@freegates.be
or Fax : +3251406325

2) Course fee: 450 €
including course syllabus and CD-ROM
Payment: Aloka Holding Europe A.G.,
Bank: Credit Suisse Bahnhofstrasse 17
CH-6301 ZUG (Switzerland)
Account No.627-92-173
SIC No.0823 (sort code)

3) Hotel registration: 340 €
including 2 nights + breakfast (24 & 25 October), lunches, seminar facilities.
P.S. accompanying person sharing the room pays additional breakfast only
Payment: AKTUA REIZEN (official travel agent) E-Mail: aktua@idnet.be
Tel +3251424050 Fax +3251424059
Bank: Fortis bank, Diestsesteenweg 430
B-3202 Aarschot (Belgium)
Account No. 230-0379902-70
SWIFT : GEBABEBB08A

GENERAL INFORMATION

The course aims to teach musculo-skeletal ultrasound and is designed to learn the practice of standard ultrasound examinations. For that purpose, the key anatomic structures are discussed for each joint and emphasis is placed on training each participant individually in pratical sessions. This is guaranteed by the presence of experienced teachers and by the limited number of participants (maximum 21) accepted for each training session. Formal lectures provide an overview of current applications .An interactive multi-media CD-ROM on US anatomy -& pathology is provided to each

practical hands-on training

We do not process registration without payment (first in , first served)

In case of written cancellation before October 10, the amount will be refunded .beyond this date: no refund.

ultrasound course

Hands on training *in* MUSCULO SKELETAL ULTRASOUND

Grand Hotel Mercure
Royal Crown*****

BRUSSELS
October 24-26 2002

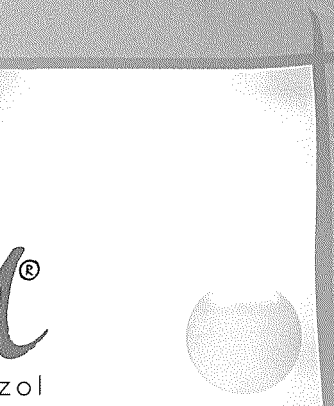
(English speaking)

STEFAAN MARCELIS
TJEERD JAGER
MICHEL DE MAESENEER

Diflazon®

kapsule

flukonazol



- *v svetu največ predpisovani sistemski antimikotik*
- *edini peroralni sistemski antimikotik za zdravljenje vaginalne kandidoze, ki ga je odobril FDA*

Skrajšano navodilo
Flukonazol je sistemski antimikotik iz skupine triazolov.

Odmerjanje pri različnih indikacijah:

vaginalna kandidoza	*150 mg v enkratnem odmerku*
mukozna kandidoza	*50 do 100 mg na dan*
dermatomikoze	*50 mg na dan ali 150 mg na teden*
sistemska kandidoza	*prvi dan 400 mg, nato od 200 do 400 mg na dan* *Največji dnevni odmerek je 800 mg.*
preprečevanje kandidoze	*50 do 400 mg na dan*
kriptokokni meningitis	*prvi dan 400 mg,* *nato od 200 do 400 mg na dan*
vzdrževalno zdravljenje	*200 mg na dan*

