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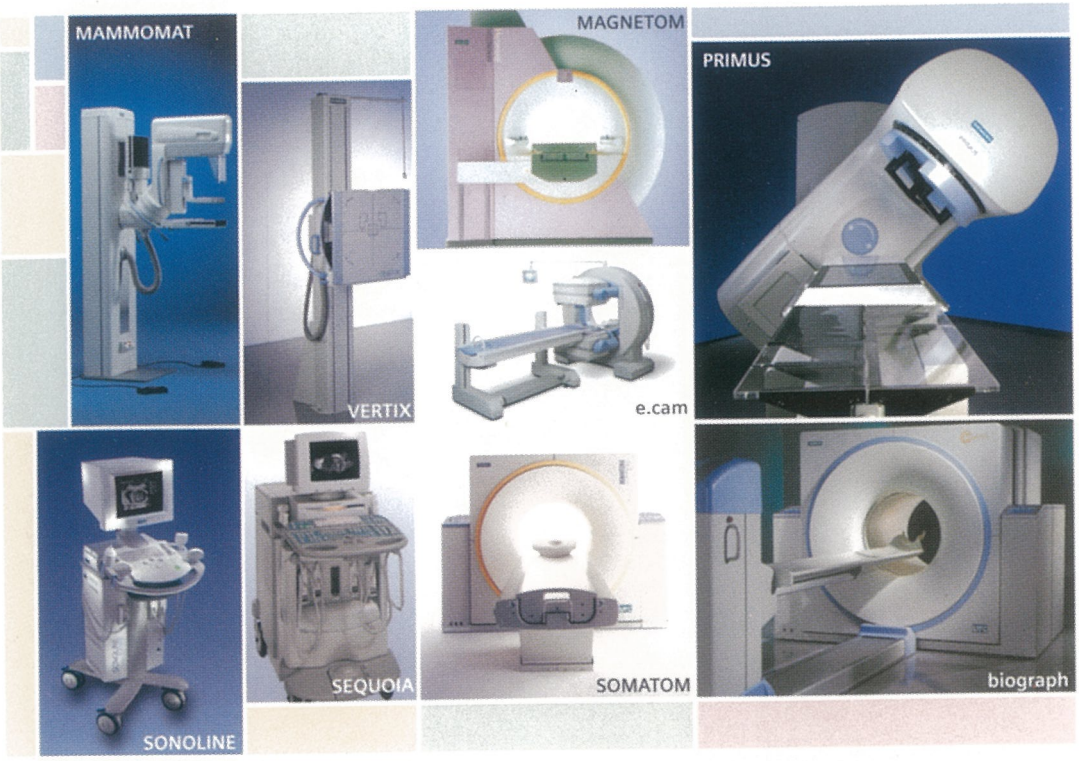

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Radiol Oncol 2003; 37(2): 67-72.

Dedicated small bowel follow-through - experience of Clinical Institute of Radiology in Ljubljana

Mateja Kropivnik and Breda Jamar

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Background. *Small bowel is a difficult part of the alimentary tract to examine. Radiologic modality most commonly used has been the conventional small bowel follow-through (SBFT), which has often been done in a cursory manner, without fluoroscopy and manual palpation. The purpose of our study is to present dedicated SBFT and to assess its sensibility and specificity.*

Patients and methods. *We analysed 35 dedicated SBFT, performed from April to September 2002, in patients. Findings were evaluated according to clinical follow-up, endoscopy and surgery.*

Results. *Our findings were consistent with clinical follow-up, endoscopy and surgery in 33 patients. In 2 patients our findings were false negative. Our results show 89.5 % sensitivity and 100 % specifity.*

Conclusions. *An adequate per-oral examination remains the most reliable tool for diagnostic evaluation of the small bowel.*

Key words: intestine, small - radiology; barium sulfate; enema; Crohn disease

Introduction

Small bowel is a difficult part of the alimentary tract to examine because of its anatomy. Proximal jejunum and the terminal ileum can also be examined by enteroscopy but the mesenteric small intestine is the part of alimentary tract in which radiologic examinations are dominant diagnostic procedures.[1]

The radiologic examinations of the small bowel include barium studies and newer imaging methods: ultrasound (US), computerised tomography (CT) and magnetic resonance imaging (MRI).

For many years, the radiologic modality most commonly used to evaluate small bowel disease has been the conventional small bowel follow-through (SBFT). After *per oral* intake of barium suspension, periodic overhead radiographs were made until the barium suspension reached the colon. Spot radiographs were obtained of the terminal ileum and any other areas of abnormality suggested on the overhead radiographs.[2] Fluoroscopy and palpation were used sparingly or not at all.

For dedicated SBFT, larger amounts of barium suspension must be taken to fill all seg-

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ments of the small bowel. Careful fluoroscopy, vigorous manual palpation and appropriate spot radiographs are routine components of the examination.

Enteroclysis, a double contrast examination needs a intubation catheter with a guidewire positioned just beyond the ligament of Treitz for direct injection of contrast medium into the jejunum.[3] The injection should be fast enough to allow moderate distension without completely abolishing peristalsis.[4] During the filling phase, the different loops are watched for mobility, distension and integrity. Spot films are taken and an overview made when the whole small bowel is filled. Several variations are possible: single contrast enteroclysis or air/water or methylcellulose insufflation for double contrast.[5,6]

Newer imaging methods are valuable tools in assessing intestinal wall and extraluminal involvement.

Gastrointestinal-related disease may be an occasional incidental observation on US examination which should lead to appropriate further investigation.[7] In experienced hands bowel US is an accurate technique for assessing extend and anatomical location of disease within the bowel.[8]

CT has the ability to depict pathology outside the intestinal lumen and in this way contributes to the imaging of the small bowel disease.[7] The accuracy of small-bowel spiral CT studies depends on the presence of well distended loops and adequate endoluminal opacification.[5]

In spiral CT enteroclysis, the administration of water soluble iodinated contrast agent by intubation during fluoroscopy is needed.[9]

MR is not a primary imaging method for the small bowel. It is limited by the mobility of the bowel and by the lack of a reliable intraluminal contrast agent.[7] Owing to excellent soft tissue contrast and multiplanar imaging capabilities, MR imaging could be the optimal imaging method for evaluation of small-bowel in the future.[10]

The aim of our study was to assess the sensibility and specificity of detailed-dedicated SBFT, as performed at our department.

Patients and methods

From April to September 2002 92 dedicated SBFT were performed at the department of gastrointestinal radiology.

Of these, we analysed 35 consecutive patients who were referred by gastroenterologists because indications were more specific and follow-up was possible.

The average age of the patients was 42 years (range, 23-64 years), 14 were male and 21 female.

The referral diagnoses were: food allergy in 1 patient, abdominal pain in 2, abdominal pain with diarrhoea in 4, coeliac disease in 2, suspected Crohn's disease in 16 and reactivation of known Crohn's disease in 10 patients.

Dedicated small bowel follow-through was performed with oral administration of 4x200 ml diluted barium suspension: 600 ml of water added to 200 ml of Micropaque. Fluoroscopy was done during the ingestion of the first 200 ml of barium. The oesophagus and the stomach were examined fluoroscopically. The first spot radiograph was obtained when suspension reached the duodenojejunal flexure.

The patient was given the second glass of barium and after 15-20 minutes an overhead film was taken, and compression and palpation of all segments, filled with barium, was done during fluoroscopy. After the ingestion of the third and fourth glasses of barium all segments of small bowel were opacified in most cases, as well as the terminal ileum and the coecum. Motility was observed during fluoroscopy and careful manual compression of the small bowel was done, before a spot film was taken, to document radiologic findings.

Our findings were evaluated according to clinical follow-up, endoscopy and surgery.

Results

We analysed 35 dedicated SBFT. Our results show 89.5% sensitivity and 100% specifity of dedicated SBFT. All of 17 patients with abnormal findings on SBFT were abnormal at confirmation method giving the proportion of correct diagnoses as 17/17 = 100% (positive predictive value). Similarly, among the 18 patients with normal SBFT the proportion of correct diagnosis was 16/18 = 88.8% (negative predictive value) (Table 1).

Table 1. The accuracy of small bowel follow-through (SBFT)

SBFT	Abnormal	Normal	Total
Abnormal findings	17	0	17
Normal findings	2	16	18
Total	**19**	**16**	**35**

The patient with food allergy has normal findings on SBFT. Endoscopic examination and clinical diagnosis was dyspepsia.

In one case of suspected coeliac disease, thickened folds in duodenum were seen on SBFT. Erosive gastritis and duodenitis were found at gastroduodenoscopy. The patient with longstanding coeliac disease had radiologic findings in the small bowel consistent with coeliac disease and lymphoma both confirmed by histology.

Four patients with abdominal pain and diarrhoea had normal radiologic findings. The absence of small bowel pathology was consistent with clinical follow-up.

Two patients had pain in the upper abdomen: in one case the compression of the third part of duodenum, caused by superior mesenteric artery, was found; in the second case, a 4 cm large diverticulum in the second part of duodenum was seen (Figures 1, 2).

Among 16 patients with suspicion of Crohn's disease, the radiologic findings were positive in 5, the diagnosis was later confirmed by endoscopy and biopsy. In 11 patients with negative radiologic findings, endoscopic findings were negative in 9, but in two coloileoscopy with biopsy showed inflammatory changes in the terminal ileum (Figure3).

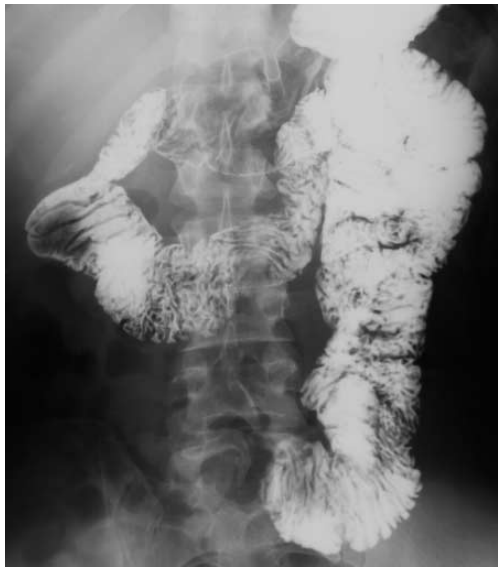

Figure 1. Compression of pars horisontalis duodeni by superior mesenteric artery.

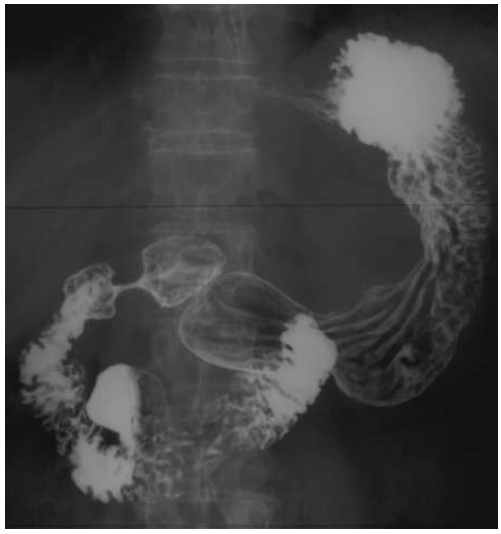

Figure 2. Duodenal diverticulum.

Ten patients had known Crohn's disease and suspected reactivation. Five of them had previous surgery. In 4 ileotransversoanasto-

mosis stenosis was found on SBFT, confirmed by surgery. One patient had a resection of terminal ileum. Inflammatory changes of distal ileum, seen on SBFT, were confirmed endoscopically (Figure 4). In 2 cases the findings on SBFT were normal; at coloileoscopy the inflammatory changes were seen in transversal part of large bowel, but terminal ileum was normal. In 3 cases radiologic changes of Crohn's disease were found in terminal ileum and our findings were confirmed on endoscopy.

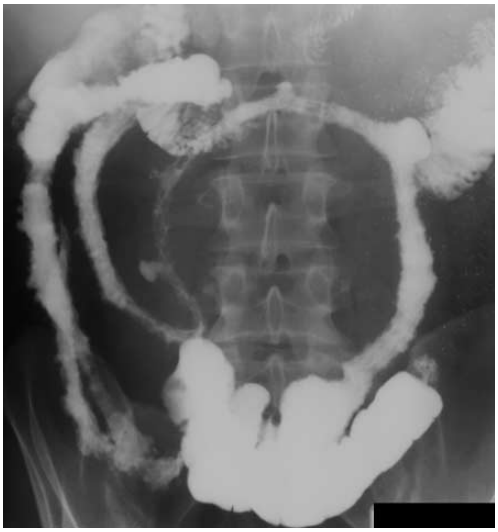

Figure 3. Crohn's disease of the small bowel.

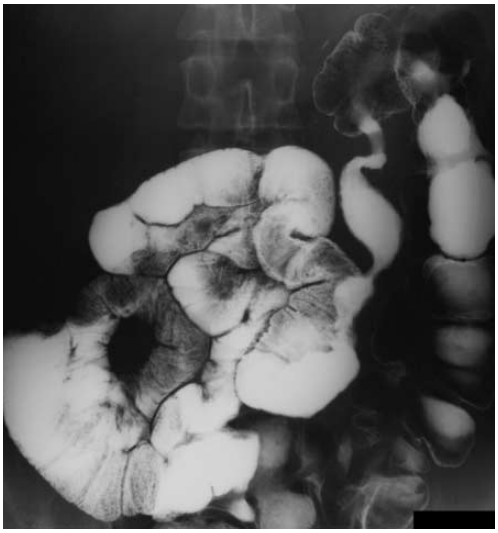

Figure 4. Crohn's disease at ileotransverso-anastomotic site.

Discussion

The prevalence of small-bowel disease is low and the clinical diagnosis is difficult by non-specific symptoms and a low index of suspicion.[1]

In patients with non-specific abdominal complaints US is often the first examining method. The main drawback of US is insufficient visualisation of intestinal lumen due to luminal collapse and presence of gas obscuring the underlying bowel.

The limitations of the radiologic investigation of the small-bowel with oral contrast material have long been recognized.[6]

The dilemma between SBFT as more acceptable to the patient, and the uncomfortable intubation-infusion method (enteroclysis) has not been resolved.[6]

Disadvantage of the conventional SBFT is a risk of overlooking an important abnormality, predominantly on overhead radiographs, which display the opacified small bowel as closely packed, overlapping loops. The major drawback is insufficient use of fluoroscopy and palpation.

Enteroclysis has been promoted as more accurate in the detection of early mucosal changes. The superiority of enteroclysis is controlled introduction of contrast material into the small bowel, luminal distension, small bowel hypotonia secondary to jejunal distension, relative rapidity of completion of the study, and the use of double contrast material.[6] The disadvantages of enteroclysis is the lack of universal availability, higher radiation dose, discomfort during intubation and inability to examine for gastroduodenal disease.

Carefully performed SBFT with frequent

fluoroscopy, manual palpation and appropriate spot radiographs has been shown to achieve results comparable to enteroclysis.[2]

It has some advantages over enteroclysis because of its simplicity, near-universal availability, a high level of patient tolerance, the opportunity to assess the gut in a relatively physiologic state of distension and distensibility, the possibility to evaluate the duodenum and a relatively low radiation dose to the patient.[11]

The sensitivity of enteroclysis in suggested inflammatory bowel disease (IBD) was found to be higher in most of the studies.[12] However, the specificity and the positive predictive value was somewhat higher concerning dedicated follow through examination. Higher sensitivity of enteroclysis probably reflects in a tendency to use it more frequently with specific symptoms of IBD. In the same way dedicated follow-through examination was probably used when symptoms were non-specific.[12]

No gross differences were found in sensitivity, specificity or predictive values, when dedicated follow-through examination and enteroclysis were compared.[4,12]

In our study specificity was 89.5%, because our findings were false negative in two patients. On retrograde evaluation the missed radiologic signs were attributed in one case to overlapping segments of distal ileum and insufficient palpation, in the second case the changes of Crohn's disease were not specific on coloileoscopy, but confirmed by biopsy.

This indicates that the dedicated follow-through technique may be used for screening purposes.[12]

Average skin entry radiation dose for enteroclysis was 1.5 times greater than that for SBFT with upper gastrointestinal examination and almost 3 times greater than for dedicated SBFT.[10]

Enteroclysis is not indicated in young patients with low suspicion of pathology.[11]

It should be borne in mind that bowel US is highly operator dependent, requiring experience and time to achieve accuracy rates comparable with those published in the literature.[8]

The major disadvantages of CT and MRI are that these modalities are expensive and not universally available.

Factors to be considered when selecting the appropriate technique include the reason for the examination, the age of the patient, time and cost involved, patient acceptance, radiation exposure and accuracy.[13]

Conclusions

An adequate per-oral examination depends largely on the use of a dedicated small bowel technique, emphasising fluoroscopic observation and spot radiographs of compression of all segments of small bowel.

The dedicated SBFT has a number of advantages for use as a screening examination, including the need for less room and radiologist time, less radiation exposure and high sensitivity when the examination is carefully performed.

Conventional SBFT has no role in present-day small bowel radiology.

References

1. Maglinte DDT, Lappas JC, Kelvin FM, Rex D, Chernish SM. Small bowel radiology: how, when, and why? *Radiology* 1987; **163:** 297-305.

2. Wills JS, Lobis IF, Denstman FJ. Crohn disease: state of the art. *Radiology* 1997; **202:** 597-610.

3. Rosenbuch G, Jansen JBMJ, Reeders JWAJ. Contemporary radiological examination of the small bowel. *Baillieres Clin Gastroenterol* 1994; **8(4):** 863-700.

4. Nolan DJ. Imaging of the small bowel. *Schweiz Med Wochenschr* 1998; **128(4):** 109-14.

5. Maglinte DDT, Chernish SM, Kelvin FM, O'Connor KW, Hage JP. Crohn disease of the

small intestine: accuracy and relevance of enteroclysis. *Radiology* 1992; **184:** 541-5.

6. Cirillo LC, Camera L, Della Noce M, Castiglione F, Mazzacca G, Salvatore M. Accuracy of enteroclysis Crohn's disease of the small bowel: a retrospective study. *Eur Radiol* 2000; **10:** 1894-8.

7. Gore RM, Levine MS. *Gastrointestinal radiology*. 2nd Edition. Philadelphia: WB Saunders 2000; 67-98.

8. Cittadini G, Giasoto V, Garlaschi G, De Cicco E, Gallo A, Cittadini G. Transabdominal ultrasonography of the small bowel after oral administration of non-absorbable anehoic solution: Comparison with barium enteroclysis. *Clini Radiol* 2001; **56:** 225-30.

9. Mazzeo S, Caramella D, Battolla L, Melai L, Masolino P, Bertoni M, et al. Crohn disease of the small bowel: spiral CT evaluation after oral hyperhidration with isotonic solution. *J Comput Assist Tomogr* 2001; **25(4):** 612-6.

10. Thoeni RF, Gould RG. Enteroclysis and small bowel series: comparison of radiation dose and examination time. *Radiology* 1991; **178:** 659-62.

11. Umschaden HW, Szolar D, Gasser J, Umschaden, Haselbach H. Small bowel disease: comparison of MR enteroclysis images with conventional enteroclysis and surgical findings. *Radiology* 2000; **215:** 717-25.

12. Baath L, Ekberg O, Borulf S, Lindhagen T, Olsson R. Small bowel barium examination in children. *Acta Radiologica* 1989; **30:** 621-5.

13. Ott DJ, Men Chen Y, Gelfand DW, Van Swearingen F, Munitz HA. Detailed small bowel examination vs. enteroclysis. *Radiology* 1985; **155:** 29-34.

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review

Early postoperative serum carcinoembryonic antigen levels in patients operated for colorectal carcinoma - a new method for following-up

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Background. *The only method of treatment offering a favourable prognosis for colorectal carcinoma is radical resection of the part of the colon or rectum including the pertaining lymph nodes and eventual radical removal of metastases. But even such presumably curative surgery does not warrant full recovery of all operated patients as recurrences are frequent and according to most analyses 5-year survival is lower than 50%. Therefore, additional treatment is attempted in some patients. Various prognostic factors of disease recurrence are helpful. One such prognostic sign is serum carcinoembryonic antigen (CEA) level measured soon after surgery.*

Conclusions. *All patients with radical R0 resection, according to their postoperative serum CEA levels and the CEA half-life fall into three groups: $_{CEA}$ R0, $_{CEA}$ R1 and $_{CEA}$ R2 resected patients. A statistically significant difference regarding survival and number of recurrences was noted among patients categorized by the stage of disease, particularly between the three groups of patients and the group having been undergone presumably curative surgery.*

Key words: colorectal neoplasms; carcinoembryonic antigen - blood; prognosis; follow-up studies

Introduction

In Slovenia there are about 850 new cases of colorectal carcinoma (CC) per year. The incidence is increasing steeply.[1,2] In about 550 cases per year in Slovenia, CC is also the cause of death. Of the newly detected cases, about 75% are treated surgically, 5% only with chemotherapy and/or radiotherapy, 20% are not receiving treatment. The major aim of the operation is R0 resection of the colon with the pertaining lymphadenectomy and radical removal of eventual metastases, i.e. complete removal of malignant cells which would lead to the full recovery of the patient.[3-16] This is logical as the classification of resections by radicality or by residual tumour is based on the surgeon's intraoperative evaluation and on the pathologist's analysis of the operative specimen.[17-25] This means that, in radical resection, the surgeon believes that no malignant cell is left in the patient's body (he is only aided by his vision, tactile sense and some-

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times intraoperative US), while the pathologist's evaluation is based only on the analysis of the operative specimen and the investigation of its margins, rarely on additional biopsy done by the surgeon during the surgical procedure. Hence, the evaluation of the residual tumour, i.e. malignant cells remaining in the body, is only approximate.[26-32] With the aid of his senses and US during surgery, the surgeon cannot exclude residual malignant cells in the body, and the pathologist can only evaluate the tissue removed.[33-42]

Radical removal of all malignant cells from the body should result in a drop in CEA level regardless of its half-life in the form of an exponential curve to normal levels.[43-57] In the patients in whom surgical treatment was not so successful, such a serum CEA drop did not occur because the residual malignant cells kept on producing CEA which is reflected in a slower drop of the serum CEA level.[57]

CEA and curative resection

In the patients in whom the CEA level dropped as expected, it is more likely that curative resection was successful. In those in whom the CEA levels were dropping more slowly than expected, an earlier detection of recurrence is possible by strict following up or the delayed drop of CEA level could indicate to carry out additional chemotherapy immediately after surgery.

For easier work and comprehension, the following new terms are recommended:

$_{CEA}$R0 resection, $_{CEA}$R1 resection and $_{CEA}$R2 resection (Figure 1).

$_{CEA}$R0 resection represents R0 resection according to surgical and histological evaluation in which the expected drop in CEA level - with regard to half-life - was noted in the serum of patients after surgery for CC.

$_{CEA}$R1 resection represents R0 resection according to surgical and histological evaluation in which a slower drop in CEA level than expected was noted in the serum of patients after surgery for CC.

$_{CEA}$R2 resection represents R0 resection in which no drop in CEA level was noted in the serum of patients after surgery.

Follow-up of patients

The follow-up of patients after surgery for CC is particularly advisable in view of the possibility to detect curable recurrences of the disease in asymptomatic patients before they become unresectable.[58-61] The major aim of such follow-up after presumably curative surgery is the detection of metachronous colorectal tumours and recurrences which are radically resectable, such as local recurrences or resectable liver and lung metastases.[58,62-64] Various protocols elaborated by expert groups are an aid to the follow-up.[3,58,62-65] We used the recommendations by the expert group of the Ministry of Health for following up the patients after surgery for CC.[3]

In most analyses in the literature, a significant prognostic sign of CC recurrence and of survival is the preoperative serum CEA level or the so-called initial CEA.[66,67] But with respect to the cut-off point, the data regarding the serum CEA differ strongly. The difference is most obvious when the cut-off point is set

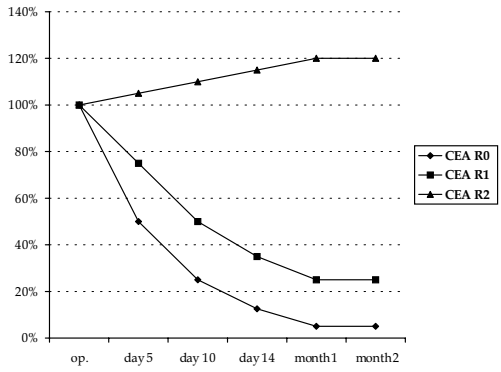

Figure 1. Graphic presentation of serum C EA value in $_{CEA}$ R0, $_{CEA}$ R1 and $_{CEA}$ R2 resections.

at 20 ng/mL CEA in the serum and when it decreases with the lowering of the cut-off point.[66,67]

In the study, the measurement early postoperative serum CEA levels proved to be a significant prognostic sign for the disease recurrence and for the survival of patient after surgery for CC, confirming completely the expectations. In the literature, several studies find the measurement of postoperative serum CEA levels imperative,[58,66-70] but with regard to CEA half-life, only a few find it significant to measure these levels soon after surgery.[71] Most studies classified the patients after surgery for CC into different groups, and practically all found that, in such patients, the postoperative drop in preoperatively increased serum CEA levels to normal levels - and remaining there (under 5 ng/mL) - was a significant prognostic sign of 5-year survival.[58,66,68,69,72-74] In the Maribor Teaching hospital, statistically significant differences were found between these groups as regards the prognostic value of two-year survival as well as disease recurrence. The results confirm that the $_{CEA}$R0 resected patients have an excellent prognosis regarding the survival and a lesser probability of recurrence. For other patients, eventual adjuvant treatment and a strict follow-up can be planned already in the perioperative period.[71]

The majority of our patients were operated in the advanced stage of disease, more than 50% in Dukes C and 8.6% in Dukes D. Although this is in accordance with the findings of some other authors,[2,16,23,75,76] it is a poor prognosis for total survival and curability of patients after surgery. Various authors describe 5-year survival after CC surgery for Dukes A as approximately 80-90%, for Dukes B 70-80%, for Dukes C 40-50% and for Dukes D 10-30%, and total survival between 40 and 60%.[1,2,4,6,7,19,66,67,70,75,76]

Conclusions

The study performed in Maribor Teaching Hospital proves that the results of early serum CEA level measurement after surgery for CC are good prognostic signs of the disease recurrence in the patients who are, according to pathohistologic criteria, assumed to be curatively operated on after having undergone R0 resection.[71]

The method, apart from being economical, is advantageous also because the patient is not exposed to any additional investigative methods, as the venous blood sample of several ml can be obtained during a regular postoperative hemogram check. Theoretically, only one measured postoperative serum CEA level, between day 3 and day 10 following the surgery, would suffice. Another advantage of the method is the possibility of repetition of measurements, if required.

The main disadvantage is that it is only suitable for about half of the patients operated for CC. Many patients exhibit no preoperative increase in the serum CEA level. In the patients requiring larger amounts of transfused blood the method is not applicable, either. However, in these patients other widely used methods would be inadequate as well.

A significant advantage of the method is that it yields the results quickly after the operative procedure is performed; giving the possibility of planning eventual adjuvant treatment and determining other methods of follow-up, such as more frequent controls of $_{CEA}$R1 and $_{CEA}$R2 resected patients.

The results of different studies, including ours, confirm the possibility of applying the method in the search for those patients who after presumably curative treatment require adjuvant therapy and/or precise follow-up - i.e. in the patients in whom we can most probably expect detecting recurrences, or metastasizing, or metachondrous intestinal tumours before they become unresectable, which is the basic aim of postoperative follow-up.

References

1. Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J, Powell J, editors. *Cancer incidence in five continents.* Vol. 6. Lyon: International Agency for Research on Cancer; 1992. (IARC Sci Publ No.120).

2. Pompe-Kirn V. Epidemiološke značilnosti raka širokega črevesa in danke v Sloveniji. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana: 1996. p. 79-85.

3. Repše S, editor. *Priporočila za celostno obravnavo bolnikov z rakom prebavil.* Ljubljana: Ministrstvo za zdravstvo R Slovenije; 1997. p. 23-32.

4. Sing S, Morgan MBF, Broughton M, Caffarey S, Topham C, Marks CG. A 10-year prospective audit of outcome of surgical treatment for colorectal carcinoma. *Br J Surg* 1995; **82:** 1486-900.

5. Harris DR, Blake JRS. Computerised audit for colorectal cancer. *Ann R Coll Surg Engl* 1993; **75:** 268-71.

6. Beart RW, Steele GD, Menck HR, Chimel JS, Ocwieja KE, Winchester DP. Management and survival of patients with adenocarcinoma of the colon and rectum: a national survey of the commission on cancer. *J Am Coll Surg* 1995; **181:** 225-36.

7. Mäkelä JT, Laitinen SO, Kairaluoma MI. Five-year follow-up after radical surgery for colorectal cancer. Results of a prospective randomized trial. *Arch Surg* 1995; **130:** 1062-7.

8. McIntosh G, Young G. Diet and large bowel cancer prevention. *Cancer Forum* 1995; **19:** 160-3.

9. Potter JD. Nutrition and colorectal cancer. *Cancer Causes and Control* 1996; **7:** 127-46.

10. Schottenfeld D, Winawer SJ. Large intestine. In: Schottenfeld D, Fraumani J Jr, editors. *Cancer epidemiology and prevention.* Philadelphia: Saunders; 1982. p. 703-27.

11. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal tumor- development. *New Engl J Med* 1988; **319:** 525-32.

12. Fenoglio CM, Pascal RR. Colorectal adenomas and cancer: pathologic relationships. *Cancer* 1982; **50:** 2601-8.

13. Winawer SJ, Zauber A, Diaz B. Temporal sequence of evaloing colorectal cancer from the normal colon. *Gastrointestal Endosc* 1987; **33:** 167-72.

14. Beahrs OH, Henson DE, Hutter RVP, Kennedy BJ, eds. *AJCC manual for staging of cancer.* 4th edition. , Philadelphia: J.B. Lippincot; 1992.

15. Hermanek P, Wittekind C. The pathologist and the residual tumor (R) classification. *Pathol Res Pract* 1994; **190:** 115-23.

16. Bračko M, Lamovec J. Patologija in klasifikacija karcinoma širokega črevesa in danke. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 86-92.

17. Jass JR, Love SB, Northover JMA. A new prognostic classification of rectal cancer. *Lancet* 1987; **I:** 1303-6.

18. International Union Against Cancer. Colon and rectum. In: Sobin LH, Wittekind Ch, editors. *TNM Classification of malignant tumors.* 5th edition. New York: Wiley-Liss; 1997. p. 66-9.

19. Krasna MJ, Flancbaum L, Cody RP, Shneibaum S, Ben Ari G. Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. *Cancer* 1988; **61:** 1018-32.

