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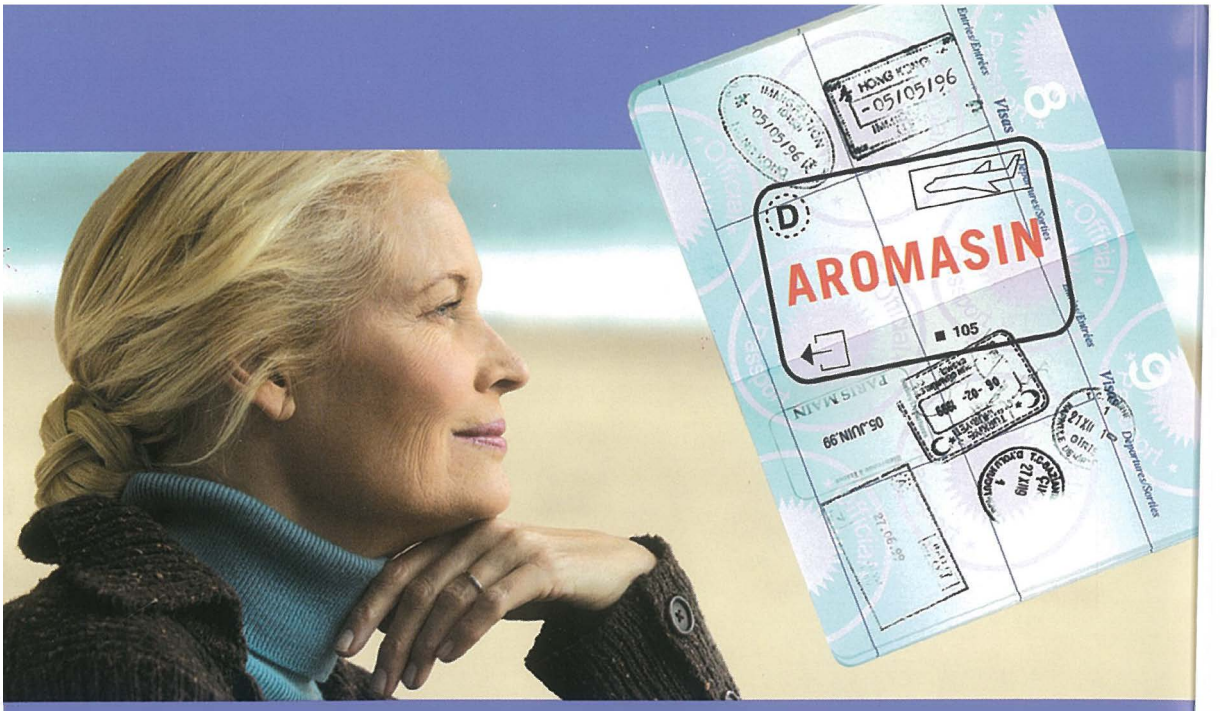

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Radiol Oncol 2006; 40(2): 67-72.

Early radiological diagnostics of gastrointestinal perforation

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Background. *The goal is to present the possibilities of radiological procedures and the early detection of gastrointestinal perforation as a common cause of acute abdomen.*

Methods. *During one year period, in emergency conditions, we evaluated 20 patients with gastrointestinal perforation. Native x-ray, ultrasound and CT of abdomen were performed on all patients, and on some of them with per os administration of 250 ml contrast, ultrasound was performed with 3, 5 MHz probe on a Siemens machine. CT scans were done on the multi row detector computed tomography (MTDC) »Volume Zoom«, Siemens with four rows of detectors and 2.5 mm width. All patients were admitted with clinical symptoms of acute abdomen.*

Results. *A group of 20 evaluated patients consisted of 8 (40%) women and 12 (60%) men of 41 as average age. The youngest patient was 14, and the eldest 67 years old. 7 (35%) had stomach perforation and 10 (50%) duodenum perforation. There was also a traumatic colon transversal perforation in one case, in the second was stitches rupture after the stomach operation and the third was the sigma perforation caused by the malign process. Out of all above mentioned cases, in 18 (90%) cases perforation occurred spontaneously and in 2 (10%) cases artificialy. Native x-ray of abdomen showed free air in the abdominal cavity in 16 (80%) cases. Ultrasound gave positive results on free liquid in 18 (90%) and CT scan revealed both free liquid and air in 20 (100%) cases.*

Conclusions. *The significance of an early and reliable discovery of gastrointestinal perforation is very important, because it usually requires the surgical intervention. Along with anamnesis, native x-ray of abdomen was and is traditionally the first procedure, especially in the detection of free air. With the development of digital techniques such as ultrasound and CT, we have a new diagnostic procedure at our disposal, especially in detecting free liquid and air as early signs of digestive perforation. According to our researches, ultrasound proves to be very useful in examining free liquid, while CT was more sensitive to the combination of liquid and minimal amount of free air, which was undetectable to ultrasound and x-ray.*

Key words: stomach-injuries-radiography; intestinal perforation-radiography- ultrasonography; CT scan

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Introduction

Gastrointestinal perforation is a common cause of acute abdomen. Spilled contents can consist of air, liquid of gastric and duodenal secretion, bile, food and bacteria.[1] Free

air or pneumoperitoneum is formed when the air leaves the gastrointestinal system. It occurs after perforation of stomach, oral part of duodenum and large intestine.[2] In case of perforation of small intestine, which in normal circumstances does not contain air, very small amount of air is released. The free air occurs in the peritoneal cavity 20 minutes after the perforation.

Causes of gastrointestinal perforation are: peptic ulcers, inflamed sigmoid colon diverticul, trauma damages, changes in case of Crohn's disease, ulcerous colitis and malign tumours in gastrointestinal system. The most common perforations are those of peptic stomach ulcer and of duodenum. Statistically, duodenum ulcers and most often in males, are the ones that perforate the most. The perforation can occur in the abdominal cavity *(perforatio libera)* or the adhesion of created pocket *(perforatio tecta)*.[2]

In 1799 clinical symptoms of perforated ulcers were recognized for the first time, although only in 1892, Ludwig Hensner, German, was the first one to perform surgery due to peptic ulcer of stomach. In 1894, Henry Percy Dean performed surgery due to perforated ulcer of duodenum small intestine.[3]

Patients and methods

This paper included 20 patients with gastrointestinal perforation, who were examined as urgent patients at our Institute in the period of one year. There were 8 women and 12 men, the youngest was 14 and the eldest was 67 years old. The average age was 41. They all had native x-ray of abdomen, ultrasound exam and native CT scan done. We applied 'Ultravist' dissolvable contrast substance on 3 patients in the amount of 250 ml orally. The exams were done with the ultrasound Siemens machine with 3, 5 MHz probe in the supine position and the position of left and right decubitus. CT scan was done on MTDC Somatom «Volume Zoom, Siemens machine with four rows of detectors and 2, 5 mm width, natively, in supine position and position of left and right decubitus.

Results

As depicted in Table 1, we can see that, out of 20, patients there were 8 women (40%) and 12 men (60%). Duodenal *bulbus* is to perforate the most 10 (50%) and stomach 7 (35%). We also had 1 (5%) sigma perforation caused by the malign process. In one case of a male child, colon transversal perforated after trauma and in other male patient there was stitches rupture after the stomach perforation surgery. With 18 (90%) of the patients the perforation was spontaneous, and with 2 (10%) patients it was a case of artificial duodenum perforation after ERCP and case of percutanous punction of pancreas pseudociste done by the ultrasound punction (Figure 1).

With 18 patients (90%) the native x-ray of abdomen in the standing position was positive on free air, and CT was positive on free air and liquid with all 20 patients (100%).

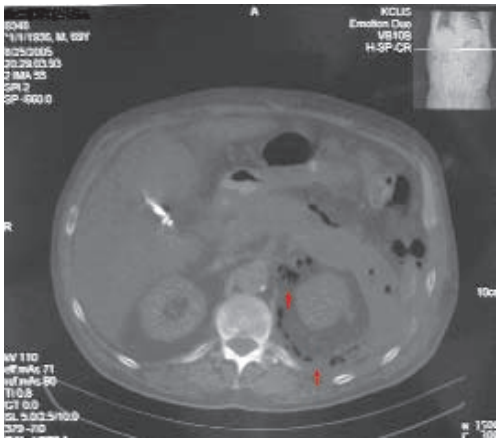

Figure 1. Artefitial perforation of duodenum with perirenal air collection.

Table 1. Frequency of gastrointestinal perforation according to localization and radiological findings

Patient	Sex	Native x-ray air	Location	Ultrasound fluid	CT Air/fluid
1.	female	neg	sigma	neg	pos
2.	female	pos	gaster	pos	pos
3.	female	neg	gaster	neg	pos
4.	female	pos	bulbus	pos	pos
5.	female	pos	bulbus	pos	pos
6.	female	neg	gaster	pos	pos
7.	female	pos	bulbus	pos	pos
8.	female	pos	bulbus	pos	pos
9.	male	pos	gaster	pos	pos
10.	male	pos	gaster	pos	pos
11.	male	pos	gaster	pos	pos
12.	male	pos	gaster	pos	pos
13.	male	pos	bulbus	pos	pos
14.	male	pos	bulbus	pos	pos
15.	male	neg	bulbus	pos	pos
16.	male	pos	bulbus	pos	pos
17.	male	pos	bulbus	pos	pos
18.	male	pos	bulbus	pos	pos
19.	male	pos	colon tr.	pos	pos
20.	male	pos	gaster-deh.	pos	pos
Total		**80%**		**90%**	**100%**

Discussion

The significance of early and reliable discovery of gastrointestinal perforation is very important, because it usually requires the surgical intervention. The radiologist has a significant role in helping the surgeon to choose the diagnostic procedure and to decide whether the patient will be operated. The detection of minimal pneumoperitoneum with patients with acute abdominal pain caused by gastrointestinal perforation is one of the most important diagnostic tasks in the urgent state of abdomen. An experienced diagnostician can, by using radiological techniques, detect such small amount of air as 1 ml. While doing so, he uses classic x-ray techniques of native abdomen in the standing position and the position of left lateral decubitus (Figure 2).

Despite of recent increased use of modern diagnostic techniques, an x-ray scan is still one of the most important initial tests

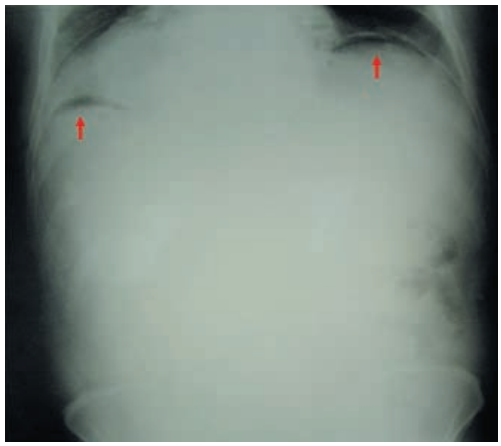

Figure 2. Subdiagraphragmal sickle- like air collection in native plain x-ray in standing position (gastrointestinal perforation).

and its analyses are sometimes a big challenge for a radiologist. Radiography is easily available, fast and cheap method. In order to see the free air and make the radiological interpretation reliable, the quality of the exposed film and correct positioning of the patient is very important. Every patient needs to take an adequate position 10 minutes before the exposition, so that, in that moment, the free air could reach the highest point in abdomen. Still, the appearance of pneumoperitoneum in case of the organ perforation can sometimes be difficult and unreliable.

Many researches show that its appearance is visible in just 75-80% of cases, but classic native x-rays of abdomen are still important procedures. Free air appears in the standing position or the position of left lateral decubitus. In case of trauma rupture, perforation can be insidious and masked by other pathological surgical conditions. The supine position reveals pneumoperitoneum in just 56% of cases.[4] About 50% of patients have collection of airs in right upper abdomen, either subhepatically or in hepatorenal space (Morison). A small oval or linear collection of air can be visible here. The small triangular collection of air is also visible between intestine meanders.

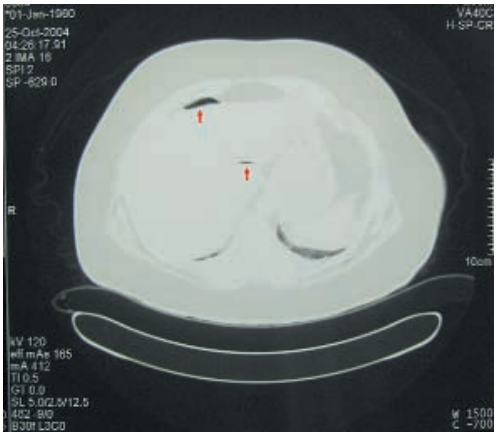

Figure 3. Air collection after gastrointestinal perforation (CT window for lung parenchyma).

Though, it is mostly visible in a shape of »dome« as a half-moons collection of air under the diaphragm in the standing position. A »football« sign represents the presence of free air above fluid collection in the middle part of abdomen. Our study has shown the presence of free air in standing x-ray of native abdomen in 80% of the cases, which is close to other authors' results (Sutton 76%). The ultrasound is an initial method for most acute abdomen conditions. It is useful for the detection of free liquid of various densities depending on the colour of grey scale, which in these cases, is very inhomogeneous because of the intestine contents.[5] It is especially precise in the detection of free liquid in small pelvis using the full urinary bladder technique. Mostly, ultrasound can not detect free air, which is not only barely detectable, but it also makes artefacts and limits this procedure. Still, some authors say that the detection of pneumoperitoneum is possible using ultrasound as the first procedure, and that they managed to see the air in the right upper quadrant when the patient is in the left lateral decubitus position. The echoes, which appear due to pneumoperitoneum, correspond to lung echoes during

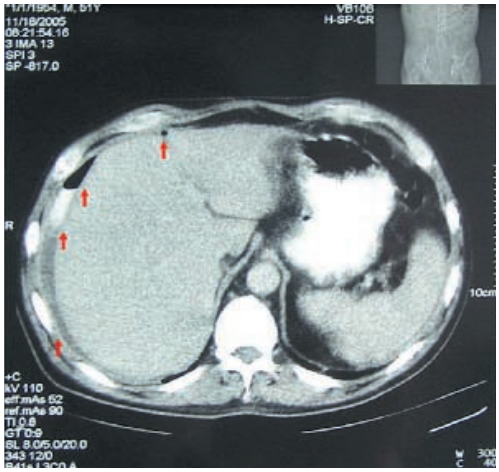

Figure 4. Air and contrast agent on CT scan after gastrointestinal perforation.

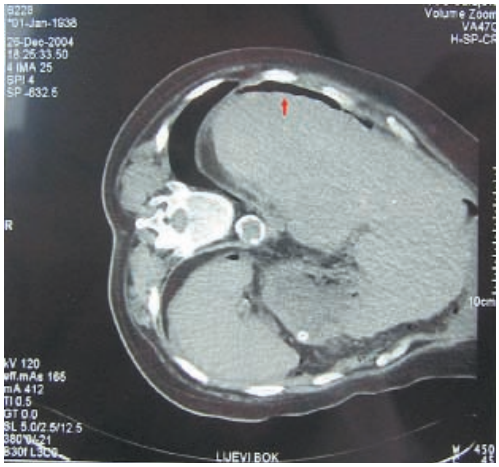

Figure 5. Movement of air collection ventraly on CT scan at lateral decubitus position.

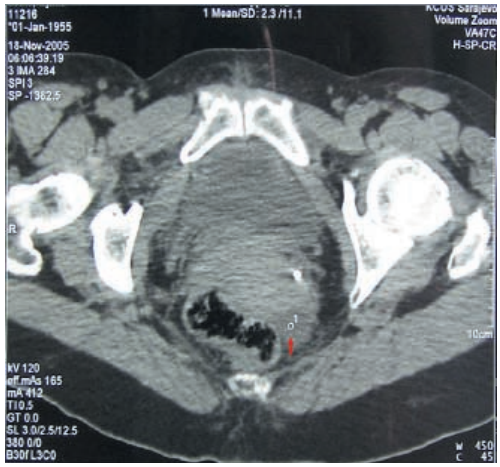

Figure 6. Fluid collection in Douglas space on CT scan of pelvis after gastrointestinal perforation.

the inspiration, but are separated during the expiration.[6] They also say that the echographic determination of perforation is possible as discontinuity of stomach wall or *bulbus* of hyper echoing aspect.[1]

In our study of 20 patients, we found free liquid in abdomen in 90% of cases, which is in coordination with other authors' researches (from 93% - 98%).[7, 8]

CT scan of abdomen is a much more sensitive method in detection of air after the perforation, even when it appears as a bubble and when the native x-ray is negative.[9] Therefore, CT is very efficient in the early detection of gastrointestinal perforation. While doing so, we need to adjust the window so that we could distinguish fat from air, because both of them appear as hipodense areas with negative densities. The window for lung parenchyma is best for solving this problem (Figure 3).

When the CT is done in the supine position, air bubbles on CT scan are mostly located on the front parts of abdomen (Figure 4). We can see air bubbles move if a patient afterwards takes the position of left decubitus (Figure 5). CT is also much better in detecting fluid collections located in bursa omentalis and retroperitoneal.[10]

Despite the great sensitivity, CT is not always necessary due to high cost and the radiation dose. In doing so, the possibility of locating the perforation is poor.[11] If we suspect that the patient has perforation, and the free air is not visible on classic native scans, we can apply nonionic contrast substance to prove our doubts. One of the ways is to apply air through nasogastrical tube 10 minutes before scanning.

The second way is to give orally the minimal 250 ml dissolvable contrast substance 5 minutes before scanning, which helps to show contrast but not the air. Barium compounds can not be given in this situation because they can cause granuloma formation and peritoneum adhesion.[1,2] CT has proved as very sensitive with our 20 patients, discovering free liquid and air in abdomen in 100 % of cases (Figure 6). Some authors claim that CT can be precise up to 95%.[12]

Conclusions

We can conclude that, along with clinical finding, complementary methods are: standard native abdomen x-ray in the standing position, ultrasound on full urinary bladder,

native CT scan and CT with orally given dissolvable contrast substance. If x-ray and ultrasound findings are uncertain, we should not hesitate to use CT, considering that it can detect fluid and very small collections of air which are undetectable by previously mentioned methods.

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review

The role of tricyclic drugs in selective triggering of mitochondrially-mediated apoptosis in neoplastic glia: a therapeutic option in malignant glioma?

Geoffrey J. Pilkington[1], James Akinwunmi[2] and Sabrina Amar[1]

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We have previously demonstrated that the tricyclic antidepressant, Clomipramine, exerts a concentration-dependant, tumour cell specific, pro-apoptotic effect on human glioma cells in vitro and that this effect is not mirrored in non-neoplastic human astrocytes. Moreover, the drug acts by triggering mitochondrially-mediated apoptosis by way of complex 3 of the respiratory chain. Here, through reduced reactive oxygen species and neoplastic cell specific, altered membrane potential, cytochrome c is released, thereby activating a caspase pathway to apoptosis. In addition, while we and others have shown that further antidepressants, including those of the selective serotonin reuptake inhibitor (SSRI) group, also mediate cancer cell apoptosis in both glioma and lymphoma, clomipramine appears to be most effective in this context. More recently, other groups have reported that clomipramine causes apoptosis, preceded by a rapid increase in p-c-Jun levels, cytochrome c release from mitochondria and increased caspase-3-like activity. In addition to clomipramine we have investigated the possible pro-apoptotic activity of a range of further tricyclic drugs. Only two such agents (amitriptyline and doxepin) showed a similar, or better, effect when compared with clomipramine. Since both orally administered clomipramine and amitriptyline are metabolised to desmethyl clomipramine (norclomipramine) and nortriptyline respectively it is necessary for testing at a tumour cell level to be carried out with both the parent tricyclic and the metabolic product. In addition, reversal of multidrug resistance in a number of solid cancers following treatment with both clomipramine and amitriptyline has been reported. This additional role for tricyclics may be of some significance in the treatment of primary and secondary brain tumours. Since a substantial number of patients with malignant glioma have already received and are receiving clomipramine, both anecdotally and within a clinical trial, we have carried out CYP (P450) gene expression studies and determined blood plasma levels of clomipramine and norclomipramine, in order to ascertain whether differences in individual patient metabolism influence clinical outcome. While the pro-apoptotic effect of norclomipramine appears to be inferior to that of the parent tricyclic, amitriptyline and nortriptyline share a similar propensity for eliciting apoptosis in neoplastic but not non-neoplastic astrocytes. The potential value of these agents as adjuvants in the management of patients with malignant glioma is apparent.

Key words: brain neoplasms – drug therapy; glioma; clomipramine; antidepressive agents, tricyclic; apoptosis

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Introduction

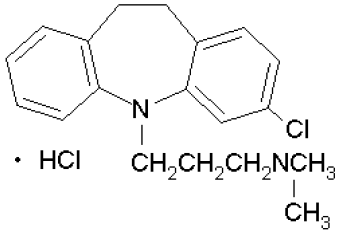
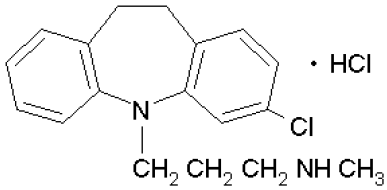
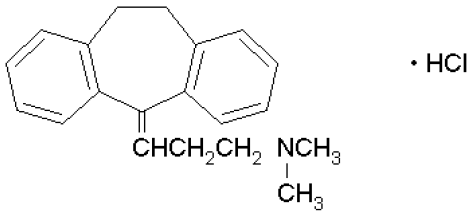
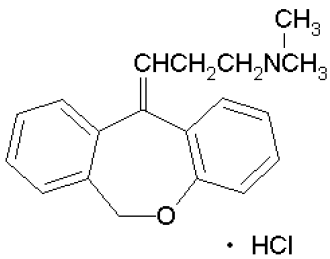
Tricyclic drugs and neoplastic cells

Tricyclic drugs, whose name is derived from their characteristic three ring nucleus (Table 1), were first thought to be useful as antihistamines with sedative properties and later as anti-psychotics. They include an important group of tricyclic antidepressants (TCAs) which have been in clinical use over 40 years. In the 1970's, it was found that TCAs showed selective inhibition of mitochondrial activity in yeast cells.[1] It was surmised that the wide range of actions shown by the TCAs *in vivo* was due to interactions with membranes and membrane bound enzymes, in particular the mitochondrial membrane[1], resulting in inhibition of cellular respiration and limitation of adenosine triphosphate (ATP) production. Further experiments showed that cancer cells were much more susceptible to the inhibitory effects of TCAs than non-transformed cells.[2] After treatment with TCAs, it was observed that the respiration rate of transformed cells was significantly less than their normal counterparts in oxygen electrode studies.[2] It was concluded that anti-mitochondrial drugs, such as TCAs, depress mitochondrial activity in cancer cells, thereby leading to cell death, whereas non-transformed cells were able to recover after treatment.[2] This mode of action of the TCAs was found to be a common feature amongst members of the group but there appears to be no clear relationship between chemical structure and pharmacological action.[3] However, the chlorine containing drugs are said to be more toxic than others to the functions of the mitochondrial membrane.[4]

Impairments of mitochondrial function may lead to ATP depletion and necrotic cell death.[5] More recently, however, mitochondria have been implicated in both the regulation of apoptotic cell death and cancer formation.[3] It has been reported that mitochondrial respiration is decreased in neoplastic tissue, along with a lowering of the cellular content of mitochondria. These findings indicate that tumour cells rely upon glycolysis as an energy source and this enables them to survive under hypoxic conditions.[6] There are at least three established mechanisms through which mitochondria can trigger apoptosis although these events may be inter-related.[7] Apoptosis may be triggered by disruption of electron transport, oxidative phosphorylation and ATP transport, release of proteins that trigger activation of caspases and alteration of cellular redox potential.[7] A number of agents appear to target the mitochondria and promote the release of cytochrome c and other pro-apoptotic proteins, which can trigger caspase activation resulting in cell death.[5] Caspases are cysteine proteases and exist in a latent state in 'healthy' cells.[8] In response to damage or a malfunction of vital metabolic processes, cells generate signals that lead to activation of caspases, which result in apoptotic cell death.[8] Figure 1 shows some of the signalling pathways involved in tricyclic-initiated, mitochondrially-mediated neoplastic cell apoptosis.

