

DOCTOR OF PHILOSOPHY, DISSERTATION THESIS

**NEW METHODS IN THE DIAGNOSIS AND THERAPY OF NPC (IN SITU
HYBRIDIZATION, POSITRON EMISSION TOMOGRAPHY AND RADIOBIOLOGICAL
INVESTIGATIONS)**

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PROGRAMME
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Director of Studies: Prof. Dr. Olga Ésik

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I. INTRODUCTION

Nasopharyngeal cancer (NPC) is not considered as a frequent disease in Hungary. 109 cases were reported in 2002 for the National Cancer Registry and 114 in 2003.

Beyond the Epstein-Barr virus (EBV) infection and genetic predisposition (certain HLA antigens), special environmental conditions (like smoking, alcohol consumption, chemicals, nutrition habits: smoked and dried foods) and chronic otorhinolaryngological diseases play important role in the development of NPC.

The disease prognosis and the selection of therapy are mainly effected by the volume of the disease before treatment (stadium, „stage”). Therefore early „staging” examinations are very important and accurately determine the anatomical extent of the disease before starting oncotherapy.

Introduction of positron emission tomography (PET) constituted a huge progress comparing to the traditional staging examinations. The method allows non-invasive observation of the biochemical and physiological processes in the living organism, offers the study of metabolic processes in the organs and tissues, and the differentiation of physiological and pathological states.

The biological and oncological characteristics of NPC confirm that both irradiation and chemotherapy contribute to the primary treatment. The average, 5-year tumour specific survival probability of the disease, including stage I-IV, is 40%.

The increasing frequency of tumours and the occurrence of more and more effective treatments resulted that more and more patients with tumorous disease may live longer. Patient survival is effected by the adequate treatment; their quality of living is influenced by the consequences of tumorous process and complications developed as an effect of the applied treatment. Serious early and late complications might occur in some of the patients treated by irradiation. The effects and possible avoidance of potential radiation injuries caused by the expanding irradiation indications are more and more highlighted.

In recent years new procedures aiming the modern, up-to-date treatment of the NPC patients were introduced in J6sa Andr6s County Hospital of Ny6regyh6za. These cover histological diagnostics (EBV detection in the sections), more accurate imaging diagnostics (PET) as well as radiobiological examinations required for planning the radiation therapy of the disease in a better way (study of radiation sensibility examination of normal tissue).

II. PURPOSE

1. To study how the histological identification of the EBV infection, which has a high importance in the development of nasopharyngeal cancer, contributes to the correct diagnosis;
2. Data were collected to determine the correct indications of PET examinations;
3. It became actual to comprehensively study that what the diagnostic benefits of the so-called „section-imaging” techniques (CT, MRI, PET) are in NPC;
4. To search for the potential prevention by radiobiological investigation of normal tissues of NPC patients suffering from late radiological injury.

III. PATIENT GROUP AND METHODS

1. Patient group

We have been treating NPC patients in the Department of Oncoradiology of Jósa András County Hospital (Nyíregyháza) since 1992. From January 1996 till May 2005, 49 patients with NPC diagnosis were registered in the department. The male/female ratio was 39:10, and the average age was 47 years (ranging from 19 to 76 years). The distribution of the histological forms was the following: undiff. cc. (type WHO III.): 33 patients, nonkeratinizing cc. (type WHO II.): 11 patients, squamous cell cc. (type WHO I.): 5 patients. The distribution according to TNM stage was: stage I.: 2 patients, stage II.: 2 patients, stage III.: 12 patients, stage IV.: 33 patients.

Description of the patient groups investigated with regard to the purpose of the dissertation

1. 1. Patients with familiar clustering of nasopharyngeal tumour

NPC was observed only in one case in a family (mother [cT2acN0cM0, stage IIA.] and son [cT2acN2cM0, stage III.]). The etiology, the clinical evolution, the treatment methods and the outcome of the disease were investigated in these cases.

1. 2. Patients examined by PET

PET examination after several unsuccessful trial excisions

In four cases (1 male: 3 females, average age: 46 years) the endoscopic examination of the nasopharynx suspected the malignant process which was accompanied by lymphadenomegaly. Conventional diagnostic methods failed to confirm this suspicion. Due to several unsuccessful trial excisions and negative cytological result of aspirational sampling, PET examination was indicated.

PET examination for staging

Since 1999 the section imaging diagnostic techniques were complemented by a whole body (WB) FDG-PET examination in the cases of 11 patients (9 males: 2 females, average age: 48

years). In case of the other patients, WB-PET examination was not completed due to their bad general condition, high age, and the extension of the process.

The dissertation presents the oncotherapeutical plan in details following throughout examination, during which depending on the extension of the process, the age or the general condition of the patient usually irradiation and platinum based chemotherapy were applied. The external radiotherapeutical technique of the nasopharynx and the exposed irradiation dose are presented. With regard to the dosing, patients were exposed to a 66 Gy (60-72 Gy) median dose of the external irradiation.

1. 3. Lhermitte's sign after irradiation

In order to investigate radiogenic injuries, FDG-, butanol-, and metionin-PET examinations of two patients with chronic radiogenic Lhermitte's sign (LS) were accomplished 26 and 7 years after irradiation. The patients were exposed to an external irradiation of 60 Gy (NPC) and of 41.8 Gy (nasopharyngeal Hodgkin-disease) sum dose, respectively.