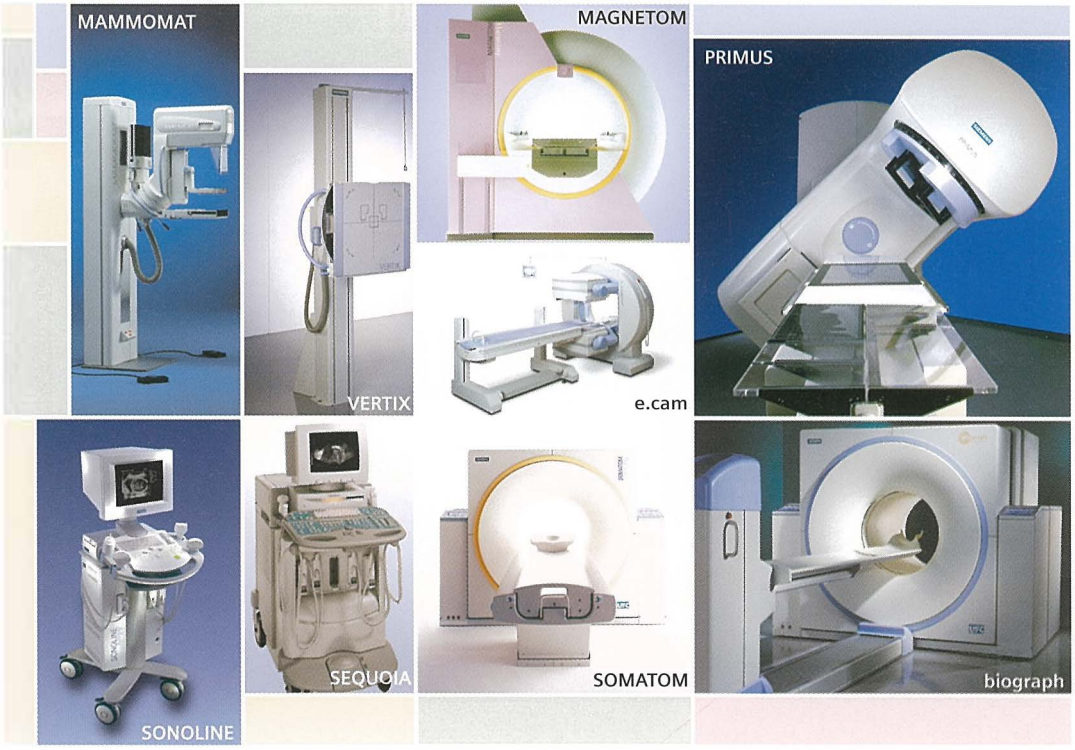
Kontraindikacije: Preobčutljivost za zdravilo ali sestavine zdravila. **Interakcije:** Pri enkratnem odmerku flukonazola za zdravljenje vaginalne kandidoze klinično pomembnih interakcij ni. Pri večkratnih in večjih odmerkih so možne interakcije s terfenadinom, cisapridom, astemizolom, varfarinom, derivati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

KRKA

Krka, d. d., Novo mesto
Šmarješka cesta 6
8501 Novo mesto

SIEMENS

SiemensMedical.com/oncology



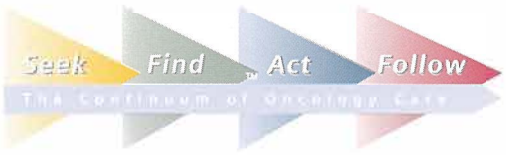
Oncology Care Systems • 4040 NelsonAvenue, Concord, CA 94520 • (925) 246-8200

