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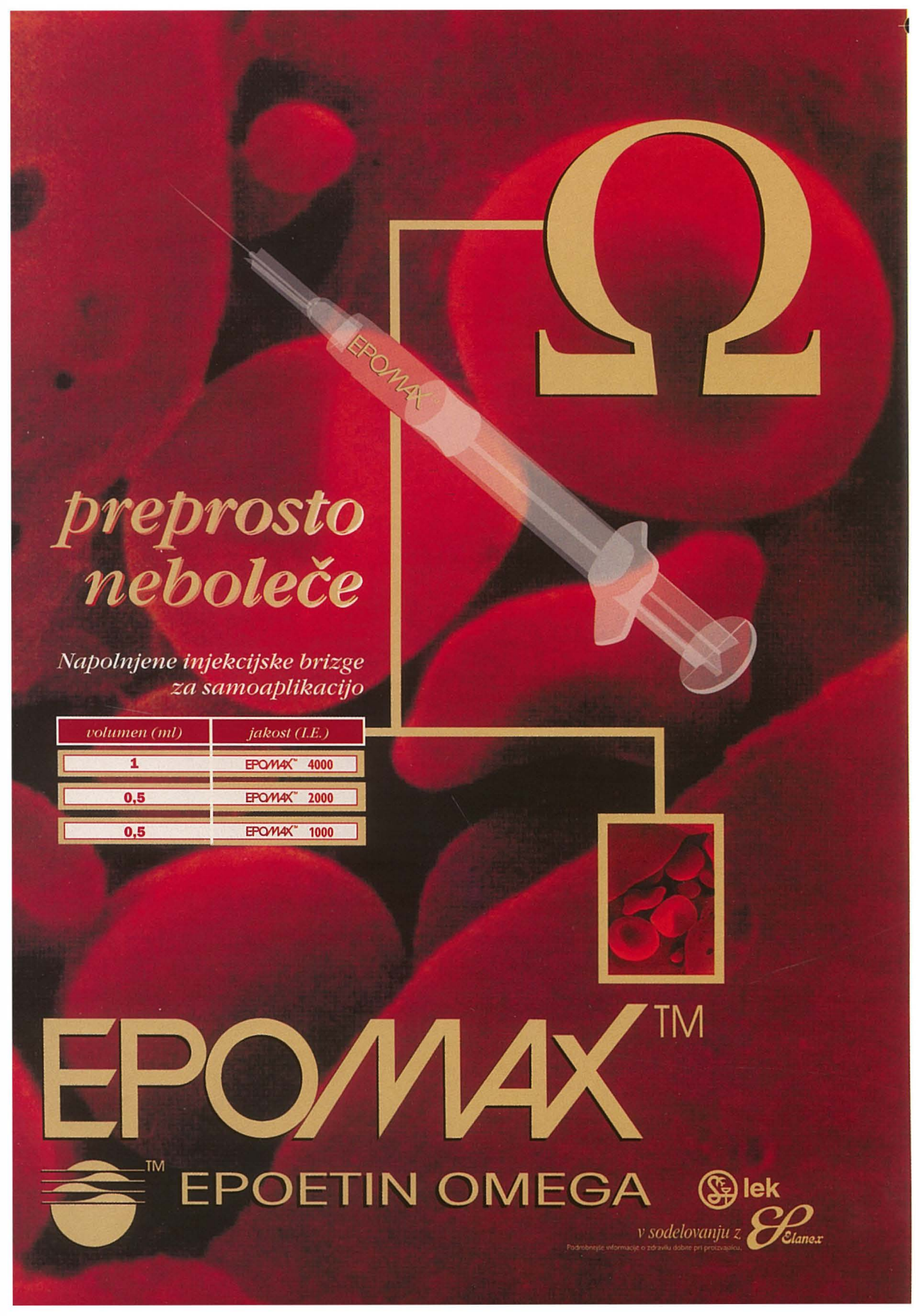
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Correspondence address
Radiology and Oncology
Institute of Oncology
Vrazov trg 4
SI-1000 Ljubljana
Slovenia
Tel: +386 61 132 00 68
Tel/Fax: +386 61 133 74 10

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Olga Shrestha

Design
Monika Fink-Serša

Key words
Eva Klemenčič

Secretaries
Milica Harisch
Betka Savski

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Radiol Oncol 1998; 32(4): 353-61.

High-resolution ultrasound and power-Doppler - advances in pre-invasive diagnosis of solid breast lesions: our one-year experience

Zoran Brnić, Ivan Drinković, Andrija Hebrang

Department of Radiology, University Hospital "Merkur", Medical School of Zagreb University, Zagreb, Croatia

The purpose of the study was to evaluate high-resolution ultrasound (HRUS) and power-Doppler (CDE) in the differentiation of malignant from benign solid breast lesions.

Patients and methods: HRUS and CDE examinations of solid breast masses were carried out in 25 women. Gray-scale criteria of malignancy and benignity were considered. The vessels of the lesion were classified as penetrant, peripheral and non-detectable with CDE. Final diagnose was obtained cytologically and/or with biopsy.

Results: HRUS detected more frequently irregularity of contours, heterogeneity and posterior attenuation than "classic" ultrasound. Lateral shadows in carcinomas were seen in a considerable number of cases, but this did not have any impact on the accuracy of diagnosis. HRUS facilitated the visualization of small carcinomas intraductal calcifications and papillomas. CDE detected flow in 15/25 lesions, of which 8 were malignant. Penetrant vessels were observed in 6/8 carcinomas and only in 2/17 benign changes;6/11 fibroadenomas were avascular, and 4/11 with peripheral vessels. In 3/6 other benign lesions, the flow was shown with CDE.

Conclusion: HRUS and CDE can successfully help in differentiation malignant from benign solid breast mass, and are a good adjunct to mammography and physical examination in the pre-invasive phase of diagnostic process.

Key words: breast neoplasms; ultrasonography, mammary; ultrasonography, Doppler; high-resolution ultrasound

Introduction

Breast sonography performed until few years ago with 5-7.5 MHz transducers was mainly confined to the differentiation between cystic and solid nature of the lesion and, to some extent, to characterizing solid mammary nodules.[1] High-frequency transducers (up to 13

Correspondence to: Zoran Brnić, M.D., B.Sc., Department of Radiology, University Hospital "Merkur", Zagreb, Medical School of Zagreb University, Zajčeva 19, 10000 Zagreb, Croatia. Phone/Fax: +385 1 233 14 40.

MHz) constructed in the last decade allow perfect spatial and contrast resolution, with the former being well lower than a millimeter. Therefore, non-palpable lesions of a few millimeters can be detected, whereas palpable ones more precisely characterized. This "high-resolution ultrasound" (high definition ultrasound, HRUS) was "born" approximately at the same time as the power-Doppler (color-Doppler energy, CDE) was introduced as a new mode of color-Doppler imaging. Although sonography has, as yet, an uncertain place among other differential diagnostic steps and is considered by many as a secondary technique,[2] HRUS and CDE available in most modern US machines enable preinvasive work-up of breast lesions with more sensitivity and specificity, sparing thus many unnecessary biopsies of benign lesions.

Tumor vascularization and power-Doppler

Each tumor larger than a few millimeters depends, during its growth, on the proliferation of new vessels in its periphery, and produces substances (angiogenetic factors) that stimulate neoangiogenesis.[3,4] In breast carcinomas, an increased number of the vessels is evident. Their diameter is enlarged. The structure of its wall, as well as the architecture are aberrant (AV-shunts, sinusoids), with a consecutive abnormal function.[5] The abnormality of such vessels is a clue to the features of Doppler signals gained from malignant breast masses, or from their close surroundings.[6]

B-mode, regardless of its high resolution, cannot visualize small intratumoral vessels because of their microscopic dimensions. The detection of such vessels on the basis of flow is thus a great advance, firstly, enabling a precise positioning of sample volume and acquisition of spectral flow signal and, secondly, imaging of their distribution and architecture, especially with CDE.

Although there are many inconsistencies and overlapping of results, and as malignancy cannot be ruled out only due to the absence of flow signal in the mass, the majority of authors agree that vessels are more numerous, and velocities higher in malignant than in benign masses. Some authors also report of the increase of pulsation indices.[6-8]

"Conventional" color-Doppler imaging (CDI, color-Doppler velocity, CDV) detects and displays blood velocity and its variance. Since 1993, a new color Doppler ultrasound technique has become available. It provides information of total amplitude or energy of signal, rather than velocity and direction of flow (Figure 1).

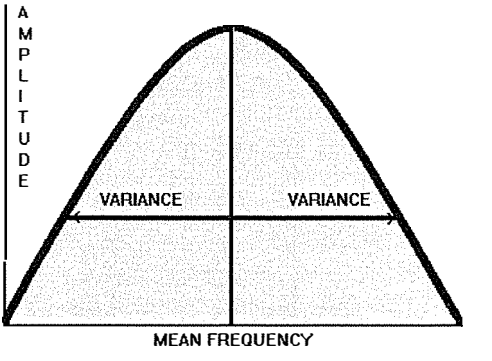

Figure 1. shows typical Doppler signal spectrum: variance is marked with arrows. Energy is proportional to the area under the curve under the curve, which is shown shadowed.

The new mode is termed power-Doppler, color Doppler energy (CDE), "ultrasound angiography" or amplitude color-Doppler sonography, which is the most exact term.[1] The flow is coded in hues of a single color, e.g. yellow, rather than blue and red (+green) as with standard CDI, and the color saturation of a pixel is related to the number of red blood cells in a unit of volume (voxel), regardless of their vector sum. When the resultant velocity is zero, as within the areas of capillaries randomly distributed in a voxel, related pixels on CDI will not be coded at all. On the contrary, CDE displays nearly all the amount

of circulating blood in these low-flow areas, which results in improved sensitivity.[9,10] Thus, directional averaging in each pixel is substituted with the summing of amplitudes, and randomly distributed tissue capillaries altogether contribute to the signal strength. Hence, an overwhelming "blush" in good vascularized organs, such as kidneys, will be apparent.[11] There is no angle-dependence of signal which is one of the basic principles (and limitations) of CDI.

Patients and methods

In the period from April to November 1997, we examined 25 women with breast lesions, aged 16 to 68 years (mean 42.2). The gray-scale morphology of the lesions was assessed with "classic 7.5 MHz" and high-resolution ultrasound machine, with their compressibility and mobility additionally evaluated, then CDI performed (searching for eventually high velocities), and finally power-Doppler (CDE) examination carried out. Some patients were seen because of breast complaints (palpable mass, discomfort, nipple discharge), others were routinely examined prior to initiation of hormone replacement therapy. All but one were out-patients. Every patient had undergone mammography, either in our hospital or in another institution, less than 30 days before ultrasound was recommended because of mammographically suspected abnormalities. Final diagnoses were obtained mainly by fine-needle aspiration cytology (FNAC) and, in 2 cases, by open surgical biopsy.

All ultrasound examinations were done and images checked by one breast radiologist (Z.B.), and occasionally reviewed by another (I.D.).

Scanning was carried out with three machines: General Electric-CGR RT 2800 equipped with linear-array 7.5 MHz transducer, and General Electric-Logic 400 MD and -Logic 500 MD ultrasonic units with multifrequent (7-13 MHz) transducers with a length of 38 and 50 mm, respectively. The latter two probes enable superior imaging in the near field, thus no distance silicone pad was required. It is especially suitable for radial examination technique, with satisfactory visualization of pyramidal architecture and ductal system. Color-Doppler velocity mode ("frequency mode") and color-Doppler energy mode ("amplitude mode", power-Doppler) are available in both.

Besides the optimization of standard gray-scale scanning parameters, special attention was given to the optimization of Doppler presets. Color Doppler velocity receiver gain was turned down until a few specks of color remained in the color box, i.e. background color "noise" was just suppressed. The color box was adjusted to include the lesion and some adjacent normal surrounding tissue. Thus defined region of interest was then scanned slowly until a persistent color signal was apparent. Power-Doppler gain was adjusted according to recommendations of Bude et al.[10-12] we would, however, like to stress that we did not strictly stick adhere to the articles referred to. We increased the gain until a clear and persistent color signal representing intralesional vessel appeared, or, if such was absent, until the background became almost uniformly colored.

The gray-scale criteria used in the evaluation of solid breast lesion were as follows: *Typical benign lesions* were smoothly marginalized, with linear borders and homogeneous fine-granulated echotexture, hypo- or hyperechoic, ovoid shaped with the long axis parallel to the chest wall (depth/width ratio, D/W>1), and with enhanced acoustic through transmission. Conversely, *typical malignant features* included ill-defined, spicular or lobulated margins, of round or ovoid shaped with the long axis perpendicular to the chest wall (D/W <1, "taller than wide"), hypoechoic and sometimes heterogeneous

echotexture, posterior acoustic shadowing, sometimes with obvious central microcalcifications, broken tissue planes or distorted breast architecture.[13,14] If any of malignant characteristic mentioned above was present, the lesion was considered malignant, until proven otherwise. When all criteria of benignity were strictly adhered to, the lesion was considered benign. Arbitrarily, when most of benign characteristics were present, the lesion was defined as "probably benign" or "indeterminate".[15]

Similar to the methodology of Raza and Baum,[16] the appearances of vascular pattern in the lesion were categorized into 3 groups: *(a) penetrating vessels* - one or more blood vessels arising at the edge of the lesion, coursing toward the center, with an irregular branching pattern, *(b) peripheral vessels* - one or more blood vessels of predominantly uniform appearance, parallel to the edge of a mass, linear or arcuate, without significant branching, and *(c) no detectable vessels* - no vessels were reliably detected, or, in other words, color signals were not so constant to distinguish them definitely from noise. Centrally located vessels were seen occasionally, but this vascular pattern was often indistinguishable from *(a)*, especially within small lesions, and therefore it was not included as a distinct category.