Defects in apoptosis signaling pathways are, however, common in cancer cells. Moreover, tumour development, progression and resistance to radiotherapy and chemotherapy are all the direct result of defects in the regulation of apoptosis in glioma[9], due to raised apoptotic thresholds. Human mitochondrial DNA (mtDNA) consists of a small circular genome of 165kb that enco-

Table 1. Chemical structure of the tricyclic antidepressants used in laboratory studies

Name Chemical	structure
Chemical structure of clomipramine hydrochloride	
Chemical structure of norclomipramine hydrochloride (N-desmethylclomipramine)	
Chemical structure of amitriptyline hydrochloride	
Chemical structure of doxepin hydrochloride	

des a complex array of proteins including 13 respiratory chain sub-units. Expression of the entire genome is required to maintain proper function of the mitochondria. The identification of the specific proteins responsible for the regulation of apoptosis may be expected to lead to the development of cancer therapies directed at altering the levels of expression of pro-apoptotic proteins and enhancing the effects of current radiotherapy and chemotherapy. The *bcl-2* proto-oncogene represses a number of cellular apoptotic pathways and is known to be expressed in increasing amounts in glial tumours with increasing degree of malignancy.[10] Transfection of glioma cells with antisense *bcl-2* has been reported to result in an increase in apoptotic cell death. This indicates that *bcl-2* plays a role in tumour progression of gliomas by acting as an oncogene and inhibition of the *bcl-2* gene could have a therapeutic effect.[10]

It has been determined that chemotherapeutic drug-induced apoptosis of human malignant glioma cells involves the death receptor-independent activation of caspa-

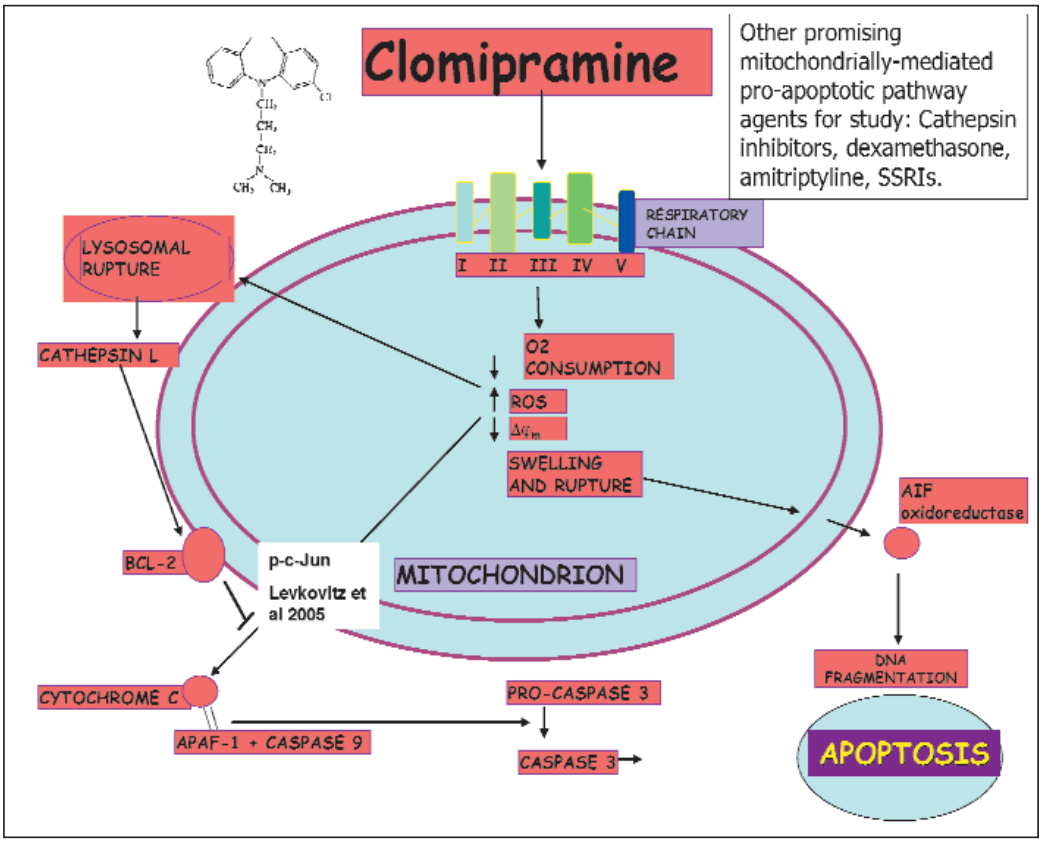


Figure 1. Flow pathway of clomipramine pro-apoptotic effect.

ses other than 3 and 8.[11] Caspases 1, 2, 3, 7, 8 and 9 are constitutively expressed in most human malignant glioma cell lines and drug-induced apoptosis involves delayed activation of caspases 2, 7 and 9 and is blocked by a broad spectrum caspase inhibitor.[11] It has also been established that the cytotoxic effects of many chemotherapeutic agents are mediated via apoptotic pathways; therefore developing drugs that target the mitochondria may provide a new strategy to induce apoptosis in tumour cells. [12]

It has been shown that the TCAs imipramine and clomipramine, and the selective serotonin reuptake inhibitor (SSRI) citalopram, induce apoptosis in cancer cells and that this process is associated with an early increase in the production of reactive oxygen species (ROS) and subsequent loss of mitochondrial membrane potential.[13] The literature suggests that TCAs can induce apoptosis in acute myeloid leukemic cells[14] and lymphomas[15] as well as gliomas.[3,16,17] The mechanism of action of clomipramine involves the inhibition of complex III of the respiratory chain, resulting in elevated levels of ROS, cytochrome c release and caspase-activated apoptosis.[16] Indeed the data presented in a study carried out by Daley *et al.* indicated that clomipramine might be useful in the treatment of patients with primary brain tumours.[16] In fact it is estimated that there are now over 300 'anecdotal' cases of patients with a range of different primary brain tumours

who are taking, or have taken, clomipramine in the UK. With respect to these cases, there have been numerous reports of survival benefit and increased quality of life. Currently, there is a clinical study in progress in which patients newly diagnosed with either an anaplastic astrocytoma or glioblastoma multiforme receive an initial dose of 25mg clomipramine, escalating to 150mg in steps of 25mg at 3-day intervals.[3]

In addition, reversal of multidrug resistance in a number of solid cancers following treatment with both clomipramine[18,19] and amitriptyline[20] has been reported. This additional role for tricyclics may, albeit at differing concentrations, provide an additional novel approach to the treatment of primary and secondary brain tumours.

In order to address some of the issues related to a possible further role of TCAs in the therapy of glioma we are carrying out the following studies:

Clinical

- Determination of blood plasma levels of clomipramine and its metabolite, norclomipramine, in patients with brain tumour taking the drug.
- Assessment of CYP (P450) gene expression in glioma patients taking clomipramine.
- Monitoring of outcome of »anecdotal« glioma patients treated with clomipramine through the Samantha Dickson Research Trust (www.sdrt.co.uk).
- Clinical trial at King's College Hospital, London in newly diagnosed patients with histologically verified anaplastic astrocytoma and glioblastoma multiforme.

Laboratory

- Assessment of viability in a dose response series of in vitro experiments in low passage, biopsy derived glioma cultures, high passage, established glioma cell lines and non-neoplastic human astrocytes to clomipramine.
- Assessment of oxygen utilisation of the above cells after treatment with clomipramine.
- Assessment of apoptosis of the above cells after treatment with clomipramine.
- Repeating the above studies with norclomipramine, amitriptyline, nortriptyline and various combinations of amitriptyline and clomipramine.
- Establishing the possible influence of different concentrations of dexamethasone on clomipramine-induced apoptosis.

Methods used in ongoing laboratory studies

Blood samples/clomipramine distribution

Blood plasma samples taken at regular intervals, from both anecdotal and trial patients taking clomipramine, are analysed using standard high-performance liquid chromatography (HPLC), to detect both clomipramine and its metabolite norclomipramine. A methodology is currently being developed for the measurement of dexamethasone via HPLC, and amitriptyline/nortriptyline can potentially be added to the range of tricyclics that we are able to offer testing for, should it be required. The data taken from the analysis of blood plasma will be used to track the metabolic progress of individual patients, and over a period of months could be used to 'tailor' the individual dose according to side effects. The preliminary studies that have been carried out are based upon the therapeutic window for patients using clomipramine as an antidepressant, however when enough data is gathered it will be possible to determine the target range for use in malignant glioma. In combination with the plasma testing of patients, it will be possible for a series of basic liver function tests (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyltransferase (GGT)) to be performed 'in-house'.

Blood samples taken from patients included in the above studies are also analysed for the presence/absence of markers related to the metabolism of clomipramine. DNA extracted from Whatman FTA cards is analysed by PCR using primers for the CYP genes 2D6 and 2C19. By determining the gene expression of the individual patient it will be possible to classify them as 'good' or 'poor' metabolisers of clomipramine and this information will be of use when clinical decisions are taken concerning the optimal daily dose.

Treatment of cells

Neoplastic and non-neoplastic glial cells are used for treatment with the following agents: amitriptyline, nortriptyline, clomipramine, norclomipramine, dexamethasone & sodium valproate (valproic acid). These experiments will show if there is any synergy, additive effect or antagonism between agents in combination.

Cell viability

Cell viability is used, in conjunction with clonogenicity assays to determine the efficacy of the drugs in vitro. Studies are also performed using normal human astrocytes (Cambrex Biosciences) to demonstrate that the tricyclic drugs affected only neoplastic cells in the brain. The MTT, Neutral Red and Alamar Blue cytotoxicity assays are used to initially determine the IC_{50} for each of the agents, and then subsequent studies are performed using pertinent concentrations of the tricyclics. Using a Beckman Coulter Vi-Cell XR trypan blue analyzer cells exposed to test agents for varying lengths of time can be analysed to determine percentage cell death, via uptake of trypan blue. The instrument also provides information about viability of different sub-populations based upon cell size after drug exposure and is used to prepare growth curves and population doubling times via its »Bioprocess« software programme.

Oxygen electrode assay

Oxygen electrode studies using Hansatech multiple Oxytherm/Oxygraph O_2 electrode apparatus are performed to establish any decrease in oxygen uptake on tumour cell exposure to the test agents. Reduction in oxygen utilisation gives an indication of the affects of test agents on mitochondrial function and is a useful indicator of events culminating in mitochondrially-mediated apoptotic cell death.

Apoptosis assays

Annexin V/Propidium iodide flow cytometry: is used to determine the mechanism of cell death subsequent to exposure to the test agent by way of a BD FACScalibur flow cytometer. The annexin V fluorochrome binds to the 'flipped' phosphotidyl serine residues of the inner leaflet of the cell membrane, after the apoptotic signalling cascade has been activated. The assay can differentiate between early and late apoptotic cells as well as necrotic cells. The protocol had to be optimised when using it to study the tricyclics in combination with dexamethasone as the propidium iodide, which is taken up by the 'leaky' cell membrane of necrotic cells, is also taken up due to the effect that dexamethasone exerts on the pores of the cell membrane via the glucocorticoid receptors.

Live cell imaging: with monolayers of tumour cells is carried out over periods of up to 72 hours of drug exposure using a Zeiss Axiovert 200M incorporating a temperature/humidity/CO_2 controlled chamber together with Improvision Openlab, Velocity and Acquisition software. Cell proliferation and apoptotic events are recorded as time-lapse DVDs.

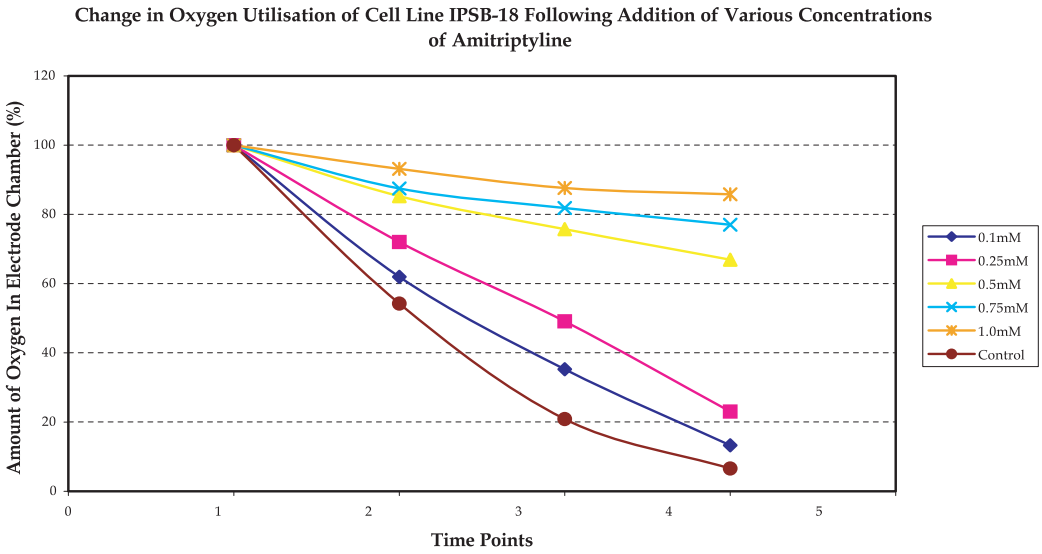


Figure 2. Graph representing the oxygen consumption of an anaplastic astrocytoma after treatment with different concentrations of Clomipramine.

Caspase 3 Activity: Caspase 3 activity is measured by its ability to cleave Ac-DEVD-AMC, whereby it produces a fluorescent AMC subunit. Cytosol extracted from cells exposed to the test agents, are read on a Mithras LB950 plate reader (Berthold Technologies) and compared to controls in which a pan-caspase inhibitor is added.

Results

Additional tricyclics and neoplastic cell apoptosis

In addition to clomipramine we have, in pilot experiments, investigated the possible pro-apoptotic activity of a range of further tricyclic drugs. Only two such agents (amitriptyline and doxepin) showed a similar (doxepin), or better (amitriptyline), effect when compared with Clomipramine. Amitriptyline has previously been reported to reduce proliferation in cancer cell lines.[21] Preliminary studies carried out using amitriptyline and nortriptyline show that it exerts a cytotoxic effect on the established anaplastic astrocytoma line (IPSB-18 p39) and the glioblastoma-derived culture (CLOM 15 p23). We have also found that Amitriptyline induces a dose-dependent reduction in oxygen utilisation in human glioma cells (Figure 2) as well as apoptosis as seen in Annexin V/PI flow cytometry. Moreover, when early passage cultures of human glioma were treated sequentially with clomipramine & amitriptyline apoptosis was initiated. Only a small proportion of cells recovered from this treatment

The possible role of dexamethasone in modulation of tricyclic drug-mediated brain tumour cell apoptosis

In the UK the great majority of patients suffering from malignant glioma receive the glucocorticoid steroid, dexamethasone, to reduce raised intracranial pressure.[22] This steroid has been reported to be both anti- and pro-apoptotic, in its own right, according to concentration, in various cancer cells.[23] In addition, it has been shown to protect established glioblastoma-derived cultu-

res from temozolomide-induced apoptosis by influence on caspase-3 activity and Bax: Bcl-2 ratio.[24,25] In our laboratories when studying concomitant dexamethasone/clomipramine treatment of glioma cells and dexamethasone pre-treatment prior to clomipramine treatment, we were able to demonstrate both inhibition and potentiation of clomipramine-mediated apoptosis.[26] These studies, however, merit greater investigation using different combinations and doses primary and early passage glioma-derived cultures as well as established cell lines.

Clinical studies with TCAs in brain tumour

Since a substantial number of patients with malignant glioma have already received and are receiving clomipramine, both anecdotally and within a clinical trial at King's College Hospital, London we are carrying out two experiments. One to determine the CYP (P450) genetic profile of individuals and the other to determine blood plasma levels of clomipramine and norclomipramine, in order to determine whether differences in individual patient metabolism influences clinical outcome. CYP (450) are hydroxylases situated on the P450 loci and are responsible for the breakdown of antidepressant in particular CYP2C19 and CYP2D6, which are highly polymorphic.

The first of these was to determine the CYP (P450) (27, 28) genotypic profile of individuals, in particular the CYP2D6 and CYP2C19 and the other was to test blood plasma levels of clomipramine and norclomipramine in order to determine whether differences in individual patient metabolism, measured by HPLC analysis, influences clinical outcome. We now wish, in collaboration with our clinical colleagues, to extend these studies in order to obtain statistically meaningful data with which to inform clinical practice.

Discussion and future studies

The pro-apoptotic role of clomipramine in neoplastic cells

Clomipramine acts by triggering mitochondrially-mediated apoptosis by way of complex 3 of the respiratory chain. Here, through reduced reactive oxygen species and neoplastic cell specific, altered membrane potential, cytochrome c is realeased thereby activating a caspase pathway to apoptosis (Figure 1).[16] Indeed, Xia *et al.*[13,14] previously reported that clomipramine induced increases in reactive oxygen species, lead to mitochondrial membrane potential alterations and increased caspase-3 activity in human acute leukaemia HL-60 cells which preceded apoptosis. Similarly, the tricyclic analog, desipramine, has also been shown to induce mitochondrially-mediated apoptosis in C6 glioma cells via increased caspase-3 gene expression and intracellular calcium homeostasis changes.[29] In addition, while we and others have shown that further antidepressants, including those of the selective serotonin reuptake inhibitor (SSRI) group, also mediate cancer cell apoptosis in both glioma and lymphoma, clomipramine appears to be most effective in this context.[15] Very recently, Levkovitz *et al.*[30] independently reported that clomipramine, in a comparative study between SSRIs and clomipramine in C6 rat glioma and human neuroblastoma cells, caused apoptosis preceded by a rapid increase in p-c-Jun levels, cytochrome c release from mitochondria and caspase-3-like activity. Significantly lower sensitivity to the drug's pro-apoptotic activity was demonstrated in primary mouse brain and neuronal cultures. The authors therefore concluded – as we had previously - that the high sensitivity of cancer cells to the drug suggested that clomipramine may have potential in the treatment of brain tumours. We have also demonstrated the role

of cathepsin L in interfering with activity of pro-apoptotic agents such as clomipramine by use of cathepsin inhibitors and anti-sense technology.[31]

Amitriptyline has previously been reported to reduce proliferation in cancer cell lines[21] and to decrease glioma cell viability.[32] In our preliminary experiments we have found that amitriptyline induces a dose-dependent reduction in oxygen utilisation in human glioma cells as well as apoptosis as seen in Annexin V/PI flow cytometry. Moreover, when early passage cultures of human glioma were treated sequentially with clomipramine & amitriptyline apoptosis was initiated. Only a small proportion of cells recovered from this treatment. In addition, reversal of multi drug resistance in a number of solid cancers following treatment with both clomipramine[19,33] and amitriptyline[20] has been reported. This additional role for tricyclics may, albeit at differing concentrations, be of some significance in the treatment of primary and secondary brain tumours.

Cancer stem cells

CD133 is a 120kDa five-transmembrane cell surface protein, originally described as a haematopoietic stem cell marker.[34,35] More recently, however, it was shown to mark normal human neural stem cells.[36] Subsequently, Singh *et al.*[37] demonstrated CD133 positivity, by both immunohistochemistry and flow cytometry, on two common forms of paediatric brain tumour; the high grade malignancy medulloblastoma and the low grade pilocytic astrocytoma. Moreover, brain tumour stem cells can be magnetic immuno-bead and fluorescence activated cell sorted by use of dissociated cell suspensions using CD133 antibodies. The subsequent CD133 positive selected sub-population of tumour cells also express nestin but fail to express markers associated with differentiated cells of neural lineage.[38] Although these CD133/nestin positive stem cells represent a minority fraction of the overall tumour cell complement, they are able to generate clonal tumour neurospheres in suspension culture. They also show increased self-renewal capacity and can be induced to differentiate into cells phenotypically similar to those seen in the original patient histology. The same group then developed an *in vivo*, serially-transplantable, xenograft model in NOD-SCID (non-obese diabetic, severe combined immunodeficient) mouse brains by injecting as few as 100 CD133-positive brain tumour stem cells. The histological appearance of the resulting tumours resembled that of the original resected tumour. Conversely, injection of as many as 10^5 CD133-negative cells failed to produce tumours.[39] We have noted that while human glioma biopsies normally grow well in standard DMEM growth conditions, cells from four clomipramine treated patients cells taken at second biopsy grow poorly in DMEM culture media. We hypothesise that cancer stem cells, as denoted by CD133 (plus CD44/CD24/nestin/Musashi-1) expression may increase in number & are resistant to clomipramine.[40] We, therefore, propose to culture these second (recurrent case) biopsies, as well as new cases of glioma in stem cell defined medium to see if yield of CD133 +ve cells has increased. Primary/*ex-vivo* cultures will be prepared from human glioma obtained from King's College Hospital (KCH) London (LREC 00-173) and Hurstwood Park Neurological Centre, Haywards Heath, Sussex (LREC applied for). Epilepsy surgical brain resection tissue from KCH will be used to provide additional non-neoplastc astrocyte cultures (LREC 02-056). Biopsied glioblastoma primary cultures taken from both newly diagnosed patients and those previously treated with clomipramine and isolated *in vitro*[41] in stem cell defined fee-

der cell conditions.[42] The monoclonal AC133 antibody (Miltenyi Biotech) will be used to identify CD133 positive stem cells and early progenitor cells. Immunocytochemistry, using fluorescence/TIRF microscopy, and flow cytometry will be used to identify and quantify CD133 antigen expression. CD133 positive cells will then be separated either by MACS/CD133 immunobeads (Miltenyi Biotech) or by sterile FACS and grown in bulk culture for subsequent testing with various drug combinations. Although we expect a low yield of CD133-positive cells we feel this would be a timely study.

Valproic acid

Many brain tumour patients also suffer from seizures and are, consequently, prescribed anti-convulsants. One particular anti-convulsant, the histone deacetylase inhibitor, sodium valproate (valproic acid), has recently attracted attention for its potential anti-cancer properties. Histone deacetylation is critical for regulation of gene expression which may affect chromatin structure and chromatin interaction with regulatory factors. In this context valproic acid has been shown to rapidly hyperacetylate histones H3 and H4 in breast cancer cells and depleted the structural maintenance of chromatin proteins, DNA methyltransferase and heterochromatin proteins with a consequent enhanced sensitivity of DNA to DNA-damaging agents, both *in vitro* and in xenograft models.[43] In addition, valproic acid has been reported to enhance radiosensitivity of human brain tumour cell lines and xenografts.[44] Combination therapy of histone deacetylase inhibitors and radiotherapy has also resulted in increased neuroblastoma cell necrosis and apoptosis compared with either single modality treatment. Interestingly, Beecken *et al.*[45] have shown that it also positively modulates neural cell adhesion molecule (NCAM) polysialylation, thereby blocking adhesion of several neuroectodermal tumour-derived cell lines to HUVEC (human umbilical vein endothelial cells) while downregulation of CD44 expression has been reported on human and rat glioma cells *in vitro*.[46] These findings may be suggestive of reduced invasion but increased tumour cell differentiation and apoptosis have also been reported in human brain tumour xenograft models.[46] Indeed, enhanced differentiated gene expression, growth inhibition, cell cycle arrest, induction of apoptosis and down-regulation of the pro-survival genes bcl-2 and bcl-xl has also been reported in thyroid cancer cells.[47,48] We are, therefore, eager to explore the potential of valproic acid in combination with tricyclics.

Current literature available on dexamethasone and its actions on glioma cells is conflicting. It has been reported that glucocorticoids have a functional role at the level of the mitochondria.[49] It has also been shown that glucocorticoids are neurotoxic and appear to play a role in neuronal cell loss following neuropathological insults.[50] Dexamethasone has been shown to enhance necrotic cell death of glioma cells induced by serum deprivation.[50] The steroid also reversibly and significantly inhibits growth of C6 glioma cells both at early and late passage.[51] Despite evidence suggesting dexamethasone exerts a necrotic type of cell death, some studies have indicated that its mechanism of action is via apoptosis and interference with apoptotic pathways. In leukaemia cells dexamethasone-induced apoptosis has been demonstrated through the mitochondria-dependent pathway.[52] Glucocorticoids are known to influence the ability of cells to undergo apoptosis, directly inducing apoptosis in thymocytes while inhibiting it in hepatoma and carcinoma cells.[23] It has been suggested that dexamethasone inhibits the induction of

apoptosis in astrocytoma cells, probably via up-regulation of *Bcl-x_L*, which could prevent cytochrome c release from mitochondria and subsequent caspase activation.[23] Dexamethasone was also shown to confer protection against the induction of apoptosis by anti-cancer agents.[23] This indicates that dexamethasone could potentially interfere with the efficacy of chemotherapeutic agents. The laboratory and clinical studies described are aimed at identifying a possible role for tricyclics in combination with standard therapies for glioma patients. It is hoped that such a combinatorial, and possibly customised, approach may enhance both quality of life and survival time for patients suffering from malignant brain tumour.

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Apoptosis of human malignant glioma-derived cell cultures treated with clomipramine hydrochloride, as detected by Annexin-V assay

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Background. *Previous research in our laboratories has shown that Clomipramine Hydrochloride (CLOM), a tricyclic antidepressant in use for over thirty years, selectively kills neoplastic glial cells in vitro whilst leaving normal brain cells unaffected. The purpose of this study was to evaluate whether a range of early passage cell cultures and established cell lines, derived from a number of patients with malignant glioma, would display different sensitivities when exposed to CLOM. The particular assay of interest, following our discovery that CLOM targets the mitochondria of tumour cells and triggers Caspase 3 mitochondrially-mediated apoptosis, was Annexin-V flow cytometry. This assay was used to determine the mechanism of cell death, either necrosis or apoptosis, according to drug concentration and period of incubation.*

Method. *Cells grown to 90% confluence in 25cm^3 flasks were incubated with concentrations of CLOM from 20μM – 100μM, for up to 6 hours. Cells were harvested and resuspended in calcium binding buffer, which triggers translocation of calcium-regulated phosphatidylserine residues to the nuclear envelope, before removing 500μl of the single cell suspension to a Facs tube. Controls used in the analysis were performed by omission of the drug incubation in one flask, and addition of 1μM staurosporine to one flask. These served as negative and positive controls respectively. Annexin-V FITC and propidium iodide were added to all tubes and incubated for 15 minutes at room temperature, in the dark. Subsequent to this, binding buffer was added to each tube and analysed using a BD FACScalibur.*

Results. *Results show that, of the five malignant gliomas tested, the two established cell lines had the lower apoptotic threshold, with a significant percentage of apoptotic cells present at 60μM and above when compared to the control sample. The three early passage cultures, developed 'in house' from biopsy, had higher apoptotic thresholds, withstanding up to 100μM CLOM incubation for six hours. Normal human astrocytes were assayed in parallel, and show that CLOM does not cause cell death at the concentrations tested.*

Conclusions. *It may be possible, in a larger study, to predict individual patient response to CLOM using the Annexin-V assay, alongside Bcl-2 analysis and CYP gene testing, on the individual patient's tumour cells. The difference in sensitivities between glioma, in this small study, indicates the importance of analysing early passage cultures, which retain original morphology and characteristics to a greater extent, alongside established cell lines.*

Key words: annexin V; apoptosis; brain neoplasms - drug therapy; glioma; clomipramine; tumour cells, cultured

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Introduction

Previous research in our laboratories has shown that Clomipramine Hydrochloride (CLOM), a tricyclic antidepressant in use for over thirty years, has the ability to induce apoptosis in malignant glioma cells *in vitro*.[1] Thus, following the initiation of a clinical trial based at King's College Hospital, London (LREC 01-235), it is important to be able to predict the outcome of a given drug treatment, especially in the case of glioma which are often characterised by a poor prognosis.

Cell death is often defined as occurring by either apoptosis or necrosis. Whilst necrosis is a relatively passive process, involving loss of membrane integrity and cell membrane rupture, which leads to the release of intracellular debris and eventual inflammatory response, apoptosis is an active process resulting in cell shrinkage, plasma membrane blebbing and chromatin condensation which produces apoptotic bodies, rapidly recognised and phagocytosed by macrophages. This 'clean' mechanism of cell death, avoiding an inflammatory response, ensures minimal tissue damage to the surrounding brain parenchyma.

Apoptosis after CLOM treatment is associated with the intrinsic pathway of mitochondrial cytochrome C release[1,2] following a rapid increase in p-c-Jun[2], and activation of caspase-3.[3] The two main pathways of apoptosis can be identified in mammalian cells, both are controlled by caspases and eventually converge on 'executioner' caspase-3[4], which is responsible for the cleavage of structural cytoplasmic and nuclear proteins, with consequent cell death and collapse.[5]

Phosphatidylserine (PS), made by the two PS synthases PSS1 and PSS2, is normally located on the inner leaflet of the plasma membrane, but undergoes transbilayer movement during apoptosis and becomes exposed on the cell surface.[6] Annexin-V FITC (BD Biosciences) binds to the exposed PS residues in a calcium dependent manner, after a rise in nuclear calcium concentration that causes the translocation of the calcium regulated proteins to the nuclear envelope.[7] This mechanism, usually a pivotal step in the recognition and removal of apoptotic cells by phagocytes[8], allows the attachment of the Annexin V-FITC antibody and allows us to visualise apoptotic cells via flow cytometry.