20. Winawer SJ, Enker WE, Lightdale CJ. Malignant tumors of the colon and rectum. In: Berk JE, editor. *Bockus gastroenterology.* 4th edition. Vol.4. Philadelphia: W.B. Saunders Company; 1985. p. 2531-74.

21. Speight VO, Johnson MW, Staltenberg PH, et al. Colorectal cancer: current trends in initial clinical manifestations. *Sought Med J* 1991; **84:** 575-8.

22. Dixon AR, Thornton HJ, Cheetan HM. General practitioners awareness of colorectal cancer, a 10 years review. *Br Med J* 1990; **301:** 152-3.

23. Žitko A. Rak širokega črevesa in danke-klinične značilnosti in naravni potek bolezni. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 93-8.

24. Filding LP, Padmanabhan A. Clinical features of colorectal cancer. In: Misiewicz JJ, Pounder RE, Venables CW, editors. *Disease of the gut and pancreas.* 2nd edition. Oxford: Blackwell Scientific Publications; 1994. 877-92.

25. Rankin GB. Indications, contraindications and complications of colonoscopy. In: Sivak MV, editor. *Gastrointestinal endoscopy.* Philadelphia: Saunders; 1987. p. 783-878.

26. Foutch PG, Mai H, Pardy K, DiSario JA, Manne RK, Kerr D. Flexible sigmoidoscopy may be ineffective for secondary prevention of colorectal cancer in asymptomatic average-risk men. *Dig Dis Sci* 1991; **36:** 924-8.

27. Bond JH. Evolving Strategies for Colonoscopic Management of patients with Colorectal Polyps. *Endoscopy* 1995; **27:** 38-42.

28. Laufer I, Levine MS. *Double contrast gastrointestinal radiology.* 2nd edition.1992. p. 423-95.

29. Hernandez-Socorro CR, Guerra C, Hernandez-Romero J, Rey A, Lopez-Facal P, Alvarez-Santullano V. Colorectal carcinomas: diagnosis and preoperative staging by hydrocolonic sonography. *Surgery* 1995; **117:** 609-15.

30. Thoeni RF. Colorectal canser: cross-section imaging for staging of primary tumor and detection of local recurrence. *AJR* 1991; **156:** 909-15.

31. Allgoewer M. Das Coloncarcinom. In: *Chirurgische gastroenterologie 2.* Allgoewer M, Harder F, Hollender LF, Siewert JR, editors. Berlin: Springer; 1981. p. 776-96.

32. Welch CE, Ottinger LW, Welch JP. Manual of lower gastrointestinal surgery. New York: Springer; 1980.

33. Gall FP, Hermanek P. Wandel und derzeitiger stand der chirurgischen behandlung des colorectalen carcinoms. *Chirurg* 1992; **63:** 227-34.

34. Beger A. Chirurgische therapie des primaeren kolonkarzinoms. In: Smola MG, Jatzko GR, editors. *ACO consensus-bericht kolonkarzinom.* Graz: ACO; 1995.

35. Herfarth Ch, Hohenberger P. Radikalitaet mit eingeschraenkter resection in der carcinomchirurgie des gastrointestinaltrakts. *Chirurg* 1992; **63:** 235-41.

36. Siewert JR, Fink U. Multimodale therapieprinzipien bei tumoren des gastrointestinaltrakts. *Chirurg* 1992; **63:** 242-50.

37. Repše S. Standardne, razširjene in multivisceralne resekcije pri raku širokega črevesa. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 131-36.

38. Žakelj B. Nizka sprednja resekcija. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. 137-40.

39. Yeatman TJ, Bland KI. Sphincter-saving procedures for distal carcinoma of the rectum. *Ann Surg* 1989; **209:** 1-17.

40. Čalić M. Abdominoperinealna ekscizija rektuma. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 141-44.

41. Rothenberger D, Douglas Wong W. Abdominoperineal resection for adeno-carcinoma of the low rectum. *World J Surg* 1992; **16:** 478-85.

42. Mac Farlane JK, Ryall RDH, Heald RJ. Mesorectal excision for rectal cancer. *Lancet* 1993; **20:** 457-60.

43. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. *J Exp Med* 1964; **121:** 439-42.

44. Krupey J, Wilson T, Freedman SO, Gold P. The preparation of purified carcinoembryonic antigen of the human digestive system from large quantities of tumor tissue. *Immunochemistry* 1972; **9:** 617-22.

45. Mai M, Takahashi Y. Prediction of recurrence of gastrointestinal cancer from standpoint of biological malignancies-tumor marker doubling time and its half life period line. *Hum Cell* 1993; **6:** 82-7.

46. Steward AM, Nixon D, Zamcheck N, Aisenberg A. Carcinoembryonic antigen in breast cancer patients: serum levels and disease progress. *Cancer* 1974; **33:** 1246-52.

47. Oehr P, Schlosser T, Adolphs HD. Applicability of an enzymatic test for the determination of CEA in serum and CEA-like products in urine of patients with bladder cancer. *Tumor Diagnostik* 1980; **1:** 40-4.

48. Reynoso G, Chu TM, Holyoke D, Cohen E, Nemoto T, Wang JJ, et al. Carcino-embryonic antigen in patients with different cancers. *JAMA* 1972; **220:** 361-5.

49. Ewing HP, Newson BD, Hardy JD. Tumor Markers. *Curr Probl Surg* 1982; **19:** 53-94.

50. Zamcheck N. CEA in diagnosis, prognosis, detection of recurrence, and evaluation of therapy of colorectal cancer. Symposiom on Clinical Application of CEA and Other Antigenic Markers Assays. Nice, France. October 1977. Amsterdam: Medica Exerpta; 1978. p. 64.

51. Martin EW Jr, Cooperman M, King G, Rinker L, Carey LC, Minton JP. A retrospective and prospective study of serial CEA Determinations in the early detection of recurrent colon cancer. *Am J Surg* 1979; **137:** 167-9.

52. Skarin AT, Delwiche R, Zamcheck N, Lokich JJ, Frei E 3rd. Carcinoembryonic antigen: clinical correlation with chemotherapy for metastatic gastrointestinal cancer. *Cancer* 1974; **33:** 1239-45.

53. Lokich JJ, Zamcheck N, Lowenstein M. Sequential carcinoembryonic antigen levels in the therapy of

metastatic breast cancer. *Ann Intern Med* 1978; **39:** 902-6.

54. Zamcheck N. Carcinoembryonic antigen. Quantitative variations in circulating levels in benign and malignant digestive tract disease. *Adv Intern Med* 1974; **19:** 413-33.

55. Go VL, Ammon HV, Holtermuller KH, Krag E, Phillips SF. Quantification of carcinoembryonic antigen-like activities in normal human gastrointestinal secretions. *Cancer* 1975; **36:** 2346-50.

56. National Committee for Clinical Laboratory Standards. *Evaluation of precision performance of clinical chemistry devices.* 2nd edition. Tentative Guideline. NCCLS Document EP5-T2, March 1992.

57. Mai M, Takahashi Y. Prediction of recurrence of gastrointestinal cancer from standpoint of biological malignancies - tumor marker doubling time and its a half life period line. *Hum Cell* 1993; **6:** 82-7.

58. Ohlsson B, Breland U, Ekberg H, Graffner H, Tranberg KG. Follow-up after curative surgery for colorectal carcinoma. *Dis Colon Rectum* 1995; **38:** 619-26.

59. Northover J. Which Type of Follow-up? *Hepatogastroenterology* 2000; **47:** 335-6.

60. Laghi L. Genetics of colorectal cancer. *Hepatogastroenterology* 2000; **47:** 315-22.

61. Rudy DR, Zdon MJ. Update on colorectal cancer. *Am Fam Physician* 2000; **61:** 1759-70, 1773-4.

62. Graham RA, Wang S, Catalano PJ, Haller DG. Postsurgical surveillance of colon cancer: preliminary cost analysis of physician examination, carcinoembryonic testing, chest x-ray, and colonoscopy. *Ann Surg* 1998; **228:** 59-63.

63. Peethambaram P, Weiss M, Lomprinzi CL, Novotny P, O`Fallon JR, Erlichman C, et al. An evaluation of postoperative follow-up tests in colon cancer patients treated for cure. *Oncology* 1997; **54:** 287-92.

64. Lucha PA Jr, Rosen L, Olenwine JA, Reed JF, Riether RD, Stasik JJ Jr, et al. Value of carcinoembryonic antigen monitoring in curative surgery for recurrent colorectal carcinoma. *Dis Colon Rectum* 1997; **40:** 145-9.

65. Omejc M. Sledenje operiranih bolnikov z rakom širokega črevesa in danke. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 196-9.

66. Behbehani AE, Dashti H, Hussain T, Szymendera JJ. Five-year survival versus initial concentrations and longitudinal serum CEA patterns in patients with colorectal carcinoma. *Supp Nutr* 1995; **11:** 614-8.

67. Dawson LA, Franssen E, Davey P. Postoperative borderline elevated CEA predicts for earlier relapse in patients with rectal cancer receiving adjuvant postoperative therapy. *Cancer J Sci Am* 1999; **5:** 374-9.

68. Szymendera JJ, Nowacki MP, Szawlowski AW, Kaminska JA. Predictive value of plasma CEA levels: preoperative prognosis and postoperative monitoring of patients with colorectal carcinoma. *Dis Colon Rectum* 1982; **25:** 46-52.

69. Filella X, Molina R, Pique JM, Grau JJ, Garcia-Valdecasas JC, Biete A, et al. CEA as a prognostic factor in colorectal cancer. *Anticancer Res* 1994; **14:** 705-8.

70. Slenz K, Senagore A, Hibbert J, Mazier WP, Talbott TM. Can preoperative and pooperative CEA predict survival after colon cancer resection? *Am Surg* 1994; **60:** 528-32.

71. Veingerl B. Serum CEA levels in patients operated for colorectal carcinoma. *Wien Klin Wochenschr* 2001; **113(Suppl 3):** 32-8.

72. Rapellino M, Piantino P, Pecchio F, Ruffini E, Cavallo A, Scappaticci E, et al. Disappearance curves of tumor markers after radical surgery. *Int J Biol Markers* 1994; **9:** 33-7.

73. Sato T, Nishimura G, Nonomura A, Miwa K, Miyazaki I. Serological studies on CEA, CA19-9, STn and SLX in colorectal cancer. *Hepatogastroenterology* 1999; **46:** 914-9.

74. Forones NM, Tanaka M. CEA and CA 19-9 as prognostic indexes in colorectal cancer. *Hepatogastroenterology* 1999; **46:** 905-8.

75. Štor Z, Repše S, Juvan R, Omejc M. Rezultati operativnega zdravljenja raka širokega črevesa in danke-naša kazuistika iz obdobja 1989-1993. In: Repše S, editor. *Kirurgija širokega črevesa in danke.* Zbornik simpozija. Ljubljana; 1996. p. 200-9.

76. Bergamaschi R, Arnaud JP. Routine compared with nonscheduled follow-up of patients with »curative« surgery for colorectal cancer. *Ann Surg Oncol* 1996; **3:** 464-9.

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Prognostic outcome of local recurrence in breast cancer after conserving surgery and mastectomy

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Background. *In our retrospective study we analysed local recurrences in breast cancer patients treated with conserving surgery (CS) followed by adjuvant radiotherapy (RT) or mastectomy (ME) with or without radiotherapy. We analysed the impact of local recurrence on overall survival.*

Patients and methods. *Between 1980-1995, 306 patients underwent conserving surgery and 1,193 patients were done mastectomy in Masaryk Memorial Cancer Institute. The patients lost to follow-up were excluded. After all, we analysed 236 patients who underwent conserving surgery (Group A), and 1,121 who underwent mastectomy (Group B). All patients with CS received adjuvant RT of the breast with or without regional lymph nodes. In 982 patients (87.6 %) with ME, we performed RT of the chest wall with or without regional lymph nodes. Median age at the time of diagnosis was 48.3 years in Group A and 52.1 years in Group B. In Group A, 149 patients (63.1 %) had T1 tumour, 86 (36.4 %) T2 and 1 (0.5 %) T3. In 24.2 % of patients, axillary node involvement was observed. In Group B, 316 patients (30.4 %) had T1 tumour, 607 (58.3 %) T2, 76 (7.3 %) T3, 33 (3.2 %) T4 and 9 (0.9 %) TX. In 46.2 % of these patients, we found axillary node involvement. Invasive ductal carcinoma was histologically proved in 67.4% in Group A and 84% in Group B. Systemic treatment was given to 133 patients (56.4 %) from Group A and to 857 patients (76.4 %) from Group B.*

Results. *Median follow-up was 100.5 months in Group A and 121 months in Group B. In Group A, we registered 22 (9.3 %) local recurrences, 5-year local control was 96.2% and median time to local recurrence was 50 months. In Group B, we registered 65 (5.8%) local recurrences; 5-year local control was 96.6%. Five-year local control in patients with T1, T2 tumours was 97.2%. In patients with adjuvant RT median time to local recurrence was 48.5 months, and in patients without adjuvant RT 51 months. Thirteen patients (8.7 %) who underwent mastectomy without RT had local recurrence . The impact of local recurrence on overall survival was statistically significant in Group B ($p = 0.002$) and not exactly statistically significant in Group A ($p = 0.062$). Patients who developed local recurrence had lover overall survival. Unambiguous linear dependence was confirmed between the time to local recurrence and overall survival.*

Conclusions. *The impact of local recurrence on overall survival was found statistically significant. Probability of local recurrence and time to local recurrence was the same in the patients treated with CS or ME. The overall survival increased with local disease free interval.*

Key words: breast neoplasms - surgery; mastectomy; neoplasms recurrence, local; prognosis; radiotherapy, adjuvant; survival analysis

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Introduction

Significance of local recurrence after breast conserving surgery (CS) or radical mastectomy (ME) has been discussed. Local recurrence after ME is supposed to have worse prognosis than after CS. The role of postoperative locoregional radiotherapy (RT) (to the chest wall or whole breast and regional lymph nodes) has been evaluated in randomised studies during the last 50 years. The results confirmed the impact of locoregional treatment on reduction of local recurrence, but the impact on overall survival (OS) is still not clear.[1] The impact on OS was proved only in the patients with positive lymph nodes and systemic therapy.[2] Breast cancer is a systemic disease. This new approach favoured breast conserving surgery and application of chemotherapy. The number of mutilating operations thus decreased. Nevertheless, locoregional RT remains an important treatment modality.

However, some questions have remained unsolved. Further studies need to be performed to explain the role of RT and its integration in multimodal therapy of breast cancer. CS followed by postoperative RT has become standard treatment modality. Prospective randomised trials have shown that the number of local recurrences after CS followed with RT is the same as after the mutilating ME.[3-8] OS rate and the risk of distant metastases development are equal in both, ME and CS followed by RT.[9-13] Contraindications for RT are the same as for CS: pregnancy, prior breast or chest irradiation (i.e. mantle technique), collagenosis. Other known contraindications for CS are: multifocal tumour, diffuse microcalcification, breast and tumour size disproportion.[14] Patients' opinion has to be respected.

The meta-analysis of 36 randomised studies compared the results of postoperative RT in early breast cancer patients (17 273 patients.) and ME alone. A treble decrease of risk of local recurrence was shown after adjuvant RT. The difference in 10-year survival was not significant.[15] Fisher's study shows similar results.[4]

The prognosis of local recurrence is still uncertain. The impact of local recurrence on overall survival is not clear.

Patients and methods

Patients with breast conserving surgery (CS)

Between January 1983 and December 1994, a total number of 306 patients underwent adjuvant RT after CS at the Masaryk Memorial Cancer Institute (Figure 1). Our report evaluates the available data of 236 patients. For statistical evaluation, SPSS, Matlab, Gehan-Wilcoxon (for survival analysis) and log-rank tests were used. Local therapy (surgery + RT) was followed by adjuvant chemotherapy or hormonal therapy in 54%. Table 1 shows the characteristic of patients. Figure 2 shows the age range. Tumour size up to 2 cm (63.1%) (Figure 3) and invasive ductal carcinoma

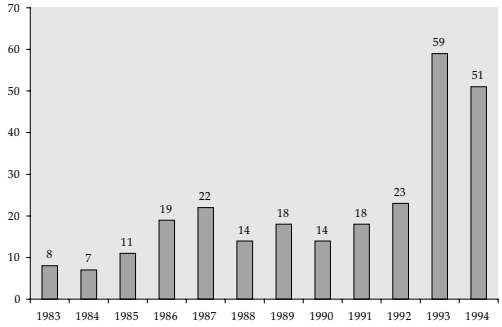

Figure 1. Number of patients treated by conservative surgery and radiotherapy.

Table 1. Characteristics of patients treated by conservative surgery (n = 236)

	Age (years)	
Mean	49.68±0.64	
Median	48.29 (25.3-80.3)	
	Stage of tumour	
T1	149	(63.1%)
T2	86	(36.4%)
T3	1	(0.5%)
	Histology	
Ductal	159	(67.4%)
Lobular	23	(9.7%)
Others	54	(22.9%)
	Margins	
Free	214	(90.7%)
Positive	22	(9.3%)
	Axillary nodes	
Positive	36	(24.2%)
Negative	118	(44.7%)
Unknown	82	(31.1%)
	Side	
Left	124	(52.5%)
Right	112	(47.5%)
	Quadrant	
Outer upper	168	(71.2%)
Outer lower	22	(9.3%)
Inner upper	37	(15.7%)
Inner lower	2	(0.8%)
Central	7	(3.0%)

(67.4%) were found in most cases. The mean follow-up was 110.4 months (median 100.5 months).

Treatment

After the elimination of distant metastases, wide local excision (23.7%) or quadrantectomy (76.3%) was performed. Axillary dissection was not performed and a limited number of axillary lymph nodes were examined in 31.1% of patients. All patients received postoperative RT to the whole breast, with or without irradiation of regional lymph nodes (axillary and supraclavicular). The mean interval between surgery and RT was 23 days (range 10-120, median 28.8 days). The entire

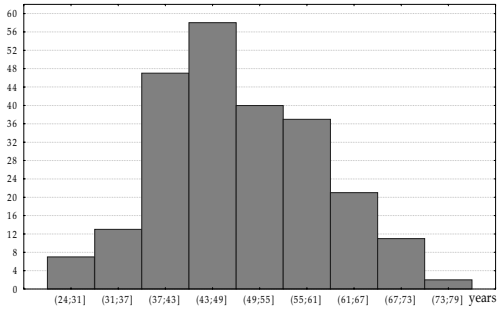

Figure 2. Age distribution of patients after conservative surgery and radiotherapy.

Stage of tumour	Number of pts.	
pT1	149	63, 1%
pT2	86	36, 4%
pT3	1	0, 5%

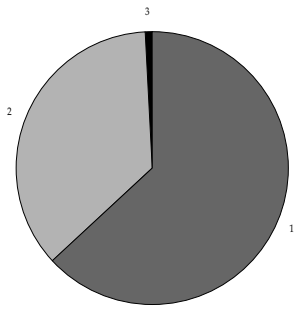

Figure 3. Stage of tumour in patients after conservative surgery and radiotherapy.

breast was included in the target volume. The superior border lay at about the level of the suprasternal notch medially and just bellows the level of the abducted arm laterally. The inferior border lay 1-2 cm below the breast. The medial border was usually in the mid-line, and the lateral border was in the mid-axillary line. Two tangential fields were used for irradiation of breast. Four fields - two tangential and two convergent - were used for irradiation of the breast and regional lymph nodes.

The lymph nodes were irradiated in 169 patients (71.6%). Sixty-two patients (22.7%) were treated with linear accelerator (photons 6MV) and 174 patients (73.7%) with cobalt unit. Electron beam of linear accelerator or caesium unit were used as boosting to tumour bed or axilla. The applied dose was prescribed according to ICRU (International Commission on Radiation Units and Measurements).

Systemic adjuvant therapy was given to 133 patients (56.4%). Hundred and nine patients (46.1%) completed 2-6 cycles of chemotherapy. Eighty-three patients (35.2%) received CMF regimen (cyclophosphamide, methotrexate, and 5-fluorouracil), 11 patients (7.6%) FAC regimen (5-fluorouracil, doxorubicin, and cyclophosphamide), and 8 patients had both regimens, CMF and FAC. Hormonal therapy (tamoxifen 20 mg per day) was given to 68 patients (28.8%). Twenty-nine patients (11%) received chemotherapy and hormonal therapy simultaneously.

All patients were routinely examined every 3-6 months. Once a year, they underwent ultrasonography of the breast or mammography, lung X-ray, ultrasonography of the liver and bone scintigraphy.

Patients with radical mastectomy (ME)

Between 1980 and 1995, a total number of 1,193 patients underwent ME at the Masaryk Memorial Cancer Institute (Figure 4). We evaluated 1,121 patients. The patients lost to follow-up were excluded. Table 2 shows the characteristics of patients. Mean age at the time of diagnosis was 53.1 years (median 52.1) (Figure 5). Tabel 3 and Figure 6 show the size of tumours. Rare occurrence of T3, T4 tumours is due to the exclusion of patients who underwent neoadjuvant RT or chemotherapy. Mean follow-up was 124.6 months (median 121).

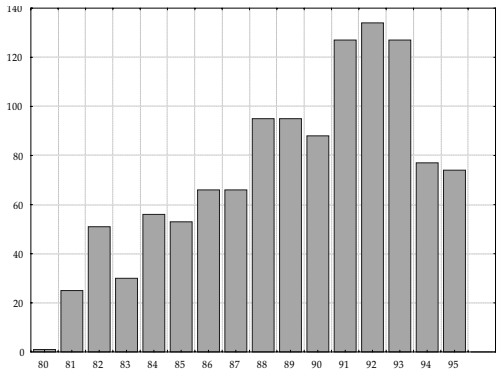

Figure 4. Number of patients treated by radical mastectomy in Masaryk Memorial Cancer Institute.

Table 2. Characteristics of patients treated by radical mastectomy, number of patients 1121

Age (years)		
Mean	53.1	
Median	52.1	
Hormonal status		
Premenopausal	488	43.5 %
Postmenopausal	633	56.5 %
Grade of tumours		
T1	342	30.4 %
T2	647	57.7 %
T3	84	7.5 %
T4	39	3.5 %
TX	9	0.8 %
Mamma		
Left	548	48.9 %
Right	573	51.1 %
Histology		
Ductal	942	84 %
Lobular	111	9.9 %
Others	68	6.1 %
Axillary nodes		
Positive	518	46.2 %
Negative	521	46.5 %
Unknown	82	7.3 %
Radiotherapy		
Yes	982	87.6 %
Only on chest wall	392	39.9 %
No	139	12.4
Chemotherapy		
Yes	466	41.6 %
No	655	58.4 %
Hormonotherapy		
Yes	381	34 %
No	740	66 %

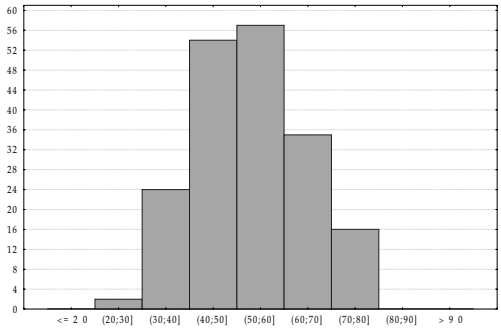

Figure 5. Age of patients after radical mastectomy.

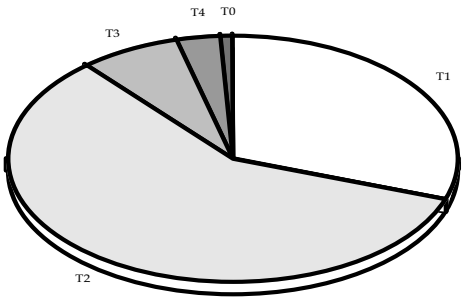

Figure 6. Stages of tumours after radical mastectomy.

Table 3. Patients after conservative surgery and radiotherapy with local recurrence, number of patients 22 (9.3%)

	Age (years)	49.1 (36-69.7)
	Surgery	
Partial mastectomy	12	(54.5 %)
Tumourectomy	10	(45.5 %)
	Histology	
Ductal	15	(68.2 %)
Lobular	1	(4.5 %)
Others	6	(27.3 %)
	Source of RT	
Cobalt Co-60	17	(77.3 %)
Linear accelerator	5	(22.7 %)
	Nodes	
Positive	8	(36.4 %)
Negative	6	(27.3 %)
Unknown	8	(36.4 %)
	Treatment of local recurrence	
Radical mastectomy	11	(35.3 %)
Exstirpation	4	(23.5 %)
Only systemic therapy (CT or HT)	7	(41.2 %)

Treatment

Radiotherapy to the chest wall with or without regional lymph nodes was performed in 982 patients (86.6%) who underwent ME. The patients were treated with linear accelerator (photons 6MV). Two tangential fields were used for the irradiation of the chest wall, two convergent fields for the regional lymph nodes. The lymph nodes were irradiated in 59.5% patients. Median dose of 44 Gy was given to the chest wall, 40 Gy to the regional lymph nodes. Systemic adjuvant therapy (chemotherapy, hormonal therapy or both) was applied to 817 patients (76.9%).

Results

Patients with conservative surgery (CS) and radiotherapy (RT)

Till the date of evaluation (December 2001), 47 patients (19.9%) died, all of them due to the progression of breast cancer.

Local recurrence occurred in 22 patients (9.3%). Table 3 shows the characteristics of patients. The mean time to local recurrence was 63.4 months (range 5 - 168, median 50 months). The mean survival of patients with local recurrence was 41.4 months (range 5 - 122). From 22 patients with local recurrence, 9 developed distant metastases (40.9%). Distant metastases occurred simultaneously or after local recurrence. The mean of follow-up of patients with local recurrence was 101.8 months (range 41 - 187). Till the date of evaluation, 8 patients (36.4%) died. Local recurrence in the primary involved quadrant occurred in 18 cases. Diffuse breast involvement was described in 4 cases. Three patients had lymphangioinvasion described in prime histology; two of them developed diffuse local recurrence. Five-year local control in the whole group of patients was 96.2%. Nine patients (3.8%) had local recurrence within 5 years. Ten-year local control was 91.9%; in 217 patients, local treatment failure was not

observed within 10 years. The histology of local recurrence corresponded to the histology of primary tumour. Among 22 patients with local recurrence, 6 patients were younger than 40 years at the time of diagnosis. Local recurrence had negative impact on survival, p = 0.06 (Figure 7).

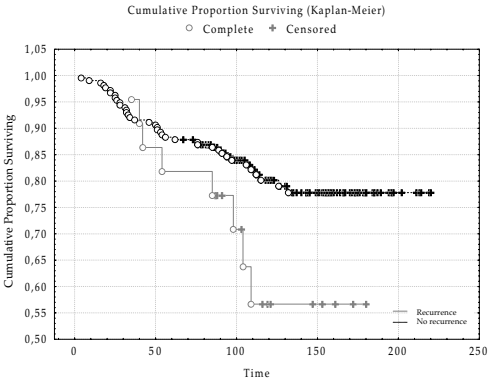

Figure 7. Influence of local recurrence on overall survival in patients after conservative surgery.

Patients with radical mastectomy (ME)

Till the date of evaluation, 65 patients (5.8%) developed local recurrence (Table 4). Five-year local control was 96.6%; in the patients with T1, T2 tumours it was 97.2%. There was no difference in the time to local progression in the irradiated patients (48.5 months) and in the patients without RT (51 months). Local recurrence developed in 13 patients (8.7%) with ME without RT, 44.6% of them had lymph nodes involvement. Lymph node involvement and local recurrence developed independently. Higher number of local recurrences associated with lobular carcinoma was not statistically significant. Patients with local recurrence developed distant metastases more frequently. The impact of local recurrence on OS was statistically significant (p=0.002) (Figure 8). Of patients without local recurrence, 12.9% died, and in the group that locally relapsed 39.1% died. Significant linear correlation between the time to local progression and OS was observed.

Table 4. Characteristic of patients with local recurrence after radical mastectomy, number of patients 65 (5.8%)

Histology		
Ductal	56	86.15 %
Lobular	4	6.15 %
Others	5	7.7 %
Nodes		
Positive	29	44.6 %
Negative	36	55.4%

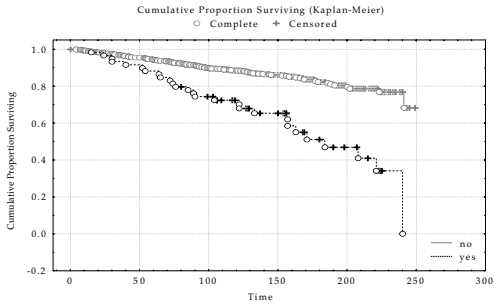

Figure 8. The Influence of local recurrence on overall survival in patients after radical mastectomy.

Discussion

In a French retrospective study of 528 patients with breast cancer, stages I and II, an attempt was made to determine predictive factors for local recurrence.[16] A multivariante analysis of this study showed 4 independent factors most important for local control: young age (up to 40 years), premenopause, bifocality and extensive intraductal component (≥25%).

The impact of local recurrence on OS is not clear. Our study showed negative impact of local recurrence on OS. The difference was on the border of statistic significance in the patients with CS (p=0.06) and significant in the patients with ME (p=0.002). The analysis of 4 prospective studies comprising more than 2000 breast cancer patients tried to evaluate the role of isolated local recurrence.[17] The impact on OS and dissemination was

proved. Elkhuizen reported similar conclusion.[18]

A very important prognostic factor is the interval between the time of diagnosis and local recurrence.[19] In our group of patients (with CS and ME), the time to local progression was equal - 50 months. The patients with longer time to local progression had longer survival. The impact of histologic type of tumour on local recurrence was not proved. Lobular carcinoma, often multifocal and multicentric, was considered not to be convenient for breast conserving surgery. The studies comparing the results of both carcinomas, ductal and lobular, did not show any difference in local control or other parameters.[20,21] The patients with local recurrent ductal carcinoma in situ had significantly better prognosis. Patients with lobular carcinoma in situ have significantly higher risk of unilateral recurrence.[22] Tamoxifen seems to decrease this risk. Histologic type of tumour did not have any impact on local recurrence in our groups of patients. Lobular carcinoma should not be a contraindication for CS.

Till the end of 1970's, postoperative RT was given to all patients with ME. As the knowledge about dissemination of tumour cells advanced, the treatment strategy changed accordingly. Positive outcome of RT after ME must be compared to potential acute and late effects of RT. Modern techniques of RT reduce the doses to the heart and the large vessels.

RT after ME is a standard in patients with high risk of local recurrence: locally advanced tumours - pT3, pT4, 4 or more axillary lymph node involvement, extracapsular invasion.[23,24] The role of postoperative RT is not clear in pT1, pT2, pN0 tumours and 1 - 3 axillary lymph node involvement. A Danish study evaluated the role of postoperative RT.[2] Combined systemic treatment with RT and chemotherapy alone (CMF regimen) were compared. OS and disease-free interval significantly increased in combined therapy. Janni's retrospective analysis evaluated the impact of RT given to the chest wall on local recurrence rate and OS.[25] The decrease of local recurrence and positive impact on OS was statistically significant in the group of patients with RT.

