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CONTENTS

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Use of computed tomography as an aid to hepatic resections

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The clinical interest in hepatic tumor resection has increased the importance of knowing the segmental anatomy of the liver. Classical descriptions of the gross anatomic divisions of the liver were based solely on surface structures. These classicall divisions of hepatic anatomy are of little help to the surgeon because the surface anatomy poorly reflects the organ's internal vascular structure. A »surgical vascular subdivision« is a valuable technique for showing abnormalities of the liver whether diffuse or focal.
Cross-sectional imaging of the liver with CT provides excellent demonstration of the hepatic vasculature and of the lesions to be removed[1-3] allowing surgeons to plan resections according to anatomic principles.

Key words: liver neoplasms-surgery; tomography, x-ray computed

Hepatic anatomy

Traditionally, the liver was divided into four lobes right, left, caudate, and quadrate, based on the external configuration of grooves on the visceral surface of the liver. The falciform ligament divides the right lobe from the left lobe. Some authors generalized even further and used the falciform ligament to divide the right lobe from the left lobe. Thus, the »right lobe« includes the right, quadrate, and caudate lobes.

However, logic and surgical need dictate dividing the liver on the basis of the vascular anatomy and not external configuration. Two commonly used hepatic segmental nomenclatures based on the branching of the portal triad have been developed. The one usually used in the American and English literature has been outlined by Goldsmith and Woodburne.[4] Based on this functional system of hepatic division, known as »American system«, the liver is divided into the right, left, and caudate lobes. The right lobe of the liver is further divided into anterior and posterior segments and left lobe into medial (formerly known as the quadrate lobe) and the lateral segments (formerly known as the left lobe) Figure 1a). The caudate lobe occupies much of the posterosuperior surface of the liver and is bounded posteriorly by the fossa of the inferior vena cava and anteriorly by the fissure of the ligamentum venosum. It is considered a separate and distinct lobe, since it receives its vascular supply and biliary drainage from both the right and left lobes.

Couinaud developed another system of hepatic anatomical description, and the French surgeon Bismuth demonstrated the practical utility of this system (»Couinaud's system«).[4] According to Couinaud's description, the three main hepatic veins divide the liver into four sectors. He terms the planes through which the hepatic veins course the *portal scissurae*. The right, main and left portal scissurae define the four sectors, each of which receives a portal pedicle (Figure 1b). The main portal scissura divides the liver into right and left livers. The right portal scissura divides the right liver into anterior and posterior sectors. Each of this sectors contains two segments. The anterior sector has segment V inferiorly and segment VIII superiorly. The posterior sector contains segment VI inferiorly and segment VII superiorly. The left portal scissura divides the left liver into superior and posterior sectors. The umbilical fissure divides the anterior sector

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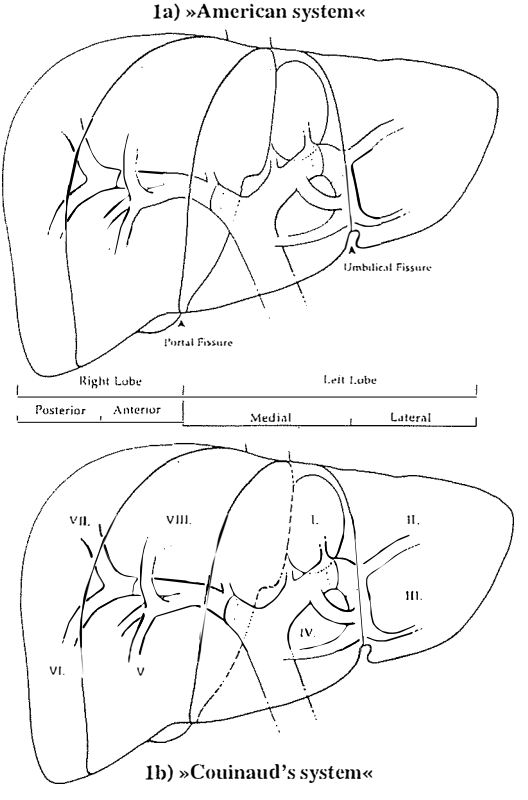

Figure 1. Segmental anatomy of the liver a) »American system«, b) »Couinaud's system«.

into two segments, medially segment IV and laterally segment III. The posterior sector has only one segment, segment II, thas forms the posterior part of left lobe. The caudate lobe comprises segment I, which is vascularized independently from portal division, receiving branches from both right and left portal vein and hepatic arteries. Its hepatic veins drain directly into the vena cava. The portal vein and hepatic arterial branches correspond to segmented anatomy. Likewise, the bile ducts provide segmental drainage.

Cross-sectional imaging

Cross-sectional imaging of the liver with computed tomography provides excellent demonstration of eight hepatic segments (according to Couinaud's system) on the basis of the vascular anatomy. They are defined principally on the position of the three major hepatic veins (right, middle and left), and are numbered spirally from the caudate lobe (segment I). From these boundaries multisegmental resections can be performed. A CT study of the liver entails imaging of the entire organ from its superior border at the dome of the diaphragm to its caudal tip. Contigious 10 mm thick slices are obtained, usually before and after intravenous injection of contrast medium. On CT the unenhanced normal liver is homogenous in density (50–70 HU) except for the portal veins, identifed as linear or circular structures of lower attenuation. The porta hepatis is visible as a fat containing horizontal cleft on the medial border of the right lobe of the liver with the quadrate lobe anteriorly and caudate lobe posteriorly.[5] Following intravenous contrast enhancement, attenuation values of liver parenchyma rise to 60–90 HU.[4]

The right and left lobes of the liver can be indicated by projecting a line from the gallbladder fossa to the inferior vena cava. The portal vein branches are largest at the level of the porta hepatis whereas the hepatic veins are larger in the more cephalad sections and are recognizable by their relationship to the normal inferior vena cava.[4] Normal calibre intrahepatic bile ducts are not demonstrable; only the major biliary radicles at the porta hepatis are identifiable as discrete structures. The common bile duct is situated anterolateral to the portal vein throuhgout its length.[5, 6]

The larger blood vessels can be seen in normodense, noncontrasted hepatic tissue as hypodense structures with a typical configuration. They may be invisible on CT scans when parenchymal density is even slightly reduced (eg. fatty infiltration) but they appear hyperdense as fatty infiltration increases.[7] Bolus administration of contrast medium ensures reliable demonstration of even small portal venous radicles which can be distinguished from parallel biliary ducts, particulary if the latter are dilated. The variable shape of the porta hepatis is extensively filled out by the vascular band of the portal vein, which is accompanied by the hepatic artery and by the common bile duct. This triad relationship is also seen in the further branches located in the liver periphery. The supply areas of tertiary rami correspond to the eight hepatic segments (according to Couinaud's system) which can be localized via computed tomography (Figures 2, 3 and 4). The three main branches of the hepatic veins drain below the diaphragm (stellate pattern) into the inferior vena cava. Therefore, left (C), mid-

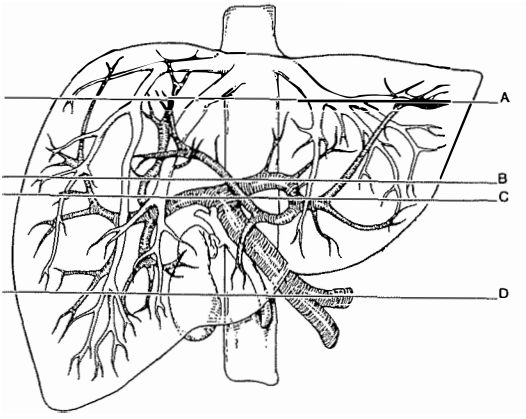

Figure 2. Lines A to D indicate the anatomic levels of images shown in Figs. 3. and 4.; A) on the level just below venous angle in the liver, B) directly above the bifurcation of the portal vein, C) on the same level as the bifurcation, D) below the bifurcation of the portal vein.

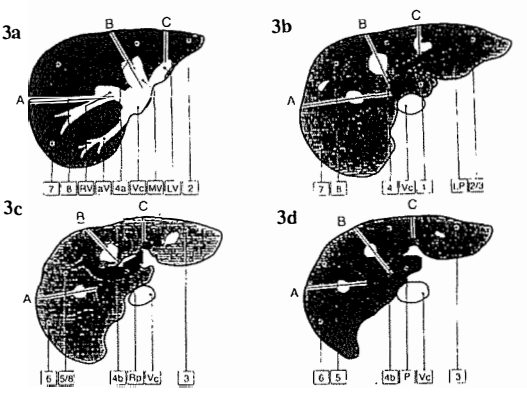

Figure 3. Segmental anatomy of the liver in transverse section. The intersegmental veins join into three cranial trunks, the right (a), middle (b), and left (c) main veins, which divide the liver into four sectors (modified according to references 7 and 8).

dle (B) and right (A) main hepatic veins can usually be demonstrated on one section. They divide the liver into four sectors. These sectors are subdivided horizontally by the plane in which the portal vein branches into fissure that cannot be seen on the surface of the liver and that contains a major hepatic vein (Figure 2). The right main hepatic vein divides the right lobe of the liver on the one side into anterior segments V and VIII, as well as into dorsally located posterior segments VI and VII. Segments VII and VIII form the dome of the liver. The caudate lobe comprises the first liver segment, which drains via smaller veins directly into inferior vena cava.[7, 8]

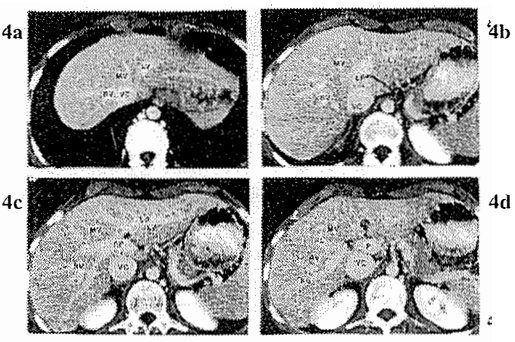

Figure 4. Hepatic segments in CT (same levels as Figure 3 and 4) (modified according to references 7 and 8).

1–8 = hepatic segments
LV = left main vein of the liver (plane C)
MV = middle main vein of the liver (plane B)
RV = right main vein of the liver (plane A)
P = portal vein
IVC = inferior vena cava
Rp = right main branch of the portal vein
Lp = left main branch of the portal vein

Resectability of the liver lesions

The indications for major liver surgery, particulary resection, continue to include predominantly focal parenchymal diseases. The decision of when and upon whom to perform major liver resection depends on the disease, the certainty of its diagnosis, the overall fitness of the patient to withstand major surgery, and, of course of the location and extent of the disease. The simpliest hepatic resection is wedge excision which consists of nonanatomic removal of a small amount of superficial hepatic tissue. The procedure is referred to as nonanatomic because the tissue removed does not correspond to a hepatic segment. Wedge excision can be performed successfully only if the vascular and biliary structures supplying the remaining hepatic parenchyma are left intact.[9] Thus, nonanatomic wedge excisions are performed only in the hepatic perifery, usually to remove small tumors.[10]

The more commonly performed hepatic resections are procedures in which one, two, or three hepatic segments are removed. The right lobe of the liver is frequently removed in a resection termed a right lobectomy.[11] The medial segment of the left lobe may be resected along with the right lobe, as described by Starzl in 1975.[11] This procedure is termed right trisegmentectomy, since the posterior, anterior, and medial hepatic segments are removed.

It is possible to remove the posterior segment of right hepatic lobe (posterior segmentectomy). However, this procedure is tehnically difficult and is rarely performed. A left lobectomy consist of removal of the medial and lateral segments of the left lobe.[11] The caudate lobe may be left in place or removed along with the left lobe.[11] The left lateral segment can be readily removed in procedure termed lateral segmentectomy.[4, 11] In 1982 Starzl described the left trisegmentectomy, in which both segments of the left lobe are resected along with anterior segment of the right lobe.[12] In general, hepatic lesion are resectable if it is tecnically feasible to remove all gross hepatic lesions and to leave a sufficient volume of viable liver parenchyma in situ to support life.[13] In order for the remaining hepatic tissue to be viable, its vascular supply and venous and biliary drainage must remain intact.[8] Lesions in the liver are easily resectable if they spare the porta hepatis are confined to the area of one of the five major resections.[14] A lesion is generally considered unresectable if it encases or invades the main portal vein, the proper hepatic artery, both intrahepatic ducts or the major branch of any of these structures contralateral to the hepatic lobe in which the lesion originates.[14, 15] Continuity of tumor with vessel wall does not necessairly mean the vessel has been invated, the attainability of a tumor-free margin in such case frequently cannot be predicted by computed tomography.[3] In patients with severe hepatic parenchymal disease and limited hepatic reserve, hepatic tumor frequently cannot be resected unless the amount of resected tissue can be minimized.[15, 16]

Conclusion

Hepatic resections are technically demanding procedures that require accurate knowlege of internal hepatic vascular anatomy which is not readily apparent to the surgeon on inspection of its surface. Cardinal rule of major hepatic resection is that the vascular supply and the hepatic venous and biliary drainage must remain intact. Classically, the liver is anatomically divided into right and left lobes by the falcirorm ligament and fissure for the ligamentum teres. This classical division is not particularly helpful for surgical planning because resection has to follow the vascular distribution. Computed tomography can detect the normal anatomy of the liver and can identify with precision focal masses. Consequently, this imaging technique play a crucial role in the resection of hepatic lesions allowint surgeons to plan resections according to anatomic principles, making the resection technically easier.

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Technetium labeled autologous polyclonal immunoglobulin G (IgG) for scintigraphy of inflammation

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Background: Radiolabeled heterologous polyclonal human gamma globulins (HIG) have been used for detection of inflammation. Autologous immunoglobulins have been successfully used for detection of inflammation in animals.

Aim of our study was to assess feasibility of directly labeled autologous IgG, separated from patients' sera, for imaging of infection.

Methods: autologous IgG were separated from patients' sera using fast protein liquid chromatography (FPLC) and directly radiolabeled with ^{99m}Tc. Planar scintigraphy was performed in 18 patients with suspected inflammation.

Results: sensitivity of autologous IgG scintigraphy was 66 % and specificity 80% when compared with other investigations and final diagnosis.

Conclusion: The method seems promising for detection of inflammation although studies comparing radiolabeled autologous IgG compared to heterologous are necessary to prove superiority of either method.

Key words: autologous IgG, Tc-labeled scintigraphy of inflammation – radionuclide imaging; IgG – diagnostic use; technetium – diagnostic use

Introduction

Radiolabeled heterologous polyclonal human gamma globulins (HIG) have been used several years for detection of inflammation[1-4] and accuracy to detect infection is excellent in selected patients.[5] Uptake of IgG in some tumors was shown as well.[2] HIG are easy to prepare and thus useful for routine clinical work although radiolabeled leukocytes are superior in the diagnosis of focal purulent disease.[6] Distribution of various HIG preparations in vivo does not always follow identical pattern, as shown in baboon experiments. Louw et al. believe that it depends on degree of damage of commercially available IgG caused during the preparation.[7]

Dormehl and coworkers[8] showed foci of inflammation with autologous labeled IgG in baboon experiments. They consider autologous IgG most closely related to intact IgG. Labeled autologous IgG are even less likely to cause allergic reaction in recipient than the heterologous ones and could therefore be useful for repeated investigations in humans.

Aim of our study was to evaluate the feasibility and possible contribution of autologous IgG, separated with fast protein liquid chromatography (FPLC) and directly labeled with ^{99m}Tc, for scintigraphy in patients with inflammation.

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Patients and methods

Separation of Ig G

Five to 10 ml of patient's blood were withdrawn and serum was separated. Serum was exposed to

0.3 % sodium cholate (Merck) for 24 hours before further manipulation to prevent contamination of the chromatographic system.[9]

For separation *of Ig* from the serum, 3.0 ml of serum were precipitated with 3.0 ml of cold saturated ammonium sulfate solution. The resulting suspension was incubated and mixed during next 30 minutes. Centrifugation at 2000 g and 4° C for 15 minutes followed. The supernatant was discarded and the precipitate redissolved in 1.0 ml of 0.15 Mol/L and pH 6.5 phosphate buffer. The resulting solution was precipitated with 1.0 ml of saturated ammonium sulfate and centrifuged under the same conditions again. The precipitate was redissolved in 1.0 ml of phosphate buffer and passed through a 0.22 μm Millipore filter.

Second step of the separation procedure was *IgG* separation. The method was originally used for IgM isolation from human serum.[10] Our modification is described in detail elsewhere.[11] In short, slower flow rate than the originally proposed resulted in improved separation of IgG from IgA. For this purpose, FPLC using strong anion exchange column Mono Q HR 5/5 (Pharmacia), HPLC Gradient Pump 2249 (LKB), ultraviolet light (UV) detector Uvicord SD 2510 (LKB), Rheodyne injector, fraction collector RediFrac (Pharmacia) and recorder REC-481 (Pharmacia) were used.

Sample of 200 ml autologous Ig solution was used with FPLC. Following solutions were used: 0.15 Mol/l phosphate buffer pH 6.5 (buffer A) and 0.30 Mol/l phosphate buffer pH 6.5 (buffer B). Mono Q HR 5/5 was rinsed with 20 % ethanol and equilibrated with buffer A before use. First elution was performed with buffer A for 10 minutes, the second elution with buffer B for 15 minutes at flow rate of 0.5 ml/min.

Different immunoglobulin classes were detected with measurement of UV absorption and separately eluted. IgG were detected at UV wavelength 277 nm and at absorbancy range 1.0 AUFS (absorbance units full scale, as defined by manufacturer). IgG were eluted at the first, IgA at the second and Ig M at the third UV absorption peak.

Confirmation of IgG was accomplished with immunoelectrophoresis. The first fraction of the eluate, containing IgG, was collected separately and its total volume of approximately 0.4 ml was used for direct radiolabeling.

Direct labeling of IgG with ^{99m}Tc

The labeling was accomplished using the method of Pettit[12] with stannous tartrate as reducing agent for pertechnetate. IgG solution was preincubated for 20 minutes at room temperature with 50 ml of 2.1×10^{-3} Mol/L stannous tartrate solution (Sigma) and 500-800 MBq $^{99m}TcO_4^-$ were added in a volume as small as possible. Incubation at 40° C for 10 minutes followed. 100 μl of saturated $NaHCO_3$ were added thereafter and incubated again at 40° C for 20 minutes. The solution was passed through a 0.22 μm Millipore filter.

Quality control

The quality control of radiolabeled IgG was performed in two systems of ascending instant thin-layer chromatography (ITLC) using silica gel strips (Gelman) of 1 x 12 cm size. Methanol (85 %) was used as solvent in the first ITLC system. Free pertechnetate moved within the solvent front, while the radiolabeled IgG and colloidal forms of technetium remained at the start.

Silica gel strips were soaked with serum albumin (5 g/L), rinsed in deionized water and allowed to dry. They were used in the second ITLC system as a supporting phase while the mixture of solvents (C_2H_5OH: NH_4OH: H_2O in 2:1:5 ratio) served as a mobile phase. The colloidal forms of technetium (hydrolyzed or reduced technetium) remained at the start, while the labeled IgG and free pertechnetate moved with the solvent's front.

The percentage of free pertechnetate was calculated from the radioactivity of the solvent's front in the first ITLC system. The radioactivity at the start of the second ITLC system represented the colloidal forms of technetium.

The difference between distribution of radioactivity in the first and in the second ITLC system gave the estimation of radioactivity bound to IgG.[13]

Radiolabeled IgG was incubated in autologous serum at 37° C for 18 hours to test in vitro stability of radiolabeled autologous IgG.

Patients

The study protocol was approved by the national ethical committee. All patients gave informed consent before entering the study.

Eighteen nonselected patients with proven or suspected infection were included. Blood sample was withdrawn one day before the scintigraphic investigation.

Autologous immunoglobulins were labeled with technetium-99m (500 - 800 MBq) on the following day and reinjected intravenously. Acquisition of scintigraphic data followed.

Scintigraphy

Planar spot view scintigrams of the whole body were acquired in anterior and posterior projections with a large field of view gamma camera (Siemens Basicam), equipped with LEAP collimator and connected to a computer (McIntosh IIfx, McLear software for nuclear imaging), at 30 minutes, 3-6 hours and 17 hours after intravenous injection of autologous radiolabeled IgG.

Data analysis

Scintigrams were analyzed by two experienced observers, blinded to the clinical data. The lesion to background uptake of ^{99m}Tc IgG was evaluated visually and the uptake intensities compared. Accumulation of labeled IgG in liver, kidneys, spleen and bone marrow as well as in the blood pool in the first phase after application was considered normal. The uptake in organs and tissues higher than the normal background was considered abnormal. Studies with nonconclusive accumulation in organs other than the normal distribution were reported as equivocal. Final decision was made by consensus in case of disagreement of separate readings of the two observers.

The results of scintigraphy with ^{99m}Tc IgG were compared with final diagnosis and with findings of all available investigations as bone scan, radiograms, computed tomography, ultrasound, erythrocyte sedimentation rate and white blood cell count.

Scintigraphy was validated using sensitivity and specificity of the new method compared with the final diagnosis for all studies. False positive or negative results were analyzed in detail.

Results

IgG separation and labeling

IgG were completely separated from other immunoglobulins in all sera as confirmed with chromatography. The labeling efficiency of isolated autologous IgG was above 95 %. Labeled ^{99m}Tc-IgG were stable in vitro. Only 2.5 % of free pertechnetate and 4.6 % of colloid was detected after 18 hours' incubation at 37 °C.

Patients

No adverse reaction was observed after intravenous application of autologous ^{99m}Tc-IgG to the patients. Seven men and 11 women, in average 46 (±15.6) years old, all with suspected inflammatory lesions were included. Two patients had spondylodiscitis, single patients had discitis and paravertebral abscess, discitis and psoas abscess, paravertebral, suspected pancreatic, gluteofemoral and paranephric abscess. Osteomyelitis was suspected in four patients, pulmonary sarcoidosis in 2 and 4 patients were investigated for suspected myocarditis (Table 1). Average duration of inflammation in patients (except in patients with sarcoidosis and myocarditis) was 2.8 (± 3.1) months. Most patients were treated with antibiotics at the time of scintigraphy. Only 2 patients with suspected osteomyelitis, 2 with sarcoidosis and 3 with myocarditis did not have antibiotic therapy.

Eight suspected inflammatory lesions were true positive and 4 true negative. In one patient with suspected inflammation in recent bone fracture the lesion was false positive. False negative were 3 lesions in patients with psoas or paravertebral abscesses and in one patient with spondylodiscitis in previously irradiated area. Results of scintigraphy in two patients with dilatative cardiomyopathy after myocarditis and in one patient with sarciodosis were equivocal.

Eight of 17 lessions in suspected inflammation were true positive, 4 were true negative, 4 were false positive and 1 false negative; calculated sensitivity of our method (without the three equivocal cases) in detection of inflammation is 66 % and specificity 80 %.

Discussion

Detection of inflammation with heterologous polyclonal human gamma globulins (HIG) is well accepted. As autologous IgG are considered to be most closely related to the intact IgG,[8] they might also have more specific affinity for patient's antigens than the heterologous ones and could therefore be suitable for investigations in humans. Radiolabeled autologous IgG are not likely to cause allergic reaction in the recipient than heterologous polyclonal HIG.

Autologous technetium labeled IgG could be therefore useful for detection and follow-up of patients with inflammation or tumors without risk of immunologic reactions and with low radiation exposure.

Our study was planned to evaluate feasibility of autologous IgG, separated with fast protein liquid

Table 1. Patient's data.

Patient. Number	Gender	Age	Therapy	Final Diagnosis	Disease Duration	Radiogram Computed Tomography	Ultrasound	Cytology	Autologous 99m Tc-IgG Scintigram	^{99m}TC-HMPAO Labeled Leucocyte	Bone Scintigram	Laboratory Tests
1	Female	34		Discitis L4/5	2 months	Discitis			Uptake	Uptake L4/5	ESR 22. L4/5	Leucocytes 8.5
2	Female	35	Anti-biotics	Paravertebral abscess. disciti	1 week	Paravertebral lesion. discitis L4/5			Uptake L4/5		Uptake L4/6	ESR 102. Leucocytes 9.1
3	Female	58	Anti-biotics	Osteosynthesis	2 months	Fragments of bone without callous			Uptake in fracture	Negative	Uptake in fracture	ESR 45. Leucocytes 8.1
4	Female	65	Anti-biotics	Spondylo discitis	10 days	L3/5 degenerative changes			Negative		Low uptake postirradition	EST 120
5	Female	59	Anti-biotics	Paranephritic abscess	2 months		Parane-phric fluid	Pus	Paranephric uptake			ESR 32
6	Female	36	Anti-biotics-	Post osteo-myelitis	5 months				Negative			
7	Female	71	Anti-biotics	Discitis. psoas abscess	6 months	Inflammation left SIS. L4/5. psoas abscess (CT)		Pus in psoas	Uptake L4/5	Negative	Uptake L4/5	ESR 60
8	Female	58	Anti-biotics	Osteomyeitis in femoral fracture	3 months	Partially healed fracture			Uptake femur		Uptake femur	Hemoculture Streptococcus viridans
9	Male	64	Anti-biotics	Gluteofemoral abscess	1 month	Discitis L4/5. pus in thigh			Uptake L4/5. gluteal	Uptake gluteal	Uptake L4/5	Positive hemoculture
10	Male	56		Pancreatic cyst	2 months	Cyst in pancreas	Cyst in pancreas		Negative	Negative		
11	Male	49	Anti-biotics	Paravertebral abscess	3 weeks	Spondylo-lysis L5	Heart. abdomen normal		Negative			Hemoculture Staphyloccocus aureus
12	Male	45		Dilatative myocardiopathy post myocarditis	2 months	Enlarged heart	No pericardial effusion	Few signs of myocar-ditis	Equivocal in myocardium	Antimyosin antibodies positive		
13	Female	20		Postpartal myocardiopathy	1 month	Enlarged heart	Intracardial thrombus		Uptake in the heart			
14	Male	25		Dilative myocardiopathy post myocarditis	18 months	Enlarged heart	No pericardial effusion	Few signs of myocar-ditis	Equivocal in myocardium	Antimyosin antibodies equivocal		
15	Male	74		Pulmonary sarcoidosis	120 months	Thorax radiogram normal			Equivocal			ESR 4
16	Female	44		Pulmonary sarcoidosis	24 months	Hilar lymph nodes			Equivocal			
17	Female	43	Anti-biotics	Sepsis. suspected myocarditis	1 month				Negative			Leucocytes 13.8
18	Male	37		Osteoarthritis	12 months	Osteoarthritis			Negative		Osteoarthritis	

L4/L5 or = fourth to fifth lumbar vertebrae. SIS = sacroiliac joint. CT = computed tomography. L = left. ESR = erythrocyte sedimentation rate (mm/h). Leukocytes = number x 10^9/L. normal 4.0–10.0. ^{99m}Tc-HMPAO = scan with ^{99m}Tc-HMPAO labeled leucocytes.

chromatography (FPLC) and directly labeled with ^{99m}Tc, for scintigraphy of inflammation. Modified flow rate with FPLC allowed complete separation of IgG from other serum proteins. In vivo instability of technetium labeled IgG was shown to be a problem with direct labeling via stannous ion reduction.[14] Hnatowich and coworkers found evidence of in vivo transchelation of ^{99m}Tc to cistein as a cause of high renal radioactivity. They also observed IgG fragmentation after stannous ion reduction. Fragments can be excreted via kidneys as well. Our studies in vivo[11] demonstrated high radioactivity in kidneys and in urinary bladder but not in the thyroid, what is consistent with stable binding of technetium. Stability of directly labeled ^{99m}Tc-IgG complex was in our study proved in vitro. Concentration of radioactivity in lesions was high enough to allow scintigraphic visualization of most inflammatory lesions in spite of shortcomings of direct labeling of autologous IgG with ^{99m}Tc.