The purpose of this study was to evaluate whether a range of both early passage cultures and established cell lines, derived from patients with malignant glioma, would display different sensitivities, with regard to apoptotic cell death, when exposed to CLOM. This, combined with other assays previously carried out by the research group, could go some way towards defining *in vitro* markers for patient response to CLOM.

Materials and methods

Cells; the following cell cultures were used:

SNB-19 – an established glioblastoma muetiforme GBM (grade IV) cell line p20-24, derived from a 47-year old male (DSMZ Cell Bank)

DK-MG – an established GBM (grade IV) cell line p23-27, derived from 67-year old female (DSMZ Cell Bank)

UPAB – a primary GBM (grade IV) cell culture set up 'in house', p11-14, derived from a 73 year-old male

UPMC – a primary GBM (grade IV) cell culture set up 'in house', p9-12, derived from a 69 year-old female

UPJM – a primary astrocytoma (grade II) cell culture set up 'in-house', p7-10, derived from 42 year-old male

CC-2565 – a normal human astrocyte cell line, p4-6, derived from an 18 year-old male (Cambrex Biosciences).

Annexin V analysis

The apoptosis assay was used to determine the mechanism of cell death according to drug concentration and period of incubation. Cells grown to 90% confluence in $25cm^3$ flasks were incubated with 10X concentrations of CLOM (20, 40, 60, 80 & 100μM), added to flasks at 1:10, for up to 6 hours. Cells were harvested by firstly removing the complete media to centrifuge tubes (to ensure that all cells are subject to analysis), before adding 1.0ml of clear Tryplexpress (a non-enzymatic rapid dissociation solution; Gibco). During the two-minute dissociation period, flasks were placed in the incubator (37°C, 5% CO_2) to maintain the optimum temperature. The Tryplexpress was removed by centrifugation at $200g_{av}$ after neutralisation with 10% complete media.

Following staining the cell pellet was resuspended in 1ml of 1X calcium binding buffer, which triggers translocation of calcium-regulated phosphatidylserine residues to the nuclear envelope, before removing 500µl of the single cell suspension to a FACS tube. Controls used in the analysis were performed by omission of the drug incubation in one flask, and addition of 1µM staurosporine to one flask. These served as negative and positive controls respectively. Five microlitres of annexin V FITC and 5µl of propidium iodide were added to all tubes, by placing a drop of the fluorochrome on the side of the tube and inverting it. The tubes were incubated for 15 minutes at room temperature, in the dark. Subsequent to this, 400µl of binding buffer was added to each tube and analysed by the BD FACScalibur within 1 hour.

Results

After a six-hour incubation with CLOM the cell lines/cultures undergoing a marked degree of apoptosis, when compared against negative controls were DK-MG and SNB-19. Because of the slow-growing nature of the lower grade astrocytoma UPJM, some samples were not achieved due to lack of cells (20,000 minimum required for analysis). Although the percentage of apoptosis in UPMC appears high, when compared to the control values it demonstrates that CLOM does not exert any effect at the concentrations tested (see Table 1 and Figure 1).

From the five malignant gliomas tested, the two established cell lines had the lower apoptotic threshold, with a significantly higher percentage of apoptotic cells present at 60µM CLOM and above. The three early passage cultures, developed 'in-house' from biopsy, had higher apoptotic thresholds, withstanding up to 100µM CLOM incubation for six hours.

The normal human astrocytes, tested in parallel, demonstrated that CLOM did not cause cell death at the concentrations tested.

The cells at the highest passage number (DK-MG) are the most responsive to Clomipramine in this assay; this could be due, in part, to the homogeneity of the sample population. Also, it is of interest to note that the CLOM was less effective at causing apoptosis in the CC-2565 cell line than the staurosporine in the positive control sample (Table 1).

		SNB-19	DK-MG	UPAB	UPMC	UPJM	CC-2565
				Apoptosis (%)			
Control Sample		*1.25*	*2.05*	*2.27*	*10.77*	*3.03*	*0.36*
Staurosporine (6hr control)		*21.47*	*71.56*	*5.04*	*47.43*	*38.94*	*1.94*
20μM	6h	1.25	1.87	2.35	9.96	1.01	0.29
	5h	0.92	1.50	3.21	10.81	3.20	4.68
	4h	0.95	1.38	2.11	12.74	0.50	3.21
	3h	1.29	1.18	3.00	11.72	0.88	2.51
	2h	0.71	1.40	1.87	10.99	0.24	4.12
	1h	1.45	1.03	1.46	10.13	1.31	2.98
40μM	6h	3.67	1.95	2.84	11.67	-	3.62
	5h	2.69	2.64	3.45	13.32	-	3.28
	4h	2.13	1.30	4.19	14.11	-	2.92
	3h	2.11	5.39	2.93	12.41	-	1.55
	2h	2.64	4.26	3.00	12.31	-	3.74
	1h	2.72	3.14	4.01	14.03	-	3.32
60μM	6h	4.84	**23.27**	3.86	14.26	-	2.86
	5h	5.14	**21.44**	3.40	12.16	-	0.03
	4h	**11.50**	**51.25**	2.99	12.80	-	5.65
	3h	**10.98**	**18.75**	3.71	13.62	-	0.32
	2h	**5.9**	**26.53**	3.66	12.33	-	4.49
	1h	**5.31**	**19.75**	3.08	13.47	-	3.52
80μM	6h	**5.89**	**27.13**	3.00	14.22	12.32	0.23
	5h	**15.91**	**20.86**	4.36	14.02	10.00	1.65
	4h	**10.55**	**38.92**	3.18	15.88	10.47	0.15
	3h	**4.98**	**42.25**	3.31	15.82	-	0.05
	2h	**9.18**	**23.76**	2.51	13.19	-	0.02
	1h	**10.27**	**23.41**	3.71	14.59	-	0.00
100μM	6h	**11.03**	**49.16**	3.91	10.97	10.73	0.36
	5h	**2.57**	**23.18**	2.77	12.91	8.64	0.01
	4h	**10.91**	**22.81**	2.97	11.64	5.99	3.21
	3h	**12.58**	**17.74**	4.98	11.94	10.28	0.04
	2h	**17.13**	**20.66**	4.68	10.76	6.50	0.05
	1h	**11.00**	**30.84**	2.79	9.41	-	0.38

Table 1. A summary of the apoptosis data obtained by Annexin V flow cytometry, highlighted are the samples at which apoptosis (defined as a sample with more apoptosis than the negative control) was achieved when compared to the negative and positive controls, which were cells with no drug and cells with staurosporine added respectively. This table illustrates the differences in apoptotic sensitivity of the cell lines; with DK-MG being the most responsive when compared to the control values.

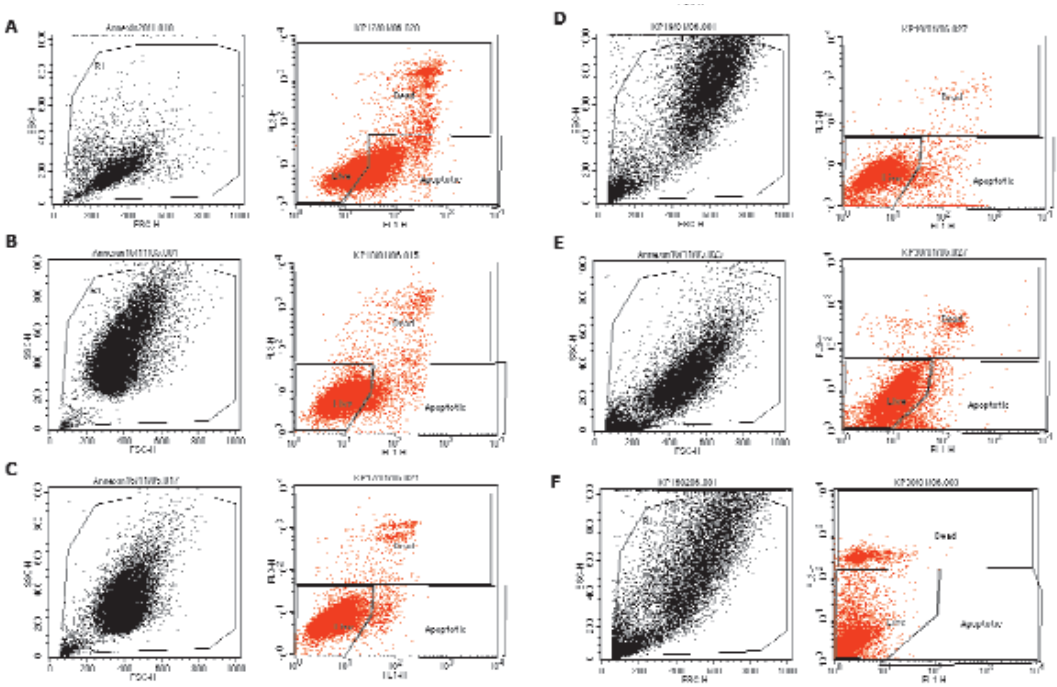

Figure 1. Annexin V Data for DK-MG (A), SNB-19 (B), UPAB (C), UPMC (D), UPJM (E) and CC-2565 (F). Plots showing the side scatter and gating selected for this assay (left) demonstrate the difference in cell size and heterogeneity of the samples. The plots of interest shown (right) are samples analysed after a 6-hour incubation with 100ĶM Clomipramine Hydrochloride. The FL-1 detector detected annexin-V binding; the FL-2 detector detected cells counterstained with isotonic propidium iodide. The percentage values (%) for apoptotic and dead cells, respectively, are as follows A = 49.16; 13.23, B= 11.03; 5.96, C=3.91; 4.76, D=10.97; 2.24, E= 10.73; 4.85, F=0.36; 11.28.

Discussion

CLOM has previously been reported to exert an apoptotic effect by Xia *et al.*[9], on human myeloid leukaemia HL-60 cells (50μM), and Levkovitz *et al.*[2] on C6 glioma cells (25μM) and human neuroblastoma SH-SY5Y cells (20μM). Significant morphological changes following incubation with 12μM CLOM, represented by red (propidium iodide) fluorescence of fragmented apoptotic nuclei, were observed by Levkovitz *et al.*[2] when compared to blue (hoechst) fluorescence of the intact nuclei treated with vehicle (saline). Similar morphological findings were presented by Daley, E[10], when human malignant glioma cells were incubated with CLOM (maximum incubation period of 4 hours) and subsequently stained with ethidium bromide and acridine orange. Internucleosomal DNA fragmentation measured by electrophoresis in glioma cell lines was also demonstrated by Daley, E[10], confirming DNA laddering and hence condensation of chromatin, the 'classic' hallmark of apoptosis. These findings, from the literature on CLOM, confirm the potent apoptotic effect that CLOM has on tumour cells. They also observe the higher resistance of primary cell cultures[11] which can be accounted for by the high proportion of non-neoplastic cells maintained in these short-term, low passage, cultures.

This was reflected in the results of this study, whereby the control normal human

brain astrocytes (CC-2565) were unaffected by CLOM. It is tempting to postulate that a population of normal astrocytes remains in the primary cultures developed 'in-house'; further subculturing (leading to increased homogeneity of cell populations) and analysis may reveal passage-dependent apoptotic sensitivity to CLOM.

It may be possible, in a larger study, to predict individual patient response to CLOM using the Annexin-V assay, alongside Bcl-2 analysis and CYP gene testing, on the patients own tumour cells. Bcl-2 analysis, performed previously in our laboratories by Daley, E[10] demonstrated that Bcl-2 expression correlated with apoptotic rate. Bcl-2 prevents cytochrome C release, and hence glioma cell lines expressing a high percentage of endogenous Bcl-2 had the lowest apoptotic rate.

A common clinical observation within a patient cohort, diagnosed with the same tumour type, is the appearance of a few 'responders' who respond very well to the test agent, and a majority of non-responders who do not gain any advantage from the test agent.[12] One explanation for this could be the multidrug-resistant phenotype of brain tumours; Andersson *et al*.[13] found a large heterogeneity in the expression of different resistance markers (P-glycoprotein, PgP; Multidrug resistance protein, MRP1; lung-resistance related protein, LRP and O(6)-methylguanine-DNA methyltransferase, MGMT). The next steps in this research are to combine CLOM with other potentially synergistic agents to enhance to apoptotic effect. It may also be possible to isolate cancer stem cells and/or other 'clones' from heterogeneous primary glioma to test the resistance of subpopulations. The significance of further studies on Bcl-2, which acts upstream of the mitochondria, is that the expression may enhance the survival of cancer cells.[3] The difference in sensitivities between glioma, in this small study, indicates the importance of analysing early passage cultures, which retain original morphology and characteristics to a greater extent, alongside established cell lines.[14]

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review

Complement resistance impairs anti-tumour therapy

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Background. *Various studies during the last two decades clearly indicate that resistance of human tumour cells to autologous complement is mainly based on the expression of membrane-bound complement regulatory proteins (mCRP) like CD59, CD55 and CD46 with good evidence for a predominant role of CD59. Beyond these in vitro findings the importance of this phenomenon for the patients` outcome now becomes evident from first clinical studies. Overcoming complement resistance of tumour cells is therefore considered a promising way to improve therapeutic options and prognosis in a variety of cancer diseases. In this short review two feasible approaches are discussed in more detail: (1) neutralisation of mCRP by monoclonal or recombinant antibodies and (2) gene silencing strategies to down-regulate mCRP by blocking the expression of these proteins on the RNA level using siRNA.*

Conclusions. *As mCRP are also present on all normal tissues like endothelial cells, parenchymatous organs (liver, kidney etc.) or blood cells, mCRP blocking strategies have to be targeted selectively to malignant cells sparing the surrounding healthy tissues from the deleterious complement attack. Despite first encouraging results, translation of mCRP inhibition to improve antibody-based immunotherapy into the clinic is still a great challenge.*

Key words: neoplasms – drug therapy; immunology; complement inactivators

Introduction

The complement system is a cascade of serin proteases that plays an important role in the immune defense, linking innate and acquired immunity.[1] Activation of complement results in the release of highly potent proinflammatory molecules, the so-called anaphylatoxins, in the formation of the lytic membrane attack complex (MAC), C5b-9, as well as in the opsonisation of pathogens and immune complexes for efficient phagocytosis. To protect themselves from unrestricted complement attack, all cells exposed to complement express various membrane complement regulatory proteins (mCRP), such as membrane cofactor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and CD59.[2] In the last years, multiple studies have shown that complement resistance of tumour cells is a widespread phenomenon that is based on various mechanisms like secretion of soluble complement inhibitors or soluble forms of mCRP, respectively, into the microenvironment[3-7], expression of sialic acid[8] or complement cleaving proteases.[9]

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Also rebinding of secreted soluble complement inhibitors to the tumour has been observed.[10,11] The most important mechanism, however, is the overexpression of one ore more of the membrane-bound complement regulatory proteins CD46, CD55 and CD59.[12,13] Although the influence of each mCRP varies between different tumour cell lines and has to be determined separately, there is strong evidence for an exceptional role of CD59, that blocks the assembly of the membrane attack complex (MAC) by interfering with the insertion of C9 thereby preventing the formation of the lytic pore. The functional importance of CD59 has been underlined by several approaches: whereas the mere number of mCRP only in part correlates with tumour cell resistance to complement-mediated lysis, transfection of CD59-negative tumour cells with CD59-cDNA increases their complement resistance considerably.[14-16] Moreover, many studies have demonstrated that neutralisation of CD59 but also of other mCRP by using monoclonal antibodies significantly increases the susceptibility of cancer cells to complement-mediated killing.[13]

Complement resistance as a prognostic factor?

There are only few clinical studies yet to underline the functional importance of complement resistance for tumour cell survival and disease progression.

Recently, Watson *et al.*[17] showed that expression of CD59 goes along with a deterioration of the patients' prognosis in colorectal carcinomas. Furthermore, the expression of CD59 correlated with local tumour progression and tissue dedifferentiation in prostate cancer.[18] High levels of CD59 are associated with an earlier biochemical relapse measured by increasing PSA levels after radical prostatectomy. However, contradicting data for other tumours do not allow to generalise about the potential impact of mCRP expression levels on disease prognosis. In a study with breast cancer patients, the loss of CD59 expression could be found to go along with a reduced over-all-survival.[19]

Also other mCRP and their association with the disease prognosis have been studied. The overexpression of CD55 seems to predict a poorer prognosis in patients with colorectal cancer.[20] The 7-year survival of patients with high expression levels of CD55 was remarkably lower than that of patients with low expression levels (24% vs. 50%). For breast tumours, Madjd *et al.*[21] found that overexpression of CD46 correlated with worse histological staging and a higher risk of tumour recurrence. Interestingly, in certain malignancies the loss of CD55 or CD59 may also result in more aggressive tumour growth (bigger tumour size, worse grading, higher rate of lymph node metastases) and a poorer prognosis.[19,22] For gastric carcinomas a correlation between CD97(EGF) and CD55, respectively, and tumour invasion into the surrounding tissue is reported.[23] High expression profiles of these two molecules go along with aggressive local tumour growth and a higher pathological and clinical staging.

All in all, overexpression of mCRP by cancer cells and its possible influence on patients' mortality seems to be rather heterogenous and has to be examined separately for each type of cancer.

Impact of complement resistance on immunotherapy

Complement resistance has gained significant importance with the introduction of anti-tumour immunotherapy. It not only influences the course of disease but also the patients' prognosis by impairing therapeutic options.

The rapid progress in molecular biology and recombinant antibody technology during the last two decades promoted immunotherapy of malignant diseases. Since then, anti-tumour antibodies successfully made their way from the laboratories to the clinic and meanwhile present a well-established adjuvant therapy regimen for a variety of cancer diseases (Table 1).[24]

Classical murine monoclonal antibodies derived from hybridomas according to Köhler and Milstein[25] could not succeed in clinical testing because of the risk of severe anaphylactic reactions and formation of neutralising human anti-mouse-antibodies (HAMA) with rapid loss of effector functions.[26] With the advent of recombinant technology, `designer' antibodies became a powerful tool in anti-cancer therapy. Beyond the well-known classical antibody effector functions such as antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), there are additional effects on the target cells that rather depend on the epitope than on the antibody itself.[27] These so-called epitope-specific antibody effects can trigger apoptosis or can modulate the auto- and paracrine secretion of tumour cells, thus influencing the tumour's microenvironment.[28] It is often difficult to determine which effect is most important for the antibody's anti-tumour response.

Despite the great success of recombinant antibodies in cancer therapy, clinical oncologists and tumour immunologists are

Table 1. Anti-tumour antibodies in clinical use

MAb name	Trade name	Target	Type	Approval date	Used to treat
Rituximab	Rituxan	CD20	IgG1, Chimeric	1997	Non-Hodgkin lymphoma
Trastuzumab	Herceptin	$p185^{neu}$	IgG1, Humanised	1998	Breast cancer
Gemtuzumab-ozogamicin*	Mylotarg	CD33	IgG4, Humanised	2000	Acute myelogenous leukemia (AML)
Alemtuzumab	Campath	CD52	IgG1, Humanised	2001	Chronic lymphocytic leukemia (CLL)
In-111/Y-90-Ibritumomab-tiuxetan*	Zevalin	CD20	IgG1, Murine	2002	Non-Hodgkin lymphoma
Daclizumab	Zenapax	CD25	IgG1, Chimeric	2002	Acute and Chronic leukemia
I-131-Tositumomab*	Bexxar	CD20	IgG2, Murine	2003	Non-Hodgkin lymphoma
Bevacizumab	Avastin	VEGF	IgG1, Humanised	2004	Colorectal cancer
Cetuximab	Erbitux	EGFR	IgG1, Chimeric	2004	Colorectal cancer

* conjugated monoclonal antibodies

confronted with limitations of this approach. Similar to the well-known phenomenon of chemoresistance of tumours, *i.e*. the capacity of certain tumour cell clones to become refractory to cytostatic agents, there is also a phenomenon of resistance to antibodies.[29] After repetitive treatment cycles, tumour cells get resistant against further antibody therapy. Several mechanisms may lead to antibody resistance, *e.g* down-regulation of the target epitope or diminished effector functions. Various studies indicate that the up-regulation of mCRP, namely CD55 and CD59, is responsible for resistance against CD20 serotherapy with rituximab.[29-32] Blocking of these regulatory molecules can restore the tumour cells' susceptibility to rituximab *in vitro*.[30,31] The cytotoxic effects of the anti-her2/neu antibody used in the therapy of metastased breast tumours could be augmented by blocking of mCRP *in vitro*.[33]

From these data mCRP appear as interesting target epitopes for new adjuvant therapeutic regimen.

Strategies for tackling complement resistance on human tumours

The significance of complement resistance of human tumours became obvious through multiple experiments applying murine monoclonal antibodies that blocked mCRPs. [4,6,12,13,34,35]

However, translation of these findings into the clinic is hampered by two major obstacles: (1) to find the most effective and secure way of mCRP neutralisiation and (2) restriction of the potentially dangerous intervention to cancer cells.

Different to the benchside situation, a therapeutic strategy must be tolerable for the patient. Blocking mCRP by murine monoclonal antibodies is not appropriate (for reasons as discribed above). Two promising approaches have been developed for the future clinical application, which, however, still require comprehensive preclinical investigation.

Bispecific mCRP-blocking antibodies

For antibody-based immunotherapy the possibility to generate bispecific antibodies that can recognize two different epitopes by their two different antigen binding sites widens the scope and improves the chances to generate truly tumour-specific »magic bullets«.[36,37] Bispecific antibodies, which allow mCRP inhibition to be restricted to tumour cells *in vitro* have been produced by various means.[38-41] Harris *et al*.[40] generated chimeric anti-CD59 x anti-CD19 and anti-CD59 x anti-CD38 antibodies by chemical linkage. B cell specific binding and lysis could be observed while sparing surrounding bystander cells. Although this work served as »proof of principle«, the chemical synthesis of bispecific antibodies is a cumbersome procedure and inappropriate for clinical testing. Blok *et al*.[41] obtained murine bispecific anti-CD55 x anti-G250 antibodies applying classical hybridoma or quadroma technology with good activity against renal cell carcinomas *in vitro*. Recently, a bispecific monoclonal anti-CD55 x anti-MHC class I antibody proved its efficacy on human colorectal and cervix carcinoma cell lines resulting in elevated C3-deposition and augmentation of complement-mediated cell lysis.[39]

For therapeutic approaches the use of humanised or at least chimeric antibodies is mandatory. These bispecific antibodies are nowadays constructed by recombinant »antibody engineering«.[42]

However, despite all progress in the field of recombinant antibody technology it remains difficult to obtain continuously sufficient amounts of bispecific antibodies for *in vivo* testing in experimental animals or even clinical studies. The best established

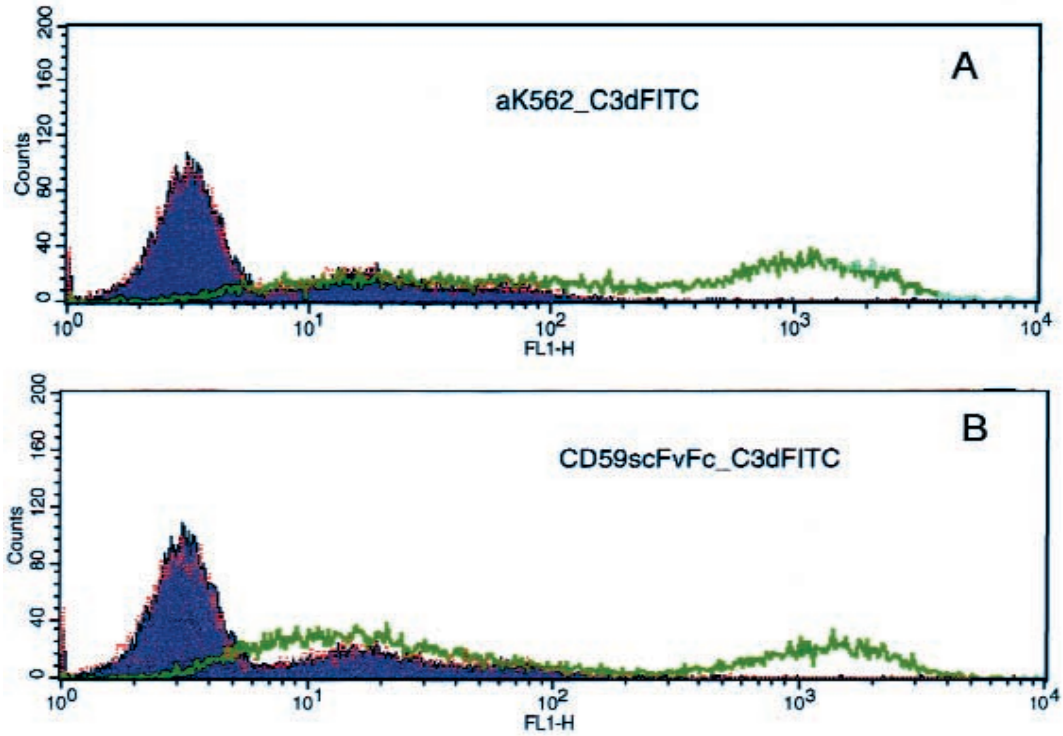

Figure 1. Blocking of CD59 augments tumour-directed complement activation: FACS-Scan for C3 (C3d) detection on human K562 erythroleukemic cell line after preincubation with polyclonal rabbit-anti-K562 or chimeric anti-CD59-miniantibody and pooled human serum as complement source. (A) Positive control with polyclonal rabbit-anti-K562 (green line), (B) Chimeric anti-CD59-scFv-Fc (green line). (Underlied curves each show two negative controls with heat inactivated serum or with irrelevant human IgG, respectively).

way to produce humanised bispecific antibodies takes advantage of expression vectors which contain the antibody genes. These vectors are commonly transfected into mammalian or insect cells that subsequently secrete the recombinant antibody into the cell culture supernatant. However, this technology still suffers from difficulties in achieving stably transfected clones, varying and vanishing protein production yields, a highly inefficient heterodimerisation of the different antibody chains, and problems with the purification of the heterodimeric bispecific antibodies. Despite the fact that there are several strategies which may help to overcome these difficulties, construction and expression of recombinant bispecific antibodies remains a »high risk challenge« far away from laboratory routine and with still unpredictable outcome.