Meta-analysis of 36 randomised studies (Early breast cancer trialist's collaboration group EBCTCG) compared postoperative RT and surgery alone in early breast cancer patients (17,273 patients with mastectomy). The risk of local recurrence after adjuvant RT was treble lower, but the difference in 10-year survival was not significant.[15] No difference was found between the patients with ME and the patients with CS and RT. Fisher's study reported similar results.[4]

The above negative results do not correlate with the results of other randomised studies: Danish 82b and 82c trials, British Columbia study.[2,24.26] Van de Steene tried to solve this contradiction. He confirmed the impact of postoperative RT on survival if modern techniques and standard fractionation of RT were used.[27] Levitt's study showed the impact of RT on OS in the patients with negative resection margin and negative lymph nodes.[28] Another meta-analysis of 6,367 patients verified that locoregional therapy of breast cancer increases disease-free interval and OS.[1]

In our study, the impact of local recurrence on OS was found statistically significant. We did not see any benefit of postoperative RT. Among 65 patients with local recurrence, 51 (78.4%) underwent irradiation of the chest wall.

In primary operated part of the breast, 65 - 80% of local recurrences occurred.[29] In selected group of patients, only tumour bed could be postoperativelly irradiated. Brachytherapy as a separate method of adjuvant treatment of breast carcinoma was described in few reports.[30-34] The studies showed that this treatment was well tolerated and had good cosmetic effect. Good cosmetic effect and reduction of treatment time are the main aim of this method.

Conclusions

There was no difference in the time to local progression found between our two groups of patients. Median time to local progression was equal - 50 months. Five-year probability of local recurrence was equal for the patients with CS (and RT) and patients with ME (without RT) - 9.3%, 8.7% respectively.

Patients with CS who developed local recurrence had equal over-all survival compared to the patients with ME due to T1, T2 tumours with local recurrence (Figure 9). Prognostic outcome of local recurrence after CS or ME does not differ.

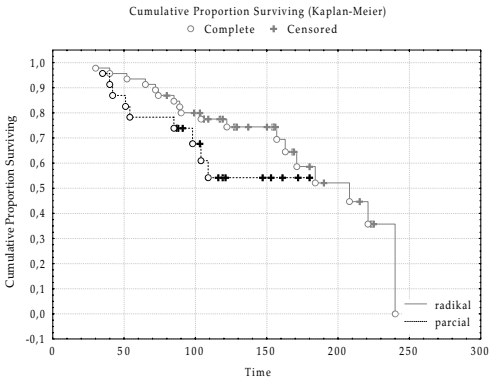

Figure 9. The overall survival of patients with local recurrence treated by conservative surgery or radical mastectomy.

References

1. Whelan TJ, Julian J, Wright J, Jadad AR, Levine ML. Does locoregional radiation therapy improve survival in breast cancer? A meta-analysis. *Clin Oncol* 2000; **18(6):** 1220-9.

2. Overgaard M, Hansen PS, Overgaard J, Rose C, Andersson M, Bach F, et al. Postoperative radiotherapy in high-risk premenopausal woman with breast cancer who receive adjuvant chemotherapy. Danish Breast Cancer Cooperative Group 82b Trial. *N Engl J Med* 1997; **337:** 949-55.

3. Clark RM, Whelan T, Levine M, Roberts R, Willan A, McCulloch P, et al. Randomized clinical trial of breast irradiation following lumpectomy and axillary dissection for node-negative breast cancer: an update. Ontario Clinical Oncology Group. *J Natl Cancer Inst* 1996; **88:** 1659-64.

4. Fisher B, Anderson S, Redmond CK, Wolmark N, Wickerham DL, Cronin WM. Reanalysis and results after 12 years of follow-up a randomized clinical trial comparing total mastectomy with lumpectomy with or without irradiation in the treatment of breast cancer. *N Engl J Med* 1995; **333:** 1456-61.

5. Forrest AP, Stewart HJ, Everington D, Prescott RJ, McArdle CS, Harnett AN, et al. Randomized controlled trial of conservation therapy for breast cancer: 6-year analysis of Scottish trial. Scottish Cancer Trials Breast Group. *Lancet* 1996; **348:** 708-13.

6. Liljegren G, Holmberg L, Adami HO, Westman G, Graffman S, Bergh J. Sector resection with or without postoperative radiotherapy for stage I breast cancer: five-year results of a randomized trial-Uppsala-Orebro Breast Cancer Study Group. *J Natl Cancer Inst Monogr* 1994; **86:** 717-22.

7. Veronesi U, Luini A, Del Vecchio M, Greco M, Galimberti V, Merson M, et al. Radiotherapy after breast-preserving surgery in women with localized cancer of the breast. *N Engl J Med* 1993; **328:** 1587-91.

8. Blichert-Toft M, Rose C, Andersen JA, Overgaard M, Axelsson CK, Andersen KW, et al. Danish randomized trial comparing breast-preserving therapy with mastectomy: six years of life table analysis. Danish Breast Cancer Cooperative Group. *J Natl Cancer Inst Monogr* 1992; **11:** 19-25.

9. Fisher B, Redmond C, Poisson R, Margolese R, Wolmark N, Wickerham L, et al. Eight year results of randomized clinical trial comparing total mastectomy and lumpectomy with or without irradiation in the treatment of breast cancer. *N Engl J Med* 1989; **320:** 822-8.

10. Jacobson JA, Danforth DN, Cowan KH, d'Angelo T, Steinberg SM, Pierce L, et al. Ten-year results of a comparison of conservation with mastectomy in the treatment of stage I and II breast cancer. *N Engl J Med* 1995; **332:** 907-11.

11. Sarrazin D, Le MG, Arriagada R, Contesso G, Fontaine F, Spielmann, et al. Ten-year results of a randomized trial comparing a conservative treatment to mastectomy in early breast cancer. *Radiother Oncol* 1989; **14:** 177-84.

12. van Dongen JA, Bartelink H, Fentiman IS, Lerut T, Mignolet F, Olthuis G, et al. Randomized clinical trial to assess the value of breast cancer , EORTC 10801 trial. *J Natl Cancer Inst Monogr* 1992; **11:** 15-8.

13. Veronesi U, Banfi A, Salvadori B, Luini A, Saccozzi R, Zucali R, et al. Breast conservation is the treat-

ment of choice in small breast cancer: Long-term results randomized trial. *Eur J Cancer* 1990; **26:**668-70.

14. Konopásek B, Petruželka *L. Karcinom prsu.* Prague: Galén; 1997.

15. Effects of radiotherapy and surgery in early breast cancer. An overview of the randomized trials. Early Breast Cancer Trialists' Collaborative Group. *N Engl J Med* 1995; **333:** 1444-55.

16. Touboul E, Buffat L, Belkacemi Y, Lefranc JP, Uzan S, Lhuillier P, et al. Local recurrences and distant metastases after breast conserving surgery and radiation therapy for early breast cancer. *Int J Radiat Oncol Biol Phys* 1999; **43(1):** 25-38.

17. Schmoor C, Sauerbrei W, Bastert G, Schumacher M. Role of isolated locoregional recurrence of breast cancer: results of four prospective studies. *J Clin Oncol 2000*; **18(8):** 1696-708.

18. Elkhuizen PH, Hermans J, Leer JW, van dE Vijver MJ. Isolated late local recurrence with high mitotic count and early local recurrences following breast-conserving therapy are associated with increased risk on distant metastasis. *Int J Radiat Oncol Biol Phys* 2001; **50(2):** 387-96.

19. Doyle T, Schultz DJ, Peters C, Harris E, Solin LJ. Long-term results of local recurrence after breast conservation treatment for invasive breast cancer. *Int J Radiat Oncol Biol Phys* 2001; **51(1):** 74-80.

20. Peiro G, Bornstein BA, Connolly JL, Gelman R, Hetelekidis S, Nixon AJ, et al. The influence of infiltrating lobular carcinoma on the outcome of patients treated with breast-conserving surgery and radiation therapy. *Breast Cancer Res Treat* 2000; **59(1):** 49-54.

21. Silverstein MJ, Lewinsky BS, Waisman JR, Gierson ED, Colburn WJ, Senofsky GM, et al. Infiltrating lobular carcinoma: Is it different from infiltrating duct carcinoma? *Cancer* 1994; **73:** 1673-7.

22. Sasson AR, Fowble B, Hanlon AL, Torosian MH, Freedman G, Boraas M, et al. Lobular carcinoma in situ increases the risk of local recurrence in selected patients with stages I and II breast carcinoma treated with conservative surgery and radiation. *Cancer* 2001; **91(10):** 1862-9.

23. Fowble B, Gray R, Gilchrist K, Goodman RL, Taylor S, Tormey DC. Identification of a subgroup of patients with breast cancer and histologically positive axillary nodes receiving adjuvant chemotherapy who may benefit from postoperative radiotherapy. *J Clin Oncol* 1988; **6(7):** 1107-17.

24. Overgaard M, Jensen MB, Overgaard J, Hansen PS, Rose C, Andersson M, et al. Postoperative radiotherapy in high-risk postmenopausal breast cancer patients given adjuvant tamoxifen: Danish Breast Cancer Cooperative Group 82c Trial. *Lancet* 1999; **353:** 1641-8.

25. Janni W, Dimpfl T, Braun S, Knobbe A, Peschers U, Rjosk D, et al. Radiotherapy of the chest wall following mastectomy for early-stage breast cancer: impact on local recurrence and overall survival. *Int J Radiat Oncol Biol Phys* 2000, **48 (4):** 967-75.

26. Ragaz J, Jackson SM, Le N, Plenderleith IH, Spinelli JJ, Basco VE, et al. Adjuvant radiotherapy and chemotherapy in node-positive premenopausal women with breast cancer. *N Engl J Med* 1997; **337:** 956-62.

27. Van de Steene J, Soete G, Storme G. Adjuvant radiotherapy for breast cancer significantly improves overall survival: the missing link. *Radiother Oncol* 2000; **55:** 263-72.

28. Levitt SH, Aeppli DM, Nierengarten ME. The impact of radiation on early breast carcinoma survival. A Bayesian analysis. *Cancer* 1996; **78:** 1035-42.

29. Liljegren G, Lindgren A, Bergh J, Nordgren H, Tabar L, Holmberg L. Risk factors for local recurrence after conservative treatment in stage I breast cancer. Definition of a subgroup not requiring radiotherapy. *Ann Oncol* 1997; **8:** 235-41.

30. Vicini FA, Chen PY, Fraile M, Gustafson GS, Edmundson GK, Jaffray DA, et al. Low-Dose-Rate brachytherapy as the sole radiation modality in the management of patients with early-stage breast cancer treated with breast-conserving therapy: preliminary results of a pilot trial. *Int J Radiat Oncol Biol Phys* 1997; **38(2):** 301-10.

31. Polgar C, Major T, Somogyi A, Fodor J, Sulyok Z, Toth J, et al. [Brachytherapy of the tumor bed after breast conserving surgery: new radiotherapeutic option in the management of early breast cancer]. [Hungarian]. *Orv Hetil* 1999; **140(26):** 1461-6.

32. Polgar C, Major T, Somogyi A, Fodor J, Toth J, Sulyok Z, et al. Sole brachytherapy of the tumor bed after breast conserving surgery: a new radiotherapeutic strategy for patients at low risk of local relapse. *Neoplasma* 1999; **46(3):** 182-9.

33. Kuske R, Bolton J, Wilenzick R, McKinnon W, Pullen B, Scroggins T, et al. Brachytherapy as the sole method of breast irradiation in TIS, T1, T2, N0-1 breast cancer. [Abstract]. *Int J Radiat Oncol Biol Phys* 1994; **30(1):** 245.

34. Fentiman IS, Poole C, Tong D, Winter PJ, Mayles HM, Turner P, et al. Iridium implant treatment without external radiotherapy for operable breast cancer: a pilot study. *Eur J Cancer* 1991; **27(4):** 447-50.

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Cathepsin L in human meningiomas

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Background. *Although meningiomas are considered as benign tumours, about 10% comprise a subgroup of atypical meningiomas, classified as WHO grade II, with greater likelihood of recurrences and/or aggressive behaviour, including the possibility of brain tissue invasion. The lysosomal cysteine endopeptidase cathepsin L plays a role in tumour cell invasion and malignant progression of cancer, and has been suggested as a prognostic marker for certain types of tumours.*

Results. *In our study, we compared the expression of cathepsin L in 30 meningiomas with their clinical invasiveness. Cathepsin L was determined by immunohistochemical analysis, quantitative real-time RT-PCR and Northern blot. We showed that expression of cathepsin L protein was significantly higher (p=0.019) in 9 atypical than in 21 benign meningiomas. Within the group of benign meningiomas, expression of cathepsin L was significantly lower in the transitional histological subtype. We measured the levels of cathepsin L A type of RNA splicing variants: LA, LAI and LAII, but not LAIII and not the LB variant, the latter being several times lower than the L A type. In contrast to protein levels, the levels of cathepsin L A, AI, AII RNA variants did not differ between histological subtypes or between benign and atypical meningiomas. The expression of total measured cathepsin L A, AI, AII RNA variants in the samples, taken from the centre and the periphery of the tumours, also showed no statistically significant differences.*

Conclusions. *These results indicate that cathepsin L protein over-expression may contribute to the development of the aggressive and possibly invasive character of atypical meningiomas and that it may be up regulated at the translational level.*

Key words: meningioma - pathology; cathepsin L; reverse transcriptase, polymerase chain reaction; neoplasm invasiveness

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Introduction

Meningiomas are tumours of the central nervous system that derive from the coverings of the brain. Meningiomas are generally slow growing, benign tumours attached to the dura matter and composed of neoplastic meningothelial (arachnoidal) cells.[1] Meningioma have a wide range of histopathological

apperances. However, of the 11 subtypes, the three most common are meningothelial, fibrous and transitional meningioma.[1,2]

Most meningiomas are benign and can be graded into WHO grade I. Certain histological subtypes are associated with a less favourable clinical outcome and correspond to WHO grades II and III classification of histological diagnosis of brain tumours. Atypical meningiomas corresponding to grade II have moderately high labelling indices of nuclear proliferation index (PI) and have likely the following features: frequent mitosis, increased cellularity, small cells with high nuclear-cytoplasmic ratio and/or prominent nucleoli, uninterrupted pattern-less or sheet-like growth and foci of 'spontaneous' or geographic necrosis.[1] These criteria have been shown to correlate with higher recurrence rates. Anaplastic malignant meningiomas (grade III) exhibit malignant histological features in excess of the abnormalities noted in atypical meningiomas. The latter constitute about 6%, whereas anaplastic malignant meningiomas account for between 1.0 and 2.8% of all meningiomas.[2]

Cathepsin L (EC 3.4.22.15) is a cysteine endopeptidase belonging to the evolutionarily well conserved clan CA/family CI proteases (http://www.merops.co.uk). It is localised mainly to lysosomes, mediating intracellular protein turnover, but under certain physiological conditions it also regulates the function of some cytosolic and extracellular proteins. Cathepsin L gene is activated by a variety of growth factors and some oncogenes. In NIH3T3 cells (mouse embryonal fibroblasts), cathepsin L gene is activated by platelet derived growth factor (PDGF), epidermal growth factor (EGF), tumour promoter phorbol 12-myristate 13-acetate (PMA), cAMP and oncogenes, including v-*ras*, v-*src* and v-*mos*.[4] Enzyme activity is regulated specifically by endogenous inhibitors of the cystatin family, thyropins and p41 fragment of invariant chain (Ii).[5]

Gottesman[3] first reported an increased synthesis and secretion of the so called »major excreted protein, MEP« in NIH3T3 fibroblasts on viral transformation, the protein later being identified as cathepsin L.[6] Many subsequent studies have supported the hypothesis that increased levels and eventual secretion of cathepsin L are related to malignant transformation. Increased expression of RNA, protein and activity of cathepsin L was found in gastric[7], colorectal, ovarian, breast, head and neck thyroid, lung endometrial carcinomas and gliomas.[8] It was shown that cathepsin L is progressively expressed with increasing tumour malignancy in breast[9] and brain tumours[10,11] and has prognostic relevance for some cancers.[8,12] The role of cathepsin L in the invasion process was confirmed in *in vitro* invasion assays by the use of selective inhibitor Click-148, which also reduced *in vitro* invasion of tumour cells[13,14] and reduced bone metastasis in an *in vivo* mouse model.[15] Collectively, this data suggests that cathepsin L plays an important role in tumour invasion.

The present study is based on the hypothesis that, due to the observed association of cathepsin L with tumour progression and invasiveness, this lysosomal endopeptidase may be a biological marker for differentiation between benign and the more aggressive atypical meningioma. This suggested the further question as to whether cathepsin L expression would be increased at the invasive edge of meningiomas.

Materials and methods

Patients

Thirty patients (19 female and 11 male) with diagnosed meningioma were selected randomly for the study. They ranged in age from 26-80 years. Immediately after surgical removal, samples from the centre and periphery of the tumours were removed and snap frozen in liquid nitrogen. The rest of the tu-

mour tissue was fixed in formaldehyde and embedded in paraffin. The histological slides were analyzed and categorized according to the WHO classification of brain tumours. The study comprised 22 benign and 8 atypical meningiomas (Table 1). The study was approved by the Ethics Committee at the Ministry of Health of the Republic of Slovenia.

Immunohistochemistry

Immunohistochemical labelling was performed using standard techniques.[16] Paraffin-embedded tissue sections were deparaffinised and rehydrated. The sections were boiled in 100mM EDTA buffer (pH 8.0) for 5 minutes for antigen retrieval. The slides were first blocked with an inhibitor of endogenous peroxidase and then incubated with primary anti-cathepsin L mouse monoclonal antibodies (CatLHLM1, clone N135) purchased by Krka d.d., Novo mesto, Slovenia, at 1:10 dilution of the stock concentration of 100μg/ml for 26min at 40°C. Subsequent IHC reaction was performed with biotinylated goat anti-mouse secondary antibodies, avidin with bound horseradish peroxidase and diaminobenzidine as chromogen. The immunohistochemistry and counterstaining with haematoxylin, was performed in an NexES IHC Staining Module (Ventana Medical Systems, USA). The intensity and frequency of immunostaining was considered independently by two pathologists for grading (0; 0.5; 1; 1.5; 2; 2.5; 3).

Proliferation index: immunolabelling with MIB-1 antibodies

Proliferation index (PI) in meningiomas was determined by the IHC procedure, described above, using MIB 1 antibodies (mouse anti-human Ki-67, clone MIB-1, No 7240, Dako Corporation). The MIB-1 solution (200μg/ml) was diluted 1:20prior to use. Positive IHC staining indicated proliferating cells and was counted under a microscope using the computer program Lexica Q Product (Leica, Germany). PI was calculated as the percentage of proliferating vs. all cells in the preparation.

Quantitative real time RT-PCR

A fluorescence-based real-time quantitative RT-PCR method, developed by Perkin Elmer ABI (TaqMan), was used to measure cathepsin L RNA levels in meningioma tissue. For each sample 1 μg of total RNA was reverse transcribed in a 50μl reaction using a High-Capacity cDNA Archive Kit (Applied Biosystem, USA). After an initial step at room temperature for 10 min, reverse transcription was performed at 37°C for 2h. Quantitative PCR reaction was carried out with a cDNA equivalent of 2ng total RNA per reaction, using the TaqMan Universal PCR Master Mix (Applied Biosystems, USA): 1x TaqMan Universal PCR Master Mix, 200nM of each primer and 100nM TaqMan (final concentration) fluorescent probe in a 10 μl final reaction volume. PCR reactions were carried out in an ABI Prism 7900 PCR machine (Applied

Table 1. Histological diagnosis of meningiomas

Histological diagnosis	WHO grade	No of tumours analysed by IHC	No of tumours analysed by RT-PCR
Benign meningioma	I		
Fibroblastic	I	6	6
Meningothelial	I	6	6
Transitional	I	8	6
Psammomatus	I	1	1
Secretory	I	1	1
Atypical meningioma	II	8	7

Biosystems, USA), with a step at 50°C for 2min for AmpErase UNG activity, hot start at 95°C for 10min, followed by 40 cycles with denaturation at 95°C for 15s and annealing/elongation at 60°C for 1min. To normalize the signal for cathepsin L A type variants, amplification of 18S ribosomal RNA was performed as an internal control in a duplex reaction together with amplification of cathepsin L cDNA. Primers and probes for 18S rRNA were purchased from Applied Biosystem in Pre-Developed TaqMan Assay Reagent Control Kit.

We designed probe and primers to measure simultaneously cathepsin LA, cathepsin LAI and cathepsin LAII RNA splicing variants, but not LAIII RNA variant.[17] The following primers were synthesized (Laboratories Eurobio, France): forward primer for cathepsin L A variants: AGC GTC TAC CCC GAA CTC TG, located at bp 158-178 in exon1 and reverse primer for cathepsin L A variants: TTG TGC ATC GCC TTC CAC T, located at bp 372-387 in exon 2. The nucleotide sequence for cathepsin L A variants probe, labelled with FAM reporter molecule, ACT CAT CCT TGC TGC CTT TTG CCT GG, is located in exon 2 at bp 372-387. Cathepsin L B variant does not have exon 1, therefore the forward primer was located in intron 1, bp 161-186, extending 4 nucleotides into exon 1, whereas the reverse primers and the probe were the same as for the cathepsin L A variants.

Northern blot of selected tumour samples

Total RNA was isolated with TRIzol (Gibco BRL, USA) according to the supplier's instructions. The RNA concentration was estimated by A_{260nm}. 20μg of total RNA per lane was mixed with ethidium bromide and electrophoresed on 1% formaldehyde-agarose gel for 3h at 70V. Northern transfer was performed overnight in 20x SSC (Standard Saline Citrate, 3M sodium chloride, 0.3M sodium citrate, pH 7.0) on a rapid downward transfer system using the turboblotter apparatus and Nytran membrane (Schleicher and Schuell, Germany). RNA was cross-linked to the membrane by UV irradiation. The membrane was photographed under UV light and the photographs used for signal normalization. The blots were pre-hybridized for 3h at 50°C in »high SDS« buffer (5x SSC, 2% blocking solution, 50mM sodium phosphate, 0.1% (w/v) lauroylsarcosine, 7% (w/v) SDS, 50% formamide) as suggested by the supplier of the blocking solution (Roche, Switzerland). Hybridization was performed overnight in the same buffer containing 30ng/ml DIG-labelled full length cDNA probe. Blots were washed twice with 2x washing buffer (2xSSC, 0.1%, w/v, SDS) and twice with 0.5x washing buffer (0.5xSSC, 0.1% (w/v) SDS). All washing steps were performed for 10min at room T. The signals were detected by chemiluminescence using the CDP Star System (Roche, Switzerland) according to the supplier's instructions. The signals of cathepsin L RNA on Northern blots were analyzed densitometrically with program Lucia G 4.21 (Laboratory Imaging Ltd, GB) and normalized to 18S rRNA signal.

Statistics

For statistical calculation we used SPSS 10.1 for Windows (SPSS Inc, USA) program. The differences in cathepsin L expression were analyzed by the Mann-Whitney and Kruskal-Wallis non-parametric test. The significance of differences between histological subtypes of meningiomas was given as p values. $P<0.05$ was considered to be significant.

Results

Immunohistochemistry

Cathepsin L immunostaining was evaluated in 30 meningiomas, characterised histologically (Table 1). Figures 1A and 1B show weak (score 1.0) and strong (score 3.0) IHC staining of cathepsin L in tumour cells of benign tran-

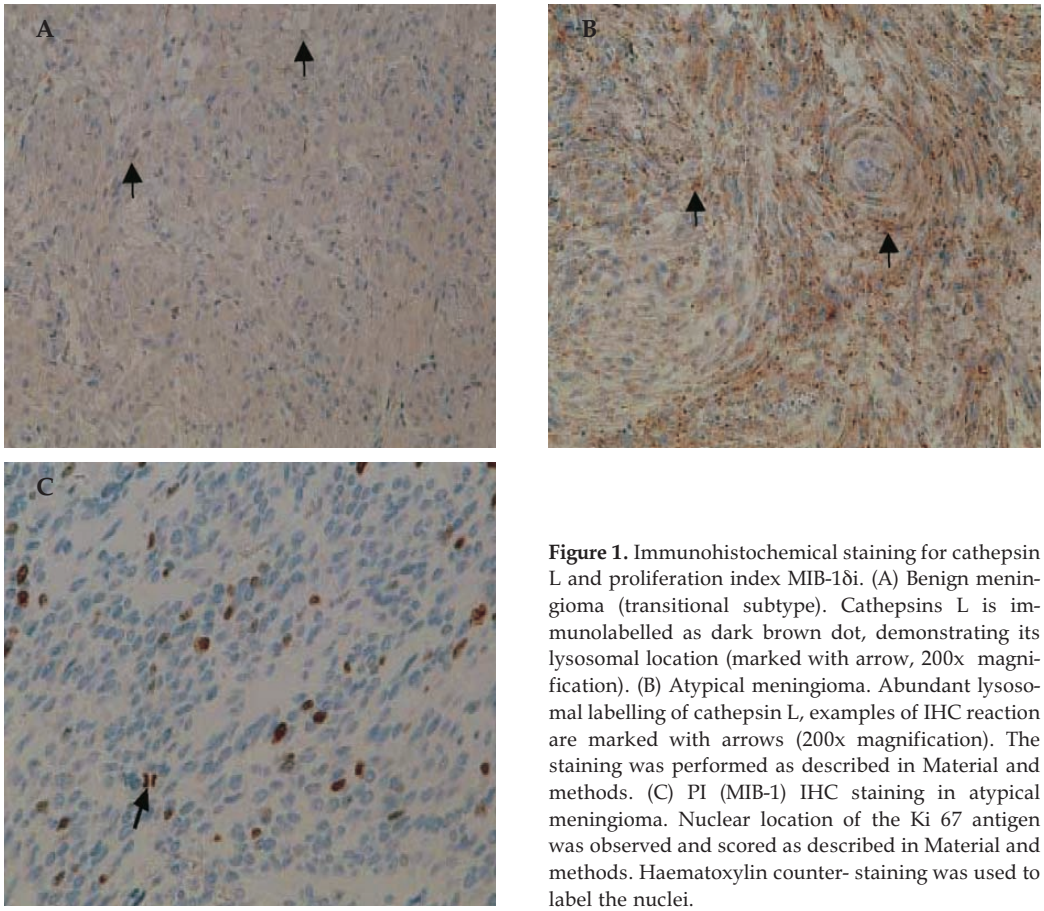


Figure 1. Immunohistochemical staining for cathepsin L and proliferation index MIB-1δi. (A) Benign meningioma (transitional subtype). Cathepsins L is immunolabelled as dark brown dot, demonstrating its lysosomal location (marked with arrow, 200x magnification). (B) Atypical meningioma. Abundant lysosomal labelling of cathepsin L, examples of IHC reaction are marked with arrows (200x magnification). The staining was performed as described in Material and methods. (C) PI (MIB-1) IHC staining in atypical meningioma. Nuclear location of the Ki 67 antigen was observed and scored as described in Material and methods. Haematoxylin counter- staining was used to label the nuclei.

sitional and an atypical meningioma, respectively. No statistically significant differences in cathepsin L levels were observed between meningothelial and fibrous meningioma, but significantly lower levels were observed in transitional meningiomas (Figure 2A). When all benign subtypes were grouped and compared with all atypical meningiomas, we found significantly higher (p=0.009) scores of IHC staining in the more invasive atypical meningiomas (Figure 2B).

Quantitative real time RT-PCR

Cathepsin L is encoded by four major RNA species: cathepsin LA, cathepsin LAI, cathepsin LAII RNAs, the newly discovered cathepsin L AIII RNA, and cathepsin LB RNA.[19] Cathepsin L RNA levels were analysed by quantitative RT-PCR using specifically designed probes, as described in Material and methods. We found that the levels of cathepsin LB RNA in meningiomas were between 3 and 5 cycles less (average about 8 times lower) than with cathepsin LA RNA variants (not shown), therefore the LB variant was not determined in this study. The primers and the probe which detects all previously mentioned cathepsin L A type RNA variants, but not the recently discovered cathepsin LAIII splice variant, were designed. No statistically significant differences were found in the amount of cathepsin L A, AI, AII type splice variants between different histological subtypes of meningiomas (Figure 3). Also, no statistically

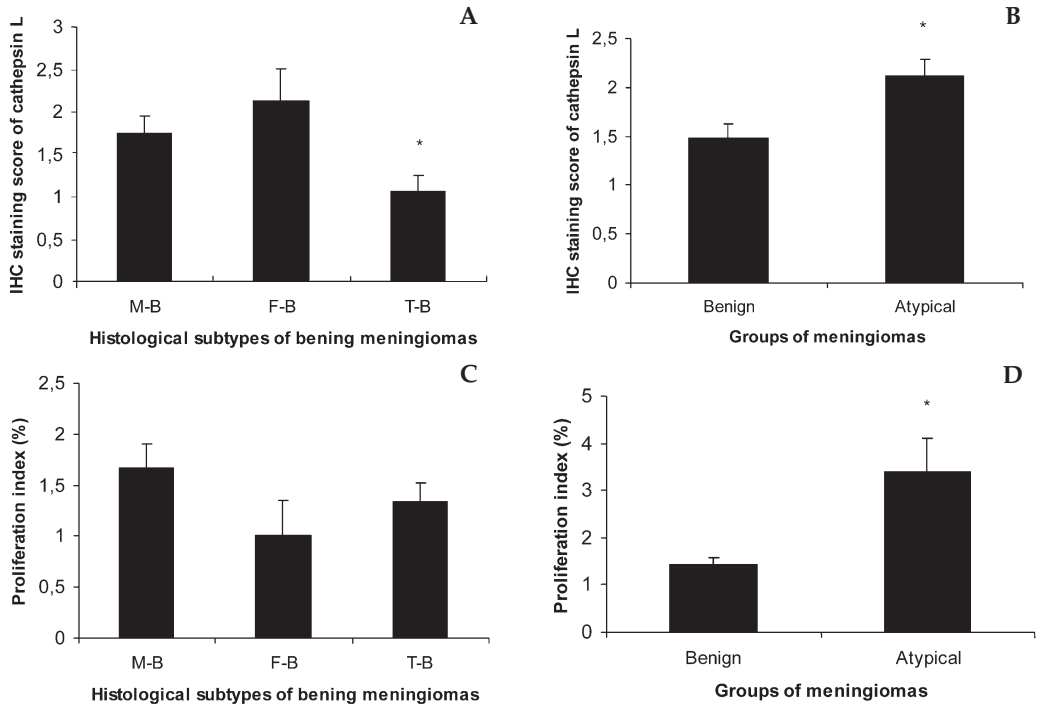


Figure 2. Immunohistochemical scoring of cathepsin L and PI in different histologycal subtypes of meningiomas. (A) Comparison of the scores of cathepsin L within benign tumours: M-B meningothelial benign, F-B fibroblastic benign and T-B transitional bening. (B) Comparison of mean IHC scores in all benign and all atypical meningiomas. IHC scoring was performed according to the frequency and intensity of cathepsin L labeling on a scale from 0 - 3, as described in Methods. (C) Immunohistochemical scoring of PI within benign tumors: M-B meningothelial benign, F-B fibroblastic benign and T-B transitional bening meningioma. (D) Immunohistochemical scoring of PI in all benign and all atypical meningiomas, presented as mean scores. Results are presented as mean ± standard error The stars indicate statistically significant diffferences ($p<0.05$) between the groups.

significant differences were found between the expression of cathepsin LA RNA variants in atypical and benign meningiomas. The comparison between the samples taken from the periphery and the centre of the tumours revealed no difference in the amount of cathepsin L A type variants (not shown).