1. 4. Control patients for PET and for investigations of radiosensitivity

Patient with hypopharyngeal tumour who received standard irradiation were examined by PET as controls for the PET examinations of the patients showing LS.

Fibroblast cultures obtained from skins of 6 healthy children by biopsy were used to determine the individual radiosensitivity. The radiosensitivity of fibroblasts of LS patients were compared to these values.

2. Methods

2. 1. Histological examinations

The histological samples, which were suitable for routine histological examination after formalin fixation, were used to prepare haematoxylin-eosin (HE) painted preparations.

The remained samples were used for immunohistochemical examination. Monoclonal antibody cocktail CS1-4, produced by DakoCytomation was used to study immunoreaction against EBV-LMP1 (L. Krenács, É. Stelkovic; Laboratory of Tumour Pathology and Molecular Diagnostics, Institute of Biotechnology, Szeged; 2004). To detect early RNAs of EBV, desoxynucleotid-oligo-probes (Novocastra) marked with FITC, monoclonal anti-FITC antibody produced in mice and anti-mouse Envision⁺HRP visualisation system were applied.

2. 2. Imaging

Radiological investigations. US examinations were accomplished to determine the condition of regional cervical lymph node groups, and the vascularisations were analyzed in Color-Doppler

operation. An instrument equipped with high-frequency transducer (ACUSON XP) was accessible for this purpose.

Native and contrast agent aided spiral CT (SOMATOM BALANCE, SIEMENS) images were recorded of the neck, and the whole chest (expanded to the lower surface of the liver) with 5mm section-width of those from the cranial base to the apexes of the lungs, and with 10 mm that of the remained parts.

T1-, T2-, and proton-weighted, transversal and coronal MRI images were taken by a SIEMENS MAGNETOM HARMONY camera (1.0 Tesla). T1-weighted measurements were repeated after administration of contrast agents (CA).

MRI examinations indicated pathological lymph node structure (e.g. central necrosis) and the tumorous infiltration of the soft tissue. During the differentiation of pathological lesions being close to bone parts, CT measurement was found to be more suitable.

To investigate distant metastases chest X-ray, abdominal US examination and bone scintigraphy were applied.

PET examinations. GE 4096 PLUS whole body camera (General Electric, Uppsala, Sweden) was applied for PET investigations. In static operation mode the instrument takes 15 simultaneous section shots with 103 mm axial vision field. The transaxial resolution is 5 mm, and in the axial direction 6 mm.

For this study positron emitting biologically active molecules (radiopharmacoons) produced in a cyclotron, marked by radioactive isotope were injected into the body, the main principal of their selection was to mark those biochemical pathways which are connected or may be connected to the relevant questions of clinical oncotherapy. After ingesting the radiopharmacoons intravenously or through inhalation, the typical equilibrium distribution often reflecting pathognomical or diagnostic meaning was developed within a short period.

Most of the oncological PET examinations were accomplished by using ^{18}F -FDG, since intracellular concentration of FDG is in connection with the sugar-consumption of the tissue.

4 hours before the WB examination, the patients were not allowed to consume any carbohydrate in order the reception of FDG due to competition with the physiologically available glucose does not decrease. 48 hours before the examination, the patients had to avoid hard physical load and consume abundant liquid hours before the examination.

In additional to the detection (^{18}F -FDG) of carbohydrate metabolism processes which have fundamental importance in neoplastic processes, aminoacid transportation was studied with ^{11}C -methionin. Circulatory changes in the central nervous system (tissue perfusion) were studied by

^{15}O -butanol tracer. The dose of ^{18}F -FDG was 5.55 MBq/kg bodyweight, that of ^{11}C -metionin was 9.25 MBq/kg bodyweight while the dose of ^{15}O -butanol was 2 GBq/scan.

The examination was started at the cranial base and measurements were completed at 0, 20 and 40 minutes after tracer ingestion. For the visualisation of ^{18}F -FDG and ^{11}C -metionin distribution data collection for 10 minutes, while for ^{15}O -butanol for 3 minutes were used in hospital patients.

PET/CT/MRI examinations were completed by using head fixation applied in radiotherapy (ORFIT). Anatomical points which are easily identifiable on the surface (e.g. ridge of the nose, tragus, lateral canthus, genial tubercle) were marked by markers (capsulated iodine contrast agent, gadolinium or FDG) according to the modality of the imaging technique. Later image fusions were completed by superposing these anatomically determined (“equivalent”) points.

Evaluation of the imaging examinations

The size of the primary tumour was determined by section imaging techniques (CT/MRI/PET). Each change was regarded as abnormal by CT/MRI/PET examinations, if the ingested, specific material (“tracer”) concentrated or if the smallest diameter of one or more lymph nodes was larger than 1 cm.

PET, CT and MRI examinations were performed on cervical central, right or left, bilateral supraclavicular, the axillary-, mediastinal-, hilar- and abdominal lymphatic regions following the lymphatic spreading route. Distant metastases were investigated by conventional imaging techniques and PET examination.

The primary stage was determined after the review of patient’s documents. If it was necessary, this was modified (TNM PET, or without PET) according to the WB PET results.

PET examinations were executed at least 90 days after the completion of the first treatment. The results of the examinations were checked during the follow-up of the disease process, this was at least 12 months in our study.