Doppler images were obtained in peak-systolic phase when the vascular signal was the most excessively enhanced, tracing vascular architecture to the largest extent.

All the examinations were performed with the lowest transmitted energies that allowed good visualization, as recommended in manuals. Scanning performed in the most erratic manner that was possible not to insonate the same tissue volume for a too long time. Medical Ethics Committee approved of the examinations referred to in this study.

Results

Of 25 solid nodules, 17 (68%) were benign and 8 (32%) were malignant. The negative-to-positive biopsy ratio was 2.13 : 1.

Table 1. shows morphologic features detected in our patients by HRUS. Certain characteristics were seen in larger percentage with HRUS and than were detectable with conventional sonography.[16,17]

CDE was performed on all masses included in this study. Six of 8 cancers showed penetrating vessels (Figure 2), one showed peripheral vessels, and in one case, no flow was detected. This latter one was a very small carcinoma (6 mm), which was diagnosed as carcinoma even with repeated FNAC. In the group of fibroadenomas, no detectable vessels were found in 6 cases. One or more peripheral vessels were seen in 4 cases and a penetrating vessel was found in one lesion. Six lesions were found to be benign breast tissue with some dysplastic changes. In 3 cases of this group, no significant flow was detected, in two cases, vessels were found at the periphery, and in one, circulation was observed arising from the intramammary lymph node.

Table 2 is a concise presentation of the results. We detected flow in the mass in 15/25 (60%) cases, while in 10/25 (40%) lesions no flow was found. Penetrating vessels were most frequent in carcinomas and in 2 benign masses; but not all cancers demonstrated vascularization. In benign lesions, the vessels, if detected, were situated predominantly peripherally. Sometimes, in a hyperechoic boundary, they remind of (pseudo) capsule. In 10/17 (59%) of nodules, no flow was detected with CDE, although they were all solid.

Table 1. Morphologic features detected with HRUS (bold figures) in comparison with "low frequency ultrasound". Some characteristics were seen in larger percentage with HRUS than with conventional sonography. These figures were marked with apostrophe (')

Diagnose %	Carcinomas	Fibroadenomas	Other benign lesions
Irregularity of contours	88 **88**	18 **36'**	33 **50'**
Inhomogeneity	75 **88'**	18 **27'**	33 **67'**
Posterior attenuation	13 **25'**	0 **18'**	0 **33'**
Lateral shadowing	13 **25'**	54 **54**	16 **16**

Table 2. CDE features of solid breast lesions

Vascular pattern	Penetrant vessels	Peripheral vesseles	Vessels undetected	Total
Carcinoma	6	1	1	8
Fibroadenoma	1	4	6	11
Other benign lesions	1	2	3	6
All diagnoses	8	7	10	**25**

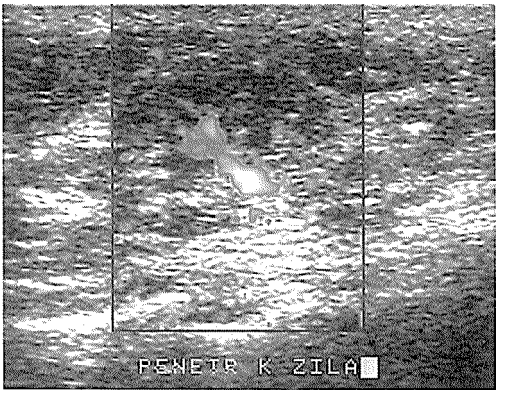

Figure 2. Penetrating vessels visualised by CDE.

Discussion

Considering the aggressiveness of aspiration tissue-sampling techniques (FNAC, core-biopsy) and open surgical biopsy as well as their costs, additional characteristics differentiating between benign and malignant lesions that can be detected in the pre-invasive phase of diagnostic process, could eliminate some financial load and patientsę sufferings. If negative predictive values (NPV) for malignant characteristics of the mass were high enough to substantiate recommendations for surveillance rather than biopsy, ultrasound would become a more capable tool in diagnostics, not restricted to distinguish only cystic from solid. Stavros et al.[15] classified prospectively 750 solid breast lesions as benign, indeterminate, or malignant using gray-scale sonography criteria, and calculated a NPV of 99.5% for malignancy. This is unexpectedly an encouraging result in spite of using state-of-the-art equipment and strict diagnostic criteria for benign masses; nevertheless, corroboration by other investigators is still required. The advantages of HRUS are undoubtedly proved [16,17] and our experiences are very similar.

In our material, we detected irregularity of contours as a typical sign of malignancy[15] in a larger number of carcinomas than other authors who employed low-frequency probes.[16,17] As the spiculation is a sign with very high positive predictive value (PPV) for malignancy,[15] its accurate sonographic disclosing is of great value. This is particularly relevant in the dense breast where mammography may be of limited value in the evaluation of the mass. However, in a considerable

number of benign lesions, irregularity of contours was displayed more frequently than with low frequency probes. This may result in false positive results, especially when dealing with fibroadenomas. But, as they predominate in younger population group and are not so characteristic for malignancy, neither is there any overt spiculation, but only microlobulation of the contour,[17] we prefer a non-aggressive (but not passive) approach, especially if no risk factors in patient's history are present.

We discovered *heterogeneity* in a large proportion of "other benign lesions", predominantly as a heterogeneous group that includes some regions of fibrocystic changes and areas of architectural distortion. Stavros et al. did not consider this feature as a separate category, but discussed about shadowing and punctate calcifications as signs of malignancy. Hence, we detected no clear (micro)calcifications in any masses analyzed: we might have not paid sufficient attention to this feature, probably also because microcalcifications were present in only one third of carcinomas. Undoubtedly, the detection of tiny calcifications has not been exclusively related to mammography; it can as reliably be done with HRUS, especially when situated in hypoechogenic mass. The lower their dimension, the lower the sensitivity for their detection.[15]

Posterior attenuation is the feature that has no decisive strength when dealing with breast tumors, unless confused with shadowing. It may serve as a clue for the diagnosis of fibrous dysplastic changes, which can explain some palpatory resistencies that are otherwise not suspected of malignancy. We were often faced with this feature, however, it was rather a practical difficulty in penetrating to deeper parts of the voluminous breasts than a reliable diagnostic sign.

Lateral shadowing was earlier referred to as a sign supporting benign diagnosis (fibroadenoma), but we detected it also in malignant tumors, and it was often asymmetric. The detection of this considerably unspecific sign is not significantly influenced by HRUS, and Stavros at al. did not even point it.[15]

Precise imaging of the ductal system is one of the leading advantages of HRUS. Although fairly time-consuming and of limited accuracy, when scrutinizingly performed radially, HRUS may compete with galactography/ductography at least in its lack of contrast agent and invasiveness. On the other hand, it may be of valuable help in the characterization of an intraductal lesion detected by the latter. Thus, it is possible to detect primary intraductal growth as well as *duct extension* of proliferative process within or/and around the lumen which courses toward the nipple. This was to a very limited extent also possible with low-frequency US. The frequencies between 10-13 MHz are optimal for this task. Moreover, scrutinize scanning, with an optimal focus adjustment, may reveal small intraductal papillomas, microcalcifications, and other intraductal masses with a possibility of precise needle guidance. In some cases, an intraductal location of calcification detected by HRUS, without presence of solid mass may be a decisive factor to consider them rather benign than suspected of malignancy. From our experience it is obvious that we should be cautious not to overestimate the significance of intraductal masses because, in many cases, they were proved to be an insignificant detritus, sites of atypical duct branching or just a tortuous duct seen in the scanned plane, as if the mass was contained within it. This may nullify advantages of HRUS by provoking many false positives resulting in additional FNACs or biopsies. When ductography cannot be obviated, the ducts dilated with contrast agent are better visualized with HRUS, and the lesion then punctured.[18]

We believe that the probe length of 38 mm, although adequate for scanning the subareolar region and axilla, may be too small for

the radial scanning technique of the breast, because of insufficient orientation in lobar/ductal anatomy; we therefore suggest a probe footprint of 50 mm as ideal for this task.

Until recently, investigations in the field of Doppler spectral analysis have not yielded unequivocal criteria for distinguishing malignant from benign solid breast lesions.[6-8] Cosgrove et al.[18] used a semiquantitative scoring system involving analysis of an average number of vessels per square centimeter and average density of color pixels. Although they found color Doppler signals in 98% of cancers, they did not prove correlation of color Doppler scores with the conventional prognostic indicators such as lymph node status or survival.[19] Birdwell et al. employing power Doppler used similar methodology.[20] They characterized breast masses with <10%, 10-25%, 25-50% and >50% of flow in a scanned area as avascular. They concluded that the presence of color in a solid breast mass was a non-specific finding, and that assessing of the extent of vascularity appears to be of limited value in the evaluation of solid breast masses. The authors found approximately equal numbers of malignant and benign masses among avascular lesions, and a quarter of malignant masses showed no color flow, although a sensitive method (CDE) was used. In their material, however, there was a significant number of small carcinomas as well as large fibroadenomas. The theory of the prevascular phase in tumor growth may explain the small amount of detected blood flow in the invasive carcinomas smaller than 2 cm, as well as good vascularity in large fibroadenomas.[21-22] We can assume that the presence of acoustic shadowing may limit the acquisition of Doppler signal and may have accounted for the avascular assessment of carcinomas with surrounding fibrosis as the dominant morphologic feature.

We studied the morphology and the pattern of distribution of vessels within the mass in an attempt to find the characteristics of malignant and benign lesions. In our study, carcinomas predominantly had penetrating vessels. Benign lesions had no detectable vessels in 59% of cases (cysts were excluded from our material) or had vessels around the periphery of the mass. In fibroadenomas vessels, if detected, were situated mainly in the peripheral parts. Sometimes, in a hyperechoic boundary, they looked like (pseudo)capsules. In one larger fibroadenoma (34 mm), an overt penetrating circulation (with a borderline spectral finding) was shown, and in one palpable intramammary lymph node with a diameter of 1 cm, hilar vascular pattern, which was categorized as "penetrant", was depicted. Later, FNA revealed the real nature of the mass, and retrospectively, when reviewing MOD recordings, we concluded that the vessel that we describe as "penetrant" was, in fact, a normal hilar vessel of the reactive lymph node. Unfortunately, we did analyze Doppler spectra in this case. In our work, we did not strictly analyze the morphology of the vessels, but in some carcinomas an examiner experienced in angiography would be amazed at a glance of a chaotic and irregularly branched vessels. The morphology was especially apparent when an individual vessel was kept well extended through the scanned plane, and the gain decreased so as to prevent the leakage of the color out of the lumen of the vessel. Such tortuous vessels depicted by standard color Doppler would be fairly confusing because of inconstant angle of insonation, and sometimes aliasing, and because of power Doppler homogeneous coding. Also, slow flow just near the wall was not eliminated and the lumen was filled with color in its real width.

We detected the flow in the mass in 15/25 (60%) cases, while in 10/25 (40%) lesions, no detectable flow was found. In comparison to some recent studies,[20] there was a considerable number of vascular lesions. Possibly, we were too rigid in eliminating some flow sig-

nals, assuming that they were related to noise. We might have falsely extended our experiences with classic color Doppler, and in some situations, decreased the gain too much. Perception of weak tiny signals of flow through an overwhelming homogenous color may sometimes be exhausting; we therefore preferred to avoid it. Another possible reason may be the attenuation of the ultrasonic beam by some lesions with abundant fibrosis, which obscure the acquisition of Doppler signal.

As we had no strict criteria to differ peripheral from penetrating vessel, we put some lesions with strong signals, even just beneath the capsule, in the latter group to avoid false negative result which we consider more dangerous than false positive one.

Motion artifacts were strong near the heart, especially when the breast was very small, and lesions situated deep close to the thoracic wall. Some patients were even unable of breathholding, or were also anxious and restless, which made the examinations more difficult. The use of ultrasonic contrast agent would theoretically help to overcome this problem, enabling the utilization of lower gain and higher pulse repetition frequency, but cost/benefit ratio remain questionable. Nevertheless, careful and patient scanning is, in the majority of cases, satisfactory to obtain images of acceptable quality.

In many centers of our country and also elsewhere, HRUS and CDE, if available, should be applied prior to FNA or biopsy, in order to further characterize the undeterminate solid lesion and to increase the specificity of the diagnostic process. It must be emphasized that this excellent sonographic technique, including radial technique, with the best scanners and transducers, as well as strict observing of relevant criteria for benign lesions, is highly recommended. With this approach, the population with benign solid breast lesions that does not require invasive work-up, can be identified with considerable accuracy. This could result in improved care and reduction of patient's discomfort, morbidity and health care cost.

Conclusions

1. HRUS can successfully help to distinguish many benign from malignant solid nodules in the breast. The chance to detect some malignant feature in a lesion are better with the application of HRUS than with conventional, lower frequency probes.
2. Assessment of internal vascular architecture of the lesion is a new approach in Doppler analysis, different from spectral waveform analysis; it may possibly add new determinants of biological nature of breast lesions. Further prospective studies on larger patient population are required.
3. When combined with, and in addition to mammography and clinical examination, HRUS and CDE increase accuracy of preinvasive differentiation of solid breast nodules.

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Hypertrophic pyloric stenosis: Ultrasound diagnosis

Goran Roić, Slavko Čop, Vesna Posarić, Suzana Ercegović

Department of Radiology, Children's Hospital Zagreb, Croatia

In the study, we analysed ultrasound (US) findings of 68 patients suspected of having hypertrophic pyloric stenosis (HPS). In 44 out of 45 patients with positive US findings, HPS was surgically confirmed. In 82.6% patients (38 pts), pyloric muscle thickness was 5-7 mm, in 10.9% (5 pts), it was 3-5 mm, and only in 6.4% patients (3 pts), we performed barium study due to doubtful values of pyloric muscle thickness (3.0-3.4 mm) detected by US; barium study confirmed HPS in one patient and excluded in two patients. The pyloric muscle thickness in all patients with HPS was at least 1.5 times thicker comparing to antral muscle thickness. Ultrasound sensitivity was 98%, and specificity 92%. Based on the results of our study, ultrasound examination of the pylorus has proved to be highly recommendable as a routine method of first choice in the diagnosis of HPS.