The mechanism of IgG accumulation in inflammatory and neoplastic lesions is not entirely understood. One of the most important reasons for IgG uptake in inflammatory lesions is increased vascular permeability and the diffusion at the site of inflammation.[15] Some authors suppose that the attraction between Fc region of IgG and Fc receptors on the leukocytes and bacteria allows accumulation of labeled substances.[16–18] The type of immunoglobulin was shown to play a role in accumulation at the site of inflammation.[19]

The results of scintigraphy in our patients with infection were comparable to the results of studies with heterologous technetium labeled IgG.[4–20] Autologous ^{99m}Tc-IgG are not equally successful in detection of all types of inflammation. Satisfactory results were achieved in patients with spondylodiscitis (Figure 1). The fact that labeled leucocytes have limitations for scintigraphy of inflammation in spinal region[5, 12] warrants further study of this specific application of radiolabeled ^{99m}Tc-IgG. On the other hand, because of high blood pool activities the results in patients with myocarditis and sarcoidosis were equivocal. False negative autologous ^{99m}Tc-IgG scintigram in paravertebral or psoas abscesses was the rule in our study. It was probably due to high background of the spine, liver and kidneys in comparison with the uptake in the abscess. The false negative scan in spondylodiscitis in a patient with previously irradiated spine was probably due to low bone metabolism and lowered perfusion of that area. False positive uptake in a recent unhealed fracture and in some osteoarthritic joints showed the low specificity of the new method. The results of our study are encouraging in patients with spondylodiscitis. Larger group of patients should be studied for more reliable evaluation of sensitivity and specificity of scintigraphy with autologous ^{99m}Tc-IgG. Furthermore, tomographic techniques would improve diagnostic accuracy of our method. Studies comparing technetium labeled autologous with heterologous IgG are necessary to prove possible differences between the two methods. Newer direct labeling techniques[22] probably have advantages for scintigraphy with autologous and heterologous polyclonal IgG.

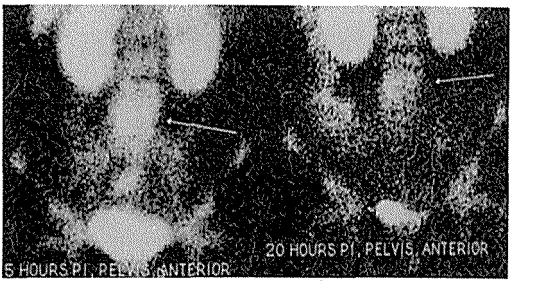

Figure 1. Scintigram with autologous IgG labeled with technetium-99m in a patient with discitis in the lumbar spine. High uptake of technetium-99m labeled autologous IgG is shown in 4th and 5th lumbar vertebrae.

Conclusions

The present study proves safe and feasible use of autologous IgG, directly labeled with technetium, for scintigraphy in patients. Convincing uptake of autologous ^{99m}Tc-IgG was shown in inflammatory lesions like spondylodiscitis. It is suitable for repeated use because there is no danger of allergic reaction in the recipient. The method of separation and labeling is to complicated to be used unselectively in routine clinical practice.

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Follow-up study of autonomous thyroid adenoma treated with I-131

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Twenty seven patients with autonomously functioning thyroid adenoma (AFTA), clinically and laboratory euthyroid were treated with I-131. A acriterion for the therapy was the scintigraphic appearance of AFTA "hot", and suppressed TRH response of the patients. The mean weight of AFTA was 50.03 ± 28.5 g, administered radioactivity was 13.3 ± 6.1 MBq per g tissue, and estimated radiation was 262.2 ± 129.9 Gy. Examinations in the follow-up period of 2-36 months included clinical and laboratory testing, TRH test and thyroid scintigraphy.

After the therapy. AFTA became unpalpable in 14 (51.8 %) patients, decreased in 7 (26.0 %) and did not change in 6 (22 %). All patients remained euthyroid by all criteria, with normal TRH test in 26 (96 %) of them. Two months after the therapy, transitory suppression or exaggeration of TRH test, was found in 3 and 1 patient resp., but spontaneous restitution occurred later in all but one, where TRH remained suppressed (3.7 % of all). AFTA appeared cold on the scan of 92.6 % of the treated patients. There was no hypothyroidism after the therapy. This and the achievement of ablation or reduction of the nodules in most of the patients, support the opinion that patients with AFTA and suppressed TRH test, even when euthyroid, should be treated with I-131.

Key words: thyroid neoplasms; adenoma-therapy; iodine radioisotopes; follow-up studies

Introduction

Plummer's disease is a thyroid disorder in which a part of the tissue is functioning autonomously and the rest of the gland is normally responding to feedback mechanisms. It is presented by a spectrum of structural and functional abnormalities which include solitary or multiple nodules, or numerous autonomous centers, and autonomous production of hormones in normal or excessive quantity.[1,2] The last is crucial for the clinical presentation of the disorder. When an autonomously functioning thyroid adenoma (AFTA) produces clinically overt hyperthyroidism, the therapy should be radically-surgical or with radio iodine, sometimes after a short-lasting medicamentous treatment.[2]

The treatment of AFTA in euthyroid patients rises many questions: is it necessary, in which cases and when, and what kind of therapy? Euthyroid clinical picture may be associated with different biological behavior of AFTA: compensated or decompensated, i.e. scintigraphically presented as isofixant or hiperfixant – "warm" or "hot" nodule, comparing to surrounding tissue.[3] Normal serum levels of T4, T3 and even TSH, may be associated with a suppressed response to TRH stimulation as a first sign of disorder.[3] Keeping in mind the slow evolution of AFTA and the possibility of spontaneous destruction of adenomatous tissue[4,5] many clinicians hesitate to choose a radical therapy. But the follow-up studies of many cases show steady progression of the disease in some patients, which may cause heart damage,[5,6] and Belfiore et al.[7] found that it happened in a higher percent of patients from

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iodine deficient regions than in those from other regions.

Here we present results of radioiodine treatment of euthyroid patients with AFTA and our attitude to that problem.

Material and methods

The study includes 27 patients with AFTA, clinically, euthyroid, who were treated with I-131 and subsequently followed-up. The diagnosis of AFTA and evaluatiion of clinical status were based on clinical, laboratory and scintigraphic examinations. Biologic behavior of AFTA was assessed by TRH-test response, as the serum thyroxine (T4) and triiodothyronine (T3) were in normal range. The therapy was accomplished by oral application of radioiodine dose, calculated according to the weight of AFTA and 24h-uptake of I-131. Follow-up evaluation included clinical and laboratory estimation of the thyroid function (T4 and T3 determination, TRH response), as well as scintigraphy of the gland.

A group of 20 patients who were healthy and with palpatory and scintigraphically normal thyroid, were tested for TRH response. They were considered as a control group in this study.

T4 and T3 were determined with RIA (kits produced by "Vinca", thyrotropine (TSH) in serum was determined with IRMA (CIS) (first generation). Normal values for T4 in T3 serum concentrations: 64–160 nmol/L resp. 1,5–3,4 nmol/L. Thyroid scintigraphy was performed mostly with I-131, after 24h of an oral dose of 1850 KBq, or with 800 MBq of Tc-99 m, 30-40 minutes after i.v. application. The weights of the nodules were determined by calculating their volumes as ellipsoids (diameters were measured by the scintigraphic imaging of the gland). This method had been proven by comparing the weights of operated nodules with calculated ones by their scans[8] (before introduction of ultrasound measurement). TRH test was performed by i.v. application of 200 µg Relefact TRH (Hoechst, AG) and serum TSH was determined in 0,30 and 60 min. after the application.

Results

Only two of the patients with AFTA were men (7 %), so that women/men ratio was 12,5/1. Age of the patients was 36–72 years, mean 53.48 ± 7.96, and the age of the control patients 40.38 ± 10.89 years. In 23 (85 %) patients there was a solitary nodule and in 4 (15 % of all) there were 2 nodules. The nodules in all patients were "hot" by scintigraphy and surrounding tissue was completely suppressed. Most of the patients were clinically normal and some of them only with mild and common complaints, as slight nervousness of fatigue. T4 na T3 serum levels were in normal range, but their mean values were statistically higher that in controls (Figure 1). TSH values were in normal range as well, ranging 0.5–0.4 µU/ml. TRH test was abnormal – unresponsive in all: no increase of TSH serum level was recorded after 30 or 60 min. of TRH injection, or the increase was minimal, not exceeding 1.5 µU/ml over basal level in 13 patients. As a normal response to TRH stimulation (positive response) it is considered an increasing of TSH level after 30 min. or more than 3 but less than 25 µU/ml, a criterion accepted by many authors.[3, 6, 9, 10] TRH test in the contol group showed an average elevation of TSH of 10.81 ± 4.2 µU/ml.

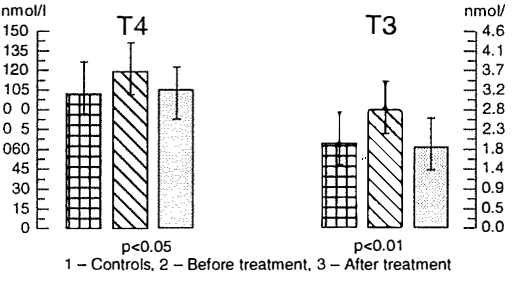

Figure 1. T4 and T3 in CONTROLS and in AFTA BEFORE AND AFTER I-131 TREATMENT

The data on the weights of AFTA and the applied radiotherapy are given on Table 1. The follow-up period after the therapy was 2–36 months (mean 14.1 ± 12.0).

Table 1. I-131 Treatment of autonomously functioning thyroid adenoma (AFTA), 27 patients.

	Mean ± SD	Range
Weight of AFTA (g)	50.0 ± 28.5	19–32
24h I-131 uptake	44.5 ± 12.1	27–78
Dose of I-131 (MBq)	1090.8 ± 244.2	740–1850
I-131/g AFTA (MBq)	12.1 ± 6.1	3.4–23
Radiation in AFTA (Gy/g)	262.2 ± 129.8	73.5–508

The clinical state in all patients remained euthyroid. The change of the size and scintigraphic appearance of AFTA is presented on Table 2. T4 and T3 levels decreased and although remained in normal range, their mean value, did not differ significantly from that of the control group (Figure 1).

Table 2. Follow-up of 27 patients with AFTA: findings of palpation, thyroid scintigraphy and TRH test (after the therapy).

	Number of patients	% of all
Reduction of AFTA size (by palpation):		
Not palpable any more	14	51.8
Reduced in size	7	26.0
Remained unchanged	6	22.2
Scintigraphic appearance:		
Cold nodule	25	92.6
Isofixant nodule	2	7.4
Hot nodule	0	0
TRH – Test:		
(Elevation of TSH levels – 30 min.)		
Normal (3–25 μU/ml)	26	96.3
Negative (3 μU/ml)	1	3.7

TRH test was performed in the follow-up period in all patients, at different times. It was normal in 22 of them and abnormal in 4: in 3 it was negative (increase of TSH less than 3 μU/ml at 30 min.), and in one, basal TSH was elevated and the test was exaggerated (Table 3). These patients were tested two months after the therapy, as were other 9 patients whose TRH test was normal. Repeated testing of those with abnormal test after 4–36 months, did not show abnormality in three of them and only one patient had a suppressed TRH response, even after 36 months. The nodule of the patient weighted 132 g and it received a lower radiation doses (86.5 Gy), but in spite of that, the nodule became unpalpable and "cold" on scan.

Table 3. Patients with abnormal TRH response two months after I-131 therapy.

Pat. No.	I-131 dosis	Radiation	No. month after I-131	TRH test	TSH (μU/ml)	
	(MBq/g)	(Gy/g)		Basal	30 min.	60 min.
J.D.	10.5	227.0	2	0.5	3.4	1.9
			22	3.2	10.4	8.1
M.A.+	4.0	86.5	2	0.5	0.6	0.6
			36	2.3	3.8	3.3
J.S.	3.6	77.9	2	2.4	5.1	4.5
			15	1.6	12.7	9.7
G.S.	14.3	309.0	2	16.0	46.0	40.0
			36	0.5	11.8	8.8

+ – the patient with persistent suppressed TRH response

Discussion

It is generally accepted that AFTA which causes hyperthyroidism should be treated radically – by surgical or radioiodine treatment. The data of many studies show that relapses and hypothyrosis after the treatment are very rare, in contrast to Graves' disease, where they occur in much higher percent.[2,5,10,11] The treatment of euthyroid patients with AFTA (solitary or multiple) is a matter of individual consideration, depending on different factors.[12] Since the early and later application of TRH test, has shown that it can discover subclinical hyperthyroidism[6,9,10] and so to help in decision-making for therapy. Discovering a disturbed thyroid function at its very beginning by TRH-test, is not of pure academic interest. Correcting it, an overt thyrotoxicosis may be prevented (which in older age is usually oligosymptomatic, manifested as a heart disease). Although progression of euthyroid AFTA to toxic nodule and hyperthyroidism is slow and not very frequent,[4,11] we observed it in 35 out of 181 euthyroid patients, followed-up during a period of 0,5–15 years, with statistical probability of 6,65 % to become hyperthyroid.[5] At last, one can not exclude the possibility of "Tissue hyperthyroidism" although not proved by laboratory findings.

All of our patients were euthyroid, with serum concentrations of T4, T3 and TSH in normal range (although statistically higher than in controls), but TRH test and scintigraphy revealed abnormal biological behavior of AFTA. Even in that case one may pose the question of the reason for therapy. Treatment of clinically and laboratory euthyroid patients if they are not young and their AFTA is large, is recommend by McConahey.[12] Our choice of radioiodine treatment for these patients was not wrong by our consideration, which could be supported by the results of the therapy:

– all treated patients remained euthyroid, with restituted physiological regulation of the thyroid, but one. None of the patients became hypothyroid. One patient who showed a transitory, subclinical hypothyroidism (exaggerated TRH response), recovered spontaneously.

– ablation of AFTA was achieved with lower doses of I-131 than those we[5] or other authors[11,13] usualy use for treatment of AFTA in hyperthyroid patients.

– the percentage of markedly reduced nodules is very high (77.8 % of all), so that this therapy offers also an esthetic effect and possible relief of com-

pressive effect of AFTA (if it were present). So, these effects, otherwise expected from surgical treatment, may be achieved by radiotherapy, without surgical risks.

A support of our approach to radioiodine treatment of euthyroid patients with AFTA, we found also in the excellent study of 87 thyroidectomized patients with Plummer's disease by Wiener.[14] He found in the follow-up period an unexpectedly high percent of postoperative thyroid autonomy in the residual tissue and this suggested that radioiodine treatment could be more effective that surgical treatment.

The amount of administered radioactivity is a matter of consideration in many studies. Although there is some relation between the dose and the success of the therapy (i.e. the incidence of hypothyroidism and failure of the therapy,[11–13,15] many authors,[11,12,15] found that the incidence of hypothyrosis is not related to the dose per gram of nodular tissue. By their experience, for its prevention is the most important: the scintigraphic appearance of the extra nodular tissue before the administration of I-131 should be sufficiently suppressed, with minimal iodine uptake. We agree with them and all of our patients were scanned shorlty before the therapy.

The finding of temporary exaggerated TSH response after the therapy in one patient, was surprising. It may be supposed that the suppressed extranodular tissue which was functioning below its normal capacity before the therapy, need more time for its functional recovery. There is a possibility that extranodular tissue was moderately irradiated and is in temporary hypofunction. These both possibilities do not exclude each other.

It is more difficult to explain the persistence of suppressed TSH response after the therapy. At two months after the therapy maybe the effect of radiation was not completed yet, but in the case when the nodule became cold, long time after the therapy the finding is unusual. As a coincidence of Grave's disease and AFTA in the same gland has been reported[16] it may be supposed that a thyroid autonomy of surrounding tissue developed during post-treatment period.

Our experience from this study is that examinations of clinically euthyroid patients with AFTA should be completed with TRH-test (or ultrasensitive measurement of TSH which is in use nowadays) and if it is suppressed the therapy may be taken in consideration.

Conclusions

1. AFTA decompensated on scan could be treated if TRH test is negative, in spite of euthyroid clinical and laboratory findings.

2. Radioiodine therapy is effective in curing the autonomy and very often in reducing the size of the nodules in doses of 3.4–23.0 MBq per g AFTA tissue.

3. About two months after the therapy a normal TSH response is achieved in most of the treated patients. Only in few patients it restores later and for evaluation of therapeutic effect TRH-test should be repeated.

4. Hypothyroidism after I-131 treatment of Plummer's disease is very rare, even in euthyrotic patients with AFTA.

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Current approaches to gene therapy in oncology: Construction of tumor vaccines

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Current conventional treatment of malignancies is based predominantly on the use of radio- and chemotherapy. The mentioned therapies are not directed against cancer tissue only and have severe dose-limiting toxic side effects accompanied with a suppressive effect on the patient's immune system. On the other hand, immunotherapy, and especially gene therapy, try to be more selective and less aggressive, having the purpose of triggering a specific immune response against tumor cells. Therefore, different approaches to the creation and application of gene therapy in oncology have been formed in the past few years, yet the aim of all of them is the same: to use extended knowledge about molecular mechanisms of the disease in order to devise a more specific mode of treatment. The major approach to present-day gene therapy of cancer is the generation of tumor vaccines as a possible future category of cancer treatment. The purpose of this article is to provide a brief overview on creation and potential applications of tumor vaccines as well as of some modes of gene therapy in oncology.

Key words: neoplasms-therapy; gene therapy; tumor vaccines

Introduction

Owing to unspecific activities of conventional therapies against cancer (radiotherapy, chemotherapy) a treatment of this kind is quite often accompanied with unrecoverable damage of the normal tissue. The tremendous increase of knowledge in immunology as well as the exponential development of recombinant DNA technology conditioned the renewal of interest for creation of different immunotherapies that were supposed to be more effective, more specific for tumor cells, and cause no or negligible toxic side effects. The goal of each immunomodulatory treatment is to stimulate (enhance) immune response and in this way alter the dynamics of host-tumor relationship to therapeutic advantage. At the same time, this treatment modality has to prevent the development of tumor cell resistance to such treatment, and cause no toxic deposition in the normal tissue. Therefore, for successful creation of immunotherapy, it is important first to understand the relationship between the host and specific tumor cells in order to choose the most appropriate approach. To induce tumor immunity more specifically and effectively, various methods of immunotherapy have emerged using different biological agents such as monoclonal antibodies, cytokines, tumor antigens, hormones, activated killer cells, immune T cells, DNA and others.[1-8]

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Vaccination against cancer

The idea of vaccination against tumor cells has been a distant goal of immunologist for many years, ever since 1909, when Paul Ehrlich suggested that tumors might express antigens that could be targets of immune system.[9] Certainly, at that time there was hardly anything known about tumor-associat-

ed antigens, B and T lymphocytes, antigen-specific receptors on lymphocytes, immunoregulatory cytokines etc. However, the observations that there is a difference in the velocity of tumor growth, and that some tumors stagnate for a longer period of time (even some years), indicate that organisms possess powerful regulation mechanisms (i.e. immune system) for tumor growth control.[5] And still, quite often tumor cells escape the control and do not trigger the immune response. Tumor vaccines were thus created with the intention to rebuild or retrigger the immune system and induce systemic immunity against tumor cells. For this purpose, irradiated autologous or allogeneic tumor cells, lysates of tumor cells, and occasionally, virally infected tumor cells were used as tumor vaccines. To intensify additionally the immune response, nonspecific immunostimulators (e.g. *Corynebacterium parvum* or *Bacillus Calmette-Guerin*) were added to most of the above mentioned preparations.[11–14] The basic working guide of all these experiments was to achieve an enhanced expression of MHC antigens on tumor cells and to increase the cytokine production.

Only the exponential development of molecular genetics and monoclonal antibody reagents, as well as the results of the latest investigations, provided enough information to allow speculation that diminished responsiveness or complete unresponsiveness of the immune system could be predominantly a consequence of the changes of tumor cells at the molecular level. Among the most important changes which enabled tumor cells to escape immune control researchers classified the following:[15,16]

- inadequate expression of MHC (major histocompatibility complex) antigens,
- prevention of tumor specific antigen presentation to T lymphocytes,
- absence of adhesion molecules which are important for the activation of immune system, and
- production of various factors (by tumor cells) which influence (change) the host immune system.

Gene therapy and tumor vaccines

The "gene therapy" term has become a new paradigm, associated with any kind of disease where the origin can be connected with the defined genes. Gene therapy involves a variety of new techniques for gene transfer, gene replacement, gene repair or gene deletion.

Although the idea of vaccination against tumor cells dates in the beginning of 19th century, the modern tumor vaccines represent just one of the approaches (major) to gene therapy of cancer. In other words, modern tumor vaccines are a form of gene therapy where, by use of different vectors, genes of interest are transferred into the tumor cells or into immunocompetent cells. This can be achieved by direct DNA transfer or by using viral vectors. The most prevalent nonviral techniques used for gene transfer are calcium phosphate transfection, microinjection, electroporation, liposomal gene transfer, injection of naked DNA, and receptor mediated gene transfer.[17–22] Among the biological delivery systems for gene transfer the cardinal ones are retroviral vectors, adenoviral vectors, adeno-associated virus vectors and other viral vectors.[23]

The first studies with genetically transformed tumor cells (that were used as tumor vaccines) confirmed that both classes of MHC antigens (MHC I and MHC II) play an important role in the process of triggering of the immune response and that the antitumor activity is predominantly a consequence of activation of cellular immunity.[24] Class I MHC antigens are recognized by cytotoxic lymphocytes (CD8+) and their presence is obligatory for the activation of these cells. On the other hand, class II MHC antigens (they are presented by antigen-presenting cells in the form of endosomes or lysosomes, respectively) take part in the activation of helper T lymphocytes (CD4+), cells which are classified as basic producers of different cytokines. Exactly, the defect in the helper arm (i.e. cytokine producing part) of the immune system is often the cause of inadequate immunogenicity of tumor cells: namely, the development of cellular immunity will fail in the case of inadequate cytokine production, regardless of the fact that MHC I antigens are normally expressed and active.[25]

Considering those facts, tumor vaccines were created predominately to achieve:

- enhanced production of various cytokines that participate in immune processes (IL-2, GM-CSF, IFN-α, TNF-α),
- expression of allogeneic human leukocyte antigens (HLA antigens) or
- enhanced concentration of products that are responsible for the expression or the activities, respectively, of oncogenes (e.g. of the product of p53 suppressor gene).

Therefore, depending on the manner chosen to fight tumor cells, quite a few different approaches

to creation of gene therapy and tumor vaccines have been established. This review will deal with some of them, i.e. those that have been found most promising and attractive.

Preparation of tumor vaccines with insertion of genes coding for allogeneic leukocyte antigens into autologous tumor cells

The purpose of such preparation of tumor vaccines is the transfer of genes encoding certain antigens (usually present on the surface of antigen-presenting cells) into tumor cells. B7 antigen is a molecule that normally functions as an activation molecule on antigen-presenting cells (macrophages, B lymphocytes, dendritic cells). B7 antigen represents a ligand for two types of T lymphocyte receptors i.e. for CD28 (present on CD4+ and CD8+) and CTLA4 (present only on CD8+) receptors. The role of CTL4 has not been determined yet, while on the other hand CD28 is well known to be the cardinal receptor for activation of T lymphocytes and for stimulation of cytokine production.[26] These data led to formation of a hypothesis about the transfer of a gene coding for B7 antigen into the tumor cells and about the potentials of such tumor vaccine to trigger systemic antitumor immunity. So Chen et al.,[13] as well as Townsend and Allison,[27] demonstrated that rejection of malignant melanoma cells expressing B7 ligand resulted from the activity of CD8+ T lymphocytes. Besides, in these experiments systemic immunity developed (in experimental animals) even against genetically unchanged melanoma cells (which were thus not expressing B7 ligand). On the basis of the cited studies we can conclude that tumor vaccines, created by transferring of B7 gene into autologous tumor cells, activate cytotoxic T lymphocytes and stimulate cytokine production in helper T lymphocytes, thus effectively triggering the development of systemic antitumor immunity. On the other hand, the best results with this kind of vaccines can be obtained (owing to the costimulatory mode of action of B7 on CD8+ and CD4+ T lymphocytes) only in the presence of MHC class I and class II antigens on tumor cells.