US examinations were performed on cervical central-, right, and left lateral lymph nodes and on bilateral supraclavicular lymphatic regions. By using Color-Doppler US examination the vascularization of the lymph nodes over 1 cm was analysable. Early stage recognition of the tumour in the lymph nodes, is highly facilitated by the fact that due to the augmented cell proliferation (like tumour-growth) the local nutritive and oxygen demands increase which is ensured by neovascularisation.

2. 3. Radiobiological investigations

Determination (Grade 1-4) of the radiogenic injury volume was performed based on the „Common Toxicity Criteria (CTC) Version 2.0”.

Individual radiosensitivity was determined by using dermal fibroblast cultures, since fibroblast activity (fibrosis, scar etc.) has fundamental impact on the development of the most frequent late side effects of irradiation. Clonogen assay was used to measure the radiosensitivity of the fibroblasts.

Dermal biopsy samples were collected from the studied patients and primer fibroblast cultures were actuated. The fibroblast cultures in the logarithmical growth phase were irradiated by ^{60}Co γ radiation. Depending on the dose, different numbers of cells were injected on a 100mm diameter Petri-dish and the number of developed colonies was determined. The radiosensitivity of fibroblasts of our patients was compared to those of healthy people.

IV. RESULTS

1. EBV detection in histological sections

The presence of EBV was successfully proven by immunohistochemical methods within one family after 14 years, from histological sections derived from NPC showed occurrence both in mother and son.

2. Expansion of traditional diagnostics with functional section imaging procedures

PET examinations after several unsuccessful trial excisions

Occult primary tumour was investigated in 4 patients as the reason of hyperplastic nasopharyngeal tissue and cervical lymph nodes. Clinical aspect suggested NPC in every case, after repeatedly negative (no detected tumour) cervical aspiration cytological examination and unsuccessful test excision, PET examination was executed for the accurate diagnosis. Following the PET examination with negative results, further follow-up of patients verified the non-neoplastic lesions. The roots of the cervical lymphadenomegaly were postoperative complaints following neck dissection, toxoplasmosis, haemangioma and inflammatory processes.

PET examinations for staging

The size of the primary tumour was determined by CT (transaxially) and MRI in case of the patients who were examined by PET. The location, number and size of the lymph nodes over 1cm were also studied by US, CT, MRI and PET. The question was reasonably arisen from the above that whether the results of the PET examination modify the determination of the range of the tumorous process (stage). In other words: from knowledge of PET results, whether the original stage category (TNM) revision is necessary, and if yes, whether the revision affects the therapeutical plan.

Histology of the samples of the patients examined by PET verified gradus WHO II. and III. except one patient (WHO I.). Among the 11 patients examined by PET for staging/restaging mediastinal metastases were identified in three patients. Bone metastases were explored in one patient. Bone metastases were also provable by other imaging procedures, however mediastinal metastases were only recognizable by PET techniques. The range of mediastinal lymph nodes are typically high, accompanied by lymph node metastases occurring in both sides of the neck. Oncotherapeutical plan was basically redefined by the identified tumour in the distant, not regional lymphatic regions (primary in mediastinum), and haematogenous dissemination (rTxNxM1). TNM classification became more serious in 5 cases (45%) and less serious in one case (9%) among the patients examined by PET. Accordingly the treatments were revised in 5 cases (45%).

In case of our two patients who obtained oncological treatment earlier, the local relapse causing the complaints and symptoms was explored only by PET. This was also proved by histological processing, thus the repeated radiation therapy was applied based on this result.

Importance of image fusion during 3D treatment planning

Radiation target volume was exactly determinable by using fused CT/PET images. Image fusion also provided assistance during determining the exact number, location and size of the lymph nodes which were detectable in the section images, therefore it was significantly beneficial during the stage determination as well as follow-up the efficiency of the therapy.

Role of PET in the measurement of oncotherapeutical efficiency

To measure the therapeutic effects, PET was applied in 9 patients, which was executed mainly after combined treatments (irradiation and cytostatic). The follow-up of the tumorous process was also assurable by the method. This was executed 17 times (after chemotherapy, before irradiation; and after irradiation) 2-36 months after the first PET examination in average.

In case of one patient, the examination was completed only during staging, the repeat was not allowed due to the far gone disease. Clinical progression became evident before the completion of the oncotherapy in two patients. The two patients died after the completion of the treatment. Mediastinal lymphatic metastases emerged in three patients during the staging. Relapse occurred within 1 year in one patient; and within 2 years in the other. No tumour occurred in three patients during the 26 follow-up months (all three patients' histological diagnoses were NPC). The patients' follow-up proved the negative PET evidences. Treatments were completed two years ago in case of two patients, at the time of my dissertation both of them were tumour less which was supported by the PET examination as well.

3. Radiobiological investigations

PET examinations of patients suffering from Lhermitte's sign with radiogen origin and of control patients

Low FDG accumulation was presented in the control patient's spinal cord before the irradiation performed in 1998. 2 months after the irradiation increased FDG uptake was measured, which definitely decreased after the next 7 months. 44 months after the irradiation, FDG uptake on the radiated myelon section was close to the base value. ¹⁵O-butanol uptake changed analogously to the FDG accumulation. ¹¹C-metionin uptake was low on both treated and untreated myelon sections, which did not change during the clinical disease process. No metionin uptake was detectable in the medulla of the irradiated vertebral bodies (C2-Th3).