Key words : pyloric stenosis-ultrasonography; hyperthrophy

Introduction

With the advent of high resolution real-time ultrasound scanners, ultrasound has become a very important imaging technique, not only in the diagnosis of solid abdominal organ pathology, but also in the evaluation of hollow gastrointestinal tract.[1,2] Hypertrophic pyloric stenosis is the best recognized use of ultrasound in the pediatric gastrointestinal tract.[1,3] Ultrasound has the advantage of providing direct visualization of the pyloric muscle, and it also allows the measurements of muscle thickness.[4-6] The ultrasonographic criteria for diagnosing pyloric stenosis vary from hospital to hospital, but most common belief is that pyloric muscle thickness of 3.5 mm or more and a pyloric canal length of 17.0 mm or more are diagnostic for HPS.[2,7,8]

Correspondence to: Dr Goran Roić, Department of Radiology, Children s Hospital Zagreb, Klaićeva 16, 10000 Zagreb, Croatia.

Patients and methods

In our study, we reviewed the findings of US performed in our hospital from 1990 to 1996 of 68 patients suspected of having HPS.

The equipment used was ALOKA 1700 i ACUSON 128 XP, using curved and linear array transducers of 5 i 7-7.5 MHz. No particular preparation of patients was needed. If the stomach was empty, patients were given fluid (tea or water) by bottle to allow adequate examination. In all patients transverse and longitudinal sonograms were made in a supine right posterior oblique position of the

patient's body aided by a rolled towel under his left side. We measured the outer diameter of the pylorus and thickness of the pyloric muscle itself on both, transverse and longitudinal sonograms, as well as pyloric canal length on longitudinal sonogram. We also considered the ratio between pyloric and antral muscle thickness. The measurements of antral muscle thickness were obtained on long axis scans with the antrum distended by fluid to avoid confusing a contracted antrum with an abnormally thickened muscle. The antral muscle thickness was measured from the outer border of the echoic submucosa to the outer border of hypoechoic muscle layer. No time limits for the examination were imposed, and diagnostic criteria of HPS called for the following criterion:

1. pyloric visualization,
2. pyloric muscle thickness > 3.5 mm,
3. pyloric canal length > 17.0 mm.

Results

During the period of 6 years, 68 patients suspected of having HPS were ultrasonographically examined; of these, we established 45 diagnoses of HPS all of which, except one /false-negative/, were surgically confirmed. In 82.6% patients (38 pts) the diameter of the pyloric muscle was 5.0-6.0 mm, in 10.9% patients (5 pts) it was 3.5-4.0 mm and in 6.5% patients (3 pts) the diameter of the muscle was 3.0-3.4 mm. In all examined patients, the length of the pyloric canal exceeded 17.0 mm. In all patients, the pyloric muscle thickness was at least 1.5 times thicker than the antral muscle thickness.

Only 3 patients underwent radiology, i.e. barium study, because of the borderline pyloric muscle thickness values of 3.0-3.4 mm. Two of these patients were false-positive and one false-negative (confirmed by barium study). Sensitivity of the US examination was 98 %, and specificity 92 %.

Discussion

The pylorus region can be easily located by US, specially when there is a positive finding of HPS. The gallblader is used as a landmark to locate the region of the pylorus. According to our experience, it is harder to locate normal pylorus and exclude the diagnosis of HPS. In our hospital, we first locate the pylorus with a curved transducer of 5 MHz and then, for more detailed examination of pyloric region and the subsequent measurements, we use a linear array transducer of 7.0-7.5 MHz.

Although the most important diagnostic criterion for HPS is the length of the pyloric canal 9-11, it is not always possible to show it in its entire length due to its awkward position. Problems in diagnosis may arise if the stomach is overdistended, because the pylorus may then be displaced which makes its identification and measurements more difficult.[1,12]

Thus, we believe that the thickness of the pyloric muscle measured on transverse and longitudinal sonograms is a better criterion for diagnosing HPS (Figure 1,2). It is also useful to follow gastric outlet ultrasonographically. In order to be more accurate in diagnosing HPS, the thickness of antral muscle can be measured and compared with the pyloric muscle thickness. Normal pyloric muscle is usually <2 mm thick, and thickness between 2 and 3 mm may be seen in pylorospasm.[13] In all patients with HPS in our study, the pyloric muscle thickness was at least 1.5 times thicker than the antral muscle thickness, ranging between 1.4 and 2.3 mm.

According to our experience, the pyloric muscle thickness of the patients with HPS is in most cases 5.0-7.0 mm and in fewer cases 3.0 - 3.4 mm. In doubtful muscle thickness values (3.0-3.4 mm), some other diagnostic criteria should be considered (the length of the pyloric canal) and, in other doubtful cases, the patient should be reexamined in

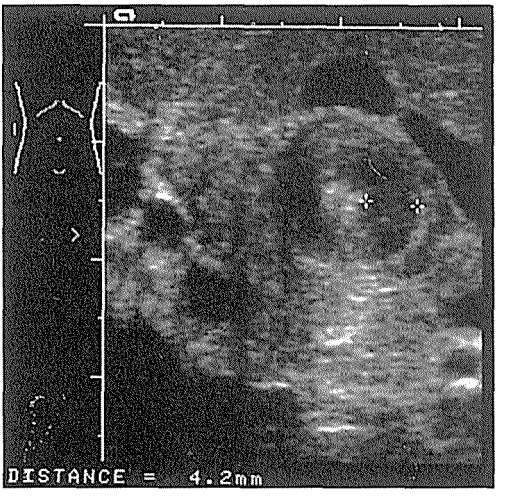

Figure 1. HPS- short-axis scan - central echogenic mucosa of pyloric canal surrounded by thickened pyloric muscle.

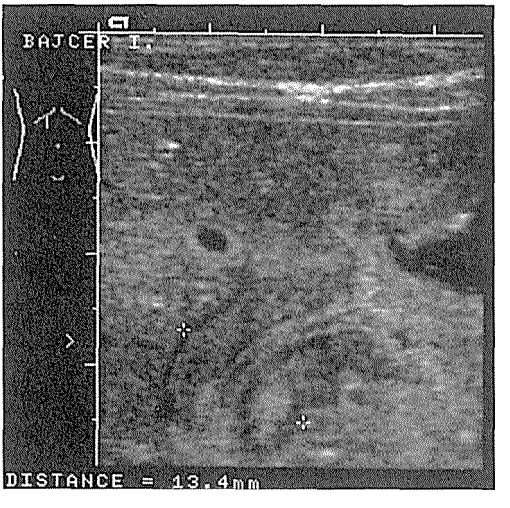

Figure 2. HPS-long-axis scan- abnormally thickened hypoechoic pyloric muscle (6mm).

24-48 hours or other diagnostic procedures should be performed (barium study).

In conclusion, the real real-time ultrasound is currently a method of choice; it is safe, painless diagnostic imaging technique for the diagnosis of HPS and, in experienced hands, it can almost completely replace conventional barium studies. The barium study should be reserved for those cases in which HPS is not considered the most likely cause of vomiting and in children with borderline measurements.

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review

Palliation of painful osseous metastases in patients with prostate cancer using Re-186-HEDP

Susanne Klutmann, Karl H. Bohuslavizki, Ulrike Schulte, Sabine Kröger, Christian Bleckmann, Janos Mester, Malte Clausen

Department of Nuclear Medicine, University Hospital Eppendorf, Germany

The skeleton is the second most common site of metastases in patients with prostate cancer. While curative means are strongly limited in these patients their life expectancy may be still several years. Therefore, it is essential to improve quality of life of these patients. Sufficient therapy of painful osseous metastases is the main goal in patients with advanced prostate cancer. The primary approach to relieve bone pain is the application of peripheral or central analgesics. In case of bone pain due to a single metastatic site local external beam radiation may provide pain relief in a reasonable amount of patients. In case of painful multilocular metastases systemic application of radiopharmaceuticals may irradiate bone metastases while normal tissue is spared from β-irradiation. Due to their physical characteristics Re-186 and Sm-153 have been developed for palliative treatment of metastatic bone pain. The response rate amounts to about 70-80% of all patients treated. Pain relief may last for 1-6 months. Due to its low grade toxicity which is mainly dominated by a transient thrombocytopenia therapy can be repeated. However, Re-186-HEDP therapy does not alter life expectancy.

Key words: prostatic neoplasms; bone neoplasms-secondary; pain-therapy; rhenium, radioisotopes, Re-186-HEDP

Introduction

Prostate cancer is the second most common malignancy in men in Western Europe. The incidence is 15-16 per 100000 habitants per year with increasing tendency. As much as 80% of patients with prostate cancer will develop bone metastases.[1] In about one third of all patients osseous metastases are detected at primary staging. Moreover, the skeleton is the only single site of metastases in a reasonable amount of patients.[2] In case of multilocular osseous metastases a complete remission of prostate cancer is nearly impossible.

Since osseous metastases are often associated with bone pain effective pain relief is the primary goal when caring for patients with prostate cancer and multiple osseous metastases.[3] Traditional therapeutic approach is the application of central or peripheral analgesics in combination with neuroleptics.[4]

Correspondence to: Dr. Karl H. Bohuslavizki, Department of Nuclear Medicine, University Hospital Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany. Phone: +49 40 4717 4047; Fax: +49 40 4717 6775. E-mail: bohu@medsph2.uke.uni-hamburg.de

Moreover, steroid medication, diphosphonates, and hormonal drugs may complete analgesics effects. However, therapy with opioids is limited in many patients due to side-effects, i.e. nausea, vomitus and gastrointestinal symptoms[5] and thus, often associated with a loss of patient's quality of life.[6]

Skeletal pain confined to single site metastases usually responds to external beam radiotherapy in 70-80 %.[4,7,8] In case of multilocular osseous metastases external beam radiation is helpful to avoid pathologic fractures or compression of the spinal cord.[9,10] However, hemibody or whole-body irradiation for pain relief is often limited by bone marrow suppression, gastrointestinal symptoms and a radiation pneumonitis.[11,12] Therefore, an effective relief of pain with low side-effects and an improvement in patient's quality of life is warranted in these patients.

Osteotropic radionuclides

The application of β-emitting osteotropic radionuclides is a promising method to selectively irradiate osseous metastases by sparing normal tissue from short-range irradiation.[13] Due to the osteoblastic character of osseous metastases the radionuclide is predominantly accumulated in malignant transformed cells which leads to a selective irradiation of bone metastases. The first agent used for this purpose, P-32-orthophosphate, was replaced by Sr-89-chloride due to its severe bone marrow toxicity. Up to now Sr-89 is still the most commonly used agent for osteotropic radionuclide therapy.[14] Sr-89 has a long physical half-life of 50,5 days with a maximum β-energy of 1,49 MeV (Table 1). Pain relief may occur 2-3 weeks after systemic application of 1,5-2,0 MBq/kg bodyweight. However, Sr-89 has no γ-emission and thus, posttherapeutic scintigraphy imaging is not feasible. Therefore, the aim of research was to develop alternative radionuclides for palliative treatment of painful osseous metastases.

Rhenium-186-HEDP

Re-186-hydroxyethylidendiphosphonate as well as Sm-153 (Table 1) have recently been developed for the palliative treatment of painful osseous metastases.[15] Re-186 has a therapeutic β-emission of 1,07 MeV associated with a γ-emission of 137 keV. Moreover, Re-186-HEDP and Tc-99m-HDP, that is commonly used for diagnostic bone scintigraphy, have an almost exactly similar bone distribution since both sorts of diphosphonates bridge to the hydroxyapatite of bone substance. Therefore, pretherapeutic and posttherapeutic scintigraphy is possible which allows a control of Re-186 distribution as shown in Figure 1. Re-186 has a short physical half-life of 3,8 days when compared to Sr-89. This allows a single application of activities of 1110 to 1850 MBq[16,17] with high tumor doses as well as an easy handling of radioactive waste, i.e. urine.

About 50 % of the activity injected are excreted via the kidneys into the urine within the first 6 hours post application. Nearly 70 % of the activity are urinary eliminated within the first 24 hours post application. Apart from the distribution in osseous structures Re-186-HEDP is not accumulated in any other structures of the body.

Pain relief is attained within two weeks after application of Re-186-HEDP and lasts for about 1-6 months. Response rates of Re-186-HEDP therapy of 70-80 % have been reported.[2,17,18] Especially in patients with oral medication of non-opioids analgesics rhenium-therapy led either to a reduction or to a stop of taking oral drug medication. Thus, the requirement for central analgesics may be delayed. Moreover, it is known that the clinical response is influenced by the size of osseous metastases. Pain relief mainly occurs

Table 1. Characters of different radionuclides used for treatment of painful osseous metases

	P-32	Sr-89	Re-186	Sm-153
Physical half-life [d}	14.3	50.5	3.8	1.9
β-emission [keV]	1710	1490	1070	810
γ-emission [keV]	ø	ø	137	103
Tracer	phosphate	chloride	HEDP	EDTMP
Scintigraphic imaging	ø	ø	possible	possible

Table 2. Inclusion criteria of patients for Re-186-HEDP therapy [20]

Four or more osseous metastatic sites (at last one single site presents with bone pain)
No diphosphonate therapy within 12 weeks
No irradiation within 3 weeks
No chemotherapy within 6 weeks
No change of dosis of hormone therapy within 8 weeks
Thrombocytes > 150000/µl
Leukocytes > 4000/µl
Creatinine < 1,3 mg/dl
No clinical sign of cerebral involvement
No heart insufficiency NYHA IV
Karnofsky-Index > 70 %
Life expectancy > 12 weeks
No level III oder level IV toxicity of previous rhenium-therapy (only in case of re-treatment)

in patients with small or medium-sized metastases, whereas large metastases with soft tissue infiltration often do not respond to radionuclide therapy.[2] Therefore, the application of bone-seeking radiopharmaceuticals is a treatment option to early complete oral drug therapy. Due to the short physical half-life of Re-186 the treatment can be repeated after 4-6 months.