Vaccines created with insertion of genes coding for different cytokines into autologous tumor cells

Insertion of genes coding for different cytokines might play a role in "overcoming" the unresponsiveness of immune system that derives from inability for normal cytokine production which is actually a consequence of complete absence or inadequate expression of MHC II antigens. In contrast to the activities of exogenous cytokines, the cytokines produced in genetically changed autologous tumor cells mimic the activities of natural endogenous cytokines (underlie to some extent the control mechanisms of the organism), which on one hand improves their effectiveness and on the other hand minimizes their toxic side effects. When preparing tumor vaccines, different researchers introduced genes for numerous cytokines or growth factors (IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IFN-α, TNF-α, GM-CSF and G-CSF), respectively, into tumor cells.[28–32] The effectiveness of vaccines tested on animal tumor models depended upon the type of cytokine produced by the cells, upon the abundance of cytokine synthesis and upon the type of tumor used in the study. Fearon et al. demonstrated that transfection of poorly immunogeneic mouse colon carcinoma cells with IL-2 gene results in reduction of tumorigenic potential of tumor cells and triggers the development of systemic immunity.[33] They confirmed that the phenomenon of systemic immunity results from the influence of IL-2 on CD4+ and CD8+ T lymphocytes (activation of T lymphocytes). Similar conclusions were made by Gansbacher et al. after the transduction of IL-2 gene into mouse fibrosarcoma cells (in syngeneic mice) and into human melanoma or renal carcinoma cells (in nude mice).[34,35] The fact that IL-2 triggers the development of systemic immunity through its action upon T lymphocytes was also confirmed by Rusell et al. in experiments with rat tumor model.[36] Namely, they transplanted transfected rat sarcoma cells either into syngeneic rats or into immunodeficient nude rats. The effect of vaccine in syngeneic rats with normal T lymphocyte production was highly superior to the one in nude rats. On the other hand, partly different results were obtained by Cavallo et al.[37] In agreement with other authors they demonstrated that vaccines prepared by IL-2 gene transduction are capable of challenging the immune response, which (according to Cavallo et al.) predominantly depends upon neutrophils activated with IL-2. Allione et al. created tumor vaccines with transfection of adenocarcinoma cells using genes encoding various interleukins (IL-2, IL-4, IL-7, IL-10), IFN-α, TNF-α or GM-CSF. The best antitumor protection was achieved with inoculation of tumor cells producing interleukins and IFN-, while the treatment outcome after application of tumor cells producing TNF-α was less favourable.[38] Quite interesting was also the comparison of the effectiveness

of the therapy with genetically changed cells, to therapy with tumor cells admixed with *Corynebacterium parvum*. Namely, the authors established that the antitumor activity of the mixture of tumor cells with *Corynebacterium parvum* approximated in its degree the antitumor activity of therapy with genetically modified cells. Similar results were observed by Hock et al., who demonstrated that tumor vaccines prepared by mixing of tumor cells with nonspecific immunostimulators exert an antitumor effect which is comparable to the effect of tumor vaccines created of genetically transformed cells.[39] In contrast to the authors, who achieved relatively modest results with tumor vaccines containing gene for TNF-α, Blankenstein presented encouraging outcomes (his own and of other authors) using the very same vaccines.[40] The antitumor activities of such vaccines were supposed to be based predominantly on an indirect effect mediated through stimulation of immune system and to a lesser extent on the direct antitumor effect of TNF-α. This kind of stimulation of immune system includes the activation of macrophages, as well as CD4+ and CD8+ T lymphocytes. Vaccines bearing TNF-α gene are also successful in the case of inhibited T lymphocyte production, but anyway, the presence of these cells enhances the antitumor effect of such treatment. The best protection from challenge with wild type tumor cells, as well as the most pronounced antitumor activity against formed tumors, has been ascribed to vaccines created of tumor cells bearing gene for GM-CSF. Mulligan and Pardoll studied the effectiveness of vaccines bearing genes for various individual cytokines or for combination of cytokines.[41] The most promising results were achieved with GM-CSF (in the group of vaccines bearing a gene for a single cytokine), while the most effective combination of genes for preparation of tumor vaccines comprised genes for IL-2 and GM-CSF. Dranoff et al. quite early discovered that tumor vaccines with GM-CSF gene are superior to vaccines prepared with genes encoding other cytokines in the case of stimulation of the antitumor immune response.[30] However, the activation of CD4+ and CD8+ T lymphocytes was obligatory for the development of systemic immunity also with vaccines bearing GM-CSF gene, regardless of the MHC II antigen expression on tumor cells. The effectiveness of tumor vaccines with GM-CSF gene was finally confirmed by Golumbek et al., since in their experiments not a single experimental animal immunized with the vaccine developed a tumor after challenge with highly tumorigenic wild type tumor cells.[42] The effect of vaccines with enhanced expression of GM-CSF gene is being ascribed to the stimulation of differentiation of the precursor blood cells and dendritic cells (important antigen-presenting cells for T lymphocytes).

Thus, the basic conclusions of these studies could be the following:

- even low concentrations of cytokines produced by transformed cells are capable of stimulating the antitumor immune response (comparable results were achieved after systemic high dosage cytokine therapy which is often accompanied with numerous toxic side effects);
- important role of cytokines in the process of activation of nonspecific leukocytes e.g. granulocytes and macrophages;
- cooperation between granulocytes, macrophages, lymphocytes, fibroblasts and endothelial cells represents the basis of immune reactions triggered by genetically transformed cells;
- degree of antitumor activity depends upon the tumor type, the type of cytokine produced by tumor cells, and upon the abundance of cytokine production;
- T lymphocyte activity is supposed to depend indirectly upon activation of macrophages and other antigen-presenting cells, as well as upon secondarily induced cytokines (which play an important role in the activation of T cells);
- sublethally irradiated genetically changed cells are capable of challenging the immune response, yet a less pronounced one in comparison to the immune response triggered by proliferating cells, since sublethally irradiated cells produce cytokine only during a limited period of time and because the abundance of tumor-associated antigens is insufficient;
- insertion of GM-CSF gene into tumor cells does not change their tumorigenic potential, yet cells modified in this way and afterwards sublethally irradiated, induce the development of a long lasting immune memory.

Application of tumor specific antigens as vaccines

The idea is to use specific antigens only, instead of intact tumor cells (as carriers of usually ill-defined tumor antigens), for the creation of tumor vaccines. In this case specific immunity can be enhanced (owing to the usage of specific antigens), and also whole work with gene transfection becomes sur-

plus. The basic condition for a successful application of vaccine is that the chosen antigen has to be expressed exclusively on the specific type of tumor cells and by no means on healthy normal cells. We are witnessing at present the identification of the first genes coding for human melanoma-associated antigens that are specifically recognized by autologous cytotoxic T lymphocytes. Mage-1 antigen represents an example of this kind, the antigen that cannot be found on normal cells of adults, but can be detected on approximately 50 % of human malignant melanoma cells.[43]

Insertion of genes coding for substances that make tumor cells susceptible to chemotherapeutic drugs

The use of tumor "suicide" genes offers an additional approach to the treatment of malignant disease. The idea is to modify genetically tumor cells, and to render them vulnerable to therapy with systemically delivered chemotherapeutic drugs. This kind of application of genetic engineering in cancer treatment represents gene therapy in a classical sense. Moolten et al. quite early formed an idea of transferring the classically described "suicide" gene, herpes virus thymidine kinase (HSVTK) gene, into tumor cells to make them sensitive to ganciclovir.[44,45] Their starting point was the fact that normal mammalian cells are insensitive to ganciclovir owing to incapability of kinases (present in normal cells) to phosphorylate ganciclovir into toxic metabolites. On the other hand, HSVTK phosphorylates ganciclovir and its toxic metabolites inhibit DNA polymerase, thus impeding the elongation of DNA molecule. Therefore, the accumulation of toxic metabolites interferes with DNA synthesis, resulting in apoptosis and cell death. The mechanism of action in tumor cells may be analogous to the one in virally infected cells, yet the effect of toxic metabolites spreads out also on genetically unchanged (not producing HSVTK) tumor cells – i.e. bystander effect. The exact mechanism of bystander effect remains questionable, but anyway, there is a hypothesis that toxic metabolites may be released from the cells (where they were produced) in form of lyposomes to enter genetically unchanged cells and affect them as described above. Besides, the antitumor activity also may be achieved through indirect mechanisms that include the activation of immune system. An affirmation derives from the observation that the effect of therapy with tumor vaccines (prepared with gene coding for HSVTK) followed by ganciclovir treatment is less pronounced in immunosuppressed animals (athymic nude mice).[24] Short et al., as well as Culver et al., demonstrated the effectiveness of such system on intracranial tumors in experimental animals.[46,47] Namely, they transferred *in vivo* HSVTK gene directly into tumors using vectors (fibroblasts) and afterwards treated the animals with ganciclovir. Even though they demonstrated that only a small number of tumor cells incorporated HSVTK gene, ganciclovir successfully destroyed both the transfected and the nontransfected cells.

Clinical trials and prospects

Preclinical studies have demonstrated that gene therapy represents a new and provocative mode of treatment with great therapeutic potentials. The insight into the mechanisms of growth and growth regulation of tumor cells has offered multiple potential methods for genetic intervention. Up till now, more than 100 trials with genetically altered tumor vaccines or gene therapy studies have received approval in humans. Most of them are using autologous tumor cells transfected with genes encoding different cytokines.

One of the first tumor vaccines applied in humans was Rosenberg's vaccine using tumor infiltrating lymphocytes stimulated *in vitro* with IL-2 and infusing them to the patient with malignant melanoma, along with additional IL-2.[48] In this case genetic manipulation was not included in the preparation of the vaccine, but exogenous biological response modifiers were applied to augment the immune response against tumor cells.

Another variant of creation of tumor vaccines was presented by Schirrmacher et al., who were employing a two-component human cancer vaccine. The purpose of such a vaccine was simply to challenge the immune system by inserting some viral antigens into tumor cells, thus rendering the cells much more immunogeneic. The idea was based on the analogy with virally induced tumors which are known to be the most immunogeneic tumors in humans. As the specific component (bearing specific antigens) they used the closest possible match to an individual cancer of a patient, namely autologous cancer cells from resected primary tumor or metastases. The non-lytic virus NDV (Newcastle Disease Virus) was applied as the second, nonspecific component for infection of tumor cells. In two clinical studies the vaccines were applied

postoperatively in patients with no macroscopic remnant of tumor, but with a high risk of developing recurrent disease (colorectal carcinoma and breast cancer), while in another three studies the vaccines were applied in combination with biological response modifiers to patients with remaining metastatic disease: renal carcinoma, metastatic breast carcinoma, and metastatic ovarian carcinoma.[49]

As it was postulated before, presently there are many clinical trials with tumor vaccines going on and the studies of Rosenberg and Schirrmacher are the illustrations of only two different approaches to creation of tumor vaccines. Also it is worth mentioning that lately Rosenberg modified his concept for generation of tumor vaccines by introducing genes coding for IL-2 or TNF-α into tumor-infiltrating lymphocytes.[50]

However, the transfer of preclinical knowledge and technology into clinical practice is accompanied with certain difficulties. For now the major concerns with tumor vaccines are inappropriate expression of the transferred gene, as well as frequent adverse immunological reactions of the organism against genetically transformed cells. Certainly, it would be highly desirable if gene expression could be regulated in time, quantity and place, yet with the current vectors this is impossible. Newer delivery systems should incorporate features that permit tissue/cell specific expression and allow the level of gene expression to be regulated by exogenous small molecules administered as a conventional pharmaceutical agent.

In addition, when autologous tumor vaccines are used, another group of questions, which have to be solved, comes to light. Namely, the basic term for development of human autologous tumor vaccines is to establish primary cell cultures from patient's tumor specimens. Since this is a procedure, which is labour and time consuming, there was an idea to use allogeneic cells, stably transfected with cDNA of choice, instead of autologous tumor cells.[51] Although the idea is attractive, conventional immunology still dictates that autologous cells are far better for triggering an effective MHC-restricted immune response than allogeneic cells.

Finally, we also have to bear in mind that Hock et al. prepared a potent tumor vaccine without any kind of genetic manipulation to tumor cells.[39] Namely, in his experiments sublethally irradiated tumor cells admixed with *Corynebacterium parvum* had an immunogeneic activity by all means comparable to the one of genetically transformed cells.

Conclusion

This article is dealing with a field of great importance, extremely fast developing, and extremely wide – a fact that makes every general conclusion (become) obsolete in a very short period of time. Anyway, if we try to stress the major points, we have to admit that new biological approaches to treatment of cancer are of central importance not only for the treatment, but also for understanding of some basic rules governing antigen immune recognition, cancer metastasizing, bystander effect etc. Apart from some classical methodological problems that remain to be solved before final assessment of gene therapy and tumor vaccines validity will be given, there are also some social conventions that have to be changed. Namely, quite often are attractive ideas for biotherapy of cancer received with scepticism by established oncologists, and in the majority of cases, such therapy is acceptable only for a patient who has failed every conventional treatment. Such patients are by no means the best candidates for establishing an active immune response, and studies of this kind can hardly prove the validity of immune therapy.

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Sarcomatoid carcinoma of the thymus – a case report

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A 20-year-old female with a sarcomatoid carcinoma of the thymus invading the left upper lobe of the lung was treated with surgical resection and adjuvant radiotherapy. We report a case of this rare histologic variant of thymic carcinoma and review the literature.

Key words: thymus neoplasms; carcinosarcoma; sarcomatoid carcinoma.

Introduction

Sarcomatoid carcinoma of the thymus is a rare histologic variant of thymic carcinoma, which was named by Snover et al in 1982.[1] That group also suggested that a thymic carcinoma should fulfill the following criteria: (1) anterior mediastinal location and (2) absence of another primary tumor. We have reported 20 consecutive cases of thymic carcinoma in a 10-year period at our institute.[2] Among these 20 cases, no histologic variant of sarcomatoid carcinoma has been disclosed. We hereby describe a case od sarcomatoid carcinoma of the thymus that, microscopically, contains both a malignant epithelial component and a sarcomatoid component. The expression of cytokeratins and epithelial membrane antigen (EMA) in tumor cells could differentiate it from true sarcomas which do not stain for these markers.[3]

Case report

A 20 year old female presented with a six month history of increasing dyspnoea and left chest pain. On admission, physical examination revealed decreased breath sounds in her left upper chest. No lymphadenopathy was found. The full blood count revealed a haemoglobin of 13.7 g/dl, a white cell count of 7.8 x 10^9/l (neutrophils 7.2, eosinophils 0.2, lymphocyte 1.7), and a platelet count of 371 x 10^9/l. A chest radiograph demonstrated a big mass in the anterior aspect of the left lung. A computed tomographic (CT) scan of the chest showed a big necrotic tumour, measuring 14 x 12 x 12 cm in size, arising from the anterior mediastinum and invading to the left upper lung field (Figure 1). The serum titre of beta-choriogonadotropin (beta-HCG), alfa feto proteim (AFP) and carcinoembrionic antigen

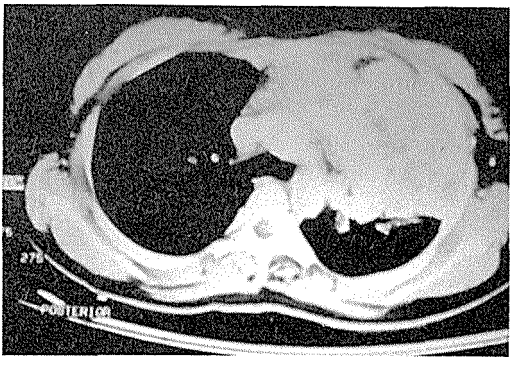

Figure 1. CT scan of the chest demonstrating a big mass arising from the anterior mediastinum and invading the left upper lung field.

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(CEA) were within normal limit. Sono-guided aspiration of the tumour was performed, and a cytological examination showed spindle cell tumour. 99m Tc-MDP whole body bone scanning and liver sonography showed no evidence of metastatic foci.

An operation was performed via standard posterolateral thoracotomy. While the tumour occupied the whole anterior mediastinum, its left lateral site invaded the left upper lobe of the lung. Removal of the mediastinal tumour with a left upper lobectomy of lung was performed. The postoperative course was uneventful, and the intercostal drain was removed on the fifth postoperative day.

Histopathological examination of the tumour revealed a custers of epithelial cells mixed with the strap-like spindle cells (Figure 2). An immunohistochemical study showed a positive staining for cytokeratin in the epithelial area and in some spindle cells (Figure 3). The patient then received radiotherapy with a 6000 Gy tumour dose. There was subjective improvement of dyspnoea and chest pain, and the patient is currently alive 10 months after surgery with no evidence of tumour recurrence or metastasis.

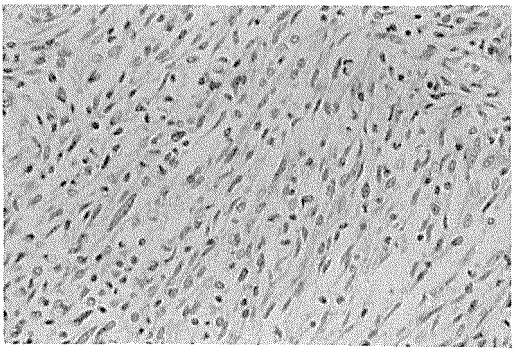

Figure 2. Cluster of thymic epithelial cells mixed with strap-like spindle cells (hematoxylin-eosin, x400).

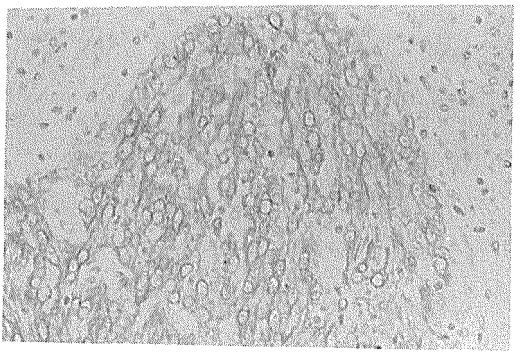

Figure 3. Sarcomatoid carcinoma of the thymus showing dark colouration in the epithelial area and in some spindle cells (peroxidase-antiperoxidase [PAP] staining with cytokeratin) (original magnification x400).

Discussion

Thymic carcinoma per se is a relatively rare tumour, with distinct pathological and clinical characteristics. There were eight histological variants of thymic carcinoma, reported in the literatures with sarcomatoid type among them.[1, 4] Various tumours showing the histological features of sarcomatoid carcinoma are seen also in other organs such as: lung,[5] pancreas,[6] kidney,[7] breast,[8] and urinary bladder.[9] However, sarcomatoid carcinoma of the thymus, as one of the histological variant of thymic carcinoma, has seldom been reported. The clinicopathologic features of the reported cases are summarized in Table 1. Clinically, this tumour mostly occurs in middle or in old age, similarly to the other variants of thymic carcinoma. To our knowledge, this is the youngest case reported in the literature.

In our previous study of 20 cases of thymic carcinoma, we found that invasion of the mediastinal stuctures is almost always present, including the inominate vein, mediastinal pleura, pericardium, and lung.[2] As compared with thymoma, thymic carcinoma has a more invasive tendency on computed tomographic scan examinations, and most of the patients have clinical symptoms caused by tumour compression of the mediastinal vital structures.[2, 4]

In general, thymic carcinoma are immunoreactive to EMA and cytokeratin, but not reactive to AFP, beta-HCG, placental alkaline phosphatase, or common leukocyte antigens.[11–13] Snover et al suggest that the presence of keratin within the spindle cell component can justify the use of the term "sarcomatoid carcinoma".[2]

In one case, initially, germ cell tumour was highly suspected, but a subsequent study of a series of tumour markers disclosed no elevation serum titre of beta-HCG, AFP and CEA.

During operation, we found that the space-occupied mediastinal tumour invaded the left upper lobe of the lung, but fortunately, the hilar structures such as the left upper lobar bronchus, superior pulmonary vein, and pulmonary artery branches to left upper lobe of lung were pushed laterally by the tumour, and total removal of the tumour with a lobectomy could be performed without difficulty.

Table 1. Reported cases of sarcomatoid carcinoma of the thymus.

Year/Author	Age/Sex	Symptoms	Location/Size/Invasion	Therapy	Follow-up
1982/Snover et al[Ref. 1]	64/M	Asymptoms	Ant. mediastinal/ 6x5x4.4 cm/–	Excision	died with metastasis at 13 months postop.
1982/Wick et al[Ref. 4]	53/M	Chest pain, dysphagia, SVC syndrome	Ant. mediastinum/?/SVC	RT & CT	died with metastasis at 28 months postop.
1992/Morita et al[Ref. 10]	53/M	Asymptoms	Ant. mediastinum/? lung, pericardium	Excision	?
1996/Hsu et al	20/F	Chest pain, dyspnoea	Ant. mediastinum/ 14x12x12 cm/ lung	Excision + RT	alive 10 months postop.

SVC – superior vena cava, RT – radiotherapy, CT – chemotherapy

There is still a limited experience in the management of thymic carcinoma. Complete resection of these tumours is sometimes difficult because of the presence of invasion of the mediastinal structures. However, surgical resection should be attempled whenever possible to decrease the tumour burden. The role of postoperative irradiation in the treatment of sarcomatoid carcinoma of the thymus is unknown because of limited experience in this field. In our previous study of thymic carcinoma, we showed that pathological stage, type of resection, postoperative radiotherapy, and cell type did not indicate a significantly favorable result.[2]

We presented a 20-year-old patient with a giant tumour, biphasic histology and with evident disease after surgery. She was believed to be at high risk of recurrence. Hopefully, complete resection and adjuvant radiotherapy in this patients can lead to a more favorable outcome.

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Influence of exogenous hormones on the recurrence and progression of cancer

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In the last few years, hormone replacement therapy (HRT) has become widely used everywhere in the world. Apart from favourable effects of HRT, such as a significant decrease in the psycho-physical menopause-related difficulties, lower mortality due to cardiovascular diseases and lower incidence of osteoporosis, some questions related to possible association between the use of this therapy and the rise, recurrence and progression of cancer have not been resolved yet. According to the majority of studies published so far, HRT is not associated with an increased risk of the onset, recurrence and progression of cancer. Moreover, some findings even indicate the possibility that HRT might exert a protective effect against the rise, recurrence and progression of cancer.

Key words: estrogen replacement therapy; neoplasms, breast-neoplasms; menopause

Introduction

In the 60's, the use of hormone replacement therapy was found to have increased considerably. However, in the 70's, this treatment again became less popular, when it was established that the use of exogenous estrogens was associated with 3-4 times higher risk of endometrial carcinoma. Later on it was found that no such risk was present when exogenous estrogens were combined with progestagens. In view of these new findings, the use of HRT is such a combined form underwent another increase in the 80's. Copious (also controversial) information provided by recent studies pointed out that possible correlation between HRT and the onset and progression of cancer should be studied in detail. Therefore, to our knowledge, the first international seminar dedicated to this pressing issue was organized in 1995 in Budapest.[1]

Our report deals with the effectiveness of HRT and the findings on its presumed influence on the recurrence and progression of cancer. The critical review has been completed with a preliminary report of our own findings, as well as with the latest recommendations related to the use of exogenous sex hormones.

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The influence of HRT on the recurrence and dissemination of cancer

In women treated for cancer, HRT in menopause is associated with two hormone-dependent entities which are important in oncology, i.e. the influence of pregnancy on the recurrence and progression of cancer, and the influence of oral contraceptives on cancer occurrence. In both instances, the concentrations of sex hormones in the blood of affected women exceed normal values (average cut-off values). It had long been believed that both situations entailed a particular danger: thus, pregnancy in women treated for cancer could accelerate an onset of recurrence and progression while oral contraceptive use were associated with a greater probability of cancer occurrence, particularly that of the breast. However, many studies published so far have discarded these hypotheses.[2]

Despite the scarce data, the influence of HRT has been most extensively studied in patients treated for breast cancer. It is an indisputable fact that breast cancer is the most frequent cancer in females. Apart from that, it is also well known that early psycho-physical symptoms of menopause, such as depression, phobias and psychic instability, which all may lead to severe disharmony in the patient's family and surroundings, as well as blushing and night sweating, are frequently more apparent in breast cancer patients than in those with other types of cancer.[3] It is believed that the more severe menopausal changes seen in patients who have undergone surgery for breast cancer could be attributable to the loss of this psychologically important and typically female external organ. We presume that these patients had more frequendy psyhyatrical treatment than others.

Considering the fact that elsewhere also HRT was indicated only in patients with severe climacteric problems, mostly owing to the suspected risk for cancer progress. The scientific evidence collected so far is relatively scarce. None of the retrospective studies published has been able to associate an increased risk of cancer recurrence or progression with the use of HRT.[4] Moreover, in the most recent report by Eden et al. published in 1996, it has been suggested that HRT might even exert a protective effect in this respect.[5]

Some epidemiological data indicate that despite

– the risk associated with a greater number of menstrual cycles and the related events,

– laboratory evidence on the influence of estrogens on the accelerated growth of mammary cells, and

– clinical effectiveness of tamoxifen (a selective estrogen antagonist) in patients with metastatic breast cancer,

The following facts should not be ignored:

– not all postmenopausal breast cancer patients have a better prognosis than premenopausal ones,

– after completed chemotherapy, the prognosis of premenopausal breast cancer patients with regular menstrual cycles is not worse than that of those without restored ovarian function;

– after two months of tamoxifen therapy, premenopausal patients present with elevated serum estradiol levels;

– among premenopausal breast cancer patients OC users do not survive worse than non-users of oral contraceptives.

It has been established that some stromal, fatty and carcinoma cells of the breast have the potential of synthesising estrogens locally from androgens, a pre-stage of estrogens. We presume that the level of local estrogens in the breast is independent of serum estrogens. The very local values of estrogens seem to be most relevant for the onset and metastasizing of breast cancer. It is also believed that tamoxifen reduces the levels of estrogens in the breast and metastatically changed cells, mainly through the competitive binding to estrogen receptors. Tamoxifen reduces cell proliferation. Given in low doses, it exerts a cytotostatic effect (increased G1 phase of the cell cycle resulting in a prolongation of cell division phase) while in high doses it is cytotoxic (arrest of G1 phase and cessation of cell division). We presume that the antiproliferative effect of Tamoxifen is also expressed through growth factors or C protein kinase inhibition.[2] Besides being estrogen antagonist, Tamoxifen is also estrogen agonist prevailingly active in the liver, bones and endometrium. While Tamoxifen reduces the risk of the onset of thromboembolic conditions and osteoporosis, it does not alleviate menopausal vasomotoric disorders. Moreover, it has been found that in 15-20 % of cases Tamoxifen might even worsen these symptoms. The results of some studies have shown that a long-term use of Tamoxifen may increase by 3-5 times the risk of endometrial carcinoma.[4]

We still cannot provide a conclusive answer to the question about the role of hormone receptors in breast cancer patients. Also, the treatment of patients with metastatic breast cancer hides many unresolved questions. The fact is that an equally long 30 % remission of the disease can also be obtained by adding some estrogens and progesterones in postmenopausal patients with positive estrogen receptors.[1]

The least known, but – according to some reports – perhaps very important is the effect of progesterone on the breast.[6, 7] We suspect that the effect, which is believed to play a protective role in the rise of breast cancer, is very complex and dependent on several factors such as the type, dose and duration of progestagen use. While a short lasting progestagen use should exert a cell-proliferative effect, a long-lasting or continuous use should result in antiestrogen, antimitotic and antiproliferative effect.[4] Most investigations are centred particularly on this.

So, let us conclude our report on hormone replacement therapy in patients treated for breast cancer with the statement made by the Breast Cancer Committee of the East Oncology Group: There are many facts which speak in favor of the belief that estrogen therapy in breast cancer patients is safe. Therefore, the time has come for a change![1]

There are but few reports on the use of HRT in patients treated for other cancers, and therefore any critical conclusions in this respect would be premature. According to the scarce preliminary reports, HRT is not associated with an increased risk of cancer dissemination; moreover, some of the reports even indicate a protective effect of HRT, which is reflected in a lower rate of progression and better survival results.[1]

HRT in patients treated for cancer at the Institute of Oncology in Ljubljana. Preliminary results.