We recently developed a chimeric mouse/human anti-CD59 miniantibody (scFv-Fc) from a murine hydridoma (MEM43) that was able to trigger C3-deposition on human tumour cells via the Fc-mediated classical pathway although it failed to significantly augment complement-dependent killing (Figure 1). [43]

Ziller *et al.*[44] generated humanised anti-CD59 and anti-CD55 miniantibodies, that were able to trigger complement-mediated lysis on human lymphoma cell lines. Furthermore, the lytic effect of rituximab could be augmented by these antibodies.

Silencing of mCRP genes

Another approach for tackling complement resistance of human tumours is RNA interference (RNAi). By using small interfering RNAs (siRNA) this technique offers great potential as a novel therapeutic strategy in tumour therapy but also in a wide field of other possible applications.

SiRNA technology, known since 2001, is based on short double-stranded RNA oligomers which cause highly specific and efficient silencing of target genes by posttranscriptional gene knockdown (Figure 2).[45] The antisense-strand of the siRNA molecule is complementary to the mRNA of the target protein.

SiRNAs induce the intracellular formation of a protein-complex, called „RNA-induced silencing complex (RISC)« consisting of helicase and nuclease-acitivity among others. The RISC-complex induces the separation of the sense and antisense strand, mediates the recognition of the target mRNA and catalyses the degradation of bound mRNA. The result is the specific inhibition of target-protein synthesis.

Although siRNA and its functionality in mammalian cells was detected just 5 years ago, plenty of studies demonstrating the therapeutic potential of siRNA have already been published. *In vivo* studies showed positive results applying siRNA for the therapy of neoplastic diseases[46-48], the treatment of sepsis[49] and the reduction of cholesterol levels.[50] Meanwhile the first clinical trial of siRNA therapy of the age-related macula degeneration (AMD) has been started.

To better exploit complement for cancer cell eradication, we tried to reduce complement-resistance of neoplastic cells by blocking mCRP function using siRNA-technology. SiRNAs targeting the mCRPs CD59, CD55 and CD46 were designed and tested concerning their downregulation efficiency *in vitro*. In this study siRNAs were either in-

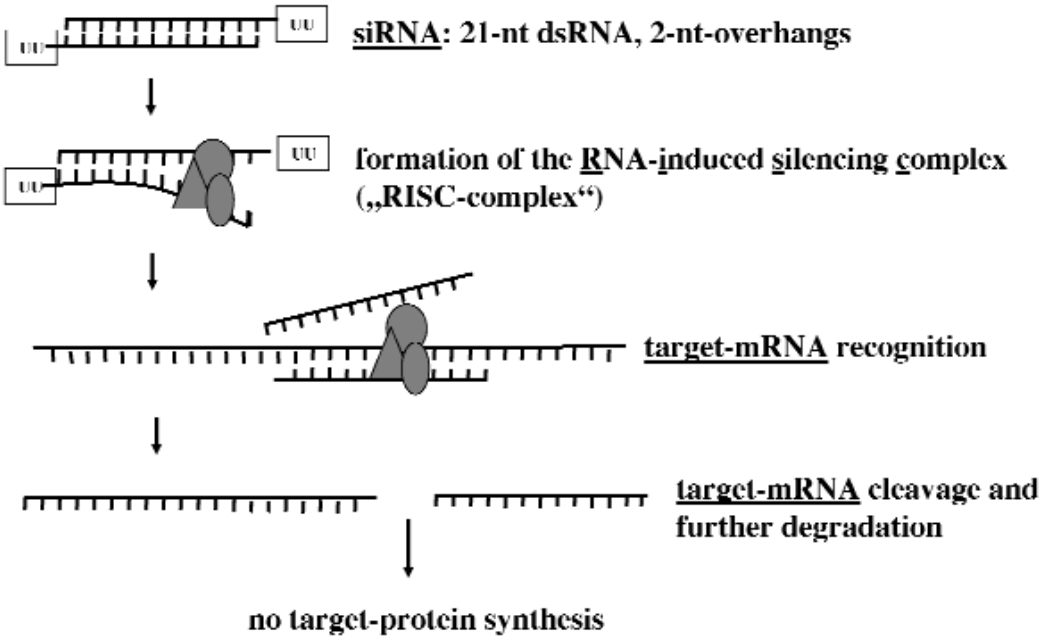

Figure 2. Schematic presentation of siRNA-induced silencing mechanism.

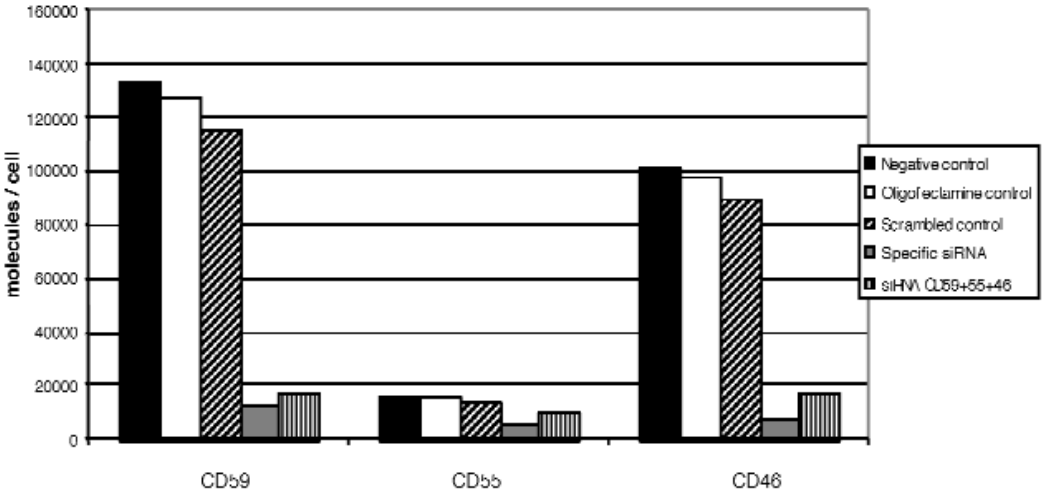

Figure 3. Cell surface expression of mCRPs CD59, CD55 and CD46 on BT-474 breast carcinoma cells after transfection of the corresponding anti-mCRP siRNA individually or in combination, respectively.

dividually or combined transfected into Du145 prostate carcinoma cells or BT474 breast carcinoma cells, respectively. The inhibition of target protein expression was analysed both on protein level by FACS analysis and on mRNA level by RT-PCR. Downregulation of mCRP up to 80% could be achieved (Figure 3). Complement-resistance of CD55-, CD46- and/or CD59-deficient tumour cells, subsequently evaluated by cytotoxicity assays and by analysis of C3 deposition, clearly indicated that siRNA-induced inhibition of mCRP expression sensitised tumour cells to complement attack.[51]

Despite these encouraging findings and the outstanding potency and selectivity of siRNA, promising to improve targeted cancer therapy, the systemic administration of aqueous siRNA, even chemically stabilized, is still limited by unspecific side effects and a lack of activity in the target tissue due to limited blood stability on the one hand and poor intracellular uptake on the other hand.[46,52,53]

The need for devices enabling systemic administration and targeted delivery to tumour tissue and disseminated metastatic lesions is obvious.

Strategies based on viral vector delivery would be a possible approach but for safety reasons they are hitherto only of limited clinical use. A feasible approach, providing tissue selectivity and safe systemic delivery is based on immunoliposome-technology.[54] Liposomes are widely investigated for their properties as site-specific drug carriers allowing higher drug doses due to fewer systemic side effects.[55,56] Liposomes are able to alter the pharmacokinetic profile of a drug, delivering the encapsulated agent preferentially to solid tumours, and acting as a slow-release depot for the drug in the diseased tissue.[57] These attributes often result in a more favourable toxicity profile and an improved therapeutic window for the use of the agent.

Though conventional liposomes allow passive tumour site targeting to some degree, the idea of conjugation of cell-specific antibodies to liposomes (immunoliposomes) has been studied for selective drug delivery.[58-60]

Tumour-associated antigens can be utilised as appropriate target molecules. Monoclonal antibodies against tumour-associated antigens have been successfully adopted for targeting to various types of cancer cells.[61]

Internalisation of immunoliposomes by receptor-mediated endocytosis into target-cells results in intracellular drug delivery.

A variety of cytotoxic drugs have been delivered to target cells *in vitro* by using immunoliposomes; *e.g.* doxorubicin, vinorelbine, methotrexate[62] and daunomycin.[63] Anti-HER2 immunoliposomal doxorubicin is awaiting Phase I clinical trials. Furthermore, immunoliposomes have been employed to deliver oligodeoxyribonucleotides (ODN) designed to specifically inhibit gene expression by blocking translation, splicing or transcription process *in vitro*, thereby providing powerful therapeutic tools against viral diseases and cancer.[64] Moreover, *in vivo* knockdown of gene expression with intravenous RNA interference (RNAi) using a small hairpin RNA (shRNA) expression plasmid encapsulated in immunoliposomes has been shown.[65]

To conclude, immunoliposomes containing siRNA combine specific antibody-mediated tumour recognition with gene-specific downregulation of target mRNAs.

Another promising approach of targeted siRNA delivery *in vivo* has been achieved by complexation of chemically unmodified siRNAs with polyethylenimine (PEI).[66,67] Self-assembling nanoparticles constructed with polyethylenimine were adapted for siRNA. Target-specific delivery can be achieved by attaching peptide ligands (*e.g.* to bind to integrins) to the nanoparticle.

Furthermore, a protamine-antibody fusion protein for systemic, cell-type specific, antibody-mediated siRNA delivery was developed recently.[68] This approach takes advantage of the non-covalent nucleic acid-binding properties of protamine, which originally nucleates DNA in sperm. In combination with the site-specific delivery properties of the antibody Fab fragment this fusion protein is a feasible device to administer siRNA systemically.

Conclusion

Complement resistance is a widespread and nowadays well examined mechanism that enables tumour cells to withstand autologous immune attack. A magnitude of *in vitro* and several *in vivo* studies support the notion that blocking of mCRP is a feasible approach for tackling cancer cells. By means of modern recombinant technologies humanised bispecific anti-mCRP-anti-tumour antibodies and siRNA based immunoliposomes for mCRP gene silencing are promising strategies that could allow transferring experimental complement research to clinical application. Encouraging results from *in vitro* and animal studies have to be reproduced and then could widen the scope of clinical anti-tumour therapy.

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review

Cysteine cathepsins and their inhibitors in head and neck cancer: an overview of research activities at the Institute of Oncology Ljubljana and ENT Department at the Clinical Center Ljubljana

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To determine the type and extent of the therapy needed for a successful treatment of cancer or to predict clinical outcome, an accurate risk stratification is required. The hypothesis on predictive and prognostic implication of individual cathepsins and their inhibitors originated in their involvement in pericellular proteolysis that participates in virtually all aspects of normal life of a cell and is involved also in the degradation of extracellular matrix barriers during the invasion and metastasizing of tumor cells. The role of cathepsins and their inhibitors in cancer may be categorized as follows: screening markers for diagnosis; predictive markers for lymph node metastasis; predictive markers for response to therapy and for recurrent disease; markers for prognosis. Although the investigations on clinical utility of cathepsins and their endogenous inhibitors in head and neck cancer are limited, the results warranted further evaluation. In the present review, we reported our experience and results gained during a decade of clinically oriented research and made comments on their predictive and prognostic value for routine clinical setting.

Key words: head and neck neoplasms; cysteine endopeptidases; cysteine proteinase inhibitors

Introduction

Head and neck cancer is the sixth most prevalent cancer worldwide. Sites of tumor origin are the organs of the upper aerodigestive tract, *i.e.* oral cavity, pharynx, larynx, salivary glands, nasal cavity and paranasal sinuses. More than 95% of tumors are of epithelial origin, with alcohol and tobacco abuse being common etiological factors.[1]

At presentation, two thirds of patients have locally and/or regionally advanced tumors, and the 5-year survival rates have not improved significantly during the last decades, remaining at 50%.[2] Conventional UICC/AJCC TNM staging system and established histopathological characteristics allow us only an approximate insight into the inherent biological aggressiveness of individual tumor. At the moment, none of the

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Table 1. Studies on cysteine cathepsins and their inhibitors in head and neck cancer: Ljubljana experience

Study details	Sample type		
	Serum	Cytosol	Tissue section
No. of patients	35, (1998, Ref. 6-8)	Group 1: 45 (1995, Ref. 6, 9, 10) Group 2: 49 (1998, Ref. 11-13) Group 3: 93 (2005)[1]	75 (2005)[1]
Tumor type(s)	OC, OP, HP, L	OC, OP, HP, L	OP
Therapy	SURG+RT	SURG+RT	RT+CT
Analytical methods	Sendwich ELISAs (KRKA dd & Institute Jožef Stefan Ljubljana, Slovenia)		

[1] Unpublished data.

OC, Oral cavity; OP, Oropharynx, HP, Hypopharynx; L, Larynx; SURG, Surgery; RT, Radiotherapy; CT, Chemotherapy.

candidate markers within the wide spectrum of biochemical and histological factors adds significantly to the prognostic information obtained from conventional prognosticators.

Cysteine cathepsins B, H and L are lysosomal proteolytic enzymes. They are implicated in virtually all aspects of normal life of a cell as well as in the degradation of extracellular matrix barriers during the invasion and metastasizing of tumor cells. Endogenous inhibitors of cysteine cathepsins constitute a cystatin superfamily, subdivided into several families (stefins, cystatins, kininogens, thyropins). In normal cells, the activation of proteolytic pathways is conducted in cascade manner and controlled by inhibitors. In the tumor tissue, the regulation of this cascade is altered as a result of the modulation of one or more mechanisms regulating the synthesis, transport and release of the involved enzymes and inhibitors.[3,4]

The predictive and prognostic value of cysteine cathepsins and their inhibitors was widely investigated in breast, lung, and colorectal carcinoma, but not also in head and neck cancer.[5] The main reasons are low incidence of the latter and its heterogeneity deriving from the diversity of possible primary sites inside the upper aerodigestive tract, each with its own natural history and treatment outcome.

The aim of the present report was to summarize the results of our research work collected during the last decade in the field of cysteine proteases and their inhibitors in head and neck cancer.

Ljubljana experience

Our experience originated in 1995. During a decade of systematic research, we tested the prognostic and predictive role of cysteine cathepsins and their inhibitors in several independent groups of patients and in different types of biological samples, *i.e.* serum, tissue cytosols, and recently also tissue sections (Table 1).[6-13]

We gained the most extensive experience from the studies on cytosols prepared from the tumor tissue of operable tumors, treated with surgery and postoperative radiotherapy. However, the main drawback of these studies was a relative heterogeneity of

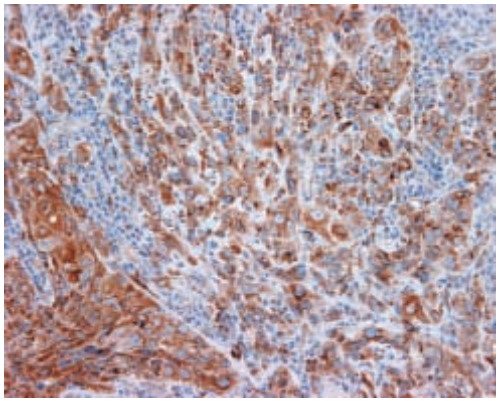

Figure 1. Cathepsin B immunohistochemistry is strongly positive in the cells of moderately differentaiated invasive squamous cell carcinoma.

the included primary tumors; one half of them were laryngeal tumors, whereas the others originated from the oral cavity, oropharynx or hypopharynx. From this viewpoint, much more homogenous was a group of patients from our recent study on tissue sections in which only those with inoperable carcinoma of the oropharynx were included; they were treated uniformly with irradiation and concomitant chemotherapy with Mitomycin C and Bleomycin. Highly positive and extensive immunohistochemical reaction in tumor cells (more than 50% of the cells with positive cytoplasmic reaction) was observed in the case of cathepsin B (Figure 1) and stefin A, whereas cathepsin L and stefin B immunohistochemistry was less pronounced (minimal, ≤10% positive cells) or modest (10-50% positive cells). Analyzing non-malignant stromal cells in the tumors, reactivity to the cathepsins and stefins were recognized also in lymphocytes and ductal cells. The immunohistochemical reaction in the former case was scored as modest and in the latter case as minimal (unpublished data).

Furthermore, in all groups of patients, the same kits for biochemical determination of studied cathepsins and stefins were used, *i.e.* the commercially available ELISEs developed at the Jožef Stefan Institute. Because the tests kits have been modified during the years as has also been the methodology for tissue cytosol preparation, the results of measurements in individual groups are not directly comparable.

Prediction of lymph node metastasis

The possibility to predict cervical lymph node infiltration with tumor cells from a primary tumor biopsy specimen would be of critical importance for treatment optimization. The presence of lymph node metastases is the single most adverse prognostic factor in head and neck cancer, reducing 5-year overall survival rate up to 50% compared to node negative patients.[14] Primary tumor-related histopathologic factors (site, T-stage, grade, growth pattern, thickness, perineural infiltration, and others) are not reliable enough in predicting lymph node metastases. Consequently, up to one third of clinically node negative necks at presentation are bearing lymph nodes infiltrated with tumor cells and, vice versa, a significant proportion of patients with palpable neck nodes or radiologically determined neck disease were actually disease free on the neck. In the latter case, nodal enlargement is caused by inflammatory processes in the affected node(s).[15]

The results of immunohistochemical studies published so far, analyzing the potential of cysteine cathepsins and their inhibitors for predicting tumor cell infiltration of regional lymphatics, were not conclusive.[16-18] We observed the same in our series for cathepsin L and both stefins. On the contrary, comparing the pattern of cathepsin B immunostaining between N_{0-1} and N_{2-3} subgroups (but not between N_0 and N_+ subgroups!) in the patients with inoperable oropharyngeal cancer treated with concomitant chemoradiotherapy, the difference

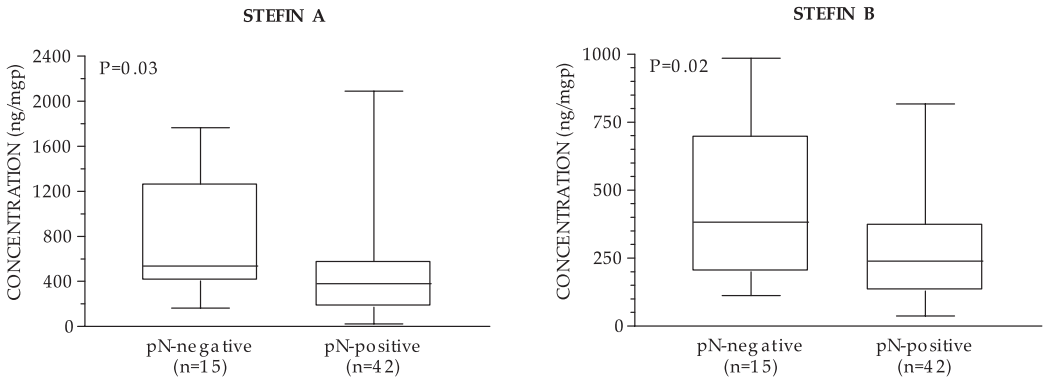

Figure 2. Distribution of tumor concentrations of stefins A and B between patients with histopathologically determined negative (pN0-stage) and positive (pN+-stage) necks, as measured in a group with clinically palpable nodes at presentation (cN+-stage). The top and the bottom of the box represent 25th and 75th percentiles, respectively and the end of the bars represents the rang. The line in the box is the median value. N, Number of patients. Reproduced by kind permission of Radiology & Oncology from Strojan P et al, Radiol Oncol 2002; 36: 145-6.

reached the level of statistical significance (Fisher exact test, P=0.03; unpublished data). However, as the key question is how to differentiate node-negative from node-positive necks, we have concluded that cathepsin B immunohistochemistry of primary tumor biopsy sample has no clinical implications in predicting the presence of metastases in the cervical lymphatics.

More encouraging were the results from the tissue cytosols. In the operated patients with clinically positive neck nodes at presentation (*i.e.* before surgery), a statistically significant difference in stefin A and stefin B cytosolic concentrations was calculated between the subgroup of patients who were actually disease-free in the neck and those with metastases confirmed on histopathological examination of the resected specimen (Figure 2).[12] This observation pointed out the ability of stefins to differentiate between the nodes enlarged due to inflammation and those with deposits of tumor cell, and raised a possibility of spearing a portion of cN_+ patients from more aggressive therapy and treatment related side effects. On the other hand, in the patients with clinically undetectable nodes at diagnosis, stefins had no potential to predict pN-stage of the disease.

Prediction of response to therapy

Tumor regression during external beam radiotherapy course is an independent predictive factor of local control in head and neck carcinomas. However, the regression is sequential: the maximum clearance rates for the primary were recorded during the treatment, whereas they were delayed for the nodes, with the maximal complete regression rate at about two months after irradiation.[19,20] One of the important mechanisms underlying tumor regression after ionizing radiation or chemotherapy is cell disintegration via apoptotic pathways in which cysteine cathepsins and their inhibitors have also been suggested to participate actively.[21]

According to our experience, only cathepsin B immunostaining showed some potential for predicting treatment failure (Fisher exact test, P=0.034; unpublished data). The latter was defined as no tumor response or only partial response (less than 100%

Table 2. Cysteine cathepsins as markers for prognosis in head and neck cancer: review of literature

Studies	Cathepsin B	Cathepsin H	Cathepsin L
Positive[1]	Russo et al, 1995 (Ref. 23) Oblak et al, 2005[3]	Strojan et al, 1999 (Ref. 6)	Budihna et al, 1996 (Ref. 9)
Negative[2]	Budihna et al, 1996 (Ref. 9) Strojan et al, 2000 (Ref. 11) Kawasaki et al, 2002 (Ref. 17) Strojan et al, 2005[3]	Kawasaki et al, 2002 (Ref. 17)	Russo et al, 1995 (Ref. 23) Strojan et al, 2000 (Ref. 11) Kawasaki et al, 2002 (Ref. 17) Oblak et al, 2005[3] Strojan et al, 2005[3]
No. of patients	404	96	387

[1]Prognostic value of cysteine cathepsins was confirmed.

[2]Prognostic value of cysteine cathepsins was not confirmed.

[3]Unpublished data.

regression) to applied chemoradiotherapy evaluated locally and regionally two months after finishing all therapies. We found low cathepsin B immunostaining being uniformly predictive (10/10 cases) for favorable clinical response; however, a substantial proportion of patients with highly positive CB staining were also complete responders (41/65 patients). It seems that cathepsin B immunohistochemistry *per se* is not specific enough and should not be used as a predictive marker of the tumor response to applied therapy independently from other markers. Evaluation in combination with other candidate markers is warranted.

The observation on low cathepsin B immunostaining being predictive for the favorable response of the tumor to radiochemotherapy directly contradicts the recognition of cathepsins as promoters of apoptosis which, in turn, leads into the reduction of cell number and, finally, the volume of the tumor. It seems that other, cathepsin B independent molecular mechanisms are involved in the irradiation induced apoptotic pathways. On the other hand, because complete tumor regression was recorded in a substantial proportion of patients with strongly positive cathepsin B tumors as well, we hypothesized that the ratio between the cathepsins and their inhibitors may also play a decisive role. For example, an evaluated inhibitor expression, blocking the intrinsic cathepsin B activity, was shown to rescue the tumor cells from TNF-induced apoptosis in experimental setting of the cell lines derived from primary and metastatic lesions of oropharyngeal squamous cell carcinoma.[22]

Markers for prognosis

In head and neck cancer, the prognostic value of cysteine cathepsins was studied much less extensively than in breast, lung, or colorectal carcinoma (Table 2).[6,9,11,17,23] With the exception of cathepsin H, the trend of higher survival probability correlates with lower levels of cathepsin B and cathepsin L. In the studies on tissue cytosols, however, no strong relationship with prognosis was established. As an immunohistochemical marker, only cathepsin B showed some association with the outcome of the disease; the latter was not confirmed on multivariate analysis (unpublished results).

We recognized stefins A and B and cystatin C as the most influential progno-

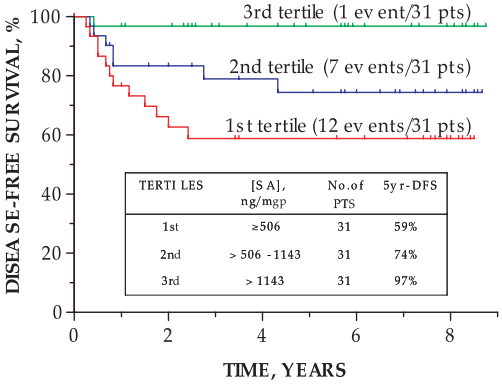

Figure 3. Disease-free survival as a function of stefin A status.

sticators in tumor cytosols. In our first two data sets from 1995 and 1998, higher cytosolic concentrations of any of the two stefins as well as those of cystatin C correlated significantly with longer disease-free interval on univariate survival analysis.[9,11,13] In multivariate model, only stefin A and cystatin C retained their independent prognostic information. However, when comparing the prognostic strength of the latter two, cystatin C lost its significant prognostic power for both survival endpoints under evaluation, disease-free survival and disease-specific survival.[13]

The prognostic strength of stefin A concentration as determined in tumor cytosol was reconfirmed recently on an independent dataset of 93 patients with operable head and neck cancer (unpublished results). After stratifying the patients according to stefin A concentration in 3 subgroups, we recognized an obvious pattern of improved survival probability with the increasing levels of stefin A (Figure 3). The maximal difference in survival rates between low and high stefin A subgroups was calculated at approximately 400 ng/mgp, which classified 29% of tumors as stefin A low and the rest as stefin A high. It is interesting that, in both historical data sets, the optimal cut-off concentration fell into the same range of measured values as it was the case in our recent group, *i.e.* around 30th percentile. On multivariate analysis, stefin A appeared as the strongest independent predictor of a disease-free survival in the model, irrespective of whether it was tested as continuously or categorically variable.

Conclusions

The results presented in this overview warranted further evaluation of cysteine cathepsins and their inhibitors as predictive and prognostic markers in head and neck cancer. In particular, this is the case when analyzing the stefin A concentrations from tissue cytosols. The latter confirmed its prognostic value in three independent data sets, given identical results in all three instances. In future, larger numbers and more homogenous (in regard to primary tumor site) populations of patients and standardization of analytical methods should be considered more rigorously to obtain maximally informative results applicable also to routine clinical practice.