SEEK-FIND-ACT-FOLLOW - the Continuum of Oncology Care™

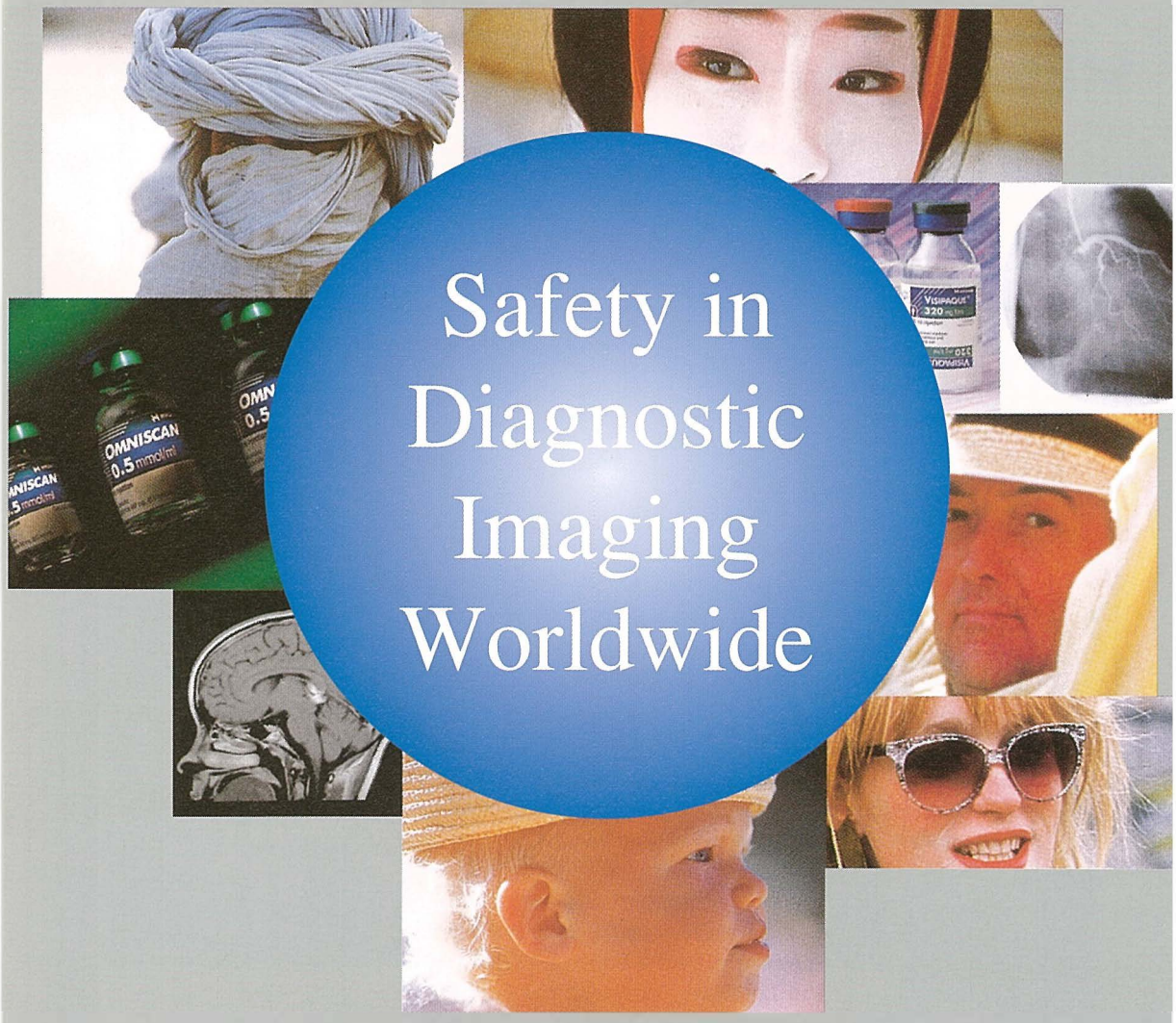
Siemens oncology portfolio comprises comprehensive workflow solutions integrating the full spectrum of care from screening/early detection and diagnosis through therapy and follow-up. All from one provider — with over 100 years history of innovation in medical technology.

Siemens proven clinical methods can help you to achieve more successful outcomes. How? Through industry-leading technology, increased productivity measures for maximized utilization potential, and patient-friendly design and features.

Every day in the United States alone, 29,000 cancer patients receive radiation therapy delivered by Siemens linear accelerators. As clinical protocols transition to include IMRT and IGRT, Siemens seamlessly integrates the diagnostic and treatment modalities. That's what we call Best Practice Oncology Care.



Siemens medical
Solutions that help



Nycomed Imaging is proud of its role in providing for the early, accurate diagnosis of disease, thus improving patients' quality of life and prospect for effective treatment. The company is commited to the continuous development of inovative imaging product to enhance diagnostic procedures.

ZASTOPA
HIGIEA d.o.o., Trzin
tel.: (01) 589 72 25
fax: (01) 589 72 26

Nycomed
Amersham

DISTRIBUCIJA
SALUS d.d. Ljubljana
tel.: (01) 589 91 00
fax: (01) 568 14 20

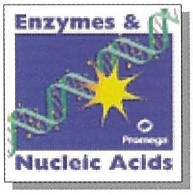
KEMOMED

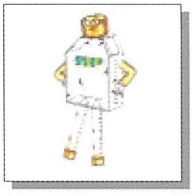
PE: Stritarjeva 5, 4000 Kranj, Slovenija
tel.: (0)4/ 2015 050, fax: (0)4/ 2015 055
e-mail: kemomed@siol.net