Northern blot of selected tumour samples

To determine whether total cathepsin L mRNA expression, using the cDNA probe of cathepsin L, differs between histological subtypes types and between the centre and the periphery of the tumours, Northern blot analysis was performed in two meningothelial (samples 2,6), one fibroblast (sample 1), three transitional (samples 3,7,8) and two atypical meningiomas (samples 4,5). Samples from the centre and periphery of the tumours were loaded separately (Figure 4A). Expression in cathepsin L RNA samples varies, even within the same subtype of tumour, for example samples 2 and 6, which are both classified as meningothelial subtype. Densitometric analysis (Figure 4B) showed slightly lower expression in fibroblastic and transitional benign subtypes, but from this we cannot deduce any significant differences between these meningiomas. As observed with cathepsin LA RNA variants, the expression is very similar in the centre and in the periphery of the tumours.

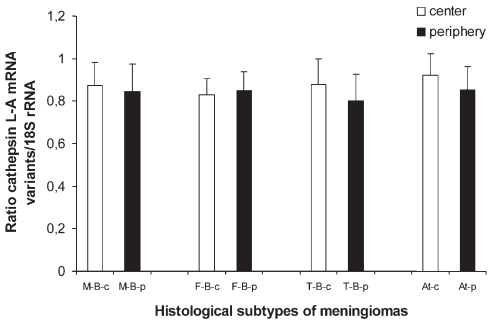


Figure 3. Cathepsin LA RNA variants determined by real time RT PCR. The expression of cathepsin LA variants was quantitated and related (normalized) to the levels of 18S rRNA in different histological subtypes of meningioma in the center (c) and at the periphery (p) of the meningiomas: M-B meningothelial benign, F-B fibroblastic benign, T-B transitional benign, At-atypical. Results are shown as mean ± standard error.

Correlation of cathepsin L measurements

Cathepsin L protein expression (IHC score) and the expression measured by cathepsin L A variants RNA (mRNA/18srRNA) levels correlated statistically significantly ($p<0.02$) regardless of the location of the sample measurements, i.e. tumour centre ($r=0.45$), tumour periphery ($r=0.43$) or total tumour ($r=0.48$).

Proliferation index (PI)

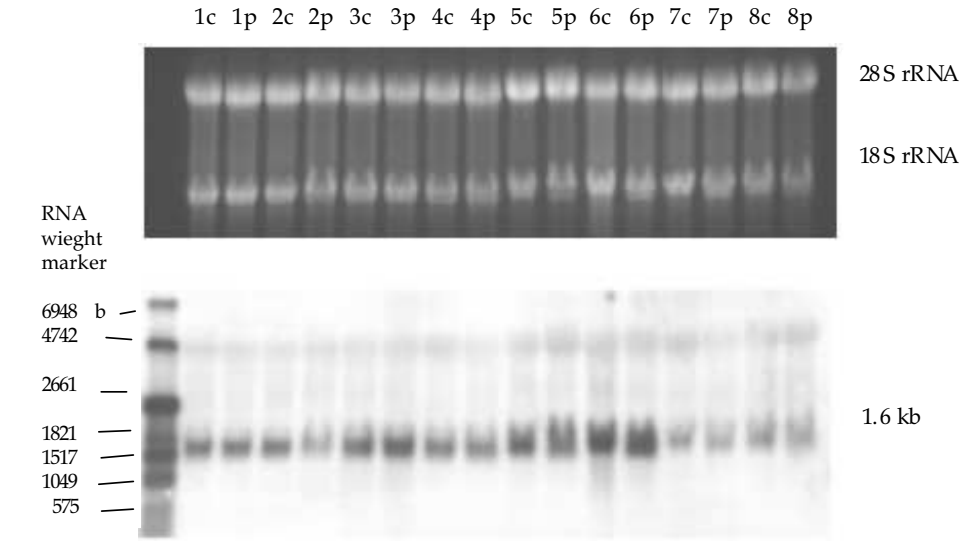
The proliferation index indicates the relative (%) expression of antibodies labelling a nuclear antigen, which is greatly increased in all phases of the cell cycle, except in Go. It is used as a histopathological marker indicating the degree of cell proliferation (Figure 1C). Our data confirm previous reports [18] that PI discriminates between benign and atypical meningiomas, but not between different histologies of benign meningioma (Figure 2 C, D). It has been also reported to be good prognostic marker for relapse of meningiomas. In our study, PI correlated moderately ($r =0.30$), although statistically significantly ($p=0.003$), with the expression of cathepsin L protein.

Discussion

Meningiomas are among the most common tumours encountered in neurosurgical practice[1,2,19] and the incidence is estimated to constitute between 13% and 26% of primary intracranial tumours, with an annual incidence of approximately 6 per 100 000 population.[1,2] Meningiomas are in 90% of cases benign (WHO grade I), whereas 6-8 % are atypical, 1-2 % malignant anaplastic meningiomas, the rest comprising other types of grade II and III meningiomas.[1] In spite of the existence of certain morphological, histopathological and a few biological parameters, discrimination between clear benign, border benign and atypical meningioma remains the key problem.[19,21] Therefore, new markers of malignant progression of atypical meningioma, which could lead clinicians towards a more informed treatment of patients, are needed.

Proliferation index is such a parameter which has been clearly shown to distinguish between benign and invasive atypical and anaplastic meningioma[18], and this has been confirmed in this study. It has been found to correlate with cathepsin L antigen in atypical *vs* benign, but not within the benign meningioma group, suggesting that cathepsin L may not be related to increased proliferation rate.

Proteolytic enzymes have long been proposed as prognostic factors for disease-free and overall survival of cancer patients (recently reviewed in 25), including those with brain tumours[26,27,28], due to their role in invasion processes and metastases. The metalloprotease stromelysin was also suggested as a marker for invasiveness of meningioma[29] and the same was confirmed for lysosomal cysteine cathepsins in our previous study.[21] We analyzed the expression of cathepsin B and cathepsin L in 67 benign and 21 atypical meningiomas and found that the protein levels of both cathepsins were significantly ele-



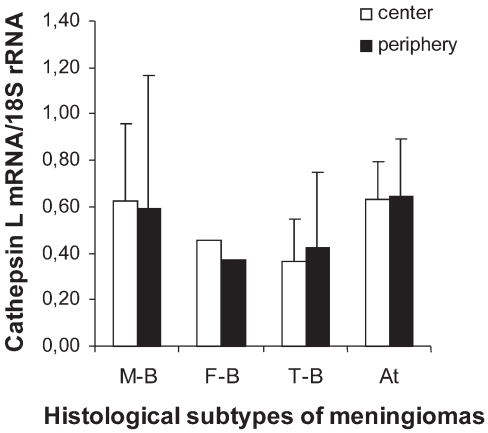


Figure 4. Detection of total cathepsin L RNA by Northern blot. The upper part of the figure indicates the total RNA (20μg) loaded on the gel and separated by electrophoresis in 28S and 18S rRNA bands. Below, 1.6 kb cathepsin L hybridised with DIG labelled full length cathepsin L cDNA probe, as described in Material and methods. The samples represent: 1, fibroblastic benign; 2 and 6, meningothelial benign; 3, 7 and 8, transitional benign; 4 and 5 atypical meningioma. Samples were loaded in pairs of tissue from center (c) and periphery (p) of the tumors. (B) The mean signal intensity of cathepsin L RNA from center and periphery of the tumor normalized to 18S rRNA signal in histological subtypes of meningothelial (M-B), fibroblastic (F-B), transitional (T-B) and atypical (At) of 8 individual meningiomas, which were analyzed by Northern blot experiment above (A).

vated in atypical tumours. Moreover, among 67 benign tumours, nine had certain features of malignancy, classified as borderline benign meningioma, and significantly higher expression of cathepsin B was also found in the borderline benign tumours compared with clear benign tumours. However, cathepsin L could only discriminate between atypical and clear benign tumours. This suggested that cathepsin L was a less selective diagnostic marker for distinguishing between histomorphologically benign but invasive, and histomorphologically clear benign tumours. However, several recent *in vitro* studies have confirmed a close link between the invasive potential of tumour cells and increased cathepsin L expression. In brain tumours, first reports that cathepsin L may be related to brain tumour invasion came from Sivaparvathi *et al.*[22], who found that cathepsin L expression paralleled increased malignancy of astrocytoma and glioblastoma cell lines. Moreover, the authors prevented glioblastoma cells from invading Matrigel by using specific cathepsin L antibodies. We have confirmed these results in an astrocytoma cell line model, using different extracellular matrices and a variety of synthetic cathepsin B and L inhibitors.[11]

Recently, we have also demonstrated stable transfection of an IPTP glioblastoma cell line with whole length cDNA of cathepsin L, which resulted in significant, 80%, inhibition of cell invasion in Matrigel compared with control cells transfected with the vector-GFP.[23] This was confirmed in other cell lines by others.[24]

In this study, protein expression of cathepsin L was significantly lower in transitional benign meningiomas than in other benign types of meningiomas. At present we cannot explain this, since the transitional subtype has features transitional between those of meningothelial and fibrous meningiomas. Strojnik *et al.*[21] also found no variations in the subtypes of benign meningioma. However, the levels of cathepsins were more related to their intracranial localisation and were the highest in parasagittal and convexity meningioma, which were also clinically more aggressive.

In our previous study of glioblastoma invasion, we detected high concentrations of cathepsin B at the invasive edges of invading tumours[27], confirming earlier reports that intra-tumour and intracellular localisation of cathepsins at the plasma membrane may be important in facilitating lytic interactions between the tumours and surrounding stroma.[25] In this study we failed to observe different expression of cathepsin L between the centre and the periphery of the tumour, either by immunohistochemistry, Northern blot or RT-PCR methods. This suggests that cathepsin L may not be directly involved in degradation of extracellular matrix proteins at the cell (tumour surface), but may play a different role in the proteolytic events leading either to tumour cell invasion and/or other features of malignancy.

In the present study we found significantly higher levels of cathepsin L protein in atypical (grade II) compared with benign meningioma (grade I), indicating possible correlation of cathepsin L with invasiveness and/or clinical aggresiveness of grade II meningiomas. However, we have not succeeded in demonstrating increased cathepsin L RNA in atypical meningioma. Although total cathepsin L RNA was only assayed in a few meningiomas, the cathepsin L A splicing variants (LA, LAI and LAII) measured in all 30 patients, also did not differ significantly between benign and atypical meningiomas. Chauhan *et al.*[30] first reported two splicing variants of the single cathepsin L gene, LA and LB variants, expressed concurrently at a similar ratio in several different cell lines, LB variant being higher than LA. This is in contrast to our results in meningioma tissue, where we found about 8 times lower L B variant and therefore did not quantify this variant. The discrepancy between messenger RNA (L A variants) and protein levels of cathepsin L can be explained either by an increased rate of translation of cathepsin L protein in atypical meningioma or by the fact, that we have not determined the LAIII splicing variant. Abula *et al.*[31] has recently demonstrated that LAIII is predominant in all tissues, including malignant tumours, and showed the highest expression and translational rate *in vitro* and *in vivo*.[17,31] It is possible, that this variant is also the most active in atypical and possibly anaplastic malignant meningioma, resulting in higher protein levels of cathepsin L, as observed in this study and previously.[21] This needs to be further investigated.

Brain invasion may occur in histologically benign, atypical or anaplastic malignant meningiomas.[1] The presence of brain invasion connotes a greater likelihood of recurrence with brain-invasive histologically benign meningioma having clinical courses similar to atypical meningioma.[1] This clearly indicates that new markers of invasiveness, such as cathepsin L, are needed to predict malignant clinical behaviour of histologically classified benign tumours.

In conclusion, we have demonstrated sig-

nificantly higher levels of cathepsin L protein in atypical meningiomas. As cathepsin L is a marker of invasion but not of cell proliferation of malignant tumours, we may hypothesize that atypical meningioma acquire invasive behaviour. In contrast to protein, the levels of cathepsin L (LA, LAI and LAII) splicing RNA variants were not higher in atypical meningioma, strongly suggesting that the rate of RNA translation increased in atypical compared to benign meningioma. Particularly, the level and translational rate of LAI-II splicing variant, may be responsible for increased protein concentration of cathepsin L in atypical meningioma, what needs to be further investigated. We confirmed our hypothesis that cathepsin L is a biological marker for differentiation between benign and the more aggressive atypical meningioma and may be used to predict clinically observed aggressive behaviour of meningiomas.

Acknowledgements

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References

1. Louis DN, Scheithauer BW, Budka H, von Deimling A, Kepes JJ. Meningeal tumors. In: Kleihaus P, Cavenee WK, editors. *Pathology and genetics of tumours of the nervous system.* Lyon: IARC press; 2000. p. 175-84.

2. Kleihues P, Burger PC, Scheithauer BWm, editors. Histological typing of tumours of the central nervous system. New York,: Springer-Verlag; 1993, pp 33-40.

3. Gottesman MM. Transformation-dependent secretion of a low molecular weight protein by murine fibroblasts. *Proc Natl Acad Sci USA* 1978; **75:** 2767-71.

4. Kane SE, Gottesman MM. The role of cathepsin L in malignant transformation. *SeminCancer Biol* 1990; **1:** 127-36.

5. Turk B, Turk D, Salvesen GS. Regulating cysteine protease activity: Essential role of protease inhibitors as guardians and regulators. *Curr Pharm Design* 2002; **8:** 1623-37.

6. Portnoy DA, Erickson AH, Kochan J, Ravetch JV, Unkeless JC. Cloning and characterization of a mouse cysteine proteinase. *J Biol Chem* 1986; **261:** 14697-703.

7. Chauhan SS, Goldstein LJ, Gottesman MM. Expression of cathepsin L in human tumors. *Cancer Res* 1991; **51:** 1478-81.

8. Kos J, Lah T. Cysteine proteinases and their endogenous inhibitors: Target proteins for prognosis, diagnosis and therapy in cancer. *Oncol Rep* 1998; **5:** 1349-61.

9. Lah TT, Čerček M, Blejec A, Kos J, Gorodetsky E, Somers R, Daskal I. Cathepsin B, a prognostic indicator in lymph node negative breast carcinoma patients: comparison with cathepsin D, cathepsin L and other clinical indicators. *Clin Cancer Res* 2000; **6:** 578 -84.

10. Levičar N, Strojnik T, Kos J, Dewey AR, Pilkington GJ, Lah TT. Lysosomal enzymes, cathepsins, in brain tumor invasion. *J Neuro-Oncol* 2002; **58:** 21-32.

11. Lah TT, Strojnik T, Levičar N, Bervar A, Zajc I, Pilkington G, Kos J. Clinical and experimental studies of cysteine cathepsins and their inhibitors in human brain tumors. *Int J Biol Markers* 2000; **15:** 90-2.

12. Harbeck N, Alt U, Berger U, Kruger A, Thomssen C, Janicke F, Hofler H, Kates RE, Schmitt M. Prognostic impact of proteolytic factors (urokinase-type plasminogen activator, plasminogen activator inhibitor 1, and cathepsins B, D, and L) in primary breast cancer reflects effects of adjuvant systemic therapy. *Clin Cancer Res* 2001; **7:** 2757-64.

13. Premzl A, Puizdar V, Zavasnik-Bergant V, Kopitar-Jerala N, Lah TT, Katunuma N, Sloane BF, Turk V, Kos J. Invasion of ras-transformed breast epithelial cells depends on the proteolytic activity of cysteine and aspartic proteinases. *Biol Chem* 2001; **382:** 853-7.

14. Yagel S, Warner AH, Nellans HN, Lala PK, Waghorne C, Denhardt DT. Suppression by cathepsin L inhibitors of the invasion of amnion membranes by murine cancer cells. *Cancer Res* 1989; **49:** 3553-7.

15. Katunuma N, Tsuge H, Nukatsuka M, Asao T, Fukushima M. Structure-based design of specific cathepsin inhibitors and their application to protection of bone metastasis of cancer cells. *Arch Biochem Biophys* 2002, 397; **2:** 305-11.

16. Perentes E, Rubinstein LJ. Recent applications of immunoperoxidase histochemistry in human neuro-oncology: An update. *Arch Patol Lab Med* 1987; **111:** 796-812.

17. Arora S, Chauhan SS. Identification and characterization of a novel human cathepsin L splice variant. *Gene* 2002; **293:** 123-31.

18. Perry A, Stafford LS, Scheithauer BW, Suman VJ, Lohse CM. The prognostic significance of MIB-1, p53 and DNA flow cytometry in completely resected primary meningiomas. *Cancer* 2002; **94:** 765-72.

19. Nakasu S, Hirano A, Llena JF, Shimra T, Handa J. Interface between the meningioma and the brain. *Surg Neurol* 1989; **32:** 206 - 12.

21. Strojnik T, Židanik B, Kos J, Lah TT. Cathepsins B and L are markers for clinically invasive types of meningiomas. *Neurosurgery* 2001, **48:** 598-605.

22. Sivaparvathi M, Yamamoto M, Nicolson GL, Gokaslan ZL, Fuller GN, Liotta LA, Sawaya R, Rao JS. Expression and immunohistochemical localization of cathepsin L during the progression of human gliomas. *Clin Exp Metastas* 1996; **14:** 27-34.

23. Levičar N, Dewey R, Daley E, Bates TE, Davies D, Kos J, Pilkington GJ, Lah TT. Selective suppression of cathepsin L by antisense cDNA impairs human brain tumor cell invasion in vitro and promotes apoptosis. *Cancer Gene Therapy* 2003; **10:** 141-51.

24. Krueger S, Kellner U, Buehling F, et al. Cathepsin L antisense oligonucleotides in a human osteosarcoma cell line: Effects on the invasive phenotypes. *Cancer Gene Therapy* 2001; **8:** 522-8.

25. Koblinski JE, Ahram M, Sloane BF. Unraveling the role of proteases in cancer. *Clin Chim Acta* 2000; **291:** 113-35.

26. Frosch BA, Sloane BF. The role of proteolytic enzymes in brain tumor invasion. In: Mikkelsen T, Bjerkvig R, Laerum OD, Rosenblum MI editors. *Brain tumor invasion: Biological, Clinical and Therapeutic Considerations. Wiley-Liss Inc.* 1998. p. 275-99.

27. Strojnik T, Kos J, Židanik B, Golouh R, Lah TT. Cathepsin B immunohistochemical staining in tumor and endothelial cells is new prognostic factor in patients with brain tumors. *Clin Cancer Res* 1999; **5:** 559-67.

29. Perret SG, Duthel R, Fotso MJ, Brunon J, Mosnier JF. Stromelysin-3 is expressed by aggresive meningiomas. *Cancer* 2002; **94:** 765-72.

30. Chauhan SS, Popescus NC, Ray D, Fleischmann R, Gottesman MM, Troen BR. Cloning, genomic organisation and chromosomal localisation of human cathepsin L. *J Biol Chem* 1993: **268, 2:** 1039-45.

31. Abudula A, Rommerskirsch W, Weber E, Gunther D, Wiederanders B. Splice variants of human cathepsin L mRNA show different expression rates. *Biol Chem* 2001; **382:** 1583-91.

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Effect of electroporation on radiosensitization with cisplatin in two cell lines with different chemo- and radiosensitivity

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Aim. *Radiosensitization with cisplatin can be enhanced by electroporation of cells and tumours. The aim of this study was to extend our previous studies on two carcinoma tumour models with different chemo- and radiosensitivity in order to evaluate whether this treatment is effective also on less chemo- and radiosensitive tumour cells.*

Materials and methods. *This in vitro study was performed on carcinoma SCK and EAT-E cells. The cytotoxicity of three-modality treatment consisting of cisplatin, electroporation and irradiation was determined by the clonogenic assay.*

Results. *The radiosensitizing effect of cisplatin on the two cell lines was greatly enhanced by electroporation. By this combined treatment, less chemo and radiosensitive EAT-E cells were rendered as sensitive as more chemo and radiosensitive SCK cells.*

Conclusion. *The enhancement of cisplatin-induced radiosensitization of cells by electroporation could be beneficially used in the treatment of intrinsically less chemo- and radiosensitive tumours.*

Key words: tumour cells cultured - drug effects; electroporation; drug delivery systems; cisplatin; radiation tolerance

Introduction

Electrochemotherapy combines administration of non-permeant or poorly permeant chemotherapeutic drug with the application of electric pulses to the tumours in order to facilitate the drug delivery into the cells.[1]

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Thus, the enhanced drug delivery locally potentiates chemotherapeutic drug effectiveness at the site of electric pulses application. So far, two chemotherapeutic drugs have proved to be effective in electrochemotherapy, bleomycin and cisplatin. Several fold increase in bleomycin and cisplatin cytotoxicity and several fold increase in antitumour effectiveness have been shown in many preclinical electrochemotherapy studies.[2-9] The increased drug delivery, both into the cells *in vitro* and tumours *in vivo*, was shown to be a predominant underlying mechanism.[4,10] Clinical trials were conducted also on the pa-

tients with different malignancies in whom electrochemotherapy proved to be effective in local tumour control.[11-19]

Based on the data that cisplatin is a radiation sensitizer when bound to DNA, several studies have been conducted using different drug delivery systems in order to increase the amount of cisplatin in tumour cells.[20-27] Our group was the first to demonstrate that the electroporation of tumours increases the radiosensitizing effect of cisplatin in tumours. Radiosensitizing effect of cisplatin increased tumour cures from 72% to 92% when cisplatin was combined with electroporation of EAT murine tumours.[26] The results of this first study were additionally confirmed on another tumour model, LPB fibrosarcoma *in vitro* and *in vivo*.[27] When electrochemotherapy with cisplatin preceded tumour irradiation, tumour curability was enhanced by a factor of 1.6 compared to tumour irradiation alone, and by a factor of 1.4 when compared to cisplatin-induced radiosensitization of tumours without tumour electroporation.[27] Furthermore, in that study, we demonstrated that the increased radiosensitizing effect of cisplatin was due to the increased electroporation-mediated cisplatin delivery into the tumours.[27]

The aim of this study was to extend our previous study in two carcinoma tumour models with different chemo- and radiosensitivity, in order to evaluate whether this treatment is effective also in less chemo- and radiosensitive tumour cells. The study was performed in EAT-E and SCK carcinoma cells with different chemo- and radiosensitivity.

Materials and methods

Tumour cell lines

In the study, two mouse-tumour cell-lines were used, SCK mammary carcinoma cells and EAT-E (Ehrlich ascites carcinoma cells) cells. SCK cells were grown in RPMI medium (RPMI, Sigma, St. Louis, USA) supplemented with 10% heat-inactivated foetal calf serum (FCS, Sigma). EAT-E cells were grown in Eagle minimum essential medium (EMEM) supplemented with 10% FCS. Both cell lines were routinely subcultured twice per week and were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

Drug

cis-Diamminedichloroplatinum (II) (cisplatin) was obtained from Pharmacia&Upjohn S.p.A. (Milan, Italy) as a crystalline powder. It was dissolved in sterile H_2O at a concentration of 1 mg/ml. Final concentration was prepared in EMEM. For each experiment, a fresh solution of cisplatin was prepared.

Irradiation of cells

The cells were irradiated using Darpac 2000 X-ray unit (Gulmay Medical Ltd, Shepperton, UK), operated at 220 kV, 10 mA, using 0.55 mm Cu filtration and 1.8 mm Al filtration. Cells ($1x10^6$ cells/ml of EMEM) were irradiated in low attachment 24-well plates (Corning, Badhoevedorp, The Netherlands) at a dose rate 2 Gy/min with graded doses (2-8 Gy) and thereafter plated in Petri dishes (Corning) for clonogenic assay. D_0 values were used as the measure of cell radiosensitivity. The data were pooled from three independent experiments and normalised to the control non-irradiated cells.

Electrochemotherapy protocol

To determine the survival of SCK or EAT-E cells after the combined treatment with cisplatin and electroporation, 90 μl of cell suspension ($2.2x10^7$ cells/ml) was mixed with 10 (μl of cisplatin of different stock concentrations, ranging from 4.0 to 800.0 μg/ml. One half of the mixture was exposed to 8 electric pulses with electric field intensity 1000 V/cm, pulse duration 100 μs, and frequency 1 Hz. These parameters were optimal for electropermeabilisation of the two cell lines and were determined by measuring the uptake of pro-

pidium iodide used as a measure of electropermeabilisation and by determining the cell survival after exposuring the cells to different electric field intensities used as a measure of electrosensitivity (unpublished data).[28] Electric pulses were generated by Jouan GHT 1287 electroporator (St. Herblain, France). Other half of cell suspension served as a control for cisplatin treatment alone. The cells were then incubated for 5 min at room temperature in low attachment 24-well plates, diluted and plated on Petri dishes for clonogenic assay. The survival of EAT-E cells treated with electric pulses alone was 84.7±3.0% and the survival of SCK cells after electroporation was 79.7±3.0%.

Electrochemotherapy combined with irradiation of EAT-E and SCK tumour cells

To evaluate whether electroporation increases the radiosensitizing effect of cisplatin *in vitro*, EAT-E and SCK cells were electroporated in the presence of cisplatin and then irradiated. After electrochemotherapy, the cells were diluted in a fresh serum-free medium and 5 minutes later exposed to irradiation (4 Gy) (Figure 1). For clonogenic assays, the cells were plated in Petri dishes. The survival of SCK cells and of EAT-E cells after electroporation combined with irradiation was 12.1±2.0% and 26.8±3.0%, respectively. All data were pooled from three independent experiments performed in triplicates. From normalised survival curves, IC_{50} value (drug concentration required to reduce cell survival for 50%) was determined for each treatment group. The statistical differences, using t-test, in sensitivity of cells to different treatments were calculated at the IC_{50} level.

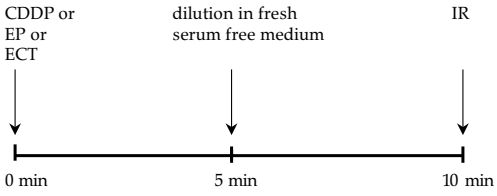

Figure 1. Treatment schedule *in vitro*. Cells were treated either with cisplatin (CDDP), electroporation (EP), electrochemotherapy (ECT), and/or irradiation (IR).

Results

Radiosensitivity of SCK and EAT-E cells

SCK and EAT-E carcinoma cells were irradiated with graded single doses (2-8 Gy) and the surviving fraction was determined by clonogenic assay. From the survival curve D_0 was determined (Figure 2). SCK cells were more radiosensitive with D_0 = 1.2 Gy than EAT-E cells where D_0 was 2.0 Gy. According to the shape of the survival curves, SCK cells were less prone to repair radiation damage than EAT-E cells. Based on these results, a dose of 4 Gy was chosen for subsequent studies to determine the effect of electroporation on radiosensitization induced by cisplatin. The treatment of SCK and EAT-E cells with 4 Gy reduced their survival to 31.8±6.0% and 53.9±6.0%, respectively.

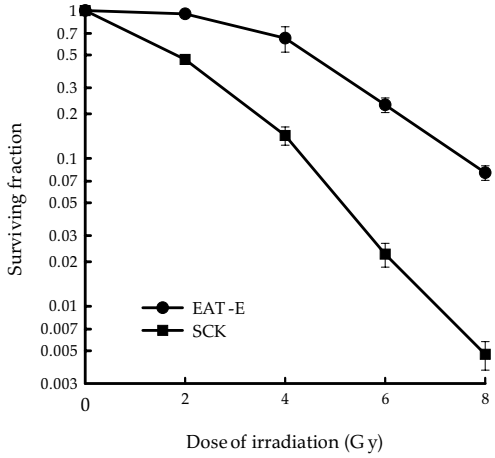

Figure 2. Surviving fraction of SCK and EAT-E carcinoma cells was determined after graded doses of irradiation by clonogenic assay. Values are mean ± SEM (n=9).

Radiosensitization with cisplatin

To determine the effect of electroporation on radiosensitizing effect of cisplatin, the cells were irradiated with 4 Gy following the pretreatment with different concentrations of cisplatin alone or combined with electroporation. SCK cells were more sensitive to the cytotoxic effects of cisplatin than EAT-E cells (Figure 3,4, Table 1). More then 3-times lower cisplatin dose was needed for the same cell kill. When the cells were irradiated 5 minutes after a 5-minute incubation with cisplatin, the cisplatin cytotoxicity was equally enhanced in both cell lines and was approximately 2-fold.

Electroporation increased cisplatin cytotoxicity (electrochemotherapy) in both cell lines. The survival curve was biphasic, which is due to the electroporation of cells. SCK cells were electroporated in 70.0±8.0%, EAT-E cells in 80.0±9.0%. The reduction in cell survival was therefore steep, declining to the level of 0.3 in SCK cells and to 0.2 in EAT-E cells, which is in accordance with the number of electropermeabilised cells. The remaining part of the cell survival curve had the same slope as the survival curve of cells treated with cisplatin alone (Figure 3,4). At the cell survival level of 0.5, the potentiation of cisplatin cytotoxicity for SCK cells by electroporation was approx 4-fold. In contrast, in EAT-E cells this potentiation was more than 20-fold, suggesting that the cell membrane is the major barrier for cisplatin cytotoxic action. Consequently, when the electroporation was used as a drug delivery system for cisplatin, IC_{50} was almost equal for both cells lines (Table 1).

The cell survival curve of the cells treated with electrochemotherapy and irradiation declined to a lower level of survival in both cell lines, thus proving the radiosensitizing effect of cisplatin (Figure 3,4).