Increased FDG uptake was shown on the irradiated cervical spinal section of patients with radiogen LS disease 25, and 7 years after irradiation comparing to the untreated areas. PET examination by ¹⁵O-butanol indicated increased blood flow on the irradiated spinal section, no uptake with ¹¹C-metionin were detectable on spinal sections C1-C3. No signs of damage in spinal cord were demonstrable in LS patients by the MRI examination after the irradiation.

Investigations of radionsensitivity

This investigation of the patients with permanent radiogen injury (LS) proved increased radiosensitivity in fibroblasts of the patient 18. and normal sensitivity in fibroblasts of the patient 19.

V. DISCUSSION

1. Etiology of nasopharyngeal cancer. Importance of EBV infection

Several reasons are responsible for the development of NPC. EBV is one of the proven responsible factors for its development independently from the patients' ethnical origin. This finding is a new paradigm in cancer diagnostics and led to the occurrence of viral serological detection of cancers. The infection is verifiable by this method in the 90% of the patients. The presence of EBV genom is also provable in the tumour, it is symptomatic particularly for the patients who live in endemic regions. The detection of EBV-DNA is well applicable in the diagnostics and the follow-up of disease activity.

After reviewing the English literature it can be stated that the 2/3 of the patients are male in the familial clustering of NPC cases living in non-endemic areas, which is consistent with the male:female rate characterising the disease. The histology is WHO III. type, this is the histological type which mainly characterises the EBV.

1/3 of the patients were younger than 30 at the time of the diagnosis. One of the possible reasons is that in the closed communities, which are typical for the young, EBV spreads easily. We can suppose that the disease develops as a complication of the catarrh of upper respiratory tract. Patognomical serological results suggested the presence of EBV.

The presence of EBV was successfully detected in the histological sections of the studied two patients. In the familial clustering cases from non-endemic areas, this was first completed.

Thus it seems to be proven that EBV infection plays an important role in familial clustering of NPC diseases occurring in non-endemic areas. The clinical progression of the disease may be modified by genetical and environmental factors. This is also supported by the fact that in our cases the disease was clinically detectable at two different dates.

The different disease processes of our two patients seem to prove that despite the EBV infection was developed (probably) at the same time, the tumour was detected 1 year later in case 2. and the far gone stage meant unfavourable prognosis.

2. Role of PET in the examination and therapy of nasopharyngeal carcinoma

Primer diagnostics, demonstration of local relapses, staging/ restaging

Today PET plays more and more important role in the diagnosis of NPC together with CT and MRI. PET examinations are also confirmed, additionally to the identification of the viable tumorous tissue and the determination of the size, by the fact that other methods are less sensible for diagnosing the lymph nodes in the regions above the diaphragm (based on their size).

Comparing the diagnostic values of CT/MRI/PET in our own patient subjects, we found that the sizes of the primary tumours defined by CT and MRI were generally the same, average size was from 5 to 47 mm. In PET images the primary lesion was smaller in 1 case and larger in the other cases (7-50 mm in average). We concluded that the lesion between the sizes of primary tumours measured by PET and CT/MRI was 1-4 mm in average.

The benefit of PET examinations is the best proven by the fact that local relapse was identified only by this method in two cases.

No essential, significant lesion was realized in the sizes of the primary tumours in our data, when the determination was executed by either CT or MRI. However the primary lesion was mostly detected higher by PET, but the deviation was not typical comparing to the results of the two previous procedures. PET examination has a significantly higher efficiency in the detection of tumour relapse than the other section imaging procedures. Based on our experiences the joint application of both radiopharmacocons (^{18}F -FDG és ^{11}C -metionin) did not significantly improve the

accuracy of the procedure, however local relapse was successfully detected by ^{11}C -methionin despite the negative FDG-PET examination in one of our patients.

The recognition of occult primaries is a constant problem and typical for the tumours at the area of head-neck, which is important as their occurrence is very frequent in the nasopharyngeal area.

In our patient subjects the result of PET examinations, which explained the increased tissue in nasopharynx and the enlarged cervical lymph nodes, following the several unsuccessful trial excisions during the detection of occult primary tumours, was negative. The other examinations during the follow-up of the patients closed out the malignant tumours and verified aspecific lesions (consequence of neck dissection, toxoplasmosis, haemangioma, infection). Based on our experiences the PET examinations were effective in the differential diagnosis of nasopharyngeal lesions. It is recommended, in case of suspicious clinical symptoms and when the results of other imaging methods are negative, to reasonably check the results by further, repeated and targeted examinations.

In order to identify the exact extension of NPC („staging/restaging”) it is important to study the spread routes.

Lymphogenous spread

Lymphogenous spread is shown the earliest in cervical lymph vessels and lymph node penetration.

We compared in our material that how many of cervical lymph nodes over 1 cm are recognizable by different examination procedures. We found that except 2 cases the numbers of lymph nodes are the same as shown by US, CT and MRI (0-4 pcs in average). The numbers of lymph nodes, which are recognizable by PET, are higher in every case than the previous (0-10 pcs in average). Analogous to the data in the literature, in our NPC patient material, FDG-PET examination is admitted as the best method for the accurate detection of regional lymph node metastasis and accordingly as essential factor in correct stage identification.

Distant metastases

Distant metastases usually develop by haematogenous spread. NPC causes such metastasis significantly more frequently than other head-neck tumours with different origin.

