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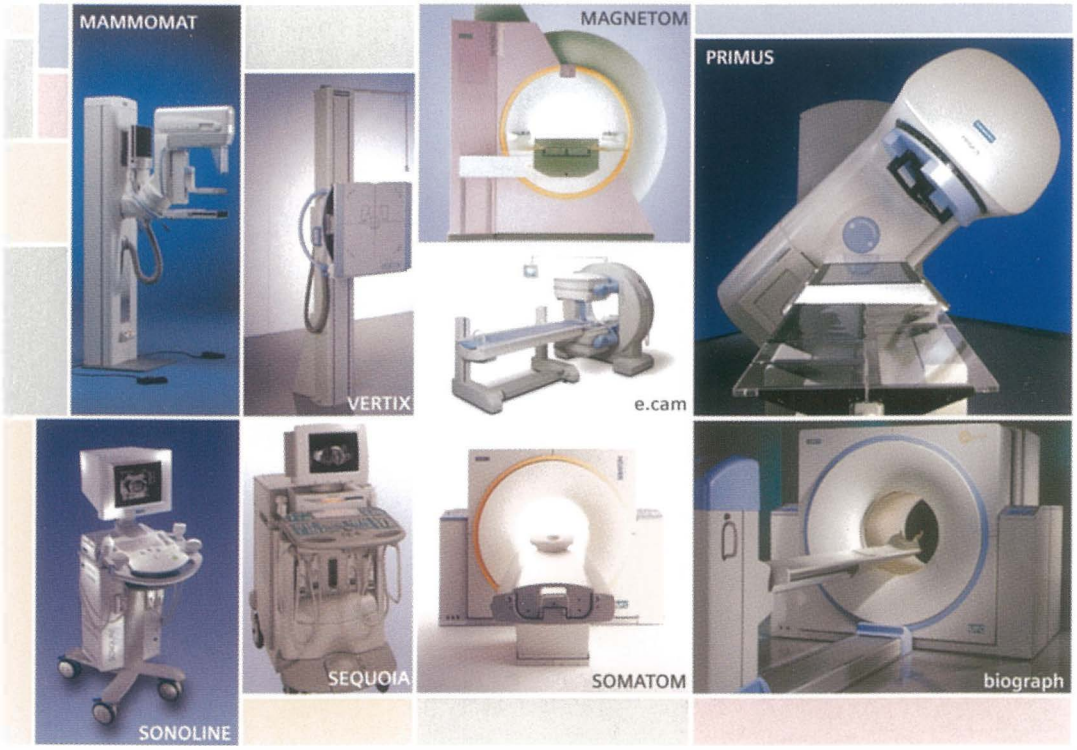

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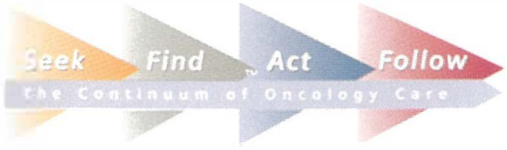

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Brain metastases in lung cancer. Impact of prognostic factors on patient survival

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Background. *Brain metastases are common patterns of dissemination in lung cancer patients. In this paper we would like to assess the pattern of brain metastases in lung cancer patients and the impact of prognostic factors on the survival of lung cancer patients with brain metastases.*

Patients and methods. *In the year 1998 there were 974 registered patients with lung cancer in Slovenia, six hundred and fifteen of them were treated at the Institute of Oncology Ljubljana and we analyzed them. Among 615 patients 137 (22.3 %) of them have had brain metastases during a natural course of disease.*

Results. *For 12 patients presenting with solitary brain metastases (most of them were undertaken metastasectomy) median survival was 7.6 months, while in patients with multiple brain metastases the median survival was 2.8 months (p = 0.0018). Of the 137 patients 45 (32.8 %) were small cell lung cancer patients, 43 (31.4 %) were adenocarcinoma patients and 19 (13.9 %) were squamous cell carcinoma patients. Patients with performance status (WHO scale) less than 2 had the median survival time 3.7 months while patients with performance status 2 or more had median survival time 2.7 moths (p=0.0448).*

Conclusions. *Patients with solitary brain metastases had better survival comparing with those who had multiple metastases. It is surprisingly that the portion of brain metastases patients with adenocarcinoma is almost equal to those with small-call lung cancer therefore, the prophylactic cranial radiation becomes actual for both groups of patients. The performance status of patients with brain metastases remains very important prognostic factor.*

Key words: lung neoplasms; brain neoplasms secondary; survival analysis

Introduction

Brain metastases are common patterns of dissemination in lung cancer patients. In the natural course of disease in around 17% of patients with squamous carcinoma, in 24% of patients with anaplastic carcinoma, in 39% of patients with adenocarcinoma and in 42% with small cell carcinoma brain metastases will develop.[1] Admittedly non-small cell car-

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cinoma of the lung patients with single resectable metastases will gain from metastasectomy followed by the external beam cranial irradiation.[2-4] In those patients a gain can be objectified as the survival. Unfortunately, those patients represent only about 10% of lung cancer patients with brain metastases. The rest 90% of lung cancer patients are either small cell lung cancer patients or patients with multiple or irresectable metastases which do not benefit from the same treatment.

The majority of patients are thus offered the choice of palliative an external beam cranial irradiation or a corticosteroid treatment as the best supportive care.[3] In last few years there is a tendency towards shorter radiation courses. There is evidence that the size and the number of fractions do not influence the outcome of treatment and duration of response.[5,6] Furthermore, it seems that the only factor influencing the response is the severity of neurological deficit and that those patients presenting with the most severe deficit usually respond better than those with only minor neurological changes. On the other hand those presenting with minor neurological changes fare better in the term of survival, which is expected.[7,8]

An acceptable approach towards the treatment of brain metastases is corticosteroid therapy alone, which is particularly appropriate for those elderly in poor physical condition.[9]

At the Institute of oncology in Ljubljana we are currently using 5 x 4 Gy, 10 x 3 Gy, 12 x 2.5 Gy and 14 x 2.5 Gy. In this paper we would like to assess the pattern of brain metastases in lung cancer patients and the impact of treatment on the outcome of lung cancer patients with brain metastases.

Patients and methods

The Cancer registry of Slovenia collects data on all cancer patients in Slovenia. In the year 1998 there were registered 974 patients with lung cancer,[10] six hundred and fifteen of them were treated at the Institute of Oncology Ljubljana. In the year 2003 we reviewed all these 615 patients who were at least once examined and managed at our Institute.

The median age of lung cancer patients was 62.66 years (min. 36.01; max. 89.54 standard deviation 10.029); male, female ratio was 4:1; histological types were: 155 (25.2 %) adenocarcinoma, 217 (35.3 %) squamous carcinoma, 32 (5.2 %) anaplastic carcinoma, 132 (21.5 %) small cell carcinoma, 45 (7.3 %) non small cell carcinoma, 10 (1.6 %) mixed carcinoma, 1 (0.2 %) bronchioloalveolar carcinoma. Twenty-one patients (3.4 %) had no histological diagnosis prior to their death.

Among 615 patients 137 (22.3 %) have had brain metastases during the natural course of disease.

Of these 137 patients 22 (16 %) presented with brain metastases at the time of a diagnosis and 115 (84 %) underwent a treatment and developed brain metastases later on.

Patients were treated for brain metastases either with radiotherapy alone (110 patients (80.3 %)) or in cases with solitary brain metastases with metastasectomy followed by radiotherapy (12 patients (8.8 %)). Due to a poor performance status 15 patients (10.9 %) received no oncological treatment except the best supportive care.

Results

The median survival for all 615 patients diagnosed with lung cancer in 1998 was 8.9 months (range: min. 2 days, max. 62.6 months; 95 % confidence interval 7.93 - 9.9 months).

For patients with brain metastases the median survival was 3.1 months (range: min 0 days, max: 52.2 months)

For 12 patients presenting with solitary

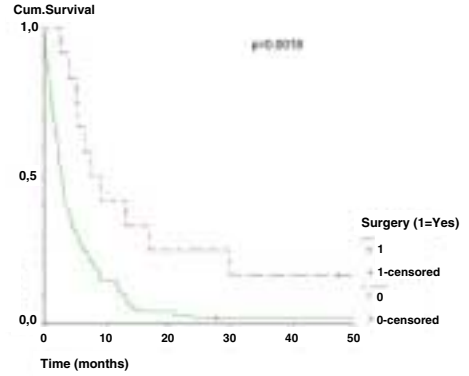

Figure 1. Surviving of patients with solitary brain metastases and patients with multiple brain metastases.

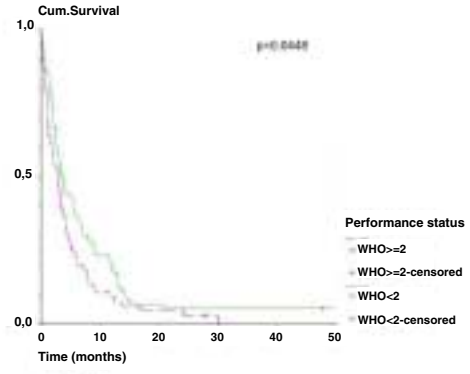

Figure 3. Survival of patients with brain metastases according the performance status.

brain metastases (the most of them were undertaken metastasectomy), median survival was 7.6 months, (range: min 2.4 months, max 52.2 months, 95% confidence interval 2.9 - 12.4 months) compared to those with multiple brain metastases for whom the median survival was 2.8 months (range: min 0 days, max 50.4 months; 95% confidence interval 2.1- 3.5 months). The difference is statistically significant (log-rank p = 0.0018) (Figure 1).

Of the 137 patients with brain metastases 45 or 32.8 % were small cell lung cancer patients, 43 or 31.4 % were adenocarcinoma patients and 19 or 13.9 % were squamous cell carcinoma patients (Figure 2).

Predictably an important factor influencing the survival in lung cancer patients with brain metastases is a performance status. In those patients with performance status on WHO scale less than 2 a median survival was 3.7 months while for patients with a performance status on WHO scale 2 or more a median survival was 2.7 moths. This small difference is statistically significant with p=0.0448 (Figure 3).

There were 18 patients with adenocarcinoma confined to chest, later on presented with brain metastases, their median survival was 16.1 months (range: min 4.7 months max: 56.6 month) and their median survival after the diagnosis of brain metastases was 2.3 months (range: min. 0 days max 50.4 months)

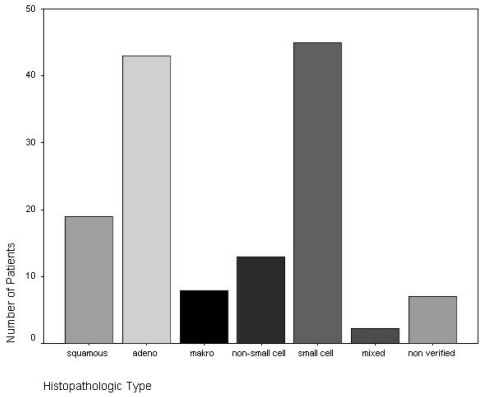

Figure 2. The histological type of lung cancer patients with brain metastases.

Discussion

As already shown elsewhere[1] a fair proportion of lung cancer patients will be, during the course of their disease, presented with brain metastases. At our Institute 22.3 % of lung cancer patients reported in 1998 presented with brain metastases in the course of disease.

There was still a prevalence of squamous cell carcinoma in all patients, but in those with brain metastases, there is almost an equal proportion of small cell lung cancer and

adenocarcinoma (32.8 % and 31.4 % respectively). In most historical studies there was a well-established prevalence of squamous cell carcinoma,[11] but in last years we are starting to recognize a rise in adenocarcinoma patients.[12] In our retrospective of 155 patients with adenocarcinoma 43 or 27.7 % presented with brain metastases which is comparable with 45 of 132 or 34 % for non small cell lung cancer and far above 19 of 217 or 8.8 % for squamous carcinoma.

Since of 43 adenocarcinoma patients with brain metastases 18 or 41 % at presentation had a disease confined to chest and either received surgery or radiotherapy with curative intent, we could assume that with the increase in adenocarcinoma incidence there will also be the increase in the number of patients who will present with brain metastases after the radical treatment. This presumption opens space for screening for brain metastases in this patients and open question of prophylactic brain irradiation in adenocarcinoma patients.[13,14]

For patients with solitary brain metastases the effective treatment is metastasectomy followed by brain irradiation, which in patients with good performance status yield a survival superior to irradiation alone.[15] However, similar results are achieved with the use of cranial stereotactic radiosurgery.[16]

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Endobronchial metastasis as first manifestation of renal cell carcinoma

Yoshiko Kaneko, Norihiro Haraguchi, Takahide Kodama, Katsunori Kagohashi, Yukio Ishii, Hiroaki Satoh, Kiyohisa Sekizawa

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Background. *In the majority of cases of endobronchial metastasis, presence of a primary tumour antedated the diagnosis of the metastasis. We showed a case of endobronchial metastasis as first manifestation of renal cell carcinoma.*

Case report. *A 61-year-old man was admitted to our hospital complaining of cough of 3 months duration. Chest CT scan showed a polypoid mass in the right upper lobe bronchus. Biopsy of the lesion was obtained, and microscopic examination showed metastatic renal cell carcinoma of the bronchial wall.*

Conclusions. *When endobronchial lesion occurs in the absence of clinical evidence of a primary tumour, appropriate diagnostic studies should be undertaken to exclude the possibility of an asymptomatic extrathoracic tumour.*

Key words: bronchial neoplasms - secondary; carcinoma, renal cell - diagnosis

Introduction

The lung is a common site of metastasis in renal cell carcinoma.[1] However, endobronchial metastasis as the first manifestation of renal cell carcinoma seems to be uncommon.[2-7] We report here clinical findings of such a rare case.

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Case report

A 61-year-old man was admitted to our hospital complaining of cough of 3 months duration. He began to smoke cigarettes at the age of 20 years and consumed two packages daily thereafter. He had no previous diseases. On physical examination, his blood pressure was 140/70 mmHg and pulse rate 80/min and regular. Enlarged lymph nodes were not detected. On percussion and auscultation of the chest, increased dullness and diminished breath sounds were noted in the upper half of the right lung field.

Routine blood tests and ECG were normal. The urine was normal. Chest X-ray revealed

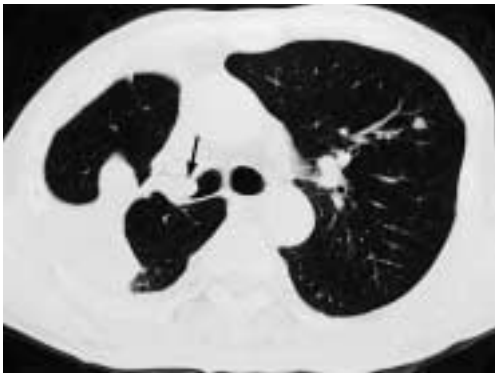

Figure 1. Chest CT scan showed a polypoid mass in the right upper lobe bronchus (arrow).

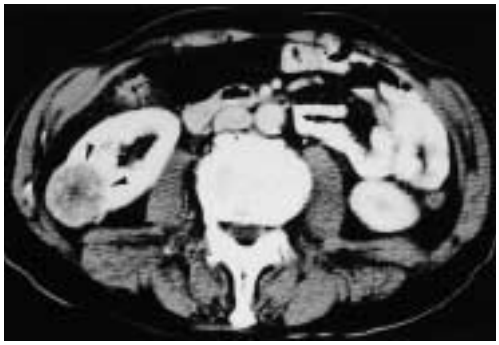

Figure 2. CT scan of the abdomen showing a 4-cm tumour in the lower pole of the right kidney (arrow head) without regional lymph node swelling.

right upper lobe atelectasis with localized pleural effusion. Chest CT scan showed a polypoid mass in the right upper lobe bronchus (Figure 1). On bronchoscopy, an obstructive, polypoid mass was found in the right upper bronchus. Biopsy of the lesion was obtained, and microscopic examination showed metastatic renal cell carcinoma of the bronchial wall. CT scan of the abdomen revealed a 4-cm tumour in the lower pole of the right kidney, but regional lymph node swelling was not observed (Figure 2). No other distant metastatic lesions than lung were found. Thereafter, nephrectomy was performed, and the tumour was confirmed as renal cell carcinoma pathologically.

Discussion

Endobronchial metastases are a late manifestation in the course of solid tumour.[8-13] In the majority of cases clinical manifestations of the presence of a primary extrathoracic tumour antedated the diagnosis of endobronchial metastasis. [8-13] Occasionally, however, clinical and roentgenographic features of endobronchial metastasis preceded recognition of the primary tumour.[2-7] In 1975, Braman and Whitcob reported 7 of the 15 renal tumours were accompanied by symptoms of a bronchial metastasis three weeks to ten months before discovery of the primary renal cell carcinoma.[14] Thereafter, however, there have been few reports of endobronchial metastasis being diagnosed before the detection of the primary extrathoracic tumour.[7,15] In the reports from our country, seven among the 37 patients with endobronchial metastasis from renal cell carcinoma preceded recognition of the primary tumour.[15] Recently, Katsimbri *et al.* reported 8 case of endobronchial metastasis from various organs. In all of the 8 patients, however, clinical manifestations of the presence of a primary extrathoracic tumour antedated the diagnosis of the endobronchial metastases, and the median interval of endobronchial metastases diagnosis from the diagnosis of the primary tumour was 41 months.[12]

Although a few, however, there are reports of endobronchial metastatic lesions being diagnosed before the detection of the primary tumour.[2-7]

In this case report, we showed that a certain type of renal cell carcinoma develops endobronchial metastasis with no regional lymph node swelling, and such hematogenous distant metastasis may not necessarily associated with enlarged size of primary lesion. When endobronchial neoplastic lesion occurs in the absence of clinical evidence of an extrathoracic primary tumour, the bronchial neoplasma is almost certain to be a

primary lung cancer. Nevertheless, if atypical clinical or pathological features as primary lung cancer are present, appropriate diagnostic studies should be undertaken to exclude the possibility of an asymptomatic extrathoracic tumour as observed in our case.

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Pubic bone metastasis as first manifestation of lung cancer

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Background. *Pubic bone metastasis as the initial manifestation of lung cancer is very rare, and it may simulate parasymphyseal insufficiency fracture of the pubic bone, which is observed in postmenopausal women and elderly people.*

Case report. *A 65-year-old woman developed pain in the right flank, which extended to the anterior aspect of the thigh. A pelvic X-ray showed osteolytic lesion in the right pubic ramus. Chest radiograph revealed a nodular mass in the right middle lobe of the lung. Transbronchal biopsy of the mass led to the diagnosis of lung adenocarcinoma. The patient was given radiotherapy of osteolytic lesion in her right pubic ramus and the pain was controlled with a combination of morphine sulfate.*

Conclusions. *When unusual bone metastasis is found in the absence of a primary tumor, investigation must include chest radiographs.*

Key words: pubic bone; bone neoplasms - secondary; lung neoplasms; adenocarcinoma

Introduction

Lung cancer is a severe disease often diagnosed a late stage when surgical resection is no longer possible because of local advancement or distant metastasis.[1] Bone metastases from lung cancer may occur early in the clinical course and are usually discovered with severe pain.[2-4]

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The spine and ribs are often the earliest sites of bone metastases, whereas the skull, femur, humerus, and scapula are involved later.[5] Pubic bone metastasis as the initial manifestation of lung cancer is very rare. We report a case of particular interest because of unusual bone metastatic site and the relatively slow progression of the neoplasm.