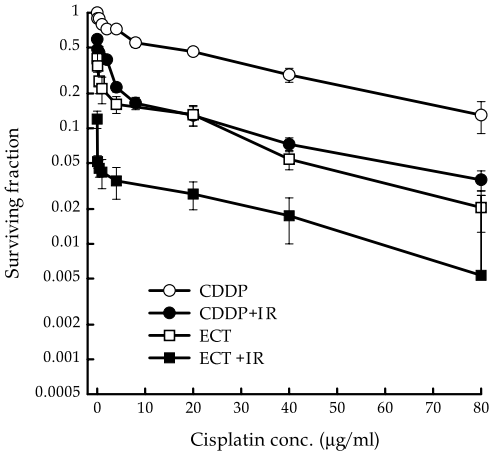

Figure 3. Survival curves of SCK cells after treatment with different cisplatin concentrations only (CDDP), cisplatin combined with electroporation (ECT) or single dose of irradiation (CDDP + IR) and combination of all the three treatment modalities (ECT + IR). Immediately after addition of cisplatin to cell suspension, cells were electroporated and 10 minutes later irradiated. Values are mean ± SEM (n=9).

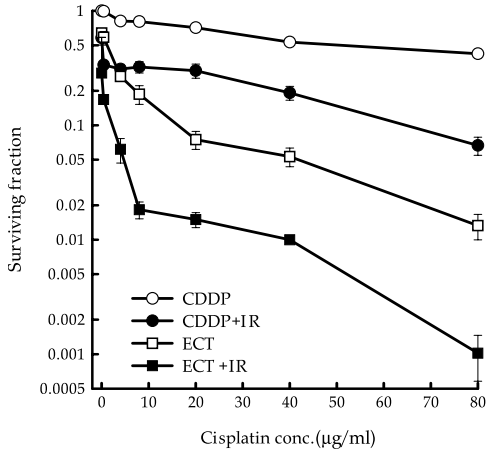

Figure 4. Survival curves of EAT-E cells after treatment with different cisplatin concentrations only (CDDP), cisplatin combined with electroporation (ECT) or single dose of irradiation (CDDP + IR) and combination of all the three treatment modalities (ECT + IR). Immediately after addition of cisplatin to cell suspension, cells were electroporated and 10 minutes later irradiated. Values are mean ± SEM (n=9).

Table 1. Cytotoxic effect of cisplatin, electric pulses, irradiation and the combination of these three treatments on SCK and EAT-E cells *in vitro*

Group	IC_{50} (μg/ml)[a]	
	EAT-E	SCK
CDDP[b]	48.5±1.5	14.8±1.0
ECT[c]	2.2±0.9	3.4±0.7
CDDP+IR[d]	22±2.0	8.0±0.9
ECT+IR[e]	0.9±0.3	0.9±0.2

[a]IC_{50} - Drug concentration to reduce cell survival for 50%.
[b]CDDP - Cisplatin. Survival of cells treated with cisplatin was normalised to the untreated control cells.
[c]ECT - Cells were treated with electroporation (EP) and CDDP. Survival of cells in this group was normalised to the effect of electroporation alone.
[d]CDDP+IR - Cells were irradiated with 4 Gy 10 minutes after incubation with CDDP. Survival of cells in this group was normalised to the effect of irradiation alone.
[e]ECT+IR - Cells were irradiated with 4 Gy 10 minutes after EP. Survival of cells in this group was normalised to the effect of electroporation in combination with irradiation.

Discussion

The results of this study show that the radiosensitization of cisplatin in the two carcinoma cell lines used was greatly enhanced by electroporation. By this combined treatment, the less chemo- and radiosensitive EAT-E cells were rendered as sensitive as more chemo- and radiosensitive SCK cells.

First reports on improvement of combined modality therapy with cisplatin and radiation using electroporation of cells and tumours have already been published.[26,27] In our first study we showed that the delivery of cisplatin into the cells by electroporation of tumours increased radiosensitizing effect of cisplatin.[26] When electrochemotherapy preceded irradiation of EAT tumours, the curability rate of the tumours increased from 27% to 92%. This combined treatment was also better than the cisplatin therapy combined with local tumour irradiation, and irradiation combined with application of electric pulses. This study confirmed that cisplatin is a well known chemotherapeutic drug with radiosensitizing effect and that with increasing cisplatin delivery into the tumour cells, radiosensitizing effect of cisplatin also increases.[26] The improved therapeutic effect was demonstrated in several studies, where intratumoural drug solution in slow release device or polymer implant were combined with irradiation of murine tumours.[20-25]

In our second study, we showed that the electroporation of tumours increased radiosensitizing effect of cisplatin also in LPB sarcoma and that the increased platinum delivery into the tumours with electroporation was a predominant underlying mechanis.[27] This was a confirmation of previous observations that radiosensitization occurs only when cisplatin is present in tumour cells in sufficient amount.[26] The study showed that, when electrochemotherapy preceded irradiation, tumour curability rate was increased compared to irradiation only by EF=1.6, as well as compared to radiosensitization of tumours treated with cisplatin alone (EF=1.4). Radiosensitization was demonstrated also in LPB cells *in vitro*. Irradiation of cells pretreated with electrochemotherapy shifted the survival curve 2-fold further to the left compared to electrochemotherapy treated cells.[27]

In the present study, we extended our previous studies on two carcinoma tumour models EAT-E and SCK with different chemo- and radiosensitivity, in order to evaluate whether this treatment is effective also on less chemo- and radiosensitive tumour cells. The results of this study are in accordance with the results of previous study on LPB sarcoma cells.[27] We found that, when electroporation is used as a drug delivery system, the same cisplatin concentration yields the same cell kill on different cell lines. Specifically cisplatin concentration, when combined with electroporation that caused 50% reduction in cell survival for EAT-E cells, was 2.2±0.9 μg/ml, 3.4±0.7 μg/ml for SCK cells, and 4.0±0.5 μg/ml for LPB cells. Furthermore, the

same effect was observed when irradiation was combined with electrochemotherapy. Electroporation-enhanced cisplatin-induced radiosensitization was approximately the same for all cell lines. If we take into account that EAT-E (IC_{50} = 48.5±1.5 μg/ml) and LPB (IC_{50} = 120.0±3.0 μg/ml) cells are less sensitive to cisplatin than SCK (IC_{50} = 14.8± 1.0 μg/ml) cells and that EAT-E cells are more radioresistant (D_0 = 2.0 Gy) than LPB (D_0 = 1.6 Gy) and SCK (D_0 = 1.2 Gy) cells, then we can conclude that the electrochemotherapy combined with irradiation radiosensitizes the cells to the approximately the same level, regardless of the intrinsic sensitivity of cells to cisplatin or irradiation.

In conclusion, by this combined treatment, the less chemo- and radiosensitive EAT-E cells were rendered equally sensitive as more chemo and radiosensitive SCK cells. Therefore, this enhancement of cisplatin-induced radiosensitization by electroporation of cells could be beneficially used for the treatment of less chemo and radiosensitive tumours.

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References

1. Mir LM, Orlowski S. Mechanisms of electrochemotherapy. *Adv Drug Deliv Rev* 1999; **35:** 107-18.

2. Belehradek J, Orlowski S, Poddevin B, Paoletti C, Mir LM. Electrochemotherapy of spontaneous mammary tumors in mice. *Eur J Cancer* 1991; **27:** 73-6.

3. Čemažar M, Milačič R, Miklavčič D, Dolžan V, Serša G. Intratumoral cisplatin administration in electrochemotherapy: antitumor effectiveness, sequence dependence and platinum content. *Anticancer Drugs* 1998; **9:** 525-30.

4. Čemažar M, Miklavčič D, Ščančar J., Dolžan V, Golouh R, Serša G. Increased platinum accumulation in SA-1 tumor cells after in vivo electrochemotherapy with cisplatin. *Brit J Cancer* 1999; **79:** 1386-91.

5. Mir LM, Orlowski S, Belehradek J, Paoletti C. Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses. *Eur J Cancer* 1991; **27:** 68-72.

6. Mir LM, Devauchelle P, Quintin-Colonna F, Delisle F, Doliger S, Fradelizi D. First clinical trial of cat soft-tissue sarcomas treatment by electrochemotherapy. *Brit J Cancer* 1997; **76:** 1617-22.

7. Ramirez LH, Orlowski S, An D, Bindoula G, Dzodic R, Ardouin P. Electrochemotherapy on liver tumours in rabbits. *Brit J Cancer* 1998; **77:** 2104-11.

8. Serša G, Čemažar M., Miklavčič D. Antitumor effectiveness of electrochemotherapy with cis- diamminedichloroplatinum(II) in mice. *Cancer Res* 1995; **55:** 3450-5.

9. Heller R, Jaroszeski M, Leo-Messina J, Perrot R, Van Voorhis N, Reintgen D, Gilbert R. Treatment of B16 mouse melanoma with the combination of electropermeabilization and chemotherapy. *Bioelectrochem Bioenerg* 1995; **36:** 83-7.

10. Belehradek J, Orlowski S, Ramirez LH, Pron G, Poddevin B, Mir LM. Electropermeabilization of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin. *Biochem Biophys Acta* 1994; **1190:** 155-63.

11. 12. Belehradek M, Domenge C, Luboinski B, Orlowski S, Belehradek J, Mir LM. Electrochemotherapy, a new antitumor treatment: first clinical phase I-II trial. *Cancer* 1993; **72:** 3694-700.

12. Heller Jaroszeski MJ, Reintgen DS, Puleo CA, DeConti RC, Gilbert RA, Glass LF. Treatment of cutaneous and subcutaneous tumors with electrochemotherapy using intralesional bleomycin. *Cancer* 1998; **83:** 148-57.

13. Heller R, Gilbert R, Jaroszeski MJ. Clinical application of electrochemotherapy. *Adv Drug Deliv Rev* 1999; **35:** 119-29.

14. Mir LM, Glass LF, Serša G, Teissie J, Domenge C, Miklavčič D, Jaroszeski MJ, Orlowski S, Reintgen DS, Rudolf Z, Belehradek M, Gilbert R, Rols MP, Belehradek J, Bachaud JM, DeConti R, Štabuc B, Čemažar M, Coninx P, Heller R. Effective treatment of cutaneous and subcutaneous malignant tumours by electrochemotherapy. *Brit J Cancer* 1998; **77:** 2336-42.

15. Serša G, Štabuc B, Čemažar M, Jančar B, Miklavčič D, Rudolf Z. Electrochemotherapy with cisplatin: potentiation of local cisplatin antitumour effectiveness by application of electric pulses in cancer patients. *Eur J Cancer* 1998; **34:** 1213-8.

16. Serša G, Čemažar M, Rudolf Z, Fras AP. Adenocarcinoma skin metastases treated by electrochemotherapy with cisplatin combined with radiation. *Radiol Oncol* 1999; **33:** 291-6.

17. Serša G, Štabuc B, Čemažar M, Miklavčič D, Rudolf Z. Electrochemotherapy with cisplatin: clinical experience in malignant melanoma patients. *Clin Cancer Res* 2000; **6:** 863-7.

18. Serša G, Čufer T, Čemažar M, Reberšek M, Zvonimir R. Electrochemotherapy with bleomycin in the treatment of hypernephroma metastasis: case report and literature review. *Tumori* 2000; **86:** 163-5.

19. Gehl J, Geertsen PF. Efficient palliation of haemorrhaging malignant melanoma skin metastases by electrochemotherapy. *Melanoma Res* 2000; **10:** 585-9.

20. Theon AP, Madewell BR, Ryu J, Castro J. Concurrent irradiation and intratumoral chemotherapy with cisplatin: a pilot study in dogs with spontaneous tumors. *Int J Radiat Oncol Biol Phys* 1994; **29:** 1027-34.

21. Begg AC, Deurloo MJ, Kop W, Bartelink H. Improvement of combined modality therapy with cisplatin and radiation using intratumoral drug administration in murine tumors. *Radiother Oncol* 1994; **31:** 129-37.

22. Deurloo MJ, Kop W, van Tellingen O, Bartelink H, Begg AC. Intratumoural administration of cisplatin in slow-release devices: II. Pharmacokinetics and intratumoural distribution. *Cancer Chemother Pharmacol* 1991; **27:** 347-53.

23. Harrington KJ, Rowlinson-Busza G, Syrigos KN, Vile RG, Uster PS, Peters AM. Pegylated liposome-encapsulated doxorubicin and cisplatin enhance the effect of radiotherapy in a tumor xenograft model. *Clin Cancer Res* 2000; **6:** 4939-49.

24. Ning S, Yu N, Brown DM, Kanekal S, Knox SJ. Radiosensitization by intratumoral administration of cisplatin in a sustained-release drug delivery system. *Radiother Oncol* 1999; **50:** 215-23.

25. Yapp DT, Lloyd DK, Zhu J, Lehnert SM. The potentiation of the effect of radiation treatment by intratumoral delivery of cisplatin. *Int J Radiat Oncol Biol Phys* 1998; **42:** 413-20.

26. Serša G, Kranjc S, Čemažar M. Improvement of combined modality therapy with cisplatin and radiation using electroporation of tumours. *Int J Radiat Oncol Biol Phys* 2000; **46:** 1037-41.

27. Kranjc S, Čemažar M, Grošel A, Ščančar J, Serša G. Electroporation of LPB sarcoma cells in vitro and tumors in vivo increases the radiosensitizing effect of cisplatin. *Anticancer Res* 2003; **23:** 275-82.

28. Čemažar M, Jarm T, Miklavčič D, Maček Lebar A, Ihan A, Kopitar NA, Serša G. Effect of electric-field intensity on electropermeabilization and electrosensitivity of various tumor-cell lines in vitro. *Electro Magnetobiol* 1998; **17:** 263-72.

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Telomerase in lung cancer diagnostics

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Background. *Telomerase is a ribonucleoprotein that looks after the telomeric cap of the linear chromosomes maintaining its length. It is over expressed in tumour tissues, but not in normal somatic cells. Therefore the aim of this study was to determine the telomerase activity in lung cancer patients as novel marker for lung cancer detection evaluating the influence of tissue/cell obtaining technique.*

Material and methods. *Using the TRAP (telomeric repeat amplification protocol), telomerase activity was determined in material obtained from bronchobiopsy (60 lung cancer patients compared with 20 controls) and washings from transthoracic fine needle aspiration biopsy performed in 10 patients with peripheral lung tumours.*

Results. *Telomerase activity was detected in 75% of the lung cancer bronchobyopsies, and in 100% in transthoracic needle washings.*

Conclusions. *Measurement of telomerase activity can contribute in fulfilling the diagnosis of lung masses and nodules suspected for lung cancer.*

Key words: lung neoplasms - diagnosis; telomerase; bronchoscopy

Introduction

Lung malignancies represent a repeated problem in the respiratory clinics constantly arousing attention due to the rapid evolution, extremely bad prognosis and frightening number of new patients every following year. Lung cancer is the most frequent pathology among all malignancies with more than 1000 000/year, and the main cause of death in 29% in this pathology. The distribution shows dominance in the developing countries with 61%, mainly due to the early presence of smoking habits.[1,2] The 5-year survival is the lowest one, compared to the most frequent cancers: (colon 63%, breast 83%, prostate cancer 93%).[2,3] It remains alarmatic low: from 8% in 1960 to 14% 2001. On the other hand there is a dramatic difference in the 5-year survival, related to the stadium of the disease. The localized form of the disease shows survival up to 40% (IA=67% IB=57%, IIA=55%, IIB=39%, IIIA=23%), compared to the extensive form of the disease -only 14%. The emergency need to deal with this highly fatal disease pointed out

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that the main interest in this field has to be: developing and using methods for early detection of lung cancer such as sputum cytology, native chest radiogram, spiral chest CT, fluorescence bronchoscopy and molecular markers for malignancy.[2,3]

One of the most important factors in establishing the cellular integrity are the telomeres, specialized structures of the chromosomal end in all eucariotic cells build of repetitive short DNA sequences (TTAGGG) and associated combining proteins. They serve as puffer zones against the chromosomal spending in the aging process and protectors in the degradation and recombinant process during the chromosomal junctions in the mitosis. The shortening of the telomere is the signal for stopping the cell division.[4-6] The enzyme telomerase is a ribonucleic protein with function of resyntetising the telomeric DNA of the chromosomal ends. It maintains the telomeric length giving the cell the opportunity for uncontrolled cellular division. Its quantity is carefully regulated, but genetic mutation and DNA damage can cause its activation or deactivation. The main characteristics of the tumour growth are avoidance of normal proliferative control so the renewal of telomeric repeats by activating the enzyme telomerase may be a path for the tumour cells to avoid senescence and death. This enzyme is normally detected only in reproductive cells and cells with self renewal capacity (bone marrow, lymphocytes, intestinal crypt cells, epidermal basal cells), but it is undetectable in normal somatic cell. The development of highly sensitive PCR-based commercial kit (TRAP) by Kim et al. in 1995 allows telomerase detection in various biosamples.[7]

The analysis of the telomerase activity in lung tumours was evaluated predominantly in surgical specimens (frozen samples of proven tumour tissues), after the diagnosis of lung cancer was already established. Therefore this study was designed to evaluate the role of telomerase in lung cancer diagnostics[6,8] in fresh specimens obtained by routine lung cancer diagnostic sampling: bronchoscobiopsy and transthoracic fine needle aspiration biopsy.

Material and methods

The study involved 60 patients with central lung tumour, and 20 pts with pneumonia as a control group. All of the pts underwent bronchoscopy and in cases of endoscopic lesion, bronchobiopsy (1 mm) was obtained for TRAP analysis. The same was performed in the control group taking 1mm sample from bronchial mucosa. Another 10 patients with peripheral lung lesion were included, and transtoracic fine needle aspiration biopsy (FNAB) was performed. The mean smear was sent to citopathology lab and the needle washings were analysed for telomerase. Analysis of telomerase was performed in total of 80 samples of bronchial mucosa, 10 samples of FNAB.

Telomerase assay

Telomerase activity was qualitatively evaluated using the TRAP (Telomerase Repeat Amplification Protocol) of Boehringer Mannheim. Practically it is a four step process: Telomerase, if present, adds multiple 6-nucleotide telomeric repeats to a biotinylated synthetic primer. Then the telomerase reaction product is amplified by PCR, using a biotinylated primer. Denaturation follows and the PCR product hybridizes to a digoxigenin-labeled probe specific for the telomeric repeat. The last step is binding of the DNA hybrid to a streptavidin-coated microtiter plate, and anti-digoxigenin-peroxidase so TMB substrate generates a coloured product measurable with a microplate reader.[9-11] Statistical analysis of the data included method of clinical test evaluation (Bayesian analysis).

Results

Telomerase positively was detected in 45 out of 60 (75%) of the positive lung cancer biopsies, compared with the controls of normal mucosa 2/20 (10%). Statistical analysis for telomerase in lung cancer biopsies showed accuracy of 80% (Ac), sensitivity of 75% (Son), specificity of 90% (Sp) positive predictive value of 95.7% (PPV) and negative predictive value of 40.4% (NPV).

Histology analysis showed predominance of telomerase positivity in small cell lung cancer (SCLC), and the lowest activity in metastatic deposits (Table 1). Telomerase activity was analyzed in needle washings after FNAB was performed in patients with peripheral lung tumour. Telomerase was detected in all 10 (100%) samples, compared to cytology where malignancy was confirmed in 8 out of 10 (80%) samples.

Discussion

This study design was performed in order to find out if the novel molecular marker of malignancy telomerase can be used in diagnosis of lung cancer. This question revealed three new points: how can we use telomerase, in what way and what type of samples in lung cancer diagnostics. The routine lung cancer investigation usually starts with bronchoscopy as a basic method for obtaining samples for histopathology. On the other hand up to 90% of the endobronhial lesions are confirmed by histopathological analysis of bronchobiopsy material, but sometimes we have to repeat the procedure in order to obtain sufficient sample for diagnosis. In our study telomerase positivity was detected in 75% in lung cancer bronchobiopsies compared to non-malignant tissue (10%). Statistical analysis showed highly significant value of $c^2=93.25$ and $p<0.0001$ that proved this method to be significant in separating malignant of non-malignant tissue. Several authors such as Hiyama et al.1995[12], Yang et al. 1998[13], Lee et al.[9] 1998 Shay et al.1999[14-16] etc reported very high telomerase activity in lung cancer tissue 75-85%.[17] These results, including those from our study impose the conclusion that telomerase is one of the leading factors in development of lung cancer. Most of the telomerase studies on lung cancer were conducted on material obtained by surgical resection or lung cancer cell lines. Statistical analysis pointed out that telomerase activity is slightly higher in such material (increasing the Sn, Sp, Ac), but the PPV is the same (Table 2). This data suggests that in the clinical practice in cases where modest endobronchial changes lack histopathological conformation, and the telomerase activity is detected, the diagnostic procedure should be persistent and directed in establishing the malignant disease.

Table 1. Histology of lung cancer and telomerase activity

Type (Total 60)	Samples	Telomerase/+/		Sn=TP/TP+FN
NSCLC	34	26	76.5%	76.5%
SCLS	15	12	80%	80%
MS deposites	11	7	63.6%	63.6%

Table 2. Telomerase activity in lung cancer: surgical sampling increases the Sn, Sp, and Ac of the method compared with bronchobiopsy sampling

Sn	Sp	Ac	PPV	NPV	
Hiyama et al./1998[12]	80%	94%	96%	96%	89%
Sen et al./1999[18]	87%	100%	88%	96%	84%
Kovkarova et al./2002	75%	90%	81%	96%	40%

In the analysis of peripheral lung lesions, transthoracic fine needle aspiration biopsy is a basic diagnostic method for tissue sampling. Telomerase activity was determined in needle washing after the preparation of material for cytology analysis. Telomerase positivity was found in all of the samples (10/10), compared to cytology were due to massive necrosis malignancy was confirmed in 8 out of 10 samples. Sen at al.[18] and Naritoku et al.[11] established telomerase sensitivity higher then the cytology (Table 3). Naritoku stresses the value of telomerase debating that this molecular marker finally determines the blurre cytological report of rare atypical cells that often confuses the pulmonologist.[12,18]

Second objective to the validation of the telomerase activity was to establish any connection with the histology type of the lung cancer (Table 1). This analysis showed no link to telomerase positivity in primary lung cancers, but the lowest rate of telomerase activity was detected in the metastatic type.

Some authors like Strovel[19] and Allbanell[20] the telomerase activity can be associated with the tumour proliferation rate, response to therapy and final outcome. Quantification of these markers according to these authors can be used as valid prognostic marker of lung cancer.

These results suggest that telomerase can be used as a complementary tool in lung cancer diagnostics especially in cases where the first line diagnosis is confuse and unprecise. The studies that evaluate the telomerase activity in more simple sampling such as sputum or plasma can open a new field in early lung cancer detection especially in high risk population. On the other hand telomerase can be used as a valid target for lung cancer treatment. The pioneer attempts are already in action, but one can not forget that the human body is very complex and therefore we have to gain more experience and knowledge in this field to achieve permanent success.

Table 3. Telomerase activity in FNAB washings of lung cancer: superior to cytology

TTAP	Telomerase/+/	Cytology
Sen et al./1999[18]	35/42 84%	68.4%
Naritoku et al./1999[11]	14/16 88%	68%
Kovkarova et al./2002	10/10 100%	80%

References

1. Hoffman PC, Mauer AM, Vokes EE. Lung cancer. *Lancet* 2000; **355:** 479-85.
2. Landis SH, Murray T, Bolden S, Wingo PA, et al. Cancer statistics, 1999. *CA Cancer J Clin* 1999; **49:** 8-31.
3. Franklin WA. Diagnosis of lung cancer: pathology of invasive and preinvasive neoplasia. *Chest* 2000; **117(Suppl 1):** 80-9.
4. Arinaga M, Shimizu S, Gotoh K, Haruki N, Takahashi T, Takahashi T, et al. Expression of human telomerase subunit gene in primary lung cancer and its clinical significance. *Ann Thorac Surg* 2000; **70:** 401-5.
5. Bacchetti S, Counter C. Telomeres and telomerase in human cancer [Review]. *Int J Oncol* 1995; **7:** 423-32.
6. Blasco MA, Gasser SM, Lingner J. Telomeres and telomerase. *Genes Dev* 1999; **13(18):** 2353-9.
7. Kim NW. Clinical implikations of telomerase in cancer. *Eur J Cancer* 1997; **33(5):** 781-6.
8. Breslow RA, Shay JW, Gazdar AF, Srivastava S.. Telomerase and early detection of cancer. *J Natl Cancer Inst* 1997; **89(9):** 618-23.
9. Lee JC, Jong HS, Yoo CG, Han SK, Shim YS, Kim YW. Telomerase activity in lung cancer cell lines and tissues. *Lung Cancer* 1998; **21:** 99-103.
10. Maniwa Y, Yoshimura M, Obayashi C, Inaba M, Kiyooka K, Kanki M, et al. Association of p53 gene mutation and telomerase activity in resectable non-small cell lung cancer. *Chest* 2001; **120(2):** 589-94.
11. Naritoku WY, Datar RH, Li P, Groshen SL, Taylor CR, Imam SA. Telomerase activity: comparison between fine needle aspiration and Biopsy Speciments for detection of tumor cells. *Cancer* 1999; **87(4):** 210-5.
12. Hiyama K, Hiyama E, Ishioka S, Yamakido M, Inai K, Gazdar AF, et al. Telomerase activity in small-

cell and non-small-cell lung cancers. *J Natl Cancer Inst* 1995; **87(12):** 895-902.

13. Yang CT, Lee MH, Lan RS, Chen JK. Telomerase activity in pleural effusions. Diagnostic significance. *J Clin Oncol* 1998; **16:** 567-73.

14. Shay J, Zou Y, Hiyama E, Wright W. Telomerase and cancer. *Hum Mol Genet* 2001; **10(7):** 677-85.

15. Shay JW, Werbin H, Wright WE. Telomerase assays in the diagnosis and prognosis of cancer. *Ciba Found Symp* 1997; **211:** 148-55.

16. Shay JW, Gazdar AF. Telomerase in early detection of cancer, *J Clin Pathol* 1997; **50:** 106-9.

17. Yashima K, Litzky LA, Kaiser L, Rogers T, Lam S, Wistuba II, et al. Telomerase expression in respiratory epithelium during the multistage pathogenesis of lung carcinomas. *Cancer Res* 1997; **57(12):** 2373-7.

18. Sen S, Reddy VG, Khanna N, Guleria R, Kapila K, Singh N. A comparative study of telomerase activity in sputum, bronchial washing and biopsy specimens of lung cancer. *Lung Cancer* 2001: **33(1):** 41-9.

19. Strovel J, et al. Plasma telomerase activity predicts response and relapse in lung cancer [Abstract]. Program and Abstracts of the American Society of Clinical Oncology. 36th Annual Meeting 2000; New Orleans, Louisiana. Abstract 1899.

20. Albanell J, Lonardo F, Rusch V, Engelhardt M, Langenfeld J, Han W, et al High telomerase activity in primary lung cancers: association with increased cell proliferation rates and advanced pathologic stage. *J Natl Cancer Inst* 1997; **89(21):** 1609-15.

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Prostate IMRT fractionation strategies: two-phase treatment versus simultaneous integrated boost

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Background. *The purpose of the study was to investigate the radiobiological effect of the number of fractions, position uncertainties and clonogen spread (microscopic disease) on two different inverse treatment planning alternatives: (a) 2-phase strategy; (b) simultaneous integrated boost (SIB).*

Material and methods. *The tumour control probability (TCP) and normal tissue complication probability (NTCP) were calculated for the 2-phase strategy, which has well defined fractionation scheme and compared to the TCP and NTCP for the SIB strategy calculated as a function of the number of fractions. For a 7-beam IMRT prostate treatment, we have performed inverse treatment planning for the two different strategies following the above method.*

Results. *When the position uncertainties and clonogen spread were accounted for in the TCP calculation a drop as large as 10% was found. A drop of 5-7% in the TCP was obtained for the SIB strategy, if delivered in the same number of fractions as the 2-phased one.*

Conclusions. *The potential of inverse planning to design tight conformal dose distributions is fully revealed in the SIB optimization process. The optimized SIB superior dose distributions require modification of the delivered dose per fraction and therefore careful selection of the fractionation regime. Hence physically optimized SIB treatments may not always lead to better tumour control and tissue sparing.*

Key words: prostatic neoplasms - radiotherapy; radiotherapy, conformal; radiotherapy dosage; dose fractionation

Introduction

The availability of inverse planning and intensity modulated radiotherapy (IMRT) technology opened the possibility of designing new treatment strategies with superior dose distributions, namely tighter treatment margins and therefore better organ sparing.

Recently Mohan et al.,[1] and Wu et al.,[2] have suggested a »simultaneous integrated boost« (SIB) IMRT approach to head and neck

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cancer, leading to better conformity and superior dose distributions in the organs at risk compared to a standard way of radiation treatment planning. The SIB strategy was proposed as an alternative to the commonly used two-phase radiation therapy. The idea itself, namely, - uniting the conventional 2-phase treatment strategy in one, is not new, it was considered by a number of authors before.[3-6] The application of IMRT to it is the important new development. In their study this approach was applied to head and neck cancer. In this paper we consider the SIB approach applied to the case of prostate cancer taking the effect of position uncertainties and possible clonogen spread into account. Further development of the investigations of Mohan et al.,[1] is conducted by more detailed radiobiological analyses. While, the main quantity under consideration in Wu et al.[2] was the »normalized total dose« NTD (similarly to Lebesque and Keus),[3] here we demonstrate the application of tumour control probability (TCP) and normal tissues complication probability (NTCP) models to evaluate the SIB IMRT plans as a function of the number of fractions. We demonstrate with this work that the SIB IMRT physical optimization, although leading to superior dose distributions in physical terms - with respect to specified dose and dose-volume criteria - may result in lower tumour control or higher probability for organ damage depending on the fractionation strategy chosen.

Material and methods

Treatment strategies

A patient who had recently undergone radiation treatment for the carcinoma of the prostate in our centre was re-planned with inverse planning for the purpose of this study (Figures 1a, b, c). The original treatment technique employed a four-field box arrangement (Plan I) with 18 MV MLC-shaped conformal beams to deliver a uniform dose of 44 Gy to planning target volume I (PTV1) in 22 daily fractions. In the second phase of the treatment (Plan II), a three-field technique with one anterior (gantry angle 0°) and two posterior lateral fields (gantry angles 260° and 100°) was employed to deliver a boost of 26 Gy to planning target volume II (PTV2) in 13 daily fractions. PTV1 encompassed the gross tumour volume (GTV) with a uniform 1.5 cm margin to account for microscopic disease, set-up uncertainties, and organ motion. PTV2 encompassed the GTV with a uniform 0.5 cm margin to account for set-up uncertainties and organ motion. In both phases the patient was treated in supine position.