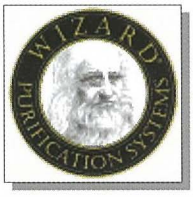
Promega

MWG AG BIOTECH

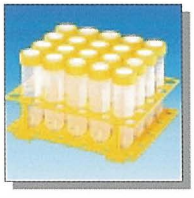
TRP

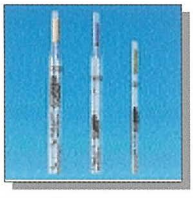






GENOMIKA - PROTEOMIKA





IZDELKI ZA MOLEKULARNO BIOLOGIJO

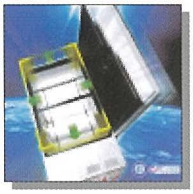
PLASTIKA ZA CELIČNE KULTURE

USF ELGA

Simply, the right solution!

SANYO

LIFE TECHNOLOGIES™

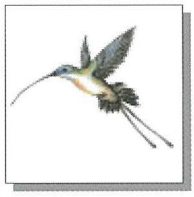


ČISTA VODA ZA LABORATORIJ

SKRINJE IN HLADILNIKI

CELIČNE KULTURE

BIOHIT



ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

LABORMED

zastopa naslednja podjetja

Köttermann (Nemčija):
laboratorijsko pohištvo, varnostne omare za kisline, luge, topila, pline in strupe, ventilacijska tehnika in digestorji

DAKO (Danska):
testi za aplikacijo v imunohistokemiji, patologiji, mikrobiologiji, virologiji, mono- in poliklonalna protitelesa

SVANOVA Biotech (Švedska):
Elisa testi za diagnostiko v veterini

NOVODIRECT BIOBLOCK (Francija):
kompletna oprema in pripomočki za delo v laboratoriju

GFL (Nemčija):
laboratorijski aparati, omare in skrinje za globoko zamrzovanje

ANGELANTONI SCIENTIFICA (Italija):
hladilna tehnika in aparati za laboratorije, transfuzijo, patologijo in sodno medicino

EHRET (Nemčija):
laminar flow tehnika, inkubatorji, sušilniki, suhi sterilizatorji in oprema za laboratorijsko vzrejo živali - kletke

INTEGRA BIOSCIENCES (Švica):
laboratorijska oprema za mikrobiologijo, biologijo celic, molekularno biologijo in biotehnologijo

CORNING (ZDA):
specialna laboratorijska plastika za aplikacijo v imunologiji, mikrobiologiji-virologiji, ipd., mehanske eno- in večkanalne pipete in nastavki

EVL (Nizozemska):
diagnostični testi za uporabo v veterinarski medicini

HÜRNER (Nemčija):
ventilacijska tehnika

CSL - Biosciences:
diagnostični testi za uporabo v veterinarski medicini

BIOMERICA (ZDA):
hitri testi za diagnostiko, EIA /RIA testi

CHARLES ISCHI (Švica):
specialna oprema za testiranje izdelkov v farmacevtski industriji;aparati za procesno kontrolo in kontrolo kvalitete

ROSYS - ANTHOS (Avstrija):
fotometri, avtomatski pralni sistem za mikrotitrine plošče

LABORMED d.o.o.
Zg. Pirniče 96/c
SI - 1215 Medvode
Tel.: (0)1 362 14 14
Fax: (0)1 362 14 15

info@labormed.si

LABORMED, razstavni salon
Bežigrajski dvor
Peričeva 29, Ljubljana
Tel.: (0)1 436 49 01
Fax: (0)1 436 49 05