Case report

A 65-year-old woman was referred to her orthopedist with pain in the right flank, which extended to the anterior aspect of the thigh for three months. A pelvic radiograph showed an osteolytic lesion in her right pubic

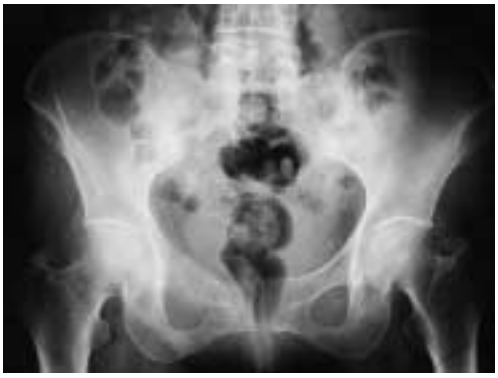

Figure 1. Pelvic radiograph showed an osteolytic lesion in the right pubic ramus.

ramus (Figure 1). Because the pain was not controlled with usual analgesic drugs and extended to the thigh, she was referred to the orthopedic division in our hospital. On admission, plain chest radiograph revealed a nodular mass in the right lung (Figure 2), therefeore she was consulted to Division of Respiratory Medicine. She had no complaints other than the pain in the right flank and thigh. Chest CT scan showed a 6 cm nodule in right middle robe with right hilar lymph node swelling. Bone scan showed hot uptake in pubic bone (Figure 3). Transbronchal biopsy of the right middle lobe led to the diagnosis of lung adenocarcinoma. A brain magnetic resonance imaging showed a 2.0 cm

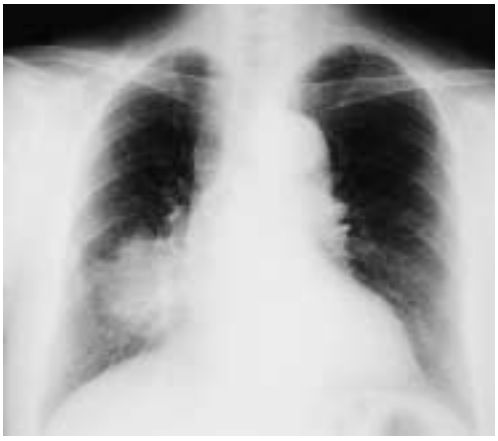

Figure 2. Chest plain radiograph on admission revealed a nodular mass in the right middle lobe.

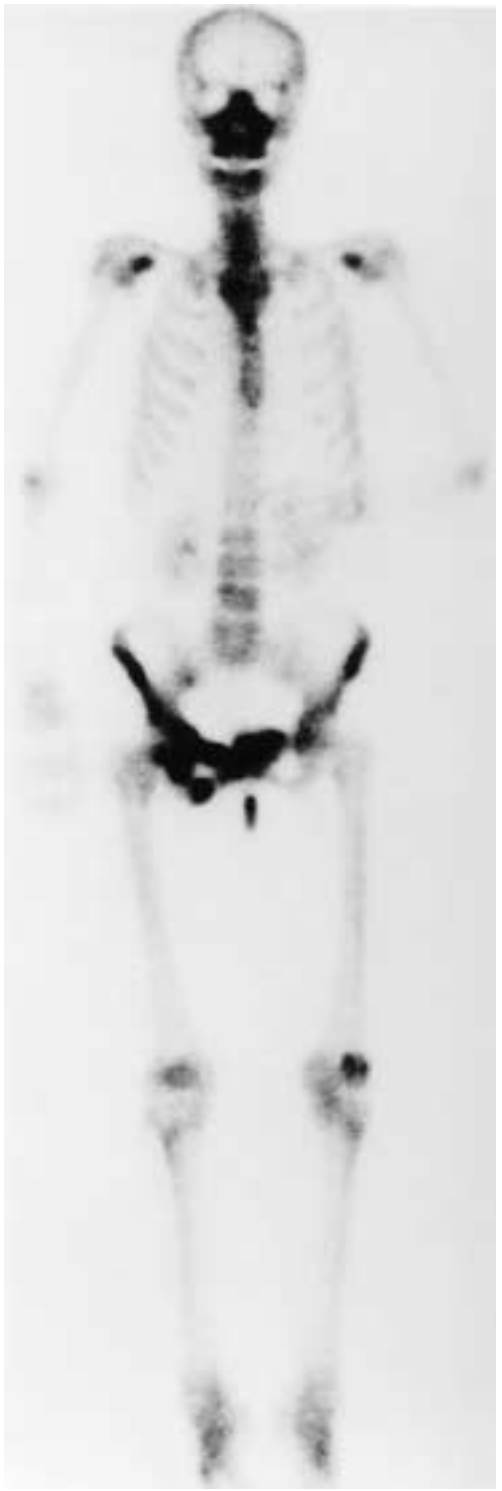

Figure 3. Bone scan showed hot uptake in pubic bone.

metastatic lesion in the right temporal lobe for which an osmotherapy and stereotactic radiosurgery were performed. The patient was given radiotherapy for osteolytic lesion in her right pubic ramus and the pain was controlled with a combination of morphine sulfate. The patient was discharged, however, she returned to our hospital because of pathological fracture of the right femur. To control the pain, total hip replacement under general anesthesia was performed. Thereafter she was discharged again. Three months later, right massive pleural effusion was developed and she was admitted to our hospital. Eleven months since the diagnosis of bone metastasis, the patient died of respiratory failure.

Discussion

Patients may first receive medical attention as the result of skeletal metastasis from an unknown primary tumor. For such individuals, imaging studies may help to identify the primary lesion.[6,7] Some primary tumors tend to result in metastases that are purely lytic in nature, whereas others tend to be associated with varying degrees of sclerosis.[8] Pubic bone metastasis may simulate parasymphyseal insufficiency fracture of the pubic bone, which is observed in postmenopausal women and elderly people.[9-11] The parasymphyseal insufficiency fracture is a commonly regarded form of stress fracture in patients with osteoporosis. Pathologically, lysis and callus formation produce a destructive malignant appearing lesion.[9] In our patient, at the time of initial diagnosis the pubic lesion was recognized on radiography as an insufficiency fracture of the symphysis pubis or a primary bone tumor because of its unusual localization of bone metastasis. Metastatic site other than pubic bone was not found at the time of initial diagnosis.

Among lung cancers, adenocarcinomas are more heterogeneous in progression than other cell types of lung cancer.[12] Therefore, some cases of lung adenocarcinoma grow very rapidly, and others are slowly progressive.[12]

Our patient had relatively slow progression despite of the first sign of distant metastatic lesion. In patients with advanced lung cancer, the major goal of treatment is recovery of the performance status of the patient and the relief of pain. In a certain percent of cases, however, intensive systemic chemotherapy would be indicated as an adjuvant to local therapy such as radiotherapy and/or surgical procedures. Although the efficacy or duration was limited, radiotherapy at metastatic bone sites and analgesics improved quality of life in our patient.

When unusual bone metastases are found in the absence of a primary tumor, investigation must include chest radiographs. Chest CT scan occasionally may be helpful in diagnosing lung cancer, which is not obvious on plain chest radiograph. Therefore, chest CT scan and bronchoscopy would be undertaken when there is a clinical indication.

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review

Detection of apoptotic cells in tumour paraffin sections

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Apoptosis is a distinct form of cell death characterised by specific morphological features and regulated by complex molecular mechanisms. Its deregulation is fundamental for tumour growth and progression and, moreover, anticancer therapies suppress tumour growth mainly by induction of apoptosis. Since the extent of apoptosis in a tumour may have prognostic as well as therapeutic implications, much effort has been invested in developing specific methods that can be routinely used to detect apoptotic cells in archival formalin-fixed paraffin-embedded tissue.

Complex molecular pathways are involved in the regulation of apoptosis. Pro-apoptotic signals trigger activation of caspases that specifically cleave target proteins. Cleavage of proteins (caspase substrates) is responsible for morphological changes of apoptotic cells and DNA fragmentation. In the last decade, detection of apoptotic cells in formalin-fixed tumour tissue sections has been based mainly on morphology and characteristic DNA fragmentation. Recently, specific antibodies to activated caspases and cleaved target proteins (including cytokeratin 18, actin and PARP) have been produced that enable accurate detection of apoptosis in paraffin sections.

Key words: neoplasms; apoptosis; caspases; DNA fragmentation

Introduction

Apoptosis, or programmed cell death, is a complex, tightly regulated and conserved process, whereby individual cells die without injuring neighbouring cells or provoking any inflammatory reaction. It is essential for the maintenance of homeostasis in multicellular organisms and is a critical component in the cellular response to injury.[1] Apoptosis is implicated in the patogenesis of various conditions, such as cancer, autoimmune disorders and neurodegenerative diseases. An imbalance between cell proliferation and apoptosis is fundamental for tumour growth and progression. Because of its wide ranging implications and possible therapeutic interventions, apoptosis is one of the most investigated areas in biological science.[2]

Since various proteins implicated in apop-

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tosis regulation have been reported to be mutated in cancer, and it has become evident that different anticancer therapeutic modalities suppress tumour growth mainly by induction of apoptosis, accurate quantification of the extent of apoptosis in tissue specimens may have therapeutic as well as prognostic implications.[3,4]

Many different biochemical, cytochemical and *in situ* methods are available for apoptosis detection,[5-8] but only a limited number of them are applicable on paraffin sections. Because of the preserved cell morphology in archival formalin-fixed paraffin-embedded tissue, there has been considerable interest in developing methods that could specifically demonstrate apoptotic cells in tissue sections. In the last decade, the morphological method and the TUNEL method (terminal transferase mediated d-UTP biotin nick end labelling) have been almost exclusively used for apoptosis detection on paraffin sections. Since great progress in understanding the mechanisms of apoptosis has been made, several new methods for apoptosis detection on paraffin sections have been introduced recently. This article deals with apoptosis detection techniques applicable to archival paraffin sections with an emphasis on tumour tissue.

Apoptotic pathway

Initially, apoptosis was recognised on morphological grounds as a well defined type of cell death.[9] During apoptosis, the cell shrinks, it detaches from the surrounding cells, the nucleus becomes condensed and fragmented, and finally, apoptotic bodies are formed. In the last two decades, the molecular basis of apoptosis has been elucidated. It has become apparent that there is a cascade of events from pro-apoptotic signalling to well defined morphological changes.

Caspases (cysteine proteases that cleave their substrates following an aspartate residue) have been recognised as key molecules in the apoptotic cascade.[5,10] At least 14 different caspases have been characterised to date. They are constitutively expressed as inactive pro-enzymes (pro-caspases) in virtually all animal cells. Pro-caspases (32 - 56 kDa) contain a large subunit (17 - 21 kDa), a small subunit (10 - 13 kDa) and an N-terminal prodomain. Pro-caspase activation requires proteolytic cleavage of the pro-caspase chain at two caspase specific cleavage sites, yielding a large and a small subunit. Active caspase is a heterotetramer composed of two large and two small subunits.

In mammalian cells, apoptosis can be initiated by either intracellular or extracellular pathways that induce initiator caspase activation (caspases 8 and 9). Activated caspase 8 and/or 9, in turn, induce cascade activation of effector caspases (caspases 3, 6 and 7), which cleave different target proteins. Demolition of the target proteins induces DNA fragmentation and is responsible for morphological changes of the apoptotic cells (Figure 1).

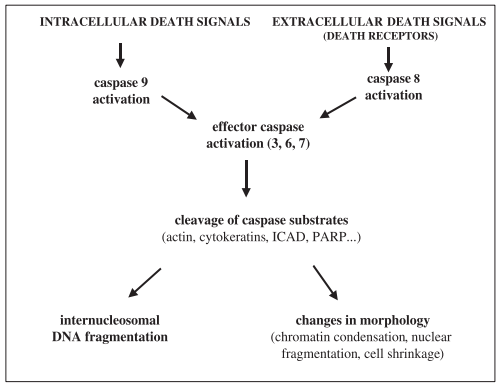

Figure 1. Apoptotic pathway. Different pro-apoptotic signals converge to induce activation of the caspase cascade. Activation of initiator caspases (8 and 9) is followed by activation of effector caspases which, in turn, specifically cleave different target proteins (caspase substrates). Cleavage of the target proteins induces DNA fragmentation and provokes morphological changes of the apoptotic cells.

Detection of apoptotic cells based on morphology

Apoptotic cells can readily be identified by means of routine histological staining methods, such as haematoxylin and eosin. Detection of apoptotic cells is based on characteristic morphological features.[11] The cytoplasm of apoptotic cells is condensed and eosinophilic. Chromatin is condensed, marginated at the nuclear membrane, and nuclear fragments are later seen (Figure 2a). Finally, the apoptotic cells desintegrate into membrane-bound apoptotic bodies, which are phagocytosed by neighbouring cells and macrophages.[12] During apoptosis, the cell membrane retains its integrity and lysosomal enzymes are not released to the surrounding tissue, so no inflammatory reaction is elicited. All morphological features are best viewed by electron microscope, but this is impractical for screening large tissue areas.[13]

Visible changes in cell morphology are the final event of the apoptotic process and are estimated to take from two to three hours, compared to 12 to 24 hours that are needed for the entire apoptotic process to be completed.[12,14] The basis for changes in the apoptotic cell morphology is caspase mediated cleavage of the target proteins (Figure 1).

The asynchronicity of apoptosis, completion of the morphological phase in a few hours and immediate clearing from the tissue usually results in a very low number of apoptotic cells in tissues.[11] Because morphological changes may be inconspicuous, the lowest numbers of apoptotic cells are usually detected by morphological criteria only.[12]

An apoptotic index is used as a measure of the extent of apoptosis, which is defined in tumour tissues as the percentage of apoptotic cells of all tumour cells. Because apoptotic cells frequently appear in clusters, enough fields need to be included in the analysis. To guarantee representativeness, at least 20 fields of 1000x magnification should be examined.[15] The identification of apoptotic cells, and thus inter-observer variation, greatly depends on the magnification, so a higher magnification should be used. Inflammatory cells that are frequently found among tumour cells may substantially influence the apoptotic index. They may be difficult to differentiate from apoptotic cells (inflammatory cells are also frequently apoptotic) as well as from the population of tumour cells analysed.[12]

Generally, assessing apoptosis based solely on its morphology is reasonably reliable and inexpensive, although tedious and fairly inter-observer dependent. It might be accurate for some tissues,[15-17] but for more accurate assessment, morphology should be used in combination with more specific methods, which detect apoptotic cells earlier in the apoptotic process. However, morphology based on routine staining methods remains, even today, a major tool of apoptosis detection and is critical for validation of new techniques.

Detection of DNA fragmentation

In situ detection of DNA fragmentation has been the most widely used method for detection of apoptosis.[12] The orderly internucleosomal fragmentation of DNA into 180 to 200 base-pair fragments is the biochemical hallmark of apoptosis.[16] It is induced by caspase mediated cleavage of ICAD (*inhibitor of CAD*) leading to activation of CAD (caspase-activated deoxyribonuclease), which in turn, cleaves DNA.[18]

DNA fragments can be detected by enzymatic labelling of the 3'-hydoxyl ends with modified nucleotides. The most sensitive and specific *in situ* method for detection of DNA fragmentation is the TUNEL method (terminal deoxynucleotidyl transferase-mediated-dUTP nick end labelling) (Figure 2b). It was introduced for use in tissue sections in 1992 by Gavrieli et al.[19] As its name implies, the TUNEL method is based on the addition of la-

belled nucleotides to free 3'-hydoxyl ends of single or double-strand DNA breaks catalysed by a terminal deoxynucleotidyl transferase (TdT) enzyme. Following *in situ* enzyme reaction, the incorporated labelled nucleotides can be detected by immunohistochemistry. Nuclear counterstains are used to facilitate recognition of apoptotic morphology.[11]

There are many commercially available apoptosis detection kits that rely on detection of DNA fragments.[16]

TUNEL labelling greatly depends on tissue fixation, tissue pretreatment and the concentration of TdT. Overlong formalin fixation and insufficient pretreatment may give rise to false negative results. On the other hand, excessive pretreatment, necrosis, autolysis or extensive DNA repair may lead to false positive results.[12] In some tissues, proteinase pretreatment may result in release of endogeneous endonucleases, which may cause false positive results with TUNEL staining.[20] Generally, TUNEL gives higher apoptotic indices than the morphological method, at least partly because DNA fragmentation slightly precedes light microscopic morphological changes.[14] TUNEL has been the most widely used method for apoptosis detection on tumour material. However, there are some drawbacks that warrant caution. TUNEL is an *in situ* enzymatic method followed by an immunohistochemical reaction and, due to its complexity, the results depend greatly on tissue preparation, pretreatment and reaction conditions.[12] Optimisation of TUNEL staining therefore requires standardisation of the above mentioned technical factors. Moreover, DNA fragmentation is a late event in apoptosis, it is not an obligatory feature of apoptosis, and may be absent.[16]

Immunohistochemical detection of cleaved caspase 3

As a result of increased knowledge about the molecular mechanisms of apoptosis, detection of apoptotic cells based on morphology and detection of DNA fragmentation is now advancing to more specific methods. Proteins selectively activated, or protein fragments generated during the process of apoptosis, have been characterised, and specific antibodies against them allow reliable detection of apoptotic cells, distinguishing them from necrotic cells.[11]

Caspase 3 is activated during most apoptotic processes and is believed to be the main effector caspase. Its activation is directly or indirectly responsible for cleavage of the target proteins, which leads to characteristic DNA fragmentation and morphological changes of apoptotic cells.[21] The appearance of the active form of caspase 3 in the cell undergoing apoptosis is an early event during apoptosis and precedes the development of classical morphological features. At the time of caspase 3 activation, the cell is fully committed to death and apoptosis is said to run beyond the 'point of no return'.[22] Detection of cleaved caspase 3 enables detection of apoptosis even before the morphological changes of apoptosis appear.

Unprocessed caspase 3 is a 32 kDa protein which is cleaved by upstream caspases into 17 kDa and 12 kDa active fragments.[5] This cleavage creates or unmasks new epitopes. Specific antibodies are available that recognise specifically the large subunit of processed caspase 3, but do not react with unprocessed caspase 3 and are therefore specific for the active caspase 3 (Figure 2c). These antibodies have been shown to work on paraffin sections.[11,23,24] Processed caspase 3 is a highly specific marker for apoptosis, since no activation of the caspase cascade has been found in necrotic cells.[11] The detection of active caspase 3 in cells can therefore be used as a discriminating criterion to distinguish apoptosis from necrosis.

Different studies have confirmed the usefulness of antibodies to cleaved caspase 3 in detecting apoptotic cells in non-tumour as well

in tumour tissues.[23-25] In breast tissue, a strong correlation between the apoptotic index assessed by morphology in haematoxilin and eosin stained sections and the apoptotic index assessed using antibodies to cleaved caspase 3 has been reported. The immunohistochemical reaction makes recognition of apoptotic cells easier and reduces subjectivity in interpretation, thus reducing inter-observer variability.[24]

One study investigating apoptosis detection in normal tissues, showed prominent immunostaining to cleaved caspase 3 in germinal centres and in neutrophilic granulocytes.[23] A prominent granulocyte infiltration can be found in tumour tissues. In our experience, many (but not all) granulocytes are positive to cleaved caspase 3. It is not clear whether this represents specific detection of apoptosis of granulocytes. However, positive granulocytes should not be confused with tumour apoptotic cells.