A 7-beam treatment technique (Figures 1b, 1c) was optimized with the Helios inverse planning option of the CadPlan treatment planning system (Varian Medical Systems, Palo Alto, CA). For the two-phase strategy, Plan I and Plan II were optimized separately with the optimization parameters listed in Table 1. In terms of target coverage and bladder/rectum sparing these parameters resulted in dose distributions superior to the ones delivered by the original forward treatment

Table 1. Inverse treatment planning dose and priority prescriptions for the different treatment strategies

	PTV1			PTV2			Bladder		Rectum	
	D_{min}[Gy]	D_{max}[Gy]	Priority	D_{min}[Gy]	D_{max}[Gy]	Priority	D_{max}[Gy]	Priority	D_{max}[Gy]	Priority
Plan I	44	44	100 %				33.9	50 %	26.4	50 %
Plan II				26	26	100 %	20.0	50 %	15.6	50 %
SIB44	44	70	100 %	70	70	100 %	53.9	50 %	42.0	50 %
SIB55	55	70	100 %	70	70	100 %	53.9	50 %	42.0	50 %

technique. Note that, in relative terms, the dose and the priority prescriptions for Plan I and Plan II in Table 1 are identical.

For the single phase, simultaneous integrated boost (SIB) strategy, both PTV1 and PTV2 were included in the optimization process and the prescription dose levels were combined to reflect the goal of the treatment in terms of total doses. Thus the SIB optimization prescribes 70 Gy to PTV2 and given the initial PTV1 objective of the 2-phase strategy - a minimum of 44 Gy to PTV1 (Table 1). However, after adding the two dose distributions optimized separately in the two-phase strategy, the minimal dose to PTV1 was found to be 55 Gy. Thus, two SIB plans with different minimum dose prescriptions to PTV1 were optimized (Table 1). Hereafter these are referred to as SIB44 and SIB55.

Biological models

In order to estimate the biological effect of a given dose distribution to the tumour or the organs at risk, the biologically equivalent dose was calculated. Based on it the TCP and NTCP were calculated using the model functions and the parameter estimates by.[7-10]

BED calculation

For each point (voxel) ijk from a certain structure the BED was calculated as:

$$[1]\quad BED_{ijk} = D_{ijk}\left(1+\frac{\beta\, d_{ijk}}{\alpha}\right);\qquad d_{ijk} = \frac{D_{ijk}}{n_{fr}}$$

where α/β ratios of 10, 6 and 3.9 for the tumour, bladder and rectum respectively,[11] were used. Following the recent suggestions of Brenner et al.[12,13] initiating the discussion[14-17] about the possible low α/β ratio for prostate, we have investigated the case of tumour α/β of 2 Gy as well.

For the two-phased approach the BED is the sum of:

$$[2]\quad BED_{ijk} = D_{ijk}^{PTV1}\left(1+\frac{\beta d_{ijk}^{PTV1}}{\alpha}\right)+D_{ijk}^{PTV2}\left(1+\frac{\beta d_{ijk}^{PTV2}}{\alpha}\right);\ d_{ijk}^{PTV1}=\frac{D_{ijk}^{PTV1}}{m_{fr}};\ d_{ijk}^{PTV2}=\frac{D_{ijk}^{PTV2}}{k_{fr}}$$

where the index PTV1 refers to the dose distribution for the treatment of the larger volume accounting for the clonogen spreads, PTV2 refers to the boost volume and m_{fr} and $k_{fr}$are the number of fractions for both consecutive phases.

Because of the higher dose uniformity in the tumour, Eq. [2] is identical to Eq. [1]: $n_{fr}=m_{fr}+k_{fr}$; $D_{ijk}=D_{ijk}^{PTV1}+D_{ijk}^{PTV2}$. However, this statement does not hold true when the normal tissue is considered due to the significant dose heterogeneity throughout volumes of normal tissues in the vicinity of the treated targets. In this case each normal tissue voxel has different dose per fraction, which changes from phase-1 to phase-2.

It should be noted here that for the purposes of the biological index estimation in the 2-phase strategy, the DVHs are rather useless tool. This is why the TCP/NTCP estimation was done on the bases of the BED distributions instead of BED DVHs. Some authors[11,18] use NTD - the dose corresponding to a standard d_f = 2 Gy dose per fraction regime:

$$[3]\quad D'_{ijk} = D_{ijk}\left[\left(\frac{\alpha}{\beta}+d_{ijk}\right)/\left(\frac{\alpha}{\beta}+d_{std}\right)\right]$$

instead BED, for the NTCP calculations.

TCP and NTCP estimation

The TCP is calculated by the following formula:

$$[4]\quad TCP = 0.5^{\frac{1}{N}\sum\exp\left[\frac{2\gamma_{50}}{\ln 2}\left(1-\frac{D_{ijk}}{D_{50}}\right)\right]}$$

The values of the parameters for prostate tumours (T3 stage) are: g50=0.95, D50=46.3 , as estimated in the work of Okunieff et al..[9]

There are several models for NTCP estimation. Here, the Lyman phenomenological model[19] and the Critical Volume (CV) population model[10,20-25] are used. The Lyman phenomenological model is given by:

$$[5]\quad NTCP = \Phi\left(\frac{D_{eff}-D_{50}}{\sigma_{D_{50}}}\right) = \Phi\left(\frac{D_{eff}-D_{50}}{m\,D_{50}}\right)$$

where, the effective dose corresponding to a given dose distribution is,

$$[6]\quad D_{eff} = \left(\frac{1}{N} \sum \sqrt[n]{D_{ijk}} \right)^{n}$$

This formula was first derived in an explicit form by Niemierko and Goitein[26] and reflects a histogram reduction algorithm proposed by Lyman and Wolbarst.[27] It was shown by Niemierko and Goitein[26] that this reduction scheme is consistent with the Critical Element model. It was also shown by Niemierko and Goitein[26], that the Kutcher and Burman reduction algorithm[28,29] is closely related to the Lyman and Wolbarst[27] one. Formula [5] was recently proposed by Niemierko[30] as a generalization of the equivalent uniform dose notion.

In the above formulae D_{ijk} denotes the dose to the *ijk*-th voxel, N is the total number of voxels in a structure. The values of the *NTCP* parameters are $n=.5; m=.11; D_{50}=80$ *Gy* (bladder) and $n=.12; m=.15; D_{50}=80$ *Gy* (rectum).[8] Recently, several authors have considered the dose delivered to the rectal wall only,[18,31-34] as a complication factor, introducing the usage of dose wall or dose surface histograms (DWH and DSH). In those works no parameter estimates are given and as far as the Burman et al.[8] parameter values were calculated having in mind the dose to the whole volume, rather than the wall volume, here the NTCP are estimated based on the whole rectal and bladder volumes. Interestingly enough,[18,31] report negligible differences in the NTCPs estimated by Lymans model and Burman et al.[8] parameters based on the wall and whole rectal volume. Same results, for rectum, are obtained by Ting et al.[32] using the critical element model.[26]

On the other hand we consider incorrect the application of the parameters given by Burman et al.[8] for estimations using the DWHs because those parameters have been obtained by fits to a »data set« that did not have such sophisticated tools to extract rectum dose wall distributions.[7]

It should be emphasized that among the parameters estimated by Burman et al.[8] there are some sets, which are considerably unreliable, like those for rectum. If one examines closely the dose-volume effects reported in the »Emami data«[35] it becomes obvious that in this case there are only 2 points for rectum and determining 3 parameter values from 2 data points is quite a long shot! Nevertheless these parameter values are used by many authors[11,18.,31-33] and are found to produce reasonable NTCP values.

For a comparison, we have also estimated the NTCP for bladder using the critical volume (CV) population model[10,25] given with the following formulae:

$$[7]\quad NTCP = \Phi\left(\frac{-\ln(-\ln \overline{\mu}_d(\gamma_{50}^{FSU}, D_{50}^{FSU}, D_{ijk})) + \overline{\ln(-\ln \mu_{cr})}}{\sigma_{-\ln(-\ln \mu_{cr})}} \right)$$

$$[8]\quad \mu_d = \frac{1}{N} \sum \Phi\left(\sqrt{2\pi}\, \gamma_{50}^{FSU} \ln \frac{D_{ijk}}{D_{50}^{FSU}} \right)$$

The parameters for bladder, estimated based on the »Emami data«,[35] are taken from Stavrev et al.[10] and are μ_{cr} =0.26±0.11, $\sigma_{\mu cr}$ = 0.07±0.03, D_{50}^{FSU} = 108 ± 24 [Gy], γ_{50}^{FSU} = 0.8 ± 0.2. However, Stavrev et al.[10] didn't bring out the parameter estimates for rectum due to the lack of data for their determination. A claim for CV model parameter estimation for rectum is made in the work of Hartford et al.,[36] based on 41 patients. Unfortunately, the parameter values are not given in this work.

In the above-presented TCP and NTCP estimation models, the response of the tissues to the variable fraction size is accounted for by a substitution of the voxel doses D_{ijk} by the biologically equivalent voxel doses BED_{ijk}.

Accounting for the position uncertainties and clonogen spread

One of the reasons behind the two phase strategy is that the slightly enlarged (in respect to GTV) volume (PTV2) is to account for

the position uncertainties, while the initial target volume PTV1 is to account for both position uncertainties and possible clonogen spread. The position uncertainties and the way to account for them have been widely discussed in the literature.[37-41] Following the ideas described in the article of Stavrev et al.[37] one can define a reduced cell density - a notion combining the initial cell density with the position uncertainties. We presume the initial cell density $H(\vec{r})$constant over the GTV and the position uncertainties being described by a 3D normal distribution $G(\vec{r})$. Here H is the Heviside step function being 1 in the GTV and 0 outside. Then the relative reduced cell density is given as:

$$[9]\quad \rho(\vec{r}) = \int_{R^3} G(\vec{r}) H(\vec{r} - \vec{\delta}) d^3\vec{\delta}$$

$$[10]\quad TCP = e^{-\int_V \rho(\vec{r}) e^{-\alpha D(\vec{r})} d^3\vec{\delta}} = e^{-N_o \sum \rho_{ijk} e^{-\alpha D_{ijk}}}$$

The parameters α, N_o could be calculated directly from γ_{50}, D_{50}.[25] The possible clonogen spread may be modelled in a similar way. One can presume that a clonogen could leave its position and relocate at another one with a given probability. If the probability is presumed normal, it could be shown that Eq. [9] describes the process of migration as well. Hence, both processes could be described as convolutions of GTV with normal distributions with different standard deviations, which is equivalent to one convolution but the variance of the normal distribution is the sum of the first ones.

The clinical practice guidelines in our institution determine the PTV2 as GTV with 5 mm margin and PTV1 as GTV with 15 mm margin. Hence, having in mind the normality (the 99 % of the possible values of the stochastic variable lay in +-3SD interval) of the position uncertainties and clonogen spread, we deduce that the first process is described with normal distribution with SD of ~1.6mm and the second one SD=3.2mm. Figure 2 demonstrates the GTV (the solid body) and the calculated reduced cell density, for the case under consideration.

Results

Figure 1 demonstrates isodose distributions for the 2-phased, SIB44 and SIB55 plans in the axial plane containing the treatment isocenter. Figure 3 illustrates the DVHs for the GTV, PTV1, bladder and rectum, as obtained for the 2-phased (solid line), SIB44

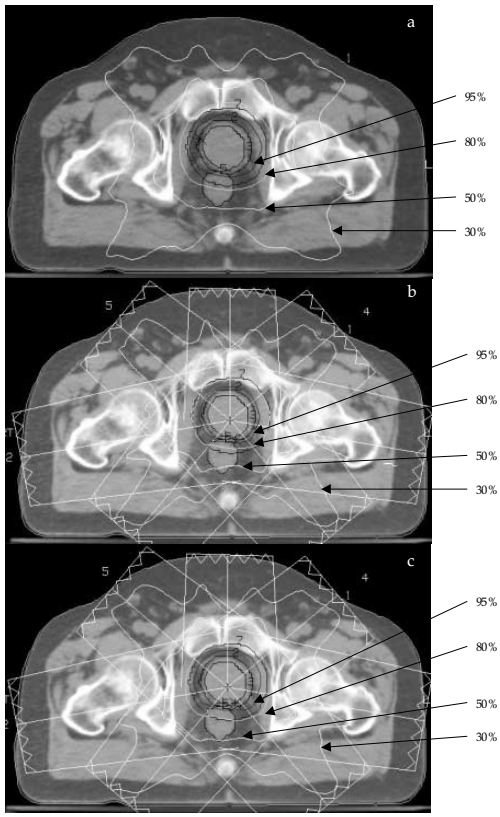

Figure 1. Set-up for 7-beam prostate treatment technique and central slice isodoses for **(a)** two-phase strategy, **(b)** - SIB 44, **(c)** - SIB 55. The PTV1 is denoted as 7, PTV2 - 2, GTV - 3, rectum - 5. The bladder is not seen on this slice. The 95 % isodose line lies between the PTV2 and PTV1 contours. The isodose distributions are normalized to 100 % at the treatment isocenter.

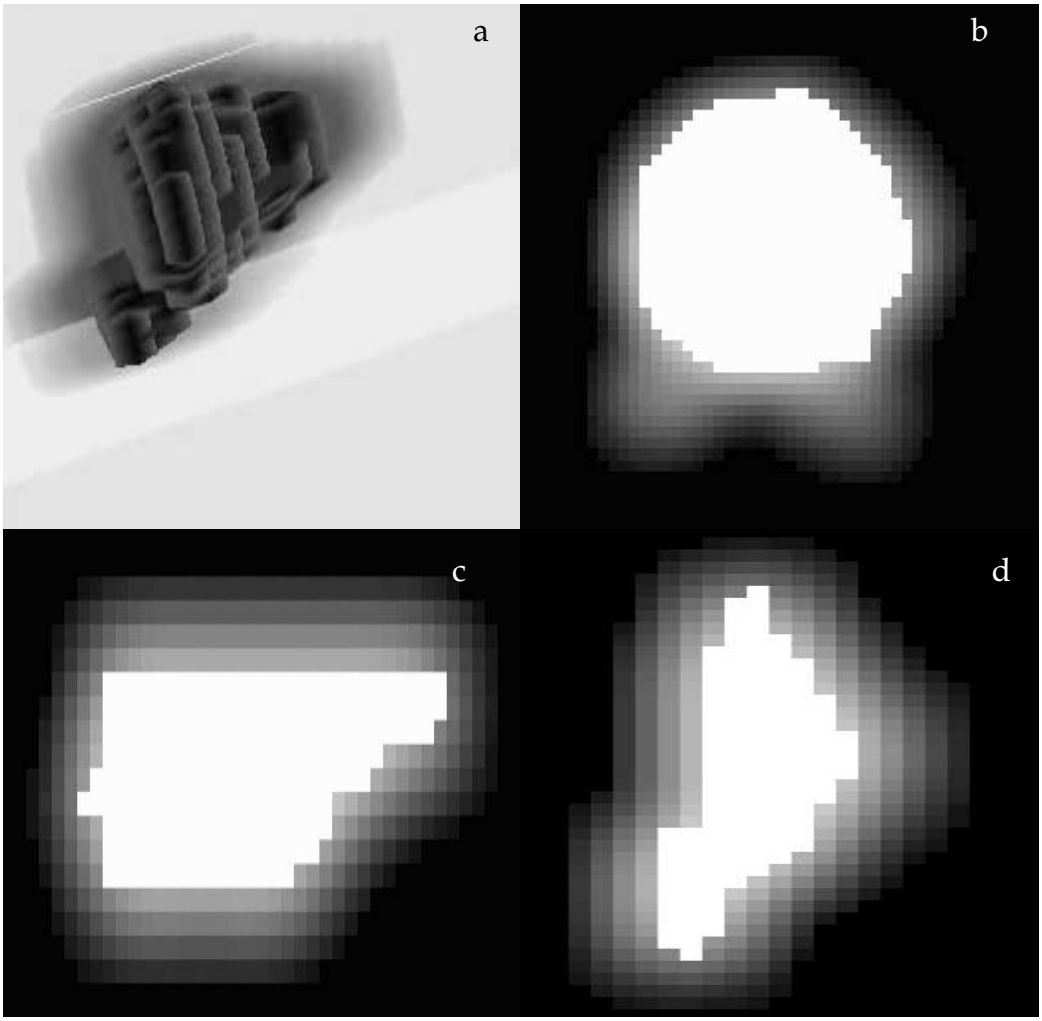

Figure 2. Illustration of the reduced cell density, accounting for the clonogen spread and position uncertainties Eq. [8]. The solid body represents the GTV surface on Figure **(a)**, the reduced cell density »hallo« is shown for two perpendicular planes. On Figures **(b)**, **(c)** and **(d)** cross-sections in three perpendicular planes (axial 2a, coronal 2b, sagital 2c) with the GTV (the white object) and the reduced cell density are shown.

(doted line) and SIB55 (dashed line) strategies. Both SIB strategies resulted in better sparing of the critical organs while satisfying the prescriptions with respect to the target volumes.

2-phased strategy

For the 2-phased strategy we have calculated the NTCPs for bladder and rectum using Eq. [2], [5], [6] and have obtained the following values $NTCP_{bladder}$ = 5.2% and $NTCP_{rectum}$ = 45%. The last represent the Lyman model *NTCP* estimates. An estimate of bladder *NTCP* was also obtained based on the CV model yielding the value of 8.25%. The *TCP* - Eq. [4] - based on the GTV BED distribution for the cases of α/β = 2 and 10 Gy respectively is 93.1% and 84.2%. If the possible clono-

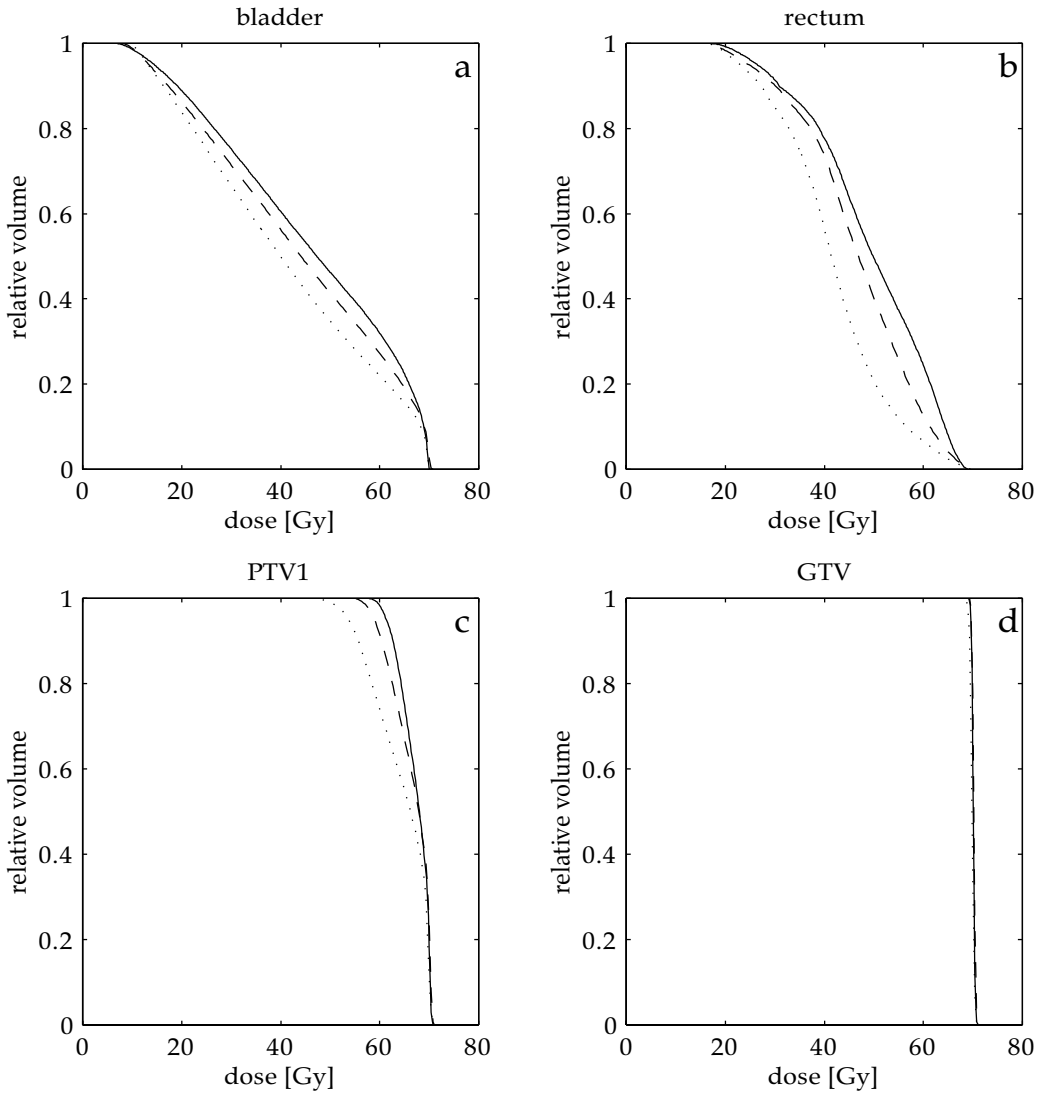

Figure 3. DVHs for the three different treatments and different structures: **(a)** bladder, **(b)** rectum, **(c)** PTV1, **(d)** GTV. The two-phase approach (solid line) prescribes 44 Gy to the first phase volume and additional 26 Gy to the boost one. The actual delivered minimal dose to the first phase volume was 55 Gy. These prompted us to investigate two SIB IMRT plans delivering minimal doses of 44 Gy (dotted line) and 55 Gy (dashed line) to the first phase volume respectively.

gen spread and the position uncertainties are taken into account using Eq. [9] and [10] one gets $TCP_{\alpha/\beta = 2}$= 86% and $TCP_{\alpha/\beta = 10}$ = 74.4%. The *TCP* and *NTCP* values for the 2-phased strategy are shown as lines parallel to the x-axes on Figures 4 a, b, c and d.

SIB strategy

The NTCPs for bladder and rectum, calculated again on the basis of Eq. [2], [5], [6], for SIB44 and SIB55 correspondingly, as a function of number of fractions are presented on

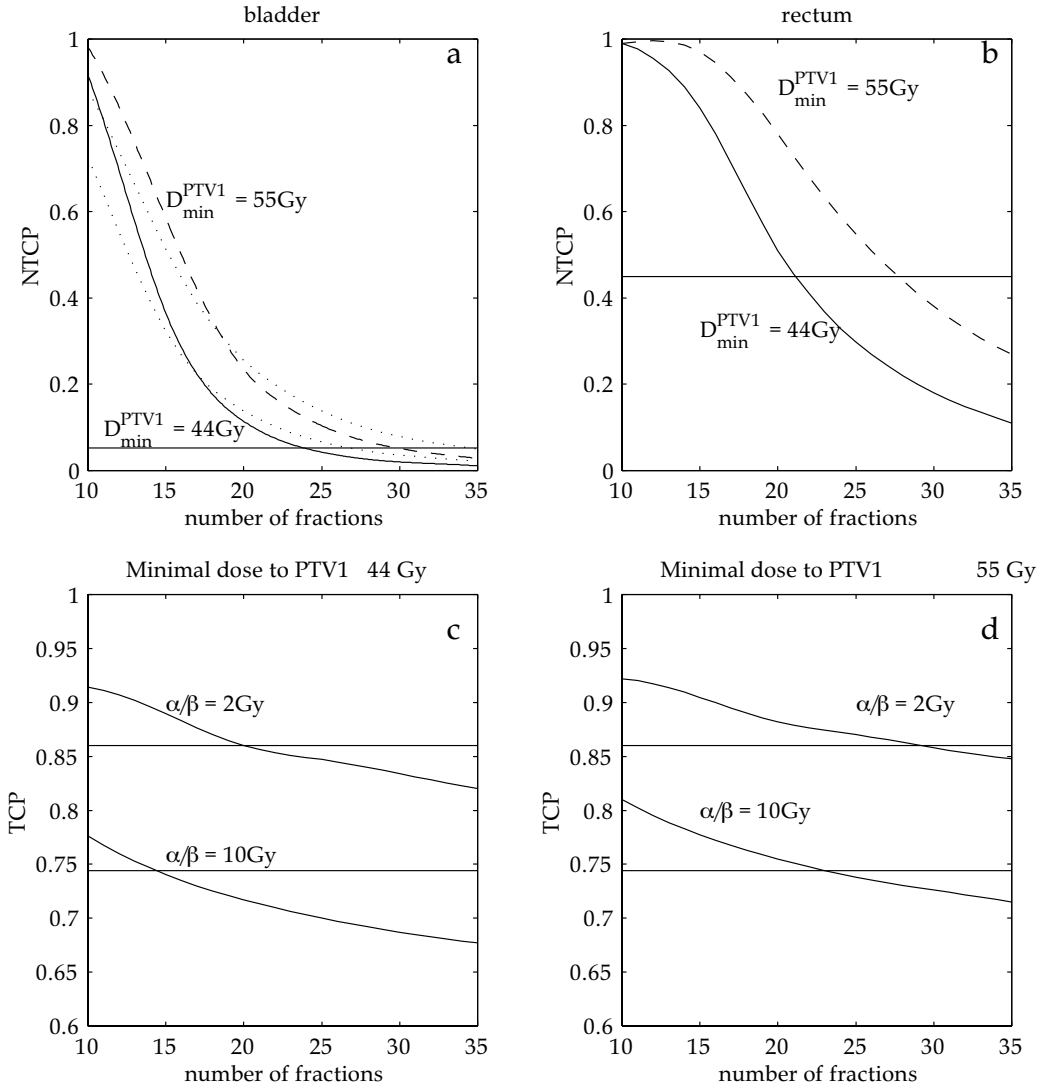

Figure 4. Figures **(a)** and **(b)** show the influence of the fractionation on the bladder and rectum NTCPs for the SIB treatment strategies. The horizontal line represents the NTCP for the two-phase treatment. Figures **(c)** and **(d)** demonstrate the dependence of the TCP, accounted for clonogen spreads and marginal uncertainties, on the number of fractions for the two different SIB treatment plans. Again the horizontal line represents the TCP for the two-phase treatment. The effect of a possible smaller α/β ratio (2 *vs.* 10 Gy) is demonstrated. Note the drop in the TCP for SIB55 fraction treatment compared to the two-phased method. Considering the SIB55, reducing the number of fractions to 25 (α/β =10 Gy) or 30 (α/β =2 Gy) in order to achieve the two-phase-equivalent-TCP will or will not reduce the bladder and rectum NTCPs below their values for the two-phase treatment (Figures 4a and 4b).

Figures 4a and 4b. Similarly the TCP, accounting for the clonogen spread and position uncertainties, as a function of number of fractions, for SIB44 is shown on Figure 4c and for SIB55 on Figure 4d. The both different curves on those plots correspond to the two different α/β ratios.

In the case when the position uncertainties

and clonogen spread are disregarded the *TCP* change with the number of fractions become negligibly small ~ 1.5% / 10 fractions.

Discussion and conclusions

First it should be said, that the TCP and NTCP values and relationships calculated here must be regarded more as relative numbers illuminating the interrelation of the dose-volume effects and fractionation on one hand and the biological outcomes on the other.

It is clear from the DVHs shown on Figures 3a and 3b that the SIB strategy results in smaller doses delivered to the organs at risk. The dose delivered to GTV is equally uniform for the 2-phase and SIB strategy. Hence, from a point of view concerning the physical dose delivery the SIB strategy results in superior dose distributions. On the other hand while the 2-phase strategy is strictly tied to a given fractionation scheme, these is not true for the SIB one. Because of the difference in the dose distributions in PTV1 the impact on the possible clonogen spread and position uncertainty would be different. As it is seen from Figures 4c and 4d there is considerable drop (up to 7%) in the TCP when the SIB strategy is applied in the same number of fractions as the 2-phased one - 35 in our case. The comparison between Figures 4c and 4d illustrates, as expected, that this difference is smaller for SIB55 compared to SIB44, and also for the α/β = 2 Gy over α/β = 10 Gy. Figures 4c and 4d also demonstrate the magnitude of the α/β ratio impact over the TCP. The difference between the TCPs calculated for 2 Gy and 10 Gy α/β ratios is about 15% for SIB44 and 11% for SIB55, almost independent of the number of fractions. The same goes for the 2-phased strategy. Thus the effect of the different α/β ratios is very significant and although more detailed studies may end up with different, than the one used here, model parameters, this would only shift up or down the TCP values but the impact on the TCP difference will be negligible. Hence, this result (Figures 4c and 4d) may be used as a hint of support for Brener's statement[12,13] in the light of the findings of Logue et al.[16] for relatively high local control.

Let us presume all the model parameters correctly estimated and consider the implication of the results shown on Figure 3. For the case α/β = 10 Gy and minimum of 44 Gy to PTV1, Figure 4c shows that if the SIB IMRT dose is delivered in 15 fractions it will result in the same TCP as the conventional 2-phase strategy. For the normal tissue, we get 24 and 21 fractions for bladder and rectum respectively. Hence in this case, regardless of the fractionation regime, either the TCP would be lower than the 2-phased strategy TCP, or the NTCP would be higher than the 2-phased strategy NTCP. This is quite natural, because the minimum dose to PTV1 in the conventional therapy was 55 Gy, not 44 Gy. When the optimization is done with the requirement - 55 Gy to PTV1, then one gets 24, 30 and 27 fractions for the tumour, bladder and rectum, respectively in order to achieve the same effect, as in the 2-phase strategy. In the case α/β = 2 Gy the situation is much better! The SIB44 plan leads to equivalent TCP at 21 fractions, and SIB55 at 29 fractions. In both cases, a 23 (or 29 for SIB55) fraction regime would lead to almost similar TCP for the tumour and NTCP for the bladder, while the NTCP for rectum will diminish.

Now we should return to the question which most probably the reader has already asked himself - *Why the value of NTCP for the rectum is so high?* The answer to this question is simple - the parameter values of the Lymans model[8,35] were calculated based on the nominal dose and in this case the usage of BED is improper. The use of Eq. [3] is equally incorrect. The NTCP values for bladder and rectum calculated using the nominal dose are .02% and 4 % correspondingly - these num-

bers are quite acceptable as expected NTCPs. If calculated on the bases of Eq. [3] the NTCP values are almost negligible. On the other hand the fractionation effect could be accounted for only if BED (or Eq. [3]) is used with Lyman's model. For the CV model the use of BED is equivalent of directly applying the LQ model of cell damage, when assessing the P_{FSU},[25] a feature intrinsic to this model.

Figure 4a - *NTCP* for bladder as a function of number of fractions - illustrates eloquently the difference between the Lyman and the CV model. The parameters for both models were estimated from the »Emami data«, but for the small probabilities (high number of fractions) $NTCP_{CV}$ is slightly higher than $NTCP_{Lyman}$ and vice-versa for higher probabilities (smaller number of fractions).

The potential of inverse planning to design tight conformal distributions is fully revealed when all relevant organs and targets along with the corresponding dosimetric constraints (in terms of total doses) are considered in a single optimization process. However, the delivery of such plans may generally require modifications of existing fractionation regimes in order to assure similar outcomes.