Mediastinal lymph node metastases were observable in our material in the lymph node states N2-3. The mediastinal lymph nodes concentrating PET tracer were detectable neither by CT nor MRI, mediastinal lymph node metastases were recognizable only by PET examination in 3 of our patients. We can state that FDG-PET examination is the most optimal method for determining the number and size of lymph nodes above the diaphragm. The execution of WB FDG-PET

examinations is also supported by the fact that developed lymphatic invasion is often accompanied by haematogenous NPC dissemination. One single PET examination allows the simultaneous identification of both types of metastasis (lymphogenous and haematogenous). Both mediastinal and bone metastases were simultaneously detected in one of our patients (histologically NPC type WHO I.). Among the patients who were examined by PET, the stage class of 5 patients (45%) was corrected to more serious and to less serious in 1 case (9%) and the treatment plan of the 5 patients needed to be revised accordingly.

Image fusion for 3D treatment planning

The treatment of NPC can be either single external irradiation or combined with chemotherapy. The most important diagnostical problem of head-neck area is the lack of anatomical details, which makes more difficult the evaluation of section images. For the equilibration CT/MRI/PET image fusion is applicable.

In our NPC subjects we completed CT/PET image fusion. In accordance with the literature the examinations were executed in masks marked by accurate markers corresponding to the characteristics of the method and applied registration and image fusion. In section images the abnormally high activity was detectable, which is typical for tumorous tissue and metastasis. Thus the contour of viable tumour tissue was accurately confinable and its effects to surrounding anatomical structure were demonstrable. At the same time the exact location, size and number of lymph nodes, which are detectable in section images, became determinable. In our subjects mediastinal metastases were diagnosed in 3 cases among the 11 patients examined by PET for staging/restaging, which were not detectable by other imaging procedures. The accurate anatomical localization of these mediastinal metastases was confirmed by the fusion of CT/PET images. Considering the data in the literature, the fusion of CT/PET images was essential during confining the viable tumour tissue and determining the expansion of the process (cervical lymph nodes, mediastinal spread) and following up the efficiency of the therapy.

By introducing and improving the 3D conformal treatment planning we would like to realize the following: (i) homogenous irradiation of the target volume; (ii) reduction of toxicity of normal tissues, (iii) prevention of late radiation injury deriving from the previous. Based on the careful analyses of the results of the section imaging procedures (CT/MRI/PET), and by applying accurate planning system and multileaf collimator (**also** in NPC) we can achieve better dose distribution and radiation load reduction with good efficiency.

Measuring therapeutical effect

PET examination was also found to be essential to measure the reaction for the complete therapy in NPC.

In our study, when establishing the oncotherapy, PET results were used in all cases and the therapeutical plan was revised in several cases based on these results. The original therapeutical conception was fundamentally changed when metastasis was found in the distant, non-regional lymphatic region (mainly in the mediastinum). The mediastinal metastases belong clearly to the “distant metastases” group. Evidently, local radiotherapy is insufficient to treat proven distant metastases; their identification supports the application of systemic chemotherapy. Confirmed haematogenous dissemination (e.g. bone metastases) also necessitates the application of irradiation combined with chemotherapy. After the diagnosis of mediastinal metastases and during the combined oncotherapeutical treatment, the mediastinal areas, where PET examination showed abnormal, high activity, also had to be irradiated.

We concluded that the growth rate of abnormal lesions (“clinical progression”) can be estimated by the visualization of the metabolic activity of the tumour tissue. The fast growing tumours of our NPC patients increasingly accumulated the glucose analogue (FDG), while tissues with low malignity had minor “glucose”-intake. This allowed to estimate the rate of malignancy (grade) or to make a careful prognostic “prediction”.

By using PET, the efficiency of the therapy was reliably analysable. This was allowed by the fact that if the area, characterized by increased FDG accumulation, is growing, the tumour clinically progrediates, while the decrease of this area indicates clinical regression. PET examination accomplished during the oncotherapeutical supports the evaluation of the therapeutic efficiency and the therapeutical reaction, since lower metabolic activity occurring before the decrease of the tumorous area reflects positive therapeutical reaction.

The effect of the therapy and the recurrence of the tumour can be isolated with high security by using PET examination. It is important and I want to emphasize that the selection of the date of the control PET examination allows avoiding the false positive diagnoses. In 90 days after the therapy there is no point to accomplish control PET examination.

PET is also an effective tool in the evaluation of the relapses of the NPC patients who were previously treated by oncotherapy. Residual (and/or relapse) tumours can be diagnosed efficiently by FDG-PET. This technique is more sensitive than other imaging techniques thus indicates the relapse earlier.

The evaluation of the conditions which developed after the previously applied treatment is even more complicated if operation scars or deformities, residual denotations of irradiation and consecutive inflammatory processes restrain the evaluation of the loco-regional status. Complaints following the therapy are not characteristic, non-pathognomical considering the existence of residue or the development of relapse.

Regarding the isolation diagnosis it is important to note that in our patients the areas with increased metabolic activity, detected by PET examination, showed increased radiopharmakon accumulation in comparison with their environment. This increased FDG accumulation can be indicative of oncological disease however it is not a characteristic, pathognomic sign by itself. All defined FDG accumulations are pathological, which are not linkable to any physiological FDG accumulation of an organ and is at least two times larger than the surrounding organs. Thus scars of earlier operation and/or radiotherapy and chronic inflamed tissues also show increased tracer accumulation! With appropriate experience these signs can be separated from those of tumour relapse mainly based on their intensity. These observations were also successfully confirmed by other analytical methods.