In our patients treated for cancer, HRT was indicated only when menopausal problems were so severe that they were regarded as life threatening.

A revision of the data on patients treated for cancer at the Institute of Oncology, and receiving HRT was started in February 1996. Up to now, complete data have been collected for 25 patients. The average duration of HRT treatment was 38 months (4-120). In 22 cases both hormones, i.e. estrogens and progesterones, were used in accordance with the well known protective role of progestagens. In 21 patients, HRT (Cyclomenorrete, Trisequens, Trisequens f tablets) was applied in four week intervals, while the remaining patients received hormones (Gynodian depot injection and Dabroston tablets) in 6-8 week intervals or even less frequently. The uterus was surgically removed in 15 patients. Twenty-four patients were free of recurrence. Progression of the disease was established six months after HRT in one patient only; she had a highly malignant leiomyosarcoma of the uterus. With respect to the histologically verified highly malignant tumor, the progression was expected, and was therefore detected relatively early, when the patient was still asymptomatic. In this patients HRT was given in 6-8 week intervals. After the diagnosis of recurrence, the patient was reoperated, and has been without evidence of the disease 10 months since the primary therapy. She was further maintained on HRT because of severe menopausal problems.

Eight of 25 patients were treated for breast cancer. Two had metastases in the axillary lymph nodes, 5/7 had negative hormone receptors while in two patients these were positive: one patient had estrogen- and the other one progesterone receptors. All those 8 patients received estrogen & progesterone based HRT in the duration of 2 years on average. The mean delay from breast surgery to the start of HRT was 4 years, after a non-hormonal treatment had failed and the menopausal problems got progressingly worse.

Other patients who received HRT had been previously treated for cancer of the reproductive organs, Hodgkin's disease, NH lymphoma or carcinomas of the thyroid and rectum.

So far, none of the patients followed up for 4-120 months has presented with progression.

Conclusion and recommendations

Recommendations for HRT in women at an increased risk of cancer, and in patients treated for cancer.

1) In women who are believed to be at a higher risk of cancer than the rest of normal female population, HRT is indicated only in the presence of menopausal problems, and not as prevention of thromboembolic conditions or osteoporosis.

2) In menopausal patients treated for cancer, HRT is indicated only in the case of severe menopausal problems.

3) In accordance with internationally accepted guidelines, HRT with estrogen alone is indicated in patients who have undergone hysterectomy or had their uterine mucosis destroyed by radical irradiation. In all others estrogen & progesterone based HRT should be used.

4) Prior to the administration of HRT, the patient should undergo a gynecological check, clinical breast examination and mammography. Regular follow up, including gynecological check and clinical examination of the breast, should be carried out every six months, while control mammographies should follow the routine set by the accepted guidelines for early detection of breast cancer.

5) In order to get a more comprehensive overview of the state of the art regarding HRT, further studies are also required in Slovenia, which would hopefully yield results that could prove useful at a national as well as international level.

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Metastases to the breast from melanoma: a rare manifestation of an unpredictable malignant disease

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Cancer metastases to the breast are not frequent. The most common among them are those originating from melanoma.

The course of this disease is often unpredictable, but the changes in the menstrual status may in some patients induce local changes in the breast that facilitate the growth of melanoma metastases. These changes are probably caused by physiological changes in serum estrogen levels, with the causes behind trafficking of melanoma cells to the breast remaining unclear. Patients with melanoma metastases to the breast will be encountered more frequently, as the incidence rate of melanoma increases worldwide.

These metastases usually manifest themselves as palpable mobile masses. Mammographic findings are of one or more rounded, well-circumscribed lesions with slightly irregular margins. The palpable and the mammographic dimensions of these lesions are usually closely correlated. Fine needle aspiration cytology of described lesions is a quick, safe and highly accurate diagnostic procedure.

Surgical excision is the appropriate treatment that provides local control with or without adjunctive chemo- and immunotherapy. Although mastectomy has not improved survival, it is sometimes required if the tumor is bulky, deep-seated, or painful.

Key words: breast-neoplasms-secondary; melanoma, melanoma-secondary; carcinoma-diagnosis; carcinoma-treatment

Introduction

Cancer metastases to the breast are not frequent, with the exception of those from contralateral breast.[1] They represent 2.7 % of all malignant breast tumors.[2] In a series of women treated for breast tumors, less than 1 % had metastases to the mammary gland from other primaries,[3,4,5] whereas in autopsy studies the overall frequency of cancer metastases to the breast ranged between 1.7 and 6.6 %.[3,6]

Melanomas are among the most common primary sites.[1,4,5,7-11] As the incidence of melanoma is increasing all over the world,[12-14] and as approximately 20 % of patients will eventually develop metastases,[15] an increase in the incidence of melanoma metastases to the breast can be expected.

It is surprising that melanoma which originates in the skin and disseminates widely throughout the body, often predominantly in the skin, rarely metastasizes to the parenchyma of the breast which is a skin appendage.[11]

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Natural history of disease

Rapid growth is an important characteristic of cancer metastases in the breast.[1,16] Furthermore, cancer metastases to the breast are generally a harbinger of wide dissemination and fulminant course of the disease. Such patients thus show very short survival, usually less than 1 year.[1,5,9,17,18]

The nature of malignant melanoma is often unpredictable and no other tumor is considered so capricious in its dissemination.[11] No clear predisposing factors correlating with the development of melanoma metastases to the breast have been identified.[9] It is not clear, whether hormonal factors have any influence on the natural history of the disease. On the other hand, some reports suggest that the changes in the menstrual status may induce some local changes in the breast that facilitate the growth of the melanoma metastases situated there.[17,18] These changes are probably caused by physiological changes in serum estrogen levels.

It is interesting to note that estrogen therapy for advanced carcinoma of the prostate may also cause the growth of metastases in the breast and nipple.[19,20] Some data suggest that changes in hormonal factors correlate with the development of breast metastases in males with various cancers[4] and in young females with rhabdomyosarcoma.[21]

However, it was reported that the patients with melanoma metastases to the breast were mostly premenopausal women and some of them were pregnant.[4,17,18] Together with the data that low levels of estrogen receptors have been sometimes observed on melanoma cells,[22-24] these reports additionally suggest that there may be some hormonal influence involved in the trafficking of melanoma cells to the breast as well.

The role of estrogens and other hormones in the natural history of melanoma remains controversial. Extremely poor prognosis was reported for patients with melanoma developed during pregnancy[25] and in postmenopausal period.[26,27] On the other hand, some recent studies show that pregnancy does not activate cutaneous melanoma or latent melanoma metastases as well.[28,29] Possible adverse or beneficial effects of oral contraceptives, estrogen and progesterone on the history of melanoma were evaluated,[22,30-32] but the results of clinical trials with hormone therapy were disappointing.[23,24,33] Additionally, it seems that the better prognosis of females with melanoma, especially of premenopausal patients,[26,34] cannot simply be explained by the presence of steroids receptors, since they were found in male patients as well.[22]

A higher proportion of thin melanoma lesions in women may contribute to an overall better prognosis for them. Nevertheless, survival rates for women were still higher than for men, even when primary lesions were of similar thickness. But, when premenopausal women were matched with men by age and location and thickness of primary lesion, a marked female superiority still exists only for those patients with very thick lesions.[27]

Despite the fact that patients with melanoma metastases to the breast are mostly premenopausal women, their prognosis remains poor. There seems to be a certain barrier against metastatic dissemination in premenopausal women,[35] but it seems it is no more effective when melanoma metastases to the breast are observed in these patients.

The occurrence of melanoma in children is uncommon.[14,36] In the report on a 14-year-old girl with melanoma metastases to the breast and brain there is no record about her menstrual status.[36] It can be presumed that her pubertal period was connected with changes in her hormonal status that may have had some influence on the course of the disease. But, the rarity of melanoma before puberty may simply reflect absence of a carcinogenic stimulus and a long latent period.

Long median intervals between the initial diagnosis of primary melanoma and involvement of breast have usually been observed[3,18] and the longest interval of 11 years was registered in a patient who was pregnant at the time of diagnosis.[18]

The most common primary sites of melanoma associated with breast involvement are on the arms and trunk. This is contrary to the most common sites in premenopausal women, namely the lower extremities. There my be a direct lymphatic and vascular drainage from these sites to the breast and this can be regarded as one of the factors that influence the natural course of this disease.[18,27,37]

The factors behind the occurrence of the melanoma metastases to the breast remain more or less unclear, but it is possible that patients with this type of metastatic dissemination will be seen more frequency as the incidence of melanoma increases worldwide.[12,13]

Diagnosis

Most patients have a known diagnosis of carcinoma at the time of presentation with breast metastases.[9,16,17] Occasionally, a breast metastasis is the first manifestation of an occult primary lesion.[1,3,4,9,38,39]

Metastases to the breast usually manifest themselves as palpable mobile breast masses that are sometimes adherent to the skin.[1,5] Diffuse skin involvement or associated subcutaneous nodules can also occur.[8] The metastases may be multiple and

can be observed in both breasts.[3,5] Tumor dimensions do not help to distinguish between primary and metastatic cancer. Although it is claimed that metastases are usually smaller than primary tumors, metastases may eventually become huge in size.[39]

The classic mammographic finding (Figure 1) consists of one or more rounded, well-circumscribed masses with slightly irregular margins.[9,16,40,41] Microcalcifications are unusual,[8,9] but it should be stressed that the presence of microcalcifications does not rule out a metastasis.[39] Since a metastatic lesion does not cause a surrounding desmoplastic reaction in adjacent normal breast, there is typically a close correlation between the palpable size of the mass and its mammographic size.[1,9,16] However, the same size seen in clinical examination of breast metastases and mammography could lead to a difficult differential diagnosis with benign breast lesions such as cysts or fibroadenomas. This contrasts with primary carcinoma of the breast, in which the mammographic abnormality is often smaller than the palpable mass.

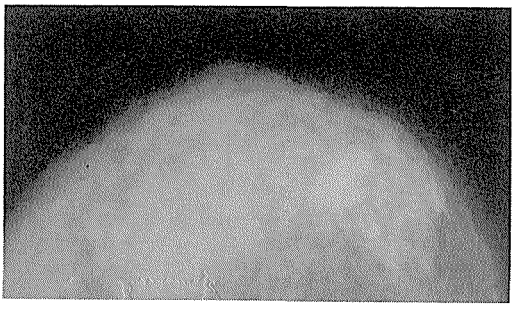

Figure 1. Patient with melanoma metastasis to the breast. Craniocaudale view of the left breast reveals two well-defined masses.

There is no doubt that mammography is the primary method to image breast tissue and evaluate specific breast lesions. As a primary technique for breast imaging, computed tomography (CT) compares poorly to mammography.[42] But, on occasion, breast lesions may be better visualized by CT than by mammography if the breasts are dense, or if the lesion is located adjacent to the chest wall.[43] The superb contrast resolution of CT allows a characterization of the density of the breast lesion and may augment the diagnostic possibilities. However, in most cases a recognition of a CT abnormality of the breast suggests the need for mammographic correlation.[42]

In young women ultrasound examination of breast should be done. We can usually see metastases to the breast as hypoechogenic nodules of different sizes, with regular margins and posterior attenuation of the posterior ultrasound beam.[40]

Particularly when the clinical evaluation is suggestive of metastatic disease, diagnostic fine needle aspiration biopsy may be confirmatory (Figure 2 and 3). The cell pattern in metastases of melanoma is generally pleomorphic.[44] Special stains (HMB45, S100, Warthin Starry)[18,44,45] and electron microscopy may be applied to this material as well to provide additional diagnostic information. Due to high diagnostic accuracy, fine needle aspiration cytology should be routinely practised as a quick and safe diagnostic procedure. It should be a very reliable method of distinguishing primary carcinoma from metastatic melanoma. Sometimes ultrasound guided and stereotactic fine needle aspiration biopsy should be applied if metastatic lesion is very small and not palpable.

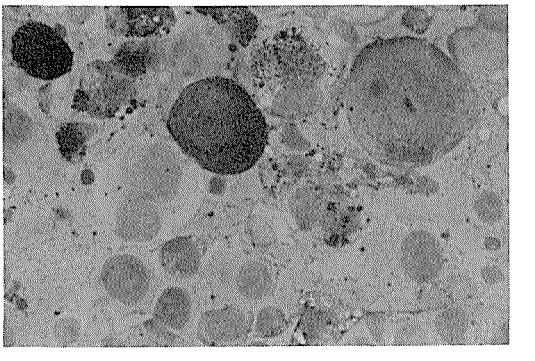

Figure 2. Fine needle aspirate of melanoma metastasis to the breast – epytheloid type (Giemsa, objective 40).

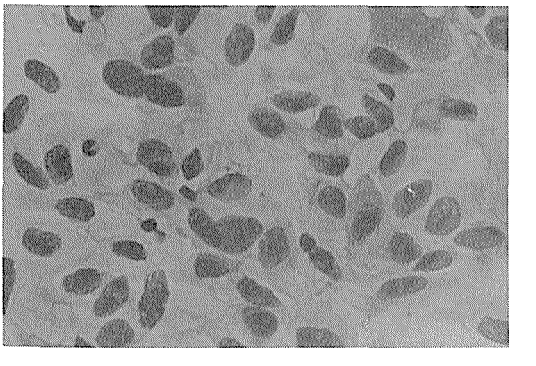

Figure 3. Fine needle aspirate of melanoma metastasis to the breast – fusocellular type (Giemsa, objective 40).

Open biopsy is rarely required for diagnosis if a primary breast tumor is not confirmed by the fine needle aspiration biopsy.[9,16,19,46-48] Until some years ago, the most helpful finding for identifying a metastatic malignancy in the breast was the recognition of the architectural pattern of the tumor, such as the presence of periductal infiltration without the coexistence of intraductal or intralobular carcinoma. But recently, immunohistochemistry has been suggested to differentiate metastatic carcinoma from a primary breast tumor in surgical specimens, and to avoid unnecessary radical surgery.[10,19] Further reports stress the importance of using a panel of immunohistochemical markers. For melanoma this should include at least two epithelial markers (i.e. BRST2, Human milk fat globulin 2 – HMFG2, CAM 5.2)[38,49] and at least two antibodies to melanoma-associated antigens (i.e. HMB45, S-100, NK1-C3).[38,50]

Occasionally, patients with breast metastases from melanoma were initially misdiagnosed.[4] Therefore for patients with a previous history of melanoma, however remote, the diagnosis of breast metastases in a premenopausal woman must be considered.[18]

Treatment

Accurate diagnosis of breast metastasis is important for avoiding unnecessary mastectomy and for implementing appropriate systemic therapy – since the metastatic disease is generally present at other sites as well.[1,3,16,51] This is particularly important if the lesion is the first sign of an extramammary malignancy.[9]

Excisional biopsy is usually the appropriate treatment and provides adequate local control with or without adjunctive chemo- and immunotherapy.[9,11,16,52] Conservative excision is recommended since mastectomy has not improved survival of patients with melanoma metastases to the breast.[11] Simple mastectomy is sometimes required if the tumor is bulky, deep-seated, or painful. Radical surgery should mostly be avoided unless needed for palliation.[1,3,5,16,17] In patients with terminal stage of disseminated melanoma, the best supportive care is mandatory.

There are no data as to in how many patients with melanoma breast metastases the axillary lymph nodes are involved. The involvement of axillary lymph nodes in patients with breast metastases from all extramammary malignancies taken together is not so rare. It was observed in about 14 % to 42 % of them, most frequently in those with lymphomas.[53,54]

In addition to surgical treatment, adequate systemic therapy for the melanoma should be instituted9 when possible in clinical trials. Although the response rate of breast metastases to systemic therapy is similar to that of other sites, the finding of breast metastases is regarded as a poor prognostic sign.[18]

The overall prognosis for patients with metastatic melanoma to the breast is poor with more than 80 % dying within one year.[5,9,16]

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Vegetarian diet and cancer

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Several components in plant foods, namely protease inhibitors, izoflavones, inositol hexaphosphate, phytosterols and saponins, inhibit a variety of tumors in various tissues.
It is clear that population consuming high levels of fruit and vegetables have low overall cancer mortality rates for the major cancers, common in the western hemisfere. There are many theories offering explanations for the variations in cancer frequency in different population.
The evidence obtained is not yet sufficient for any specific dietary recomendation and further work is needed to establish the role of these natural compounds in human health and disease.

Key words: vegeterianism, vegetarian diet; neoplasms

Introduction

Epidemiologic studies have suggested that diet is an important environmental factor involved in the etiology of the most prevalent forms of cancer.[1] There is enough evidence from case -- control, correlation and cohort studies to show that high meat and fat consumption increase the risk of developing cancer, whereas consumption of diets high in cereals, fruits and vegetables reduces the risk.[2]

Over the last few years there has been an increasing body of literature suggesting that vegetarian diet may be protective against cancer.

Studies of diet and cancer are limited by the fact that cancer is a group of several different diseases developing over decades in a multistage process involving both initiation and promotional events.[3]

All epidemiological studies are dependent on variability in both risk factor and rates of disease as evidence for environmental influences on diseases. Especially important are variations among migrant groups and rapid changes over time, because changes within a few decades are unlikely to be caused by genetic mutations. For instance, rates of breast cancer, thought to be related to high fat diets, are lower in Japan than in the United States and increased among Japanese migrating to Hawaii. The rapidity with which cancer rates change is thought to reflect the time in life at which the risk factor is influential. Thus, the risk of colon cancer changes within a few decades of migration, while the risk of breast cancer does not increase until the second generation implying a dietary effect early in life.[4]

Even where diet can be measured prospectively in population with sufficient variation over time, cross – correlations among dietary variables have rendered indentification of specific dietary risk factors extremely difficult. Thus, ecological and migration studies relating fat to risk of breast cancer rates have been unable to separate effects of total fat from cholesterol, saturated fat and low fiber intake or from protective factors present in fruit and vegetables.[5,6]

Many epidemiological studies, including migrant studies, support the view that the Western diet is one of the main factors causing the high incidence of the so – called Western diseases, including the major hormone – depending cancer, colon cancer and coronary heart disease.[1,2,4-6]

Western diet, compared with the vegetarian or semivegetarian diet in developing and Asian Coun-

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tries, may alter hormone production, metabolism or action at the cellular level by some biochemical mechanisms. The compounds, which mainly occur in legumes, fruits, vegetables, whole – grain products and various seeds, have now been shown to influence not only sex hormone metabolism and biological activity but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation and angiogenesis supporting their role as cancer protective compounds.

It is clear that population, consuming high levels of fruits and vegetables, have low overall cancer mortality rates for the major cancers, common in the western hemisfere. There are many theories offering explanations for the variations in cancer frequency in different populations.[7]

Lee et al[8] compared 200 Singapore Chinese women with histologically confirmed breast cancers with 420 matched control subjects. In the premenopausal women, high intake of red meat was associated with increased risk for breast cancer. He concluded that decreased risk was associated with intake of soya products. It has been known that diets rich in legumes and fiber[8] decrease cancer risk relative to those diets which include animal products.[9]

Recently, the consumption of soy products has been associated with low rates of hormone dependent and hormon – independent cancers. Asians, who consume 20-50 times more soy per capita than Americans, have lower incidence and death rates from breast and prostate cancer.[8,10]

The current discussion will outline general issues in epidemiologic studies of diet and cancer relevant to vegetarian diet, summarizing the current epidemiologic literature relating cereals, fruits and vegetables intake and cancer risk.

Food constituents known to have anticarcinogenic activity.

Certain normal dietary components have the potential to affect carcinogenesis,[11] either by action as a carcinogen or tumor promoter or by modifying the action of such agents. Although deliberate addition of carcinogenic agents into the diet is legislatively forbidden, various agents, such as fats, and components produced during cooking, notably pyrolysis products, or storage, oxidized fats, have some carcinogenic or promoting potential. However, many more agents in the diet appear to inhibit carcinogenesis. Some of these reduce metabolic activation or enhance detoxification of carcinogens, other may protect against the attack of electrophilic carcinogens on DNA and still other seem to have an antitumor – promoting effect on cells. The role of chemoprevention appears to lie in reversal of the premalignant process rather than supression of malignant growth.[12]

Epidemiological evidence has related decreased cancer risk to increased consumption of phytoestrogen and lignans in a vegetarian diet.

The lignans and isoflavonoid phytoestrogens are normal constituents of human urine, plasma and feces and occur in large amounts in plasma, urine and feces, particularly in vegetarians, in subjects consuming large amount of whole – grain products, vegetables, berries, fruits, linseeds and sesame seeds[13]. The same findings can be applied to the Chinese and the Japanese, whose traditional diet contains similar food.[13] Plasma levels are higher in vegetarian women compared with omnivarous women, correlating negatively with rates of breast cancer risk.[14]

Plant food contains, in addition to the traditional macronutrients, a wide variety of microcomponents such as enzyme inhibitors, phytosterols, indoles, flavones and saponins. These micro components are known to be biologically active. Their role in the prevention of chronic diseases is currently being investigated. [4,7,15]

According to one hypothesis, the anticancer effect of soy products and other legumes is due to the presence of the isoflavone genistein in legumes. Isoflavones are weak estrogens and can function both as estrogen agonists and antagonists depending on the hormonal milieu and the target tissue and species under investigation. Genistein, one of the two primary isoflavones in soybeans, whole – grain products and various seeds, has attracted much attention from the research community, not only because of its potential antiestrogenic effect, but because it inhibits several key enzymes thought to be involved in carcinogenesis.[16,17]

The list of the tumors, responding to hormonal influence, is usualy limited to breast. endometrium, prostata, thyroid, pituitary and ovary, and to certain categories of leukemias and lymphomas.

The list should be considered temporary, because many specific growth factors (insulin, insulin – like growth factor, platelet – activating factor) have been shown to influence the growth and biological evolution of many normal and neoplastic cells and tumors.[17] Receptors for hormones (steroid hormone) and factors are found in many tumors but their precise role in modulating tumor growth is not yet fully and completely understood. Phytoestrogens

and, in particular isoflavones in soybean, possess binding capacity to many of these receptors and complete with the physiologic hormones and/or growth factors bind to them. By doing so isoflavones may, by virtue of this competitive celular binding, play a role in the modulation and evolution of neoplastic growth.[18] Lignans and isoflavonoides also seem to stimulate sex hormone binding globulin synthesis in the liver and in this way they may reduce the biological effects of sex hormones.[13,19]

They decrease the relative amount of free testosterone and free estradiol and reduce both the albumin – bound and the free fraction of the sex hormones. This reduces the metabolic clereance rate of the steroids and thereby lowers their biological activity. Subjects with breast cancer or those at high risk of breast cancer, omnivorous women living in Boston, excrete low amount of lignans and isoflavonoids.[20]

In Finland the lignan excretion is mainly associated with the intake of grain fiber or whole – grain products. Intake of fruits and berries in Finnish women also has a positive correlation with lignan excretion. Berries contain the seeds of the plant and these may be rich in lignan precursors.

In Japanese subjects lignan excretion shows the strongest correlation with the intake of whole soybeans.[13,20]

The significantly positive association between lignan excretion and intake of whole – grain products and total fiber is altered by the intake of various seeds with relatively low fiber but high content of lignan precursos. This occurs particularly in vegetarians.

Another factor, affecting the association between intake of whole – grain products and lignan excretion, is the fact that many subjects consume purified grain fiber directly or in the form of whole – meal bread which is a mixture of bran and white meal containing only small amount of meal from the aleurone layer of the grain where the lignan precursors occur. Also the preparation of tofu products seems to eliminate the lignan precursors from the beans.

Intake of fiber – rich food definitely affects the plasma levels of estrogen, increases phytoestrogen intake, but also reduces energy intake which may be an important risk – reducing factor.[13]

The well known therapeutic effect of estrogens in prostatic cancer suggests that phytoestrogens may inhibit prostatic cancer cell growth during the promotional phase of the disease or they may influence differentation as shown for genistein with leukemic cells and other cancer cells. Despite high fat intake, the prostatic cancer incidence in Finland is much lower than in USA but higher than in Japan.[21] The higher production of lignan in the gut, due to relatively high intake of whole – grain products, particularly rye bread, in the low – incidence rural areas in Finland, may perhaps explain this phenomenon.

Lignan excretion is also higher in Finish subjects living in areas with lower colon cancer risk.[21]

Epidemiological evidence obtained in Japan points to lower colon cancer incidence in areas with high tofu consumption.[22]

High concentrations of genistein in urine of vegetarians suggest that genistein may contribute to the preventive effect of plant – based diet on chronic diseases, including solid tumors, by inhibiting neovascularization (angiogenesis, angiogenesis diseases) and tumor cell proliferation.

Genistein reduces the incidence of tumors or other angiogenic diseases like rheumatoid arthritis, psoriasis, and diabetic retinopathy.[22]

Soy products that contain isoflavonoids and lignans may also play a role in the prevention of several types of cancer.

The concentration in plasma of these compounds may easily reach biologically active levels without toxic effect. By inhibiting the effect of growth factors and angiogenesis, genistein may be a general inhibitor of cancer growth. By modulating drug transport, genistein may prove to be a good addition to the established cancer therapy. The described biological effect may also be used as a preventive strategy for other western diseases not discused in this connection, such as cardiovascular diseases and osteoporosis, due to the estrogenic and antioksidative effect.

Saponins, which are present in plants, have been suggested as possible anticarcinogens. Legumes such as soybeans and chickpeas are a major source of saponins in the human diet.[23] They possess surface – active characteristics that are due to the amphiphilic nature of their chemical structure. The proposed mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune – modulatory effect, bile acid binding and normalization of carcinogen – induced cell proliferation[24].

The biological activity in *ginseng* is largely attributed to the triterpanoid saponins (ginsenoides), which constitute 2-4 % of ginseng's dry weight. Ginseng is widely used in Oriental medicine for treatment of cancer, diabetes and hepatic and car-

diovascular diseases. Growth inhibition and reverse transformation of B_{16} melanoma cells were observed with ginsenosides treatment.[25,26]

Phytic acid, inosital hexaphosphate is ubiquitous in the plant kingdom and is abundant in cereals and legumes. Because phytic acid is high in high – fiber diet, the epidemiologic observations showing high – fiber diet, are associated with a lower incidence certain kinds of cancer.

It reduces cell proliferation and increases differentiation of malignant cells often resulting in reversion to the normal pheno type.[27]

Studies report that consumption of soy protein diets inhibits the growth of various tumors in rats. The inhibitory effect has been attributed to the phytoestrogens or protein kinase inhibitor in soy protein products.