For the case of SIB prostate IMRT, accounting for position uncertainties and possible clonogen spreads, we have estimated the radiobiological effect of various fractionation schemes using existing TCP/NTCP models. Although, some of the model parameters are not quite reliable, the models illuminate the major TCP/NTCP interrelations as a function of the number of fractions. It has been demonstrated that the optimized SIB superior dose distributions may not always lead to better tumour control and tissue sparing and therefore careful selection of the fractionation regime is required.

References

1. Mohan R, Wu Q, Manning M, Schmidt-Ullrich R. Radiobiological considerations in the design of fractionation strategies for intensity-modulated radiation therapy of head and neck cancers. *Int J Radiat Oncol Biol Phys* 2000; **46:** 619-30.

2. Wu Q, Manning M, Schmidt-Ullrich R, Mohan R. The potential for sparing of parotids and escalation of biologically effective dose with intensity-modulated radiation treatments of head and neck cancers: a treatment design study. I*nt J Radiat Oncol Biol Phys* 2000; **46:** 195-205.

3. Lebesque JV, Keus RB. The simultaneous boost technique: the concept of relative normalized total dose. *Radiother Oncol* 1991; **22:** 45-55.

4. Knee R, Fields RS, Peters LJ. Concomitant boost radiotherapy for advanced squamous cell carcinoma of the head and neck. *Radiother Oncol* 1985; **4:** 1-7.

5. Heukelom S, Lanson JH, Mijnheer BJ. Quality assurance of the simultaneous boost technique for prostatic cancer: dosimetric aspects. *Radiother Oncol* 1994; **30:** 74-82.

6. Essers M, Lanson JH, Mijnheer BJ. In vivo dosimetry during conformal therapy of prostatic cancer. *Radiother Oncol* 1993; **29:** 271-9.

7. Stavrev PV, Hristov DH, Seuntjens JP. On the consistent use of organ definitions and radiobiological models for the evaluation of complication probabilities of tubular organs. I*nt J Radiat Oncol Biol Phys* 2002; **52:** 1150-2.

8. Burman C, Kutcher GJ, Emami B, Goitein M. Fitting of normal tissue tolerance data to an analytic function. *Int J Radiat Oncol Biol Phys* 1991; **21:** 123-35.

9. Okunieff P, Morgan D, Niemierko A, Suit HD. Radiation dose-response of human tumors. *Int J Radiat Oncol Biol Phys* 1995; **32:** 1227-37.

10. Stavrev P, Stavreva N, Niemierko A, Goitein M. The Application of biological models to clinical data.. *Phys Medica* 2001; **17:** 71-82.

11. Dale E, Hellebust TP, Skjonsberg A, Hogberg T, Olsen DR. Modeling normal tissue complication probability from repetitive computed tomography scans during fractionated high-dose-rate brachytherapy and external beam radiotherapy of the uterine cervix. *Int J Radiat Oncol Biol Phys* 2000; **47:** 963-71.

12. Brenner DJ. Toward optimal external-beam frac-

tionation for prostate cancer. *Int J Radiat Oncol Biol Phys* 2000; **48:** 315-6.

13. Brenner DJ, Hall EJ. Fractionation and protraction for radiotherapy of prostate carcinoma. *Int J Radiat Oncol Biol Phys* 1999; **43:** 1095-101.

14. King CR, Mayo CS. Is the prostrate alpha/beta ratio of 1.5 from Brenner & Hall a modeling artifact. *Int J Radiat Oncol Biol Phys* 2000; **47:** 536-9.

15. Brenner DJ, Hall EJ. In response to Drs. King and Mayo: Low alpha/beta values for prostate appear to be independent of modeling details. *Int J Radiat Oncol Biol Phys* 2000; **47:** 538-9.

16. Logue JP, Cowan RA, Hendry JH. Hypofractionation for prostate cancer. *Int J Radiat Oncol Biol Phys* 2001; **49:** 1522-3.

17. Brenner DJ. Hypofractionation for prostate cancer - In response. Int J Radiat *Oncol Biol Phys* 2001; **49:** 1522-3.

18. Dale E, Olsen DR, Fossa SD. Normal tissue complication probabilities correlated with late effects in the rectum after prostate conformal radiotherapy. *Int J Radiat Oncol Biol Phys* 1999; **43:** 385-91.

19. Lyman JT. Complication probability as assessed from dose-volume histograms. *Radiat Res* 1985; **8(Suppl 1):** S13-9.

20. Niemierko A, Goitein M. Modeling of normal tissue response to radiation: the critical volume model. *Int J Radiat Oncol Biol Phys* 1993; **25:** 135-45.

21. Jackson A, Kutcher GJ, Yorke ED. Probability of radiation-induced complications for normal tissues with parallel architecture subject to non-uniform irradiation. *Med Phys* 1993; **20:** 613-25.

22. Yorke ED, Kutcher GJ, Jackson A, Ling CC. Probability of radiation-induced complications in normal tissues with parallel architecture under conditions of uniform whole or partial organ irradiation. *Radiother Oncol* 1993; **26:** 226-37.

23. Jackson A, Ten Haken RK, Robertson JM, Kessler ML, Kutcher GJ, LawrenceTS. Analysis of clinical complication data for radiation hepatitis using a parallel architecture model. *Int J Radiat Oncol Biol Phys* 1995; **31:** 883-91.

24. Niemierko A. Present and future of the clinical use of radiobiological models in radiation therapy treatment planning. *Phys Medica* 1997; **13:** 34-8.

25. Stavrev P, Stavreva N, Niemierko A, Goitein M. Generalization of a model of tissue response to radiation based on the idea of functional subunits and binomial statistics. *Phys Med Biol* 2001; **46:** 1501-18.

26. Niemierko A, Goitein M. Calculation of normal tissue complication probability and dose-volume histogram reduction schemes for tissues with a critical element architecture. *Radiother Oncol* 1991; **20:** 166-76.

27. Lyman JT, Wolbarst AB. Optimization of radiation therapy, III: a method of assessing complication probabilities from dose-volume histograms. *Int J Radiat Oncol Biol Phys* 1987; **13:** 103-9.

28. Kutcher GJ, Burman C, Brewster L, Goitein M, Mohan R. Histogram reduction method for calculating complication probabilities for three-dimensional treatment planning evaluations. *Int J Radiat Oncol Biol Phys* 1991; **21:** 137-46.

29. Kutcher GJ, Burman C. Calculation of complication probability factors for non-uniform normal tissue irradiation: the effective volume method. *Int J Radiat Oncol Biol Phys* 1989; **16:** 1623-30.

30. Niemierko A. A generalized concept of Equivalent Uniform Dose. [Abstract]. 41th AAPM Annual Meeting, Nashville, 24-29 July. *Med Phys* 1999; **26:** 1100.

31. Lebesque JV, Bruce AM, Kroes AP, Touw A, Shouman RT, van Herk M. Variation in volumes, dose-volume histograms, and estimated normal tissue complication probabilities of rectum and bladder during conformal radiotherapy of T3 prostate cancer. *Int J Radiat Oncol Biol Phys* 1995; **33:** 1109-19.

32. Ting JY, Wu X, Fiedler JA, Yang C, Watzich ML, Markoe A. Dose-volume histograms for bladder and rectum. *Int J Radiat Oncol Biol Phys* 1997; **38:** 1105-11.

33. Boersma LJ, van den Brink M, Bruce AM, Shouman T, Gras L, te Velde A, et al. Estimation of the incidence of late bladder and rectum complications after high-dose (70-78 GY) conformal radiotherapy for prostate cancer, using dose-volume histograms. *Int J Radiat Oncol Biol Phys* 1998; **41:** 83-92.

34. Jackson A, Skwarchuk MW, Zelefsky MJ, Cowen DM, Venkatraman ES, Levegrun S, et al. Late rectal bleeding after conformal radiotherapy of prostate cancer. II. Volume effects and dose-volume histograms. *Int J Radiat Oncol Biol Phys* 2001; **49:** 685-98.

35. Emami B, Lyman J, Brown A, Coia L, Goitein M, Munzenrider JE, et al. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991; **21:** 109-22.

36. Hartford AC, Niemierko A, Adams JA, Urie MM, Shipley WU. Conformal irradiation of the

prostate: estimating long-term rectal bleeding risk using dose-volume histograms. *Int J Radiat Oncol Biol Phys* 1996; **36:** 721-30.

37. Stavrev PV, Stavreva NA, Round WH. A new method for optimum dose distribution determination taking tumour mobility into account. *Phys Med Biol* 1996; **41:** 1679-89.

38. Lind BK, Kallman P, Sundelin B, Brahme A. Optimal radiation beam profiles considering uncertainties in beam patient alignment. *Acta Oncol* 1993; **32:** 331-42.

39. Lof J, Lind BK, Brahme A. An adaptive control algorithm for optimization of intensity modulated radiotherapy considering uncertainties in beam profiles, patient set-up and internal organ motion. *Phys Med Biol* 1998; **43:** 1605-28.

40. Mageras GS, Kutcher GJ, Leibel SA, Zelefsky MJ, Melian E, Mohan R, et al. A method of incorporating organ motion uncertainties into three-dimensional conformal treatment plans. *Int J Radiat Oncol Biol Phys* 1996; **35:** 333-42.

41. Rudat V, Schraube P, Oetzel D, Zierhut D, Flentje M, Wannenmacher M. Combined error of patient positioning variability and prostate motion uncertainty in 3D conformal radiotherapy of localized prostate cancer. *Int J Radiat Oncol Biol Phys* 1996; **35:** 1027-34.

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Radioactive sources in brachytherapy

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Background. *In modern brachytherapy, a great step forward was made in the 1960s in France with the introduction of new radioactive isotopes and new techniques. These innovations spread rapidly across Europe, though no single dosimetry standard had been set by then. In the new millennium, the advances in brachytherapy are further stimulated by the introduction of 3-D imaging techniques and the latest afterloading irradiation equipment that use point sources. The international organization ICRU (International Commission on Radiation Units) worked out brachytherapy techniques and standardized them in 1985 and in 1997. Due to rapid development of new techniques, the revision is required in order to set new international standards in dosimetry and brachytherapy techniques that will fit to the changed conditions in radiotherapy.*

Conclusions. *This is an outline of radioactive sources that are currently used in brachytherapy, such as Cs-137, Ir-192, Sr-90, Ra-226, Rn-222, Co-60, I-131, I-125, Pd-103, Tu-106 and Cf-252.*

Key words: brachytherapy; radioisotopes; radiation protection

Introduction

In brachytherapy, the radiation dose is applied to tumor by sealed sources. The sources are implanted to the tumor tissue itself or in its close vicinity. The institution in which the therapy is being performed should follow the rules of safe and accurate implantation of the source; this implies that the therapy should be carried out in compliance with international regulations on ionizing radiation, thereby also assuring the accuracy of prescribed doses. In all irradiation techniques,[1,2] the principles of ALARA *(As Low As Reasonably Achievable)* should be observed, indicating that the minimum exposure of the personnel, which may be achieved by the institution at moderate costs, is allowed. In brachytherapy, the patient can be irradiated nonintermittently for several days if the therapy is applied at low dose rate (LDR). Hence, special concern should be focused on the radiotherapists and nursing staff who care for the irradiated patients. The measurements showed that the doses to which the personnel working in a brachytherapy unit is exposed are higher than those that are considered as average rates at other oncology departments.

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Techniques and materials

Radioactive sources Cs-137 and Ra-226, used in brachytherapy, are coated with stainless steel, whereas the iridium wire Ir-192 is coated with platinum alloy that covers the radioactive core. If a crack happens in this shielding coat, there is a great risk of extensive contamination; radioactive material may enter the respiratory and alimentary canals of anyone involved in the treatment process. Therefore, special caution and attention are required from the personnel in charge of regular control of mechanical and radiation properties of the sources. Safety tests need to be performed especially on the gamma-radiation source as beta-rays are largely absorbed in by the coat of the source while alpha-rays are not applied in brachytherapy. Eye irradiation applicators have beta-source Sr-90 and are applied in the irradiation of superficial tumors of the eye. In this case, a few millimeters of a substance with low atomic number would serve as a protective shield against irradiation. Alpha-particles, emitted by Ra-226 and Rn-222, are absorbed by the coat of such source. Radioactive gas Rn-222 is being accumulated in the source as waste product; therefore, regular tests of Ra-226 sources should be urgently performed. If the coat of the source is damaged, the leakage of radioactive gas can occur. The Ra-226 source is practically not in use any more and is replaced by Cs-137 and Ir-192. These sources are implanted into the patients automatically by remote control, thereby allowing the application of high dose rate techniques at higher activity. The high dose rate (HDR) techniques are those that are performed at dose rates over 12 Gy/h, whereas the low dose rate (LDR) techniques are performed at dose rates below 2 Gy/h. As the high specific activity of the source is required, only Co-60 and Ir-192 can be applied as sources in HDR. The required activity is as high as 370 GBq, which speaks in favor of remote afterloading of the sources that should be performed in a specifically protected room. In the recent decades, sequential irradiation with Ir-192 as point source, with the activity of 37 GBq, has been widely investigated. This technique is known as pulsed dose rate technique (PDR) and is actually a type of hyperfractionation in brachytherapy. Irradiation restarts every hour for 10 minutes throughout 24 hours. The afterloading equipment allows the interruption of irradiation when necessary, e.g. if patients need nursing care or if they are having a visitor. Statistic analyses have confirmed that the nursing staff at the department where the afterloading irradiation equipment is installed is receiving markedly lower doses. The operating instructions of the irradiation equipment should be accessible to the users and the equipment should be regularly tested for safety reasons.

Irradiation with radioactive sources

The brachytherapy irradiation dose is defined as the ratio between activity of the source, irradiation time, and distance from the source. The total air KERMA $_{\text{dose}}$ is computed from the equation

$$K_a = \frac{\dot{K}_r * t}{r^2}$$

in which t denotes the irradiation time and r the distance between the source and irradiation point. $\dot{K}r$ is the reference air KERMA rate, measured in μGy/h at the distance of 1 m. Activity of the source is measured in MBq. The effect of the source coating on the dosage around the source is taken into account by the correction factor. Photons get absorbed also into the tissue of the irradiated patient, which is expressed by the transmission factor, depending on the duration of transit passage of the photons through the tissue, on the electron density of the tissue, and on the energy of gamma rays. Figure 1 shows a graphi-

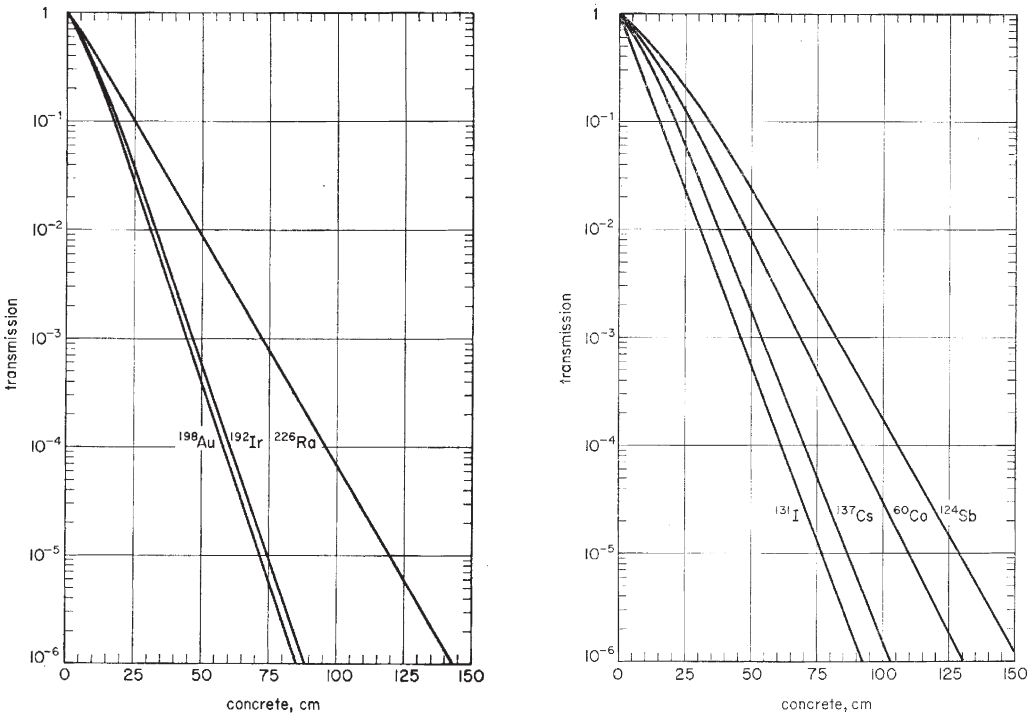

Figure 1. Transmission factors for concrete:
- y axis: transmission factor
- x axis: concrete (in cm)

cal presentation of the transmission factor of concrete.[4] Transmission factor is the percentage of photons (axis of ordinate *y*) that reach at a particular depth of a substance (axis of abscissa *x*). An important data required for radiation protection is half value layer (HVL). This is the depth at which gamma ray looses one half of its initial intensity. A similar definition was adopted for tenth value layer (TVL). This is the depth of the absorption substance at which γ-ray looses one tenth of its initial intensity. Table 1 is a collection of all key data 1 that are relevant for the radiation protection against the isotopes most frequently used in brachytherapy. The worker who is in charge of the preparation of the

Table 1. Data relevant for radiation protection in brachytherapy

Nuclide	Mean energy (in MeV) of emitted photons	Half life	First HVL in Pb (in mm)	TVL in Pb (in mm)	TVL in concrete (in cm)
^{198}Au	0.42	2.7 day	2.5	9	18
^{60}Co	1.25	5.3 years	13	45	27
^{137}Cs	0.66	30 years	6.5	21	22
^{125}I	0.029	60 days	0.025	-	-
^{103}Pd	0.021	17 days	-	-	-
^{192}Ir	0.35	74 days	2.5	11	19
^{226}Ra	0.78	1620 years	12	45	25

sources to be implanted into the patient must strictly follow the following basic rules that are imperative:

- to minimize as much as possible the time spent in the area of radiation exposure;
- to maximize the distances from the sources;
- to minimize the activity of the sources

Even though the staff is capable of handling the sources safely, the risk of contamination at the department storing up and applying radioactive material is not excluded. If it does happen, it spreads over the objects and the staff. The most exposed parts of the body are the skin, respiratory and alimentary tracks. The contamination of other organs depends on the chemical properties of the source, *e.g.* Sr-90 affects the bones. The decontamination should be carried out strictly according to the prescribed regulations. After each application of radioactive sources, the irradiation levels should be necessarily tested by radiation detectors and remove eventual residual radioactive substance in compliance with the standards. Throughout the year, regular testing of the sources should be carried out following manufacturer's instructions. Handling with radioactive sources should only be entrusted to specifically trained personnel, who have obtained the required knowledge and skills of radiation protection and who regularly attend refreshment courses on radiation protection as prescribed by law.

Handling with radioactive sources

Radioactive sources are kept in safety box, made from radio-protective material. At the distance from the box of 10 cm, the exposure should not exceed 1 μSv/h. The safety box and the shelter where it is kept under lock should be tagged with noticeable radiation warning label. A record book, keeping records of implanted and returned radioactive sources, should necessarily be kept beside the safety box. The shelter in which the safety box is stored should be equipped with ventilation system that provides good airing to the room as gas products may be generated due to radioactive decay of the sources. Regular checking and cleaning of the sources should be performed in an area shielded by a lead wall and using a system of mirrors in order to avoid direct strike of photons on the eye lenses. The sources should be transported by special carriage under radiation protection from the shelter to the patient. The eventual leakage on the surface of the transport trolley should not exceed the value of 2 mSv/h. If the implantation of the source is not carried out manually, but mechanically with the afterloader, the radiation protection is much safer. The source can be automatically removed out of the patient whenever nursing personnel is entering the room. The communication between the patient and nursing staff should be provided through audiovisual media in order to allow the patient to ask for help or for service, whenever necessary. No visitors are allowed during irradiation and the cleaning-up of the patient's room is reduced to a minimum. After the therapy is completed, the whole room, including bedclothes, should be examined for eventual contamination with radioactive substance. The risk of contamination is particularly high if irradiation is performed with unsealed radiation sources, such as I-131 that is administered in pills. The sources permanently implanted into the prostate, such as the seed sources I-125 and Pd-103 [5], do not require special protection because these are the sources with low energy, viz. 29 keV and 21 keV, for I-125 and Pd-103, respectively. Upon discharge from the hospital, the patients with implanted sources receive the necessary instructions on radiation protection and are also warned that the first few days after the implantation there is a risk of losing the implanted source with the secretion of the urine. If this occurs with the implanted I-125 with the half-life period of 60 days, special protection instruction should be

followed. If the patient dies the first year after the implantation, cremation of the body is not allowed. The isotope Sr-90 applied in the irradiation of eye malignancies is currently by and large replaced by beta-radiant, *e.g.* Ru-106. The highest beta-energy that strontium (Sr-90) emits is 2.3 MeV, which means that it can penetrate 12 mm deep into the water. The beta-energy generated by ruthenium (Ru-106) amounts to 3.54 MeV; this implies that application of Ru-106 requires more severe safety measures. The safety containers are therefore made of substances with low atomic number in order to avoid braking radiation. Alpha-radiants are not used in brachytherapy. If the source emits them, as it is the case with Ra-226 and Rn-222, their particles are completely absorbed in the wall of the safety coating. Radium is a highly radioactive element with an immensely long half-life period of 1620 years. The contamination with this isotope poses a serious problem because decontamination is very costly and demanding as all the surfaces that were contaminated need to be completely and permanently removed. Californium (Cf-252) is one of the few neutron radiation sources whose application in brachytherapy is still restricted as long we are not able to yield a higher radiobiological effect in poorly oxygenated tumor tissues. In radiation protection, particular concern should be paid to neutrons as their biological efficiency, at the same dose as in photons, is ten times that of photons. Therefore, the containers where Cf-252 is kept should be extremely carefully protected. Primary shielding should be made of a substance with high atomic number, e.g. lead (Pb), which is additionally layered with a substance with low atomic number, e.g. polystyrene.

References

1. Dutreix A, Marinello G, Wambersie A. Dosimetrie en curietherapie. Paris: Masson ed.; 1982.
2. ICRU, International Commission on Radiation Units and Measurements. Dose and volume specification for reporting intracavitary therapy in gynecology. *Report 38 of ICRU.* Bethesda: ICRU Publications; 1985.
3. Nath R, Anderson LL, Luxton G, Weaver KE, Williamson JF, Meigooni AS. »Dosimetry of interstitial brachytherapy sources: Report of the AAPM Radiation Therapy Commitee Task Group No. 43«. *Med Phys* 1995; **22:** 209-34.
4. ICRP Publication 33, International Commission on Radiological Protection. Protection against ionizing radiation from external sources used in medicine. Pergamon Press, 1982.
5. Beyer D, Nath R, Butler W. American Brachytherapy Society recommendations for clinical implementation of NIST-1999 standards for Palladium-103 brachytherapy. *Int J Radiat Oncol Biol Phys* 2000; **47:** 273-5.

Radiol Oncol 2003; (2): 67-72.

Natančna radiološka preiskava ozkega črevesa - izkušnje na Kliničnem radiološkem inštitutu v Ljubljani

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Izhodišča. Ozko črevo je del prebavnega trakta, ki ga je najteže pregledati. Največkrat uporabljena radiološka preiskava je jejunoileografija, ki pa je pogosto narejena brez fluoroskopije in pritiska na vse segmente ozkega črevesa. Rezultat preiskave je odvisen le od sprememb, prikazanih na rentgenogramih. Namen naše študije je prikazati natančnejšo radiološko preiskavo ozkega črevesa.

Bolniki in metode. Od aprila do septembra 2002 smo na našem oddelku opravili 35 radioloških preiskav ozkega črevesa pri bolnikih, ki so jih napotili iz specialistične gastroenterološke ambulante. Rezultate naše preiskave smo primerjali s kliničnim potekom, endoskopskim izvidom in izvidom operativnega posega.

Rezultati. V 33 primerih so bili rezultati naše preiskave potrjeni z drugimi preiskavami. V 2. primerih je bil rezultat naše preiskave lažno negativen. Senzitivnost preiskave je bila pri naši skupini pacientov 89,5% in specifičnost 100 %.

Zaključki. Natančno opravljena radiološka preiskava ozkega črevesa s fluoroskopijo in pritiskom na vse segmente ozkega črevesa je zanesljiva diagnostična metoda.

Radiol Oncol 2003; 37(2): 73-8.

Določanje zgodnjega postoperativnega nivoja karcinoembrionalnega antigena v serumu pri bolnikih, ki so bili operirani zaradi raka širokega črevesa in danke - nova metoda sledenja bolnikov

Veingerl B

Izhodišča. Pri bolnikih z rakom debelega črevesa in danke le kirurško zdravljenje omogoča njihovo ugodnejšo prognozo. Potrebno je narediti radikalno resekcijo zajetega debelega črevesa ali danke ter odstraniti pripadajoče limfne bezgavke in morebitne metastatske spremembe. Kljub radikalnemu kirurškemu posegu, ki bi naj bil domnevno ozdravilen, pa takšno zdravljenje ne zagotavlja popolne ozdravitve. Pogosta je ponovitev bolezni in kakor kažejo različne analize, je 5-letno preživetje manjše kot 50%. Tako je pri veliko bolnikih potrebno dodatno zdravljenje. Pri tem nam pomagajo različni napovedni dejavniki, med njimi tudi nivo karcinoembrionalnega antigena (CEA) v serumu, ki ga določimo takoj po operaciji.

Zaključki. Vse bolnike, pri katerih je bila narejena radikalna R0 resekcija, lahko glede na vrsto resekcije in na njihov pooperativni nivo serumskega CEA (ob upoštevanju razpolovne življenjske dobe CEA) razdelimo v 3 skupine: $_{CEA}$R0, $_{CEA}$R1 in $_{CEA}$R2. Ob upoštevanju stadija bolezni smo med temi skupinami ugotovili statistično značilno razliko v preživetju in pogostnosti ponovitve bolezni. Razlika je bila najbolj očitna pri bolnikih, pri katerih je bil kirurški poseg domnevno ozdravilen.

Radiol Oncol 2003; 37(2): 132-6.

Radiol Oncol 2003; 37(2):79-88.

Prognostična vrednost lokalne ponovitve bolezni pri raku dojke po ohranitveni kirurgiji in mastektomiji

Soumarová R, Horová H, Šeneklová Z, Horová I, Budíková M

Izhodišča. V retrospektivni študiji smo analizirali lokalne ponovitve bolezni pri bolnicah z rakom dojke. Bolnice so bile zdravljenje z ohranitveno kirurgijo (CS), ki ji je sledila adjuvantna radioterapija (RT) ali pa so bile zdravljenje z mastektomijo (ME) in nekatere od njih tudi z RT. Ugotavljali smo vpliv lokalne ponovitve bolezni na preživetje.

Bolniki in metode. Med leti 1980-1995 smo v naši ustanovi zdravili 306 bolnic z ohranitveno kirurgijo in 1193 bolnic z mastektomijo. Izključili smo bolnice, pri katerih nismo uspeli slediti poteka bolezni. Tako smo analizirali 236 bolnic po CS (skupina A) in 1121 bolnic po ME (skupina B). Vsem bolnicam s CS smo obsevali operirano dojko, nekaterim pa tudi regionalne limfne bezgavke. Pri bolnicah z ME pa smo le pri 982 (87,6%) obsevali dojko in nekaterim od njih tudi regionalne bezgavke. Srednja starost v času diagnoze je bila 48,3 let v skupini A in 52,1 let v skupini B. V skupini A je imelo 149 bolnic (63,1%) tumor T1, 86 (36,4%) T2 in 1 (0,5%) T3. Pri 24,2% bolnic se je rak razširil v pazdušne bezgavke. V skupini B je imelo 316 (30,4%) bolnic tumor T1, 607 (58,3%) T2, 76 (7,3%) T3, 33 (3,2%) T4 in 9 (0,9%) TX. Pri 46,2% bolnic se je rak razširil v pazdušne bezgavke. Invazivni duktalni karcinom je bil histološko potrjen pri 67,4% bolnic v skupini A in 84% v skupini B. Sistemsko smo zdravili 133 (56,4%) bolnic iz skupini A in 857 (76,4%) bolnic iz skupine B.

Rezultati. Srednji čas sledenja bolnic je bil 100,5 mesecev pri skupini A in 121 mesecev pri skupini B. V skupini A smo ugotovili 22 (9,3%) lokalnih ponovitev bolezni, 5-letna lokalna kontrola bolezni je bila 96,2% in srednji čas do ponovitve bolezni je bil 50 mesecev. V skupini B smo ugotovili 65 (5,8%) lokalnih ponovitev bolezni, 5-letna lokalna kontrola bolezni je bila 96,6%, pri bolnicah s T1 in T2 pa je bila 97,2%. Bolnice, ki smo jih tudi obsevali, so imele srednji čas do ponovitve bolezni 48,5 mesecev, bolnice brez obsevanja pa 51 mesecev. 13 bolnic (8,7%) z ME, ki niso bile obsevane je imelo lokalno ponovitev bolezni. Vpliv lokalne ponovitve bolezni na preživetje je bil v skupini B statistično značilen ($p = 0,002$), v skupini A pa ne ($p = 0,062$). Celokupno preživetje bolnic z lokalno ponovitvijo bolezni je bilo tako nižje. Ugotovili smo linearno odvisnost med časom do lokalne ponovitve bolezni in celokupnim preživetjem.

Zaključki. Vpliv lokalne ponovitve bolezni na celokupno preživetje bolnic je bilo statistično značilno. Verjetnost lokalne ponovitve bolezni in čas do ponovitve je bil pri bolnicah s CS in ME enak. Celokupno preživetje se veča ob daljšem času brez lokalne ponovitve bolezni.

Radiol Oncol 2002; 37(2): 89-99.

Katepsin L pri meningiomu

Trinkaus M, Vranič A, Dolenc V V, Lah T T

Izhodišča. Čeprav so meningiomi benigni tumorji, jih okoli 10% sodi v skupino atipičnih meningiomov, klasificiranih kot WHO stopnja II, ki imajo večjo verjetnost recidiva in/ali bolj agresivno obnašanje, vključno z večjo verjetnostjo metastaziranja v možganovino. Lizosomalna cisteinska endopeptidaza katepsin L igra določeno vlogo v invaziji tumorskih celic in v malignem napredovanju raka in je prognostičen faktor za izid.