In some tumours, no cleaved caspase 3 could be detected. This might reflect a low level of apoptotic activity, but the expression of uncleaved caspase 3 may nevertheless be downregulated in tumours.[26]

Immunohistochemical detection of caspase-cleaved target proteins

It has become evident that caspase mediated cleaveage of target proteins at specific consensus sequences represents a unique feature of apoptosis. At least 100 different caspase substrates have been identified. Among them are structural proteins, such as actin and cytokeratins, cell signalling molecules, regulators of cell cycle and DNA repair, such as PARP (polyADP-ribose polymerase), regulators of cell-cell interactions, inhibitors of endonucleases, such as ICAD, and others.[5,27] Their cleavage can result in their inactivation or activation, leading to morphological changes of apoptotic cells and DNA fragmentation.

Cleavage of target proteins may generate or unmask new epitopes. The generation of antibodies to such epitopes, which recognise cleaved but not uncleaved target proteins, has been reported for several target proteins.

Cytokeratins are intermediary filaments of epithelial cells. Different cytokeratins are found in different epithelial cells as well as in epithelial tumours, so they are considered differentiation markers for epithelia and epithelial tumours.[28] Cytokeratin 18 is expressed in simple nonstratified, ductular and pseudostratified epithelia (hepatocytes, renal tubular cells, ductular epithelia, mesothelium, respiratory epithelium).[16,29] Caspase mediated cleavage of cytokeratin 18 leads to exposure of an epitope that can be recognised by the binding of specific antibodies. The M30 antibody recognises cleaved but not uncleaved cytokeratin 18.[5,29] This antibody works on paraffin sections and has been shown to detect apoptotic cells in neoplastic simple epithelia as well in lung and colonic carcinoma. Detection of cleaved cytokeratin 18 precedes TUNEL positivity, indicating that cleavage of cytokeratin represents an early event, but in the late phase of apoptosis, the M30 epitope is lost. Its use is, however, limited to detecting apoptotic cells in epithelial tissues or tumours expressing cytokeratin 18.

Actin filaments are present in all human cells. During apoptosis, caspase-mediated cleavage of actin generates an actin fragment, which is specifically recognised by a polyclonal antibody, called fractin.[30] Positive fractin immunostaining of apoptotic cells has been shown in several neoplastic and non-neoplastic tissues. Fractin preferentially stains apoptotic bodies, indicating that caspase cleavage of actin filaments occurs late in the apoptosis.

PARP (poly ADP-ribose polymerase) is a 116 kDa nuclear protein that is implicated in DNA repair. During apoptosis, it is one of the earliest proteins cleaved (and thus inactivated) by caspases.[31] PARP cleavage generates an 89 kDa C-terminal fragment and a 24 kDa

N-terminal peptide. The 89 kDa fragment can be detected by the use of specific antibodies (Figure 2d).[32] PARP degradation may precede DNA cleavage, as evidenced by the presence of the 89 kDa fragment in TUNEL negative cells. Therefore, in addition to late apoptosis (TUNEL positive), antibodies against cleaved PARP can detect early apoptosis before DNA cleavage.[33]

Conclusions

Three decades ago, based on the morphology, apoptosis was recognised as a special type of cell death. Later, fragmentation of nuclear DNA was recognised as a biochemical hallmark of apoptosis, and methods for detection of DNA fragments were developed. Recently, the apoptotic pathway has been elucidated and caspases have been recognised as key regulatory and executioner molecules of apoptosis. Their activation, followed by the cleavage of target proteins, precedes and is responsible for DNA fragmentation and morphological changes of apoptotic cells. Based on this knowledge, new more specific and sensitive *in situ* immunohistochemical methods for the detection of apoptotic cells have been developed that enable accurate and rou-

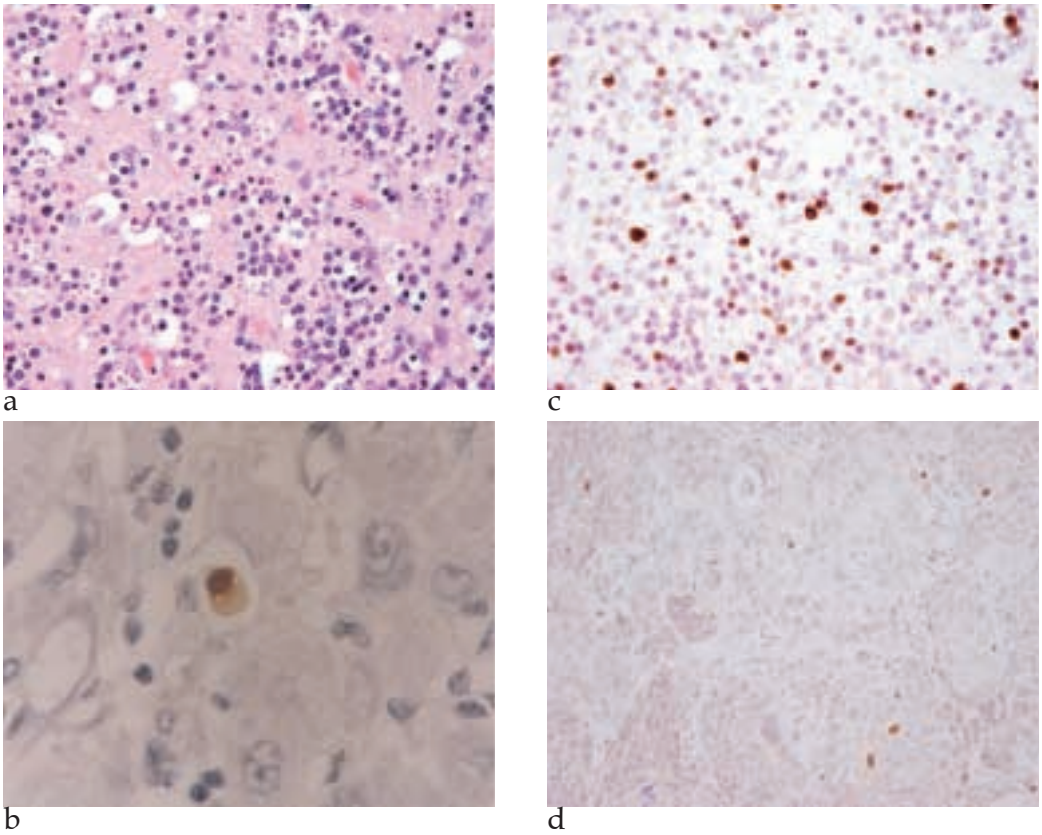

Figure 2. Apoptotic cells in tumour tissues. (a) Numerous apoptotic cells in multiple myeloma, as revealed by condensed eosinophilic cytoplasm and condensed and fragmented nuclei in haematoxylin and eosin sections. (b) An apoptotic cell in hepatocellular carcinoma labelled by the TUNEL method. (c) Apoptotic cells immunostained against active caspase 3, the same tumour tissue as in (a). (d) Apoptotic cells in squamous cell carcinoma immunostained against cleaved PARP. Magnification 400x a,c,d and 1000x b.

tine detection of apoptosis on formalin-fixed and paraffin-embedded tissue.

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Expression of cathepsin B is related to tumorigenicity of breast cancer cell lines

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Background. *The lysosomal cysteine proteases cathepsins B (CatB) and L (CatL) and their endogenous inhibitors, stefins A (StA) and B (StB), are widely thought to be involved in the progression of human breast carcinoma. Previously we showed that, in model breast carcinoma cell lines, the reported tumorigenicity was not directly related to their in vitro invasive potential.[1] However, CatL expression was positively related to the invasiveness of the cells and inversely related to the levels of StA. Here we challenge the hypothesis that imbalance between CatB and the two stefins is associated either with the invasiveness or the reported tumorigenicity of the panel of selected breast carcinoma cells.*

Results. *We investigated levels of mRNA, protein and activity for CatB in the panel of human breast carcinoma cell lines whose tumorigenicity in vivo increased in the order MCF-7 < MDA-MB468 < MDA-MB231 < MDA-MB435, the most invasive being MDA-231. Levels of expression of mRNA, protein and activity for CatB were highly correlated and increased progressively with cell tumorigenicity. The ratio of CatB to stefins was shifted in favour of CatB in the more tumorigenic cell lines.*

Conclusions. *Since CatL has been shown previously to be associated with invasive potential and, in this study, CatB expression was found positively associated with the tumorigenicity of the same breast carcinoma cell lines, the two cathepsins in these cells do not appear to be regulated in a coordinated manner. CatB expression and the ratio between CatB and stefins increased progressively with tumorigenicity of the cells and suggests a similar situation in human tumours in vivo.*

Key words: breast neoplasms; cathepsin B; neoplasm invasiveness; tumor cells, cultured

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Introduction

Lysosomal cathepsins comprise a variety of peptidases of different clans, among which ClanA (CA) includes the cysteine peptidases cathepsins (Cats) B and L, which belong to so-called papain family.[2] The association of the two cathepsins with tumour progression is

well established in the literature.[3,4] In clinical studies of breast carcinoma patients, elevated levels of CatB are associated with bad prognosis of patient survival.[5-9]

The activities of cysteine cathepsins are regulated by their endogenous inhibitors, a large superfamily of cystatins.[10] The stefin (St) family comprises intracellular inhibitors, of which levels of StA and StB were found to be altered in tumour cells[11] and in clinical samples from cancer patients.[3,7,9,12-13] Therefore, the molecular mechanisms responsible for the biological roles of CatB and CatL in tumour cells, together with their inhibitors, StA and StB, need to be elucidated at the molecular level.

Tumour metastasis is a multi-step process, starting by detachment of tumour cells from the primary tumours, invasion through the extracellular matrix and/or basement membranes of vasculature, to reach the blood flow, which carries the tumour cells to distant organs where organ selective invasion and growth of tumour cells into the secondary site takes place. Invasion is therefore the common denominator of many metastatic steps, a process which is associated with tissue remodelling. Presumably, this is induced by tumour cells which are triggered to express, secrete and/or activate a battery of proteolytic enzymes at their cell surface. Extracellularly and at the plasma membrane, metalloproteinases and the plasminogen activator/plasmin system may initiate the extracellular matrix degradation. However, it has been proposed that Cats B and L initiate the proteolytic cascade by specific activation of pro-urokinase and/or metalloproteinases.[14] Furthermore, intracellular degradation of extracellular matrix components occurs during the invasion process[15-16] which involves activation of lysosomal cathepsins, including CatB.[17] Moreover, recent reports show that the invasion of tumour cells is significantly impaired when the intracellular activity of cysteine cathepsins is blocked.[16-18]

Tumorigenicity is a key characteristic of the malignant cancer cell, although the potential to form tumours at the secondary site may not directly reflect its invasive potential. In the two models of breast carcinoma cell lines, we have demonstrated, that the tumorigenicity of the cell lines was not strictly related to their *in vitro* invasiveness in Matrigel.[1,19] Here, we used a model of four selected human breast cancer lines: MCF7, MDA-MB468, MDA-MB231 and MDA-MB435, which have been reported to differ in tumorigenicity and metastasis *in vivo*.[20,21] Their phenotype varies from epithelial (MCF7) to mesenchymal (MDA-MB435), with highly increased expression of vimentin and downregulated expression of estrogen receptors.[22] However, not much is known about the expression of lysosomal proteinases, except our recent report on the positive association of CatL mRNA and protein expression, and an inverse correlation of StA expression, with the invasiveness of the cells in this model.[1] In the present study we used the same cell model with the aim (a) to determine mRNA, protein and activity levels for CatB, (b) to relate protein expression of CatB to those of StA and StB and (c) to relate these to the invasiveness and progressive tumorigenicity of the human breast carcinoma cell lines.

Materials and methods

Cells and their characteristics: tumorigenic and invasive potentials

Human breast carcinoma cell lines were obtained from the ATCC cell bank and cultured under conditions recommended by the supplier. The cells range from poorly to highly tumorigenic and metastatic in the order, MCF7 < MDA-MB468 < MDA-MB231 < MDA-MB435.[20] The MCF7 cell line is poorly tumorigenic and non-metastatic, MDA-MB468 cells exhibit low tumorigenic and low metastatic activities, while MDA-MB231 and

MDA-MB435 cell lines are both highly tumorigenic and metastatic, the latter producing the highest number of distant metastases.[21] The invasiveness of this panel of cell lines was determined in vitro, using the Matrigel assay.[1] Invasiveness ranged from MCF7, MDA-MB468, MDA-MB435 to MDA-MB231 which was the most invasive cell. Thus invasiveness does not parallel tumorigenicity in the two most tumorigenic cell lines.

RNA analysis - Northern analysis and real time PCR

Total RNA was extracted from cells by TRIzol Reagent, according to the instructions of the supplier (Gibco, UK). CatB mRNA was determined by Northern analysis and by quantitative RT-PCR. For Northern analysis, 15μg of RNA was electrophoresed through agarose/formaldehyde gel and hybridized with full length CatB cDNA probe that was non-radioactively labelled with digoxygenin, according to the instructions of the supplier. The cDNA probe was kindly provided by Dr. Boris Turk, Josef Stefan Institute, Ljubljana, Slovenia. The signals were detected by chemiluminescence, using CDP Star™ System (Boehringer, Germany).

A fluorescence-based real-time, quantitative RT-PCR method developed by Perkin Elmer ABI (TaqMan), was used to measure CatB RNA levels in cell extracts. 1μg of total RNA was reverse transcribed using High-Capacity cDNA Archive Kit and PCR amplified with TaqMan Universal PCR Master Mix according to the instructions of the supplier (both Applied Biosystems, USA). The sequence of the CatB forward primer was 5′-CTCTATGAATCCCATGTAGGGTGC-3′, 5′-CCTGTTTGTAGGTCGGGCTG-3′ for the reverse primer and 5′-CCCTGTGAGCAC-CACGTCAACGG-3′ for the TaqMan probe. Amplification of 18S ribosomal RNA was performed as an internal control.

Protein concentration

Cells were homogenised in Tris buffer (50mM Tris, pH 6.9, 0.05% Brij 35, 0.5mM DTT, 5mM EDTA, and 10μM pepstatin A) at 4°C using Tissue Tearor™ (Biospec Products Inc., USA). The homogenates were centrifuged at 12,000 rpm for 15 min and supernatants (cell lysates) were stored at -20°C. Total protein concentration was determined by Bio-Rad protein assay (Bio-Rad, USA) using bovine serum albumin as standard. Protein concentration of CatB was measured in cell lysates using a specific ELISA kit (Krka d.d., Slovenia). The ELISAs recognised total CatB protein, i.e. the precursor and the active forms as well as CatB complexed with the inhibitors.

Cathepsin B activity

The activity of CatB was determined using the fluorogenic substrate Z-Phe-Arg-AMC (modified from *Lah*[12]), which is not selective for CatB, but also measures other cathepsins, such as CatL. First, the total cysteine peptidase activity was determined as the difference between the total activity and the background activity of the non-cysteine peptidases; this was determined using the general cysteine peptidase inhibitor, E64c, at a final concentration of 16μM. CatB activity was measured by adding its selective inhibitor, 10μM CA-074, and determining the residual activity. CatB activity was obtained as the difference between total cysteine protease activity and residual activity. One enzyme unit (EU) is defined as the quantity releasing 1μmol of AMC per min. Specific activity is expressed as mEU/mg of total protein in the cell lysates.

Statistical significance between measurements was determined by the two tailed t-test and $p<0.05$ was considered as significant.

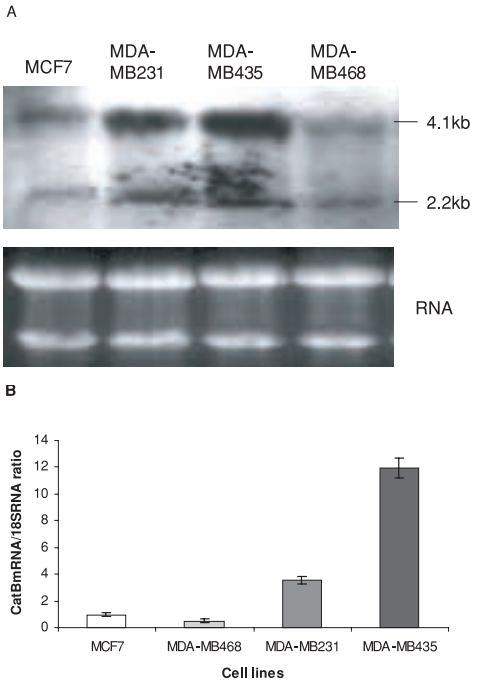

Figure 1. CatB mRNA expression in MCF7 and MDA-MB cell lines determined by Northern analysis (A) and quantitative RT-PCR (B). (A) For Northern analysis the total RNA was electrophoresed and hybridised with full length CatB DNA probe, as described in Material and methods. Total RNA stained with EtBr served as loading and transfer control (shown underneath). CatB expression is shown typically as two bands, one at 4.1kb and the other by 2.2.bp. (B) For quantitative analysis the total RNA was reverse transcribed and PCR amplified using CatB specific primers, as described in Material and methods. The results were normalised to 18S RNA. Error bars depict standard error of the mean. CatB mRNA levels were similar in MCF7 and MDA-MB468, but significantly higher in MDA-MB231 and MDA-MB435 cells.

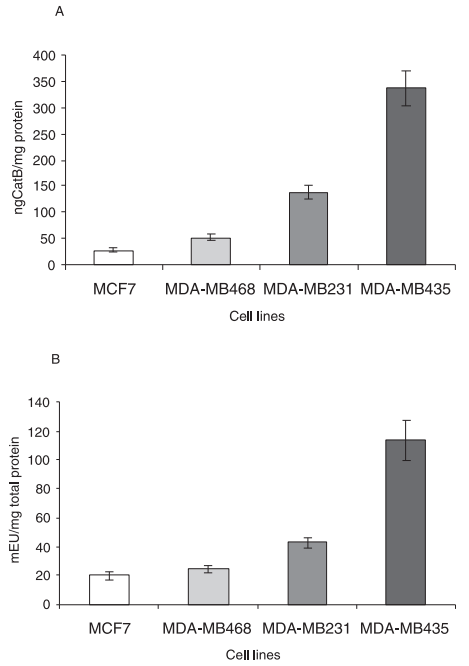

Figure 2. Protein concentration (A) and enzymatic activity (B) of CatB in MCF7 and MDA-MB cell lines. Cell lysates were prepared as described in Material and methods. (A) CatB protein was measured by ELISA, and expressed as ng/mg total protein. The differences in protein concentration were statistically significant. (B) CatB activity was determined as described in Materials and methods.. CatB enzymatic activities were significantly different, except between MCF7 and MDA-MB468 cells. Error bars depict standard error of the mean.

Results

Expression of cathepsin B in four human breast carcinoma cell lines

Expression of CatB was determined in the four breast cancer cell lines that differ in their tumorigenicity and invasive potential. Levels of CatB mRNA, protein and enzyme activity correlated highly (r>0.99). All three levels increased with the degree of tumorigenicity of these breast carcinoma cells (Figures 1 and 2).