In order to measure the effect of the therapy and follow the process, 17 PET examinations were completed in 9 patients in our study. In the average 2-36 months follow-up time 7 patients live without while 2 live with the tumour. 2 patients died of the tumour.

Summarized, PET examination appropriately sensitive and characteristic tool in primary diagnosis of NPC, analogously in the diagnosis of lymphogenous spread and the detection of distant metastases, the occult primaries, evaluation of residues and relapses or in the measurement of the therapeutic efficiency. PET application is also recommended when CT/MRI imaging is unsuccessful or ambiguous.

The achievable results of the oncological therapy can be further improved if before its application PET examinations also applied to determine the size of tumour tissue and to evaluate the extension of the metastases, since with the aid of the examination one can more effectively decide how to combine different therapies appropriately.

The determination of tumorous tissues with active metabolism by PET examination supports the evaluation of the tumorous processes extension, staging/restaging, the determination of the places of biopsy, in planning the operation, determining the objective volume of the irradiation (macroscopic tumour), and in the identification/determination of residual lesions and relapses.

The image fusion supports effectively 3D treatment planning by more accurate determination of the extension of the viable tumour tissue, thus more effective tumour destruction and at the same time reduced side effects are achievable.

In NPC the identification of the primary tumour and the occult lesions, the more accurate stage determination, the measurement of the reaction to the complex oncotherapy, early stage identification of the residues and relapses allow more rational treatment strategy, which will prospectively improve the survival results.

3. Radiobiological investigations

The role of PET examination in the study of radiogenic side effects

Two radiogenic myelopathic patients showing clinical advance during the disease process and one receiving standard radiotherapy with no radiogenic damage have been followed in time.

A comprehensive literature review proved that previous to our study no publication was available on PET results of irreversible deformations effected by irradiation of the spinal cord. Our PET records of the 3 patients registered after irradiation showed similar (almost identical) image: FDG accumulation and ^{15}O -butanol perfusion significantly increased in the irradiated spinal cord (compared to spinal cord with no irradiation), while the ^{11}C -metionin accumulation of the affected region was unimportant. In the patients received standard irradiation, the symptoms normalized in the 4th follow-up year, while were detectable still in the 25th and 7th years after the irradiation in the case of patients with Grade 3 and Grade 2 radiation damages.

Our studies are in accordance with the earlier data, which show that similarly to the brain, the blood perfusion and the metabolism of the spinal cord are coupled (.,BF-LCGU coupling). In our patients the increased FDG accumulation, reflecting higher energy-demand, (and the parallely changing, increased spinal cord perfusion) was connected to regeneration. This was detectable long in the patients showing chronic radiogenic damage, while the increased FDG accumulation was found to be temporary in the asymptomatic patients, who had received irradiation in a standard dose.

In order to interpret the PET results, we supposed that the increased metabolism and perfusion were caused by the increased energy demand of the action potential conduct. This may be connected to the fact that in demyelinated axons (opposite to normal saltatoric impulse conduction through Ranvier-nots) the transmission of action potential is ensured by Na-channels expressing with higher density than normal, the operation of which requires higher energy to operate.

Investigations of radiosensitivity

Clinical estimation of the stage of normal tissues is essentially required before the second irradiation treatment. If the examination of intact tissues shows normal results, no further action is required. However, if increased personal radiosensitivity is confirmed, the decrease of the dose exposing normal tissues can be indicated or might necessitate the use of other therapies.

In LS patients in our study, the symptoms caused by irradiation persisted for years instead of disappearing in cca. 6 months in accordance with earlier findings. By completing the radiosensitivity investigation of fibroblast cultures, increased radiosensitivity was found in one case. It is assumable that this had a role in the development of the radiogenic damage in the spinal cord.

VI. SUMMARY

In the present study we have examined the factors, which contribute to the development of nasopharyngeal cancer, concluded that the EBV infection plays a central role in the development of familial clustering of NPC diseases in non-endemic areas, which is also modified by genetical and environmental factors.

We have studied the role of PET examinations in our NPC patients with hyperplastic nasopharyngeal tissue therefore they were highlighted during the examination in diagnosis and also research of occult primary tumours.

In case of NPC we have studied its importance in primary staging/restaging, 3D treatment planning, measurement of reaction to the complete oncotherapy, patient follow-up, identification of residual and relapsing tumours and during studying the side effects of irradiation.

We concluded that the size and number of tumorous focuses identified by PET method are higher than those identified by other conventional imaging methods, however no significant lesion was experienced in the determination of primary tumour size comparing to the section imaging procedures.

Conversely FDG-PET examinations were admitted to be significant in investigation of metastases in regional lymphatic regions and distant, non regional lymphatic areas. This lesion was outstanding in cervical, supraclavicular and mediastinal lymphatic regions. The determination of number and size of the lymph nodes above the diaphragm is the most optimal by PET examinations. By analysing the lymphatic region concern we pointed out that lymphogenous metastasis development follows a defined route, which is to be considered in diagnostics, examination and therapeutical planning.