www.labormed.si

Instructions for authors

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

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

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

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

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

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

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

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

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

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

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

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

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

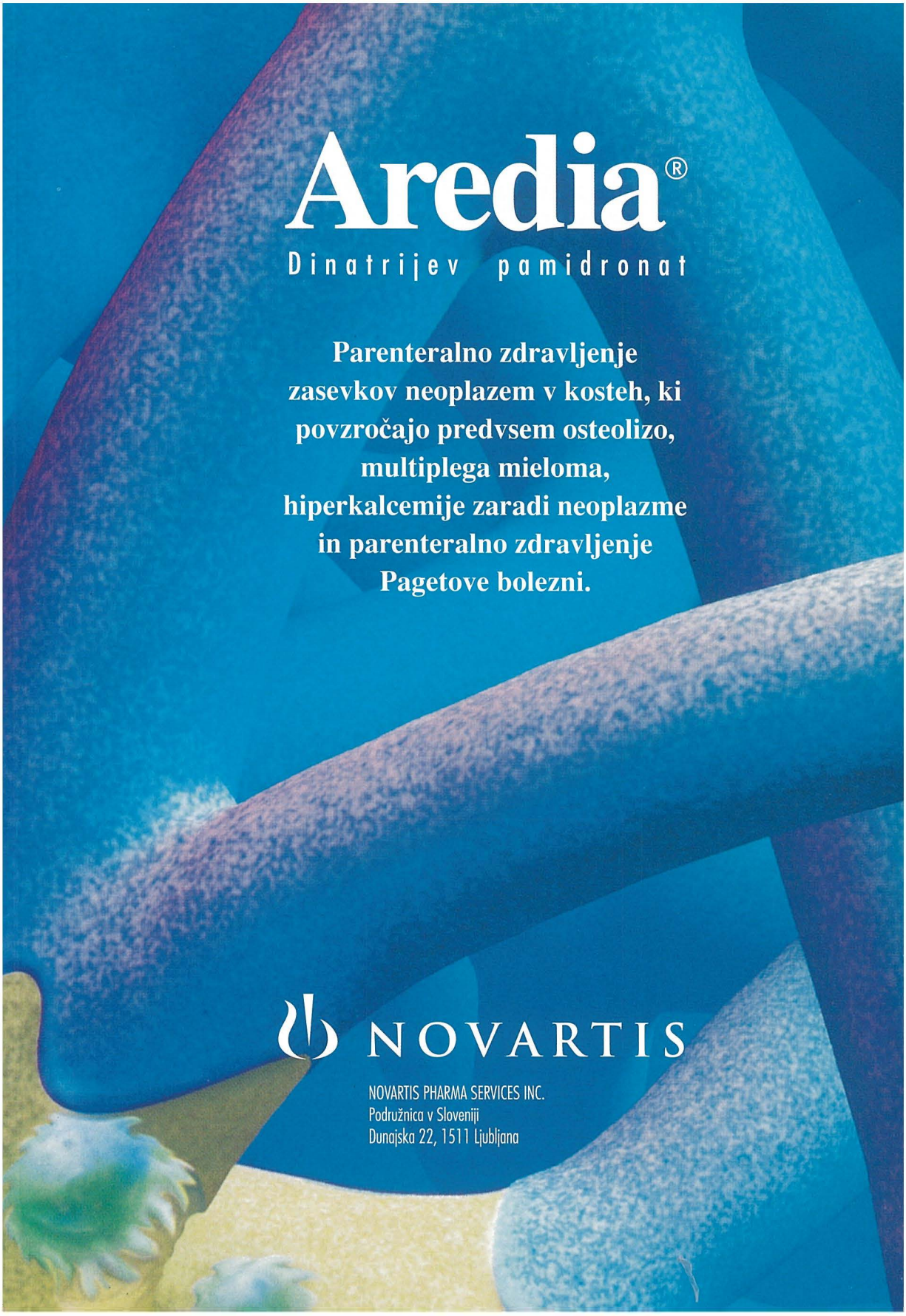
Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall; 1986.

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

Page proofs will be faxed to the corresponding author whenever possible. It is their responsibility to check the proofs carefully and fax a list of essential corrections to the editorial office within 48 hours of receipt. If corrections are not received by the stated deadline, proof-reading will be carried out by the editors.

Reprints: Fifty reprints are free of charge, for more contact editorial board.

For reprint information contact: International Reprint Corporation, 287 East "H" Street, Benicia, CA 94510, USA. Tel: (707) 746-8740; Fax: (707) 746-1643; E-mail: reprints@intlreprints.com


Aredia®
Dinatrijev pamidronat
Parenteralno zdravljenje zasevkov neoplazem v kosteh, ki povzročajo predvsem osteolizo, multiplega mieloma, hiperkalcemije zaradi neoplazme in parenteralno zdravljenje Pagetove bolezni.
NOVARTIS
NOVARTIS PHARMA SERVICES INC.
Podružnica v Sloveniji
Dunajska 22, 1511 Ljubljana