Recent studies indicate that additional factors in soy protein products may also contribute to the inhibition of tumorgenesis, namely the deficiency of the essential amino acid methionin. Metastatic growth of a primary rhabdomysarcoma lung tumor was inhibited by adopting a soy protein diet.[12]

Conclusion

In conclusion, it has been established that several components in plant foods, namely protease inhibitors, izoflavones, inositol hexaphosphate, phytosterols and saponins, inhibit a variety of tumor in various tissues in the animal model. Current studies also indicate that the lower amount of methionin in soy protein compared with casein may be important in selectively retarding the growth of tumors.

The evidence obtained is not yet sufficient for any specific dietary recomendation and further work is needed to establish the role of these natural compounds in human health and disease.

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Prevention of fertility disturbances in oncological male patients

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The modern therapeutic approaches to oncological patients are not only aimed at cure but also ensuring the least possible side effects and the optimal quality of life which naturally includes preserved fertility.
The most effective measure to prevent the occurrence of surgery-related damage of fertility in males is by replacing radical retroperitoneal lymphadenectomy with selective or unilateral lymphadenectomy; radiotherapy-related fertility damage can be prevented by shielding the remaining testicle from the scattered radiation; and chemotherapy-related fertility damage can be prevented by choosing the chemotherapeutic regimen which doesn't contain alkylating agents.
We have to consider other modalities of prevention, including eliminating lifestyle and environment factors which can influence fertility, adequate sexual behaviour, early treatments of cryptorchidism and treatments of infections.

Key words: infertility, male - prevention; neoplasms; radiotherapy - adverse effects; antineoplastic agents - adverse effects; lymph node excision - adverse effects

Introduction

The modern therapeutic approaches to oncological patients are not only aimed at cure but also ensuring the least possible side effects and the optimal quality of life which naturally includes preserved fertility.[1]

The best approach to reduce the sterility problems is to decrease the chance of occurrence of infertility[2] since the efficiency of treatment is uncertain and unpredictable.[3]

In case of fertility impairment several methods of treatment exist. Following cancer treatment fertility status is also frequently unpredictable, it is therefore advisable for young male patients to store their deep-frozen sperm in a sperm bank prior to oncological therapy.[4-8]

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Prevention of surgery-related damage

It is important that patient with testicular tumours are operated transinguinally but not transscrotally, otherwise the ipsilateral scrotal sac and inguinal lymph nodes must be postoperatively irradiated, which also means more scattered radiation to contralateral testis.[1,9]

Radical retroperitoneal lymphadenectomy was usually performed on patients with testicular nonseminomatous tumours and Hodgkin's diseases[10-12] and the most effective measure to preserve fertility in males is by replacing radical retroperitoneal lymphadenectomy with selective or unilateral lymphadenectomy.[13,14] In patients with such operations it is possible to preserve emission and ejaculation, most of semen analyses in majority of patients are considered to be in normal range and most of them are potentially fertile.[13] In this way the modified retroperitoneal lymph node dissection preserves the sympathetic outflow, retaining ejaculation in 75% of these men.[15]

In spite of the fact that fertility is usually not the central problem in patients with prostate cancer because of their advanced age, early detection of this disorder allows less extensive surgery, with fewer complications. For example, nerve-sparing radical retropubic prostatectomy, which preserves erectile function in many men by avoiding injury to the cavernosal neurovascular bundles, can be performed and prevent fertility disturbances.[16,17]

Prevention of radiotherapy-related damage

The most effective measure to prevent the occurrence of radiotherapy-related fertility damage can be by shielding the remaining testicle from scattered radiation.[1,9,18]

Having already had unilateral semicastration prophylactically the retroperitoneal nodes only are treated and the irradiation of the remaining testicle should be avoided. The testicle is in any case exposed to the scattered radiation causing a degree of spermatogenesis impairment.[19-21] By shielding the remaining testicle gonadal dose should be kept lower than 1 Gy in order to avoid prolonged and frequently permanent azoospermia or hypozoospermia.[10,22,23]

In Hodgkin's disease, when we irradiate the infradiaphragmatic region, the shielding of both testicles from scattered radiation is necessary. Contemporary radiotherapy techniques recommend the protection from scattered radiation, in case of gonadal doze higher than 0,5% .[18]

Attempts were made to reduce postirradiation injury by reducing the size of target volume[24,25] and thus decreasing the scattered radiation and in the same time increasing the distance between the target volume and testes.[19,26] Additionally, reducing the tumour dose or even by the omission of the postoperative irradiation[24,25] radiation side-effect can be avoided altogether.[27] Disease free survival is reduced and further studies are necessary to find patients where management by surveillance alone should be sufficient.[27-31] Nevertheless, it is important to irradiate patients with high energy photons because scattered radiation is reduced in such treatment.[19]

Prevention of chemotherapy-related damage

Chemotherapy-related fertility damage can be prevented by choosing the chemotherapeutic regimen which doesn't include alkylating agents.[32,33]

Chemotherapy with alkylating agents is associated with fertility problems in 60% of patients regardless whether it is given in combination with radiotherapy or not.34 Cisplatin, one of the most efficient agent, has fortunately only moderate damaging effect on spermatogenesis.[5,35]

Limiting the number of cycles administrated to the minimum for achieving remission is also beneficial for preserving male reproductive ability.[5,36]

Cytoprotective techniques to limit testicular injury from the damaging effect of chemotherapy are currently ineffectual. The gonadal toxicity caused by chemotherapy and radiation was attempted to be reduced by luteinizing hormone releasing hormone (LHRH) analogues. It has been shown that non-pulsatile, chronic treatment with supraphysiological doses of LHRH analogues results in the suppression of the pituitary-gonadal axis and the suppression of spermatogenesis. Furthermore, there has been suggested that the inhibition of spermatogenesis during exposure to cytotoxic drugs and radiation might reduce or prevent gonadal toxicity. Experimental studies were encouraged,[37] but in none of clinical trials the influence on severity and duration of spermatogenesis impairment has significantly been shown.[38-40]

The administrations of some other drugs (i.e. antioxidants N-acetylcystein and ascorbate) before the administration of procarbasine have been effective by preserving spermatogenesis in an animal model. But the analogous studies in humans have not been published.[5,41]

During the oncological treatment there were also attempts to reduce spermatogenesis by administrating of testosterone which can suppress gonadotropin secretion and in this way protect testicular function. There is no clinical relevance up to now as well.[42]

Others principles of prevention of fertility disturbances

As the causes of fertility disturbance are manifold[10] we have to consider all other modalities of prevention, including the elimination of lifestyle and environment factors which can affect fertility, more appropriate sexual behaviour, early treatments of cryptorchidism and treatments of infections.

Elimination of lifestyle factors

One of the most important steps of the prevention of fertility disturbances is to eliminate all factors

that can affect fertility, such as bad nutrition,[43,44] cigarette smoking,[45] alcohol abuse,[46] use of illicit drugs such us marijuana and cocaine,[43,44] and some drugs as anabolic steroids, antihypertensive medications, cimetidine, antiholinergic drugs, etc.[10,47]

Elimination of another environment factors
Oncological patients should not work with arsenic and lead. They should avoid exposure to heat, such as in saunas or working as plumbers and cooks.[10,48]

Sexual behaviour
According to Howards, sexual intercourse is recommended every 48 hours in the middle of woman's cycle or at the time of ovulation.[43] Lower frequency of intercourse can result in missing the ovulation time, and also diminish the quality of the sperm (over 5 days of abstinence). In view of contradicting reports, namely, that frequent intercourse diminishes sperm concentration[47] or even improve it,[49] it should be cautiously recommended more frequent intercourse during the time of woman's ovulation if their libido is adequate.[4,49]

Treatments of cryptorchidism
Cryptorchidism is first treated with gonadotropines (HCG, LH-RH) and later with orhiopexy.[47,50] Orhiopexy must be performed between the age of 5 and 9 or else as soon as possible. Some argue that cryptorchidism should be cured by the age of 2, before histological changes occur,[50,51] but at any rate prior to puberty.[47] Adequate treatment reduces the chances of sterility, and also the incidence of malign alteration of testicles.[52,53] Only 75% and 50% fertility rate is reported for patients with successful unilateral and bilateral orhiopexy. The results, however, considerably improve if the procedure is performed before the age of 2. Hormonal treatment is recommended to start at the age of 10 months.[50]

Treatments of infections
It is imperative that uro-infections are treated effectively, preferably with regards to antibiogram of the agent.[43] Trichomonas or gonorrhoea require that the sexual partner is treated as well.

Conclusion

Multimodality treatment has increased the survival of cancer patients in recent years. The quality of life should also be taken into consideration during the cure. The maintenance of the reproductive capacity is of great concern to many young patients. The cause of sterility was attributed to the long-term side effects of oncological treatment in spite of the fact that the step of fertility disturbances can be decreased by the selected treatment. One of the most important steps of prevention of fertility disturbances is also, if possible, to eliminate all factors that can influence the fertility.

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Monte Carlo Simulations of a metal/a-Se Portal Detector

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The detector response, modulation transfer function (MTF) and quantum detective efficiency (DQE) of four amorphous selenium (a-Se) based image receptors have been calculated through Monte Carlo simulations of x-ray absorption. As part of a preliminary study on electrostatic portal imaging, the effects of receptor geometry and composition on the imaging characteristics of a-Se in the megavoltage range have been investigated. Our results indicate that the DQE increases as the a-Se thickness while the MTF decreases slightly, and a front metal plate can enhance detector response, DQE and MTF of a-Se receptors at the detail level relevant to portal imaging.

Key words: Monte Carlo method, portal detector; selenium; radiography-methods, metal/a-Se detector

Introduction

Electrostatic (xeroradiographic) imaging is a process in which the intensity pattern of a photon beam is transformed to a charge distribution on the surface of a photoconductor.[1-2] With the development of novel methods for extracting the latent image, such as photoinduced discharge with laser[3-5] and electrostatic coupling, xeroradiography is regaining its vitality. Recent studies have shown that by using amorphous Selenium (a-Se) and digital readout has various advantages: higher contrast, wider dynamic range and improved quantum detective efficiency.[6,7] A latent image on the a-Se surface is formed via local neutralization of the uniform charge distribution achieved through some charging procedure before irradiation. In diagnostic radiology, the selenium is directly exposed to x-rays transmitted through a patient. One would naturally ask if a-Se can be introduced into portal imaging where beam energy is much higher.

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In portal imaging, an image is acquired with a therapy beam with high penetrating ability which reduces detection efficiency. A metal plate is usually combined with a portal image receptor. For example, a portal film is placed in a cassette with a copper plate on the beam entrance side. Because of the high attenuation coefficient of the metal, a significant portion of the incident photon beam is converted to secondary electrons. It is the interaction of the electrons with the receptor that is responsible for image formation. Metal plates are also employed in fluoroscopic EPIDs and matrix ion chamber EPIDs to enhance detector response.

Droege and Bjarngard reported that a metal plate can significantly reduce scatter to primary ratio when used with portal films.[8] Jaffray et al reported that a copper plate can reduce quantum noise associated with x-ray absorption in phosphor screens thus improve the detective quantum efficiency.[9]

The objective of this study is to measure the radiation discharging curve of metal/a-Se receptors, and to calculate their detector response, modulation transfer function and the detective quantum efficiency in the megavoltage range with the Monte Carlo technique.

Mathematical simulations

Theory

The measurement of the line spread function $l(x)$ can be modelled as a smoothing process followed by sampling process. Mathematically, the measured line spread function $l_m(x)$ can be expressed as

$$l_m(x) = \left[l(x) \otimes rect\left(\frac{x}{a}\right)\right] \cdot comb\left(\frac{x}{b}\right)$$

where the convolution with

$$rect\left(\frac{x}{a}\right) = \begin{cases} 1, & |x \le \frac{a}{2}| \\ 0, & |x > \frac{a}{2}| \end{cases}$$

represents the averaging effect of the aperture size a, and the multiplication with

$$comb\left(\frac{x}{b}\right) = \sum_{n=-\infty}^{n=+\infty} \delta(x - nb)$$

represents the sampling with a spacing of b. The measured modulation transfer function is then given by the modulus of the Fourier transform of $lm(x)$:

$$MTF_m(u) = |\mathcal{F}\{l_m(x)\}|$$
$$= |[MTF(u) \cdot sinc(au)] \otimes comb(bu)|$$

which contains truncation error introduced by the multiplication with $sinc(au)$ and aliasing artifact introduced by the convolution with $(comb(bu))$. Precautions have to be taken in the selection of aperture size and sampling rate in order to keep systematic errors under an acceptable limit. The upper limit of spatial frequency at which the modulation transfer function can be measured is determined by the Nyquist criterion:

$$u_{max} = \frac{1}{2b}.$$

The convolution in Eq. (4) causes overlapping of adjacent cycles. This overlap can be reduced by increasing the aperture size. A larger aperture reduces the amplitudes of the sidelobes of $sinc(au)$ but at the same time increases truncation error. As a trade-off of aliasing reduction, the measured modulation transfer function will deviate more from the true value. The current convention used in modulation transfer function measurements is $a = 2b$, i.e., the aperture should be at least twice the size of the sampling interval. This convention ensures a less than 2 % systematic error in the sampled data.[10]

Quantum noise in x-ray imaging originates from the fluctuation of the incident photon flux characterized by Poisson statistics and the randomness of the amount of energy deposited by each x-ray photon in the receptor. While the former determines the noise level of the input, the latter is the reason for the degradation of the signal to noise level introduced by the receptor. This degradation is usually characterized by the detective quantum efficiency defined as

$$DQE = \left(\frac{SNR_{out}}{SNR_{in}}\right)^2.$$

For an amorphous selenium receptor, the energy deposited by an incident photon is used to create electron-hole pairs which are responsible for the formation of the electrostatic image. The number of these charge carriers resulted from E incident photons of energy is given by

$$\frac{\int_0^{E_{in}} n(E, E_{in}) E dE}{W}$$

where $n(E, E_{in})$ is the average number of photons that deposited the amount of energy E and W is the average energy required to generate one electron-hole pair. The fluctuation of $n(E, E_{in})$ is $\sqrt{n(E, E_{in})}$. Considering the absorbed energy distribution, the total uncertainty is

$$\frac{\sqrt{\int_0^{E_{in}} \left(\sqrt{n(E, E_{in})}E\right)^2 dE}}{W}.$$

Therefore,

$$SNR_{out} = \frac{\int_0^{E_{in}} n(E) E dE}{\sqrt{\int_0^{E_{in}} n(E) E^2 dE}}$$

$$SNR_{in} = \frac{N}{\sqrt{N}}$$

$$DQE = \left(\frac{\int_0^{E_{in}} \frac{n(E, E_{in})}{N} E dE}{\sqrt{\int_0^{E_{in}} \frac{n(E, E_{in})}{N} E^2 dE}}\right)^2$$
$$= \frac{M_1^2(E_{in})}{M_2(E_{in})}$$

where

$$M_i(E_{in}) = \int_0^{E_{in}} \frac{n(E, E_{in})}{N} E^i dE$$

is the ith moment of the normalized pulse height spectrum $\frac{n(E,E_{in})}{N}$ from incident photons of energy E_{in}. Eq(11) is the DQE at zero spatial frequency because spatial information transfer is not considered. DQE at a non zero spatial frequency is lower as the receptor can not fully transfer the information at that detail level. DQE as a function of spatial frequency can be expressed as

$$DQE(f) = DQE(0) \cdot MTF^2(f)$$

provided that quantum noise is white noise. This is justifiable since the input noise is determined by the Poisson statistics and the output noise is determined by the fluctuation in the energy deposited by a photon. Neither of them depends on the spatial frequency of the input under the assumption that x-rays are photons and the detector is a large continuum.

Monte Carlo Simulations

Image acquisition in transmission radiology starts with the detection of x-rays transmitted through a patient. The change of some physical observable caused by the interaction between the x-rays and the detector is then extracted as the output signal by a certain means. The incident photon generates a photon-electron "shower" in the detector introducing an uncertainty in the spatial location of the incident point resulting in receptor blur. Due to the stochastic nature of the coupled photon-electron transport, energy deposition introduces fluctuation in the output signal or quantum noise.[11-13] The magnitude of receptor blur and quantum noise depend on the energy of the x-rays and the composition and geometry of the receptor.

The coupled photon-electron transport within the detector was simulated with the Electron Gamma Shower (EGS4) code[14] which has been extensively used for radiation dose calculation in the energy range of 1~10 MeV and has been proven to produce reliable results. As a general purpose software package, EGS4 (National Research Council, Canada) consists of two major parts: the system code that handles the physics of coupled photon-electron transport and the user code that defines the geometry and type of the medium/media. The user code also specifies which physical observable(s) will be scored. This package also provides two general purpose programs, XYZDOS and DOSRZ, to define simulation geometry in Cartesian and polar coordinate systems which include the Parameter Reduced Electron Step Transport Algorithm (PRESTA) that reduces the dependence of charged particle transport on user-selected parameters.[15] Density effect corrections were also included in the collisional stopping powers. The K fluorescence production was not considered since it is not significant in the megavoltage energy range.[9] The parameters controlling the transport were set as the following: ECUT=AE=0.521 MeV, PCUT=AP=0.01 MeV, where ECUT is the minimum total energy of electrons that are transported, PCUT is the minimum total energy of photons that are transported, AE and AP are the energy thresholds for creation of secondary electrons and photons, respectively. Monoenergetic photons (0.1~6 MeV) were used in all simulations. The results of simulation runs are consistently well within 1 % of each other.

A layer of amorphous selenium is coated on an 8 x 8 in^2 metal plate: the metal-plate is in the beam-entrance side of the detector. As a build up material, the metal plate converts the incident photons into electrons. Intuitively, the optimal thickness of the metal plate should be the depth d_{max} where electron equilibrium is reached. Beyond this depth, energy absorption decreases because the primary photon beam is attenuated and electrons do not travel over a certain range. Three of the four receptors (Noranda Advanced Materials Inc., Pointe Claire, QC) have a 2 mm thick aluminum plate with different thickness of a-Se: 150 μm, 300 μm and 500 μm. The other receptor consists of 1 mm copper and 300 μm a-Se.

The simulation of the line spread function was run with the user code XYZDOS. A 2 μm x 20 cm^2 parallel beam of monoenergetic photons is incident at the center of a 20 x 20 cm^2 receptor. The a-Se layer of the receptor is divided into a series of 5 μm wide strips inside which the deposited energies are scored. Every two adjacent points are then averaged:

$$l_m(x_i) = \frac{1}{2}[E(x_i) + E(x_{i+1})]$$
$$x_i = (i-1)h, \quad i = 1, 2, 3, \cdots, N.$$

to satisfy the requirement of the adequate aperture size. According to the Nyquist criterion, the sampling rate gives a cutoff frequency of 100 mm^{-1}. The selection of the bin width must also ensure that multiple scattering can be modelled accurately by the EGS4 Monte Carlo code. The rule of thumb to estimate the number of multiple scattering events is

$$N_{ms} = density(g/cm^3) \cdot (Z/8)^{\frac{1}{3}} \cdot stepsize(\mu m).$$

where N_{ms} is the number of multiple scattering events, Z is the atomic number of the material considered. For a 5 μm step size in amorphous selenium, N_{ms} is approximately 35 which is sufficient. The MTF is obtained by applying the Fast Fourier Transform (FFT) to the discrete LSF. To ensure the accuracy of the results, 30 millions of photons were used in each simulation resulting in a statistical uncertainty less than 5 % in each strip. This requires calculation times ranging from 7 to 24 hours on an SGI workstation (IRIS INDIGO, Silicon Graphics, Mountainview, CA).

In order to calculate the absorption efficiency and the detective quantum efficiency, the energy absorbed in the entire sensitive volume and its pulse height spectrum need to be scored. Unfortunately, XYZDOS does not include the option of pulse height spectrum. The simulations had to be run with the more versatile and more user friendly DOSRZ. A pencil beam of monoenergetic photons is incident at the center of the circular detector with a radius of 10 cm. The effect of the detector shape is negligible since the radius is sufficiently large for a pencil beam. Equal energy bin width was used in the pulse height spectrum: 0.01 MeV for incident photons of energy less than 3 MeV and 0.03 MeV for 3 MeV and above. Simulation were terminated only when the uncertainty in the pulse height spectrum became less than 10 % in each bin. Approximately 72 hours were required for each run.

Results

Three of the four receptors have a common front metal plate (2 mm Al) but different a-Se layer (150 μm, 300 μm and 500 μm thick) while the other has a 1 mm thick Cu front plate and 300 μm thick a-Se. The calculated MTFs are shown in Figure 1. Error bars are not plotted because they are smaller than the symbols.

For each plate, the MTF degrades as energy increases and becomes relatively constant from 2 MeV up to 6 MeV. This indicates that there is a transition of the dominant interaction from one type to another between 1 and 2 MeV. The MTFs were also calculated for the a-Se/Cu receptor when the Cu plate was used as back plate. Degradation was also observed as the photon energy was increased.

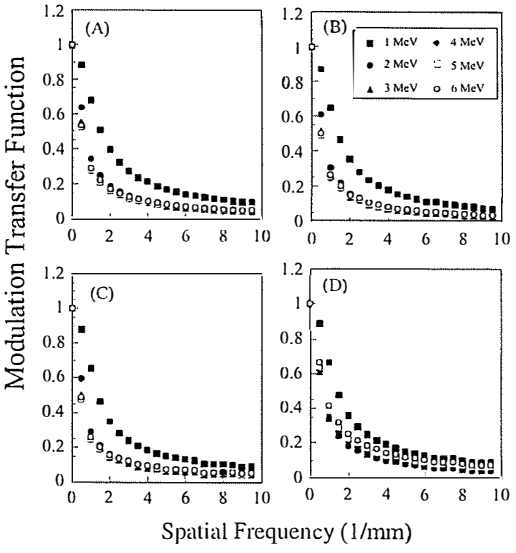

Figure 1. The modulation transfer functions for different energies for various receptors (A) 2 mm Al/0.15 mm a-Se, (B) 2 mm Al/0.30 mm a-Se, (C) 2 mm Al/0.50 mm a-Se, (D) 1 mm Cu/0.3 mm

The data of all receptors at each individual energy were plotted in Figure 2. It can be seen that for the Al plate receptors, the MTF decreases as the thickness of the a-Se increases at all energies (1~6 MeV). For 300 μm thick a-Se layer, a 2 mm Al plate and a 1 mm Cu plate lead to the same modulation transfer function at 1 MeV. As the photon energies increases, the Cu plate improves the MTF considerably. When a back Cu plate is used, the MTF is the lowest at 1 MeV but highest for 2 MeV and above.

The quantum absorption efficiency is defined as the ratio of the photons that have deposited energy in the sensitive volume of the detector to all the incident photons. Figure 3 (A) shows the calculated quantum absorption efficiencies of four receptors. The error bars are too small to be shown in the plots. The quantum absorption efficiency increases as the a-Se layer becomes thicker when the same front plate is used. At 1 MeV, a front metal plate reduces the probability of absorption due to the attenuation of the primary beam. For energies ≥ 2 MeV, the 1 mm Cu front plate increases the absorption more than the 1 mm Cu back plate. A 1 mm Cu back plate is more effective in absorption than a 2 mm Al front plate.

The output signal of a receptor is determined by the average energy deposited by an incident photon. Figure 3 (B) shows the responses of four receptors to

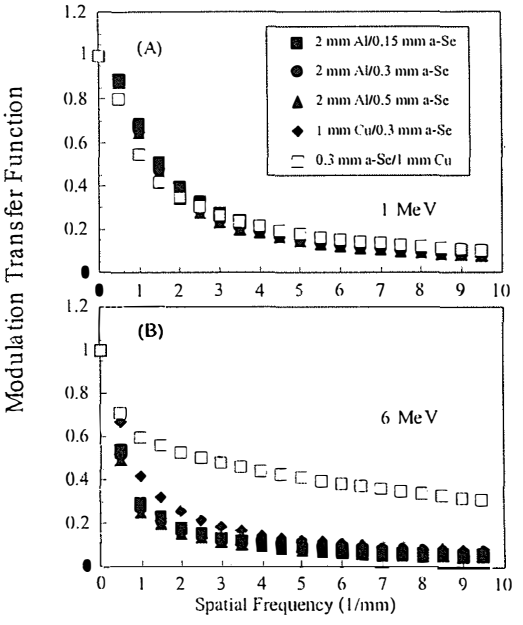

Figure 2. The modulation transfer functions of four receptor at incident photon energy of (A) 1 MeV and of (B) 6 MeV.

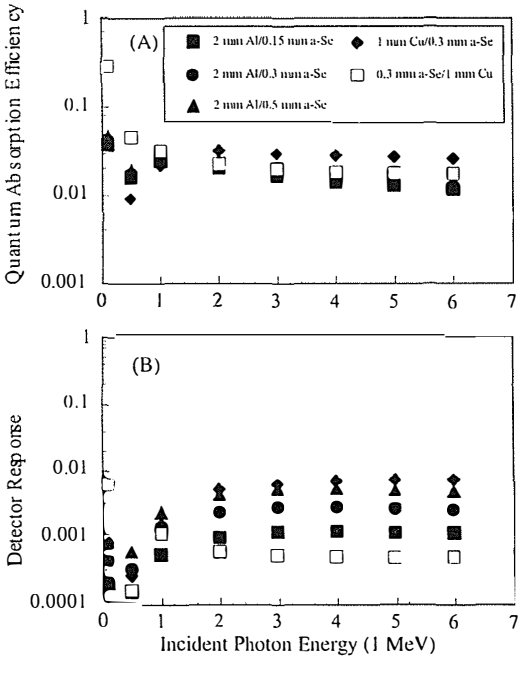

Figure 3. (A) Quantum absorption efficiencies and (B) Detector response of four receptors at different incident photon energies.

monoenergetic photons at different energies. For the three Al plates, detector response increases with the thickness of a-Se. For the same thickness of the a-Se layer (300 μm), a 1 mm Cu front plate results in a much greater detector response than a 2 mm Al front plate. The comparative detector response of the Cu with respect to Al increases at higher energies. For 2 MeV and above, it becomes even greater than that of the Al receptor with a thicker a-Se layer (500 μm). Among all the receptors, the one with a 1 mm Cu back plate has the lowest detector response.

The statistical factor describes the loss in DQE due to the incomplete absorption of an interacting photon. As shown in Figure 4 (A), the statistical factor of a front metal plate receptor decreases as the x-ray energy increases. With the front metal plate as an electron convertor, the pulse height spectra of energy deposition in the a-Se layer have similar shapes at different energies. But the width increases with energy. The drop of the statistical factor is due to this widening. For a back metal-plate receptor, however, the pulse height spectrum becomes narrower when x-ray energy increases. The smaller variation in the amount of the energy deposited per interaction photon is responsible for the slight increase of the statistical factor of the 1 mm Cu back plate receptor.