Figure 1 shows CatB mRNA expression in MCF7 and the three MDA-MB cell lines determined by Northern analysis (A) and quantitative RT-PCR (B). The highest CatB mRNA level was detected in the most tumorigenic MDA-MB435 cells, slightly less in the most invasive MDA-MB231 cells, whereas both poorly tumorigenic cell lines MCF7 and MDA-MB468 expressed significantly lower levels of CatB. However, the difference between the latter two cell lines was not statistically significant.

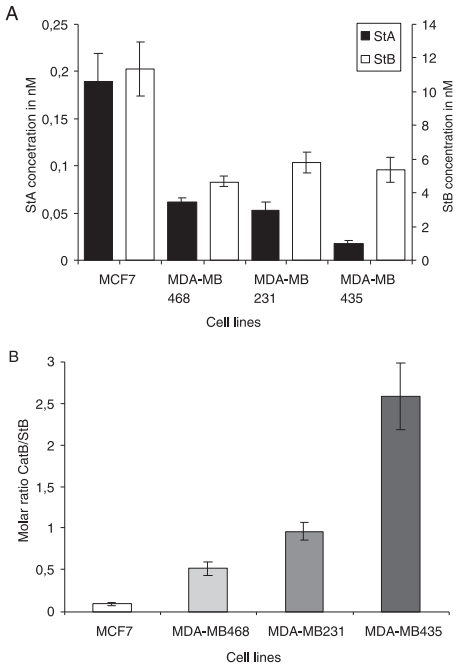

Figure 3. The molar concentrations of StA and StB (A) and the molar ratio between CatB and StB (B) in MCF7 and MDA-MB cell lines. Protein concentrations of StA, StB and CatB were measured in cell lysates with ELISA (*see above*) and their molar concentrations were calculated. (A) Both stefins were the highest in MCF7 cells and significally lower in metastatic MDA-MB cell lines. Note the difference in scale. (B) The molar ratio between CatB and StB increased progressively with tumorigenicity of the cells. All the differences between the cell lines were statistically significant. Error bars depict standard error of the mean.

Concentrations of CatB protein in MCF7 and the three MDA-MB cell lines are presented in Figure 2A. The lowest concentration was observed in the non-metastatic and poorly tumorigenic MCF7 cells and increased progressively from MDA-MB468, MDA-MB231 to MDA-MB435, the most tumorigenic cell line. All the differences were statistically significant. The protein concentration of CatB in cell lysates increased more than 14 fold, from 25 ng/mg (cca 1 nM) in MCF7 cells to 350 ng/mg protein (14 nM) in MDA-MB435 cells.

Figure 2B shows CatB proteolytic activities in MCF7 and the three MDA-MB cell lines. As for protein concentration, specific activity of CatB was the lowest in MCF7 cells and increased progressively with tumorigenicity of the cells. The difference is statistically significant between the high and low tumorigenic lines, but not between the poorly tumorigenic cell lines MCF7 and MDA-MB468.

Correlation between cathepsin B and stefins in four breast carcinoma cell lines

Protein concentrations of StA and StB in the same breast cancer cell lines have been shown to be lower in cells with higher tumorigenicity.[1] As shown in Figure 3A, StB in the cell lysates was 2.4 fold lower in MDA-MB435, the most tumorigenic cell line, (59 ng/mg; 5,4 nM) than in MCF7 (about 126 ng/mg; 11 nM), whereas the levels of StA were about 10 fold lower, at 2 ng/mg protein (0.19 nM) and 0.2 ng/mg protein (0.018 nM) respectively. The molar ratio of the protein concentration of CatB to that of StB in the lysates of MCF7 and the three MDA-MB cell lines was determined (Figure 3B). This ratio was lowest in the MCF7 cell line and increased with tumorigenicity of the cells, as was observed with CatB levels alone.

Discussion

Cathepsin B and CatL were initially considered to be products of single copy housekeeping genes, their expression being necessary for normal protein turnover in the cells. Surprisingly, homozygous CatB-deficient mice have an apparently normal phenotype, [23] suggesting redundancy of the genes, whereas CatL-deficient mice have periodic shading of fur and abnormal skin, but are otherwise viable,[24] suggesting cell-specific functions of this enzyme. Their expression is regulated at the gene level,[25-26] by mRNA splicing[27-28] and by posttranslational modification (reviewed *by Frosch*[29]). In the present

study, we have determined the expression of cathepsin B at the mRNA, protein and activity levels in MCF7, MDA-MB468, MDA-MB231 and MDA-MB435 breast cancer cell lines of increasing tumorigenicity *in vivo*.[20] CatB expression at all three levels is highly correlated. This suggests that the initial regulation of CatB occurs at the genetic level, the regulation of CatB transcription during development of the tumorigenic phenotype being most probably modulated through multiple GC boxes.[25] This needs to be elucidated further. However, in a model comprising cells derived from MCF-10A manipulation to result in distinct invasive and tumorigenic phenotypes, we observed that CatB was significantly related to invasiveness but not tumorigenicity,[19] although again all three levels of CatB expression correlated well. Presumably, expression of other gene profiles in the two panels of breast carcinoma cell lines is responsible for up regulation of CatB in relation to the invasive and/or tumorigenic potential.

There are also differences in the regulation of CatB and CatL. We found previously that, in contrast to CatB, CatL was highly increased at mRNA and protein levels, but was lowered at the activity level in the most invasive of the four cell lines, MDA-MB231.[1] This may be due to selective inhibition of CatL activity by endogenous inhibitors, or by another defect in its intralysosomal processing. Similar differences in expression of CatL between different levels were observed in the MCF10A model, suggesting that, in contrast to CatB, CatL regulation of expression also occurs posttranslationally. This supports our previous observation on differential regulation of cathepsins in breast cancer cell lines[30] and in clinical samples of breast tumours.[7,8]

The ultimate regulation of CatB and CatL activities in the cells results from binding by the endogenous inhibitors, the stefins.[31] Alteration of their levels in tumours, presumably downregulation, was reported.[3,11] In the cell line model used here, we have shown previously that StA levels decrease significantly with increasing tumorigenicity, in line with the hypothesis that imbalance between cysteine proteinases and their inhibitors facilitates tumour progression. StB levels were higher in the MCF-7 cells than in the other three invasive and tumorigenic cell lines.[1] It is noteworthy that the molar concentration of stefins was higher in low tumorigenic cells lines, but was lower than that of CatB in the most invasive and tumorigenic cells. This would suggest insufficient inhibition of CatB activity, since the complexes between cysteine cathepsins and cystatins are equimolar, as shown by crystallography[31] and kinetic measurements.[32]

Although measurements in cell lysates may not completely parallel the situation in living cells, the result is a good indication that the balance between cathepsins and stefins is drastically altered in this panel of cell lines. We conclude that, in this model of breast carcinoma cells, upregulation of CatB is a characteristic of the highly tumorigenic cell phenotype. Together with our previous studies on this cell model, the results presented here confirm that cathepsins B and L are important in the processes of tumorigenicity and invasiveness of the cells, respectively, but are not regulated in a coordinated manner. Furthermore, the imbalance between proteolytic enzymes and their inhibitors may facilitate the development of a malignant phenotype in breast cancer. If confirmed by further studies, both cathepsins could constitute potential targets for anti-invasive therapy.

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Cysteine and aspartic proteases cathepsins B and D determine the invasiveness of MCF10A neoT cells

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Background. *Lysosomal cathepsins B and D have been reported to play a role in various processes leading to progression of malignant disease. In ras-transformed MCF10A neoT cells both enzymes show similar vesicular distribution in perinuclear and peripheral cytoplasmic regions.*

Results. *The co-localization of cathepsins B and D in some vesicles as defined by confocal microscopy supports their co-ordinate activity in the proteolytic cascade. On the other hand, we showed that stefin A, an endogenous intracellular inhibitor of cysteine proteases, did not co-localize with cathepsin B and is presumably not involved in regulation of its enzymatic activity within the vesicles. Intracellular localization of both enzymes was confined to similar vesicles as the fluorescent degradation products of DQ-collagen IV either in individual cells or cell spheroids. The capability of these two enzymes to degrade collagen and other components of extracellular matrix is further supported by the results of Matrigel invasion assay. We showed that specific intracellular (CA-074 Me) and extracellular (CA-074) inhibitors of cathepsin B and pepstatin A, an inhibitor of cathepsin D, significantly reduced invasion of MCF10A neoT cells. Our results also show that in contrast to some other studies the activation peptide of pro-cathepsin D exhibited no mitogenic effect on MCF10A neoT, MCF-7 or HEK-293 cells.*

Conclusion. *We conclude that lysosomal cysteine proteases cathepsins B and D predominantly participate in degradation of extracellular matrix and facilitate invasion of tumour cells.*

Key words: tumor cells, cultured; cathepsin B; cathepsin D; neoplasms invasiveness

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Introduction

Dissemination of malignant cells of primary tumours to distant tissues and the formation of secondary tumours is the primary cause of treatment failure and death of cancer patients. Besides serine and metallo proteases, lysosomal cysteine proteases cathepsins B and L and aspartic protease cathepsin D have

been shown to participate in processes of tumour progression, including tumour cell growth, invasion and metastasis.[1-3] Alterations in expression, processing and subcellular distribution of these three cathepsins have been linked with more aggressive tumour behaviour, increased risk of relapse and shorter survival of cancer patients.[1, 4]

In numerous studies it has been reported that proteolytic activity of cysteine proteases is involved in degradation and remodelling of extracellular matrix (ECM), a crucial step in tumour cell invasion.[5] Degradation of ECM in living tumour cells was localized either extracellularly or intracellularly, following endocytosis of partially degraded ECM proteins. Recently, we have demonstrated that tumour cells can use both proteolytic pathways simultaneously.[6] Cathepsin B has been suggested to facilitate ECM degradation and subsequent tumour cell invasion directly by intra- or extracellular degradation of ECM components or indirectly, by activation of other enzymes of the proteolytic cascade mediating this process.[7]

In contrast to cathepsins B and L, which are believed to be mainly involved in the invasion of tumour cells, the importance of cathepsin D in cancer progression is less clear. In breast cancer catalytically inactive cathepsin D pro-peptide was proposed to act as a mitogen, promoting tumour cell proliferation.[8] Additionally, we showed that active enzyme can promote tumour invasion *in vitro*.[9] This is in line with clinical studies, revealing the correlation of the mature form of cathepsin D with poor prognosis of cancer patients.[10]

In this study, cathepsins B and D have been simultaneously evaluated in MCF10A neoT cells with regard to their impact on cell proliferation, degradation of ECM and tumour cell invasion. Additionally, the localization of these two cathepsins and stefin A was determined in this cell line by immunofluorescence labeling and confocal microscopy.

Materials and methods

Cell cultures

Ras-transformed human breast epithelial cell line MCF-10A neoT was obtained from Prof. B. F. Sloane (Wayne State University, Detroit, USA). Human cell lines HEK-293 and MCF-7 were obtained from the American Tissue Culture Collection. MCF-10A neoT cells originate from MCF-10, a human breast epithelial cell line derived from a patient with fibrocystic breast disease that underwent spontaneous immortalization in culture and grows attached as MCF-10A cell line. Co-transfection of MCF-10A cells with plasmid containing the neomycin resistance gene and human T24 mutated c-Ha-ras oncogene using the calcium phosphate method resulted in MCF-10A neoT cells.[11,12] MCF-10A neoT cells were cultured as monolayers in DMEM/F12 medium supplemented with Hepes, 5% FCS, antibiotics and growth factors. Human breast cancer cell line MCF-7 and transformed human embryonic kidney cell line HEK-293 were cultured in MEM supplemented with 2 mM L-glutamine, Earle's BSS and adjusted to contain 1.5 g/l sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and 10 % FCS. Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Cysteine protease inhibitors

The natural reversible tight-binding protein inhibitor chicken cystatin[13] and synthetic irreversible epoxysuccinyl inhibitor E-64 (Sigma, St. Louis, USA) were used as general cysteine proteinase inhibitors. Cathepsin B was inhibited with selective membrane impermeable epoxide derivative CA-074 (Bachem, Bubendorf, Switzerland) and membrane-permeable pro-inhibitor CA-074Me (Sigma, St. Louis, USA). Cathepsin L was inhibited by CLIK-148[9] (a gift from Prof. Nobuhiko Katunuma, Tokushima Bunri University, Japan) and cathepsin D by pepstatin A (Sigma, St. Louis, USA).

Cell viability and proliferation assays

Cytotoxic and/or proliferative effects of cysteine protease inhibitors on MCF-10A neoT cells were tested as described.[14] Cells were added to a final concentration of 5 x 10^4 cells/200 μl per well of a 96 well microtiter plate. Appropriate concentrations of inhibitors or control media were added. Plates were incubated for 24 hours at 37°C and 5% CO_2. The medium was carefully removed, 200 μl of 0.5 mg/ml MTT (3-4,5-dimethylthiazol-2,5 diphenyl tetrazolium bromide, Sigma, St. Louis, USA) was added and incubated for three hours at 37°C and 5% CO_2. The medium was carefully removed and formazan crystals dissolved in 200 μl/well of isopropanol. Absorbance was measured on an ELISA microplate reader at 570 nm, reference filter 690 nm. All tests were performed in quadruplicate.

Cell invasion assays

Effects of protease inhibitors on invasion were tested using the modified method described by Holst-Hansen et al.15 Transwells (Costar Corning, New York, USA) with 12 mm polycarbonate filters, 12 μm pore size, were used. 25 μl of 100 μg/ml fibronectin were applied on the lower side of the filters, which were left for one hour in a laminar hood to dry. The upper side of the filters was coated with 100 μl of 1 mg/ml Matrigel (Becton Dickinson, San Diego, USA) and 100 μl of DMEM/F12 was added. The Matrigel was dried overnight at room temperature in a laminar hood and reconstituted with 200 μl of medium for one hour at 37°C. The upper compartments were filled with 0.5 ml of cell suspension, final concentration 4 x 10^5 cells/ml, containing the appropriate concentration of the inhibitor. The lower compartments were filled with 1.5 ml of medium containing the same concentration of the inhibitor. The plates were incubated for 24 hours at 37°C and 5% CO_2. MTT was added to a final concentration of 0.5 mg/ml to the upper and lower compartments and plates were incubated for an additional 3 hours. Media from both compartments were transferred separately to Eppendorf tubes, and centrifuged at 6200 rpm for 5 minutes. Supernatants were discarded and the formazan crystals which remained dissolved in 1 ml of isopropanol. The colour intensity of the dissolved formazan crystals was measured as described above. As controls, cells were incubated with medium containing the appropriate volumes of distilled water, ethyl acetate and DMSO, the solvents used for preparation of concentrated solutions of the inhibitors and monoclonal antibody. Invasion was recorded as the percentage of cells that penetrated the Matrigel coated filters compared to controls, and was calculated as OD_{lower} / OD_{lower}+OD_{upper} x 100. All tests were performed in triplicate.

Mitogenic effect of procathepsin D activation peptide

18 amino acids long cathepsin D activation peptide[8] (IAKGPVSKYSQAVPAVTE) as a part of cathepsin D pro-peptide, was synthesised by NeoSystems (Calgary, Canada) at 95% purity. The mitogenic effect of the peptide was tested as described for cytotoxicity and proliferation assays, with minor modifications. Cells were added to a final concentration of 5 x 10^3 cells/200 μl per well of a 96 well microtiter plate. The activation peptide was added in a range of 0.001-10 μM concentrations. Plates were incubated for the period of 1-5 days at 37°C and 5% CO_2 and analysed as described above by MTT assay.

Immunocytochemistry

Cathepsins B and D, and stefin A were localized in MCF-10A neoT cells fixed in ice-cold methanol and permeabilized in 0.1% saponin in PBS, pH 7.4. Non-specific staining was blocked with 0.2% BSA in PBS, pH 7.4. Primary antibodies used were rabbit anti-human cathepsin B polyclonal antibody, mouse

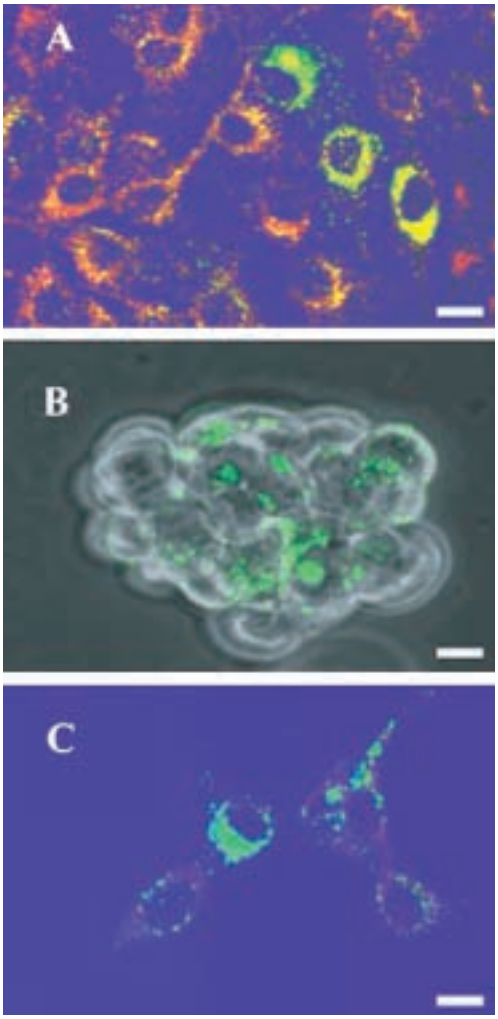

Figure 1. Immunolocalization studies in MCF-10A neoT cells. (A) Co-localization (yellow) of cathepsin B (red fluorescence) and cathepsin D (green fluorescence). (B) MCF-10A neoT spheroids of living cells. Green fluorescent degradation products are predominantly localised within the cells. (C) Localization of cathepsin B (green fluorescence) and stefin A (red fluorescence). Bars, 20 ?M.

anti-human cathepsin D D101 monoclonal antibody and mouse anti-human stefin A C5/2 monoclonal antibody (all KRKA d.d., Novo mesto, Slovenia). Antibodies recognize precursors, mature forms and enzyme/inhibitor complexes of all three antigens. Secondary antibodies were goat anti-rabbit labelled with Alexa Fluor 488 and goat anti-mouse labelled with Alexa Fluor 546 (Molecular Probes, Eugene, USA). Controls were run in the absence of primary antibodies, but in the presence of secondary antibody and pre-immune goat serum. Pro Long Antifade Kit was used for mounting coverslips on glass slides. A Zeiss LSM 510 confocal microscope was used to observe the cells.

Proteolysis assays

Pre-cooled glass coverslips were coated with 25 μg/ml of the quenched fluorescent substrate DQ-collagen IV (Molecular Probes, Eugene, USA) suspended in Matrigel, 10 mg/ml, for 10 minutes at 4 °C. Cells were plated and grown for a period of 72 hours. A Zeiss LSM 310 confocal microscope with 40 X water immersion objective was used to observe the cells or cell spheroids for fluorescent degradation products of DQ-collagen IV.

Results

Immunolocalization

Cathepsins B and D showed similar vesicular distribution in MCF-10A neoT cells, but the level of their expression varied between cells. They were localized both in the perinuclear and in the peripheral cytoplasmic region (Figure 1A) and both enzymes co-localized in some of the vesicles. Stefin A, an endogenous intracellular inhibitor of cysteine proteases, localized evenly in the cell cytoplasm, but no co-localization with cathepsin B was observed (Figure 1C).