Side-effects

The main radiobiological side-effect of bone seeking radionuclides is their potential bone marrow toxicity. In contrast to Sr-89 which is associated with a prolonged bone marrow suppression, Re-186 has a relatively mild hematological toxicity. Thrombocytopenia plays the major role in its bone marrow suppressing effect. The decline of thrombocytes presents with a nadir about 3 weeks post application. Prior to treatment the decrease of platelet count can be estimated for an individual patient presenting for rhenium-therapy.[19] Thus, severe hematological side-effects can successfully be avoided. In general, a control of platelet counts in a two-week interval for the duration of two months is sufficient in posttherapeutic follow-up.

Patient management

Several days before the therapeutic administration of Re-186-HEDP the patients undergo conventional bone scintigraphy with labeled diphosphonates, e.g. 600 MBq Tc-99m-HDP. In case of multilocular, osseous metastases with at least four metastatic sites and at least one single painful lesion rhenium-therapy is indicated if the patient fulfills inclusion criteria[20] as given in Table 2. Due to its potential bone marrow suppression

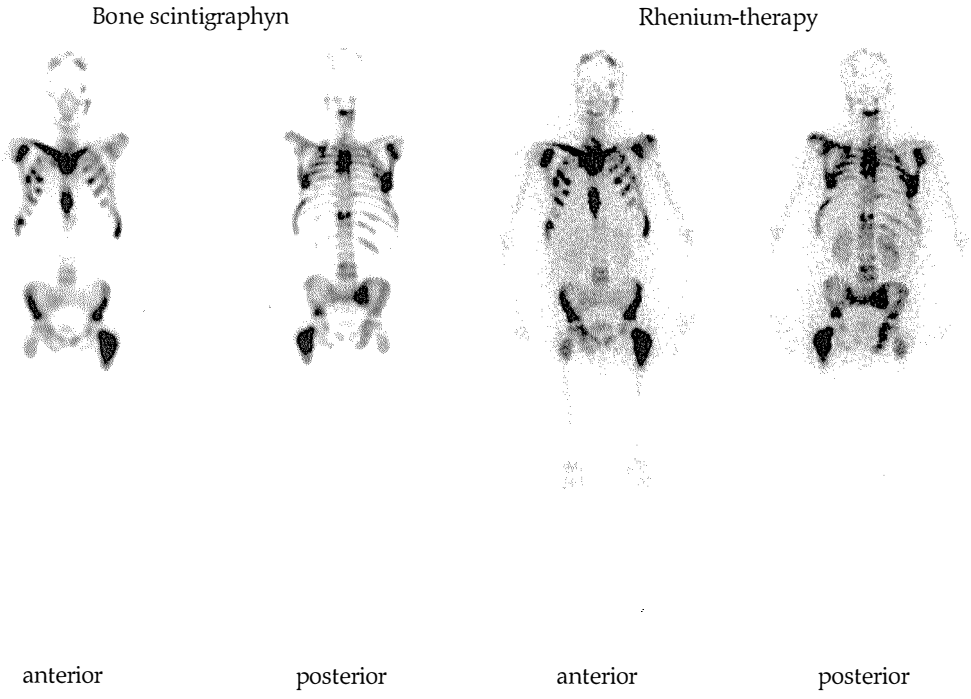

Figure 1. 64-year-old patient with multilocular bone metastases of a primary prostate cancer. The patient claimed about pain of the left femur and both scapulas. Left scintigram: Pretherapeutic conventional whole-body bone scintigraphy 3 hrs after application of 600 MBq Tc-99m-HDP i.v. Note tracer accumlations of the skull, the ribs, the sternum, the bassin, both proximal femurs, and the spinal column corresponding to sites of osseous metastases. Right scintigram: Posttherapeutic whole-body scintigraphy 48 hrs after application of 1.3GBq Re-186-HEDP i.v. Note distribution of Re-186-HEDP corresponds to osseous metastatic sites.

patients with thrombocytes below 150000/µl have to be excluded from therapy. In clinical routine, the blood count is defined directly prior to rhenium-application. If there are no contraindications, 1.3 GBq Re-186-HEDP with a total volume of about 2 ml are administered via an intravenous line. 48 hrs post application a whole-body scintigraphy with a scan-speed of 6 cm/min is obtained in order to evaluate the distribution of the bone-seeking Re-186-HEDP.

Conclusion

Palliative Re-186-HEDP therapy of multilocular, painful osseous metastases in patients with prostate cancer is a sufficient therapeutic modality for pain alleviation with low toxicity, thereby increasing patient's quality of life. However, life expectancy will not be affected.

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review

p53 - the paradigm of tumor-suppressor genes?

Barbara Jezeršek and Srdjan Novaković

Institute of Oncology, Ljubljana, Slovenia

p53 is a tumor-suppressor gene the alterations of which are among the most frequent genetic changes detected in human neoplasms. Its product - p53 protein is a component of several biochemical pathways that are central to carcinogenesis: DNA transcription, genomic stability, DNA repair, cell cycle control, and apoptosis. The analysis of the spectrum of p53 mutations and insight into the p53 mediated biochemical pathways of programmed cell death and cell cycle arrest, provide clues to the understanding of molecular pathogenesis of cancer and of mechanisms related to p53 mediated tumor suppression. The purpose of the present article is to summarise the most important facts concerning p53 since understanding of the above listed processes might provide the potential molecular targets for the development of a rational cancer treatment.

Key words: neoplasms; genes, suppressor, tumor; genes, p53; protein p53

Historical background

In 1979, Lane and Crawford,[1] as well as Linzer and Levine[2] independently discovered p53 as a nuclear 53kd phosphoprotein tightly associated with the large T antigen in the SV40 tumor virus-transformed cells. Originally, p53 protein came to be classified as a tumor antigen since it was suggested that the interaction of p53 with the large T antigen was important for transformation.[1,2] The p53 cDNA constructs isolated in this period were all derived from tumor cells[3] and were found to cooperate with the ras oncogene to transform rat fibroblasts in cell culture.[4,5] So, p53 came to be classified as an oncogene. Finally, in late 1980s, all the transforming p53 cDNA clones were discovered to be mutant forms of p53, while the wild-type gene isolated from normal cells failed to induce neoplastic transformation and even inhibited tumor cell growth or blocked the neoplastic transformation.[6-10] Now p53 looks like being a tumor-suppressor gene, negatively regulating the cell cycle and requiring loss-of-function mutations for tumor formation. However, unlike other classical tumor-suppressor genes, at least some mutated p53 forms act as dominant transforming oncogenes.[11]

Correspondence to: Barbara Jezeršek, Dept. of Tumor Biology, Institute of Oncology, Zaloška 2, 1000 Ljubljana, Slovenia, Tel: +38661 323 063 ext. 29 33, Fax: +38661 1314 180; E-mail: snovakovic@onko-i.si

Structure and regulation

The p53 gene spans a moderately-sized segment of DNA, located on the short arm (17p13) of chromosome 17 and is ultimately

translated to a phosphoprotein consisting of 393 amino acids contained in 11 exons, the first of which is noncoding.[12] Five evolutionary conserved domains within the coding regions are supposed to be essential to the functional activity of p53.[12,13]

The N-terminal domain (residues 1-42) interacts with the subunits of the general transcription factors TFIID and TFIIH and acts as a transcriptional activator. This domain also binds the MDM-2 protein - a negative regulator of p53, and adenovirus E1B protein. The core domain (residues 100-300) harbors the sequence specific dsDNA binding function of p53, and encodes the binding site for SV40 large T antigen and, possibly, for the papillomavirus E6 protein. The C terminal domain (residues 300-393) has multiple functional activities, including nonspecific DNA binding and reannealing of complementary ssDNA oligonucleotides. Residues 320 to 355 are involved in oligomerization, and the very terminal C domain (residues 360-393) binds ssDNA ends and regulates specific DNA binding by the core domain.[14-18]

It appears that p53 alone assembles into inactive forms and requires activating factors to confer an effective sequence-specific DNA binding capacity. Such a regulation is exerted by the C-terminal end of p53 itself. Hupp *et al.* proposed a model according to which the C-terminus negatively regulated specific DNA binding by interacting with a region in another p53 molecule within the tetramer.[19] This locked the tetramer in a conformation that was incapable of specific DNA binding.

p53 contains multiple phosphorylation sites located at both the C and N-termini of the molecule. Eight different protein kinases are involved in p53 phosphorylation: p34cdc2 kinase, DNA-activated protein kinase, mitogen activated protein kinases, protein kinase C, casein kinase I and II, Raf-1 kinase, and Jun kinase.[20-26] p34cdc2 kinase (an A- and B-cyclin dependent kinase) phosphorylates at serine 315 and thus stimulates the specific binding of DNA to the consensus sequence of p53 and also causes a specific conformational change of the protein.[20] DNA-activated protein kinase and mitogen activated protein kinases are involved in the phosphorylation of p53 at the N terminal domain, influencing the transcriptional activity and the half-life of the protein.[21,22] Protein kinase C-dependent, direct or indirect, phosphorylation of serine residues 372-381 at the C terminal of the p53 tetramer is a critical event for the transition from the latent to the active form of p53. Namely, the "open" configuration of the four phosphorylated C ends of the tetramer is a necessary prerequisite for the nonspecific DNA binding which, in turn, allows the consequent specific DNA binding to p53 consensus motifs.[19,23,27] The phosphorylation of the serine 392 is dependent on casein kinase II, however, this site is less critical for p53 activation.[28]

Another form of p53 regulation is exerted at the level of p53 protein stability. In normal cells, p53 shows a relatively short half-life (about 20 minutes) due to its rapid turnover, yet its half-life can be extended to hours following some kinds of cellular stress or as a consequence of mutations involving the core domain. Stability of the protein is affected by its complex formation with a number of cell proteins that are capable of slowing-down or preventing its ubiquitin-pathway degradation.[29,30]

It is still uncertain which physiological signal activates p53 after an appropriate stimulus. Possible candidates are p300 and the closely related transcription factor CBP that bind to N-terminal domain of p53. p300 acetylates conserved lysine residues in the p53 C-terminal domain which results in the activation of specific DNA binding of p53.[31,32]

Intracellular localisation, concentration, and state of phosphorylation of p53 are cell-cycle dependent. Activity of wild-type p53

protein demands nuclear localisation of the protein which occurs close to the beginning of S phase. Following the beginning of DNA synthesis, p53 accumulates again in the cytoplasm.[33,34]

Function

p53 protein is implicated in nearly all forms of cell growth stimulation and inhibition. It may be required early in the induction of cell proliferation and is also a transcriptional regulatory protein, capable of both stimulating and repressing gene expression.[35] p53 binds DNA in a sequence-specific manner and also influences gene expression indirectly by interacting with other transcription factors.[35,36] In certain cell types, over-expression of p53 induces apoptosis.[23,37,38] p53 may regulate in vitro cellular senescence and, under the influence of certain cytokines, it cooperates in the induction of differentiation.[39,40]

Transcription dependent pathway

Several genes were found to be transcriptionally activated by p53, including MDM-2, p21, GADD45, cyclin G1, BAX, FAS, transforming growth factor-alpha, muscle kreatinine kinase, and insulin-like growth factor-binding protein 3.[10,41-48]

Following DNA damage, p53 protein rapidly accumulates in the nucleus. The C-terminal domain of p53 recognises the damaged DNA, and the accumulation of p53 is probably a consequence of conformational change of the protein which leads to reduced degradation by ubiquitin degradation pathway or, less likely, a consequence of increased synthesis of p53 protein.[33,38,49] At the same time, no changes in p53 mRNA levels are observed.[50]

The p53 protein, in turn, activates downstream genes whose products are involved in growth inhibition, e.g. p21 and GADD45. p21 is a cyclin-dependent kinase inhibitor that inhibits the activity of cyclin D-cdk 4/6 causing a hypophosphorylation of retinoblastoma protein (Rb), thus preventing the release of E2F and blocking the G1-S transition.[51,52] Transactivation of GADD45, the protein product of which binds proliferating cell nuclear antigen and inhibits S phase entry, may contribute to the p53 dependent cell cycle arrest pathway.[53] Insulin-like growth factor-binding protein 3 gene which encodes a protein that binds insulin-like growth factor and thus inhibits its growth signalling is another p53 target gene that may function in this pathway.[48] p53 also regulates the G2/M checkpoint of the cell cycle, yet the mechanism of p53 mediated G2/M control is unknown.[54,55]

The expression of MDM-2 protein is regulated by the level of wild-type p53 protein. The MDM-2 protein, in turn, forms a complex with p53 and decreases its ability to act as a positive transcription factor - which represents a negative feedback loop to buffer changes in p53 levels.[56,57]

Transcription independent pathway

Modulation of cellular processes goes often via the mechanism of protein-protein interactions. In agreement with its multifunctional qualities, p53 protein associates with a group of viral and cellular proteins that may play an important role in the p53 mediated and transcription independent pathway (Table 1).[35,36]

Several basic transcription factors, including TATA binding protein, TATA binding protein-associated proteins, TFIIH-associated factor p62 form a complex with p53.[58-61] Binding of TATA binding protein to p53 protein has been implicated to be responsible for p53 mediated transcriptional repression. The list of genes reported to be transrepressed by p53 consists of proliferating cell nuclear antigen, interleukin 6, Rb gene, multidrug-resitance (MDR) gene, p53, BCL-2, inducible