Image fusion supported the definition of viable tumour tissue contour, the determination of exact number, location and size of lymph nodes detectable in section images, therefore had an important role in both stage determination and measurement of therapeutic efficiency. The fusion method also confirmed the accurate anatomical localization of mediastinal metastases.

During the establishment of oncotherapeutical plan the results of PET examinations were always considered. In our material the local irradiation of proven distant metastasis was not sufficient; chemotherapy was also required.

The efficiency of the therapy was reliably assessable by applying PET, as based on the increase of the area showing increased FDG image progression, while based on its reduction regression was deducible.

PET was also an efficient tool in the decision of relapses in NPC patients treated previously with oncotherapy, as residual tumour was identifiable and relapse was earlier indicated comparing to other imaging methods.

We proved by PET examination in our LS patients that similarly to the brain, metabolism and perfusion progress paralelly also in spinal cord. Increased metabolism and perfusion were pointed out by PET examination, which was found to be in connection with the increased energy demand of spinal cord action potential conduct.

By having completed the radiation sensibility examination of fibroblast cultures in LS patients, we confirmed that it might have a role in the development of radiogenic injury in spinal cord.

LIST OF PUBLICATIONS

1. LECTURED PUBLICATIONS IN THE SUBJECT OF THE DISSERTATION

Olajos J, Erfán J, Lengyel Zs, Emri M, Füle E, Erdélyi L, Lengyel E, Ésik O, Trón L. Epipharynx-daganatok PET-vizsgálata. Orvosi Hetilap 2002; 143 (Suppl 3): 1275-1278.

Olajos J, Erfán J, Lengyel Zs, Emri M, Füle E, Erdélyi L, Lengyel E, Ésik O, Trón L. Újabb lehetőségek a rosszindulatú orrgarati daganatok kivizsgálásában és terápiájában. Fül-Orr-Gégegyógyászat 2002; 48: 12-17.

Lengyel E , Baricza K, Somogyi A, Olajos J, Pápai Z, Gödény M, Németh G, Ésik O. Az epipharynxrák lokális kiújulásának ismételt sugárkezelése. Orvosi Hetilap 2002; 143: 2343-2350.

Ésik O, Csere T, Stefanits K, Szakáll Sz jr., Lengyel Z, Sáfrány G, Vönöczky K, Lengyel E, Olajos J, Bajzik G, Trón L. Increased metabolic activity in the spinal cord of patients with long-standing Lhermitte's sign. Strahlenther Onkol 2003; 179: 690-693.

IF: 3. 121

Lengyel E, Baricza K, Somogyi A, Olajos J, Pápai Z, Gödény M, Németh G, Ésik O. Reirradiation of locally recurrent nasopharyngeal carcinoma. Strahlenther Onkol 2003; 179: 298-305.

IF: 3. 121

Olajos J, Füle E, Erfán J, Krenács L, Stelkovic É, Francz M, Lengyel E, Al-Farhat Y, Ésik O. Familial clustering of nasopharyngeal carcinoma in a non-endemic geographical region. Report of two Hungarian cases and a review of the literature. Acta Oto-Laryngologica 2005; 125: 1008-1013.

IF: 0. 870

2. Issued lectures / abstracts in the subject of the dissertation

Olajos J., Erfán J, Lengyel Z, Trón L, Csejtej A, Ésik O. Whole-body PET examinations in patients with multiple cancers. 17th Annual ESTRO Meeting, Edinburg, 1998. szept. 20-24. Radiother Oncol 1998; 48: (Suppl 1) S165.

Olajos J., Erfán J, Lengyel Z, Emri M, Major T, Sziklai I, Trón L, Ésik O. Combined therapy of patients with advanced tumors of epipharynx. Magyar Sugárterápiás Társaság 4. Kongresszusa, Szombathely, 1999. máj. 15-16.

Olajos J., Erfán J, Füle E, Erdélyi L, Trón L, Ésik O. Az epipharynx daganatok korszerű kivizsgálása és kezelése. Magyar Fül-Orr-Gégeorvosok Egyesületének Tudományos Ülése, Budapest, 1999. máj. 28.

Olajos J., Erfán J, Füle E, Erdélyi L, Trón L, Ésik O. Epipharynx-daganatok PET-vizsgálata. „Öt éves a Magyar PET Program”, Debrecen, 1999. szept. 22.

Olajos J., Erfán J, Lengyel Z, Emri M, Füle E, Erdélyi L, Trón L, Ésik O. PET-Investigation of epipharynx tumors (Epipharynx daganatok PET-vizsgálata). Magyar Fül-Orr-Gégeorvosok Egyesületének Jubileumi 36. Nemzeti Kongresszusa, Hévíz, 2000. okt. 24-28.

Olajos J. Epipharynx daganatok PET-vizsgálata. Egészségügyi Szakdolgozók 4. Tudományos Napja, Nyíregyháza, 2000. nov. 15.

Olajos J., Erfán J, Lengyel Zs, Emri M, Füle E, Erdélyi L, Ésik O, Trón L. Epipharynx daganatok PET-vizsgálata. Magyar Radiológusok Társasága Tiszántúli Szakcsoportjának Tudományos Ülése, Nyíregyháza, 2000. nov. 18. „Gammamed Users Meeting”, Szombathely, Claudius Hotel, 2001. ápr. 5-6.