Degradation of DQ-collagen IV by living MCF-10A neoT spheroids

The ability of MCF-10A neoT cells to degrade extracellular matrix was analysed by the novel confocal assay desribed by Sameni et al.[16] using quenched fluorescent substrate DQ-collagen IV. MCF-10A neoT cells plated on a DQ-collagen IV/Matrigel matrix formed sphe-

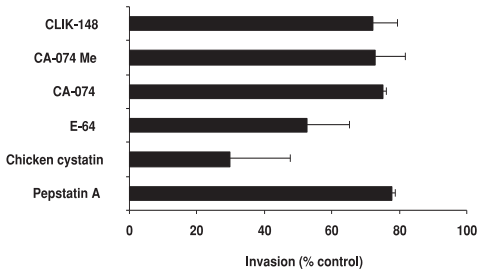

Figure 2. Effect of cysteine and aspartic protease inhibitors on Matrigel invasion by MCF-10A neoT cells. Concentration of chicken cystatin was 2 mM, all other inhibitors were used at 10 mM concentration. Data are represented as mean (S.D. of two independent determinations performed in triplicate.

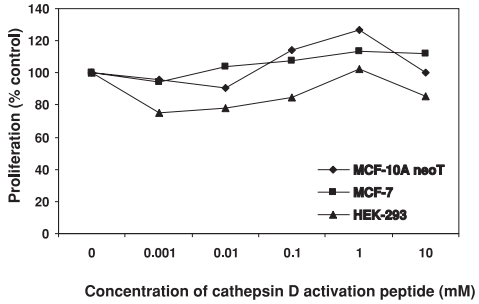

Figure 3. Effect of pro-cathepsin D activation peptide IAKGPVSKYSQAVPAVTE on the growth of cancer cell lines. After five days incubation the number of cells was determined by MTT method and compared to controls

roids after 72 hours. Fluorescent degradation products seen as green fluorescence were observed inside the cells forming the body of the spheroid (Figure 1B). They were localised in vesicular structures as observed in individual cells.[6] Individual spots of degradation were observed also in the pericellular regions.

Effect of protease inhibitors on the viability and proliferation of MCF-10A neoT cells

Prior to the invasion assays, the selected protease inhibitors were tested for their possible cytotoxic and proliferative effects. No effect on cell viability and/or proliferation was observed within the same concentration range as used in invasion assays (data not shown).

Effect of protease inhibitors on Matrigel invasion of MCF-10A neoT cells

The roles of cathepsins B and D in the invasion of MCF-10A neoT cells were determined with selected natural and synthetic inhibitors of cysteine and aspartic proteases. All inhibitors decreased cell invasion after 24 h (Figure 2). Inhibition of aspartic protease cathepsin D with 10 μM pepstatin A resulted in 22.1 ± 0.8 % decrease in invasion of MCF-10A neoT cells. The effect of pepstatin A was much lower compared to general cysteine protease inhibitors chicken cystatin (70.2 ± 17.8 % at 2 μM) and E-64 (47.5 ± 12.7 % at 10 μM). To assess the contribution of cathepsin B, CA-074, a specific inhibitor of extracellular cathepsin B and membrane-permeable analogue CA-074 Me were used. CA-074 and CA-074 Me reduced invasion by 24.9 ± 1.2 % and 27.0 ± 8.7 % at 10 μM, respectively. For comparison, 10 μM concentration of CLIK-148, a specific synthetic inhibitor of cathepsin L resulted in 27.7 ± 7.1 % decrease in cell invasion.

Effect of cathepsin D activation peptide on tumour cell proliferation

To investigate the mitogenic effect of the activation peptide of pro-cathepsin D, a corresponding synthetic peptide was used in MTT proliferation assay. In contrast to results published by Vetvicka *et al.*[8] and Fusek and Vetvicka[17], no significant mitogenic effect was observed on MCF-10A neoT and MCF-7 cell proliferation within the comparable concenration range after five days (Figure 3). Additionally, no proliferative activity was observed in human embryonic kidney cells HEK-293 used as negative control.

Discussion

Metastatic process depends on the ability of tumour cells to invade through ECM. This process is facilitated by proteolytic degradation of various ECM proteins, including different types of collagens, laminin and fibronectin by proteases. The common believe is, that this process takes place extracellularly at the invasive front of tumour cells. New techniques, like confocal laser scaning microscopy enable analysis of proteolytic degradation of quenched fluorescent protein substrates, like DQ-collagen IV, in living cells and the results show that cells differ in their sites of matrix remodeling, located either extracellularly or intracellularly.[5,16] We found that invasive MCF-10A neoT cells accumulate fluorescent products of digested DQ-collagen IV inside the cells, in vesicle-like structures.[6] This result was confirmed in this study by using 3-dimensional spheroid cultures of MCF-10A neoT cells. Again, positive vesicular staining was found in most of the cells forming the body of the spheroid. In addition, individual spots of pericellular fluorescence observed both in individual cells and in spheroid cultures suggest, that MCF-10A neoT cells can simultaneously use both pathways of ECM degradation.

Several studies have implicated cysteine proteases in invasion and tumour progression. Therefore, we tested the impact of general and specific cysteine protease inhibitors on invasiveness of MCF10A neoT cells in the concentration range not affecting cell viability and proliferation. Both general inhibitors, i.e. the reversible tight-binding protein inhibitor chicken cystatin[13] and the irreversible inhibitor E-64 were the most effective in Matrigel invasion assay, with 70.2 ± 17.8 % (2 μM concentration) and 47.5 ± 12.7 % (10 μM) inhibition of invasion, respectively. CA-074, an inhibitor of extracellular cathepsin B, decreased invasion by 24.9 ± 1.2 % at 10 μM, a result is comparable to the effect of intracellular cathepsin B inhibitor CA-074 Me (27.0 ± 8.7 %). We may expect that the inhibitor, capable to impair the activity of both, intracellular and extracellular fraction of cathepsin B would be even more effective to decrease tumour cell invasion. Further, we demonstrated that cathepsin L also participates in this process. However, none of the inhibitors completely blocked MCF-10A neoT cell invasion, suggesting the involvement of other, presumably serine and matrix metallo proteases in invasion process.

Our finding, that aspartic protease inhibitor pepstatin A also reduced Matrigel invasion of MCF-10A neoT cells, suggests that beside cysteine cathepsins B and L, active aspartic protease cathepsin D is implicated in invasion process as well. This is in contrast with reports of Johnson et al.[18] that the irreversible peptide inhibitor pepstatin A was ineffective in inhibiting MCF-7 tumour cell invasion *in vitro.* In our case treatment of MCF-10A neoT cells with 10 μM pepstatin A resulted in 22.1 ± 0.8 % decrease in invasion. Reduction of invasion was even higher when SQAPI-like natural protein inhibitor of aspartic proteases was used.[9]

Besides direct degradation of ECM, cathepsin D was suggested to act as an initiator protease upstream in a proteolytic cascade activating pro-cathepsin B7. Activated cathepsin B can further convert serine protease prourokinase type plasminogen activator (uPA) into active enzyme uPA, which, in turn, is able to activate plasmin and matrix metallo proteases. These enzymes can then actively degrade various ECM components. Our results support the involvement of cathepsin D in the proteolytic cascade. Cathepsin D was co-localized with cathepsin B in the same cytoplasmic vesicles in MCF10A neoT cells and is, therefore, able to activate procathepsin B. However, we have to be aware that antibody used for localization of cathepsin B recognises besides precursor also other forms of the enzyme[19] and that other assays and experiments, including specific fluorogenic sub-

strates are needed to determine the ratio between pro and active forms of cathepsin B and to assess the rate of its activation.

The effect of endogenous protease inhibitors on degradation of ECM in MCF10A neoT cells remains to be evaluated. Whereas no endogenous inhibitor of cathepsin D is known so far, the activity of cysteine proteases cathepsins B and L are regulated by their endogenous inhibitors, the cystatins.[1] In MCF10A neoT cells we localised an intracellular inhibitor stefin A, which was difusely distributed throughout cell cytoplasm. It was not co-localised with cathepsin B what indicates that at least inside the cells it is not important for regulation of cathepsin B dependent degradation of ECM. The results are similar to that of Calkins and Sloane[20] in hepatoma cells, reporting differetial intracellular distribution of cathepsins and stefins.

In conclusion, our results show that lysosomal cathepsins B and D, overexpressed in most of malignant tumours, predominantly participate in degradation of ECM and facilitate tumour cell invasion. Regulation of their enzymatic activities by exogenous inhibitors represents a new possibility for therapeutic intervention in cancer patients.

Aknowledgement

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Experiencing professional strains of nurses, radiation engineers and physicians working at the Institute of oncology in Ljubljana

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Background. *Since 1974 the term burnout is used in psychology. Burnout describes the end result of stress and has been described by Maslach comprising three basic components: emotional exhaustion, depersonalization and low personal accomplishment.*
In this paper we would like to describe some aspects of burnout experiences of the employees of the Institute of Oncology in Ljubljana.
Subjects and methods. *We used Questionnaire of professional stress, created by Žunter Nagy and Kocmur. In our research 137 health workers from four professional groups participated: physicians, graduated nurses, nurses and radiation engineers, representing 38% of all employees.*
Results. *We found out that in the experience the professional stressof all four professional groups is relatively equalized. The most prominent feelings are of fatigue, irritability and work overload. There were no signs of depersonalization - as described by Maslach - reported in our group. In nurses and in radiation engineers a distress is significantly more often displayed due to poorer personal income and poorer material status. Nurses reported significantly more often the intention to change work position (51%), institution (57%) or job (47%).*
Conclusions. *Workstress impacts on the experience and on the thought patterns in those participating in the study. We can describe those signs as burnout signs. However, there are more new questions opening in the future as well as the need to a longitudinal approach to the research of this more and more prominent field.*

Key words: medical oncology; stress, psychological; burnout, professional

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Introduction

The term »burnout« was first used by Herbert Freudenberger in 1974 when he described problems in so the called »help professions«. He described problems appearing when one encounters situations that are above his ability, power and energy. A decade later

Christine Maslach defined »burnout« with the help of three basic dimensions - emotional exhaustion, depersonalisation (non compassionate way of treating the patients, increased emotional distance from the patients) and reduced personal accomplishment.[1]

The development of the burnout syndrome is influenced by the intertwined influence of many factors. Some personal types are more prone to the burnout development as others.[2] Maslach especially described the following personal factors: low level of hardiness, poor self-esteem, an external locus of control and avoidant coping style. She found out that females, younger employees and singles are more prone to the burnout development.

Beside the individual factors the burnout is also dependent upon the organization and nature of the job. Among those night work and work in shifts, lack of the personal and poor shift organisation stand out in the health system. The good team serves as an important source of emotional support and as such the safety factor; on the other hand conflicts at work are important risk factors for the burnout.

The problem of burnout is common in health care workers in every speciality. The research finding shows that the burnout prevalence among health care workers varies from 28% (Ramirez et al, 1996)[3] to 56% (Whippen and Canellos, 1991).[4] In the field of oncology the work with incurable patients was described as the most important risk factor. The therapy often has a limited impact; health care workers are confronted with the difference between real state of the affairs and expectations - theirs as well as patients' and patient's relatives. Due to a constant contact with dying and the constant loss of patients some thought that the burnout is more pronounced in workers in the field of oncology than in other medical fields; however, some researches discarded this hypothesis.[5,6] Research findings confirmed the Maslach findings that women, younger and single health care workers are more prone to the burnout.[4,7]

The aim of this paper is to present findings considering health care workers at the Institute of Oncology in Ljubljana and shed light to how physicians-oncologists, nurses and radiation engineers are experiencing the burdens of professional and personal life.

Subjects and methods

When carrying out this study, there was no standardized questionnaire for measuring burnout in Slovenia. Therefore, we decided to use Questionnaire of professional stress created by Žunter Nagy and Kocmur, which was fashioned for health care workers in the field of psychiatry.[8]

Participants ranged items on 5-degree scales, some questions were dichotomized. For a statistical analysis we used chi-squared test with Yates correction and in the interval type of results we used Student t-test.

All the physicians-oncologists, nurses, graduated nurses and radiation engineers working at the Institute of Oncology in Ljubljana (which is the only comprehensive cancer centre in Slovenia) received the questionnaire. We got 137 usefully filled questionnaires, representing 38% of those sent, which is comparable with the data from the literature.[4] Response rate was the greatest among the nurses (59.3 %), followed by physicians (21 %) and radiation engineers (19.3 %).

Results

Demographics

The average age of the participants was 32 years for radiation engineers and 40 years for physicians; the combined average age for all groups was 35 years. Like the age the average work time was the lowest for the group of radiation engineers (8.8 years, and for the other

Table1. Demographics of medical oncology staff

	Physicians (n=30)	nurses (n=57)	graduated nurses (n=26)	radiation engineers (n=25)
Age				
under 25 yr	0	36 %	24 %	29 %
26 - 35 yr	37 %	13 %	31 %	37 %
36 - 45 yr	27 %	33 %	20 %	13 %
46 and more yr	37 %	16 %	23 %	21 %
Average work time	14.1 yr	13.9 yr	13.6 yr	8.78 yr
Gender				
Male	27 %	11 %	7 %	31 %
Female	73 %	89 %	93 %	69 %
Marital status				
Single	23 %	32 %	19 %	60 %
Married	73 %	65 %	81 %	36 %
divorced/widowed	3 %	3 %	0	4 %
Children				
None	34 %	43 %	24 %	50 %
one or more	66 %	57 %	76 %	50 %
Direct work with patients	83 %	96 %	100 %	100%

three groups between 13 and 14 years, respectively). The majority of participants were females (83 %); the proportion of females was the greatest among nurses and graduated nurses (90 %). Three quarters of physicians and graduated nurses were married and had at least one child; in radiation engineer's two thirds were singles and half of all without children (Table 1). For the most of working time 83 % of the physicians and almost all participants from other professional groups were working directly with patients.

Work and professional life

Participants from all professional groups are in general satisfied with their profession and their relation to patients (average mark >4). They are less satisfied as regards the professional relations with their colleagues as well as their superiors; their position within the work organization, amount of responsibility they are trusted with; the possibilities of continuous education and possibilities of advancement. The results differ significantly according to groups in the item of satisfaction with salary ($F = 3.519$, $p = 0.017$), where physicians and graduated nurses showing significantly higher mark in comparison to other two groups. What all have in common is the feeling of being overloaded and emotionally too involved in their work.

There were no statistically significant differences between groups in describing their relations toward patients. In their description there a positive, almost idealistic view is dominant. Participants are »almost all the time« understanding, patient, considerate and »almost never« impatient, indifferent, they are never rude (Figure 1). Although there are no statistically significant differences between groups, there is an interesting feeling of guilt in the group of physicians, which could be attributed to their responsibility for treatment, as well as to their adoption of the responsibility for disease outcome.

Participants estimated that their attitude towards patients did not change in the past years of professional work and remained »almost always« the same as their attitude towards patients at the beginning of their work.

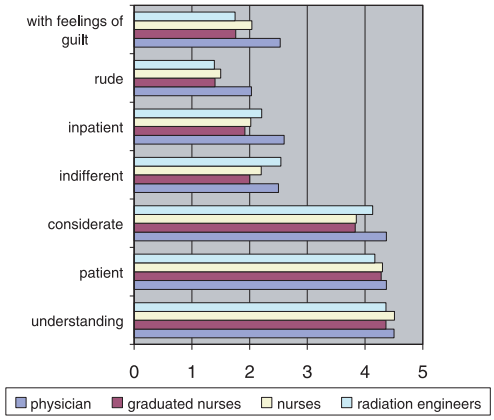

Figure 1. The attitude towards patients of four professional groups.

Results are the same also when comparing their marks in relation to the work time of participants.

The failure of treatment was a prominent factor influencing the experience of work stress in all professional groups. Among organization factors there is a pre-eminent lack of labourers and as a consequence too crammed timetable (Figure 2). Differences between average item marks are small; following the described three items there are pretentiousness of work with the patients and close contact with dying.

We found statistically significant differences among professional groups in items concerning organization matters. Physicians and both groups of nurses estimate more often as radiation engineers that their distress is connected to the lack of personnel ($F = 3.30$, $p = 0.022$). Shift work is more burdensome to nurses as to radiation engineers ($F = 5.149$, $p = 0.002$); radiation engineers work in shifts, but they have a shorten work time; radiation engineers also most often estimate that there is enough personnel. The other two groups do not have a shift work but have instead of this 24 hours duty.

Physicians and graduate nurses are on duty on average 2-3 days per month. As a rationale for taking duty they are citing in the first place the needs of the institution, while physicians more often cite the material needs ($F = 3.073$, $p = 0.049$). Nevertheless, half of graduate nurses and one third of physicians do not want to be on duty less often, which is in both groups linked with bonus allowance.

Among the ways of settling the problems in work place the discussion is pre-eminent in all professional groups, with colleagues, superiors and discussions at home. Recreation is important in the same measure. A statistically significant difference between professional groups is evident only in one item. When encountering problems in work place nurses are more likely to enter sick leave even for minor physical strains ($F = 2.796$, $p = 0.043$).

Statistically significant differences are showing in the item about thinking on work problems at home ($F = 4.237$; $p = 0.007$). Transfer of work situation in the home environment is most pronounced in physicians who could be linked to their responsibility for treatment. This transfer is the least pronounced in radiation engineers.

On the question about considering the change of profession, specialization or work field/work position within the institution there are statistically significant differences in all items ($F = 2.67$, $p = 0.050$; $F = 3.71$, $p = 0.012$; $F = 6.11$, $p = 0.001$). While physicians and radiation engineers are never or almost never considering this, there is a group of nurses with the marked deviation in the positive direction, while in the meantime their answers to questions on the contentment with profession and work place were no different from the others.

Family life

All professional groups estimated that a partner ″almost always″ understands the nature of their work (average mark 4.03). While they are satisfied with the emotional relationship they are having with a partner, the emotional

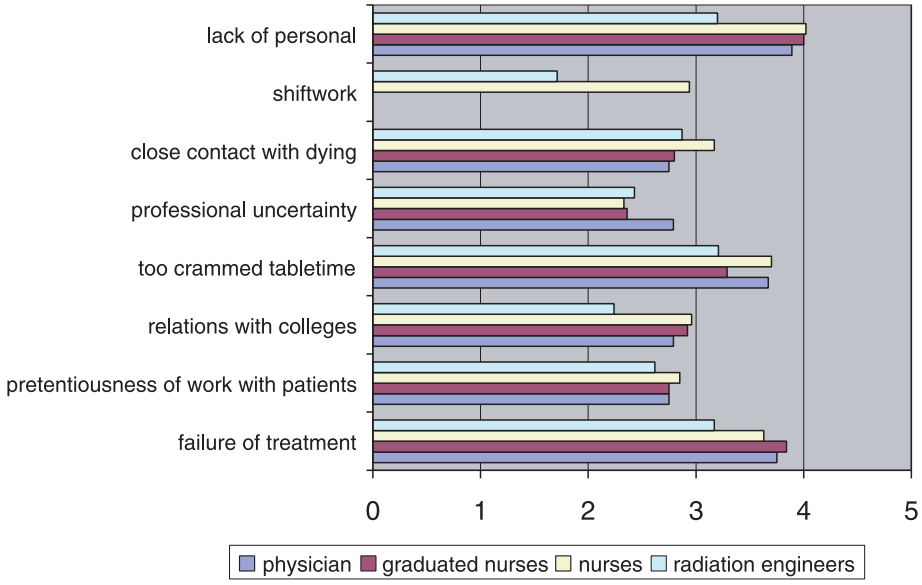

Figure 2. The estimated stress sources on work places of four professional groups.

satisfaction with children in all groups is estimated between ″most of the time″ and ″always″. The relationship with a partner is -like in the all complex of questions regarding personal life - presented in the very positive light - and we can assume idealized. The relation with a partner is almost always loving and comprehensive, items with a negative connotation such as impatient, indifferent, burdened with guilt are estimated as ″almost never″. A statistically significant difference between groups was found for the item indifferent ($F = 3.245$, $p = 0.025$), which is least expressed in the group of physicians.