Table 1. Some of viral and cellural proteins that associate with p53

Viral proteins	Cellular proteins
human papilloma virus E6	heat-shock protein 70
simian virus 40 T antigen	MDM-2
Epstein-Barr nuclear antigen	ubiquitin-ligase E6-AP
adenovirus E1B	transcription factor WT-1

nitric oxide synthase-2.[62-67] The binding of p53 to replication protein A also alludes to the possible direct role of p53 in DNA replication and nucleotide excision repair.[68]

p53 in the nucleotide excision DNA repair

The observations that p53 can selectively bind to several DNA helicases, including XPB and XPD, which are a part of transcription factor TFIIH, led to the hypothesis that p53 may play a direct role in modulating DNA nucleotide excision repair.[69,70] Furthermore, p53 can also recognise several forms of damaged DNA (mismatched DNA, ssDNA ends).[71] So, a new model emerged in which p53 may act as a sensor that binds to damaged parts and recruits the nucleotide excision repair machinery by trapping TFIIH (i.e. the major component of the repair complex) at regions where it is needed which, in turn, facilitates the constitution of a functional "repairosome".[72]

p53 mediated apoptosis

The molecular mechanisms behind p53 induced apoptosis are only partially explained. The current idea is that DNA damage induces stabilization of the p53 protein which promotes DNA repair by assembling the repair machinery.[70,71] In case the DNA damage is unrepairable, p53 triggers cells to undergo apoptotic death to prevent propagation of the cells carrying a mutation. Several activities of p53 have been identified that could participate in the process of programmed cell death. Namely, p53 upregulates the expression of BAX and downregulates expression of BCL-2, all of which have been implicated in modulation of apoptosis.[73] Another possible explanation for the induction of apoptosis could be that the transactivation of insulin-like growth factor-binding protein 3 gene and thus increased insulin-like growth factor-binding protein 3 levels may presumably block an insulin-like growth factor mediated survival signal and lead to apoptosis.[48] Finally, a whole series of new p53 induced genes related to redox control have been discovered that lead to the formation of reactive oxygen species, oxidative degradation of mitochondrial components and apoptotic cell death.[74] Beyond this, a transactivation independent function of p53 in the triggering of the apoptotic pathway has been implicated and may well be performed by a proline rich region located between residues 64 and 91 in p53 molecule. The proline rich region may provide a crucial accessory apoptotic signal, perhaps by interacting with a cellular SH3-domain-containing partner protein.[75,76]

Briefly, the major role of p53 is being a monitor of cellular proliferation (guardian of the genome) and a determinant of response to DNA damage.

Mutations

The p53 mutations are found in the preponderance of human tumors and the functional p53 is lost in approximately half of all human malignancies.[52,77]

The majority of p53 mutations are missense point mutations giving rise to single

amino acid substitutions that abrogate the specific DNA binding activity.[18] Concomitantly, the half-life of p53 extends from normal 20 minutes (wild-type protein) to approximately 48 hours (mutant protein) resulting in nuclear accumulation of the mutant protein.[78,79]

Most of the mutations are clustered in the most highly conserved domains of the gene spanned by four to nine exons. There are at least three mutation "hot spots" affecting the residues 175, 248, and 273.[80] Although mutations of the p53 gene are most frequently acquired, they can also be inherited through the Li-Fraumeni syndrome. In these families, one mutant p53 allele is inherited, and the second allele acquires a mutation.[81]

p53 is not inactivated only through mutation, but also at the protein level through complexing with DNA tumor viral oncoproteins like the SV40 large T antigen, the adenovirus E1B protein, and the human papilloma virus E6 protein[82] or cellular protein MDM-2.[57]

Detection of p53 mutations

In respect to the fact that inactivation of p53 in tumor cells leads to the increased cellular proliferation and inhibition of apoptosis and concerning the observations that mutations of p53 gene are associated with advanced disease, poor response to chemotherapy or radiotherapy, and short survival,[83,84] it is of great importance to determine the p53 status in every patient prior to treatment. Various approaches to the detection of p53 mutations have evolved in the last 19 years and each of them has certain advantages and certain disadvantages.[77,85]

The most informative method for the study of p53 mutations is the determination of the nucleotide sequence with either direct sequencing or indirect molecular analysis.[85] Molecular sequencing is the only way to evaluate the mutational event that inactivates the gene (there is no accumulation of the mutant protein) and allows for the unequivocal detection of alterations. On the other hand, the indirect molecular methods as denaturing gradient electrophoresis, single-strand conformational polymorphism analysis or variants as hydroxylamine and osmium tetroxyde chemical cleavage, and pulse field gel electrophoresis, are more suitable for screening and easier to perform. Yet, both methods share some drawbacks - namely, they cannot be at the moment performed in routine diagnosis, tumor tissue is required, and care must be taken to avoid contamination from an excess of normal tissue.[85-87]

Immunohistochemical and immunocytochemical methods, under optimum conditions, are capable of detecting most missense mutations (which result in nuclear accumulation of the mutant protein) and can also identify p53 stabilization without mutations (a consequence of the alteration of pathways regulating p53 expression). On the contrary, the mutations which do not induce p53 overexpression (nonsense mutations, frame-shift mutations, splice mutations, gene deletions, promoter mutations) will go undetected. Immunohistochemical results can be affected by the degradation of antigen during tissue processing and by the specificity of the antibodies used. Tumor tissue is needed, but the contamination by normal tissues is not a critical factor. In sum, p53 immunostaining is still an imperfect reflection of the prevalence of p53 mutations.[85,86,88-90]

It remains controversial whether the mutant p53 protein can be detected in patients sera, since the results of various determinations are opposing. Namely, two groups of authors determined the serum levels of mutant p53 in patients with malignant lymphomas using a commercially available ELISA kit,[91,92] while another group of authors failed to do so using the same ELISA method in patients with lung cancer.[87] Simi-

larly, Hassapoglidou using immunofluorimetric method could not detect mutated p53 protein in sera of patients with cancer.[93]

Although p53 is a cellularly encoded protein, it has been found to be immunogenic and capable of eliciting a p53 specific antibody immune response. About one third of patients (the percentage varies for different types of cancer) with tumors that carry p53 missense mutations develop circulating p53 antibodies. These antibodies are not seen if there is no p53 accumulation in the tumor cell and, in case of lung carcinoma, they can appear before the cancer is detectable. The p53 protein may either be released during tumor cell necrosis, or otherwise translocates to the surface of the cell, inducing a B-cell response as a result of the breakdown of the immune system tolerance. Methods used for the determination of p53 antibodies include ELISA, immunoblot, and immunoprecipitation techniques. These methods can be performed routinely, they do not require tumor tissue, and can be used for follow up. Therefore, assessment of serum p53 antibodies is quite specific, but has low sensitivity (some mutations do not induce the production of p53 antibodies) in the detection of p53 mutations.[85,87,94-99]

Therapeutic approaches

Several therapeutic approaches are currently being assessed against the growth advantage and resistance to chemotherapy and radiotherapy observed in tumor cells with p53 mutations. The first approach is the investigation of active immunization against the potential tumor antigens carried by mutated p53, and indeed, it has been shown that it is possible to generate p53 specific CD8+ cytotoxic T lymphocytes by immunizing mice with mutated p53 protein.[100] Furthermore, it was observed that a monoclonal antibody to p53, PAb 421, and a small peptide derived from p53 (the C-terminal domain) are able to restore the sequence specific DNA binding as well as growth suppression function of at least some mutant p53 proteins (by inducing a change in the configuration with a return to the active wild-type configuration).[19,101-103]

Among the recently proposed approaches, two are quite interesting. The first uses an adenovirus defective for E1B gene, which replicates only in the cells lacking functional p53 but not in the cells with wild-type p53, leading to selective destruction of tumor cells with mutant p53.[104] The second, on the other hand, utilizes the transfer of a cytotoxic gene which is only activated in the presence of a mutant p53, resulting in a selective killing of tumor cells with p53 mutation.[105]

However, the most promising approach is p53 gene transfer in tumor cells carrying a p53 mutation. In tumor cells lacking functional p53, such a transfer can lead to tumor regression, as well as improve the cytotoxicity of antineoplastic agents, and the response to ionizing radiation.[106-108] The most frequently used vectors for p53 gene transfer in animal models have been recombinant adenoviruses, and less often retroviruses, which have a lower capacity of gene transfer *in vivo*. Interestingly, some tumor regressions were more important than expected. They were indicated by the percentage of p53 transfected cells, suggesting a possible "bystander" effect, with destruction of non-transfected cells in the vicinity of transfected cells, as for suicide gene transfer.[109]

And finally, another idea was to try to identify the drugs that may trigger programmed cell death through a p53 independent pathway. It has been suggested that taxol could be one of them, however, the clinical results with taxol were poorer in patients with mutant p53.[110,111]

Latest findings

Even though p53 seems to play a central role in nearly all forms of cell growth stimulation and inhibition and was termed as the "guardian of the genome", it is becoming obvious that other proteins, as for example the recently discovered p33 and p75, also take an important part in the regulative mosaic.

The nuclear protein p33 (a product of the tumor-suppressor gene ING1) forms a complex with p53 and cooperates in the negative regulation of cell proliferation by modulating p53 dependent transcriptional activation.[112,113]

p73 is a protein that is closely related to p53, both structurally and functionally; however, it is induced by different signals and thus plays a fundamentally different role in the maintenance of cell homeostasis. It can, at least when overproduced, activate p53 responsive genes and act as a growth suppressor.[114,115]

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Micronuclei in cytokinesis-blocked lymphocytes as an index of occupational exposure to antineoplastic drugs

Vera Garaj-Vrhovac and Nevenka Kopjar

Institute for Medical Research and Occupational Health, Zagreb, Croatia

In order to investigate possible DNA damaging effects of antineoplastic drugs, the micronucleus assay combined with Giemsa, DAPI and silver staining was performed. Blood samples were taken from nurses working without adequate protection in oncological department on preparing and administration of different antineoplastic drugs. Lymphocytes were cultivated in vitro at 37°C. To prevent cytokinesis, at 44h cytochalasine-B at a final concentration of 3μg/ml was added. The results obtained indicate statistically significant increases in the total number of micronuclei in the exposed subjects compared to controls. DAPI staining has revealed signal-positive and signal-negative micronuclei while silver staining has revealed Ag-NOR$^+$ and Ag-NOR$^-$ micronuclei. Compared to controls, the number of signal-positive and Ag-NOR+ micronuclei in the exposed subjects were increased, indicating a greater susceptibility of particular chromosomes to damage caused by antineoplastic agents.

Key words: antineoplastic agents-adverse effects; occupational exposure; lymphocytes; micronucleus test

Introduction

Antineoplastic drugs are inhibiting or preventing growth of neoplasms, by checking the maturation and proliferation of malignant cells. They are risk factors for different categories of workers who are occupationally exposed during the stocking, preparation, administration and disposal of such agents.[1-4] Many of these, commonly used in cancer chemotherapy have proved carcinogenic, mutagenic and teratogenic in experimental animals and *in vitro* test systems.[5-9] Careless handling of cytotoxic agents may lead to exposure of the personnel to amounts detectable by chemical or biological methods in the body fluids or cell samples of the subjects. The exposure is typically to mixed compounds over a longer period, and to low exposure levels with accidental peaks. Therefore, the use of biological exposure markers is appropriate for monitoring such exposure patterns. The biological markers/methods for exposure assessment are either non-specific (e.g. cytogenetic damage, point mutations or ^{32}P-post-labelling adducts in peripheral blood lymphocytes, urinary mutagenicity) or specific for a given compound (immunological methods, specific analytical methods).[3,10-15]

Correspondence to: Vera Garaj-Vrhovac PhD, Nevenka Kopjar, MSc. Institute for Medical Research and Occupational Health, Ksaverska c. 2, HR-10001 Zagreb, Croatia. Tel:++385 1 467 31 88 (264); Fax: ++385 1 467 33 03; E-mail: vgaraj@mimi.imi.hr nkopjar@mimi.imi.hr

The *in vitro* micronucleus assay is used widely as a useful endpoint in biomonitoring, and ecotoxicology, as well as for the assessment of *in vitro* and *in vivo* damage of chromosomal material caused by different mutagens.[16-21]

The aim of this study was to investigate the cytogenetic damage resulting from the exposure to different antineoplastic drugs in nurses working in oncology department under inadequate protection. Genotoxic damage in lymphocytes was evaluated by a micronucleus assay. For sensitive detection of genome damage conventional Giemsa staining was compared to DAPI and silver staining techniques.

Materials and methods

Subjects

Samples of peripheral blood were taken from 20 healthy never-smoking subjects aged from 24 to 50 years (mean age 39.5 years). Ten of them were controls and ten were nurses working in oncological department of one hospital. Nurses were daily involved in the preparation and administration of different antineoplastic drugs for an average period of 17.3 years. Both experimental groups were previously interviewed to document a history of radiation exposure, chemical exposure and viral infection within one month before the study.

Micronucleus assay

Blood samples were cultured at 37°C *in vitro* in F-10 medium (Gibco) supplemented with fetal bovine serum (Biological Industries, Israel), phytohaemagglutinin (Murex) and antibiotics (penicillin and streptomycin). Cultures were harvested at 72 h.

To prevent cytokinesis, at 44 h Cytochalasin-B (Sigma) in the final concentration of 3 μg/ml was added to each sample, and the cells were harvested after a further incubation of 28 h. The slides for scoring micronuclei were prepared according to the modified method of Fenech and Morley.[22] After a brief treatment with physiological saline, cells were fixed with 3:1 mixture of methanol and acetic acid. They were dropped onto clean slides, dried at room temperature and afterwards stained using conventional Giemsa staining, DAPI and silver staining technique.

Staining techniques

Giemsa staining was performed by means of 5% buffered solution of Giemsa for 10 minutes. After staining, the slides were washed and air-dried.