Acknowledgement

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Complete yearly life tables by sex for Slovenia, 1982-2004, and their use in public health

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Life tables are an important tool in statistical analysis in many branches of science including public health and epidemiology. In Slovenia, they are recently mostly used in relative survival analyses. For this purpose, we need complete period life tables for each calendar year. Since such life tables have not been available for Slovenia, we calculated our own life tables for years in 1982-2004, stratified by sex. In the article we describe the methodology used for calculation and present some examples on the use of these life tables. The complete life tables are freely available by contacting register@onko-i.si or through the international Human Life-Table Database (http://www.lifetable.de/). We intend to produce life tables for following years as soon the necessary data will be available.

Key words: public health; life tables; survival analysis

Introduction

Life tables are the oldest demographic tool and still among the most important instruments for mortality analysis and other investigations concerning the length of life.[1] As suggested by some classical papers, already in Babylonian civilization individuals understood the idea of likelihood of death assessment.[2] First simple life tables were composed by the roman perfect Domitius Ulpianus in the third century. His technique of life table calculation was in use in the northern Italy till 1814.[2] English insurer Milne computed first totally regular life tables for the period 1779-1787.[3] In Slovenia this spadework was performed by dr. Ivo Lah who computed the life tables for Drava province for the years 1931-1933.[4] After the Second World War, life tables were produced by Yugoslavian and Slovenian statistical office. Today the life tables are used for statistical analysis in numerous branches of science as: demography, insurance, judiciary, public administration, public health, epidemiology, biology and other.[5]

Various forms of life tables are known. According to the age groups used they are divided into complete and abridged life tables.[6] The first are calculated for one-year age groups, from the age of zero to the last

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defined age. On the contrary, the abbreviated life table combines several years, as they presume the mortality rates in adjacent age groups are similar.[7] The second classification is distinguishing between cohort and period life table.[6] During the preparation of cohort life table a selected cohort is followed from its first birth till its last death. The procedure is very time consuming, and on the other hand, such data are rarely available, so cohort life tables are mostly produced for historical purposes. Instead of following one cohort life-long, we model what would happen to a hypothetical cohort if a certain set of mortality conditions pertained throughout its life in period life table. There are many additional forms of life tables, as exact and approximate life tables, adjusted and unadjusted life tables or life tables for population and subpopulations (by gender, occupation, social status etc.).[6]

This paper is focusing on the complete period life tables for the population of the Republic of Slovenia for each year from 1982 to 2004. Currently, the complete period life tables are available for four three-year periods only: 1980-1982, 1990-1992, 1993-1995 [5] and 2000-2002.[8] In addition, there are abbreviated life tables (five-year age groups) for all two-year periods between 1981 and 2001.[5,9] All these life tables were prepared by the Statistical Office of the Republic of Slovenia (SORS). They combine several years, as the Slovenian population is rather small, hence the probability of dying in certain age group tends to fluctuate. For the same reason SORS's life tables are adjusted as well.[5] Further on, World Health Organization published the abbreviated gender stratified life tables for Slovenia for years 2000 and 2001 separately.[10]

In public health, especially for the purpose of relative survival analysis, complete, unadjusted and gender specific life tables for single year are needed. These life tables cannot be used to evaluate the demographic attributes of Slovenian population, but only to compare a group of patients with their origin population. Therefore the exact probability of dying is required in relative survival analysis. For this purpose the complete, unadjusted and gender specific period life tables for Slovenia for each year in the period 1982-2004 were prepared in our study. The methodology applied is identical for all the tables provided, which makes them intercomparable.

Material and methods

The data needed for calculating the life tables were obtained from SORS:

- aggregated number of deaths by age, sex, year of birth and year of death for deaths in period 1982-2004 and
- aggregated number of residents by age and sex at the beginning of each year in period 1982-2004.

Information regarding data collection and population definition is published in Statistical Yearbook of the Republic of Slovenia by SORS.[11]

Probability of dying (q_x) is the basic indicator for mortality of population. This is conditional probability for person aged x years at the beginning of the year to die during the year conditionally on surviving x years in the first place. Probability of dying for age x is calculated as the ratio between the number of people that died during the observed calendar year and were aged x at the beginning of the year and the number of all living aged x at the beginning of the same calendar year.[7,12] Probability of dying is always one for selected highest age interval, which is hundred years and more in our case.

All the other variables in life tables are calculated from the probability of dying. Standard methods and notations were used

that are well known and easily found in literature [2,6,7,13], so they will not be explained here, but listed only. The notations used in life tables are given in brackets after the name of the variable.

Probability of surviving (p_x) is the probability of a person aged x years to survive exact age x+1. The number of persons surviving (l_x) is the number of persons who reach age x out of 100,000 live births. The number of deaths (d_x) is the annual number of deaths between ages x and x+1. The number of person–years (L_x) is the number of persons alive at any point in time between ages x and x+1. The total number of person–years (T_x) is the total number of years lived from age x to death. Life expectancy (e_x) is the average number of years a person aged x years can expect to live assuming that mortality rates by age will remain unchanged since the year of observation. Life expectancy at birth (e_0) is the mean age at death for persons dying in any particular year and is the most important indicator for population mortality.[1]

Probability of dying, probability of surviving, number of persons surviving and number of deaths are frequency (or intensity) measures since they show frequencies of events (deaths or survival). They are all defined within elementary age interval [x, x+1). Number of person–years, total number of person–years and life expectancy at birth are duration measures since they show amounts of lifetime and are measured in person-years.[13]

Results

The results of our calculations are complete, unadjusted and gender specific period life tables for Slovenia for each calendar year in the period 1982-2004. We calculated life tables separately for men and women, as generally there is significant difference in mortality by sex.[7] As an example, there are life tables for men and women for calendar year 2004 in Appendix. The life tables for all calendar years are freely available by contacting register@onko-i.si. Since our methodology is consistent with the methodology of Human Life-Table Database[13], our life tables are also included in their database available on Internet (http://www.lifetable.de/). We intend to produce life tables for following years as soon as we get the necessary data.

When calculating life table, special caution should be given to the age group zero years. In order to illustrate this problem we take a closer look at the year 2004. As there are no data on the number of residents at the beginning of 2004 available, we use instead the number of residents on December 31, 2003, published in the Statistical Yearbook of the Republic of Slovenia 2004.[11] However, newborns born for example in April 2004 and died in May 2004 are not accounted for in this report and have to be added extra in the denominator when calculating probability of dying for age group zero years. Data on newborns provided by Institute of Public Health of the Republic of Slovenia (IPH) cannot be applied in life table analysis since IPH has different population definition - only the number of babies born to Slovenian mothers in the territory of the Republic of Slovenia is reported by IPH. [14] However, in the SORS database the number of children aged zero to one year also includes all emigrant newborn babies and those who were born to Slovenian mothers abroad.[11] The difference is about 300 (or about two percent) newborn children each year.

Demographic data by age, year of birth and year of death are properly presented on Lexis diagram [15]. A cut out from Lexis diagram for males in 2004 is presented on Figure 1. We can see that there were 8909 males aged less than one year out of which 30 newborns were born in 2004 and also died in 2004. Additionally, 2 children were

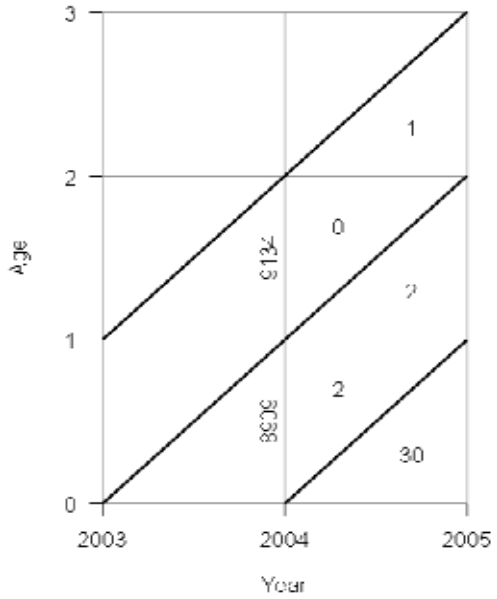

Figure 1. Lexis diagram with data on alive and deceased for males by age, year of birth and year of death.

born in 2003 and died in 2004 still aged zero years. However, 2 children of those born in 2003 had a birthday before their death. From this data we can calculate probability of dying for 2004 for children aged zero years as $q_0 = (30+2+2)/8909 = 0.003816$. From data on Figure 1 we can also calculate probability of dying for children aged one year as $q_1 = (0+1)/9134 = 0.000109$.

For general public, the most interesting function is life expectancy. It is also the most important indicator for population mortality[1] and is consequently the population health estimation. On Figure 2, one can observe how life expectancy is improving with time in Slovenia. Moreover, one can observe how the difference between men and women gets smaller with age.

Discussion

The methodology of life tables computation and their reliability

The important advantage of our life tables is the precision of data applied in their calculation. In the Republic of Slovenia birth and death certificates are automatically gathered in the Government Centre for Informatics database in digital form, so complete and updated birth and death dates are available for every period.[16]

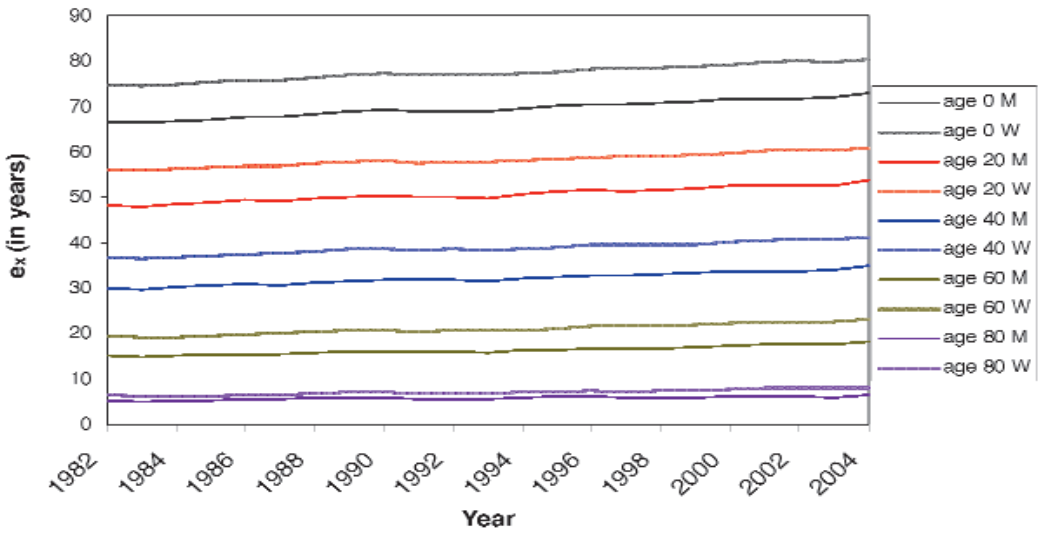

Figure 2. Life expectancy in years for selected age groups by sex (M stands for men and W stands for women), Slovenia 1982-2004.

It is of special importance to pay attention on population definitions, which are not consistent in Slovenian official statistics over different databases or time periods. By comparing the IPH and SORS population definitions, one of such inconsistencies has already been mentioned regarding newborns. Moreover, the SORS population definition by itself is inconsistent across different time periods. It was changed after the Republic of Slovenia's independence; since June 30, 1995 population data are reported according to the new definition.[11]

Data quality and complete understanding of their definitions are of major importance in all medical investigations. One of the essentials is to assure maximal feasible coherency among medical (patients) and official (population) data. Errors or discordances of official data cannot be abolished, however it is possible to adjust the analysis and minimize the possible biases by applying the adequate methodology.

The application of life tables in public health

From public health point of view life tables are basic tool for population health estimation. World Health Organization is preparing life tables for each of its state members and uses them for the major health indicator computation (e.g. health life expectancy – HALE). Life tables are indispensable also in most cost-benefit analyses, as the assessment of the effectiveness of screening programmes or some other public health interventions.

In addition to above mentioned fundamental public health research, life tables are an essential component in relative survival analysis. The relative survival analysis is not applied frequently in Slovenia. The unavailability of life tables in a proper format is certainly one of the main reasons for that. Only SORS's life tables were available until now for calculation of relative survival of cancer patients in Slovenia.[17,18] In these analyses the same life tables were used for several years. Data on Slovenian cancer patients are included in the second and in the third European study of cancer patient's survival EUROCARE.[19,20] In the EUROCARE project some census data were used for the interpolation of SORS's abbreviated life tables. Moreover, in the field of relative survival methodology, Slovenian authors recently developed a unique statistical approach and illustrated it by the investigation of survival in a myocardial infarction patient cohort.[21,22] Life tables are also required in public health studies evaluating years of potential life lost. Such studies gained on its applicability in Slovenia recently.[23,24] We believe that annual releases of life tables in applicable format will smooth the way for public health investigations in the future.

Cancer survival data provide comprehensive and complex measure of cancer burden in the observed population. They reflect the impact of all measures in cancer control programmes, from mass screening to treatment, follow-up and rehabilitation of cancer patients. There are several options and methods of the survival rates calculation. Observed and relative survival rates are the two fundamental forms. The observed survival indicates the actual mortality in a patient group. The causes of death other than cancer may differ from group to group and depend on cancer site, patient's age, sex, socio-economic position and the health care provided. Thus younger patients usually live longer in comparison with older patients with the same cancer or on the contrary survival of patients with certain cancers is short regardless of their age at diagnosis.[18]

Death notification in Slovenia is precisely prescribed. Rule of the coroner's inquests (Official Gazette of RS, No. 56/93 - 25) strictly defines among other also coroner's duties with documentation structure and its arrangement. Data protection laws are

implemented in the rule as well. In spite of all the efforts, death certificate data are often inaccurate as the primary cause of death is often indeterminable. Primic Žakelj with co-authors investigated the accuracy of official causes of death in a cohort of cervical cancer patients between 1985 and 1999. They concluded that the official Slovenian mortality rate of cervical cancer is underestimated for more than 25%.[26] Obviously the cause specific observed survival rate would be underestimated in that example as well. In this case, the relative survival analysis, which takes into account only dates of death and no causes of death, will lead us to more adequate result.

Because of strict personal data protection laws, collection of vital status or date of death data is very limited for individual investigators in Slovenia. Consequently, a simultaneous linkage between population diseases registries and Central Population Registry of Slovenia for patient's vital status update is of special importance and is protected by law.[27]

Relative survival analysis provides rather unbiased estimation of population disease burden even if the cause of death is unavailable. Anyway, relative survival is not applicable in all occasions. Observed survival should still be used as a golden standard in all clinical studies as they deal with selected population which characteristics are not necessary in accordance with general population attributes. An application of population life tables would in such a case bias the results. That is why relative survival analyses are limited to population studies and apply data from population based disease registries.

In relative survival analysis the disease unrelated death risks are removed by the usage of population life tables.[28] These tables are based on official mortality data stratified by age and sex, so only these two diseases unrelated death risks can be omitted by the relative survival analysis in Slovenia. If the influence of some other demographic characteristic on survival rates is supposed to be of practical importance, the life tables applied in relative survival analysis should be stratified by this attribute. Life tables stratified by socio-economic status are available in Finland.[29] A relative survival analysis was performed by Finish investigators to examine the influence of social class on the survival of cancer patient cohort. In comparison with observed or cause specific survival, relative survival adjusted to social class gave the most adequate results.

Laura M. Woods with co-authors [30] confirmed that geographical patterns of life expectancy identified for England and Wales in 1998 are mainly attributable to variations in deprivation status. Life expectancy is highest in most affluent groups with clear north-south gradient. For conducting this analysis they first had to construct life tables describing age specific mortality rates and life expectancy at birth for (a) quintiles if income deprivation, (b) each government office region and (c) every combination of deprivation index and geography.

Medical example

To understand the implications of relative survival techniques we look at the results of a study of survival of patients after myocardial infarction.[21] Having taken into account the age and calendar year, the observed survival after infarction does not differ significantly ($p = 0.15$) with respect to sex (Figure 3).

However, the problem of this study is that we do not have information on cause of death (a common situation in all the long term studies) and we are forced to consider all deaths as events. But as the observed group was on average 62 years old at diagnosis, we can expect that many of these deaths we-

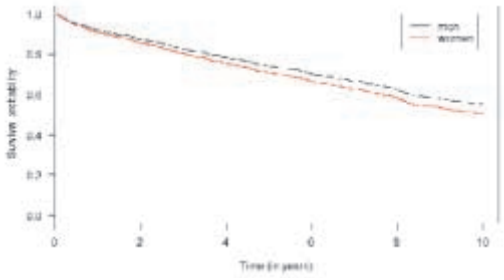

Figure 3. Observed survival by sex, adjusted for age and calendar year (age 62, year 1984).

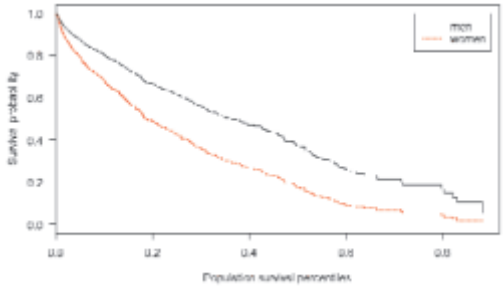

Figure 4. Relative survival by sex, adjusted for age and calendar year (age 62, year 1984)

re not necessarily due to infarction. When considering all deaths as events, we will thus always notice a strong effect of age, regardless whether the age is connected with the disease in question or not. The same is true for the year of diagnosis. As the population survival is constantly improving, this will be reflected in any long term study that doesn't have information on cause of death.

The relative survival comes as a solution whenever we wish to get information on the specific disease risk of a variable that has a known effect on the population risks. In our myocardial infarction study, sex is such an example. While men and women have an equal observed survival, the population hazards tell us that the women of this age should actually do much better, and we can therefore conclude that the mortality after infarction is connected with sex. The results of the relative survival are shown on Figure 4. We can see that sex (taking into account age and year) is strongly significant ($p < 0{,}001$). We can conclude that the hazard of dying of infarction related causes are much larger for women than for men (the hazard ratio is 1.77).

Conclusion

Slovenia is comparable to Scandinavian countries by its register orientation.[16] Population and mortality data are up to date thanks to its electronical collection in Central Register of Population so we don't have to wait for yearbooks to obtain necessary data for calculating life tables.

We calculated life tables presented exclusively for needs of relative survival analyses. In order to promote this and other already mentioned statistical analyses in public health where life tables are an essential tool, we have presented them in this article and put them available for public use. The warning should be given at this point for all potential users of our life tables. They contain crude probability of dying and so they require some adjustment regarding the purpose of use. For example smoothing of crude probability of dying is needed for demographic use.[5,6] If needed, one can also calculate abbreviated life tables or tables for several years combined from our exact life tables.

In very specific medical research separate life tables for occupational (social status, religious etc.) groups would be useful. However, such data are not collected at population level, as registering them is very costly and laborious.

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Appendix

Table 1. Complete life table for men, Slovenia 2004

x	q_x	l_x	d_x	L_x	T_x	e_x
0	0,003816	100000, 0	381, 6	99675, 6	7302946, 1	73, 03
1	0,000109	99618, 4	10, 9	99612, 9	7203270, 5	72, 31
2	0,000544	99607, 5	54, 2	99580, 4	7103657, 6	71, 32
3	0,000315	99553, 3	31, 3	99537, 6	7004077, 2	70, 36
4	0,000109	99521, 9	10, 9	99516, 5	6904539, 7	69, 38
5	0,000213	99511, 0	21, 2	99500, 4	6805023, 2	68, 38
6	0,000211	99489, 9	21, 0	99479, 3	6705522, 7	67, 40
7	0,000101	99468, 8	10, 1	99463, 8	6606043, 4	66, 41
8	0,000102	99458, 8	10, 1	99453, 7	6506579, 6	65, 42
9	0,000099	99448, 6	9, 9	99443, 7	6407125, 9	64, 43
10	0,000097	99438, 8	9, 7	99433, 9	6307682, 2	63, 43
11	0,000096	99429, 1	9, 5	99424, 3	6208248, 3	62, 44
12	0,000000	99419, 6	0, 0	99419, 6	6108823, 9	61, 44
13	0,000349	99419, 6	34, 7	99402, 2	6009404, 3	60, 44
14	0,000580	99384, 9	57, 7	99356, 0	5910002, 1	59, 47
15	0,000230	99327, 2	22, 9	99315, 8	5810646, 1	58, 50
16	0,000383	99304, 4	38, 1	99285, 3	5711330, 3	57, 51
17	0,001092	99266, 3	108, 4	99212, 1	5612045, 0	56, 54
18	0,001049	99157, 8	104, 0	99105, 8	5512832, 9	55, 60
19	0,001036	99053, 8	102, 6	99002, 5	5413727, 1	54, 65
20	0,000647	98951, 2	64, 0	98919, 2	5314724, 6	53, 71
21	0,001365	98887, 2	135, 0	98819, 6	5215805, 4	52, 75
22	0,001368	98752, 1	135, 1	98684, 6	5116985, 8	51, 82
23	0,001417	98617, 0	139, 7	98547, 2	5018301, 2	50, 89
24	0,000875	98477, 3	86, 2	98434, 2	4919754, 0	49, 96
25	0,000997	98391, 1	98, 1	98342, 1	4821319, 8	49, 00
26	0,000765	98293, 0	75, 2	98255, 4	4722977, 7	48, 05
27	0,001263	98217, 8	124, 0	98155, 8	4624722, 3	47, 09
28	0,001298	98093, 8	127, 3	98030, 2	4526566, 5	46, 15
29	0,000787	97966, 5	77, 1	97928, 0	4428536, 4	45, 20
30	0,001054	97889, 4	103, 2	97837, 8	4330608, 4	44, 24
31	0,000931	97786, 2	91, 1	97740, 7	4232770, 6	43, 29
32	0,001154	97695, 2	112, 8	97638, 8	4135029, 9	42, 33
33	0,001143	97582, 4	111, 5	97526, 6	4037391, 1	41, 37
34	0,000826	97470, 9	80, 5	97430, 6	3939864, 5	40, 42
35	0,000937	97390, 3	91, 3	97344, 7	3842433, 9	39, 45
36	0,001588	97299, 1	154, 5	97221, 8	3745089, 2	38, 49
37	0,001985	97144, 5	192, 9	97048, 1	3647867, 4	37, 55
38	0,002633	96951, 7	255, 3	96824, 1	3550819, 3	36, 62
39	0,002317	96696, 4	224, 0	96584, 4	3453995, 2	35, 72
40	0,002168	96472, 4	209, 2	96367, 8	3357410, 8	34, 80
41	0,002697	96263, 2	259, 6	96133, 5	3261043, 0	33, 88
42	0,003833	96003, 7	368, 0	95819, 7	3164909, 5	32, 97
43	0,003811	95635, 6	364, 5	95453, 4	3069089, 9	32, 09
44	0,003573	95271, 2	340, 4	95101, 0	2973636, 5	31, 21
45	0,003497	94930, 8	332, 0	94764, 8	2878535, 5	30, 32
46	0,005372	94598, 8	508, 2	94344, 7	2783770, 7	29, 43
47	0,005003	94090, 6	470, 8	93855, 2	2689426, 0	28, 58
48	0,005701	93619, 9	533, 8	93353, 0	2595570, 7	27, 72
49	0,006444	93086, 1	599, 9	92786, 2	2502217, 8	26, 88
50	0,005466	92486, 2	505, 6	92233, 5	2409431, 6	26, 05
51	0,006952	91980, 7	639, 4	91661, 0	2317198, 1	25, 19
52	0,009189	91341, 3	839, 4	90921, 6	2225537, 2	24, 37
53	0,008580	90501, 9	776, 5	90113, 6	2134615, 6	23, 59
54	0,008780	89725, 4	787, 8	89331, 5	2044502, 0	22, 79
55	0,010874	88937, 6	967, 1	88454, 0	1955170, 5	21, 98
56	0,011733	87970, 5	1032, 2	87454, 4	1866716, 4	21, 22
57	0,012437	86938, 3	1081, 2	86397, 7	1779262, 0	20, 47
58	0,013764	85857, 1	1181, 8	85266, 2	1692864, 3	19, 72
59	0,014149	84675, 3	1198, 0	84076, 3	1607598, 1	18, 99
60	0,012695	83477, 3	1059, 7	82947, 4	1523521, 8	18, 25
61	0,016026	82417, 6	1320, 8	81757, 1	1440574, 4	17, 48
62	0,018685	81096, 7	1515, 3	80339, 1	1358817, 2	16, 76
63	0,020545	79581, 4	1635, 0	78763, 9	1278478, 1	16, 07
64	0,020086	77946, 5	1565, 7	77163, 6	1199714, 2	15, 39
65	0,022566	76380, 8	1723, 6	75519, 0	1122550, 6	14, 70
66	0,026644	74657, 2	1989, 2	73662, 6	1047031, 6	14, 02
67	0,026450	72668, 1	1922, 1	71707, 0	973368, 9	13, 39
68	0,029052	70746, 0	2055, 3	69718, 3	901661, 9	12, 75
69	0,031774	68690, 7	2182, 6	67599, 4	831943, 6	12, 11
70	0,039502	66508, 1	2627, 2	65194, 5	764344, 2	11, 49
71	0,040692	63880, 9	2599, 4	62581, 2	699149, 7	10, 94
72	0,047181	61281, 4	2891, 3	59835, 8	636568, 5	10, 39
73	0,044080	58390, 1	2573, 8	57103, 2	576732, 7	9, 88
74	0,053367	55816, 3	2978, 8	54326, 9	519629, 5	9, 31
75	0,057874	52837, 5	3057, 9	51308, 6	465302, 6	8, 81
76	0,065789	49779, 6	3275, 0	48142, 1	413994, 0	8, 32
77	0,076589	46504, 6	3561, 8	44723, 8	365851, 9	7, 87
78	0,071369	42942, 9	3064, 8	41410, 5	321128, 1	7, 48
79	0,087725	39878, 1	3498, 3	38128, 9	279717, 6	7, 01
80	0,090664	36379, 8	3298, 3	34730, 6	241588, 7	6, 64
81	0,102819	33081, 5	3401, 4	31380, 7	206858, 1	6, 25
82	0,100904	29680, 0	2994, 8	28182, 6	175477, 3	5, 91
83	0,125664	26685, 2	3353, 4	25008, 5	147294, 7	5, 52
84	0,128308	23331, 9	2993, 7	21835, 0	122286, 2	5, 24
85	0,122419	20338, 2	2489, 8	19093, 3	100451, 1	4, 94
86	0,153310	17848, 4	2736, 3	16480, 2	81357, 8	4, 56
87	0,135827	15112, 1	2052, 6	14085, 8	64877, 6	4, 29
88	0,161417	13059, 4	2108, 0	12005, 4	50791, 8	3, 89
89	0,202786	10951, 4	2220, 8	9841, 0	38786, 4	3, 54
90	0,216891	8730, 6	1893, 6	7783, 8	28945, 4	3, 32
91	0,257732	6837, 0	1762, 1	5956, 0	21161, 5	3, 10
92	0,238411	5074, 9	1209, 9	4470, 0	15205, 6	3, 00
93	0,289340	3865, 0	1118, 3	3305, 9	10735, 6	2, 78
94	0,262411	2746, 7	720, 8	2386, 3	7429, 8	2, 70
95	0,276316	2025, 9	559, 8	1746, 0	5043, 4	2, 49
96	0,254545	1466, 1	373, 2	1279, 5	3297, 4	2, 25
97	0,343750	1092, 9	375, 7	905, 1	2017, 9	1, 85
98	0,411765	717, 2	295, 3	569, 6	1112, 8	1, 55
99	0,437500	421, 9	184, 6	329, 6	543, 2	1, 29
100+	1,000000	237, 3	237, 3	213, 6	213, 6	0, 90