In estimating which factors of the home environment are connected with stress in participants the estimations are in all groups centred ″mostly″ on ″almost never″, which means that they almost never connect their stress with factors at home. The highest rankings are material problems (between ″almost never″ and ″sometimes″) with statistically significant differences between groups ($F = 5.425$, $p = 0.002$); material problems are highest ranking by nurses and lowest by physicians. The item comparison according to age failed to show statistically significant differences.

Personal life

In the last three years more than 90 % of nurses and radiation engineers and 73 % of physicians completed the physical exam. Ten percent of physicians and 15 % of nurses have a chronical disease, most often hypertension, spine problems (nurses), asthma and migraines. Among regular smokers there are mostly nurses (20 % of participants), and less physicians (6.7 %); but the differences in the frequency allocations are not statistically significant. According to gender among regular smokers there are 48 % males and 13% females. Of the psychoactive substances the participants do not use benzodiazepines, anti-depressants or anti-psychotics; they also almost never consummate alcohol. The differences between groups are not statistically significant. 2-3 % of participants sometimes think of suicide, and the same proportion

cites the attempt in the past; there are no differences between groups. Bad psychical condition is in all groups most often expressed as fatigue and irritability (median estimate 2.5-3) and less often as anxiety, weakness or loneliness (equal or lower than 2). Considering this there are no significant differences between groups. In all groups there is sometimes felling of overworking.

Discussion

Burnout is a complex phenomena and is a result of mutual co-influence of work stress (related to type and nature of work combined with its organization) and underlying personality; with return negative implications for a person with worse attitude toward a patient and decreased efficiency of work as well as for patients one is in care of and organization.

Mutual co-influence of those factors requires a complex research, which had quite expanded in last few years; nevertheless there is a lack of longitudinal researches in research settings.[2] Despite findings that underlying personality could have greater influence on the burnout development than work stress, the research of personality characteristics is still a challenge for the further research.[5]

This study has not by-passed those faults (while there the possibility of longitudinal setting is still open). To investigate one - only institution is related with smaller number of participants, and the descriptive nature of approach enables the investigation of specific social and organizational aspects useful in planning future strategies for the prevention of burnout at the level of a particular organization.

The comparison of all four professional groups shows that in the experiencing the professional stress all four are relatively equalized. In all included professional groups, the most stressful are those situations where the treatment was unsuccessful, which is related to encounter with mortality. The organizational factors are represented even more - poor labourers covering resulting in stuffed schedule. Professional and personal stress result thus above all in feelings of fatigue, irritability and work overload.

Beside this nurses and radiation engineers display distress due to poorer personal income and poorer material status. Shift work decreases their presence in family life and creates conflicts between their professional role and other life roles. Beside this many young nurses not only more frequently think about changing their profession but also to change their work place fairly quick.

Signs of depersonalization as defined by Maslach were not encountered - on the contrary, there is explicitly positive, even idealistic view on e.g. one's attitude towards patients. We can not therefore rule out a hypothesis that this can express phenomena of a denial and no criticism to the state of affairs.

There are two so called burnout protection factors pointed to by the results. The first one is the expressed satisfaction at work and the other social support experienced by the employees when the relationships within the team are satisfactory and in situations, when troubles occur and co-workers help to get out of them.[9]

Due to burnout negative consequences on health professionals, as well as patients and a health organization, it is important to recognize early signs of burnout and act preventively. The factors we can use for this are incorporation of appropriate contents in the educational process, communication skills training, appropriate work organization and unburdening of negative emotions and finally raising consciousness on burnout with appropriate recording.

This study of course is only a static picture of a current situation and warrants further longitudinal approach.

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An outline of the history of radiotherapy at the Institute of Oncology in Ljubljana from its beginning till 1980s

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Background. *The article presents the milestone events in the history of radiotherapy at the Institute of Oncology since its establishment till 1980s. It reviews the facts deduced from various jubilee publications, seminar reports and staff interviews of the Institute of Oncology. The aim of the article is to present the chronological history of radiotherapy at the Institute of Oncology, and to supplement the fragmented and incomplete records written in the past.*

Conclusions. *Available records are occasionally discrepant, but the most significant events in the history of the Institute of Oncology and its Radiotherapy Ward can nevertheless be ascertained.*

Key words: radiotherapy - history - trends; medical oncology; Ljubljana

Introduction

The inception of the Institute of Oncology goes back to October 1917, when Dr Josip Cholewa (Figure 1), Chief Physician of the regional hospital in Brežice, used his modest resources to found an oncological laboratory. He successfully performed experiments in it, and published results in national and foreign scientific literature. Cholewa had studied medicine at the University of Krakow, and later specialized in surgery. While practising the latter, he became interested in oncology. His experimental inducement of cancer was based on the realization that cancer in humans did not differ significantly from cancer in other mammals.[1] He maintained contacts with prominent oncologists in the World, and attended many congresses abroad. In September 1921, he lectured on induced blastoma in a white mouse in Zagreb. His work aroused keen interest abroad. He was the initiator of the »*Yugoslav Committee for Cancer Abatement*«, and instrumental in the establishment of the *Institute for Research and Treatment of Neoplasms of Dravska banovina* (an administrative unit of the pre-war

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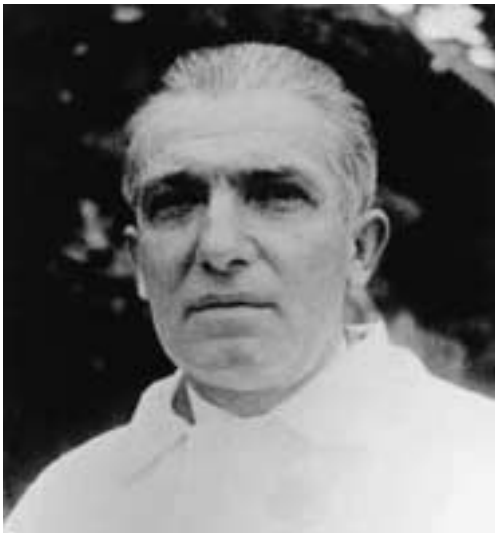

Figure 1. Dr. Josip Cholewa, who found an Oncological Laboratory and who was instrumental in the establishment of the *Institute for Research and Treatment of Neoplasms of Dravska banovina* (an administrative unit of the pre-war Yugoslavia, corresponding largely to the present-day Slovenia) in1937.

Yugoslavia, corresponding largely to the present-day Slovenia) in1937.[2]

Other people, besides Cholewa, must be given credit for the establishment of the Institute. One of them was Dr. Gerlovič, Principal of the State Hospital for Mental Disorders in Ljubljana, whose rich experience was always at the disposal of the Institute. Janko Dolžan, Senior Inspector of *Dravska banovina*, was a regular visitor to the Institute. Ever since the lecture of Professor Blumental in Ljubljana in 1935, the establishment of the Institute was strongly supported by Dr. Mayer, while Dr. Pogačnik, Head of the Department of Otology at the General Hospital in Ljubljana, supplied resources for radium treatment as early as 1928.

The Institute for Research and Treatment of Neoplasms (IRTN)

Prior to the establishment of the Institute for Research and Treatment of Neoplasms in *Dravska banovina* (IRTN), oncological patients had been treated in surgical, gynecological, otological, ophtalmological and other wards in hospitals throughout Slovenia, and the treatment consisted of surgical operations for the most part. Surgeons invested great efforts in saving operable patients, but their interest rapidly ebbed in case of recurring disease, metastases, or inoperable tumours. The new institute filled the void in the cancer treatment of the time.

The IRTN was opened in the southeastern extension of the military hospital in Šempeter. On the ground floor, a boiler room and facilities for test animals were installed. Four patient rooms with 30 beds, an office, a library, the principal's office, a tea kitchenette, toilets, two bathrooms, a waiting room, an outpatient surgery, roentgen - and radiumtherapy facilities, and an operating theatre were provided on the first floor.[3]

The radiotherapy equipment of the IRTN consisted of three units.[4] The *Siemens Stabivolt* (Figure 2) was used for deep therapy, and its orthovoltage roentgen tube worked at 15 mA and 200kV. The tube was mounted on a special casing, which functioned as the shield against high voltage and secondary radiation. The tube was adjustable along all three axes, so that the rays could reach any part of the body. At the time, the *Stabilivolt* was a state-of-the-art radiotherapeutical de-

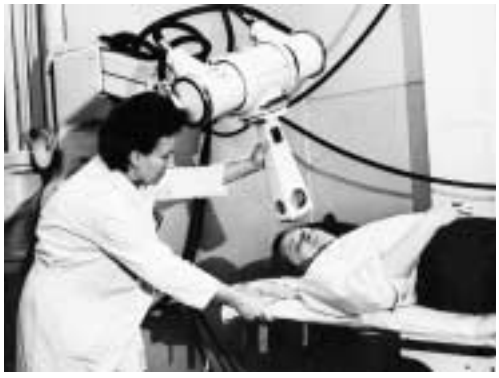

Figure 2. Stabilivolt, a 200 kV device for radiotherapy, made by Siemens.

vice. Its high quality enabled the Oncological Institute to use it until the year 1977, for no less than four decades.

The second unit of the radiotherapy equipment was a *Schafer-Witte*, used for contact intravaginal irradiation. It worked at the maximum voltage of 100 kV and with maximum amperage of 5 mA. A special x-ray tube irradiated neoplasms with straight beams or at an angle, so that cervical cancer could be treated without damaging healthy tissue.

The third device was a *Chaoul*, similar to the *Schafer-Witte* in construction, but working at a lower voltage of 60 kV and maximum amperage of 5 mA. The apparatus was used for contact irradiation of superficial neoplasms. The roentgen tube was cooled with water.

All three pieces of radiotherapy equipment had timers on their respective switch boards, which controlled the duration of irradiation. The control unit was separated from the devices with lead-coated partitions and lead-glass windows, shielding the staff from radiation. The dosimetry was performed with an electrostatic *Hammer dosimeter* with two ionization cells.

The southeastern extension of former military barracks in Šempeter, where the IRTN was set up, was short of room, but an operating theatre with adjoining scrubbing facilities was nevertheless installed. It was small, but fully equipped for major surgeries. The attic accommodated separate rooms for patients' wardrobe, sterilization, chemical, chemobiological and histopathological laboratories, pharmacy, filing cabinets and storage, three rooms for physicians, a room for orderlies, three rooms for nurses, a bathroom and toilets.

Although the IRTN was fully equipped for research and treatment of neoplasms, the shortage of room soon became an impediment, since, in addition to the Institute's own patients, outpatients were sent there for radiotherapy by other medical institutions. Meals were delivered from the nearby hospital for mental diseases, where the laundry was done as well.

The IRTN's resources depended largely on external sponsors. The purchase of 300 mg of radium, for example, was made possible by the *Savings Bank of Dravska banovina*. The material lasted for quite some time, since the *Schafer-Witte* and the *Chaoul* substantially relieved and partly replaced the radium-therapy.[3]

The Principal of the IRTN was Dr Cholewa, the Chief Physician was Dr Lev Šavnik, a gynecologist, who had previously been in charge of radiotherapy at the Roentgen Department of the State General Hospital in Ljubljana. The two of them were assisted by three younger physicians. The histopathological laboratory was lead by Professor Alija Košir, the roentgen equipment, radium and other physical appliances were taken care of by France Avčin, an electroengineer, the scientific chemical laboratory was lead by Professor Vladimir Premru.

From World War II to the Institute of Oncology

During the Italian occupation (1941-1943), the IRTN of *Dravska Banovina* was replaced, to a limited degree, by the *Institute for Neoplasms of the Ljubljana Province* (since *Dravska banovina* no longer existed). After the death of Dr Cholewa in 1943, the management was taken over by Professor Šavnik.

In August 1945, the Institute merged with the Roentgen Institute of the former General Hospital, and became the *Institute of Roentgenology and Radiology* of the newly established *University Hospitals* in Ljubljana.[5] It was headed by Dr. Josip Hebein. Already in April 1946, the two institutes were separated again, and the name of the former changed to *Institution of Oncology.*

In December 1948, the *Chair of Oncology and Radiotherapy* was opened and the same

time the name *Institution of Oncology* changed to *Institute of Oncology*. The management of the Institute, which was still attached to the University Hospitals, was taken over by Professor Šavnik, who remained its principal until the year 1963.

During the first few years after the war, radiotherapy was the fastest developing activity, propelled by the newly acquired equipment. Until 1949, radiotherapy was performed by only one radiologist, who was in charge of x-ray diagnostics as well. The shortage of manpower made it necessary to engage physicians who were trained as radiotherapists on the job. After the year 1949, the Institute started to educate radiotherapists systematically. In 1955, two physicians (Dr. Danica Žitnik and Dr. Majda Mačkovšek-Peršič) successfully passed the specialist exam in radiotherapy. This achievement was the beginning of radiotherapy as an independent branch of medicine in Slovenia. By the year 1963 six other physicians had passed the same exam.[4]

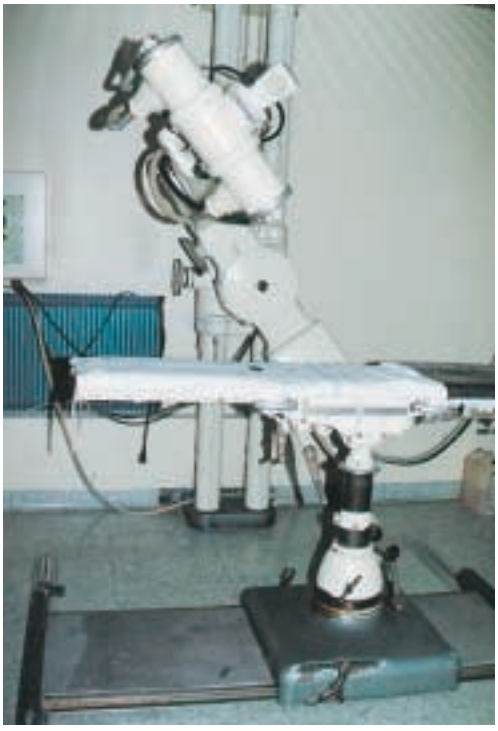

Figure 3. Stabilipan, a 300 kV device for radiotherapy, suitable for superficial irradiation, and allowing 3 to 5 combinations of voltage, made by Siemens.

Figure 4. Betatron, the 31 MeV circular accelerator, enabling supervoltage radiotherapy.

At that time, radiotherapy was the most important therapeutic activity at the Institute. The equipment was relatively modest in the beginning, but it improved considerably over the first few years. In addition to the Stabilivolt and two other out-dated roentgen devices, the Institute acquired, in 1952, a *Siemens Stabilipan* (300 kV), suitable for superficial irradiation, and allowing 3 to 5 combinations of voltage (Figure 3). It was used primarily in the treatment of superficial and semi-deep seated tumours. The focus of source-skin distance and the size of the field were regulated by a special accessory unit, an *applicator*, which reduced the scattering of rays and adjusted the field of irradiation. In those years, the Institute received 600 mg of radium from the

United Nations Relief and Rehabilitation Agency (UNRRA).

In 1955, the irradiation of patients with a *31 MeV Betatron* (Figure 4) started, which was another milestone event. The Betatron was the first circular accelerator and, due to its high energy potential, the first device enabling supervoltage radiotherapy. It was bought by the Institute of Physics *Jožef Stefan*, and also installed there. It was used both for research and for treatment. Unfortunately, patients had to be transported all the way through the city, which made the therapy even more complicated. During the irradiation, patients were placed on an adapted operating table. The position of the patient had to be adjusted to the horizontal emanation of beams - patients with lung cancer were treated in sitting position.

In 1958, the idea of complete autonomy of the Institute was reborn. The aspirations resulted in the Institute of Oncology becoming and independent institution on August 1, 1961, exactly after 23 years of its existence.

The development of radiotherapy at the Institute of Oncology from 1961 till 1980

The period between 1961 and 1970 was marked by the efforts to build new facilities and acquire larger premises, as well as to expand the research activity. The Institute obtained some new radiotherapy equipment, but the shortage of room hindered its efficiency. Towards the end of the 1960s, the blueprints of a new building for teleradiotherapy were designed. By the year 1968, the bulk of resources necessary for the construction of the new Institute of Oncology had been accumulated in the *fund for the construction of the new Institute of Oncology* (set up in 1965). The building was to be erected on the right side of the Ljubljanica river. The facilities for the teleradiotherapy were the first to be built, but the plans fell through because of the construction of the new University Clinical Centre.

Figure 5. Gamatron, supervoltage device with sealed radioactive source for deep irradiation, the source of Co60 with activity of 111 TBq (3000 Ci), made by Siemens.

The first supervoltage device with sealed radioactive source used at the Institute of Oncology (in 1962) *was a Siemens Gamatron* (Figure 5), a *Co60* unit, with the initial activity of 111 TBq (3000 Ci). The activity of the cobalt source (Co60) was 111 TBq, and its head made of wolfram, which shielded the environment from radiation, weighed more than half a ton. It was used for deep irradiation, and soon nick-named *the Cobalt Bomb*. In addition, two roentgen diagnostic devices were bought. They were used for tracing the localizations of irradiated fields and the position of brachytherapeutic sources of radium, as well as for diagnostic purposes.[5]

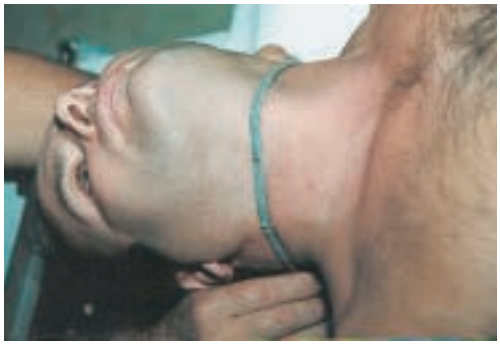

Figure 6. The lead wire for tracing the body contour, in which the position of the tumour and of other organs were marked while devising the irradiation plan,

Figure 7. Needle contour device with radially arranged metal needles for tracing the body contour.

The body contour, in which the position of the tumour and of other organs was marked while devising the irradiation plan, was traced with the help of a lead wire (Figure 6). It was wrapped around the patient, then carefully removed and copied to tracing paper. The method was very imprecise, since distortions of the wire were inevitable during its removal from the patient.

In the middle of the 1960s the body contours were traced with a special *needle contour device (NCD)*, with radially arranged metal needles (Figure 7). The tips of the needles were pressed against the patient's body, and then the dots were copied to the tracing paper and connected into a line.

In the beginning, the protection of healthy tissue around the irradiated area consisted of

Figure 8. The standardized lead blocks of different shapes for protection the healthy tissue around the irradiated area.