DAPI (4',6-diamidino-2-phenylindol-dihydrochloride) staining was performed according to Schweizer.[23] Prior the staining, slides were preincubated in Mc Ilvaine's buffer (citric acid - disodium hydrogenphosphate) pH 7.0 for 10 minutes. The staining solution contained 1 μg of DAPI / ml of Mc Ilvaine's buffer (pH 7.0). The staining procedure was carried out in dark and lasted for 10 minutes. Afterwards, slides were rinsed in Mc Ilvaine's buffer, air-dried and mounted in 1:1 mixture of glycerol and Mc Ilvaine's buffer. The preparations were observed under short-wave-length blue light using an UG 1 filter for excitation.

For the silver staining of NORs the method of Howell and Black[24], which uses gelatine as colloidal protector, was employed. To prepare colloidal developer, gelatine was dissolved in deionized water by stirring and gentle heating. When the gelatine was dissolved, formic acid was added. To prepare silver nitrate solution, silver nitrate was dissolved in deionized water. To stain NORs, a colloidal developer and $AgNO_3$ solution (50%) were mixed and pipetted onto the micronuclei preparations. The slides were covered with coverslips and

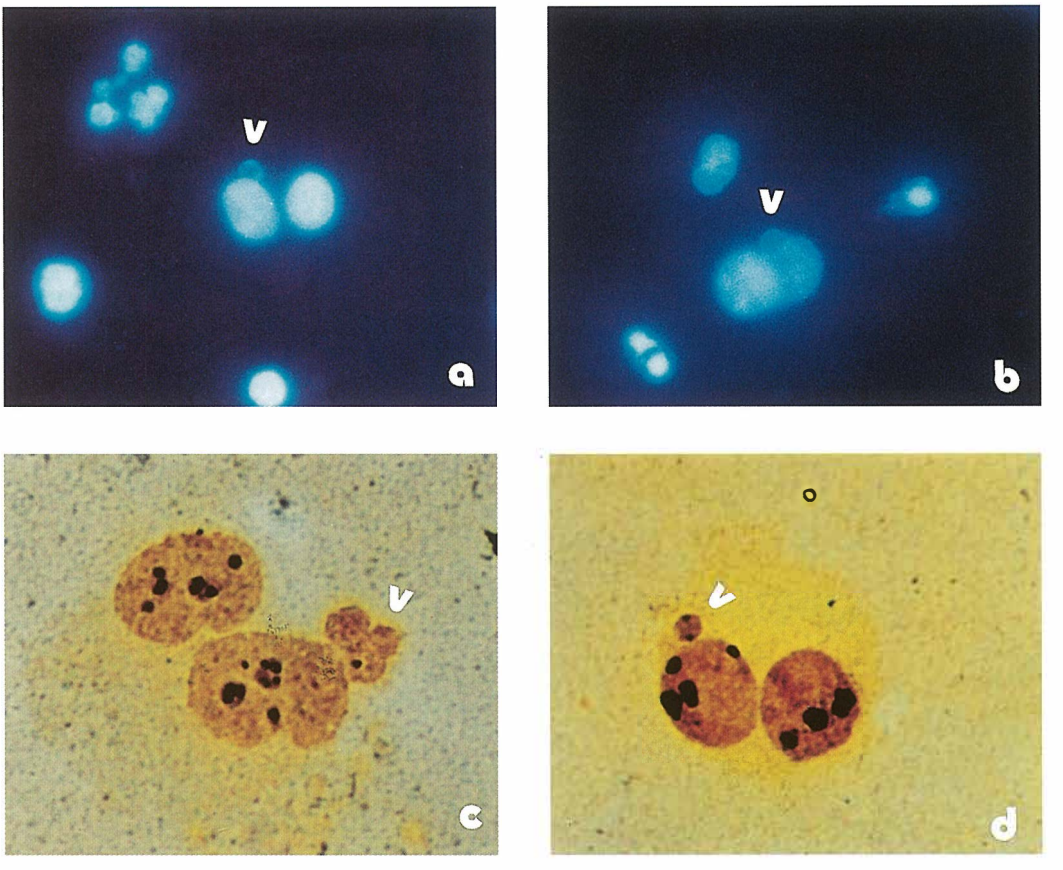

Figure 1. Binucleated lymphocytes of nurses occupationally exposed to antineoplastic drugs. (a) signal-positive micronucleus stained with DAPI, (b) signal-negative micronucleus stained with DAPI, (c) cell with two Ag-NOR$^-$ and one Ag-NOR$^+$ micronuclei after silver staining, (d) Ag-NOR$^+$ micronucleus with two signals after silver staining

placed on a hot plate pre-heated to 70°C. After 1-2 minutes the solution turned yellow and then golden brown. At this stage slides were washed off, rinsed with deionized water and air-dried.

Scoring of the slides

Slides from the exposed and control subjects were scored by the same scorer.

For the incidence of micronucleated lymphocytes and determination of the frequency of micronuclei 500 binucleated cells per subject were scored.

The published criteria for MN determination were followed: (1) binucleated cells containing any number of MN were scored; (2) fluorescence intensity per unit area of scorable MN was either equal in intensity or more or less intense than that of the main nuclei; (3) only MN that were distinctly separate from the main nuclei and located within binucleated cells with intact cytoplasmic and nuclear membranes were scored. Micronuclei were located for their DAPI fluorescence and NOR activity and successively classified as signal-positive and signal-negative as well as Ag-NOR$^+$ and Ag-NOR$^-$ MN.

Table 1. Total number and distribution of micronuclei in lymphocytes of control group after Giemsa, DAPI and silver staining

Subject No.	1	2	3	4	5	6	7	8	9	10
No. of	496	498	497	496	498	498	498	497	497	498
binucleated	496	497	497	497	496	498	498	497	498	497
cells										
without MN	495	497	497	497	496	497	497	498	496	497
No. of	3	2	2	4	4	2	2	3	3	2
binucleated	4	3	3	3	3	2	2	2	2	3
cells										
with 1 MN	5	3	3	3	4	3	3	2	4	3
No. of	1	0	1	0	1	0	0	0	0	0
binucleated	0	0	0	0	1	0	0	1	0	0
cells										
with 2 MN	0	0	0	0	0	0	0	0	0	0
Total No. of	5	2	4	4	6	2	2	3	3	2
MN per 500	4	3	3	3	5	2	2	4	2	3
binucelulated	5	3	3	3	4	3	3	2	4	3
cells										

Giemsa staining technique

DAPI staining technique

Silver staining technique

Statistical analysis

The statistical significance of the results was determined using the χ^2 test.

Results

The results regarding the frequency, distribution and total number of micronuclei (MN) for the control group are reported in Table 1, and for the exposed group in Table 2.

All staining techniques have revealed statistically significant increases in total number of micronuclei in the exposed group ($P<0.05$ using χ^2 test) compared to the controls. The distribution of micronuclei per 500 binucleated cells in all the exposed subjects was also disturbed compared to the controls.

Considering the presence or absence of bright DAPI, flourescent dots inside of MN signal-positive and signal-negative MN have been detected. Their total number and distribution for the exposed and control groups are shown in Table 3. In all the exposed subjects we have observed an increased total number of micronuclei as well as an increased number of signal-positive micronuclei compared to control.

Related to the patterns of nucleolar organizer (NOR) activity, Ag-NOR$^+$ (MN that contain one or more NOR parts) and Ag-NOR$^-$ micronuclei (MN without NOR parts) have been noticed. Their total number and distribution of in control and exposed subjects are shown in Table 3. In all the exposed subjects an increased total number of micronuclei as well as increased number of Ag-NOR$^+$ micronuclei, compared to the controls, is observed.

Figure 1 shows signal-positive and signal-negative micronuclei obtained after staining with DAPI (a,b) and Ag-NOR$^+$ and Ag-NOR$^-$ micronuclei (c,d) obtained after silver staining in subjects occupationally exposed to antineoplastic drugs.

Table 2. Total number and distribution of micronuclei in lymphocytes of exposed group after Giemsa, DAPI and silver staining

Subject No.	1	2	3	4	5	6	7	8	9	10
No. of	477	482	488	490	489	490	488	491	462	485
binucleated	488	478	480	482	485	480	490	483	474	484
cells										
without MN	488	482	483	477	486	486	480	485	487	483
No. of	13	16	12	10	10	8	11	8	32	12
binucleated	11	17	19	13	14	17	11	15	24	15
cells										
with 1 MN	8	14	15	17	14	12	18	12	19	15
No. of	0	1	0	0	1	2	1	0	5	2
binucleated	1	4	1	3	1	2	0	2	1	1
cells										
with 2 MN	4	4	2	5	0	2	1	2	3	5
No. of	0	1	0	0	0	0	0	1	1	0
binucleated	0	0	0	1	0	1	0	0	1	0
cells										
with 3 MN	0	0	0	1	0	0	1	1	0	0
No. of	0	0	0	0	0	0	0	0	0	0
binucleated	0	0	0	2	0	0	0	0	0	0
cells										
with 4 MN	0	0	0	0	0	0	0	0	0	0
Total No. of	13	21	12	10	12	12	13	11	45	18
MN per 500	13	25	21	30	16	24	11	19	29	17
binucelulated	16	22	19	30	14	16	23	19	25	19
cells										

Giemsa staining DAPI staining Silver staining

Discussion

The primary source of human exposure to antineoplastic drugs results from their use in cancer therapy. However, persons involved in the manufacture, preparation and administration of drugs to patients and in nursing care of patients may also be exposed.

The results of numerous studies on cytogenetic endpoints performed on medical personnel exposed to antineoplastic drugs are conflicting, probably due to different degrees of exposure in different occupational settings, as well as due to different sensitivity of the indicators, to their different persistence and different safe handling measures. [10,13,17,25,26]

Based on their mode of action, antineoplastic agents are divided into several categories. Since most of these drugs exert their effects during a certain phase of the cell cycle (cell growth phase, cell division phase, resting phase etc.), many treatment regimens combine two or more of these agents. This is the reason why are nurses usually exposed to a mixture of different antineoplastic drugs used in their daily preparation and administration procedures. The most frequently handled antineoplastic drugs in our study were: bleomycin, vinblastine, cyclophosphamide,

Table 3. Total number and distribution of DAPI signal-positive and signal-negative micronuclei (MN) as well as Ag-NOR$^+$ and Ag-NOR$^-$ MN for control and exposed subjects

Subject No.	1	2	3	4	5	6	7	8	9	10
Exposed group										
No. of signal-positive MN	8	10	13	15	10	14	8	8	7	4
No. of signal-negative MN	5	17	8	12	6	10	3	11	22	13
Total No. of MN	13	27	21	27	16	24	11	19	29	17
No. of Ag-NOR$^+$MN	8	15	15	13	12	10	14	15	15	12
No. of Ag-NOR$^-$MN	8	7	4	17	2	6	9	4	10	7
Total No.of MN	16	22	19	30	14	16	23	19	25	19
Control group										
No. of signal-positive MN	2	1	2	1	2	0	1	1	0	1
No. of signal-negative MN	2	2	1	2	2	2	1	2	2	2
Total No. of MN	4	3	3	3	4	2	2	3	2	3
No. of Ag-NOR$^+$MN	2	2	1	2	2	1	2	1	1	2
No. of Ag-NOR$^-$MN	1	3	2	2	1	2	1	1	3	1

DAPI staining technique Silver staining technique

cisplatinum, 5-fluorouracil, adriamycin and mitomycin C.

The results of our study have clearly indicated that occupationally exposure to antineoplastic drugs caused cytogenetic damage. The in vitro micronucleus assay combined by Giemsa, DAPI and silver staining techniques has revealed a significant increases in the number of micronuclei as well changes in their distribution in all the exposed subjects compared to control.

It is known that micronuclei originate from either whole chromosomes or acentric chromosome fragments due to chromosomal breakage, or from lagging chromosomes which consequently are excluded from the main nuclei. Therefore, enumeration of MN can provide an index of chromosome loss from the main nuclei if whole chromosomes can be identified within them. The incidence of micronuclei observed could result from clastogenic as well as aneugenic effect on peripheral blood lymphocytes. It has been shown that lymphocytes are an extremely sensitive indicator of induced chromosome structural damage both *in vivo* and *in vitro*. Approximately 90% of lymphocytes have a half-life of three years and thus can reflect damage incurred over a long period.

Among the antineoplastic drugs used in our study some are known aneuploidy-inducing agents with spindle damaging effects (vinblastine) while others are clastogens with direct DNA damaging effects (bleomycin, mitomycin C). Considering the average duration of occupational exposure to those agents, an increased number of micronuclei observed in all the exposed subjects, compared to controls, is not surprising. These results are consistent with previous reports on the use of different endpoints with different antineoplastic drugs in vivo and *in vitro*.[8,12,17,27,28,29,30]

In this study, the micronucleus assay was performed in combination with conventional Giemsa staining and more specific DAPI and silver staining. Giemsa technique was compared to DAPI and silver because it is known that both techniques exhibit a considerable specificity in detecting particular chromosomal regions or distinct chromosomes. Therefore, they allow us to speculate about the ori-

gin of micronuclei. It is known that DAPI staining produces intense fluorescence of the paracentromeric regions of chromosomes 1,9,16, of the distal part of the long arm of Y chromosome, and also of a region of the short arm of chromosome 15. On the other hand, silver staining make visible nucleolar organizing regions (NORs), which are loops of chromatin containing rRNA gene clusters. In normal human cells, NORs are localized on the secondary constrictions of the 10 acrocentric chromosomes of D and G groups.

Previous reports have shown that the frequency of micronuclei detected by special staining techniques was generally higher than with conventional May-Grünwald Giemsa staining technique.[29] Our results are consistent with this observation (Tables 1,2).