Csere T, Stefanits K, Szakáll Sz jr, Lengyel E, Olajos J., Vönöczky K, Ésik O. Permanens radiogen myelopathia 26 éve sugárkezelt epipharynx-tumoros betegben. A Magyar Sugárterápiás Társaság 5. Kongresszusa, Nyíregyháza, 2001. jún.15-16.

Csere T, Sáfrány G, Stefanits K, Szakáll Sz jr, Lengyel E, Olajos J, Vönöczky K, Ésik O.

Permanens radiogen myelopathia epipharynx tumoros betegeknél. Magyar Onkológusok Társaságának 24. Kongresszusa, Budapest, 2001. nov. 22-24. Magyar Onkológia 2001; 45: 255.

Emri M, Trón L, Repa I, Bogner P, Berényi E, Olajos J, Lengyel Zs, Ésik O. Képfúziós és képregisztrációs módszerek alkalmazása a sugárterápiában. Magyar Sugárterápiás Társaság 5. Kongresszusa, Nyíregyháza, 2001. jún. 15-16.

Olajos J, Erfán J, Lengyel Zs, Emri M, Füle E, Erdélyi L, Lengyel E, Ésik O, Trón L.

Epipharynx daganatok korszerű kivizsgálása és terápiája. Magyar Sugárterápiás Társaság 5. Kongresszusa. Nyíregyháza, 2001. jún. 15-16. Magyar Fül-Orr-Gégeorvosok Egyesületének Északkelet-Magyarországi Regionális Tudományos Ülése, Nyíregyháza, 2001. okt. 5.

Emri M, Ésik O, Repa I, Bogner P, Berényi E, Olajos J, Lengyel Zs, Trón L. Képfúziós és képregisztrációs módszerek alkalmazása az onkológiában. Magyar Onkológusok Társaságának 24. Kongresszusa, Budapest, 2001. nov. 22-24. Magyar Onkológia 2001; 45: 259.

Lengyel E, Baricza K, Somogyi A, Olajos J, Pápai Z, Gódey M, Ésik O. Reirradiation of locally recurrent nasopharyngeal carcinoma. 21st Annual ESTRO Meeting, Prága, 2002. szept.17-21. Radiother Oncol 2002; 64: (Suppl 1) S 240.

IF: 2, 815

Acknowledgment

Olajos J, Erfán J, Lengyel Zs, Emri M, Füle E, Erdélyi L, Lengyel E, Ésik O, Trón L. Újabb lehetőségek a rosszindulatú orrgarati daganatok kivizsgálásában és terápiájában.

Jósa András Pályázat, Nyíregyháza, 2002. I. kategória 1. díj

Olajos J, Füle E, Erfán J, Krenács L, Stelkovic É, Francz M, Lengyel E, Al-Farhat Y, Ésik O. Familial clustering of nasopharyngeal carcinoma in a non-endemic geographical region. Report of two Hungarian cases and a review of the literature. Jósa András Pályázat, Nyíregyháza, 2005. I. kategória 1. díj Kórházunk 2006; 8: 6.

3. Publications in other subjects

Erfán J, Olajos J, Bellyei Sz, Farkas R, Liposits G, Ésik O. The state of Hungarian radiotherapy. Rep Pract Oncol Radiother 2005; 10 (4): 209-216.

Ésik O, Koiss R, Kneffel P, Al-Farhat Y, Bellyei Sz, Farkas R, Szigeti A, Strassz A, Olajos J, Erfán J. Műtét előtti, kizárólagos üregi sugárkezelés méhnyak- és méhtestrákos kórképekben: bizonyítékok és nemzetközi szakértői vélemények. Nőgyógyászati Onkológia 2005; 10: 168-172.

4. Lectures / abstracts in other subjects

Bráz K, Kóródi L, Erfán J, Jenei P, Fülöp I, Olajos J. Fül-orr-gégészeti daganatok brachyterápiás kezelése osztályunkon (poszter). Magyar Sugárterápiás Társaság Kongresszusa, Kaposvár, 2005. okt. 13-15. Magyar Onkológia 2005; 49 (3): 260.

Nagy V, Vargáné Elischer É, Kóródiné Karászi K, Erfán J, Olajos J. Fej-nyak tájéki daganatos betegek lokalizálása osztályunkon. Magyar Sugárterápiás Társaság Kongresszusa, Kaposvár, 2005. okt. 13-15. Magyar Onkológia 2005; 49 (3): 272.

Szakács M, Tóth M, Vargáné Elischer É, Kóródiné Karászi K, Erfán J, Olajos J. Emlődaganatok szimulációja osztályunkon (poszter). Magyar Sugárterápiás Társaság Kongresszusa, Kaposvár, 2005. okt. 13-15. Magyar Onkológia 2005; 49 (3): 275.

Fülöp I, Olajos J, Jenei P, Bráz K, Erfán J, Füle E. Fej-nyaksebészeti daganatok brachyterápiás kezelése kórházunkban. Magyar Fül-Orr-Gégeorvosok Egyesületének Északkelet-Magyarországi Regionális Tudományos Ülése, Debrecen, 2005. nov. 11.

Fülöp I, Olajos J, Jenei P, Lengyel Cs. Gy, Erfán J, Füle E. Fej-nyak daganatok magas aktivitású intersticiális sugárkezelésével szerzett tapasztalataink (poszter). A Magyar Fül-Orr-Gégeorvosok Egyesülete 39. Nemzeti Kongresszusa, Debrecen, 2006. szept. 6-9.