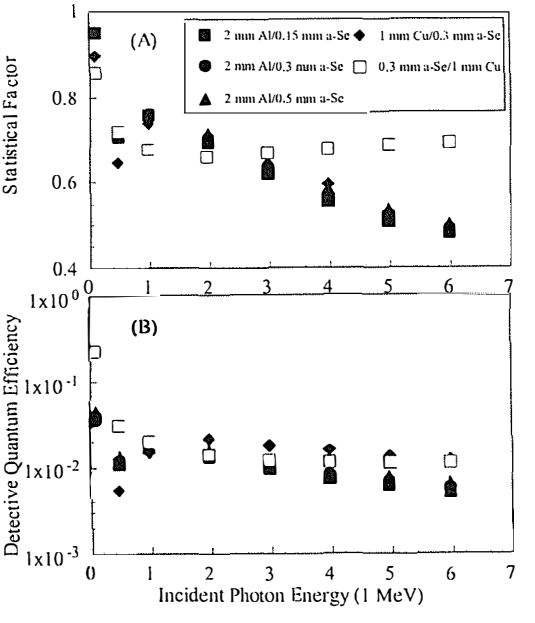

Figure 4. (A) Statistical factors, and (B) Zero spatial frequency detective quantum efficiencies of four receptors at different incident photon energies.

The zero spatial frequency DQE of the four receptors are shown in Figure 4 (B). Except for the 1 mm Cu receptor at 1 MeV, the DQEs of all four receptors decrease as incident photon energy increases. For the same front metal plate, a larger sensitive volume results in a higher DQE due a more complete absorption of the incident photon. For the same a-Se layer, a front metal plate decreases the DQE at 1 MeV due to the attenuation of the primary photons (Figure 3 (A)). But for 2 MeV and above, the 1 mm Cu front plate, increases the DQE more than the 2 mm Al front plate. Figures 3, 4 (A) and (B) indicate that DQE is dominated by quantum absorption efficiency. The DQE as a function of spatial frequency can be calculated from Eq(13). For 2 MeV and above, both the zero frequency DQE and the MTF of the Cu receptor are greater than those of the Al receptor. The Cu front plate, therefore, will lead to higher DQE at any detail level. At 1 MeV, the DQE of the Cu receptor at higher spatial frequencies will be compensated by its higher MTF.

Discussion

Image formation in a xeroradiograhic system has three stages: x-ray absorption, electron-hole pair production and charge collection. In our simulations, only the first is modeled. Another simplification is that only monoenergetic x-ray beams were considered.

Our calculations of zero frequency DQEs have followed the approach taken by Jaffray *et al*[9] to calculate zero frequency DQEs of metal plate/phosphor screen combinations. Since the physical process modeled by the simulations is the same: energy absorption, the results could be compared. In fact, similar trends are observed in the way zero frequency DQE changes with respect to incident photon energy although the behavior of the DQE(●) of the metal plate/a-Se detector is not as simple. Since the Monte Carlo results are the higher limit, comparison of the overall performance of the two type of detection systems (metal/a-Se versus metal/phosphor) must include the later stages in the imaging chain. The metal/phosphor system requires an additional component that converts light into an electronic signal which may seriously affect the resultant DQE. This critical component is theoretically not required for metal/a-Se system, where the information is already stored in charge form. Intuitively, it should be simpler to read charge information directly in the metal/a-Se system than it would be with a metal/phosphor system. However, the optimum technique for reading a metal/a-Se has not been found yet.

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Incidence of spontaneous cytogenetic changes in peripheral blood lymphocytes of a human population sample

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Three mutagenetic methods – conventional structural chromosomal aberration analysis (CA), sister chromatid exchange (SCE) method and micronucleus test (MN) were carried out on a population sample of 350 test subjects aged 18 to 65 years.

The spontaneous incidence of these aberrations in the first in vitro metaphases of lymphocytes was 0.83 % chromatid breaks, 0.43 % isochromatid-chromosome breaks, 0.21 % acentrics fragments and 0.01 % dicentric chromosomes per test subject.

The mean value for SCE per cell amounted to 6.52 ± 0.70, while the frequency of micronuclei was 5.8 ± 2.1 per 500 binuclear lymphocytes. These results represent the mutagenetic background for the Slovenian population and can be used for the assessment or in case of suspect exposure to clastogenic agents.

Key words: Lymphocytes; chromosome aberrations; sister chromatid exchange; micronuclei

Introduction

Intensive industrialisation over the past few decades has resulted in the production and use of numerous genotoxic chemicals and sources of ionising and non-ionising radiation. The need to identify the mutagenic and carcinogenic effects of these agents on the human population exposed environmentally, professionally or accidentally is therefore on the increase. There are several methods with which it is possible to prove changes occurring in DNA molecules. Unfortunately, there are few direct methods for the identification and assessment of the degree of mutations.

Among methods for the detection of large changes in the genome of human somatic cells which are used routinely for mutagenetic monitoring, the International Commission for Protection against Environmental Mutagens and Carcinogens recommends the detection of chromosomal aberrations, the micronucleus test and sister chromatid exchange technique.[1]

The analysis of chromosomal aberrations in peripheral blood lymphocytes has gained the widest use to date. The methodological and technical conditions for this technique and the procedure for the analysis of specimens are well defined, largely owing to the use of specific chromosomal aberrations in biological dosimetry.[2]

It is well known, however, that ionising radiation and the majority of chemical genotoxic agents have different effects on cellular DNA which is directly included in the formation of chromosomal changes.

The frequency of SCE is a sensitive indicator of the effects of chemical genotoxic agents and high linear energy transfer (LET) radiation, but a poor indicator of the exposure to low LET ionising radiation.[3,4]

The micronucleus test has almost universal application in the detection of changes in the cellular genome. Micronuclei may originate from acentric fragments resulting from two-chain breaks of DNA after its exposure to radiation, and have shown very

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good dose-response relationships. They may also contain several chromosomes which were not distributed equally to daughter cells due to a non-functional cell-division spindle or kinetochores. The latter phenomenon is most frequently caused by chemical agents.[5] It therefore seems that all three tests should be used on parallel specimens in order to be able to assess the type of exposure: to a physical or chemical agent, or even possibly simultaneously to both categories of clastogens.[6]

To enable the evaluation and correct performance of cytogenetic population monitoring, it is necessary to know as much as possible about the frequency of normal, spontaneous chromosomal aberrations, as well as about the factors which exert an influence on their occurrence.[7]

Considering the institutions in which the authors of this paper work and the legal obligation of mutagenetic monitoring for defined groups of people who are professionally exposed to ionising radiation, the purpose of this study was:

– to standardise laboratory conditions of in vitro cultivation of lymphocytes in accordance with the Protocol of the International Atomic Energy Agency,

– to determine the mutagenetic background in a population sample of persons who are not professionally exposed to ionising radiation by using the structural chromosomal aberration analysis, micronucleus test and SCE frequency per cell.

Subjects and methods

Subjects

The study included 350 subjects, of whom 153 were students prior to their enrolment at the High School of Radiology, and 197 persons prior to the assumption of their duties in radiation zones. It is important to emphasise that such a selection of the population sample enables comparative analyses regarding mutagenic effects in conjunction with age, and even more, lifestyle factors.

The group of students included secondary school students who had just graduated, aged 18 to 20 years. The majority of them were non-smokers and persons with an extremely low previous influence of alcohol, coffee, drugs and ionising radiation used for diagnostic and treatment purposes.

The second group of test subjects had not been professionally exposed to genotoxic substances before taking of blood samples for mutagenetic tests. However, the age dispersion in this group (20 to 65 years) was significant, and the lifestyles of its subjects had undoubtedly brought along more numerous factors with mutagenic characteristics. At the time when blood samples for mutagenetic tests were taken, none of the test subjects showed any subjective troubles nor objective signs of acute illness, and none of them had been previously subject to major diagnostic or therapeutic irradiation. Test subjects were both men and women, of whom 34 % were smokers.

Methods

Data of subjects was collected by means of filling in Personal Health Questionnaires. Peripheral blood lymphocytes from subjects were used as cellular material. Blood samples were taken simultaneously for all three tests.

Structural chromosomal aberrations (CA)

Standard *in vitro* lymphocyte cultures were used for structural chromosomal aberration analysis. 0.3 ml of heparinised whole blood was added to 5 ml of the culture medium (GIBCO BRL Chromosome med 1A with Phytohaemaglutinin). The first in vitro cell division cycle was established with an addition of 5 mg/ml of BrdU (Sigma). 0.075 mol/l potassium chloride was used for the hypotonic procedure. Fixation was performed in a mixture of ice acetic acid and methyl alcohol at a ratio of 1:3. The cell suspension was pipetted onto cold glass slides, specimens were air-dried and stained with Giemsa-Sigma. For every test subject, the first 200 *in vitro* metaphases were analysed at 1000X magnification on a Nikon LABOPHOT2 microscope. Structural damage to chromosomes was categorised as chromatid breaks, isochromatid-chromosomal breaks, acentric fragments or dicentric and ring chromosomes. Gaps were not included in the total number of chromosomal aberrations.

Sister chromatid exchange (SCE)

The same culture medium was used as for the first test. 72-hour lymphocyte cultures with the addition of 10 mg/ml BrdU were prepared in dark conditions. The procedure was performed according to the Kato (1974) method.[8]

50 cells per subject were analysed, SCE were counted and presented as average numbers per cell. The range of SCE frequencies was also recorded for every subject.

Micronucleus test (MN)

For this test, 3 mg/ml of cytochalasin B (Sigma) was added to each in vitro lymphocyte culture in the 43rd hour of cultivation. The Fenech-Morley (1985) method was used.[9]

Hypotonic procedure was omitted, and specimens were stained according to May-Grünwald and Giemsa. Cells with clearly blocked cytokinesis (CB cells), i.e. binuclear cells, were analysed. 500 cells per person were inspected and the results were presented as the number of micronuclei per 500 CB cells.

Statistical data processing

Data was processed using standard methods of parametric statistics. The differences between the average values for individual groups were tested using the variance analysis method with the SPSS computer program.

Results

Structural chromosomal aberrations (CA) were analyzed in 153 students or 30,600 their first in vitro metaphases, and in 197 technicians pending employment or 39400 their first in vitro metaphases (Table 1). The average value of acentric fragments for technicians pending employment is 0.58 and for students only 0.24. Acentric fragments were found

Table 1. Mutagenetic testing of subjects – Structural chromosomal aberrations.

No.		Tests		% aberations All subjects		ACENTRICS All subjects		Subjects with acentrics		
	Groups of test subjects	No. of subjects	No. of exam. cells	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Technicians	197	39400	1,812	1,028	0,584	1,045	39,090	1,494	1,199
2	Students	153	30600	1,101	0,580	0,242	0,473	22,880	1,057	0,338
3	Total	350	70000	1,501	0,861	0,434	0,860	32,000	1,357	1,029
	Variance analysis	FR		58,647		14,133			4,455	
		FP		0,000		0,000			0,037	

RINGS All subjects		RINGS Subjects with rings			DICENTRIC CROMOSOMES All subjects		DICENTRIC CROMOSOMES Subjects with dicentric chrom.		
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
11	12	13	14	15	16	17	18	19	20
0,005	0,071	0,508	1,000		0,041	0,283	2,538	1,600	0,894
0,000	0,000	0,000			0,000	0,000	0,000	0,000	0,000
0,003	0,054	0,286	1,000		0,023	0,211	1,429	1,600	0,894
0,776					3,153				
0,379					0,077				

CHROMOSOMAL BREAKS All subjects		CHROMOSOMAL BREAKS Subjects with chromosomal br.			CHROMATID BREAKS All subjects		CHROMATID BREAKS Subjects with chromatid br.		
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
21	22	23	24	25	26	27	28	29	30
1,157	0,980	73,100	1,583	0,798	1,838	0,928	97,460	1,885	0,891
0,497	0,660	41,180	1,206	0,446	1,464	0,726	93,460	1,566	0,634
0,871	0,856	59,143	1,469	0,729	1,675	0,860	95,714	1,749	0,806
51,418			12,358		16,801			13,313	
0,000			0,001		0,000			0,000	

in 22.88 % of students (1.05 on average) and in 39.09 % of technicians pending employment (1.49 on average). In one case, the presence of a ring chromosome was found in a technician pendingemployment, while such aberrations were not found in students. The average value for rings is therefore 0.0051 per subject for technicians. In five persons same population, dicentric chromosomes were found. The average value of dicentric chromosomes for this group is 1.6, and 0.0406 for all technicians.

The number of chromosomal breaks in technicians is 1.15 per subject and only 0.49 in students; for chromatid breaks, it is 1.83 per subject and 1.46 in students.

Chromosomal breaks were present in 41.18 % of students (1.20 on average) and in 73.10 % of technicians (1.58 on average); chromatid breaks were present in 93.46 % of students (1.56 on average) and in 97.46 % of technicians (1.88 on average). The differences between the groups of students and technicians pending employment were found to be statistically significant for the percentage CA test, the number of acentric fragments, chromatid and chromosomal breaks. The SCE and MN tests were performed only on a smaller number of test subjects. The average result of the SCE test for students was 6.28 and 6.52 for technicians per 50 cells; for MN test the average results were 4.00 for students and 5.89 per 500 CB cells for technicians. The differences between the two compared groups were not statistically significant (Table 2).

It can be seen from mutagenetic questionary that during the present length of service examined subjects had not worked with sources of ionising radiation. There is a considerable difference in the total duration of service between the two groups, since the technicians have in average up to 7 years of service, while the majority of students have none. The age differences are also significant, since the technicians are on average 11 years older than students.

With the comparison of mutagenetic tests for the two groups we have established that the percentages of CA, the number of acentrics, chromosomal and chromatid breaks increase almost linearly with age. The differences in age groups are statistically significant in % CA, acentrics and chromosomal breaks (Table 4).

While examining the influence of smoking on our results it was established that there were no significant differences in mutagenetic tests between smokers and non-smokers, except for the values for leukocytes (which are higher than average both in technicians and in students who smoke). In these two groups, the SCE test was performed only on a small number of subjects. In their smoking history is very short (smoking history for students is 1.5 years). Student smokers (26 %) smoke on average only 8 cigarettes per day, while for technician smokers (41 %) the average duration of smoking is 6.8 years and they smoke on average 14 cigarettes per day).

Table 2. Mutagenetic testing of subject – SCE and MN test.

No.	Tests / Groups of test subj.	SCE TEST: No. of subjects	SCE TEST: No. of exam. cells	SCE TEST: Mean – per 50 cells	SCE TEST: SD	MN TEST: No. of subjects	MN TEST: No. of exam. cells	MN TEST: Mean – per 500 CB cells	MN TEST: SD
0	1	2	3	4	5	6	7	8	9
1	Technicians	115	5750	6,528	0,714	113,000	56500,000	5,896	2,122
2	Students	5	250	6,268	0,292	5,000	2500,000	4,000	1,581
3	Total	120	6000	6,517	0,702	118,000	59000,000	5,815	2,132
	Variance FR			0,651				3,880	
	analysis FP			0,421				0,051	

Table 3. Length of service and age of test subjects.

No.	Variable / Groups of test subj.	Length of service – at present (years): No. of subjects	Mean	SD	Lenth of service – total (years): No. of subjects	Mean	SD	Age (years): No. of subjects	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Technicians	197	0,000	0,000	197	6,944	8,587	197	32,906	9,395
2	Students	153	0,059	0,367	153	0,059	0,367	153	21,597	1,983
3	Total	350	0,026	0,244	350	3,917	7,279	350	27,934	9,092
	Variance FR		5,066			98,182			214,057	
	analysis FP		0,025			0,000			0,000	

Table 4. Mutagenetic tests according to age of groups of subjects – Structural chromosomal aberrations.

No.	Groups of test subj. ects / Tests		% CA		ACENTRICS			
			All subjects		All subjects		Subjects with acentrics	
	Age groups of subjects (years)	No. of subjects	No. of exam. cells	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
0	1	2	3	4	5	6	7	8	9	10
1	Till 18	5	1000	1,000	0,354	0,200	0,447	20,000	1,000	0,000
2	19–25	183	36600	1,195	0,666	0,236	0,487	21,311	1,103	0,384
3	26–35	102	20400	1,639	0,693	0,446	0,640	36,274	1,216	0,417
4	36–45	39	7800	2,218	1,455	0,923	1,345	51,282	1,800	1,399
5	46–55	13	2600	2,269	1,666	1,308	2,394	61,538	2,125	2,800
6	56–65	8	1600	2,438	0,729	1,250	0,707	87,500	1,429	0,535
7	Total	350	70000	1,504	0,849	0,437	0,812	32,000	1,357	1,030
	Variance analysis FR			15,255		9,695			2,421	
	Variance analysis FP			0,000		0,000			0,040	

RINGS					DICENTRIC CHROMOSOMES				
All subjects		Subjects with rings			All subjects		Subjects with dicentric chrom.		
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
11	12	13	14	15	16	17	18	19	20
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,000	0,000	0,000	0,000		0,011	0,105	1,093	1,000	0,000
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,000	0,000	0,000	0,000		0,128	0,570	12,821	2,500	0,707
0,077	0,277	7,690	1,000		0,077	0,277	7,692	1,000	0,000
0,000	0,000	0,000	0,000		0,000	0,000	0,000	0,000	0,000
0,003	0,054	0,286	1,000		0,023	0,211	2,286	1,600	0,500
5,487					2,492			5,400	
0,000					0,031			0,156	

CHROMOSOMAL BREAKS					CHROMATID BREAKS				
All subjects		Subjects with chromosomal br.			All subjects		Subjects with chromatid br.		
Mean	SD	Share of subj. (%)	Mean	SD	Mean	SD	Share of subj. (%)	Mean	SD
21	22	23	24	25	26	27	28	29	30
0,600	0,548	60,000	1,000	0,000	1,200	0,447	100,000	1,200	0,447
0,571	0,745	44,262	1,300	0,560	1,571	0,803	94,536	1,663	0,727
1,099	0,878	72,549	1,500	0,667	1,733	0,747	97,059	1,786	0,692
1,436	1,071	87,179	1,647	0,981	1,949	1,297	97,436	2,000	1,273
1,231	0,927	76,923	1,600	0,699	1,846	0,801	100,000	1,846	0,801
1,625	1,598	62,500	2,600	1,140	2,000	1,069	87,500	2,286	0,756
0,871	0,856	59,143	1,469	0,729	1,675	0,860	95,714	1,749	0,806
11,091			4,322		2,047			2,333	
0,000			0,001		0,072			0,042	

Discussion

The determination of the frequency of chromosomal aberrations in peripheral blood lymphocytes as a biological indicator of the effect of genotoxic agents has been used in practice for a long period of time. During this time, a large number of papers were published which presented data on the frequency of spontaneous aberrations, especially those of the chromosomal type. With regard to the fact that the spontaneous incidence of genome damage is essentially influenced by individual non-identified agents and regional, ecologically specific features, we are interested in comparing the recent situation with values from 10 or more years ago. The most systematic analysis of this type was performed by Lloyd in 1984. Even though he focused only on the spontaneous occurrence of dicentric and acentric fragments, he also analysed other available data on chromatid and chromosomal lesions.[10] He analysed

Table 5. Mutagenetic testing of subjects – SCE and MN tests.

No.	Age groups of subj. (years) / Tests	SCE TEST: No. of subjects	SCE TEST: No. of exam. cells	SCE TEST: Mean – per 50 cells	SCE TEST: SD	MN TEST: No. of subjects	MN TEST: No. of exam. cells	MN TEST: Mean – per 500 CB cells	MN TEST: SD
0	1	2	3	4	5	6	7	8	9
1	19–25	23	1150	6,228	0,658	23	11500	4,913	2,151
2	26–35	62	3100	6,466	0,551	61	30500	5,817	1,873
3	36–45	23	1150	6,737	1,021	22	11000	6,509	2,516
4	46–55	7	350	6,763	0,434	7	3500	6,286	1,976
5	56–65	5	250	7,096	0,664	5	2500	6,600	2,702
6	Total	120	6000	6,517	0,702	118	59000	5,815	2,132
	Variance analysis FR			2,816				1,921	
	Variance analysis FP			0,029				0,112	

Table 6. Length of service and age of test subjects according to age groups.

No.	Age groups of subj. (years) / Variable	Length of service – at present (years): No. of subjects	No. of exam. cells	SD	Length of service – total (years): No. of subjects	No. of exam. cells	SD	Age (years): No. of subjects	No. of exam. cells	SD
0	1	2	3	4	5	6	7	8	9	10
1	do 18	5	0.000	0,000	5	0,000	0,000	5	18,367	0,070
2	19–25	183	0.044	0,326	183	0,412	1,142	183	21,885	1,760
3	26–35	102	0.010	0,100	102	4,525	4,711	102	29,390	2,635
4	36–45	39	0.000	0,000	39	11,811	8,366	39	39,537	2,720
5	46–55	13	0.000	0,000	13	16,615	14,245	13	49,730	3,033
6	56–65	8	0.000	0,000	8	22,000	15,062	8	59,913	3,168
7	Total	350	0.026	0,244	350	3,917	7,279	350	27,934	9,092
	Variance analysis FR		0.427			70,792			1074,692	
	Variance analysis FP		0.830			0,000			0,000	

a total of 65 different mutagenetic studies with over 2000 test subjects, i.e. 211,660 examined cells. The values for dicentric fragments are mainly in the range from 1 to (more rarely) 2/1000. Since his studies included test subjects from a wide age interval, our data differ somewhat from his. Not one dicentric fragment or ring chromosome was found in our group of students, while in the group of technicians (which was composed of subjects of greater age differences and longer smoking periods, as well as the influence of other lifestyle factors), dicentric and centric ring chromosomes were found in 0.011 %. Acentric fragments were found in both groups in 0.217 %. Lloyd stated that in the majority of publications he analysed, the value for acentric fragments was about 3×10^{-3}. In a study which included 304 subjects, Galloway et al. (1986) found a frequency of dicentrics of 2.1×10^{-3} and 3.2×10^{-3} for acentric fragments.[11] Bender et al. (1988) found 1.6×10^{-3} dicentrics and 4.6×10^{-3} acentric fragments in a mixed black-white American population.[7] In addition to individual standard aberrations, Awa and Neel (1986) also state data on the presence of a certain number of "rogue" cells with multiple dicentric, tricentric and acentric fragments and breaks. No explanation for this phenomenon is given.[12] Such types of changes were not found in this study, even though they were present in certain other of our studies.

The comparative analysis of the frequency of chromatid aberrations revealed certain problems. This type of aberrations involves changes which usually do not originate from the circulating Go population of lymphocytes, but are formed either during or after the phase of DNA synthesis, i.e. *in vitro*.

There are also differences regarding the classification of chromosomal changes: true chromatid breaks with larger or smaller dislocations of the broken fragment of one chromatid, and gaps – achromatic regions on chromatids. The latter are not a true damage to the genome, but most often merely a change in the condensation of the protein part of chromosomes, while DNA continuity is preserved.

Galloway found 0.64 % of chromatid deletions in the range of 0 to 6 and chromatid exchanges in 0.11 %.[11] In 7000 examined metaphases of the con-

trol population, Karačić et al. (1995) found 0.48 % chromatid breaks, 0.27 % isochromatid chromosome breaks and 0.23 % acentric fragments. Dicentric and centric rings were not found.[13]

Our data with 0.837 % chromatid breaks per subject not including gaps is comparable to the data of other authors. At the same time, these values can serve as a good indicator of the conditions of in vitro cultivation. This type of damage differs for different authors, since it can be caused by the conditions of in vitro cultivation, including the quality of culture medium, serum, temperature, centrifugation, etc. Isochromatid-chromosomal breaks, whose presence in the first in vitro division indicates G1 damage, was found in 0.248 % of the student group and in 0.5787 % of the technician group. The difference between the two groups is significant (p= 0.00) and may indicate the influence of age. It does not only include physiological differences caused by age, but especially lifestyle factors, which are expressed at older age.

With all the simplicity and quickness of the technical procedure, as well as the possibility of machine (automatic) processing, the micronucleus test as the universal indicator of exposure to genotoxic substances shows a large variability in the number of micronuclei per 500 or 1000 analysed cells prepared according to the same protocol.[14] Certain authors found 20 or more micronuclei per 1000 CB lymphocytes, while others state 3 to 5 per thousand.[5,9,15] This non-uniformity of data and the resulting non-availability of universal reference values dictate the need for collecting one's own data for the general population.

Our results per 59000 CB cells indicate the frequency of micronuclei of 5.815 ± 2.132 (CB cells). This data is therefore the background value for the professionally non-exposed Slovene population.

The reference value for the SCE test which is nowadays regularly used in all population mutagenetic studies is 6.52 ± 0.702 per metaphase for professionally non-exposed Slovenian population, which is similar to data stated by numerous authors.[5,16,17] No significant differences in the incidence of SCE per metaphase with regard to age were noticed. This test which can be considered as a mutagenetic method of choice for the detection of exposure to chemical genotoxic agents showed very small individual deviations in our study. For this reason it is considered to be a reliable indicator for the assessment of combined exposure to chemical and physical agents.

The stated values of mutagenetic tests carried out on 350 test subjects provide a good orientation value for mutagenetic monitoring and large ecological studies, or for the analysis of specific groups professionally exposed to genotoxic agents.

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Introduction to ethical analysis

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The complexity of modern medicine and of the related ethical issues is reflected in progressively more detailed ethical codes and guidelines. We believe that an equal emphasis should be devoted to ethical analysis, a method which permits every physician to define, analyse, and solve an ethical dilemma. Ethical dilemmas are best approached using the common morality theory with its four principles: autonomy, nonmaleficence, beneficence, and justice. A person, or a group of persons, experience either ethical benefits or ethical costs by an action resulting in a greater or diminished respect of any of the four principles. The same action may bring both ethical benefits and costs: lying about the diagnosis of a serious disease may be occasionally beneficial but violates the principle of patient autonomy. Ethical analysis may be divided into three steps. In the first step, ethical benefits or costs are ascribed to the involved individual or collective subjects before any action is undertaken. In the second step, potential actions of changing the present situation are discussed; for each of these actions, a comparison with the present situation will reveal a net ethical benefit or cost for the affected subjects. The third step is a recommendation for the most appropriate action. This final step of ethical analysis is an interdisciplinary task: a discussion among physicians, psychologists, sociologists, economists, or polititians will hopefully lead to a balanced and realistic proposal.

Key words: medical; ethics, ethical analysis; ethics, institutional; public policy

Introduction

The times when moral dilemmas were resolved by adhering to simple rules do belong to the past. Today's world is one of increasing complexity, of breaking the traditional social structure and of individual responsibility. New information networks and global marketing are reaching the most remote sites of the world; at the same time, however, new technologies remain an illusion for majority of mankind.