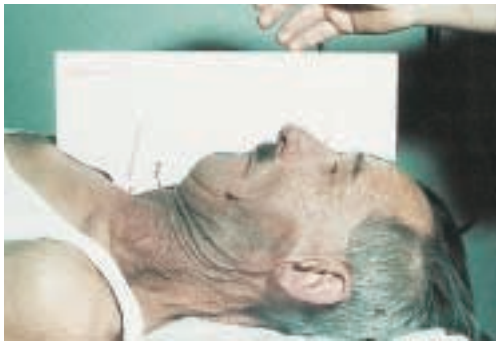

Figure 9. A special cardboard profile, which allowed the resumption of the patient's position during the planning and during the irradiation process.

standardized lead blocks of different shapes (Figure 8), attached to an acrylic plate (or inserted between two such plates). This method is still applied with sealed sources of radiation (cobalt), but for linear accelerators it was replaced by customized shields in the 1990s. The only exception in the use of standardized shields was the treatment of patients with Hodgkin's disease. Custom shields were made for them at an early date. Depending on the shape of the part of the body that needed shielding, holes were cut out of a Styrofoam board with a hot wire, filled with protective lead pellets, and sealed with paraffin.

It is crucial that the position of the patient remains the same throughout the therapy, especially when the patient's head and neck with many sensitive organs are within the irradiation field. It is the only way to make irradiation effective, and the protection reliable.

In the beginning, patients with tumours in the head and neck were secured in their position with the help of a special cardboard profile (Figure 9), which allowed the resumption of the position during the planning and during the irradiation process. The profile was taken with the help of a special device made of metal needles. Once the position of the patient was secure, the metal needles were arranged along the patient's profile and fixed. The contour was copied to the cardboard and the profile was cut out of it.

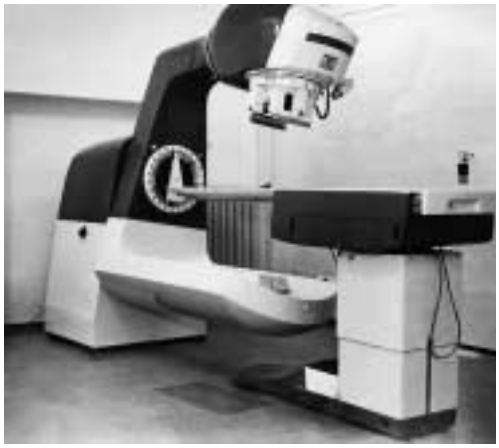

Figure 10. *Theratron 80*, the supervoltage device with sealed radioactive source for deep irradiation, the source of Co60 with activity of 222 TBq (6000 Ci), made in Canada.

In 1969, another sealed source apparatus (Co60) joined the slightly out-dated and overloaded *Siemens Gamatron*. It was a *Theratron* 80, 222 TBq (6000 Ci), made in Canada (Figure 10). It was provisionally set up in the adapted former garage near the isotopic laboratory. Its specialty was the so-called »beam stopper«, which intercepted the exiting radiation, so that the room needed considerably less shielding than in the case of devices with no such accessory.

Still, it was yet another proof of how the shortage of space impeded the development of radiotherapy.

Towards the end of the 1960's, body contour tracing was performed with a pantograph (Figure 11), a mechanical device which traced the contours of the body on paper while a radiographer moved its antenna along the surface of the patient's body. Several types of pantographs were used, from mechanically very simple ones, to complex and precise devices. A pantograph is still part of the standard equipment of the Teleradiotherapy Department. Being a mechanical instrument, it can hardly ever fail. It serves as a back-up device to more modern and complex appliances (such as CTs or lasers).

In spite of the fact that everything pointed to the Oncological Institute moving to a new location towards the end of the 1960s, the early 1970s clearly revealed that other projects had priority. The *Act on the Construction of the University Clinical Centre* (passed by the Assembly of the SR Slovenia in May 1981) stipulated the construction of the TRT on the left bank of the Ljubljanica river, on the premises of the old auxiliary units of the Clinical Centre. The postponement was a huge blow to the Oncological Institute, curtailing its spatial perspectives. To compensate for that, the Oncological Institute was given the old building of the Internal Clinic, which was renovated with the funds for the construction of the new Oncological Institute - the renovation cost 500 million dinars.[3]

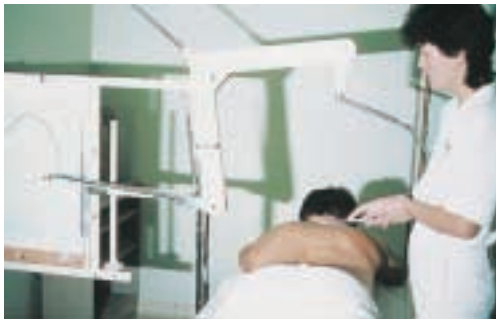

Figure 11. A pantograph, a mechanical device, which traced the contours of the body on paper while a radiographer moved its antenna along the surface of the patient's body.

Figure 12. The simulator *Ximatron*, used for the preparation and planning of radiotherapy, made by *TEM* in United Kingdom.

The Teleradiotherapy Department moved to the renovated building of the Internal Clinic, which became the new building C of the Oncological Institute. All existing equipment was transported there, except the *Gamatron*, which went out of use. Two new roentgen devices were bought. The first one was the simulator *Ximatron TEM* (Figure 12), used for the preparation and planning of radiotherapy. The apparatus simulated the conditions on irradiation devices, determined the irradiation field in the patient, and tested the shielding. One of the major advantages of simulators is that the irradiation field is immediately visible on the screen. Since the table with the patient is movable as well, any aberration can be immediately detected and rectified, without the time-consuming radiography. This must be done only at the end, for verification, possible fabrication of shields, and records.

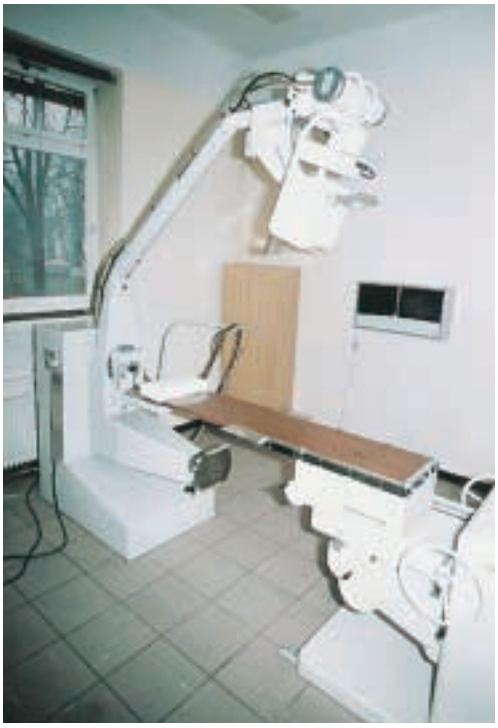

Figure 13. *Transversal Tomograph*, which enables the contactless tracing of body contour.

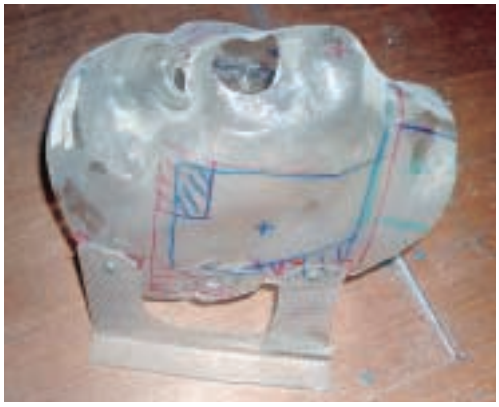

Figure 14. The *fixation mask*, which secures the patient's position during the treatment.

The second new device was a Japanese *Toshiba Transversal Tomograph* (Figure 13).

It changed completely the treatment planning and the body contour tracing procedure. It made it possible to locate the precise site of the tumour in relation to all internal organs in the vicinity, which was of crucial importance for the treatment planning. The body contour tracing became contactless, the wrapping of the patient with a wire, or pressing metal needles against the patient's body became superfluous.

In the middle of the 1970s *fixation masks* replaced cardboard profiles in securing the patient's position during the treatment (Figure 14). The procedure was long, uncomfortable and difficult for the patient. The masks were made in a special workshop, and their fabrication took one whole day.

Figure 15. The cornerstone for the new teleradiotherapy building was laid.

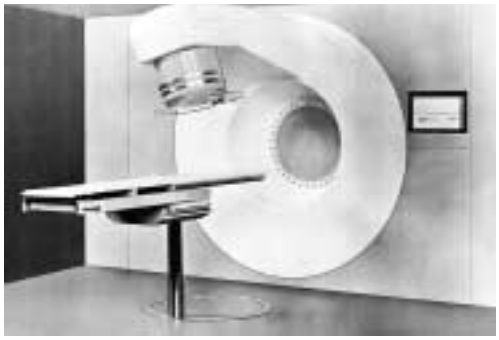

Figure 16. *The linear accelerator* SL75/20, a supervoltage radiotherapy device made by *Phillips*. It was used until the year 2000.

After the disappointment in 1971, the struggle to overcome the shortage of space continued. The staff of the Institute of Oncology became more optimistic on November 1974, when the cornerstone for the new teleradiotherapy building was laid (Figure 15). The construction took three years, and the Teleradiotherapy Department moved to new premises in 1977.

Figure 17. A sealed source radiation (Co60) device made by *Phillips*. It is still being used.

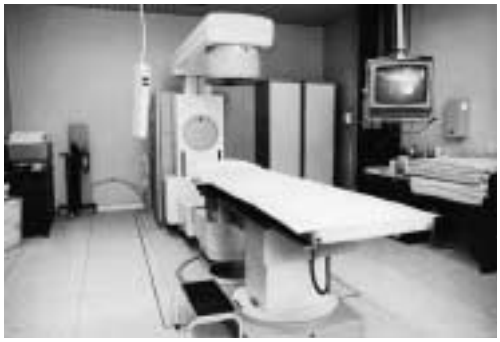

Figure 18. The Phillips simulator, which went out of use in 1999.

All the existing equipment, except the Gamatron, were moved to the new building. Two new supervoltage radiotherapy devices were installed there as well - a new *Phillips linear accelerator SL75/20* (Figure 16), which was used until the year 2000, and a sealed source radiation (Co60) apparatus of the same producer, which is still being used (Figure 17),. In addition to the *Ximatron* simulator, a new Phillips simulator was installed (Figure 18), which went out of use in 1999.

The Teleradiotherapy Department of the Institute of Oncology is still confined to the same premises, although it has outgrown them already. The number of patients has greatly increased since the 1980s, and so has the staff. The purchase of new equipment promotes the efficiency of the Institute, but the finalization of the new Institute of Oncology will have to be next step. Although the teleradiotherapy will remain in the same building, some additional room will be provided in the new building, reducing the shortage of space, especially in view of the imminent purchase of three new linear accelerators.

Acknowledgement

We thank all the staff of the Institute of Oncology for the help rendered in the collection of historical data. A special thank-you goes to Boris Sekereš.

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Radiol Oncol 2003; 37(4): 213-6.

Možganske metastaze pri bolnikih s pljučnim rakom. Vpliv prognostičnih dejavnikov na preživetje

Smrdel U, Zwitter M, Kovač V

Izhodišča. Pljučni rak pogosto zaseva v možgane. V prispevku smo ugotavljali, pri katerih bolnikih s pljučnim rakom se pojavljajo možganske metastaze in kako različni napovedni dejavniki vplivajo na preživetje bolnikov.

Bolniki in metode. V letu 1998 je bilo v Sloveniji ugotovljenih 974 novih bolnikov s pljučnim rakom, 615 med njimi je bilo obravnavanih tudi na Onkološkem inštitutu v Ljubljani. Med potekom bolezni smo pri 137 (22,3 %) od 615 bolnikov odkrili možganske metastaze.

Rezultati. Srednje preživetje pri 12 bolnikih s solitarnimi možganskimi metastazami (pri večini je bila narejena metastazektomija) je bilo 7,6 mesecev, srednje preživetje pri bolnikih z multiplimi možganskimi metastazami pa je bilo 2,8 meseca (p = 0.0018).

Od 137 bolnikov jih je 45 (32,8 %) imelo drobnocelični pljučni rak, 43 (31,4 %) žlezni rak in 19 (13,9 %) skvamoznocelični pljučni rak. Bolniki, ki so imeli stanje splošne zmogljivosti po WHO-ju manj kot 2, so imeli srednje preživetje 3,7 meseca, bolniki s stanjem splošne zmogljivosti 2 ali več pa 2,7 meseca (p=0.0448).

Zaključki. Bolniki s solitarnimi možganskimi metastazami imajo statistično značilno boljše preživetje kot tisti z multiplimi možganskimi metastazami. Preseneča velik odstotek bolnikov z žleznim pljučnim rakom, pri katerih odkrivamo možganske metastaze skoraj v enakem odstotku kot pri bolnikih z drobnoceličnim pljučnim rakom. Tako moramo ponovno razmisliti, ali ni indicirano profilaktično obsevanje glave tudi pri bolnikih z žleznim pljučnim rakom? Tudi pri naših bolnikih se je pokazalo, da je stanje splošne zmogljivosti odločilen napovedni dejavnik za preživetje.

Radiol Oncol 2003; 37(4): 217-9.

Endobronhialna metastaza kot prvi znak ledvičnega karcinoma

Kaneko Y, Haraguchi N, Kodama T, Kagohashi K, Ishii Y, Satoh H, Sekizawa K

Izhodišča. Najbolj pogosto najdemo endobronhialne metastaze po diagnosticiranju primarnega tumorja. Prikazujemo pa redek primer, ko je bila endobronhialna metastaza odkrita pred primarnim tumorjem oz. je bila prvi znak ledvičnega karcinoma.
Prikaz primera. 61-letni bolnik se je ob sprejemu v našo bolnišnico pritoževal zaradi 3 mesece trajajočega kašlja. CT preiskava prsnega koša je pokazala polipoidno maso v bronhiju za desni zgornji pljučni reženj. Odvzeli so material za biobsijo in mikroskopski pregled je pokazal, da ima bolnik metastazo ledvičnega karcinoma na steni bronhija.
Zaključki. Ko odkrijemo endobronhialne tumorozne spremembe brez kliničnih znakov primarnega tumorja, moramo pomisliti tudi na asimptomatski primarni tumor izven prsnega koša. V takšnih primerih so potrebne vse ustrezne diagnostične preiskave.

Radio Oncol 2003; 27(4): 221-4.

Metastaza sramne kosti kot prvi znak pljučnega raka

Kodama T, Satoh H, Ueno T, Homma S, Sekizawa K

Izhodišča. Metastaze sramne kosti so kot prvi znak pljučnega raka zelo redke. Napačno jih lahko ocenimo kot parasimfizealne osteoporotične frakture, ki so pogoste pri postmenopauzalnih ženah in pri starostnikih.
Prikaz primera. 65-letna bolnica je tožila zaradi bolečin v predelu desnega boka, ki so se širile v prednji del stegna. Z roentgenskim slikanjem medenice smo odkrili osteolitično spremembo v predelu zgornjega ramusa sramne kosti. Roentgenska slika prsnih organov pa je pokazala nodularni infiltrat v predelu srednjega režnja desnih pljuč. S pomočjo transbronhialne biobsije smo ugotovili, da ima bolnica bronhialni adenokarcinom. Bolnici smo obsevali prizadeti del kosti, bolečine pa dodatno umirili z morfinskim zdravilom.
Zaključki. Ob odkritju neobičajne kostne metastaze, ko še nismo odkrili primarnega tumorja, moramo najprej pomisliti na pljučni karcinom in narediti rentgensko slikanje prsnih organov.

Radiol Oncol 2003; 37(4): 225-32.

Prikaz apoptotičnih celic v tumorskih parafinskih rezinah

Pižem J, Cör A

Apoptoza je oblika celične smrti z značilnimi morfološkimi spremembami, ki jo uravnavajo zapleteni molekularni mehanizmi. Motnje v uravnavanju apoptoze so pomembne za rast tumorjev, hkrati pa različne metode zdravljenja tumorjev zavirajo njihovo rast pretežno s sprožanjem apoptoze tumorskih celic. Ugotavljanje apoptotske aktivnost v tumorju je lahko pomembno za napoved poteka bolezni in njenega zdravljenja, zato je pomemben razvoj metod za rutinski prikaz apoptotičnih celic v tumorskem tkivu, ki je bilo fiksirano v formalinu in vklopljeno v parafin.

Pri uravnavanju apoptoze sodelujejo zapletene molekularne poti. Različni proapoptotični signali sprožijo aktivacijo kaspaz, slednje pa cepijo tarčne beljakovine. Njihova cepitev je odgovorna za morfološke spremembe apoptotičnih celic in značilno cepitev jedrne DNK. V zadnjem desetletju je prikaz apoptotičnih celic v tumorskem tkivu fiksiranem v formalinu temeljil pretežno na morfoloških značilnostih in značilni fragmentaciji DNK. V zadnjem času omogoča zanesljiv prikaz apoptotičnih celic imunohistokemična reakcija na prikaz aktiviranih kaspaz in cepljenih tarčnih beljakovin (citokeratin 18, aktin, PARP).

Radiol Oncol 2003; 37(4): 233-40.

Izražanje katepsina B je povezano s tumorigenostjo celičnih linij raka dojke

Zajc I, Frangež L, Lah TT

Izhodišče. Menimo, da lizosomski cisteinski proteazi katepsina (Cat) B in L ter njuna endogena inhibitorja stefina (St) A in B sodelujejo pri napredovanju raka na dojki pri človeku. V prejšnji raziskavi na celičnem modelu raka dojke smo pokazali[1], da v literaturi opisana tumorigenost teh celičnih linij ne sovpada neposredno z njihovo invazivnostjo in vitro. Izražanje CatL je naraščalo z invazivnostjo celic, in bilo v obratnem sorazmerju z izražanjem StA. V tej raziskavi preverjamo hipotezo, da je porušeno ravnotežje med CatB in stefinoma povezano bodisi z invazivnostjo bodisi s tumorigenostjo izbranih celičnih linij raka dojke.

Rezultati. Raziskovali smo izražanje CatB na nivoju mRNA, proteina in njegove aktivnosti v celičnih linijah humanega raka dojke, katerih in vivo tumorigenost narašča v vrstnem redu MCF-7 < MDA-MB468 < MDA-MB231 < MDA-MB435, najbolj invazivna od omenjenih pa je MDA-MB231. Izražanje CatB na vseh treh nivojih je medseboj dobro sovpadalo in naraščalo hkrati z rastočo tumorigenostjo celic. Razmerje med CatB in stefini je bilo na strani CatB v bolj tumorigenih celičnih linijah.

Zaključki. Ker smo predhodno pokazali, da je CatL povezan z invazivnostjo, v tej raziskavi pa ugotovili, da je izražanje CatB povezano s tumorigenostjo istih celičnih linij, menimo, da izražanje obeh katepsinov v teh linijah ni regulirano na enak način. Izražanje CatB, kot tudi razmerja med CAtB in stefinoma, narašča s tumorigenostjo celic, kar morda zrcali podobno situacijo v človeških tumorjih in vivo.

Radiol Oncol 2003; 37(4): 241-8.