Table 2. Complete life table for women, Slovenia 2004

x	q_x	l_x	d_x	L_x	T_x	e_x
0	0,004193	100000, 0	419, 3	99643, 6	8026359, 0	80, 26
1	0,000116	99580, 7	11, 5	99574, 9	7926715, 4	79, 60
2	0,000233	99569, 2	23, 2	99557, 6	7827140, 5	78, 61
3	0,000112	99546, 0	11, 1	99540, 4	7727583, 0	77, 63
4	0,000000	99534, 9	0, 0	99534, 9	7628042, 5	76, 64
5	0,000000	99534, 9	0, 0	99534, 9	7528507, 7	75, 64
6	0,000223	99534, 9	22, 2	99523, 8	7428972, 8	74, 64
7	0,000108	99512, 7	10, 7	99507, 3	7329449, 1	73, 65
8	0,000107	99501, 9	10, 7	99496, 6	7229941, 7	72, 66
9	0,000000	99491, 3	0, 0	99491, 3	7130445, 1	71, 67
10	0,000307	99491, 3	30, 6	99476, 0	7030953, 8	70, 67
11	0,000000	99460, 7	0, 0	99460, 7	6931477, 8	69, 69
12	0,000189	99460, 7	18, 8	99451, 3	6832017, 1	68, 69
13	0,000091	99441, 9	9, 1	99437, 4	6732565, 8	67, 70
14	0,000441	99432, 9	43, 8	99410, 9	6633128, 4	66, 71
15	0,000081	99389, 0	8, 1	99385, 0	6533717, 5	65, 74
16	0,000159	99380, 9	15, 8	99373, 0	6434332, 5	64, 74
17	0,000244	99365, 1	24, 2	99353, 0	6334959, 5	63, 75
18	0,000394	99340, 9	39, 1	99321, 3	6235606, 5	62, 77
19	0,000307	99301, 7	30, 5	99286, 5	6136285, 1	61, 79
20	0,000300	99271, 3	29, 7	99256, 4	6036998, 6	60, 81
21	0,000290	99241, 5	28, 8	99227, 1	5937742, 2	59, 83
22	0,000630	99212, 7	62, 5	99181, 5	5838515, 1	58, 85
23	0,000134	99150, 2	13, 3	99143, 6	5739333, 6	57, 89
24	0,000408	99136, 9	40, 5	99116, 7	5640190, 1	56, 89
25	0,000273	99096, 5	27, 1	99082, 9	5541073, 3	55, 92
26	0,000274	99069, 4	27, 1	99055, 8	5441990, 4	54, 93
27	0,000268	99042, 3	26, 5	99029, 0	5342934, 6	53, 95
28	0,000544	99015, 8	53, 8	98988, 9	5243905, 6	52, 96
29	0,000142	98961, 9	14, 0	98954, 9	5144916, 7	51, 99
30	0,000280	98947, 9	27, 7	98934, 1	5045961, 8	51, 00
31	0,000422	98920, 2	41, 7	98899, 4	4947027, 7	50, 01
32	0,000433	98878, 5	42, 9	98857, 1	4848128, 4	49, 03
33	0,000729	98835, 6	72, 1	98799, 6	4749271, 3	48, 05
34	0,000999	98763, 6	98, 6	98714, 2	4650471, 7	47, 09
35	0,000210	98664, 9	20, 7	98654, 5	4551757, 5	46, 13
36	0,001045	98644, 2	103, 0	98592, 6	4453103, 0	45, 14
37	0,000834	98541, 1	82, 2	98500, 0	4354510, 3	44, 19
38	0,000754	98458, 9	74, 2	98421, 8	4256010, 3	43, 23
39	0,000458	98384, 7	45, 0	98362, 1	4157588, 5	42, 26
40	0,000853	98339, 6	83, 8	98297, 7	4059226, 4	41, 28
41	0,001325	98255, 8	130, 2	98190, 7	3960928, 7	40, 31
42	0,001614	98125, 6	158, 3	98046, 4	3862738, 0	39, 37
43	0,001315	97967, 2	128, 8	97902, 8	3764691, 6	38, 43
44	0,001611	97838, 4	157, 6	97759, 6	3666788, 8	37, 48
45	0,001824	97680, 8	178, 1	97591, 7	3569029, 2	36, 54
46	0,001941	97502, 7	189, 3	97408, 0	3471437, 4	35, 60
47	0,003889	97313, 4	378, 5	97124, 2	3374029, 4	34, 67
48	0,002561	96935, 0	248, 2	96810, 9	3276905, 2	33, 81
49	0,002339	96686, 7	226, 2	96573, 7	3180094, 3	32, 89
50	0,003577	96460, 6	345, 0	96288, 1	3083520, 7	31, 97
51	0,003979	96115, 6	382, 4	95924, 3	2987232, 6	31, 08
52	0,003785	95733, 1	362, 4	95551, 9	2891308, 3	30, 20
53	0,004388	95370, 7	418, 5	95161, 5	2795756, 4	29, 31
54	0,004619	94952, 3	438, 6	94733, 0	2700594, 8	28, 44
55	0,004199	94513, 7	396, 9	94315, 2	2605861, 9	27, 57
56	0,004746	94116, 8	446, 7	93893, 5	2511546, 6	26, 69
57	0,005811	93670, 1	544, 4	93397, 9	2417653, 2	25, 81
58	0,004805	93125, 7	447, 5	92902, 0	2324255, 3	24, 96
59	0,006698	92678, 3	620, 8	92367, 9	2231353, 2	24, 08
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61	0,006248	91530, 2	571, 9	91244, 3	2047191, 5	22, 37
62	0,006914	90958, 4	628, 9	90643, 9	1955947, 2	21, 50
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64	0,008423	89604, 4	754, 7	89227, 0	1775336, 3	19, 81
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66	0,010050	87986, 6	884, 3	87544, 5	1597691, 2	18, 16
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78	0,040628	67888, 8	2758, 2	66509, 7	638382, 9	9, 40
79	0,046940	65130, 6	3057, 2	63602, 0	571873, 2	8, 78
80	0,056879	62073, 4	3530, 7	60308, 0	508271, 3	8, 19
81	0,069948	58542, 7	4094, 9	56495, 2	447963, 2	7, 65
82	0,070994	54447, 7	3865, 5	52515, 0	391468, 1	7, 19
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85	0,100148	42410, 2	4247, 3	40286, 6	246083, 3	5, 80
86	0,108792	38162, 9	4151, 8	36087, 0	205796, 7	5, 39
87	0,121715	34011, 1	4139, 7	31941, 3	169709, 7	4, 99
88	0,149133	29871, 4	4454, 8	27644, 0	137768, 4	4, 61
89	0,142495	25416, 6	3621, 7	23605, 8	110124, 4	4, 33
90	0,179487	21794, 9	3911, 9	19838, 9	86518, 7	3, 97
91	0,178519	17883, 0	3192, 4	16286, 8	66679, 7	3, 73
92	0,214885	14690, 5	3156, 8	13112, 2	50393, 0	3, 43
93	0,210300	11533, 8	2425, 6	10321, 0	37280, 8	3, 23
94	0,226860	9108, 2	2066, 3	8075, 1	26959, 8	2, 96
95	0,268617	7041, 9	1891, 6	6096, 1	18884, 8	2, 68
96	0,271889	5150, 3	1400, 3	4450, 2	12788, 6	2, 48
97	0,305882	3750, 0	1147, 1	3176, 5	8338, 5	2, 22
98	0,250000	2603, 0	650, 7	2277, 6	5162, 0	1, 98
99	0,557692	1952, 2	1088, 7	1407, 8	2884, 4	1, 48
100+	1,000000	863, 5	863, 5	1476, 5	1476, 5	1, 71

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3T MR-based treatment planning for radiotherapy of brain lesions

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Purpose. *The aim of this work is to develop a complete treatment planning procedure for radiation therapy of intracranial lesions based solely on 3T magnetic resonance imaging (MRI), i.e. MRI simulation.*
Methods. *The proposed 3T MR-based radiotherapy treatment planning procedure consists of converting the MR images into CT-like images by assigning electron density information (related to CT values) to organ structures. Firstly, the 3D distortion field present in the MR volumes is determined and rectified by using an in-house developed distortion correction method. The MR volumes are segmented into anatomical structures, i.e. brain, bone and scalp, by using a combination of the »Profile« and »Autocontouring« tools available on Pinnacle (Philips Medical Systems) treatment planning system (TPS). Bulk electron density values are assigned to the 3D volumes in Pinnacle by overriding their default MR values. Once the MR images contain the target volume along with the electron density information, they are ready to be used for dose calculations. The resulting CT+MR and MR only based plans were compared in terms of isodose distributions and dose-volume histograms (DVHs). For plan ranking we use a tumor-control probability (TCP)-based procedure for heterogeneous irradiation, which does not require the knowledge of radiobiological parameters.*
Results. *For all patients investigated, the 3T MR only and CT+MR-based plans are in good agreement in terms of isodose distributions, DVHs and TCPs (within 1%) following our clinical criteria.*
Conclusions. *The proposed 3T MR only based treatment planning procedure performs as good as the standard clinical procedure that relies on both CT and MR studies. MRI simulation can significantly reduce the patient treatment cost and save staff and machine time, and avoid any errors that may be associated with the image fusion process.*

Key words: brain neoplasms – radiotherapy; radiotherapy planning, computer-assisted

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Introduction

Magnetic resonance imaging (MRI) is the imaging modality of choice for the delineation of target volumes used for radiation treatment planning (RTP) due to its superior soft-tissue contrast. Presently, knowledge of electron density of the images is requi-

red for treatment planning dose calculations. In practice, the electron density of a limited number of tissue types (*e.g.*, lung, bone, soft-tissue) is required in this process. For intracranial lesions, due to lack of electron density information in the magnetic resonance (MR) images, image fusion of CT and MR data sets along with CT-based dose calculations have become a standard treatment planning procedure. Ideally, the treatment planning process should rely solely on the information generated by the MR image studies, *i.e.* MRI simulation.[1] Using such a procedure, the CT imaging sessions and the image fusion process would become redundant. This would significantly reduce the patient treatment cost and save staff and machine time. Furthermore, the patient would not be exposed to unnecessary radiation (as insignificant as it may be when compared to doses received in radiation treatment) and the errors associated with the image fusion process would be avoided.

It is known that the MR images are affected by distortions that alter the accurate representation of anatomical structures, *i.e.* spatial location and relative intensity. Image distortions are due to system-related and object-induced effects. The system-related distortions are generated by inhomogeneities in the main magnetic field and gradient non-linearities whereas the object-induced distortions are sourced in susceptibility and chemical shift variations in the sample. To be used for MRI simulation, the images have to be corrected to a degree that is acceptable for RTP, *i.e.* spatial resolution accuracy less than 2 mm.

The data on MRI simulation for intracranial lesions are rather scarce. Beavis *et al.*[2] used a basic approach for 1.5T MR images-based RTP. The authors considered no inhomogeneities corrections and the distortions corresponding to a typical field of view of a brain patient as being negligible. To accurately measure the 3D distortions, it is required to have a phantom that contains a large number of control points to properly sample the volume of interest and a robust algorithm to map the control points and determine the distortions along all three axes. Beavis *et al.* used a phantom with a design that gives a limited number of control points and would allow to determine 2D distortion only, *i.e.* (x,y) plane. Recently, Wang *et al.*[3] performed a MR distortion correction study on various 1.5T MRI scanners and found that the total 3D distortion can be up to 6 mm in a sphere with a radius of 100 mm (relevant to brain studies). Therefore, more studies need to be performed to develop an accurate and robust MR-based RTP for intracranial lesions that takes into account the distortions and inhomogeneities present in the MR images. MRI simulation was also investigated for prostate patients by different authors.[4,5] The authors showed that MR data sets that are corrected for distortion and assigned bulk densities to organ structures can successfully replace the CT images for treatment planning. The advent of 3T MR systems offers superior image quality to facilitate delineation of tumor and organs at risk.

In the present study, we investigate a 3T MR-based treatment planning procedure that relies on converting the MR images into CT-like images by assigning electron density information which is typically associated to CT values, to organ structures. The first step in the process is to correct the raw MR images for 3D geometrical distortions by applying a novel distortion correction procedure. The next step is to segment the volumes of interest into anatomical structures by using a semi-automatic method, *i.e.* brain, bone and scalp, required for dose calculations. Each volume is assigned a particular electron density before the data is used for dose calculations. The resulting CT+MR and MR only based plans

are compared in terms of isodose distributions, dose-volume histograms, and tumor-control-probability (TCP) modeling.

Materials and methods

We have evaluated the proposed MR-based treatment planning procedure by using 3T MR clinical studies to compare MR and CT+MR-based treatment plans. The flowchart of the procedure is presented in Figure 1.

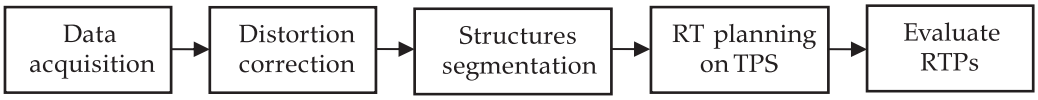


Figure 1. MR only based treatment planning procedure for RT of intracranial lesions.

Data acquisition

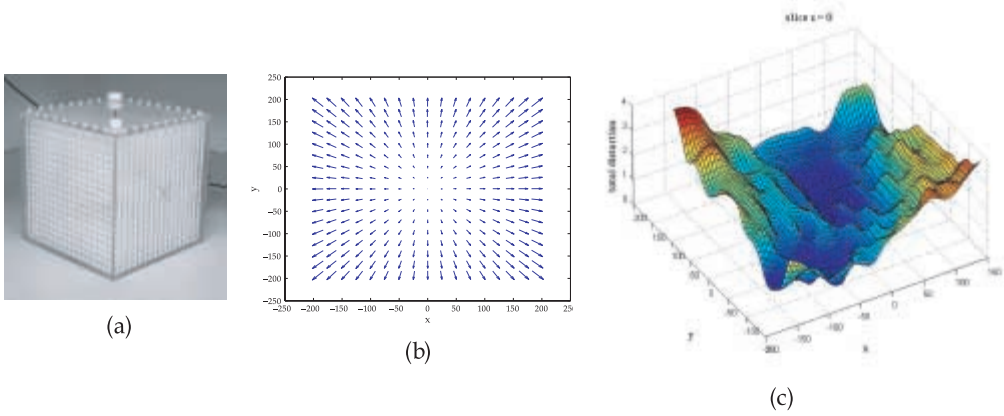
Data was acquired for each subject on a PQ 5000 CT (Philips Medical Systems) and a 3T Intera (Philips Medical Systems) MR scanner. The 3T clinical sequence consists of a 3D T1 TFE protocol with TE/TR/α 4.1/8.8/8°, field of view 240x240 mm^2 scanned on a 256x256 matrix in-plane, 125 partitions, each 1 mm and no gap. This MR sequence is used clinically for diagnostic and treatment planning of brain patients.

Distortion correction

Our technique is based on acquiring and comparing CT and MR scans of a 3D phantom filled with mineral oil consisting of parallel plastic grids 1 cm equally distributed inside the phantom (Figure 2a). We took three MR axial scans using the T1-weighted typical clinical sequence with the phantom positioned in such a way that the grid sheets were parallel to the transversal, saggital and corronal planes, respectively. The data sets were reconstructed using the scanner's software in the transversal, saggital and corronal plane to resemble grid-like structures in the MR images. CT axial scans (PET-CT Gemini, Philips Medical Systems) of the phantom was also acquired and reformatted to generate 3 data sets, *i.e.* transversal, saggital and corronal that would match the corresponding MR data sets. The 3D CT datasets are considered distortion-free, an accepted assumption in the field. To correct for object-induced distortions such as susceptibility, we acquired additional MR scans with reversed read gradient as per the technique described by Chang *et al.*[6]

The image analysis of all 3D data sets is performed automatically using our software developed in Matlab. Our algorithm determines the CT and MR control points, defined by the intersection of the grid crosses with the planes of the sheets surface. This is done sequentially by a) setting a threshold on the histogram for each image low enough to resemble the entire grid structure, b) applying 1D Gaussian blurring kernels along the x and y-axis to generate control point »blobs«, *i.e.* areas containing the control points, c) applying a watershed technique to isolate each »blob« in the images and d) determining the center of mass of each »blob« to obtain the coordinates of each control point.

The resulting CT and MR 3D matrices of control points are registered to a common system of reference. The 3D CT control points matrix is considered to accurately describe our volume of interest as there is no spatial distortion in the CT images. We can estimate the distortion by determining the displacement of the MR points from the corresponding CT ones. As an example, Figure 2b and Figure 2c show a typical distortion vector distribution and total distor-



(a) (b) (c)

Figure 2. (a) phantom design; (b) typical distortion vector distribution; (c) sample graph of total distortion values.

tion values, respectively. Once we determined the 3D distortion field matrix, we can correct the raw images by applying spatial and pixel intensity interpolations.

Structures segmentation

We converted the MR data sets into CT-like images by assigning electron density information to organ structures. Namely, the head image slices were segmented into scalp, bone and brain by using a set of contouring tools available on Pinnacle (Phillips Medical Systems) treatment planning system. We found that the best structure delineation method was based on a combination of the »Autocontouring« and »Profile« tools. Threshold values of the structures of interest interfaces are quickly assessed using the »Profile« tool and inputted into the »Autocontouring« tool. Contours are automatically generated by placing a seed point reasonably close to the boundary of the region that needs to be delineated. These contours can be subsequently adjusted as desired using manual tools. The scalp-air interface of the entire volume, the scalp-bone and the bone-brain interfaces corresponding to the upper part of the skull can be automatically generated with little manual adjustment. For the lower part of the skull, due to a higher gradient of anatomical structures more manual adjustment of the automatically generated contours is required. Bulk electron density values, relevant to the delineated structures, were assigned to the 3D volumes in Pinnacle by overriding their MR default values *i.e.* 1 g/cm^3 to brain and scalp and 1.47 g/cm^3 to bone.

RT planning on TPS

We generated and compared CT+MR and MR only based treatment plans using clinical data. The treatment planning process was performed on Pinnacle. At the Cross Cancer Institute (CCI), the standard clinical procedure for radiotherapy of intracranial lesions consists of acquiring CT and MR studies and performing image fusion. In the image fusion process, the contours of the planning target volume (PTV) and organs-at-risk are drawn on the T1-weighted MR images and automatically generated on the corresponding CT images. These contours are required in order to use the CT images for treatment planning purposes. In our study, we had data available for 4 GBM (glioblastoma multiforme) patients scanned

on CT and 3T MR units. To compare the CT+MR and the MR only based plans, we built typical treatment plans using the CT+MR datasets and applied these plans to the MR images only by using the same beam arrangements, dose constraints and optimization parameters. To perform dose calculations on the MR images all structure contours (*i.e*. brain, bone, scalp and all other delineated structures) were assigned relevant bulk electron density values by using Pinnacle's override density feature.

Evaluate RTPs

The resulting CT+MR and MR only based plans were compared in terms of isodose distribution and DVHs. For plan ranking, we use a TCP-based procedure for heterogeneous irradiation, which does not require the knowledge of radiobiological parameters. Here we give a brief description of the method, which will be published in details in another study and was applied for plan ranking in.[7]

The Poisson based TCP model $TCP = e^{-N_3}$ is used, where N_s is the number of surviving clonogens, estimated by the single hit cell dose-response model as $N_s = N_o e^{-\alpha D}$ where N_o is the initial clonogen number and α is the radiosensitivity. As pointed out by Brahme[8], the mathematical form of the single hit model becomes identical to the LQ model in the case of the standard fractionation schemes (*n* fractions each delivering a dose *d*): $NS = N_s = N_o e^{-(\alpha D+\beta nd)D} = N_o e^{-\hat{\alpha}D}$, where is $\hat{\alpha}$ called adjusted radiosensitivity. Recently, it was shown that the adjusted radiosensitivity takes into account the repopulation as well.[9] For the plan ranking purposes, it is better to use the TCP model in terms of the survival fraction at 2 Gy (SF_2), because this parameter is confined in the interval [0,1].

$$TCP = e^{-N_o e^{-\hat{\alpha}D}} = e^{-N_o SF_2^{0.5D}}$$

In the case of heterogeneous irradiation one obtains:

$$TCP = e^{-\rho\sum V_i e^{-\alpha D_i}} = e^{-\rho\sum V_i SF_2^{0.5D_i}}$$

where represents the differential DVH using the absolute (not the relative) volume. The tumor cell density is presumed to be 10^9 cells/mm^3. This number is actually not very important because the plans ranked are for one and the same tumor site, hence having one and the same tumor cell density.

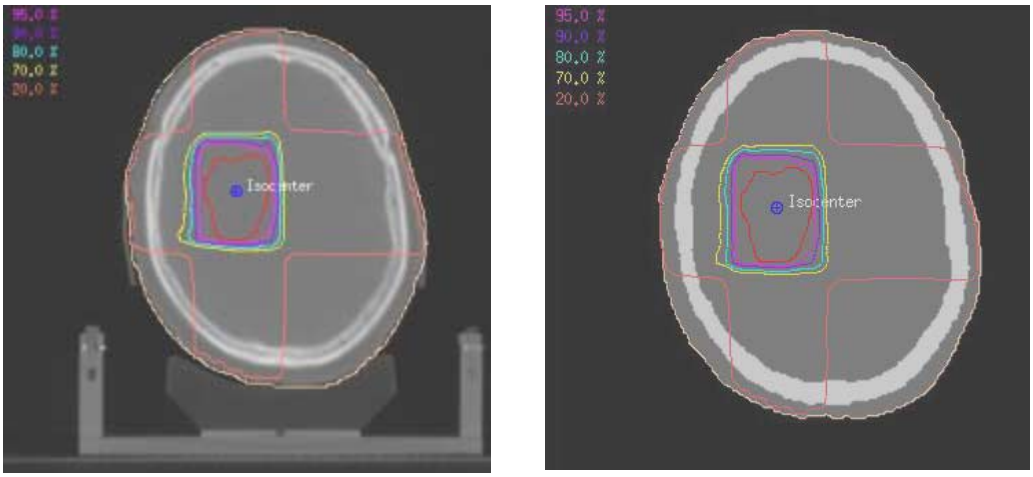
Let us have two plans, defined by a set of DVHs $\{V_i,D_i\}^I$ and $\{V_i,D_i\}^{II}$. The plan for which the tumor control probability is higher for each value of the parameter SF_2 is obviously the better one $TCP^I(SF_2 \mid \{V_i,D_i\}) > TCP^{II}(SF_2 \mid \{V_i,D_i\}) \forall SF_2$. The method is easily visualized graphically. Curves $TCP^I(SF_2 \mid \{V_i,D_i\})$ and $TCP^{II}(SF_2 \mid \{V_i,D_i\})$ are calculated and plotted for both plans. The far right curve will correspond to the better RT plan, producing the highest TCP.

Results and discussion

The total distortion for the standard 3T MR sequence used for brain patients in a volume relevant to brain studies, *i.e*. 20x20x20 cm^3, was found to be about 4 mm. Considering that the requirement for image spatial accuracy in radiation treatment planning is 2 mm, our distortion correction is applied to correct the patient MR images.The residual distortion determined after applying these transformations was found to be within one pixel resolution, *i.e*. 0.94 x 0.94 mm^2.

Figure 3a shows an example of the planning target volume (PTV) isodose distributions of RT plans based on CT+MR and MR only images, respectively. It can be seen that the two plans look very similar in terms of PTV isodose distributions and they are all in agreement with our clinical

(a)



CT+MR-based RT plan MR only based RT plan

(b)

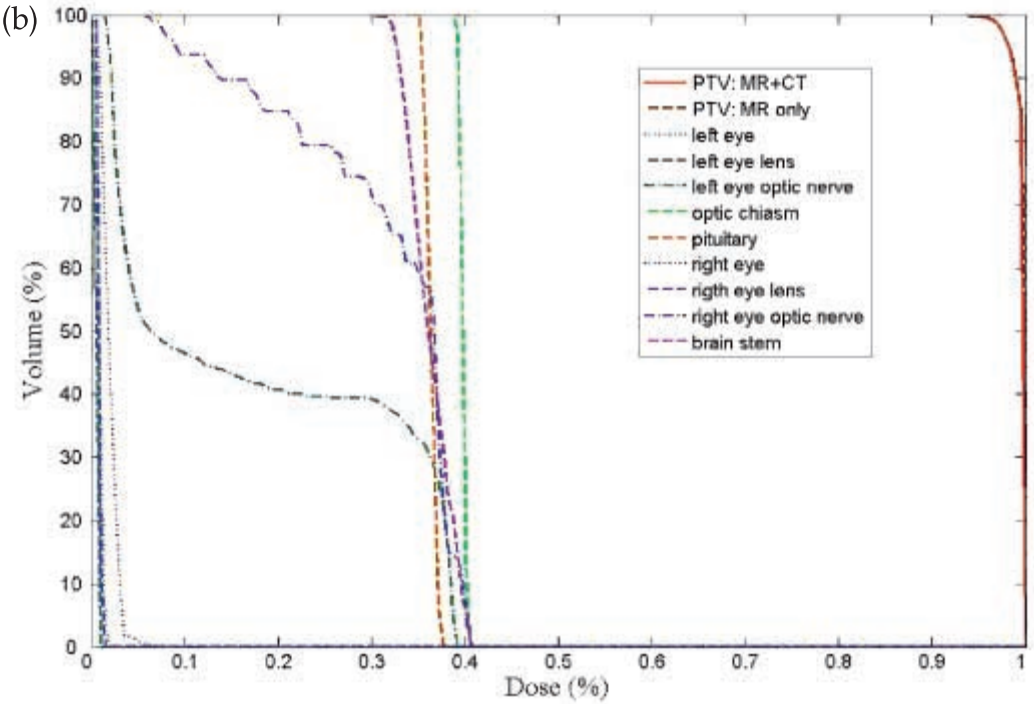


Figure 3. (a) Comparison of isodose distributions; (b) dose-volume histograms (DVHs) for the CT+MR and MR only based radiation therapy plans.

criteria, *e.g.* 95% isodose line coverage of the PTV. For all patients, we also compared the CT+MR and MR only based RT plans in terms of DVHs. Figure 3b depicts sample DVHs corresponding to the two plans of the same patient. For visualization purposes, we displayed only the DVHs of the organs-at-risk (*i.e.* eyes, eye lenses, optical nerve, pituitary gland, optic chiasm and brain stem) corresponding to the CT+MR-based plan only as they overlap with the corresponding DVHs generated for the MR only based plan. For all patients, we found that the differences are clinically insignificant (within 1%).