Medicine is not an exception to these global social changes and to the related moral dilemmas. We all feel the pressure of a widening gap between technological development on one side, and restrictions due to limited resources on the other. Problems of distributive justice are often linked to uncertainties regarding life-sustaining treatments. The declared autonomy of patients in decisions concerning their life, treatment and death may be in sharp contrast with the principles and rules of beneficence and non-maleficence of the medical community. Even preventive medicine is not free of moral dilemmas: to what extent may we limit individual autonomy in order to explore patterns of occurrence of human diseases, and how far should we go in imposing medically beneficial behaviour in society?

The increasing importance of ethical issues in medicine is beyond doubt. Less clear is the way to greater ethical awareness. Should we teach young physicians detailed codes and rules as seems to be the prevailing practice, or should we rather teach them to define, analyse and solve ethical dilemmas? Do we need consultants in medical ethics –

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yet another discipline of medicine, or is ethics really everybody's business?

First, we will point to a much needed distinction between law, ethical codes, and expert ethical opinions. The main part of our discussion will be devoted to the presentation of ethical rules, principles and theories and to ethical analysis as a method of approaching an ethical dilemma.

Possibly the most unfortunate consequence of recent trends in medical ethics, with increasingly detailed and hardly understandable guidelines, is the passive role taken by most physicians when approaching an ethical question. In order to alleviate this deficiency in medical education and practice, we here present the tools for ethical analysis and the three-step process of formulating and solving an ethical dilemma.

Intuition, ethical codes, detailed guidelines and ethical analysis

Any dilemma forces us to choose among mutually exclusive actions. In everyday life, we rarely follow a systematic approach: as Brewin[1] noted, the most caring doctor may be totally ignorant of academic ethics. Intuition functions well on an individual basis and in simple situations. However, intuition is of limited usefulness in complex situations and in arguments between often widely differing views.

From the times of Egyptian papyri, of Hammurabi and Hippocrates[2] to the present day, physicians, their associations and rulers or representatives of society have tried to codify the guidelines of medical ethics into an obligatory system of rules for members of the medical profession. No other profession has devoted so much attention to ethical issues: this proves how delicate the field of medical ethics is and at the same time reflects an inability to govern physician's behaviour exclusively by law.

Still, centuries-old ethical codes could not provide an answer to many dilemmas from the increasingly complex medical practice of today. A response to this apparent obsolescence of codes and resolutions has been recent trend towards their inclusivity. The result is their progressive complexity and a vanishing distinction between law, ethical code, and expert ethical opinion. In international documents on medical ethics, vague expressions as a reflection of a compromise among distinct cultures are a further limitation to their practical use.

Legislation covers the most obvious and easily defined patterns of our behaviour. In addition, we need a simple code of medical ethics that all physicians will understand and remember and which will not be subject to revision with every new technological development: Hippocrates' oath still remains a beautiful masterpiece of eternal value.

Tools for ethical analysis: considered judgements, ethical rules, principles and ethical theories

The practical use of ethical analysis by those who have little insight into philosophy or academic ethics demands that we keep the discussion as simple as possible. Nevertheless, we can not avoid a brief and admittedly incomplete presentation of the main elements of ethical discussion.

Considered judgements

These are moral convictions in which we have the highest confidence and believe to have the lowest level of bias.[3] Wrongness of racial discrimination, religious intolerance, torture, or slavery are such widely accepted considered judgements. All ethical theories include such considered judgements which are as fundamental to ethics as axioms are to mathematics.

Ethical rules

These appear similar to considered judgements. Such rules are "Speak the truth", "Do not kill", "Help another human being". An important difference from considered judgements is that in a process called *balancing,* reality of life may force us to abandon one rule in order to comply with another. We may decide to override the rule "Speak the truth" and not reveal a positive pregnancy test to an overtly aggressive father of a teenager. The rule "Do not kill" may be disregarded in self-defence or, if ethical analysis permits us to do so, in helping a terminally ill patient to die with dignity. The rule "Help another human being" has its limitations: without them everybody would be obliged to give most of the belongings to the poor and physicians would be obliged to work regardless of working hours and payment.

Ethical principles

These are more abstract than rules and are a bridge between rules and ethical theories. An ethical theory, with its philosophical background defines the number and the list of principles needed for its construction. As we will see when discussing the utilitarian and Kantian ethical theories, a single principle leads to an unrealistic simplification; too many principles do not contribute to explanatory power and clarity of a theory. Following the arguments presented by Beauchamp and Childress[4] and Gillon[5], it seems that an ethical dilemma may be defined with four basic principles:

1. *Respect for autonomy:* a principle demanding the respect of the decision-making capacities of autonomous persons. An integral part of this principle is the right to be informed: incomplete understanding of a situation frequently leads to dependence, inferiority and loss of autonomy.

2. *Nonmaleficence:* a principle of avoiding the causation of harm. Although similar to the principle of beneficence, the principle of nonmaleficence covers a broader range of people: we are obliged not to harm unknown people to whom we have no obligations of beneficence.

3. *Beneficence:* a principle of providing benefits and balancing benefits against risks and costs. For its practical application, the principle of beneficence has to be specified: towards whom, in what circumstances and for which personal sacrifices are we obliged to act beneficently? An important element in these specifications are traditional relations: our obligations are much greater towards our own children, parents, or friends than towards unknown persons.

4. *Justice:* a principle for distributing benefits, risks and costs fairly. Limited resources invariably lead to a conflict and a balancing between the principles of beneficence and justice. The principle of justice demands that the rules for such a process of balancing are clearly defined in advance.

Ethical theories

Ethical theories define a system of ethical principles, rules and guidelines. A good theory satisfies eight conditions: [4]

1. *Clarity*: without obscurity and vagueness.

2. *Coherence*: internal consistency and devoid of contradictory statements.

3. *Completeness and comprehensiveness:* focused to cover all potential dilemmas.

4. *Simplicity*: a few basic norms are preferrable to more norms but no additional content.

5. *Explanatory power*: adequate insight to understand moral life.

6. *Justification power*: a good reason for the justification of a decision and also for the rejection of unacceptable options.

7. *Output power*: analysis also for new dilemmas not considered in the construction of the theory.

8. *Practicability*: not demanding actions beyond physical or social capabilities of most normal individuals.

A survey of all theories which have been proposed as a guide through ethical dilemmas, or of related literature would clearly be beyond the scope of this short presentation; the work of Beauchamp and Childress [4] is a classical text offering a comprehensive and balanced coverage of the topics. We will only briefly describe three groups of ethical theories: consequence-based utilitarian theories, obligation-based Kantian or deontological theories, and common morality theory based on the four aforementioned ethical principles.

Utilitarian ethical theories

These hold that actions are right or wrong according to the balance of their good and bad consequences. The question of whether we need rules in between the ethical theory and judgement about an action, or whether we should simply skip the rules and follow the end result divides utilitarians into "rule utilitarians" and "act utilitarians". The former strive to identify rules which, if always observed, will lead to overall maximal utility although the result in a particular case may be suboptimal; the latter simply observe each particular action which should produce maximal balance of positive value over disvalue, or the least possible disvalue if only undesirable results can be achieved.

The weakness of utilitarian theories is apparent when we realise that the actions leading to the goal – maximum balance of positive value over disvalue – are ethically unacceptable. For example, torturing prisoners may reveal a network of criminals; medical experiments on mentally incompetent persons may lead to important discoveries; the limitation of nursing care, or even active killing of elderly or incurable patients could save resources for treatment of young patients with curable diseases.

Kantian, obligation-based or deontological theory

This theory views and judges actions as right or wrong exclusively through moral obligations on which these actions are based. According to the categorical imperative of Immanuel Kant, "I ought never to act except in such a way that I can also will that my maxim become a universal law." The consequences of our actions are irrelevant; our desires or reasoning based on emotions may indeed annihilate the moral value of an action. In addition, Kant stressed the unique value and respect for every human being: "One must act to treat every person as an end and never as a means only."

Critics of Kantian deontological ethics maintain that the theory cannot offer advice in practical life, where we often have to choose among several mutually exclusive obligations. Beyond responsibility to a single patient, a physician's obligations may include the institution, the rules of a health insurance company and to his or her family. The stress on law and obligations on one side, and ignoring motivation originating from emotions, friendship, or family relations on the other, is the weak part of Kantian ethics.

Principle-based, common morality theories

These are not based on a single ethical principle. While the principle of beneficence is a basis for utilitarian ethics, and the principle of autonomy may be regarded as fundamental to Kantian ethics, the common morality theory seeks a maximal practically achievable balance among the four principles: autonomy, nonmaleficence, beneficence and justice. No priority is attributed to any of these principles; rather, we try to balance between ethical "costs" and "benefits" of each of the prospective possibilities for action.

A weakness of the common morality theory is its latitude: by choosing appropriate ethical principles, many opposing actions may be ethically defendable. The common morality theory is somehow in between a true, philosophical ethical theory and a method of ethical analysis. While this theory will be unsatisfactory for those who are seeking the deep philosophical foundations of morality, it may be very helpful in solving practical dilemmas.

The three steps of ethical analysis

We believe that the common morality theory, with its four principles, offers the best background in ethical analysis, and we will refer to it in this section. However, the three steps which we now describe are applicable also in conjunction with any other ethical theory.

1. Ethical assessment of the situation prior to action

This is the first step. All individual and collective subjects who are affected by the problem are recorded. For each subject, an assessment is made of a balance between *ethical benefits* resulting from respect of the principles of autonomy, nonmaleficence, beneficence and justice, and the *ethical costs* as a result of the violation of these principles. The same action may bring both ethical benefits and costs to the same individual: lying about the diagnosis of a serious disease may be sometimes beneficial but violates the principle of patient autonomy.

2. Possible actions with their ethical implications

It is important that all actions (here including a choice of no action) which could influence the present situation are recorded. In preparing such a list, advice from an expert may be needed. For each action, its influence upon the respect or violation of the four principles for all subjects involved is assessed. Some actions may bring new individuals under consideration.

3. Balancing among ethical costs and benefits and recommendation for action

The third step is often interdisciplinary. A discussion between physicians and such people as philosophers, psychologists, technical experts, economists, ecologists, or politicians will hopefully lead to a consensus regarding the best course of action.

Ethical analysis frequently begins with a question regarding the ethical acceptability of a certain action. Even in such a case, however, all three steps can not be avoided. One can not judge the ethical consequences of a certain action without an insight into the present state, a state which is often not ideal. A proposal for the strict control of private clubs advertising sexual pleasures may be easily rejected on the grounds of limitation of personal autonomy. Nevertheless, such a proposal can only be properly assessed in view of the costs of a liberal policy on the sexual abuse of children or adolescents. Likewise, the ethical acceptability of animal experiments in the screening of new drugs depends

on the weight of the problem to be solved, and on the existence of alternative methods. The serious clinical problem of an incurable disease will find more support than an initiative motivated solely from commercial interests, or the more so if the same results could be obtained from cell cultures.

Our recent discussion on the ethics of genetic screening for breast cancer illustrates how ethical analysis is applied to a particular problem.[6]

Conclusion

Our aim was to present ethical analysis in a way understandable to a professional without training or a deep interest in philosophy. The narrowing of our professional interests should not lead us to leave medical ethics to a few specialists in yet another medical speciality. It is critical that we all participate in discussions which play a decisive role in the shaping our future as professionals and as citizens.

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Ethical principles of autonomy and beneficence in genetic screening for breast cancer

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For a patient with breast cancer and for her adult relative, genetic counselling will usually increase their autonomy and may be beneficial. No benefit for autonomy and markedly negative influence regarding beneficence may be attributed to genetic screening in a young relative who is not yet in the age group at risk for developing breast cancer. For a woman without family history of breast cancer, we may expect an insignificant benefit with respect to her autonomy and beneficence, and potential cost from her false perception of a low risk for breast cancer. These considerations lead to a conclusion that at the present state of knowledge, genetic screening for breast cancer should be restricted to relatives of patients with breast cancer who are already in the age group at risk for developing the disease.

Key words: breast neoplasms-genetics; genetic screening, ethics medical

Introduction

For many decades, familial predisposition towards breast cancer has been recognised as one of the risk factors. Recent research has linked this predisposition to mutation of particular genes, thus allowing us to understand and much more precisely estimate the risk.

We start this paper with a brief summary of current understanding about genetic predisposition towards breast cancer. Then the ethical issues are presented and discussed. We conclude by proposing some practically-oriented ethical guidelines for genetic screening of breast cancer.

Genetic predisposition towards breast cancer

The familial breast cancer syndromes include the site-specific breast cancer, breast cancer with extremely early onset, the breast-ovarian cancer syndrome, the Li-Fraumeni syndrome and some other cancers and rare hereditary conditions which are associated with an increased incidence of breast cancer.[1] About 5 % of all cases of breast cancer and 25 % of those occurring under 35 years of age are due to inheritance of mutations in dominant susceptibility genes which confer a high lifetime risk of the disease.[2]

A number of molecular abnormalities with a loss of heterozygosity have been described in familial and also in sporadic breast cancer.[1] A mutation of BRCA 1 gene on chromosome 17, normally serving as a negative regulator of mammary epithelial cell growth, is at present considered as the most important cause for a genetic predisposition to breast cancer.[3-5] Mutation of this gene has been found in most families with multiple cases of breast and ovarian cancers, and in about half of the families with the early-onset breast cancer.[6] BRCA 2 gene on chromosome 13 has also been implicated in the etiology of some familial breast cancers,[7] and other mutated genes, linked to development or progression of breast cancer, have been recently described.[8]

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Ethical issues in genetic testing and screening

In order to give structure to our discussion of the ethical issues we will use the method of ethical analysis[9] with a simple framework in two dimensions.[10] The first dimension specifies the persons affected, i.e. the patient with breast cancer, her adult relative (who is already in the age group at risk for developing breast cancer), her minor relative, and an adult woman without a family history of breast cancer. The second dimension specifies the relevant value-premises. We will use the two principles of autonomy and beneficence. The first principle states the moral obligation to respect the right to self determination, while the second one states the moral obligation to benefit others, especially not to harm them. Issues of justice are at the present state of knowledge less relevant.

1. A patient with breast cancer

Should the physician offer genetic testing to a patient with breast cancer? Gene diagnostics offers additional information on prognosis and on probability for contralateral disease, and may benefit the patient and help her reach a rational decision regarding follow-up and eventual preventive measures.[5] It therefore seems reasonable to assume that she would like the physician to inform her about the possibility of testing.

However, a patient who has been informed about her genetic predisposition has hardly any choice but to forget about the privacy of her disease and feel a strong responsibility for other women among her relatives. She might feel guilty because of the implications for her daughters, and would feel obliged to inform and perhaps even advise them. But we doubt that she, in this situation, would be the best advisor for her relatives concerning genetic screening or preventive measures.

To sum up: genetic testing offered to a patient with breast cancer would principally be to her advantage, both from the point of view of the principle of autonomy and the principle of beneficence. However, a positive result of screening may also imply ethical costs such as guilt and a loss of privacy (Table 1, first row).

2. Adult female relative of a patient with breast cancer

A woman who is in the age group at risk for developing breast cancer and who has a close relative with the disease is nowadays often aware of the familial predisposition towards the disease. Testing for genetic predisposition will allow her either to dismiss her fears, or to reach a rational approach towards future preventive or screening activities.

A positive result of screening for genetic predisposition may also have adverse effects. The permanent threat of breast cancer may be an unbearable burden and a source of extreme concern. Probable involvement of psychological factors in the multifactorial etiology of breast cancer[11,12] is explained by studies of molecular mechanisms for stress-induced alterations in susceptibility to cancer:[13] we may therefore speculate that genetic screening, with its possible negative effect on the woman's emotional stability could in fact increase the chances that the woman will actually develop the disease.

To sum up: to offer testing to the adult relative would provide her with an opportunity for more rational decisions about screening and eventual preventive measures, but a positive result would almost certainly adversely affect the quality of her life (Table 1, second row).

3. Minor female relative of a patient with breast cancer

At present, we can offer no practical advice to a child or a woman younger than the age group at risk for developing breast cancer. Whether she undergoes screening or not and regardless the result of the test, no activity seems advisable.

There are two possible outcomes. If the tests turn to be negative, the young relative will be relieved of her fears. But a positive result of testing starts a life-long and increasing anxiety. One may speculate that the relief of fears is insignificant when compared to potential harmful effects of the news about a positive genetic predisposition: while a teenager tends to accept positive news as granted, she will probably show extreme concern about even minor negative aspects of her body image, or of predictions about future life.

It is natural to share our secret fears with those whom we love. Unfortunately, however, a young woman who has told this news to her boyfriend may soon realise how weak and full of prejudices is the human nature: the news may spread and adversely affect her social life.

To sum up: testing a young woman for genetic predisposition will result in information which has,

at present, no practical implications. If the test is negative she will experience relief, but a positive test would almost certainly have a serious negative impact on her emotional stability and social life (Table 1, third row).

4. Adult woman without a family history of breast cancer

The likelihood of detecting a mutation of BRCA1 or of another gene predisposing to breast cancer among women without a family history is very low: less than one among 500 will be positive.[2] On the other hand, breast cancer is a frequent disease, affecting close to 1 in 10 women in Western Europe and North America.

In the rare instance when the test is positive, the benefits and costs to such a woman will be the same as discussed under the category of an adult relative of a patient with breast cancer. If the result is negative, however, it will be very difficult not to leave the woman with a feeling that her risk of developing breast cancer is small – when, in fact all what a negative test says is that she is not among those very few women who, in spite of a negative family history, develop breast cancer as a result of their genetic predisposition. Such a false feeling of safety might affect the compliance to the established, cost-effective and life-saving programmes of self-examination and mammographic screening.

To sum up: testing a woman without family history of breast cancer will most probably yield a negative result, implying an insignificant ethical benefit with respect to the woman's autonomy and beneficence. At the same time, potential serious cost may arise from her false perception of a low risk for breast cancer (Table 1, last row).

Discussion and conclusions

Many women, and especially relatives of patients with breast cancer, are now aware of a high incidence of breast cancer and of the possibility that the risk is genetically determined, and the demand for genetic counselling and the related screening and prevention strategies is increasing.[14-16] A survey among first-degree relatives of ovarian cancer patients revealed that 75 % would definitively want to be tested for a mutation of BRCA1 gene.[17] Still, in spite of the efforts to convey an objective information, the perception of the true risk for developing the disease is often very unprecise;[18] compliance with the recommended programmes for early diagnosis is poor;[19] and psychological distress is often so severe that professional counselling is needed.[20, 21]

It seems that for a patient and for her adult relatives, the advantages of testing often outweigh the potential disadvantages. This opinion is in concordance with a high level of interest for genetic screening among adult members of families with an elevated risk for breast or ovarian cancer.[17, 22] On the other hand, few advantages and severe negative effects shift the balance to the opposite side in a young woman. The potential harm induced by genetic screening in this age group far outweighs the benefits. Until something can be done to remove the genetic defect, we believe that genetic screening should not be done to persons younger than the earliest age when the disease may be detected. We also see few advantages, and possible costs in testing women without a family history of breast cancer: there may be no public health benefit in screening the general population for genetic susceptibility to common, multifactorial disorders.[23]

According to a Statement of The American Society of Human Genetics,[24] genetic testing for breast

Table 1. Ethical costs and benefits of testing for genetic predisposition for breast cancer. As a base-line, no such testing is assumed.

	AUTONOMY	BENEFICENCE
A patient with breast cancer	Benefits and possible costs	Benefits and possible costs
An adult female relative	Benefits	Benefits and possible costs
A minor female relative	Neither benefits nor costs	Minor benefits or severe costs
Adult woman without a family history of breast cancer	Insignificant benefits	Insignificant benefits and potential serious costs

cancer predisposition is not a routine, and it is premature to offer population screening. A clear indication for testing can be found only in families with a mean age at diagnosis of less than 45 years, and controlled studies are urgently needed to assess the value of the recommended screening protocols.[25,26] An important issue in testing for a genetic predisposition is the psychological harm caused by an embarrassing information which is accompanied by an unprecise practical advice.[20, 21] While we share this concern, the aim of this report is to show that neither of the two extreme positions regarding genetic screening may be generally acceptable. Careful weighting of the ethical costs and benefits in applying these procedures may identify groups of women for whom the procedure seems advisable, and others in whom the ethical cost is prohibitive. This clearly applies to the present state of knowledge: if removal of the genetic defect becomes possible or if targeted methods of prevention become available, ethical evaluation will lead to a different conclusion.

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Radiol Oncol 1996; 30: 314-6.

Seventh international symposium on Neutron Capture Therapy for cancer

September 4-7, 1996, Zurich

The Symposium was organized by the Paul Sherer Institute and University of Zurich on behalf of the International Society for Neutron Capture Therapy and has gathered some 300 scientists and clinicians from some 30 countries. Due to the very selected topic of the symposium, the majority of the presentations either oral or posters were devoted to different aspects of Neutron Capture Therapy (NCT). The scientists were gathered dealing with physical, chemical, biological and clinical problems associated with NCT.

In its principle NCT is bimodal therapy. It is based on selective accumulation of the agents which become cytotoxic only when activated by some form of radiation. Therapy could be confined to the chosen region of the activating irradiation, independent of the distribution of the targeting agent. The best known example of this principle is boron neutron capture therapy (BNCT). The beginnings of BNCT are in 1936 when Lochner suggested that neutron capture reaction on boron-10 could be used in cancer treatment. Boron-10, which is a stable isotope could be linked or incorporated into a substance that has affinity to the tumor cells and the tumor area irradiated with thermal neutrons. The neutron capture reaction in boron leads to the prompt emission of lithium and helium particles, which release their kinetic energy with less than 10 mm from the reaction site. Hence, if boron carrier with high tumor-specific uptake can be found, this technique may provide a "magic bullet" that kills only the tumor cells with boron uptake, while sparing the surrounding boron-free healthy tissue.

According to these principles of NCT it is evident which are the major obstacles to broader clinical application of the therapy. Major problems are tumor targeting of the boron-10 containing substances and their activation with adequate neutron sources.

From the centers that are involved in NCT Japanese have the most experience. They have performed already several clinical trials and demonstrated that NCT is feasible for treatment of neuroblastoma and malignant melanoma tumors. Following their pioneering work, centers in USA and in Petten in Europe have done a lot of work in this field, which has or will soon lead into the treatment of the first patients on trans-national scale. However, except these reactors already mentioned there are 11 centers all around the world that have pursued to start with the NCT. Slovenia is also one of them, preparing the TRIGA Mark II reactor for the NCT studies. The aim of the project is to develop some new approaches in targeting the boron-10 containing substances to the tumors, in order to increase therapeutic gain of the NCT.

New developments in preclinical studies on NCT are according to the reports on the symposium in the fields of biology, chemistry and physics. Biology of the NCT is largely dependent on biodistribution of boron containing substances. One of the directions mentioned was to search for new compound that are more specific for tumor cells, such as the boronophenylalanine (BPA). This is a substance that is specific for the melanoma cells, but the problem with the substance is that it is not readily soluble. Therefore, new analogues are sought to find the analogue with high water solubility and good specific accumulation in tumor cells. In treatment of glioma tumors sulfhydril boron hydride (BSH) is already well established. It is well water soluble but still there is not enough its accumulation in the tumor tissue. In order to increase the boron uptake in the brain tumors new strategies are sought. On of them is to invasively approach with intracerebral infusion. This approach was already reported, but with limited success, because distribution within the tumors was not even. The other invasive approach is to disrupt blood brain barrier, which is the major limiting factor for the delivery of boron compounds into the brain tumors. Another possibility is to inject substances into carotid

artery. The pharmacological approach could be to incorporate the boron containing substances into the tumors, or to search for smaller molecules. Neither of the two approaches has produced any positive results. Biology in NCT is directed also to radiation oncology. Predictive assays for the NCT studies are highly needed. Unfortunately in this field, similarly as in radiation biology, many attempts were undertaken but with limited success. Important are also imaging assays, to develop a system that would with high accuracy detect boron distribution in the tumors. This is important because relative biological effectiveness of NCT is proportional to the boron content in the tumors. Some computer programs, coupled to boron detection in the tumors *in vivo*, are already operable. These programs help with treatment planning of the patients and are in essence similar to classical treatment planning in oncology. Very few were the contributions from the field of molecular oncology in NCT. Fortunately our contribution of dr. Đ. Novak was one of them. She has produced monoclonal antibodies with high specificity to breast carcinoma cells, with cross reactivity for melanoma cells. These monoclonal antibodies coupled with BSH proved to be very effective in targeting boron to tumor cells, and also proved effective in NCT study *in vitro*. It was stressed in the symposium that it is necessary to attract more molecular biologists and radiobiologists into the field to further develop this new treatment modality.

In the field of chemistry there were few original contributions. Therefore in the concluding remarks it was stressed that this is the field that is not developing fast enough, and does not follow the needs of the NCT. Search for the analogues of the already known compounds continues, as already mentioned, but there is little effort put into development of new compounds that would be specific also for other tumors.

In the field of physics the following topics were discussed: neutron sources (reactors, accelerators, cyclotrons), dosimetry and microdosimetry, dose planning. The requirements for an acceptable neutron source for NCT are rather scarce in terms of the need to provide sufficient epithermal neutrons (0.4 eV to 10 KeV, flux 0.84 x 10^9 nepi/cm^{2}sec^{-1}) to a patient's accessible location in a reasonable time with minimal thermal neutrons, fast neutrons or gamma ray background. Besides the epithermal neutrons from high flux reactors applied for the medical treatment, thermal neutrons from the much smaller and less expensive low power research reactor (as TRIGA Mark II 250 KW) have an important role in the further development and application of NCT, was stressed in concluding remarks. The research group from Finland reported how they succeeded to adapt TRIGA Mark II research reactor. The neutrons are moderated into the epithermal range using a patented material. The facility does not have any beam shutter between the core and the moderator, so the output of the beam always follows the power of the reactor. Therefore, the alternative looks as follows: simple, cheap, safe reactor of low power with expensive filter or powerful, expensive and potentially more dangerous reactor with simple and cheap filter. For reasonable choice between these variants a deeper technical and economical study is necessary. In the poster session our research group from TRIGA Mark II reactor presented the development of the radiation facility using Monte Carlo simulation code MCNP4A. With information on symposium our project team is encouraged to continue the research with small program corrections.