Cisteinski in aspartatni proteazi katepsina B in D določata invazivnost MCF10A neoT celic

Premzl A in Kos J

Izhodišča. Znano je, da imata lizosomska katepsina B in D pomembno vlogo v različnih procesih, ki vodijo do napredovanja malignih bolezni. V ras-transformiranih MCF10A neoT celicah oba encima kažeta podobno vezikularno razporeditev, tako v perinuklearnih kot v perifernih citoplazemskih regijah.

Rezultati. V nekaterih veziklih je bila s pomočjo konfokalne mikroskopije določena njuna kolokalizacija, kar potrjuje odvisno delovanje v proteolitski kaskadi. Za stefin A, endogeni znotrajcelični inhibitor cisteinskih proteaz, pa smo pokazali da se ne kolokalizira s katepsinom B in predvidoma ni udeležen pri regulaciji njegove aktivnosti znotraj veziklov. Znotrajcelična lokalizacija obeh katepsinov se ujema z lokalizacijo razgradnih produktov DQ-kolagena IV, bodisi v posameznih celicah ali v celičnih sferoidih. Sposobnost katepsinov B in D, da razgrajujeta kolagen in druge komponente zunajceličnega matriksa, potrjujejo tudi rezultati testa razgradnje matrigela.

Zaključki. Pokazali smo, da specifična inhibitorja katepsina B (znotrajcelični CA-074 Me in zunajcelični CA-074) in pepstatin A, inhibitor katepsina D, značilno zmanjšajo invazijo MCF10A neoT celic. Naši rezultati tudi kažejo, da v nasprotju z nekaterimi prejšnjimi študijami aktivacijski peptid pro-katepsina D ni mitogen na MCF10A neoT, MCF-7 in HEK-293 celice.

Radiol Oncol 2003(4): 249-55.

Doživljanje preobremenjenosti medicinskih sester, radioloških inženirjev in zdravnikov na Onkološkem inštitutu v Ljubljani

Škufca Smrdel AC

Izhodišča. Izraz sindrom izgorevanja uporabljamo v psihologiji od leta 1974. Predstavlja možni končni izid delovanja stresogenih dejavnikov. Maslachova ga je opredelila s pomočjo treh osnovnih dimenzij: čustvena izčrpanost, depersonalizacija ter zmanjšana osebna vpletenost.
V prispevku želimo opredeliti nekatere značilnosti sindroma izgorevanja, kolikor se kažejo pri zaposlenih na Onkološkem inštitutu v Ljubljani.
Subjekti in metode. Uporabili smo vprašalnik poklicnih obremenitev, avtoric Žunter Nagyeve in Kocmurjeve. Sodelovalo je 137 zdravstvenih delavcev iz 4 poklicnih skupin: zdravniki, diplomirane oz. višje medicinske sestre, srednje medicinske sestre ter radiološki inžinirji; kar predstavlja 38% vseh povabljenih k sodelovanju.
Rezultati. Izsledki raziskave kažejo, da sodelujoči iz vseh štirih poklicnih skupin na zelo podoben način doživljajo poklicno obremenjenost. V ospredju so občutja izčrpanosti, razdražljivosti in preoobremenjenosti z delom. Znakov razoseblenja, kot jih opisuje Maslachova, nismo zasledili. Pri srednjih medicinskih sestrah ter radioloških inženirjih je stiska statistično pogosteje povezana z manjšim osebnim dohodkom ter slabšim materialnim stanjem. Medicinske sestre tudi statistično pogosteje izražajo namero, da bi zamenjale delovno mesto (51%), ustanovo (57%) ali poklic (47%).
Zaključki. Obremenitve na delovnem mestu že vplivajo na doživljanje in mišljenje sodelujočih v študiji. Izražene znake lahko opišemo kot znake sindroma izgorevanja. Odpirajo pa se tudi nova vprašanja ter potreba po longitudinalnem pristopu k raziskovanju tega vse bolj pomembnega področja.

Radiol Oncol 2003; 37(4): 257-66.

Pregled razvoja radioterapije na Onkološkem inštitutu v Ljubljani od pričetkov do osemdesetih let 20. stoletja

Oklješa Lukič A, Hübscher K

Izhodišča. Članek obravnava dogajanja na radioterapevtskem oddelku Onkološkega inštituta od njegovega nastanka do osemdestih let dvajsetega stoletja. Podatki o razvoju oddelka so pridobljeni predvsem iz publikacij, ki jih je Onkološki inštitut izdajal ob obletnicah svojega delovanja, iz različnih poročil s strokovnih seminarjev in s pomočjo pogovorov z zaposlenimi na Onkološkem inštitutu. Cilj zgodovinske raziskave je bil kronološko predstaviti razvoj radioterapevtskega oddelka Onkološkega inštituta, saj so dosedanja zgodovinska poročila razdrobljena in nepopolna.

Zaključki. Ugotovili sva, da si nekateri zapisi iz dosegljivih virov celo nasprotujejo, kljub temu pa je bilo moč iz zbranega gradiva izluščiti najpomembnejše prelomnice v razvoju Onkološkega inštituta in njegovega radioterapevtskega oddelka.

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.*

Radiation oncology

March, 2004

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

See http://www.isro.be

Tobacco counters health

March 7-11, 2004

The conference »3rd World Assembly on Tobacco Counters Health (3rd WATCH - 2004)« will take place in New Delhi, India.

Contact Major General Dr. Avnish K. Varma, World Assembly on Tobacco Counters Health, M 38 A, Rajouri Garden, New Delhi 110027, India; or call +91 11 2544 7395; or fax +91 11 2510 9397; or e-mail cancerak@del6.vsnlnet.in; or see http://www.watch-2000.org

Radiotherapy

March 7-11, 2004

The ESTRO course »Radiotherapy Treatment Planning: Principles and Practice« will take place in Dublin, Ireland.

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

Brachytherapy

March 21-25, 2004

The ESTRO course »Modern Brachytherapy Techniques« will take place in Bled, Slovenia.

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

Radiology

March 26-28, 2004

The seminar »Annual Advanced Topics in CT Scanning: CT Angiography, 3D Imaging, Virtual Imaging« will take place in Los Angeles, California, USA.

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

Surgical oncology

March 31 - April 3, 2004

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

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

Radiotherapy

April 1-3, 2004

The »2nd ESTRO Meeting on Radiotherapy for Non-Malignant Diseases« will take place in Nice, France.

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

Oncology

April 15-17, 2004

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

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

Radiotherapy

April 18-22, 2004

The ESTRO course »Imaging for Target Volume Determination in Radiotherapy« will take place in München, Germany.

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

Radiation oncology

May 2-6, 2004

The ESTRO course »Radiation Oncology: a Molecular Approach« will take place in Giardini Naxos, Italy.

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

Radiotherapy

May 2-6, 2004

The ESTRO course »Dose Determination in Radiotherapy: Beam Characterisation, Dose Calculation and Dose Verification« will take place in Nice, France.

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

Brachytherapy

May 13-15, 2004

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

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Radiology

June 6-8, 2004

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

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

Radiation oncology

June 13-18, 2004

The ESTRO course »Evidence-Bases Radiation Oncology: Methodological Basis and Clinical Application« will take place in Moscow, Russia.

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

Radiotherapy

June 20-24, 2004

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

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Prostate cancer

June 27-29, 2004

The ESTRO course »Brachytherapy for Prostate Cancer« will take place in Leeds, U.K..

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Oncology

July 3-6, 2004

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

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

Gynaecological cancer

August 26-28, 2004

The ESTRO advanced teaching course on »Brachyherapy for Gynaecological Cancer« will take place in Vienna, Austria.

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

Medical physics

August 29 - September 2, 2004

The ESTRO course »Physics for Clinical Radiotherapy« will take place in Leuven, Belgium.

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

Paediatric oncology

September, 2004

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

See http://www.siop.nl

Radiobiology

September 19-23, 2004

The ESTRO course »Basic Clinical Radiobiology« will take place in Lausanne, Switzerland.

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

Lung cancer

September 23-25, 2004

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

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

Radiation therapy

October 3-7, 2004

ASTRO Annual meeting will be held in Atlanta, USA.

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

Therapeutic radiology and oncology

October 24-28, 2004

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

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

Medical oncology

October 29 - November 2, 2004

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

See http://www.esmo.org

Radiation oncology

November 7-12, 2004

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

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

Radiation oncology

November 25-28, 2004

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

See http://www.isro.be

Radiation oncology

March, 2005

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

See http://www.isro.be

Lung cancer

July 3-6, 2005

The »11th World Conference on Lung Cancer« will be offered in Barcelona, Spain.

Contact Heather Drew, Imedex, Inc., 70 Technology Drive, Alpharetta, GA 30005 USA; or call +1 770 751 7332, or fax +1 770 751 7334; or e-mail h.drew@imedex.com, or see www.imedex.com/calenders/oncology/htm

Radiation oncology

September - October, 2005

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

See http://www.isro.be

Oncology

October 30 - November 3, 2005

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

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

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

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

Author Index 2003

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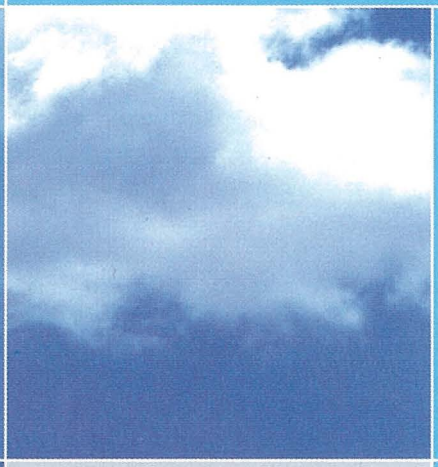
Activity of »Dr. J. Cholewa« Foundation for Cancer Research and Education - A Report for the Final Quarter of 2003

In the final report for the year 2003 it is important to emphasise some other aspects of the meeting of the Administrative and Supervising Boards of the Dr. J. Cholewa Foundation for Cancer Research and Education Foundation and the report by the Health experts Commission of the Foundation. As was stressed in our previous report, several new members joined the Foundation, research activity with the cooperation of a major pharmaceutical company was discussed, and the graceful and important donation to the Foundation by Dr Ana Hinterlechner Ravnik was gratefully acknowledged. Other topics were also discussed, especially those concerning the day-to-day activity of the Foundation and were also presented at the annual meeting of the general assembly. As reported, the reports also included the detailed overview of the Foundation's activity in supporting and sustaining research in cancer in Slovenia. With this in mind, the Foundation will continue to support the regular publication of »Radiology and Oncology« international scientific journal, which is edited, published and printed in Ljubljana, Slovenia. The Foundation will also strive to continue in its activity to promote cancer biology research, research in cancer epidemiology and clinical cancer research in their many different pathways.

The Dr. J. Cholewa Foundation for Cancer Research and Education is optimistic about the prospects in the coming year 2004. Republic of Slovenia is one of the ten accessing countries that will join the European Union in 2004, and this important fact may help the Foundation to gain more information, to expand its existing framework of activities and to find and deal with new challenges in the new economic and political surroundings. All of these changes will probably have a serious impact on the cancer research and education activity in Slovenia and will present it with new challenges. All this may help the Foundation to find the ways to collaborate with similar institutions all over Europe and elsewhere, and the Foundation may in this way further expand its scope and goals.

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

Glivične okužbe



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Datum priprave besedila: marec 2003

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zastopa naslednja podjetja

Köttermann (Nemčija):
laboratorijsko pohištvo, varnostne omare za kisline, luge, topila, pline in strupe, ventilacijska tehnika in digestorji

DAKO (Danska):
testi za aplikacijo v imunohistokemiji, patologiji, mikrobiologiji, virologiji, mono- in poliklonalna protitelesa

SVANOVA Biotech (Švedska):
Elisa testi za diagnostiko v veterini

NOVODIRECT BIOBLOCK (Francija):
kompletna oprema in pripomočki za delo v laboratoriju

GFL (Nemčija):
laboratorijski aparati, omare in skrinje za globoko zamrzovanje

ANGELANTONI SCIENTIFICA (Italija):
hladilna tehnika in aparati za laboratorije, transfuzijo, patologijo in sodno medicino

EHRET (Nemčija):
laminar flow tehnika, inkubatorji, sušilniki, suhi sterilizatorji in oprema za laboratorijsko vzrejo živali - kletke

INTEGRA BIOSCIENCES (Švica):
laboratorijska oprema za mikrobiologijo, biologijo celic, molekularno biologijo in biotehnologijo

CORNING (ZDA):
specialna laboratorijska plastika za aplikacijo v imunologiji, mikrobiologiji-virologiji, ipd., mehanske eno- in večkanalne pipete in nastavki

EVL (Nizozemska):
diagnostični testi za uporabo v veterinarski medicini

HÜRNER (Nemčija):
ventilacijska tehnika

CSL - Biosciences:
diagnostični testi za uporabo v veterinarski medicini

BIOMERICA (ZDA):
hitri testi za diagnostiko, EIA /RIA testi

CHARLES ISCHI (Švica):
specialna oprema za testiranje izdelkov v farmacevtski industriji;aparati za procesno kontrolo in kontrolo kvalitete

ROSYS - ANTHOS (Avstrija):
fotometri, avtomatski pralni sistem za mikrotitrine plošče

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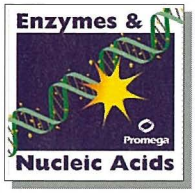
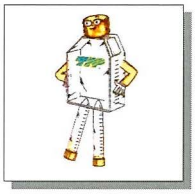
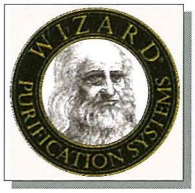
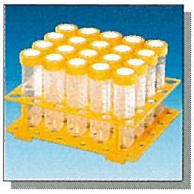








IZDELKI ZA MOLEKULARNO BIOLOGIJO

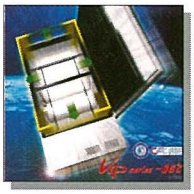
DOKUMENTACIJA IN ANALIZA GELOV

PLASTIKA ZA CELIČNE KULTURE

ELGA LABWATER

SANYO

Invitrogen life technologies



ČISTA VODA ZA LABORATORIJ

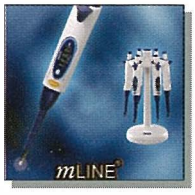
SKRINJE IN HLADILNIKI

CELIČNE KULTURE, GELI IN MOLEKULARNA BIOLOGIJA

BIOHIT

minerva biolabs

LI-COR Biosciences



ELEKTRONSKE IN MEHANSKE AVTOMATSKE PIPETE

DIAGNOSTIKA MIKOPLAZEM IN LEGIONEL

SEKVENATORJI

3rd Conference on Experimental and Translational Oncology

Kranjska gora, Slovenia, March, 18-21, 2004

Organised by:
Tamara Lah, Gregor Serša and Janko Kos

Topics:

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

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

Correspondence:
Conference Secretary:
Phone: +386 1 423 1867
Fax: +386 1 423 5038
Email: milena.kisovec@nib.si
http://www.onko-i.si/radiolog/rno.html

Organised under patronage
of Association of Radiology and Oncology

ESTRO

European Society for Therapeutic Radiology and Oncology

The GEC-ESTRO Teaching course on

Modern Brachytherapy Techniques

Bled, Slovenia 21-25 March, 2004

Grand Hotel Toplice

Course Director:	E. Van Limbergen
Vice Director:	J.J. Mazeron
Teaching Staff:	C. Haie-Meder, J.J. Mazeron, R. Pötter, E. Van Limbergen, J. Venselaar
Guest Lecturer:	A.Gerbaulet
Local Organiser:	H. Lesnicar, Matjas Zwitter, Primos Strojan, Janez Burger.
Course Co-ordinator:	Roselinne Nisin

ESTRO Office, Ave. E. Mounierlaan 83, 1200 Brussels, Belgium
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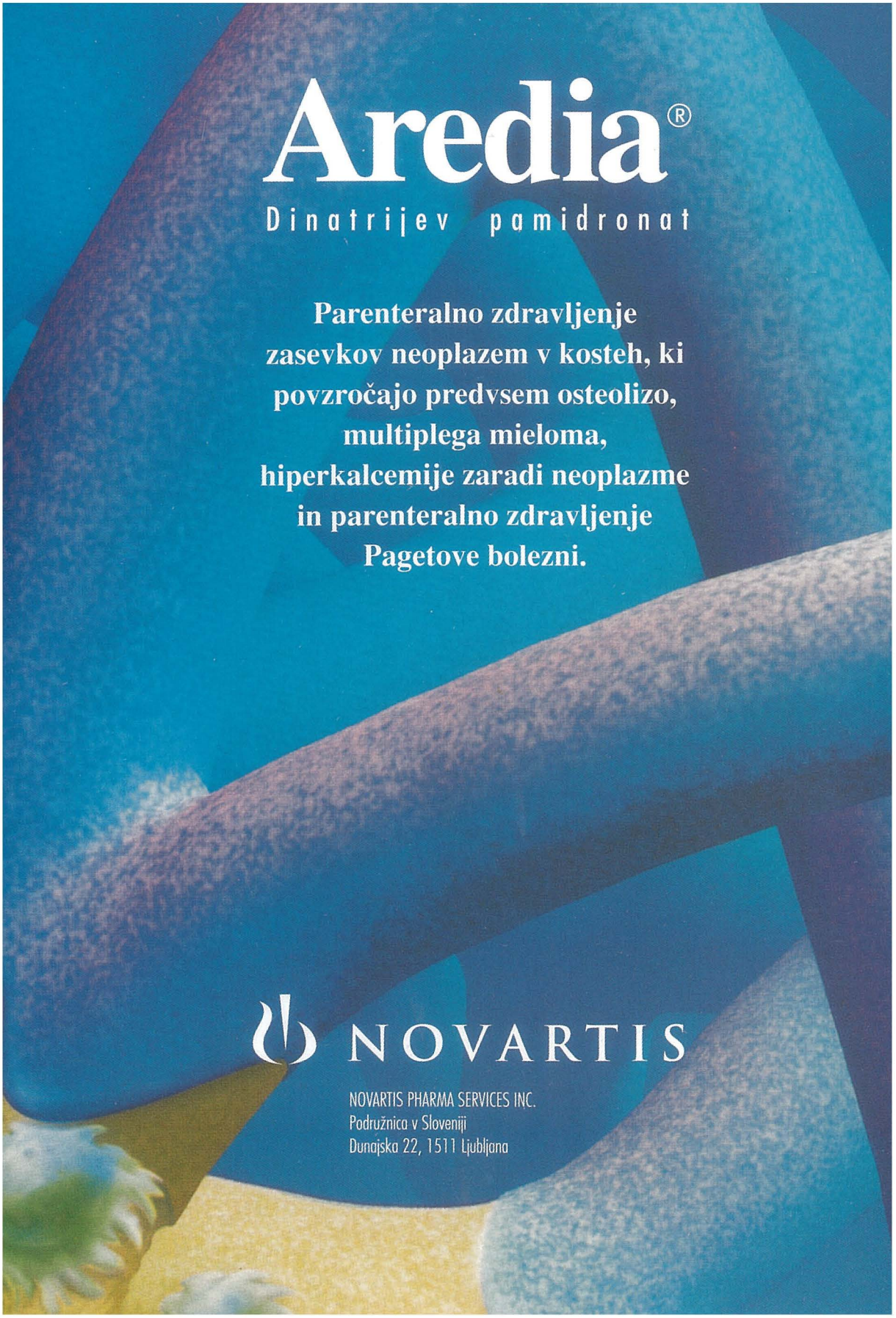
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