To evaluate the impact of the inhomogeneities on the treatment planning process, we compared the standard CT+MR based plans with and without non-homogeneity correction. The 3D skull contours were assigned bulk water electron density values, *i.e.* 1g/cm^3, for the plans that used non-inhomogeneity corrections. In the case of 3 patients, we found that the difference between the plans with and without the non-homogeneity correction was within 2%. For the 4th patient the discrepancy was 3% due to a large tumor volume and its location near the vortex, therefore the beams passed through a thicker layer of bone.

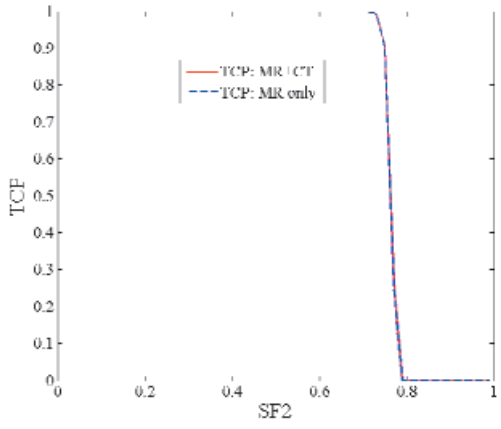


Figure 4. TCP-based radiation treatment plan ranking for CT+MR and MR only based plans.

Figure 4 shows a typical TCP-based RT plan ranking for the CT+MR and MR only based plans. It can be seen that there is a good agreement between the two plans. The differences are clinically insignificant (within 1%) for all patients investigated.

In this study, we investigated a treatment planning procedure for intracranial lesions based solely on 3T MRI data sets that consists of converting the MR images into CT-like images by assigning bulk electron density to segmented structure volumes, *i.e.* scalp, bone and brain. Before being used in the treatment planning process, the MR images were corrected for 3D geometrical distortions. We found that the MR-based treatment planning procedure performed as good as the current clinical procedure based on both the CT and MR data sets.

MRI has proven to be the best imaging modality for RTP target delineation. Increasing the magnetic field strength form 1.5 to 3 T results in an increase in the signal-to-noise ratio, which not only, simplifies the task of target delineation, but could improve the accuracy in delineating the 3D tumor and structures volumes.

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Zgodnje radiološko ugotavljanje gastrointestinalne perforacije

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Izhodišča. Namen raziskave je bil predstaviti radiološke preiskave pri zgodnjem odkrivanju gastrointestinalne perforacije, ki je pogost vzrok akutnega abdomna.
Metode. V obdobju enega leta smo nujno obravnavali 20 bolnikov z gastrointestinalno perforacijo. Pri vseh bolnikih smo opravili rentgensko slikanje ter ultrazvočno in CT preiskavo. Nekateri bolniki so opravili rentgensko preiskavo tudi z zaužitjem 250 ml kontrasta. Ultrazvočno preiskavo smo naredili z 3,5 MHz sondo in Siemensonovim aparatom; CT preiskavo pa s štiri listnim računalniškim tomografom «Volume Zoom« in 2,5 mm širine. Vsi bolniki so imeli klinične znake akutnega abdomna.
Rezultati. V skupini 20 obravnavanih bolnikov je bilo 8 (40%) žensk in 12 (60%) moških, povprečna starost je bila 41 let (od 14 do 67). 7 (35%) jih je imelo predrtje želodca in 10 (50%) dvanajsternika. V enem primeru smo ugotovili predrtje transverzalnega dela debelega črevesa po poškodbi, v enem predrtje želodca po operaciji in v enem predrtje sigmoidnega črevesa zaradi malignega procesa. Pri 18 (90%) bolnikih je predrtje nastalo spontano. Rentgenska preiskava trebuha je pokazala nivoje prostega zraka v 16 (80%) primerih, ultrazvočna preiskava prosto tekočino v 18 (90%) in CT preiskava oba znaka bolezni v vseh primerih.
Zaključki. Zgodnje prepoznavanje gastrointestinalne perforacije je izjemno pomembno, saj običajno zahteva kirurško zdravljenje. Ob anamnezi je še vedno nativno rentgensko slikanje trebuha prva preiskava. Z razvojem novejših digitalnih aparatov, kot sta ultrazvok in CT, pa lahko natančno opredelimo zgodnje znake gastrointestinalne perforacije. V naši raziskavi smo ugotovili, da je ultrazvočna preiskava zelo koristna pri odkrivanju proste tekočine, s CT-jem pa smo ugotovili prosto tekočino in nivoje zraka v trebuhu tudi v tistih primerih, kjer jih ultrazvok in rentgensko slikanje nista pokazala.

Radiol Oncol 2006; 40(2): 73-85.

Pomen tricikličnih zdravil pri selektivnem proženju mitohondrijske apoptoze pri neoplastični gliji. Nova možnost zdravljenja malignih gliomov?

Pilkington G J, Akinwunmi J, Amar S

Izhodišča. Naše raziskave so že pokazale, da ima triciklični antidepresiv klomipramin *in vitro* specifičen pro-apoptotičen učinek na humanih malignih gliomskih celicah. Učinek je odvisen od koncentracije klomipramina in ga ni opaziti na normalnih humanih astrocitih. Zdravilo sproža apoptozo tako, da deluje na mitohondrije, kjer učinkuje na dihalno verigo. Čeprav so ob naših tudi druge raziskave pokazale, da imajo različni antidepresivi (vključno s selektivnimi zaviralci ponovnega prevzema serotonina – SSRI) vpliv na apoptozo pri limfomih in gliomih, je klomipramin najbolj učinkovit. Ugotavljali smo tudi pro-apoptotično aktivnost drugih tricikličnih zdravil in odkrili, da imata le dve takšni zdravili (amitriptilin in doksepin) enako ali boljšo učinkovitost kot klomipramin. *Per os* zaužiti zdravili klomipramin in amitriptilin se metabolizirata v desmetil klomipramin (norklomipramin) in nortriptilin. Menimo, da bi bilo potrebno testirati tumorske celice na omenjeni zdravili in na njuna metabolita. Učinkovitost obeh zdravil je namreč lahko močno zmanjšana zaradi odpornosti na zdravila (multidrug resistance), ki pa je pri obeh zdravilih različna. Ugotovili smo tudi, da ima metabolit klomipramina norklomipramin slabši pro-apoptotični učinek, medtem ko ima metabolit amitriptina nortriptilin enak učinek kot amitriptin.

Zaključki. Menimo, da bo potrebno v kliničnih raziskavah ugotoviti učinkovitost tricikličnih antidepresivov pri malignem gliomu, najprej kot dopolnilno zdravljenje.

Radiol Oncol 2005; 40(2): 87-93.

Klomipramin hidroklorid in ugotavljanje apopotoze na celičnih kulturah humanih malignih gliomov s pomočjo pretočne citometrije ob uporabi Annexina-V

Parker K, Pilkington GJ

Izhodišča. Predhodne raziskave v našem laboratoriju so pokazale, da klomipramin hidroklorid (CLOM), triciklični antidepresiv, ki ga uporabljamo že 30 let, *in vitro* selektivno ubija neoplastične glialne celice in pri tem ne prizadene normalnih možganskih celic. Namen naše raziskave je bil oceniti celične kulture malignega glioma, ki smo jih odvzeli različnim bolnikom. Želeli smo ugotoviti, ali so različno občutljive na CLOM. Posebno nas je zanimala apoptoza, saj CLOM deluje na mitohondrije tumorskih celic in na ta način sproži apoptozo. Pri tem smo uporabljali pretočno citometrijo in Annexin-V. Glede na koncentracijo zdravila in čas inkubacije smo želeli ugotaviti mehanizem celične smrti, ali ta nastane predvsem zaradi nekroze ali zaradi apoptoze.

Metode. Celice smo inkubirali do 6 ur z različno koncentracijo CLOM-a (20μM – 100μM). Sledila je priprava celic za pretočno citometrijo, kjer smo uporabili tudi Annexin-V FITC in propidium iodid.

Rezultati. Preiskavo smo naredili s petimi malignimi gliomi. Pri dveh so imele celice manj apoptoze, koncentracija CLOM-a je bila 60μM ali več. Pri treh, kjer smo uporabili zgodnje celične linije, pa smo opazili zelo izrazito apoptozo, koncentacija CLOM-a je bila do 100μM, inkubacija pa 6 ur. Vzporedno smo preiskovali normalne humane astrocite in ugotovili, da CLOM v omenjenih koncentracijah ni povzročil njihove smrti.

Zaključki. Preiskava z Annexinom-V bi lahko služila testiranju posamičnih bolnikov – ob analizi Bcl-2 in genskem CYP preiskovanju – ugotavljali bi lahko, ali so njihove tumorske celice občutljive na CLOM.

Radiol Oncol 2006; 40(2): 95-105.

Odpornost na komplement ovira onkološko zdravljenje

Konatschnig T, Geis N, Scultz S, Kirschfink M

Izhodišča. Različne *in vitro* raziskave, ki so bile narejene v zadnjih dveh desetletjih, jasno kažejo, da je odpornost človeških tumorskih celic na avtologni komplement pogojena z na membrano vezanimi regulatornimi proteini komplementa (mCRP). Takšna proteina sta CD55 in CD46, najpomembnejšo vlogo pa ima CD59. Ta imunska dogajanja zelo vplivajo na potek bolezni, kar potrjujejo novejše klinične raziskave. Odpraviti odpornost na komplement obeta izboljšanje zdravljenja bolnikov z različnim rakom, s tem pa tudi izboljšanje napovedi izhoda bolezni. V pričujočem kratkem preglednem članku podrobneje predstavljamo: (1) nevtralizacijo proteinov mCRP z monoklonskimi ali rekombinantnimi protitelesi in (2) strategijo »utišanja« genov za proteine mCRP z delovanjem na nivoju RNA ob uporabi siRNA.
Zaključki. Ker so proteini mCRP prisotni v vseh normalnih tkivih endotelnih celic parenhimskih organov (jetra, ledvica, itd...) in v krvnih celicah, je zelo pomembno, da je blokiranje delovanja proteinov mCRP selektivno in da tako ne prizadene zdravega tkiva. Čeprav so prvi rezultati ohrabrujoči, je vplivanje na delovanje proteinov MCRP, da bi izboljšali imunoterapijo, še vedno velik izziv v klinični praksi.

Radiol Oncol 2006; 40(2): 107-13.

Katepsini cisteinske skupine in njihovi inhibitorji pri raku glave in vratu: pregled raziskovalnega dela na Onkološkem inštitutu Ljubljana in Kliniki za otorinolaringologijo Kliničnega centra Ljubljana

Strojan P

Za odločitev o vrsti in intenzivnosti terapije, potrebne za uspešno ozdravitev raka, kot tudi za napoved izida bolezni je potrebna natančna ocena agresivnosti bolezni. Hipoteza, ki predpostavlja napovedni in prognostični pomen posameznih katepsinov in njihovih inhibitorjev, temelji na vpletenosti enih in drugih v obcelične proteolitične procese. Ti so sestavni del večine aktivnosti, povezanih z življenjem normalne celice, kot tudi procesov, povezanih z razgradnjo zunajceličnega matriksa med procesom invazije in zasevanja tumorskih celic. Vlogo katepsinov in njihovih inhibitorjev pri raku lahko razčlenimo na naslednje skupine: markerji za presejanje; markerji za napoved prisotnosti zasevkov v področnih bezgavkah; markerji za napoved odgovora na zdravljenje in ponovitev bolezni; prognostični markerji. Čeprav je raziskav s področja katepsinov in njihovih endogenih inhibitorjev pri raku glave in vratu malo, rezultati opravičujejo nadaljna preučevanja. V pričujočem pregledu smo predstavili naše izkušnje in rezultate iz desetletnega obdobja klinično usmerjenega razsikovalnega dela in podali mnenje o njihovi napovedni in prognostični vlogi za potrebe vsakodnevne klinične prakse.

Radiol Oncol 2006; 40(2): 133-8.

Radiol Oncol 2006; 40(2): 115-24.

Popolne letne tablice umrljivosti za Slovenijo po spolu, 1982-2004, in možnosti uporabe v javnem zdravju

Žagar T, Zadnik V, Pohar M, Primic Žakelj M

Tablice umrljivosti se uporabljajo kot osnova za statistične izračune v mnogoterih znanstvenih strokah; tudi v javnemu zdravju in epidemiologiji. V zadnjih letih jih v Sloveniji uporabljamo predvsem v analizah relativnega preživetja, za kar potrebujemo popolne momentne tablice umrljivosti za posamezna koledarska leta in ločene po spolu. Ker takšne tablice umrljivosti za Slovenijo še niso na razpolago, smo jih pripravili sami za obdobje 1982-2004. V pričujočem prispevku je opisana metodologija po kateri smo tablice izračunali in primeri, v katerih so takšne tablice umrljivosti uporabne. Tablice so bralcu na razpolago, če pošlje prošnjo na naslov register@onko-i.si. Objavljene so tudi v mednarodni bazi tablic umrljivosti (angl. Human Life-Table Database), ki je dosegljiva na internetu (http://www.lifetable.de/). Tudi v prihodnje nameravamo računati tablice umrljivosti, takoj ko bomo dobili potrebne podatke.

Radiol Oncol 2005; 40(2): 125-32.

Načrtovanje obsevanja možganskih sprememb s pomočjo 3T MR tehnike

Stanescu T, Hans-Sonke J, Stavrev P, Fallone BG

Izhodišča. Namen pričujoče raziskave je bil oblikovati postopek za načrtovanje obsevanja možganskih sprememb s pomočjo 3T magnetne resonančne preiskave (MRI), t.i. MRI simulacije.

Metode. Pri načrtovanju obsevanja s 3T MR načinom spremenimo MR slike v CT-ju podobne slike in pri tem uporabimo podatke elektronske gostote organov. Ker pri MR volumnih ugotavljamo 3D izkrivljanje, smo razvili posebno korektivno metodo. Najprej smo MR volumne razdelili glede na anatomske strukture kot so možgani, kosti in koža ter pri tem uporabili orodja programa Pinnacle (Philips Medical Systems), ki je del sistema za načrtovanja obsevanja (treatment planning system – TPS). Izračun smo naredili, ko smo MR slikam določili tarčne volumne z elektronsko gostoto. Primerjali smo načrte obsevanja s pomočjo CT+MR tehnike in samo MR simulacije. Zanimala nas je razporeditev izodoz in doza-volumen histogrami (dose-volume histograms – DVHs). Ob ugotavljanju heterogenosti obsevanja smo ocenjevali verjetnost tumorske kontrole (tumor-control probability – TCP), kar ni zahtevalo poznavanje radiobioloških parametrov.

Rezultati. Pri vseh bolnikih smo ugotovili, da so obsevalni načrti s CT+MR tehniko in 3T MR tehniko podobni, bodisi ob primerjavi izodozne distribucije, bodisi DVHs in TCP. Razlike niso bile večje kot 1%, če smo upoštevali naše klinične kriterije.

Zaključki. Uporaba predlagane 3T MR tehnike načrtovanja obsevanja je enako natančna kot uporaba kombinacije CT+MR tehnike. MRI simulacija obsevanja lahko poceni obravnavo bolnikov in prihrani čas obravnave, če jo primerjamo s CT+MR tehniko, prav tako pa se lahko izognemo napakam, ki bi lahko nastale pri združevanju CT in MR slik.

Radiol Oncol 2006; 40(2): 133-8.

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.*

Cancer research

July 1-4, 2006

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

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

Gynaecological malignancies

August 31 - September 1, 2006

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

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

Radiotherapy

September 3-7, 2006

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

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Oncology

September 8, 2006

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

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

Radiobiology

September 17-21, 2006

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

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

Lung cancer

September 25-26, 2006

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

Contact Dr. Alessandra Crippa, Senior Account, CCI Centro Congressi Internazionale, Via Cervino, 60, 10155 Torino or call + 39 011 2446916; or fax +39 011 2446900 – 2446944; or e-mail: a.crippa@congressiefiere.com; or see http://www.congressifiere.com

Otorhinolaryngology

September 27-30, 2006

The 11th Danube Symposium 2006 »International Otorhinolaryngological Congress« will take place in Bled, Slovenia.

Contact Albatros Bled, Ribenška 2, 4260 Bled, Slovenia; or call +386 4 5780 350; or fax +386 4 5780 355; or e-mail info@albatros-bled.com; or see http://www.albatros-bled.com

Oncology

October 8-12, 2006

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

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

Radiation oncology

October 22-27, 2006

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

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

Lung and head & neck

October 26-28, 2006

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

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

Oncology

October 26-29, 2006

The »2nd Congress of the Polish Oncology« will take place in Poznan, Poland.

See http://www.kongresonkologii.pl

Lung cancer

November 8-12, 2006

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

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

Head & neck

November 16-18, 2006

The »5th European Workshop on Basic Biology of Head & Neck Cancer« will take place in Poznan, Poland.

See http://www.orl.amp.wdu.pl/5workshop

Radiotherapy

November 19-23, 2006

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

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Surgical oncology

November 30 – December 2, 2006

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

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

Radiotherapy

December 3-7, 2006

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

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Toxicology

July 15-19, 2007

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

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

Lung cancer

September 2-6, 2007

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

Contact Conference Secretariat; e-mail WCLC2007@ncc.re.kr; or see http://www.iaslc.orgIumages/12world-confannounce.pdf

Oncology

September 23-27, 2007

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

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

Lung cancer

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

Contact Conference Secretariat; e-mail WCLC2007@ncc.re.kr; or see http://www.iaslc.orgIumages/12worldconfannounce.pdf

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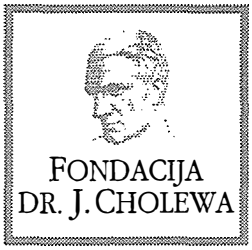
Please send information to the Editorial office, Radiology and Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia.

Radiol Oncol 2006; 40(2): 139-41.



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Activity of »dr. J. Cholewa« Foundation for Cancer Research and Education - a report for the second quarter of 2006

The Dr. J. Cholewa Foundation for Cancer Research and Education continues to support activities associated with cancer research and education in Slovenia. A number of different grants and other forms of financial support were bestowed to experts from various scientific fields and disciplines of cancer research and education in Slovenia. All the requests for such grants were being dealt with responsibly by Foundation members with clinical and research experience in cancer and by members with important experience in finance.

The Dr. J. Cholewa Foundation for Cancer Research and Education continues to support the regular publication of »Radiology and Oncology« international scientific journal, which is edited, published and printed in Ljubljana, Slovenia, as it has done over the last couple of years. This support is considered to be one of its more important commitments and with this in mind, the Foundation will also continue to support the publication of the results from research it supported in respectable international scientific oncology journals and other novel electronic forms of dissemination of scientific information dealing with cancer research and education.

is noteworthy that many study and research grants have been bestowed to researchers and experts from various scientific fields associated with oncology in Slovenia and that many of them were also given grants to attend scientific meetings, conferences and symposia dealing with oncology worldwide. The information and knowledge of cancer in general and problems associated with caner research have thus been spread in Slovenia as a result of these activities The Dr. J. Cholewa Foundation for Cancer Research and Education thus has every reason to respectfully acknowledge the importance of the commitment of various public companies and private individuals to its cause.. goes without saying that the Foundation also remains active in promoting cancer education in general, especially in general population, among medical and nursing students and among all the others with a particular interest in cancer research and education.

Borut Štabuc, MD, PhD
Tomaž Benulič, MD
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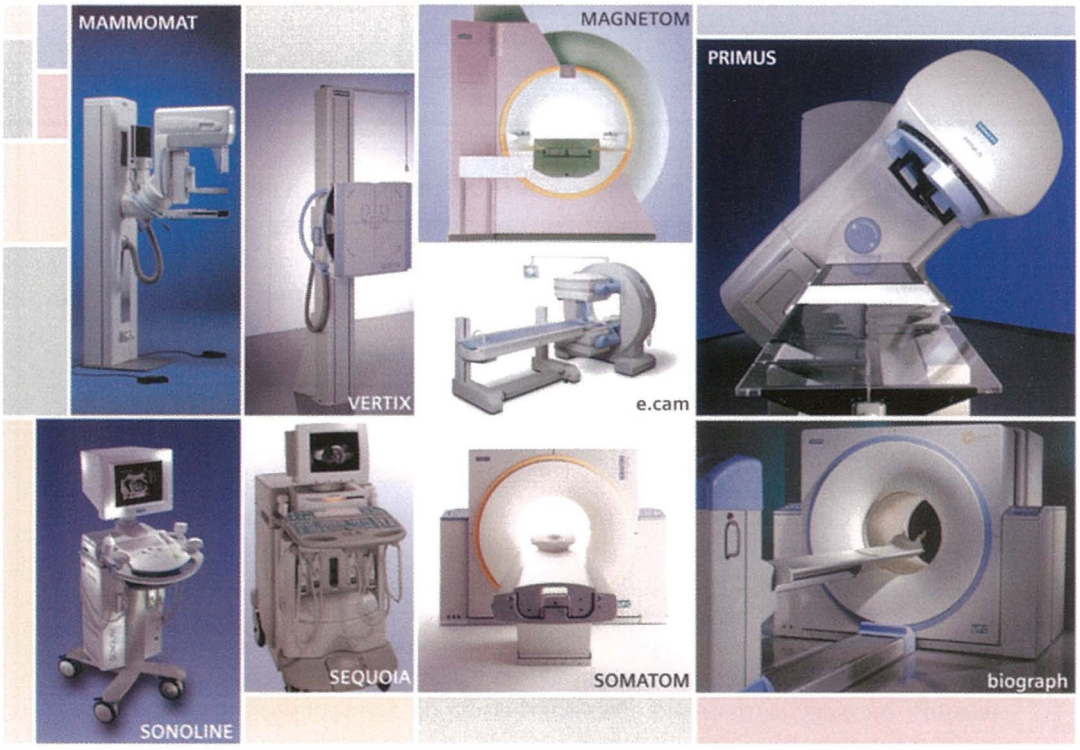

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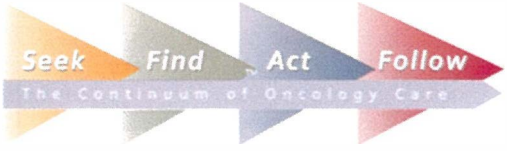

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Sakura finetek (Evropa):
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ERBITUX®
CETUKSIMAB

Zavira EGFR - odpira nove možnosti

Ciljno usmerjeno zdravljenje metastatskega raka debelega črevesa in danke

- Visoko specifično monoklonsko IgG1 protitelo, ki kompetitivno inhibira receptorje za epidermalni rastni faktor (EGFR)
- Učinkovitost je dokazana v kombinaciji z irinotekanom[1]

Merck v onkologiji | biološko zdravljenje za boljšo kakovost življenja

Erbitux 2 mg/ml raztopina za infundiranje (skrajšana navodila za uporabo)

Cetuksimab je himerno monoklonsko IgG1 protitelo, ki je usmerjeno proti receptorju za epidermalni rastni faktor (EGFR). **Terapevtska indikacija:** Zdravilo Erbitux je v kombinirani terapiji z irinotekanom indicirano za zdravljenje bolnikov z metastatskim rakom debelega črevesa in danke z ekspresijo receptorjev za epidermalni rastni faktor (EGFR), in sicer po neuspešni citotoksični terapiji, ki je vključevala tudi irinotekan. **Odmerjanje in način uporabe:** Zdravilo Erbitux infundirajte enkrat na teden z intravensko infuzijo prek linijskega filtra. Začetni odmerek je 400 mg/m^2 telesne površine. Naslednji tedenski odmerki so vsak po 250 mg/m^2. Priporočljivo je, da z zdravljenjem s cetuksimabom nadaljujete do napredovanja osnovne bolezni. **Kontraindikacije:** Zdravilo Erbitux je kontraindicirano pri bolnikih z znano hudo preobčutljivostno reakcijo (3. ali 4. stopnje) na cetuksimab. **Posebna opozorila in previdnostni ukrepi:** Pojav hude preobčutljivostne reakcije (3. ali 4. stopnje) zahteva takojšnjo in stalno ukinitev terapije s cetuksimabom. **Neželeni učinki:** Pri približno 5% bolnikov se lahko pojavijo preobčutljivostne reakcije. Približno polovica teh reakcij je hudih. Pri približno 5% bolnikov lahko pričakujemo konjunktivitis. O dispneji so poročali pri 25% bolnikov z rakom debelega črevesa in danke v zadnjem stadiju. Hude kožne reakcije se pojavijo v približno 15%, predvsem nastopijo v obliki aknam podobnega izpuščaja in/ali motenj nohtov. Če se pri bolniku pojavi huda kožna reakcija (stopnje 3), smete zdravljenje nadeljevati le, če se je reakcija pomirila do 2. stopnje. **Pakiranje:** 1 viala po 50 ml. Vsak ml raztopine vsebuje 2 mg cetuksimaba.