Rezultati. V tem delu smo primerjali izražanje katepsina L v 30 meningiomih z njihovo klinično invazivnostjo. Za določevanje katepsina L smo uporabljali imunohistokemijsko reakcijo, kvantitativno RT-PCR analizo in hibridizacijo Northern. Ugotovili smo, da obstajajo značilne razlike v vsebnosti proteina katepsina L (p=0.019) med 9 atipičnimi in 21 benignimi meningiomi. Vsebnosti katepsina L v prehodnem tipu so bile nižje kot v ostalih vrstah benignih meningiomov. Merili smo tudi vsebnosti RNA cepitvenih različic katepsina L A vrst: LA, LAI in LAII, ne pa tudi LAIII in ne LB različice, ki je v meningiomih nekajkrat nižja kot so L A,AI, AII različice. V nasprotju s proteinsko koncentracijo, se skupne vsebnosti merjenih RNA različic katepsina L A, AI, AII med benignimi in atipičnimi meningiomi niso razlikovale. Ko smo primerjali skupne vsebnosti RNA katepsina L A različic med vzorci, vzetimi iz sredice in tistimi z roba tumorja, se te tudi niso statistično značilno razlikovale.

Zaključki. Ti rezultati nakazujejo, da utegne povišana proteinska vsebnost katepsina L prispevati k razvoju agresivnosti in morebitne invazivnosti atipičnih meningiomov in da se njegovo izražanje verjetno poviša na nivoju prevoda RNA v protein.

Radiol Oncol 2003; 37(2): 101-7.

Učinek elektroporacije s cisplatinom na radiosenzibilizacijo dveh celičnih linij z različno občutljivostjo za kemoterapevtike in obsevanje

Kranjc S, Čemažar M, Grošel A, Pipan Ž in Serša G

Izhodišča. Z elektroporacijo celic in tumorjev lahko povečamo njihovo občutljivost za cisplatin. Namen raziskave je bil razširiti našo predhodno raziskavo na dva tumorska modela karcinoma z različno občutljivostjo za kemoterapijo in obsevanje, ter ugotoviti, ali je takšno zdravljenje učinkovito tudi pri celicah, ki so manj občutljive za kemoterapijo in obsevanje.
Materiali in metode. V *in vitro* raziskavi smo uporabili celični liniji karcinoma SCK in EAT-E. Citotoksično delovanje kombiniranega zdravljenja s cisplatinom, elektroporacijo in obsevanjem smo določili s testom klonogenosti.
Rezultati. Z elektroporacijo se je radiosenzibilizirajoči učinek cisplatina na testiranih celičnih linijah zelo povečal. Manj kemo- in radio-občutljive celice EAT-E so po kombiniranem zdravljenju postale enako občutljive kot celice SCK, ki so bolj občutljive za kemoterapijo in obsevanje.
Zaključki. Povečano občutljivost celic za cisplatin po elektroporaciji lahko koristno uporabimo tudi za zdravljenje manj občutljivih celic za kemoterapijo in obsevanje.

Radiol Oncol 2003; 37(2): 109-13.

Telomerasa pri ugotavljanju pljučnega raka

Kovkarova E, Stefanovski T, Dimov A, Naumovski J

Izhodišča. Telomeraza je ribonukleoprotein, ki je prekomerno izražen v tumorskem tkivu, ne pa v zdravih somatskih celicah. Tako je bil namen te študije določiti telomerazno aktivnost pri bolnikih z rakom pljuč, saj lahko telomeraza predstavlja nov označevalec pri ugotavljanju pljučnega raka. Želeli smo tudi ugotoviti, ali je telomerazna aktivnost različna pri različnih načinih odvzema materiala za raziskavo.
Material in metode. Material smo odvzeli z bronhoskopijo pri 60 bolnikih s pljučnim rakom in pri 20, ki so bili v kontrolni skupini, pri 10 bolnikih, ki so imeli periferni pljučni tumor pa smo naredili transtorakalno aspiracijsko biobsijo s tanko iglo. Uporabljali smo telomerni amplifikacijski test (TRAP).
Rezultati. Telomerazno aktivnost smo ugotovili pri 75% bolnikov s pljučnim rakom, kjer smo naredili bronhialno biosijo in pri 100% bolnikih, kjer smo opravili transtorakalno punkcijo.
Zaključki. Merjenje telomerazne aktivnosti lahko prispeva k natančnosti diagnosticiranja tumorskih sprememb, ki so sumljive za pljučni rak.

Radiol Oncol 2003; 37(2): 115-26.

Radiobiološka primerjava dveh optimiziranih načinov obsevanja (IMRT) prostate: obsevanja v dveh delih in sočasnega visokodoznega obsevanja na manjše polje

Stavrev P, Hristov D

Izhodišča. Namen raziskave je bil proučiti radiobiološki učinek števila frakcij obsevanja, natačnost namestitve bolnika med obsevanjem in klonogenost raka prostate glede na dva različna optimizirana načina obsevanja: obsevanja v dveh delih in sočasnega visokodoznega obsevanja na manjše polje (simultaneous integrated boost -SIB).
Material in metode. Verjetnost kontrole tumorja (TCP) in verjetnost poškodbe normalnega tkiva (NTCP) smo izračunali za obsevanje v dveh delih z natačno določenim frakcioniranjem obsevanja in ju primerjali s TCP in NTCP pri obsevanju s SIB načinom, pri čemer smo upoštevali odvisnost od števila frakcij. Za oba načina obsevanja smo uporabili metodo inverznega planiranja in obsevali prostate s 7 polji (IMRT).
Rezultati. Ob upoštevanju natančnosti namestitve bolnika med obsevanjem in klonogenosti raka prostate smo izračunali, da se je TCP znižal za 10%. Če smo pri obsevanju s SIB načinom uporabili enako število frakcij kot pri obsevanju v dveh delih, se je TCP zmanjšal za 5-7%.
Zaključki. Pri obsevanju s SIB načinom je razvidna prednost inverznega planiranja obsevanja za dosego konformne dozne porazdelitve. Metoda pa zahteva spremembo doze na frakcijo in tako tudi ustrezno prilagoditev frakcioniranja obsevanja. S fizikalnim optimiziranjem doze torej ne dosežemo vedno boljše kontrole tumorja pa tudi ne boljše ohranjanje zdravega tkiva.

Radiol Oncol 2003; 37(2): 127-31.

Uporaba radioaktivnih izvirov v brahiterapiji

Burger J

Izhodišča. V moderni brahiterapiji je bil napravljen velik korak naprej v šestdesetih letih v Franciji z vstopom umetnih radioizotopov in novih radioterapevtskih tehnik. Nove metode so se hitro razširile po Evropi, četudi uporaba doze še ni bila standardizirana. V novem tisočletju brahiterapija doživlja nov zagon z vstopom trodimenzionalnega (3D) načrtovanja obsevanja in novih obsevalnih aparatur, ki uporabljajo točkaste obsevalne vire. Mednarodna organizacija ICRU je zaokrožila in standardizirala brahiterapevtske postopke leta 1985 in 1997. Zaradi hitrega razvoja novih tehnik pa bo kalu spet potrebno priporočilo, ki bo mednarodno predpisalo način predpisovanja doze in brahiterapijskih postopkov v novih razmerah.
Zaključki. V članku je podan pregled radioaktivnih izvirov, ki se danes uporabljajo pri obsevanju v brahiterapiji. Opisana je uporaba Cs-137, Ir-192, Ra-226, Sr-90, Ra-226 in Rn-222, Co-60, I-131, I-125, Pd-103, Ru-106 in Cf-252.

Radiol Oncol 2003; 37(2): 132-6.

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.*

Radiology

July 17-20, 2003

The seminar »11th Annual Advanced Topics in Multidetector-Row CT Scanning: The 2003 Edition« will take place in Resort at Squaw Creek, Olympic Valley, Lake Tahoe, CA, USA.

Contact Conference Coordinator, Office of Continuing medical Education, John Hopkins University School of Medicine, Turner 20/720 Rutland Avenue, Baltimore, Maryland 21205-2195, USA; or call +1 410 955 2959; or fax +1 410 955 0807; or e-mail cmenet@jhmi.edu; or see http://www.hopkinsmedicine.org/cme

Oncology

August 3-8, 2003

The »12th World Conference on Tobacco or Health« will be offered in Helsinki, Finland.

Contact Ms. Aira Raudesoja, CongCreator CC Ltd., P.O. Box 762, FIN-00101 Helsinki, Finland; or call +358 9 454 2190; or fax +358 9 4542 1930; or e-mail secretariat@congcreator.com

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

Prostate cancer

August 31 - September 2, 2003

The ESTRO teaching course »Brachytherapy for prostate Cancer« will take place in Kiel, Germany.

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

Radiotherapy

August 31 - September 4, 2003

The ESTRO teaching course »Physics for Clinical Radiotherapy« will be held in Leuven, Belgium.

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

Cancer immunology and immunotherapy

September 8-13, 2003

The European cancer immunology and immunotherapy summer school will be offered in Ionian Village, West Coast of the Peloponese, Greece.

Contact Dr. M. Papamichail, Center for Immunology, St. Savas Cancer Hospital, 171, Alexandras Ave, Athens 115 22, Greece; or call +30-210-6409 624/5; or fax +30-210-6409 516; or e-mail papmail@netor.gr

Radiotherapy

September 13-18, 2003

The 7th Biennial ESTRO Meeting on Physics for Clinical Radiotherapy / ESTRO Meeting on Radiation Technology for Clinical Radiotherapy will take place in Geneva, Switzerland.

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

Oncology

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

Radiobiology

October 12-16, 2003

The ESTRO teaching course »Basic Clinical Radiobiology« will be offered in Santorini, Greece.

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

Pleural mesothelioma

November 7-8, 2003

The international conference will be offered in Como, Italy.

Contact ASK, International Conference Como 2003, Via Tabacchi, 20, 21056 Induno Olona (VA), Italy; or call +39 0332 840650; or fax +39 0332 204028; or e-mail ask@skylink.it

Lung cancer

November 8, 2003

The international conference »Lung Cancer Screening and Early Diagnosis« will be offered in Como, Italy.

Contact ASK, International Conference Como 2003, Via Tabacchi, 20, 21056 Induno Olona (VA), Italy; or call +39 0332 840650; or fax +39 0332 204028; or e-mail ask@skylink.it

Radiation oncology

November 9-14, 2003

The ESTRO teaching course »Evidence-Based Radiation Oncology: Methodological Basis and Clinical Application« will take place in Lisbon, Portugal.

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Radiation oncology

March, 2004

The ISRO international teaching course on »Radiation Oncology in the 21st Century« will take place in Cape Town, South Africa.

See http://www.isro.be

Surgical oncology

March 31 - April 3, 2004

The 12th ESSO Congress will be held in Budapest, Hungary.

See http://www.fecs.be/conferences/esso2004

Oncology

April 15-17, 2004

The European Oncology Nursing Society EONS Spring Convention will be held in Edinburg, UK.

See http://www.fecs.be/conferences/eons4

Brachytherapy

May 13-15, 2004

The Annual Brachytherapy Meeting GEC-ESTRO will take place in Barcelona, Spain.

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

Radiology

June 6-8, 2004

The UK Radiological Congress will be held inManchester, U.K.

Contact Ms. Rebecca Gladdish, UKRC 2003 Secretariat, PO Box 2895, London W1A 5RS, U.K., or call +44(0) 20 7307 1410/20, or fax +44(0) 20 7307 1414; or e-mail exhibition@ukrc.org.uk; or see www.ukrc.org.uk

Oncology

July 3-6, 2004

The 18th EACR (European Association for Cancer Research) Congress will be held in Innsbruck, Austria.

See http://www.fecs.be/conferences/eacr18

Paediatric oncology

September, 2004

The International Society of Paediatric Oncology - SIOP Annual Meeting will be held in Oslo, Norway.

See http://www.siop.nl

Lung cancer

September 23-25, 2004

The »9th Central European Lung Cancer Conference« will be offered in Gdansk, Poland.

Contact Conference Secretariat, »9th Central European Lung Cancer Conference«, Via Medica, ul. Swietokrzyska 73, 80 180, Gdansk, Poland; or call/fax +48 58 349 2270; or e-mail celcc@amg.gda.pl; or see www.lungcancer.pl

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

Therapeutic radiology and oncology

October 24-28, 2004

The 23rd ESTRO Meeting will be held in Amsterdam, the Netherlands.

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Medical oncology

October 29 - November 2, 2004

The 28th ESMO Congress will be held in Vienna, Austria.

See http://www.esmo.org

Radiation oncology

November 25-28, 2004

The ISRO international teaching course on »Practical Radiation and Molecular Biology with Mayor Emphasis on Clinical Application« will take place in Chiangmai Thailand.

See http://www.isro.be

Radiation oncology

March, 2005

The ISRO international teaching course on »Palliative Care in Cancer Treatment« will take place in Dar es Salaam, Tanzania.

See http://www.isro.be

Radiation oncology

September - October, 2005

The ISRO international teaching course on »Rational Developments from developing to developed Contries« will take place in Lombok, Indonesia.

See http://www.isro.be

Oncology

October 30 - November 3, 2005

The ESTRO 24 / ECCO 13 Conference will take place in Paris, France.

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

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

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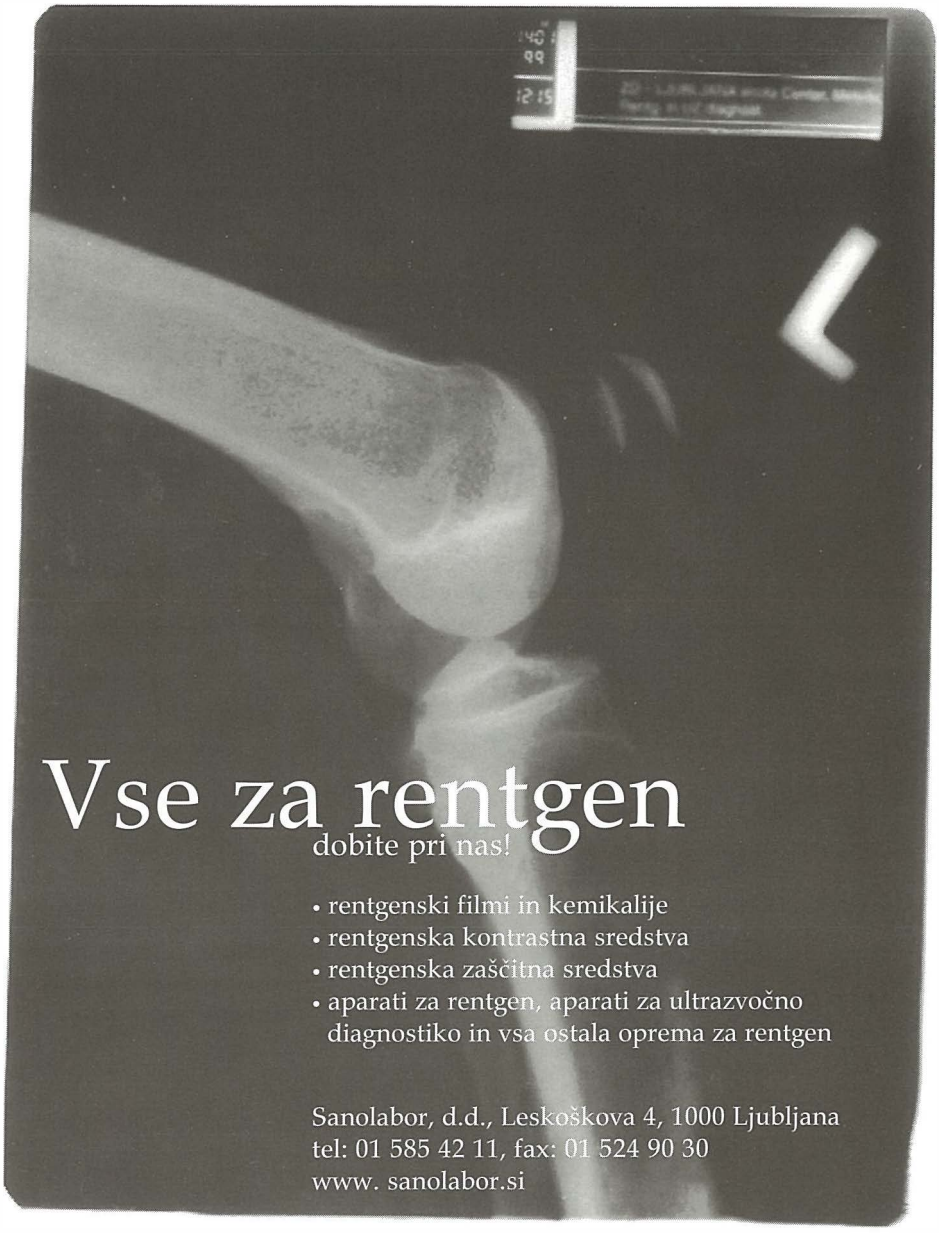
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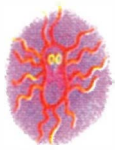
Activity of "Dr. J. Cholewa" Foundation for Cancer Research and Education – A Report for the Second Quarter of 2003

The members of the »Dr. J. Cholewa Foundation« for Cancer Research and Education are convinced that some of the new approaches in contacts and communications with future research grants applicants, experts in the various fields of oncology, general public and prospective donors may in the future contribute to the wider diffusion of information and knowledge about cancer in Slovenian society. Some of these new ways are being considered by the Foundation members and its Boards, and the Foundation's activity may thus gain new and important impetus. However, the overall goals of the Foundation's activity remain the same and it is essential that the members and all the people, whose activity is any way associated with the Foundation, do not disgress from achieving these goals.

These new approaches are also believed to hopefully enable the Foundation's members and its Boards to achieve more important results with regard to the financial means needed in the future. In this context, the ongoing changes in the circumstances and emergence of new and unexpected problems, associated with maintaining regular contacts with the donors, have been taken into consideration. These topics have now been regularly discussed by the Foundation's members and Boards. It is salutary to know that the results of cancer research, supported by the Foundation, may have found its way to the practical application in hospital wards across Slovenia in a significantly easier manner in recent years than before. In addition, the support of the Foundation to present these research results on international meetings, conferences, symposia and any other events of scientific importance will continue and receive further support and encouragement. Most importantly, the Foundation will try to continue to support the publication of the results from research it sponsored and supported in respectable international scientific oncology journals and in other, more novel forms of dissemination of scientific information.

It is commonly agreed among the members of the Foundation and its Boards, that cancer research should be further encouraged in all parts of Slovenia where the interest for such research exists. With this in consideration, the Foundation continues to support the regular publication of »Radiology and Oncology« international scientific journal, which is edited, published and printed in Ljubljana, Slovenia. The Foundation continues in its activity to promote cancer biology research, research in cancer epidemiology and clinical cancer research. It is also active in promoting cancer education in general and especially to increase its impact in general population and among scientists with a particular interest in cancer research.

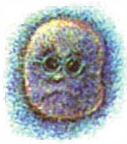
Borut Štabuc, MD, PhD
Andrej Plesničar, MD
Tomaž Benulič, MD



Escherichia coli

Proteus

Pseudomonas



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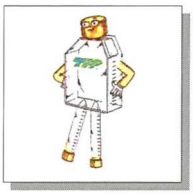

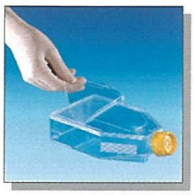

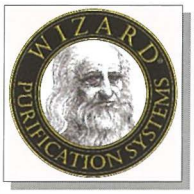

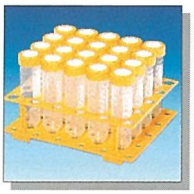

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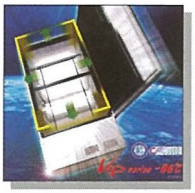

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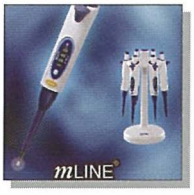

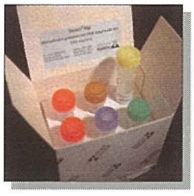

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EHRET (Nemčija):
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fotometri, avtomatski pralni sistem za mikrotitrine plošče

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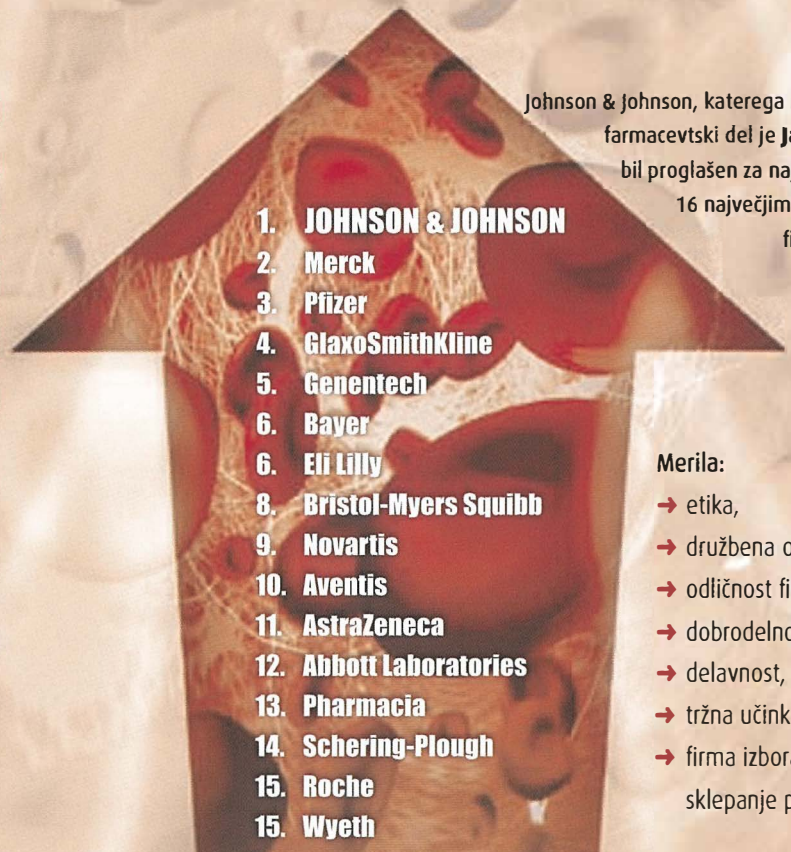

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Vir: J.T. Resnick, A matter of reputation; Pharmaceutical Executive Magazine, Advanstar Publications, 2002

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Indikacije: Zdravljenje anemije zaradi kronične ledvične odpovedi pri otrocih in odraslih na hemodializi, odraslih bolnikih na peritonealni dializi ter odraslih bolnikih z zmanjšanim ledvičnim delovanjem, ki se še ne zdravijo z dializo. Zdravljenje anemije in zmanjšanje potreb po transfuziji pri odraslih bolnikih, ki prejemajo kemoterapijo. Povečanje izkoristka avtologne krvi bolnikov, ki so vključeni v program shranjevanja krvi ali zmanjšanje izpostavljenosti alogenim transfuzijam krvi pri odraslih bolnikih pred večjim elektivnim ortopedskim posegom.
Odmerjanje in način uporabe
Bolniki s kronično ledvično odpovedjo na hemodializi: Zdravilo injicirajte i.v. Ciljna koncentracija Hb: 10-12 g/dl pri odraslih in 9,5-11 g/dl pri otrocih. Odmerek povečajte, če se koncentracija Hb ne povečuje za najmanj 1 g/dl na mesec. Faza korekcije: 50 i.e./kg trikrat na teden, i.v. Odmerek prilagajamo postopno, za 25 i.e./kg, trikrat na teden. Faza vzdrževalnega zdravljenja v zdržujemo ciljno koncentracijo Hb. Odrasli bolniki z zmanjšanim ledvičnim delovanjem, ki se še ne zdravijo z dializo Faza korekcije: 50 i.e./kg trikrat na teden, i.v. Odmerek prilagajamo postopno, za 25 i.e./kg, trikrat na teden. Faza vzdrževalnega zdravljenja: vzdržujemo koncentracijo Hb 10 – 12 g/dl.
Odrasli bolniki z rakom, ki se zdravijo s kemoterapijo: Začetni odmerek je 150 i.e./kg, 3-krat na teden, s.c. Odmerek prilagodimo na osnovi spremembe koncentracije Hb in št. retikulocitov.
Kontraindikacije: Nenadzorovana arterijska hipertenzija, preobčutljivost za katero od sestavin zdravila, kontraindikacije v povezavi s programom avtologonega zbiranja krvi. Subkutano injiciranje pri bolnikih s kronično odpovedjo ledvic. Bolniki, pri katerih se med zdravljenjem z epoetinom pojavi čista aplazija eritrocitne vrste, bolniki s hudo koronarno, cerebrovaskularno, karotidno ali periferno arterijsko boleznijo, po nedavno prebolelem miokardnem infarktu ali cerebrovaskularnem inzultu pri katerih je predviden večji neurgenten ortopedski kirurški poseg in niso vključeni v program avtologonega zbiranja krvi, bolniki, ki iz kateregakoli razloga ne morejo prejemati ustrezne tromboprofilakse.
Neželeni učinki: Predvsem na začetku zdravljenja se lahko pojavijo gripi podobni simptomi. Poročali so o nespecifičem kožnem izpuščaju in trombocitozi, ki je zelo redka. Najpogosteje se pojavi od odmerka odvisno zvišanje krvnega tlaka ali poslabšanje že obstoječe hipertenzije. Lahko se pojavi hipertenzivna kriza s simptomi, podobnimi encefalopatiji in generalizirani tonično-klonični krči. Pojavijo se lahko tromboze fistul. Pri bolnikih s kronično ledvično odpovedjo so po več mesecih ali letih zdravljenja z Eprex-om ali drugimi eritropoetini, v zelo redkih primerih poročali o eritroblastopeniji.
Posebna navodila za shranjevanje: Shranjujte zaščiteno pred svetlobo, pri temperaturi od 2° do 8 °C. Zdravila ne zamrzujte ali stresajte.

EPO-SLO-A-019-060303

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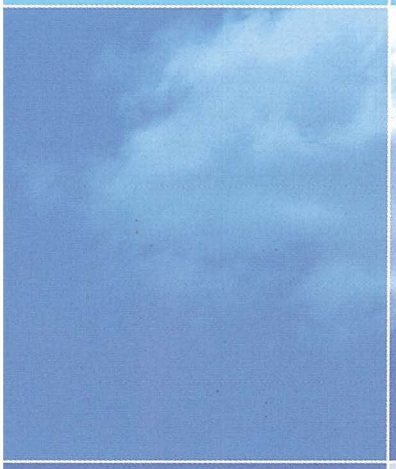
Glivične okužbe



- sistemske kandidoze
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Stranski učinki: Lahko se pojavijo slabost, napenjanje, bruhanje, bolečine v trebuhu, driska. Možni so glavobol, krči in alopecija. Zelo redke so preobčutljivostne reakcije. Pri bolnikih s hudimi glivičnimi obolenji lahko pride do levkopenije, trombocitopenije, povečane aktivnosti jetrnih encimov ter hujše motnje v delovanju jeter.

Oprema in način izdajanja: 7 kapsul po 50 mg, 28 kapsul po 100 mg, 1 kapsula po 150 mg – samo na zdravniški recept. 1 viala s 100 ml raztopine za intravensko infundiranje (200 mg/100 ml) – uporaba samo v bolnišnicah.

Datum priprave besedila: marec 2003

Podrobnejše informacije so na voljo pri proizvajalcu.

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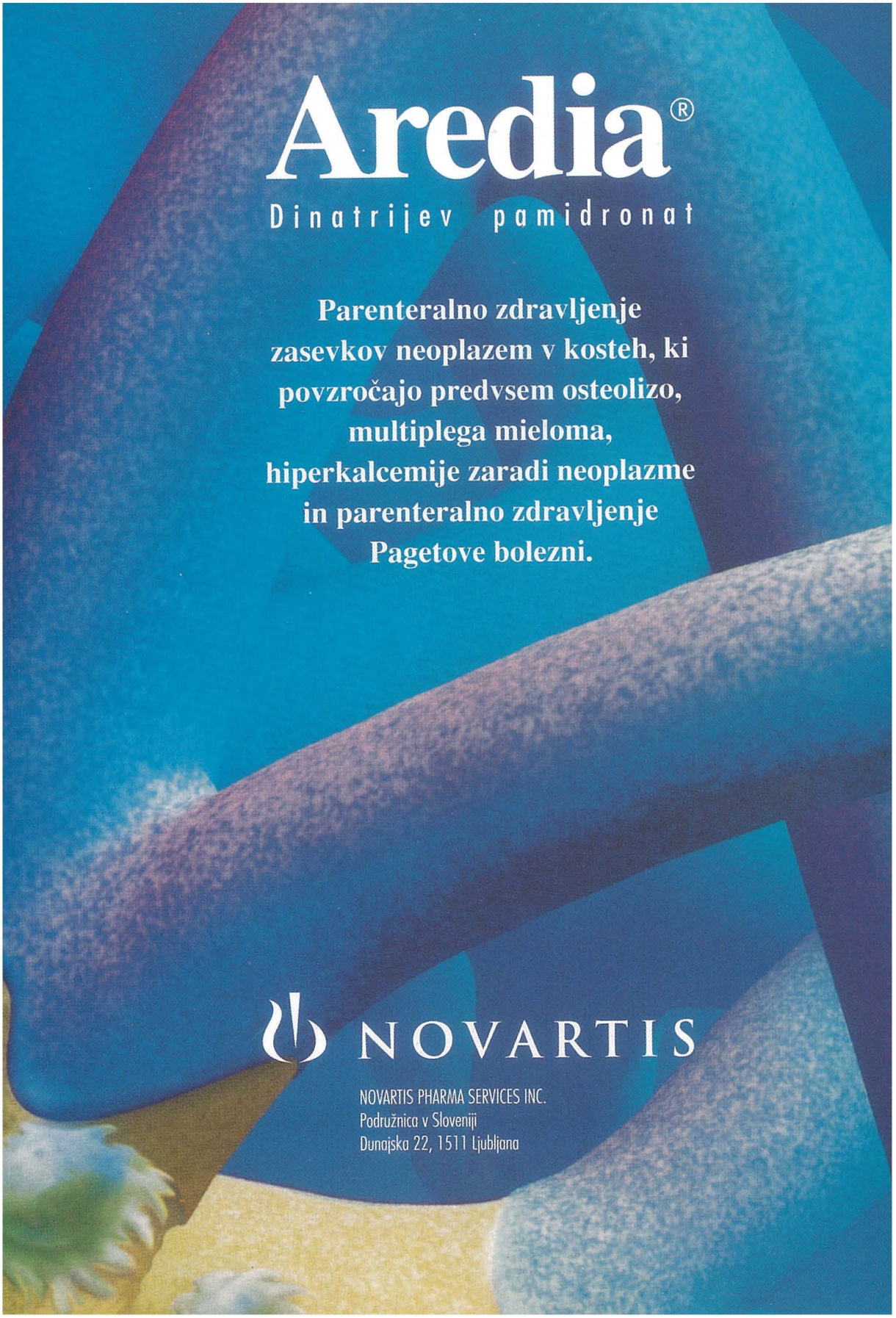
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