The incidence of DAPI signal-positive and Ag-NOR$^+$ micronuclei lead us to a conclusion that parts of regions which they detect specifically are involved in acentric fragments, or in whole chromosomes excluded as micronuclei from the main nucleus due to clastogenic or aneugenic effect of antineoplastic drugs mixture.

In the exposed subjects an increased number of both types of micronuclei compared to controls was observed. Thus, it is reasonable to assume that chromosomes of D and G groups, respectively 1,9,16, 15 and Y, are more susceptible to DNA damage caused by antineoplastic drugs, as compared to other human chromosomes.

The results of the present investigation show that inadequate protection in handling antineoplastic drugs leads to a significant cytogenetic damage. Our study confirms the suitability of micronucleus assay, combined with special staining techniques for the assessment of risk of occupational exposure to antineoplastic drugs.

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The role of thyroxin in thyroid radiation carcinogenesis in rats

Katarina Koritnik[1] and Andrej Cör[2]

[1]*Radiotherapy Deparment, Institute of Oncology Ljubljana, Slovenia*
[2]*Institute of Histology and Embriology, Medical Faculty,Ljubljana Slovenia*

The aim of this study was to test the hypothesis on the protective role of thyroxin administration before and during irradiation on the occurrence of thyroid carcinoma in rats. Application of thyroxin before and during irradiation was expected to decrease production of thyrotropin by the hypophyseal feedback mechanism, caused by radiation damage of thyroid tissue. Stabilizing the thyroid cells in this way during irradiation would thus make them less radiosensitive.

In the experiment, we first divided 81 three to four week old Wistar strain rats of both sexes into two groups, i. e. thyroxin (T_4) and water (H_2O). The T4 rats were injected 1% thyroxin solution (0.01 mg / 100 g body weight) twice a day for 15 days, while the H_2O rats received saline in the same way. After ten days, the two main groups were divided each into two subgroups. The rats from both irradiated subgroups (T_4/X and (H_2O/X) received 10 Gy to the neck area. They were irradiated with a telecobalt machine for five consecutive days with one direct field. During a two years follow - up, all moribund animals were sacrificed and their thyroid glands taken. The rest of the thyroid glands were taken at the end of the experiment. All glands were pathohistologically analysed. Besides, all suspicious and enlarged extrathyroid organs and tissues were examined and the occurrence of tumors was noted. Pathohistological examination revealed the occurrence of 8 thyroid carcinomas and 7 adenomas in the H_2O/X group, and 3 adenomas in the T_4/X group. In the irradiated group of rats without thyroxin, significantly ($P = 0.01$) more thyroid carcinomas occurred than in the irradiated group without thyroxin.

The experiment confirmed the hypothesis about a protective role of thyroxin administration before and during the irradiation in postirradiation thyroid carcinogenesis in rats.

Key words: neoplasms, radiation induced; thyroid neoplasms; thyroxine; rats

Introduction

The carcinogenic effect of ionizing radiation in human thyroid gland has been well established.[1,2] Thyroid cancer was the first solid tumor that showed an increased incidence among the Japanese A-bomb survivors,[3] and a great increase of thyroid carcinoma has been recently reported among the children exposed to nuclear fallout in the areas around Chernobyl.[4] Long ago, the data implicating radiation as an etiologic factor in thyroid cancer rendered the practice of irradiat-

Correspondence to: Katarina Koritnik, M.D., M.Sc., Radiotherapy Deparment, Institute of Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia.

ing benign childhood disorders obsolete,[5] but in radiotherapy of malignant tumors in the head and neck region, the exposure of the thyroid gland usually cannot be avoided. Subclinical or overt hypothyroidism, diffuse thyroid enlargement and benign nodules were frequently observed in patients after the irradiation of the thyroid region for Hodgkin's disease,[6] and most outsatnding was the occurrence of thyroid carcinoma. The relative risk of thyroid cancer after irradiation for Hodgkin's disease was 15.6%.[7] In the study of late effects after radiotherapy for childhood malignancies, the relative risk for secondary thyroid cancer was 53%.[8] Careful evaluation of thyroid status is required in all patients who were irradiated in the neck region and, in cases of elevated TSH level, substitutional therapy with thyroxin is recommended.[9]

Thyroid tumours can also be induced in experimental animals by the administration of radioactive iodine or external radiation. The induction of thyroid carcinoma by exposure to X-rays in rodents was first described by Frantz *et al.*[10] Lindsay and Sheline[11] studied the development of thyroid tumors in rats after the glands had been irradiated with 5, 10 and 20 Gy. Benign nodules and adenomas were frequently observed in irradiated rats and appeared to have originated as foci of nodular regeneration and hyperplasia. Thyroid carcinomas occurred in 22 - 45% of irradiated animals and apparently arised from pre-existing benign adenomas. Similar incidence of thyroid carcinomas in rats after external irradiation was reported from experiments on laboratory animals by Christov[12] and Lee *et al.* [13]

Clinical evidence that TSH has an important role in thyroid carcinogenesis was clearly confirmed by experimental studies. If the irradiation was followed by long-term goitrogen treatment, the yield of tumours was higher[14] and; on the other hand, if animals were treated with thyroxin after the irradiation, no thyroid tumours occurred.[15] In the experiments on tissue cultures, the frequency of cancer expression from initiated thyroid cell was greatly increased by certain chemicals, growth factors and hormones associated with elevated TSH level.[16-19] It is suggested that, in addition to radiation, elevated TSH promotes carcinogenesis by increased number of cell divisions and stimulation of growth.[20,21]

The aim of our study was to test if reduced TSH level *before and during* irradiation could protect the thyroid glands from the development of radiogenic cancer. Thyroxin application would decrease production of thyrotropin by impeding the hypophyseal feedback mechanism[22,23] before exposing the thyroid gland to radiation. If the thyroid cells were stabilized by thyroxin they would be expected to be less susceptible to growth stimulation caused by radiation damage. Reducing the number of mitoses would diminish the possibility of radiogenic mutations and thus make the thyroid tissue less sensitive to carcinogenic initiation.

Material and methods

In the experiment 81 three to four week old Wistar strain rats of both sexes were used. They were first divided into two groups according to thyroxin administration, *i. e.* thyroxin (T_4) and water (H_2O). Each of the main groups was further divided into two subgroups according to irradiation (X and sham: X) (Table 1).

Thyroxin administration

The T4 rats were injected 1 % thyroxin solution (0.01 mg / 100 g body weight) twice a day for 15 days, while the H_2O rats received saline in the same way. The effectiveness of thyroxin mediated TSH suppression was tested in a preliminary study. Four rats were used, 1 % thyroxin solution or water was

Table 1. Number of rats in subgroups

Irradiation	Application	
	Water	Thyroxin
Irradiated	H_2O/X	T_4/X
	(n=37)	(n=20)
Sham irradited	$H_2O/\not{X}$	$T_4/\not{X}$
	(n=12)	(n=12)

H_2O/X - water injection + irradiation, T_4/X - T_4 injection + irradiation, H_2O/X - water injection + sham irradiation, T_4/X - T_4 injection + sham irradiation.

administered twice a day for 10 days; after that, they were sacrificed and the serum TSH level was measured. The mean value was 0.015 mU/L in thyroxin group and 0.31 mU/L in control group ($P < 0.01$), which was accepted as sufficient suppression.

Irradiation

After 10 days, the rats from both irradiated subgroups (T_4/X and (H_2O/X) received 10 Gy to the neck area. They were irradiated with a telecobalt machine for five consecutive days with one direct field. Daily dose was 2 Gy defined at a depth of 1.5 cm, FSD of 80 cm, and field dimension of 5 cm x 5 cm.

Follow up

The animals were kept in cages, 4 - 5 of the same subgroup together, with food and water ad libitum. They were monthly weighed and regularly examined. Special attention was paid to the animals' coats and general appearance indicative of altered thyroid function. The animals which looked unhealthy or had an obvious neoplasm were sacrificed for immediate post mortem examination. Two years later, the survivors were also sacrificed by chloroform.

Histopathology

At autopsy, the trachea with the whole thyroid gland was removed, fixed in Bouin's fixative, embedded in paraffin and sectioned serially. All sections were stained with hematoxylin and eosin and pathohistologically examined. In search for metastases, specimens from the lungs and lymph nodules of the neck region were obtained, as well as from all other organs displaying pathological changes. The diagnoses were made according to Murthy's classification.[24] All lesions were classified as follicular cysts and hyperplasia, follicular adenomas or follicular carcinomas. Carcinomas were diagnosed on the basis of nuclear pleomorphism, anaplasia and dedifferentiation, but the main criteria for malignancy were capsular and/or vascular invasion and tumor cell emboli in the vasculature.[25]

Results

During the initial 18-month latent period, 12 rats had to be sacrificed and further material of 11 animals was not diagnostically due for postmortem autolysis. Therefore, 58 animals were assigned for final analysis.

The incidence of thyroid lesions in different groups are shown in Table 2. Pathological changes were the most numerous in the H_2O/X subgroup, where also carcinomas occurred; a smaller number of lesions, but no carcinomas, were noticed in the T_4/X subgroup, while pathological changes in the $H_2O/\not{X}$ and $T_4/\not{X}$ subgroups were rare.

Table 3 shows the incidence of thyroid carcinomasin rats sacrificed in the last four months of follow-up. The statistical analysis was made by modified t - test for small samples by Bonferroni[26]; the difference between both irradiated subgroups was significant ($P < 0.01$).

The cumulative incidence of tumors in irradiated subgroups is shown in Figures 1 and 2. The first carcinoma occurred in the H_2O/X subgroup 20 months after irradiation.

Table 2. Number (and relative portion) of thyroid lesions in 58 rats by subgroups

Group	Carcinoma	Adenoma	Other lesions	Normal glands	Sum
H_2O/X	8 (0.33)	7 (0.29)	5 (0.20)	7 (0.29)	24
f	3 (0.25)	4 (0.33)	2 (0.16)	4 (0.30)	12
m	5 (0.41)	3 (0.25)	3 (0.25)	3 (0.25)	12
T_4/X	0	3 (0.27)	2 (0.18)	6 (0.54)	11
f	0	2 (0.40)	0	3 (0.60)	5
m	0	1 (0.16)	2 (0.33)	3 (0.50)	6
H_2O/✱	0	1 (0.08)	1 (0.08)	10 (0.83)	12
f	0	1 (0.16)	1 (0.16)	4 (0.66)	6
m	0	0	0	6 (1.00)	6
T_4/✱	0	0	0	11 (1.00)	11
f	0	0	0	6 (1.00)	6
m	0	0	0	5 (1.00)	5
	8	11	8	34	58*

H_2O - water, T4 - thyroxin, X - irradiation, ✱ - sham irradiation, f - female, m - male, Other Lesions - diffuse hyperplasia, nodular hyperplasia, follicular cyst; *as more than one sort of lesion may have occurred in the thyroid gland of one rat, the sum of all lesions is different from the number of rats.

Table 3. Number (and relative portion) of thyroid carcinomas in rats by subgroups - sacrificed in the last four months of observation

Group	H_2O (n=27)	T_4 (n=19)	p<
X (n=24)	8/16 (0.50)	0/8 (0.00)	0.01
✱ (n=22)	0/11 (0.00)	0/11 (0.00)	N.S.

H_2O - water, T_4 - thyroxin, X - irradiation, ✱ - sham irradiation.

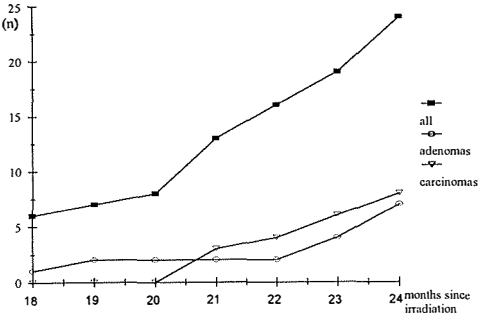

Figure 1. Cumulative incidence of thyroid tumors in the H_2O/X subgroup (water + irradiation).

Discussion

In thyroid carcinogenesis, the moment of induction is followed by a latent period before the first thyroid tumors occur.[13] It is assumed that the latent period after irradiation is one to one and a half years long.[11,12] In our material, the first carcinoma was observed in the rat sacrificed 21 months after irradiation, while the overall time of observation was 24 months. Statistical analysis of carcinoma incidence for the period of the last three months revealed significant differences between the two irradiated groups. In the irradiated subgroup without T_4, the number of carcinomas was significantly higher than in the subgroup treated with T_4, where no carcinomas were found. Based on the results

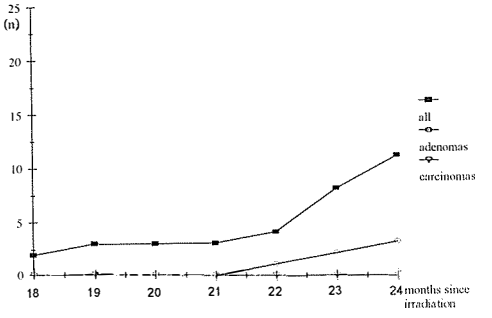

Figure 2. Cumulative incidence of thyroid tumors in the T_4/X subgroup (thyroxin + irradiation).

of our experiment, the hypothesis of radioprotective role of thyroxin in thyroid carcinogenesis was confirmed.

Similar findings are reported from the experiment on animals by Doniach.[14] The author studied histological changes in the thyroid glands of irradiated rats after administration of T_4 and water, respectively. In the animals which received single doses of 1 Gy, 2.5 Gy or 5 Gy and were after that given every day 20 μg T_4 until they were sacrificed 20 months later only one adenoma occurred. On the contraty, in the irradiated group drinking water 5 adenomas and two carcinomas occurred. The small number of tumors in the whole group was probably due to a relatively low dose of irradiation.