Clinical aspect of NCT was dominated by the groups that already perform NCT studies. Japanese groups have the treatment already as established treatment modality and are therefore leading groups in this field. They treat both, glioma and melanoma patients, with high success rate. However, the success of the treatment is still not very convincing compared to standard radiotherapy treatment. They have demonstrated that in treatment of malignant melanoma patients they have more success than with glioma patients, especially because the tumor lesions are easier to access with radiation in melanoma patients than in glioma patients, and because of the development of the BPA substance that is highly specific for melanoma cells. The American groups have so far treated 19 cases of glioma tumors. Therefore, their experience in NCT is much smaller, but their clinical trials are more strict and well planned. The discussion about the fractionation in NCT is contradictory. The American groups argue that fractionation is not necessary, however the European study has foreseen in protocol fractionation. In Europe first clinical trial will begin soon in EORTC protocol as phase I clinical trial. Before the first patients will be irradiated in Petten, pharmacological studies on BSH in patients have been done. The purpose of the trial is to treat glioma patients from several centers in Europe in Petten. After tedious administrative obstacles, which

have been overcome lately, the first patients are scheduled to be irradiated in Petten by the end of this year. Slovenia has not succeeded to enter into the phase I trial, but will most probably enter the phase II trial and send some of their patient to be treated in EORTC protocol.

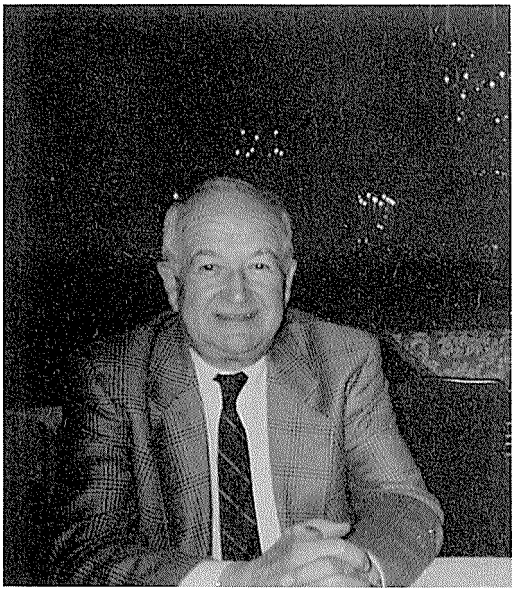
In conclusion, the developments in NCT are predominantly in radiophysics, radiobiology and treatment planning, however less in the chemistry. The NCT is still in its developmental phase but is attracting many new scientists that feel this is an topic which has perspectives. In the light of this developments, also Slovenian project has high hopes to contribute some insights into NCT. We all hope that in the next meeting in USA in 1998, progress in NCT will be seen.

Gregor Serša Ph.D.
prof. Janez Škrk Ph.D.
Institute of Oncology, Ljubljana

Radiol Oncol 1996; 30: 317-8.

In memoriam

Prof. Krsto Kolarić



Prof. Krsto Kolarić, Ph.D., left us forever on April 28, 1996.

The severe illness which he had bravely fought for several years finally gained the upper hand. He left us almost imperceptibly, as if he had come to terms with the inevitable.

He was born in Koprivnica on May 22, 1928. He finished high school in Zagreb, where he also graduated from the School of Medicine of the University in 1953. In 1963 he completed his specialization in internal medicine at the Military Hospital in Zagreb, where he later became Head of the Hematology Division of the Medical Department. In 1972 he joined the Central Institute for Tumors and Allied Diseases.

His efforts were instrumental in the establishment, over the past 24 years, of the medical oncology nucleus which has had a major impact on the development of this medical subspeciality in Croatia. He founded the Chemotherapy and Medical Oncology Service of the Institute for Tumors in line with modern principles. He introduced solid tumor polychemotherapy, and modern clinical testing principles in the treatment of solid tumor patients, into Croatian medicine. The Chemotherapy and Medical Oncology Service and the Central Institute for Tumors (now the University Hospital for Tumors) became the venue of many European polycentric clinical trials, many of which were designed in Zagreb.

Prof. Kolarić was a member of a dozen European and overseas oncological associations, among which particular mention should be made of the American Society of Clinical Oncologists (elected member), the European Society for Medical Oncology and the European Association for Cancer Research. He was an honorary member of the Hungarian Oncological Society. Prof. Kolarić has coauthored ten foreign books on solid tumor chemotherapy, and was one of the editors and authors of the first book on solid tumor chemotherapy to appear in Croatia, *The Chemotherapy of Malignant Solid Tumors*, published in Zagreb in 1982. He was a consultant of the World Health Organization. He took part as invited lecturer in many international scientific meetings and conferences. Prof. Kolarić was also the M.S. and Ph.D. thesis supervisor of quite a few young researchers. From 1991 to his death he was the editor-in-chief of the Croatian oncological journal *Libri Oncologici*. In 1986 he was appointed Professor at the Internal Medicine Chair of the School of Medicine, University of Zagreb.

Prof. Kolarić published more than 200 scientific and technical papers, half of them in leading foreign journals. He was one of the most frequently quoted Croatian scientists in international scientific publications. There was hardly a European country in which he did not have close contacts with leading oncologists, and he also exchanged his experience with many American oncologists. He travelled throughout the world promoting the achievement of Croatian medicine and contributing to the scientific image of Croatia. He helped many colleagues, also beyond Croatia's frontiers, in their scientific and professional advancement, and he fostered particu-

larly close relations with his colleagues in Slovenia. He was a masterly scientist with a particular feeling for detecting the essence of a scientific problem and designing a new research project on that basis. He was obliging, cordial, well-meaning, ready to help, to teach, but also to reprimand when he thought it necessary. He always welcomed the success of his younger associates.

He devoted the last years of his life to *Clinical Oncology*, a project into which he had invested all his effort and knowledge; unfortunately, he did not live to see the publication of this major work of Croatian oncology.

We have lost so much with the premature death of our esteemed colleague, teacher and dear friend, but his professional and scientific work has left an indelible trace in medical onoclogy.

Zagreb, July 1996 Anton Roth

Notices

Notices submitted for publication should contain a mailing address, phone and/or fax number of a contact person or department.

English for cancer conference

April 3-7, 1997.

The ESTRO endorsed teaching course will take place in Edinburgh, Scotland, UK.

Contact John Maclean, Institute for Applied Language Studies, University of Edinburgh, 21, Hill Place, Edinburgh EH8 9DP, Scotland, UK; or call +44 131 650 6200; or fax +44 131 667 5927.

Tumour biomarkers

April 14-16, 1997.

The ESO advanced course "Tumour Biomarkers: Methodology and Clinical Significance" will be held in Venice, Italy.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Paediatric Oncology

April 23-25, 1997.

The International congress of the UK Children's Cancer Study Group "Childhood Cancer into the 21st Century" will take place in Birmingham, UK.

Contact UKCCSG; or call +44 1794 511 331; or fax +44 1794 511 455.

Molecular genetics

April 23-27, 1997.

The ESO advanced course "Molecular Genetics in Solid Tumours for Clinical Oncologists" will be offered in Ascona, Switzerland.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Genito-urinary tract tumours

April 25-26, 1997.

The ESO training course will be held in Buenos Aires, Argentina.

Contact ESO Latin America, Dr.A.Rancati, Av. Las Heras 1666, 1018 Buenos Aires, Argentina; or call +54 1 8141 129; or fax +54 1 8141 129.

Genito-urinary tract tumours

April 27-28, 1997.

The ESO training course will be held in Montevideo, Uruguay.

Contact ESO Latin America, Dr.G.Farante, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Oncology

May, 1997.

The ESO training course "Diagnostic and Therapeutic Radiology in Oncology" will be held in Moscow, Russia.

Contact ESO Russia and Community of Independent States, c/o CSC Ltd., Mrs. Mira Vukelic, Heiligenstaedter Strasse 395b, 1190 Vienna, Austria; or call +43 1 3188 466; or fax +431 3188 466-20.

Brachytherapy

May 5-7, 1997.

The "Annual Brachytherapy Meeting GEC-ESTRO" will be held in Stockholm, Sweden.

Contact the ESTRO office, Radiotherapy Department, University Hospital Gasthuisberg, Herestraat 49, B-3000 Leuven, Belgium; or call +32 16 34 76 80; or fax +32 16 34 76 81.

Gynecological cancer

May 7-9, 1997.

The ESO training course will be offered in Nicosia, Cyprus.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 Ioannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

AIDS

May 8-10, 1997.

The ESO advanced course "AIDS Related Malignancies" will be offered in Milan, Italy.

As a service to our readers, notices of meetings or courses will be inserted free of charge. Please sent information to the Editorial office, Radiology and Oncology, Vrazov trg 4, SI-1105 Ljubljana, Slovenia.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Medical oncology

May 15-16, 1997.

The ESO training course "Practical Problems in Medical Oncology" will be held in Miami, USA.

Contact ESO US Office, Mrs. Gilda Zane, AICF, 872 Madison Avenue, Suite 2B, New York, NY 10021, USA; or call +1 212 6289 090; or fax +1 212 5176 089.

Oncology

May 17-20, 1997.

The "ASCO Spring Meeting" will be offered in Denver, CO, USA.

Contact ASCO Headquarters, 435 North Michigan Av., Suite 1717, Chicago, USA; or call +1 312 644 0828; or fax +1 312 644 8557.

Paediatric pathology

May 22-28, 1997.

The ESO training course will be offered in Ioannina, Greece.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 Ioannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Paediatric oncology

May 26-30, 1997.

The ESO training course will be offered in Halkidiki, Thessaloniki, Greece.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 Ioannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Breast cancer

May 29-31, 1997.

The ESO advanced course "Breast Reconstructive Surgery" will be held in Paris, France.

Contact European School of Oncology, Via Ripamonti, 66, 20141 Milan, Italy; or call +39 2 57 305 416; or fax +39 2 57 307 143.

Psychology - oncology

May 30 - June 1, 1997.

The ESO training course will be offered in Ioannina, Greece.

Contact ESO Balkans and Middle East Office, c/o Egnatia Epirus Foundation, 7A Tzavella St., 453 33 Ioannina, Greece; or call +30 651 72315/76992; or fax +30 651 36695.

Imaging diagnosis in breast cancer

May 31 - June 1, 1997.

The ESO training course will be held in Buenos Aires, Argentina.

Contact ESO Latin America, Dr.A.Rancati, Av. Las Heras 1666, 1018 Buenos Aires, Argentina; or call +54 1 8141 129; or fax +54 1 8141 129.

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Editors greatly appreciate the work of the reviewers who significantly contributed to the improved quality of our journal.

Author Index 1996

Subject Index 1996

International Conference

LIFE SCIENCES 1997

and

SLOVENIAN - CROATIAN MEETING ON MOLECULAR ONCOLOGY TODAY

Gozd Martuljek, Slovenia
16th-19th October, 1997

ORGANIZED BY

Slovenian Biophysical Society
and
Institute of Oncology, Ljubljana

IN COOPERATION WITH: Physiological Society of Slovenia, Slovenian Genetic Society, Slovenian Immunological Society, Slovenian Medical Association, Oncology Section, Slovenian Pharmacological Society, Slovenian Society for Medical and Biological Engineering, Society for Stereology and Quantitative Image Analysis, Faculty of Electrical Engineering, University of Ljubljana, Institute Rudjer Bošković, Zagreb

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Biophysics and Biology of Tumors

MAIN TOPICS

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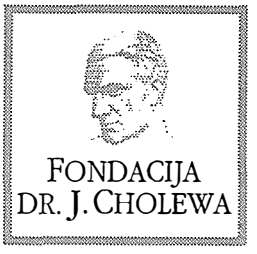

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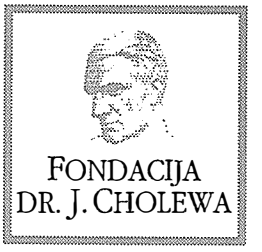

Activity of "Dr. J. Cholewa Foundation" for cancer research and education – report for the first and second quarter of 1996

"Dr. J. Cholewa Foundation" for cancer research and education continues its activity in the third quarter of 1996 as outlined at the meetings of the executive and scientific councils of the Foundation at the end of 1995, albeit at a slightly slower pace, as it can be expected in the summer months.

The meeting of the assembly of the Foundation took place in the end of June, 1996. The Foundation is proud to announce that several new members of the executive council were present at this meeting. All new members of the executive council were unanimously elected to this position, and in this way the council is now composed of experts in various fields of oncology from almost all major and important institutions in Slovenia that spend at least part of their activity in cancer research and education. Members of the executive council also confirmed the report on the financial situation in the Foundation, as presented by the President of the Foundation.

Another important milestone in the development of the activity of the Foundation is represented by the meeting between its representatives and high level officials from European School of Oncology from Milan. The meeting took place slightly after the assembly of the Foudation, and specific points of the collaboration between these two institutions were discussed. Several interesting initiatives were investigated, one of the more important being the possible intensification of some of the publishing activity of the European School of Oncology in Ljubljana. Details of the various initiatives in the collaboration between these two institutions will be further discussed in the coming months of the final quarter of 1996.

From the activity of the Foundation in the summer months of 1996 it is clear that despite the summer recess it continues to follow its stated goals.

Tomaž Benulič, MD
Borut Štabuc, MD; PhD
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Symposium on

ORGAN SPARING TREATMENT IN ONCOLOGY

Ljubljana, Slovenia
19–21 June 1997

Organized by:

Institute of Oncology, Ljubljana, Slovenia

Under the auspices of:

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Design and Content

The Symposium is designed for medical doctors and other specialists involved in cancer treatment who wish to exchange experiences with oncologists and to contribute to upgrading the knowledge in organ sparing treatment. It will cover the following topics:

- Breast Conserving Therapy
- Bladder Sparing Treatment in Muscle Invasive Bladder Carcinoma
- Organ Sparing Treatment in Head and Neck Carcinoma
- Organ Sparing Treatment in Soft Tissue Tumors
- Quality of Life after Organ Sparing Treatment

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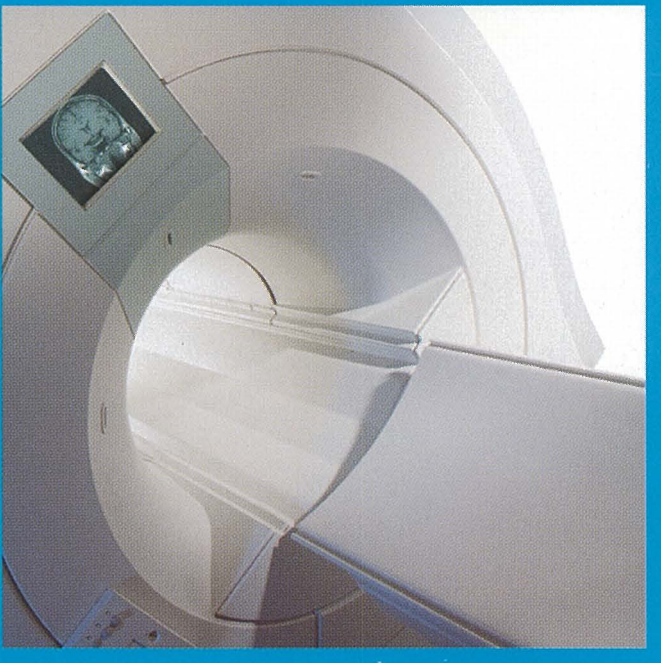

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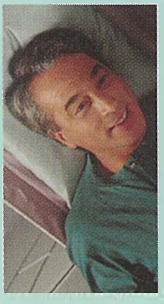

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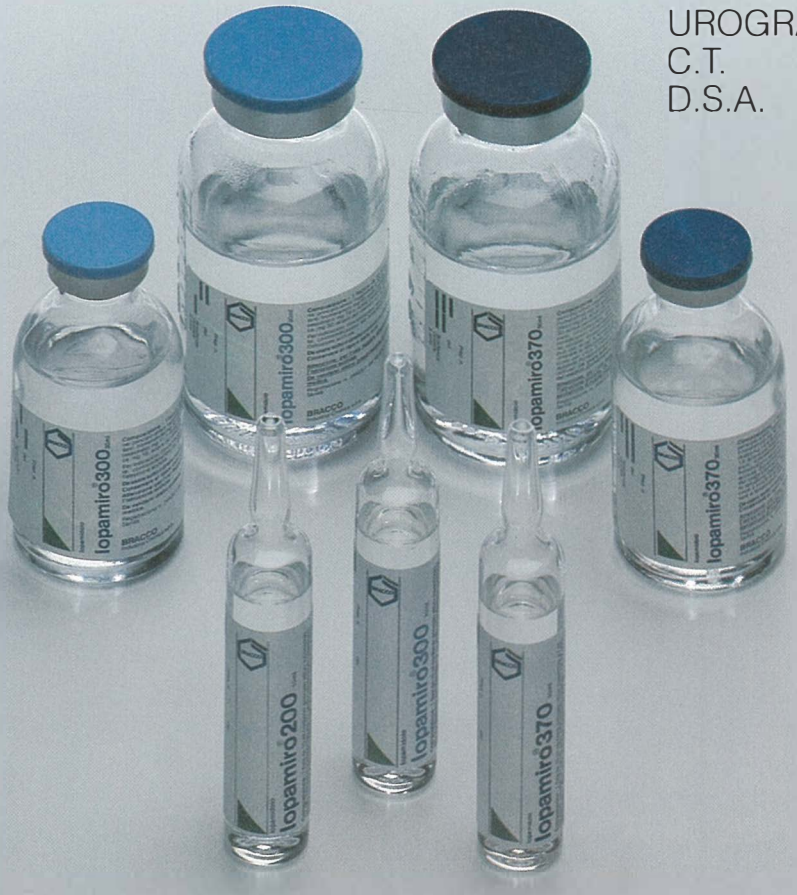

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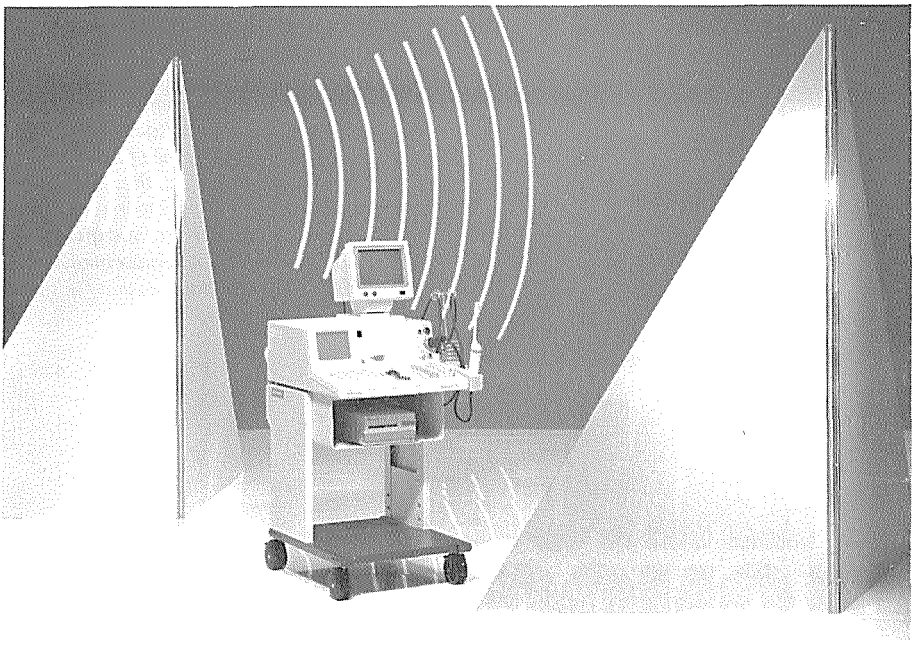

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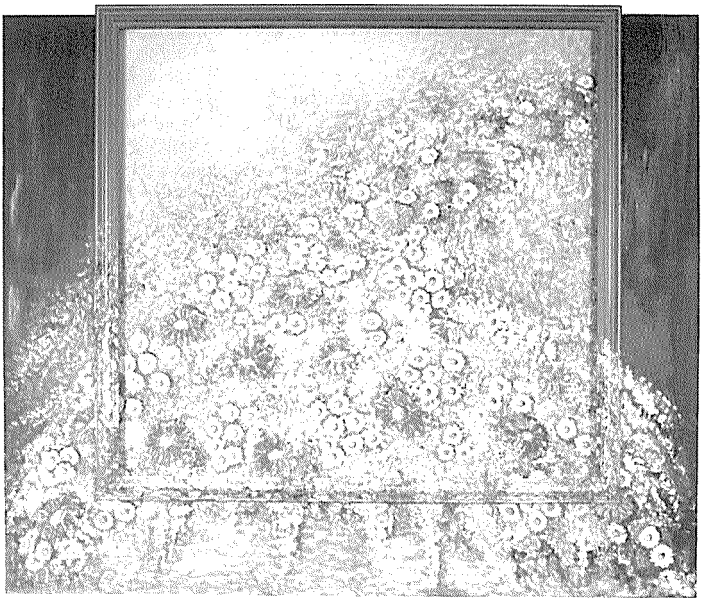

– centralno delujoči analgetik za lajšanje zmernih in hudih bolečin

– učinkovit ob sorazmerno malo stranskih učinkih

– tramadol je registriran tudi pri ameriški zvezni upravi za hrano in zdravila (FDA)

Kontraindikacije: Otroci do 1 leta starosti, akutna zastrupitev z alkoholom, uspavali, analgetiki in drugimi zdravili, ki vplivajo na CŽS, zdravljenje z inhibitorji MAO. **Interakcije:** Pri sočasni uporabi zdravil, ki delujejo na osrednje živčevje, je možno sinergistično delovanje v obliki sedacije pa tudi močnejšega analgetičnega delovanja. **Opozorila:** Pri predoziranju lahko pride do depresije dihanja. Previdnost je potrebna pri bolnikih, ki so preobčutljivi za opiate, in pri starejših osebah. Pri okvari jeter in ledvic je potrebno odmerek zmanjšati. Bolniki med zdravljenjem ne smejo upravljati strojev in motornih vozil. Med nosečnostjo in dojenjem predpišemo tramadol le pri nujni indikaciji. Bolnike s krči centralnega izvora skrbno nadzorujemo. **Doziranje:** *Odrasli in otroci, starejši od 14 let:* 50 do 100 mg 3- do 4-krat na dan. *Otrokom od 1 leta do 14 let* dajemo v odmerku 1 do 2 mg na kilogram telesne mase 3- do 4-krat na dan. **Stranski učinki:** Znojenje, vrtoglavica, slabost, bruhanje, suha usta in utrujenost. Redko lahko pride do palpitacij, ortostatične hipotenzije ali kardiovaskularnega kolapsa. Izjemoma se lahko pojavijo konvulzije. **Oprema:** 5 ampul po 1 ml (50 mg/ml), 5 ampul po 2 ml (100 mg/2 ml), 20 kapsul po 50 mg, 10 ml raztopine (100 mg/ml), 5 sveč po 100 mg.

Podrobnejše informacije so navoljo pri proizvajalcu.

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Discussion should explain the results, and not simply repeat them, interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

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Dent RAG, Cole P. *In vitro* maturation of monocytes in squamous carcinoma of the lung. *Br J Cancer* 1981, **43:** 486-95.

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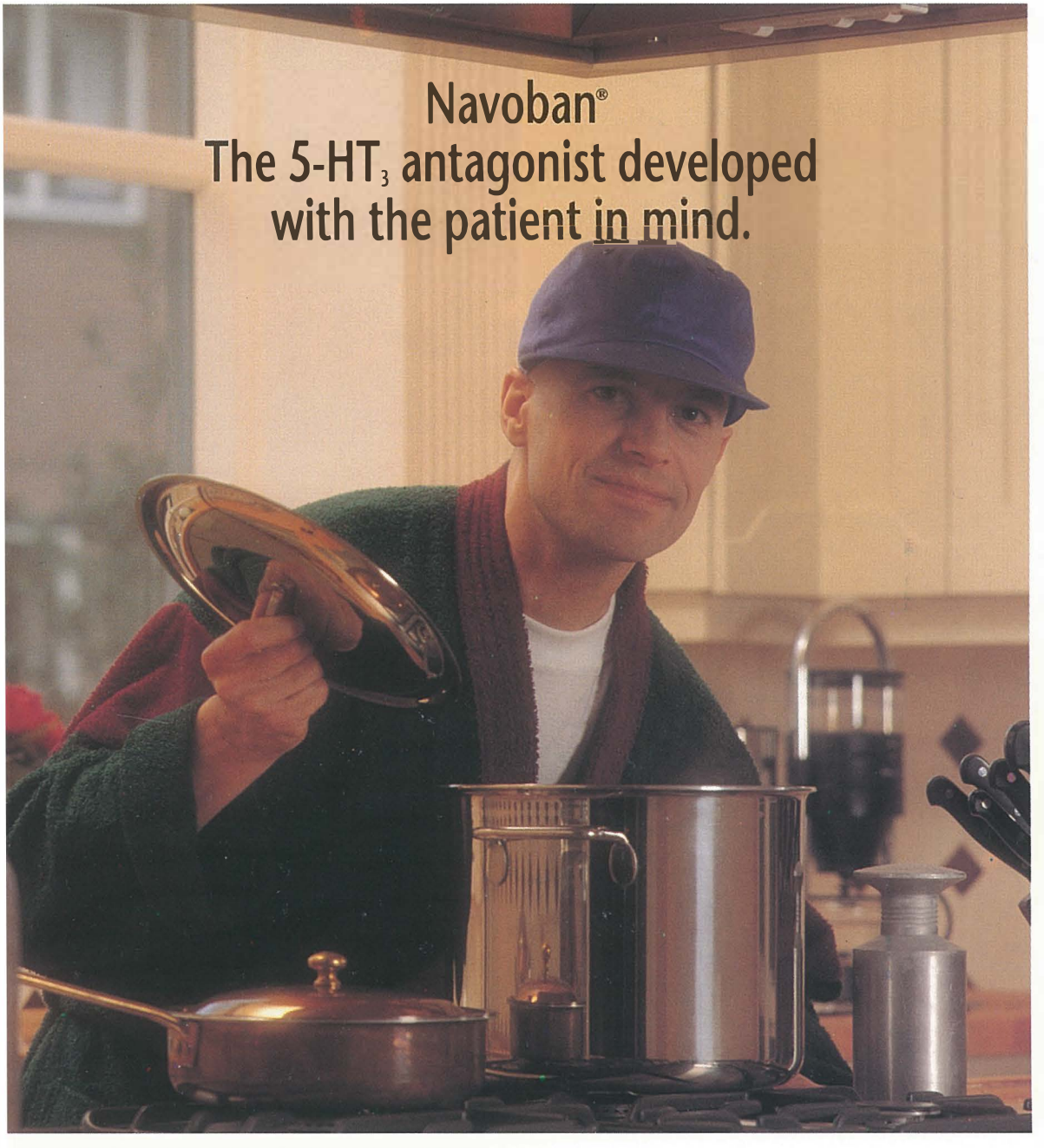

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