Vse nadaljne informacije so vam na voljo pri: Merck, d.o.o., Dunajska cesta 119, 1000 Ljubljana, tel.: 01 560 3810, faks: 01 560 3831, el. pošta: info@merck.si

1) Cunningham D et al., Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer. New ENG J Med 2004; 351(4): 337-345

www.oncology.merck.de

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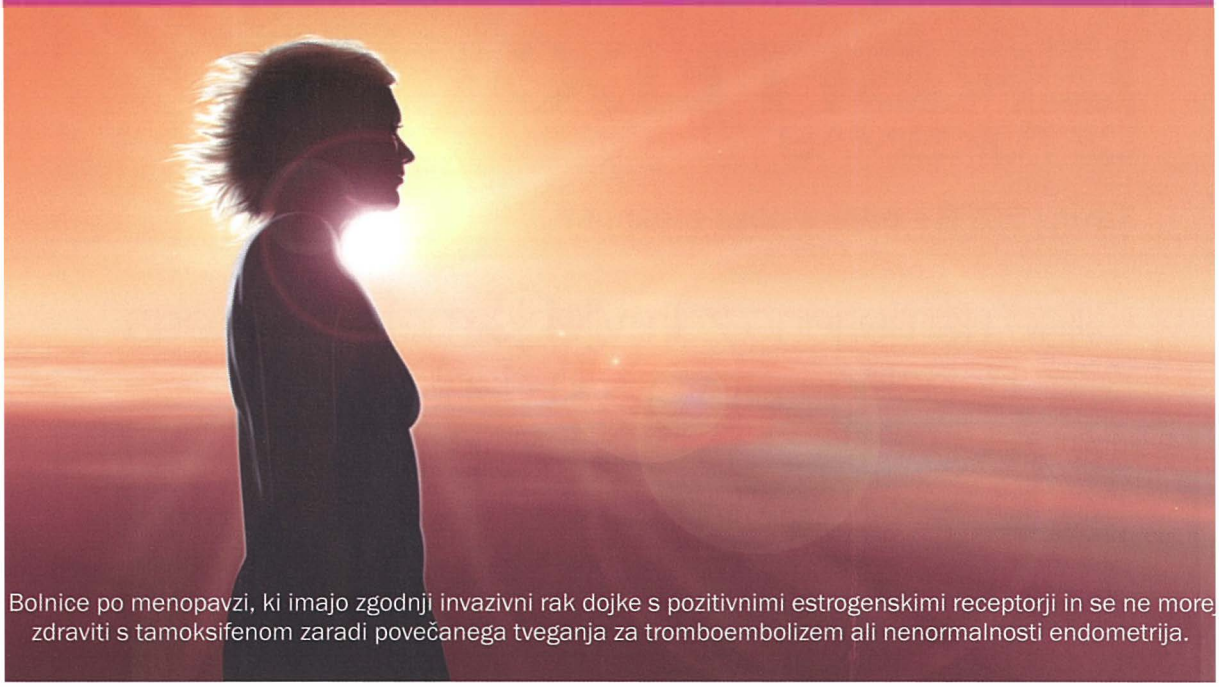
dopolnilno zdravljenje*



Arimidex vodilni zaviralec aromataze

anastrozol

Kratka informacija o zdravilu Arimidex 1 mg

Sestava: Filmsko obložena tableta vsebuje 1 mg anastrozola.

Indikacije: Adjuvantno zdravljenje žensk po menopavzi, ki imajo zgodnji invazivni rak dojke s pozitivnimi estrogenskimi receptorji in se ne morejo zdraviti s tamoksifenom zaradi povečanega tveganja za tromboembolizem ali nenormalnosti endometrija. Zdravljenje napredovalega raka dojke pri ženskah po menopavzi. Učinkovitost pri bolnicah z negativnimi estrogenskimi receptorji ni bila dokazana razen pri tistih, ki so imele predhodno pozitiven klinični odgovor na tamoksifen.

Odmerjanje in način uporabe: 1 tableta po 1 mg peroralno, enkrat na dan. Pri zgodnjem raku je priporočljivo trajanje zdravljenja 5 let.

Kontraindikacije: Arimidex je kontraindiciran pri: ženskah pred menopavzo, nosečnicah in doječih materah, bolnicah s hujšo ledvično odpovedjo (očistek kreatinina manj kot 20 ml/min (oziroma 0,33 ml/s)), bolnicah z zmernim do hudim jetrnim obolenjem in bolnicah, ki imajo znano preobčutljivost za anastrozol ali za katerokoli drugo sestavino zdravila. Zdravila, ki vsebujejo estrogen, ne smete dajati sočasno z Arimidexom, ker bi se njegovo farmakološko delovanje izničilo.Tamoksifena ne smete uporabljati skupaj z Arimidexom, ker lahko pride do zmanjšanja njegovega delovanja.

Posebna opozorila in previdnostni ukrepi: Uporabe Arimidexa ne priporočamo pri otrocih, ker njegova varnost in učinkovitost pri njih še nista raziskani. Menopavzo je potrebno biokemično določiti pri vseh bolnicah, kjer obstaja dvom o hormonskem statusu. Ni podatkov o varni uporabi Arimidexa pri bolnicah z zmerno ali hudo jetrno okvaro ali hujšo ledvično odpovedjo (očistek kreatinina manj kakor 20 ml/min (oziroma 0,33 ml/s)). Ni podatkov o uporabi anastrozola z analogi LHRH. Te kombinacije zdravil se ne sme uporabljati zunaj kliničnih preskušanj. Pri ženskah z osteoporozo ali pri ženskah s povečanim tveganjem za razvoj osteoporoze je treba določiti njihovo mineralno gostoto kosti z denzitometrijo, na primer s slikanjem DEXA na začetku zdravljenja, pozneje pa v rednih intervalih. Po potrebi je treba začeti z zdravljenjem ali preprečevanjem osteoporoze in to skrbno nadzorovati. Ni verjetno, da bi Arimidex zmanjšal bolničino sposobnost za vožnjo ali upravljanje s stroji. Ker pa so med uporabo Arimidexa poročali o splošni oslabelosti in zaspanosti, je potrebna previdnost pri vožnji in upravljanju strojev, dokler simptoma trajata.

Nosečnost in dojenje: Arimidex je med nosečnostjo in dojenjem kontraindiciran.

Neželeni učinki: Najpogostejši neželeni učinki so navali vročine, suhost vagine in redčenje las. Ostali neželeni učinki vključujejo gastrointestinalne motnje (anoreksija, slabost, bruhanje, diareja), astenijo, bolečine/okorelost v sklepih, zaspanost, glavobol in izpuščaje. Občasna poročila navajajo krvavitev iz nožnice, ki se pretežno pojavlja pri bolnicah z napredovalim obolenjem raka na dojki v prvih tednih po prehodu z dotedanjega hormonskega zdravljenja na zdravljenje z Arimidexom. Če krvavitev traja dlje časa, so potrebne dodatne preiskave. Hiperholesterolemija, običajno blaga do zmerna. O povišanih nivojih gama-GT in alkalne fosfataze so poročali le občasno. Vzročna povezanost omenjenih sprememb ni bila ugotovljena.

Medsebojno delovanje z drugimi zdravili: Zdravila, ki vsebujejo estrogen, ne smete dajati sočasno z Arimidexom, ker bi se njegovo farmakološko delovanje izničilo. Tamoksifena se ne sme uporabljati skupaj z Arimidexom, ker lahko pride do zmanjšanja njegovega delovanja.

Vrsta ovojnine in vsebina: Pretisni omoti iz PVC in aluminija, ki vsebujejo 28 tablet v škatlici.

Režim izdaje zdravila: Rp/Spec

Datum priprave informacije: oktober 2005

Pred predpisovanjem, prosimo, preberite celoten povzetek temeljnih značilnosti zdravila.

Dodatne informacije in literatura so na voljo pri:
AstraZeneca UK Limited, Podružnica v Sloveniji, Einspielerjeva 6, Ljubljana
www.breastcancersource.com

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KEMOMED

PE: Stritarjeva 5, 4000 Kranj, Slovenija
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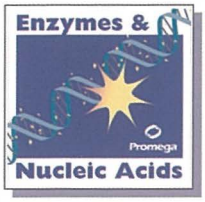
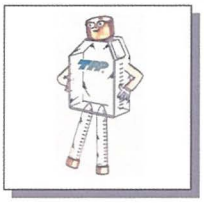
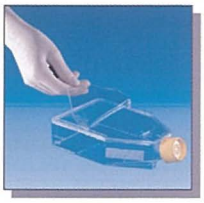
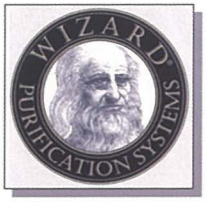
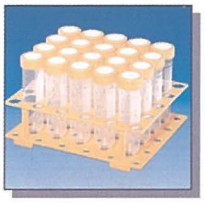
Promega











IZDELKI ZA MOLEKULARNO BIOLOGIJO

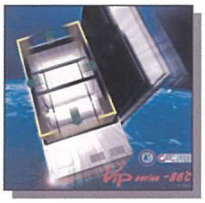
DOKUMENTACIJA IN ANALIZA GELOV

PLASTIKA ZA CELIČNE KULTURE

ELGA LABWATER

ČISTA VODA ZA LABORATORIJ

SANYO



SKRINJE IN HLADILNIKI

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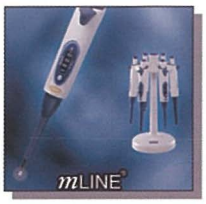
CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA

BIOHIT



ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

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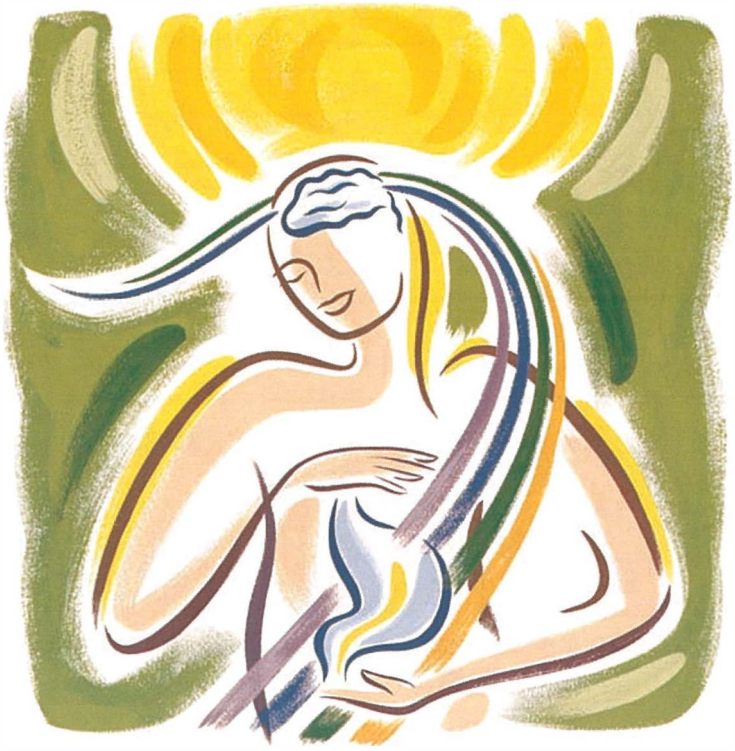
DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL

HPLC in GC POTROŠNI MATERIAL

PRVI IN EDINI ANTAGONIST NEVROKININ-1 (NK_1) RECEPTORJEV[1]



EMEND®†
(aprepitant)

Preprečevanje akutne in zapoznele slabosti in bruhanja

NOVA indikacija

- **Preprečevanje navzeje in bruhanja, povezanih z zmerno emetogeno kemoterapijo raka[1]**
- **Preprečevanje akutne in zapoznele navzeje in bruhanja, povezanih z zelo emetogeno terapijo raka s cisplatinom[1]**

MSD

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Tel.: 01/ 52 04 201, faks: 01/ 52 04 349, 52 04 350

Prosimo, da pred predpisovanjem preberete priložen Povzetek glavnih značilnosti zdravila.

Zdravilo se izdaja le na zdravniški recept (H/Rp).

Literatura: 1. Arhiv MSD, Slovenija.

†Zaščitena blagovna znamka MERCK & Co., Inc., Whitehouse Station, N. J., ZDA.

EMEND®† (aprepitant)

EMEND 80mg trde kapsule
EMEND 125 mg trde kapsule
EN-EMEA/HC/0527/II 11

SKRAJŠAN POVZETEK GLAVNIH ZNAČILNOSTI ZDRAVILA

Pred predpisovanjem, prosimo, preberite celoten Povzetek glavnih značilnosti zdravila, ki ga dobite pri našihstrokovnih sodelavcih!

Sestava:
EMEND 125 mg trde kapsule in EMEND 80 mg trde kapsule.
Ena 125 mg kapsula vsebuje 125 mg aprepitanta.
Ena 80 mg kapsula vsebuje 80 mg aprepitanta.
Pomožne snovi: saharoza, mikrokristalna celuloza (E460), hidroksipropilceluloza (E463), natrijev lavrilsulfat, želatina, titanov dioksid (E171) - 125 mg kapsule pa še rdeči železov oksid (E172), rumeni železov oksid (E172), šelak, kalijev hidroksid, črni železov oksid (E172).

Terapevtske indikacije:
Preprečevanje akutne in zapoznele navzeje in bruhanja povezanih z zelo emetogeno kemoterapijo raka s cisplatinom.
Preprečevanje navzeje in bruhanja, povezanih z zmerno emetogeno kemoterapijo raka.
EMEND se daje v sklopu kombiniranega zdravljenja (glejte poglavje 4.2 v Povzetku glavnih značilnosti zdravila).

Odmerjanje in način uporabe:
EMEND je na voljo v obliki 80 mg in 125 mg trdih kapsul.
EMEND se daje 3 dni po shemi zdravljenja, ki vključuje kortikosteroid in antagonist 5-HT_3.
Priporočeni odmerek zdravila EMEND je 125 mg peroralno prvi dan ter 80 mg enkrat na dan drugi in tretji dan.
Podatkov o učinkovitosti pri kombiniranju z drugimi kortikosteroidi in antagonisti 5-HT_3 ni dovolj.
EMEND se lahko jemlje s hrano ali brez. Trdo kapsulo je treba pogoltniti celo.
Starejši bolniki: Pri starejših bolnikih odmerka ni treba prilagajati.
Okvara ledvic: Pri bolnikih z okvaro ledvic in pri bolnikih s končno ledvično odpovedjo, ki se zdravijo s hemodializo, odmerka ni treba prilagoditi.
Okvara jeter: Pri bolnikih z blago okvaro jeter odmerka ni treba prilagajati. Pri bolnikih z zmerno okvaro jeter so podatki omejeni, podatkov pri bolnikih s hudo okvaro jeter ni na voljo.
Otroci in mladostniki: Varnost in učinkovitost pri otrocih in mladostnikih nista znani. Uporabe pri bolnikih, ki so mlajši od 18 let, zato ne priporočamo.

Kontraindikacije:
Preobčutljivost za zdravilno učinkovino ali katero koli pomožno snov.
Zdravila EMEND se ne sme uporabljati sočasno s pimozidom, terfenadinom, z astemizolom ali s cisapridom.

Posebna opozorila in previdnostni ukrepi:
Podatki o uporabi pri bolnikih z zmerno okvaro jeter so omejeni. Podatkov o uporabi pri bolnikih s hudo okvaro jeter ni na voljo. Pri teh bolnikih je treba aprepitant uporabljati previdno.
EMEND je treba uporabljati previdno pri bolnikih, ki sočasno jemljejo zdravila, ki se primarno presnavljajo s CYP3A4. Zato je treba previdno uporabljati kemoterapevtike, ki se presnavljajo s CYP3A4. Še posebej je previdnost potrebna pri sočasnem dajanju irinotekana, saj lahko kombinacija poveča toksični učinek.
Pri sočasni uporabi EMEND-a z alkaloidi rženega rožička (ergot alkaloidi), ki so substrat za CYP3A4, se lahko zviša plazemska raven teh zdravil.
Sočasna uporaba EMEND-a z varfarinom zmanjša protrombinski čas, izražen kot INR *(International Normalised Ratio)*. Pri bolnikih, ki se neprenehoma zdravijo z varfarinom, je treba INR skrbno spremljati med zdravljenjem z zdravilom EMEND in 2 tedna po vsakem 3-dnevnem ciklusu zdravljenja z EMEND-om.
Med jemanjem EMEND-a se lahko zmanjša učinkovitost oralnih kontraceptivov. Med zdravljenjem z EMEND-om in dva meseca po zadnjem odmerku EMEND-a je treba uporabljati alternativna ali dodatna kontracepcijska sredstva.
Sočasnemu jemanju EMEND-a in zdravil, ki močno inducirajo aktivnost CYP3A4 (npr. rifampicin, fenitoin, karbamazepin, fenobarbital), se je treba izogibati, ker kombinacija povzroči zmanjšanje plazemskih koncentracij aprepitanta. Sočasna uporaba EMEND-a in šentjanževke ni priporočljiva.
Potrebna je previdnost pri sočasni uporabi EMEND-a in zdravil, ki zavirajo aktivnost CYP3A4 (npr. ritonavir, ketokonazol, klaritromicin, telitromicin), ker kombinacija povzroči zvišanje plazemskih koncentracij aprepitanta.
Bolniki z redkimi dednimi motnjami fruktozno intoleranco, malabsorpcijo glukoze in galaktoze ali insuficienco saharaze-izomaltaze ne smejo jemati tega zdravila.

Medsebojno delovanje z drugimi zdravili in druge oblike interakcij:
Aprepitant je substrat, zmerni zaviralec in induktor CYP3A4. Aprepitantje tudi induktor CYP2C9.
Kot zmerni zaviralec CYP3A4 lahko aprepitant zviša plazemske koncentracije sočasno uporabljenih zdravil, ki se presnavljajo s CYP3A4. AUC peroralno vzetih substratov CYP3A4 se lahko poveča do približno 3-krat. Pri sočasnem jemanju s substrati CYP3A4 svetujemo previdnost.
EMEND-a se ne sme uporabljati skupaj s pimozidom, terfenadinom, z astemizolom ali s cisapridom. Aprepitant zavira CYP3A4, zaradi česar bi se lahko zvišale plazemske koncentracije teh zdravil, kar bi lahko povzročilo resne ali življenje ogrožajoče reakcije.
Kot zmeren induktor CYP2C9 in blag induktor CYP3A4 in glukuronidacije, lahko aprepitant zniža plazemske koncentracije substratov, ki se izločajo po teh poteh. Ta učinek se lahko pokaže šele po koncu zdravljenja z EMEND-om. Indukcija substratov CYP2C9 in CYP3A4 je prehodna, maksimalen učinek pa je dosežen v 3-5 dneh po koncu 3 dnevnega zdravljenja z zdravilom EMEND. V tem obdobju svetujemo previdnost pri dajanju peroralnih zdravil, ki se presnavljajo s CYP3A4.
Pokazano je bilo, da aprepitant inducira presnavljanje S() varfarina in tolbutamida, ki se presnavljata s CYP2C9. Sočasno dajanje EMEND-a in teh ali drugih zdravil, ki se presnavljajo s CYP2C9, denimo fenitoina, lahko zniža plazemske koncentracije teh zdravil, zato svetujemo previdnost.
EMEND nima medsebojnega vpliva z digoksinom, zato verjetno ne intereagira s P-glikoproteinskim prenašalcem.
Kortikosteroidi:
Deksametazon: Pri sočasnem jemanju z EMEND-om je treba običajni peroralni odmerek zmanjšati za približno 50 %.
Metilprednizolon: Pri sočasni uporabi z EMEND-om je treba običajni intravenski odmerek metilprednizolona zmanjšati za približno 25 %, običajni peroralni odmerek metilprednizolona pa za približno 50 %.
Kemoterapevtiki: V kliničnih raziskavah so EMEND uporabljali skupaj z naslednjimi kemoterapevtiki, ki se predvsem ali delno presnavljajo s CYP3A4: etopozid, vinorelbin, docetaksel in paklitaksel. Odmerkov teh zdravil niso prilagajali glede na morebitno medsebojno delovanje zdravil. Svetujemo previdnost; pri bolnikih, ki dobivajo taka zdravila, je lahko potreben dodaten nadzor.
Midazolam: Pri sočasni uporabi z EMEND-om je treba upoštevati možne učinke zvišanih plazemskih koncentracij midazolama in drugih benzodiazepinov, ki se presnavljajo predvsem s CYP3A4 (alprazolam, triazolam).
EMEND poveča AUC midazolama, ki je občutljiv substrat za CYP3A4.
Varfarin: Pri bolnikih, ki se dolgotrajno zdravijo z varfarinom, je treba protrombinski čas (INR) skrbno nadzorovati med zdravljenjem z zdravilom EMEND in 2 tedna po vsakem 3-dnevnem ciklusu zdravljenja z EMEND-om.
Tolbutamid: EMEND je prijemanju po shemi 125 mg prvi dan ter 80 mg/dan drugi in tretji dan zmanjšal AUC tolbutamida (ki je substrat za CYP2C9), ki so ga bolniki prejemali v enkratnem odmerku 500 mg per os pred začetkom 3-dnevne sheme odmerjanja EMEND-a ter 4., 8. in 15. dan, in sicer za 23 % 4. dan, za 28 % 8. dan in za 15 % 15. dan.
Oralni kontraceptivi: Med jemanjem EMEND-a se lahko zmanjša učinkovitost oralnih kontraceptivov. Med zdravljenjem z EMEND-om in dva meseca po zadnjem odmerku EMEND-a je treba uporabljati alternativne ali dodatne kontracepcijske metode.
Antagonisti 5-HT_3: V kliničnih raziskavah medsebojnega delovanja aprepitant ni imel klinično pomembnih učinkov na farmakokinetiko ondansetrona in granisetrona.
Vplivi drugih zdravil na farmakokinetiko aprepitanta
Potrebna je previdnost pri sočasni uporabi EMEND-a in zdravil, ki zavirajo aktivnost CYP3A4 (npr. ritonavir, ketokonazol, klarítromicin, telitromicin), ker se zaradi kombinacije zvišajo plazemske koncentracije aprepitanta.
Sočasnemu dajanju EMEND-a in zdravil, ki močno inducirajo aktivnost CYP3A4 (npr. rifampicin, fenitoin, karbamazepin, fenobarbital), se je treba izogibati, saj se pri kombiniranju zmanjšajo plazemske koncentracije aprepitanta, zaradi česar se lahko zmanjša učinkovitost EMEND-a. Sočasno uporabo EMEND-a in šentjanževke odsvetujemo.
Ketokonazol: Pri enkratnem odmerku 125 mg EMEND-a 5. dan 10-dnevnega zdravljenja s ketokonazolom (ki je močan zaviralec CYP3A4) 400 mg na dan, se je AUC aprepitanta povečal za približno 5-krat, povprečni terminalni razpolovni čas aprepitanta pa se je povečal približno za 3-krat.
Rifampicin: Pri enkratnem odmerku 375 mg EMEND-a 9. dan 14-dnevnega zdravljenja z rifampicinom (ki je močan induktor CYP3A4) 600 mg na dan, se je AUC aprepitanta zmanjšal za 91 %, povprečni terminalni razpolovni čas aprepitanta pa se je za 68 % zmanjšal.

Neželeni učinki:
Varnost aprepitanta so ocenjevali pri približno 3800 preiskovancih.
O kliničnih neželenih učinkih, ki so jih raziskovalci opredelili kot dogodke, povezane z zdravilom, so poročali pri približno 17 % bolnikov, zdravljenih z aprepitantom, ter pri 13 % bolnikov, zdravljenih z običajno terapijo (pri bolnikih, ki se zaradi raka zdravijo z zelo emetogeno kemoterapijo). Zaradi neželenih učinkov so zdravljenje prekinili pri 0,6 % bolnikov, zdravljenih z aprepitantom, ter pri 0,4 % bolnikov, zdravljenih s standardno terapijo. V klinični raziskavi bolnikov, ki so dobivali zmerno emetogeno kemoterapijo, so o kliničnih neželenih učinkih poročali pri približno 21 % bolnikov, zdravljenih z aprepitantom, ter pri približno 20 % bolnikih, zdravljenih s standardno terapijo. Zdravljenje z aprepitantom so zaradi neželenih učinkov prekinili pri 1,1 % bolnikov, zdravljenih z aprepitantom, ter pri 0,5 % bolnikov, zdravljenih s standardno terapijo.
Najpogostejši neželeni učinki, o katerih so pri zdravljenju z aprepitantom pri bolnikih, ki so dobivali zelo emetogeno kemoterapijo, poročali pogosteje kot pri običajni terapiji, so bili: kolcanje (4,6 %), oslabelost/utrujenost (2,9 %), zvišanje alanin-aminotransferaze (ALT) (2,8 %), zaprtje (2,2 %), glavobol (2,2 %) ter anoreksija (2,0 %). Najpogostejši neželeni učinek, o katerem so pri bolnikih, ki so dobivali zmerno emetogeno kemoterapijo, poročali pogosteje kot pri bolnikih, ki so bili zdravljeni s standardno terapijo, je bila utrujenost (2,5 %).
Pri bolnikih, zdravljenih z aprepitantom, so opazili naslednje neželene učinke, ki so se pojavljali pogosteje kot pri običajni terapiji:
Pogoste (>1/100, <1/10): anoreksija, glavobol, omotica, kolcanje, zaprtje, driska, dispepsija, eruktacija, oslabelost/utrujenost, zvišanje ALT, zvišanje aspartat-aminotransferaze (AST).
Občasne(>1/1.000, <1/100): kandidiaza, okužbe s stafilokoki, anemija, febrilna nevtropenija, pridobivanje telesne teže, polidipsija, dezorientacija, evforija, anksioznost, nenormalne sanje, motnje mišljenja, konjunktivitis, tinitus, bradikardija, navali vročine, faringitis, kihanje, kašelj, zatekanje izcedka iz nosu v žrelo, draženje žrela, navzeja*, bruhanje*, refluks kisline, motnje okusa, neugodje v epigastriju, zaprtje, gastroezofagalna refluksna bolezen, predrtje razjede dvanajstnika, bolečine v trebuhu, suha usta, enterokolitis, vetrovi, stomatitis, izpuščaji, akne, fotosenzitivnost, prekomerno potenje, mastna koža, srbenje, lezije kože, mišični krči, bolečine v mišicah, poliurija, dizurija, polakisurija, bolečine v trebuhu, otekanje, zardevanje, nelagodje v prsnem košu, letargija, žeja, zvišanje alkalne fosfataze, hiperglikemija, mikrohematurija, hiponatremija, znižanje telesne teže.
*Navzeja in bruhanje sta bila parametra učinkovitosti prvih 5 dni po kemoterapiji; o njih so kot o neželenih učinkih poročali šele po tem času.
Profil neželenih učinkov je bil v podaljšku raziskave z več ciklusi zdravljenja (do 5 dodatnih ciklusov kemoterapije) na splošno podoben tistemu po prvem ciklusu. Pri enem bolniku, ki je dobival aprepitant ob kemoterapiji zaradi raka, so poročali o Stevens-Johnsonovem sindromu kot o resnem neželenem učinku. Pri enem bolniku, ki je prejemal aprepitant, vendar ne v raziskavi CINV (Cisplatin-Induced Nausea and Vomiting), so poročali o angioedemu in koprivnici kot o resnem neželenem učinku.
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