In our experiment, the parenteral administration of T4 was started before the irradiation to prevent an enhanced growth and mitotic activity during the action of mutagen. Thyrotropin suppression was measured in the preliminary test. Since no carcinomas occurred after irradiation, we can conclude that the stabilization of thyroid cells was achieved and cocarcinogenic stimulation of thyrotropin in the phase of initiation was abolished.

Bause *et al.*[27] reported a significant reduction of tumor incidence by an immunostimulation with xenogenic, lyophilized fetal cells administered twice after whole body irradiation of rats. The incidence of carcinomas was 25% in the immunized group versus 55% in the controls. In spite of the promising results, this method was never described in a clinical trial.

The use of T_4 as radioprotective agent was tested in humans by Bantle *et al.*[28] They have administered exogenous T_4 to lymphoma patients receiving radiation therapy, in an attempt to suppress serum thyrotropin and prevent radiation induced thyroid damage. Twenty patients in experimental group were treated with 200 μg T_4 1 to 13 days (average 5 days) before the beginning of mantle radiation. The level of thyrotropin suppression was documented by measuring T_4 index in the serum and by performing TRH (thyrotropin realising hormone) test. In all 20 patients of the experimental group, T_4 index rise was achieved and, in 19 of 20 patients, TSH (thyrotropin) response to TRH was demonstrated before radiotherapy was initiated; only in one case, the test was not performed. T_4 application was discontinued after the completion of the radiation and the 20 patients without T_4 served as controls. After a mean follow-up of 19 months, 35% of patients in the experimental group had a higher level of serum thyrotropin and, in the control group, only 25% of patients developed hyperthyrotropinaemia. From these results, it may be concluded that the suppression of serum thyrotropin during neck irradiation should not prevent subsequent thyroid dysfunction. This finding is contrary to the results of our study, but the end-point observation in this clinical trial, *viz.* thyroid dysfunction, was different from ours, *viz.* the detection of thyroid tumors.

In addition to carcinomas, other lesions were observed in the thyroid glands of our experiment. The histopathologic examination revealed the occurrence of adenomas, diffuse or nodular hyperplasia and follicular cysts. The largest proportion of these lesions was found in the irradiated rats not receiving T_4.

A smaller number of such changes occurred also in the irradiated subgroup of rats which were given T_4. In the non-irradiated subgroups, such lesions were rare. This additionally confirms the protective role of T_4 in thyroid radiation carcinogenesis.

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Systemic Lupus Erythematosus diagnosed with extreme skin reaction during radiation therapy: a case report

R. Jeffrey Lee, Gary M. Proulx, Craig W. Donaldson, James B. Orner

Roswell Park Cancer Institute, Department of Radiation Medicine, Buffalo, USA

The use of breast conservation therapy in patients with collagen vascular disease is controversial. Initial reports demonstrated severe skin reactions in patients receiving radiotherapy who also had a diagnosis of systemic lupus erythematosus or sclererderma. However, a more recent case-control study found no increased incidence of complications in these patients compared to patients without collagen vascular disease. We report a case of a patient diagnosed with systemic lupus erythematosus after developing a severe skin reaction early during her course of breast radiotherapy. The clinical course of the patient is reviewed along with the controversies surrounding this clinical dilemna.

Key words: breast neoplasms-radiotherapy; lupus erythematosus, systemic

Introduction

Breast conserving surgery is gaining popularity in the treatment of stage I and II breast carcinoma. Lumpectomy, with or without, axillary dissection and radiation therapy results in a similar local control and survival rate when compared to modified radical mastectomy. Many of the contraindications to such therapy are clearly outlined.[1] Collagen Vascular Disease (CVD) remains a controversial issue in the field of radiation therapy at this time. CVD is considered a relative or absolute contraindication to the utilization of radiation in breast conserving therapy. The literature was reviewed following this case presentation to illustrate current beliefs. The questions raised by this case warrants a discussion of what course to take when the diagnosis of Systemic Lupus Erythematosus (SLE) is made during treatment with external beam irradiation.

Correspondence to: R. Jeffrey Lee, M.D., Roswell Park Cancer Institute, Department of Radiation Medicine, Elm and Carlton Streets, Buffalo, N.Y.14263, USA. Tel: (716) 845-3172 Fax: 845-7616; E-mail: jlee@sc3101.med.buffalo.edu

Case history

A 38 year old Caucasian woman was evaluated following a routine mammography which showed calcifications of the left breast. This suspicious lesion was evaluated with fine needle guided biopsy. The official pathology revealed a foci of lobular carcinoma in situ measuring 1.5 cm in greatest dimension. She subsequently underwent left breast lumpectomy with axillary node dissection. Patholog-

ic examination revealed multi-centric lobular carcinoma in situ. There was a questionable component of invasive carcinoma. The axillary node dissection revealed no evidence of metastases.

History revealed a recent diagnoses of "Chronic Fatigue Syndrome" and extreme allergies in the past year. This included allergic reactions to formaldehyde, fabrics and many new synthetic substances. She denied being diagnosed with CVD and blood tests were apparently negative.

Physical exam revealed three incisions in her left breast. One periareolar, horizontal in the inferior medial quadrant and in the left axilla. There were no abnormalities other than induration at the incision sites.

Because of the possible foci of invasive disease, external beam irradiation to the entire breast with a boost to the tumor bed was recommended.

She was treated with 6 MeV photons utilizing medial and lateral tangents to the left breast. Thirty degree wedges were used and computer planning confirmed a homogenous dose distribution. Field size was 16.4 x 20.8 cm. A daily dose of 180 cGy with a total dose of 5040 cGy was prescribed.

Signs and symptoms early in the treatment (900 cGy) with breast erythema, tenderness and constitutional symptoms with extreme fatigue, prompted us to pursue a detailed information regarding chronic fatigue syndrome.

Because of the severity of her symptoms, we performed additional testing. An antinuclear antibody and rheumatoid factor were negative, however, the erythrocyte sedimentation rate (ESR) was 49 (with normal being 0-20) and the antibodies to double stranded DNA with Farr assay (anti DNA-FARR) of 89 u/ml (normal 0-3.5u/ml) is highly indicative of SLE. Following a one week treatment break, persistent moist desquamation in the upper outer quadrant of the left breast and inframammary fold was noted. Erythema around the areola and inner quadrants had improved. Because of the severe reaction and diagnosis of SLE, it was recommended that radiation therapy be terminated at 3780 cGy and close follow-up with evaluation every three months during the first year and mammography every six months.

She returned one month later with significant improvement in the irradiated site with a small area of moist desquamation and persistent hyperpigmentation and moderate erythema (Figure 1).

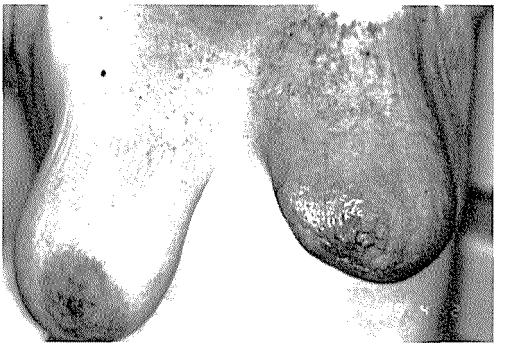

Figure 1. Area of moist desquamation and persistent hyperpigmentation and moderate erythema two weeks following 3780 cGy to the left breast.

Discussion

Systemic lupus erythematosus (SLE), a chronic inflammatory disease, appears to result from an immunoregulatory disturbance brought about by the interplay of genetic, hormonal, and environmental factors.[2] It is a disease of unknown etiology in which tissues and cells are damaged by deposition of pathogenic autoantibodies and immune complexes. Ninety percent of cases are in women, usually of child-bearing age, but children, men and the elderly can be affected.[3] Patients with SLE typically have disease that affects multiple organ systems. Not all systems, however, are involved simultaneously. The characteristic clinical course is one of exacerbations and remissions, the lat-

ter often lasting for many years. Criteria for classification of SLE were revised in 1982.[4]

This discussion is not centered around the diagnoses of SLE but, the suspicion of this disease in regards to radiation therapy and the appropriate course of action should the disease be determined during treatment.

Wallach describes two cases of lupus-like syndrome developing after therapeutic irradiation for locally advanced carcinoma of the breast.[5] This was characterized by pleuritis, pneumonitis, positive fluorescent antinuclear antibody reaction and lupus erythematosus preparation. Both patients responded to Prednisone for an extended period of time and had no evidence of radiation related disease or tumor progression. There were no abnormal acute treatment reactions noted in the article. The first patient was treated with 5000 cGy to the breast with a 2000 cGy boost to the mass. The second patient was treated with 3900 cGy to the breast, supraclavicular and axillary areas. Both patients had a history of non-deforming arthritis involving the hands, wrists, knees and ankles. It was proposed that radiation may have initiated an immunologic response leading to full blown SLE. Pleural effusions, pericarditis, or sever respiratory distress may have been induced from a lupus-like syndrome rather than a metastatic tumor radiation-induced disease.

Ransom *et al.*, discusses a case presentation of a 55 year old with scleroderma.[6] The patient received 45 Gy in 25 fractions to the breast, 45 Gy in 20 fractions to the supraclavicular fossa and apex of the axilla and 40 Gy in 25 fractions to the internal mammary chain. In addition, the tumor bed received 25 Gy in 54 hours by Iridium implant. No unusual acute skin reaction was noted. Three months following radiation, unusual induration developed and rapidly proceed to dense fibrosis in the treated area. In one year the shoulder movement was limited to a few degrees, there was marked arm edema and severe retraction and fibrosis of the breast. They conclude that the unusual reaction was caused by an unusual reaction of scleroderma with radiation.

Olivotto *et al.*, present a case report of a 25 year old woman with SLE who suffered fatal pelvic necrosis two years following radiation for carcinoma of the cervix.[7] An autopsy revealed no evidence of a residual tumor. There was pelvic ischemic necrosis with extensive pelvic fibrosis and fistulization. She was treated with three intracavitary tandem and ovoids. Point A received 1680 cGy with each insertion. The total rectal dose was 3830 cGy. This was followed with 40 Gy in 20 fractions with 10 MV photons with AP/PA fields and a 5 cm midline block. There were no technical difficulties with the insertion and minimal morbidity. They reviewed 158 consecutive patients with invasive carcinomas of the cervix and there were no increases in complications. They believed the endarteritis of SLE was either aggravated by or potentiated the effects of radiation leading to progressive pelvic fibrosis and necrosis. They believe that patients with connective tissue diseases do not tolerate radiation well.

Fleck *et al.*, retrospectively reviewed 5682 patients from 1959-1985 and discovered nine cases with CVD.[8] All patients received 40-50 Gy in the intact breast or chest wall at 1.8 to 2 Gy with boosts of five to 15 Gy at standard fractionation. Of these nine patients, five underwent breast conservation. Five of the nine patients received chemotherapy, three before irradiation and two after irradiation. Four of the patients had known pre-existing CVD prior to radiation and three of them developed exaggerated acute and late effects within two years of treatment. The five women who developed CVD three months to ten years following treatment had no complications. Of the four patients with pre-existing CVD, two were specifically diagnosed with SLE. One suffered moist desquamation, lymph edema of the arm, flap necrosis, severe lung damage, a bronchopleural-cuta-

neous fistula and osteoradionecrosis of the clavicle, sternum and rib cage. The authors conclude that pre-existing CVD appears to represent a relative contraindication for any elective high or moderate-dose radiation therapy.

Robertson *et al.*, reports two cases of CVD which exhibit extremely poor cosmetic results.[9] One patient had scleroderma and the second rheumatoid arthritis (RA). The first patient (RA) received 5251 cGy at 210 cGy fractions followed by a 1600 cGy iridium-192 implant boost. She exhibited no unusual acute reactions, however, 11 months post treatment she developed breast swelling, erythema and severe pain. The breast was hard, fibrotic and fixed to the chest wall. The appearance was similar to an inflammatory carcinoma. However, a simple mastectomy revealed no malignancy. Two years following the mastectomy she remains free of recurrence and her chest wall is symptom free, however, her RA has progressed. The second patient (scleroderma) received 5040 cGy in 180 cGy fractions to her left breast, supraclavicular region and a posterior axillary boost. During treatment, she developed a brisk skin reaction and worsening of skin and joint symptoms. A diagnosis of scleroderma was made six months following therapy. At 16 months the breast was severely fibrotic and retracted. There were also rib fractures in the treatment field. They concluded that both patients exhibited an acceleration of systemic disease either during RT or shortly thereafter. They believe that CVD must be active before or during RT for severe fibrosis and severe late injury to occur.

Varga *et al.*, presents four patients with systemic sclerosis (SSc).[10] Two of the patients had breast cancer. The first patient was a 38 year old status post MRM who received 50 Gy to the chest wall and draining nodes followed by adjuvant chemotherapy (CMF). Two months following RT she developed Raynauds phenomenon and swelling of the hands with subsequent increasing skin tightness. She had a right hip metastasis treated four years following this with 40 Gy in 2.5 Gy fractions. She developed an extreme fibrotic reaction in the treatment area within four months of treatment and subsequently developed small bowel obstruction (SBO). She died two years following her second course of radiation. The second patient with invasive ductal adenocarcinoma of the right breast underwent a radical mastectomy followed by RT and CMF chemotherapy. She received a 9 MeV electron beam with 20 Gy at 1 cm depth in 25 fractions through a 10 x 10 cm field. Three months following RT she noted swelling of the right hand, forearm, and arm. During the next three months she developed rapidly progressive asymmetrical skin induration and thickening involving the right arm and right side of her trunk and contracture of the fingers of the right hand. The authors concluded that the areas of radiation induced fibrosis extended well beyond the confines of the radiation portals. Three of the four patients died of complications of the fibrotic process without evidence of recurrent malignant neoplasms. They concluded some patients with SSc develop a markedly exaggerated fibrotic response following localized ionizing radiation. Caution should be used in the delivery of RT to patients with SSc.

Ross *et al.*, has the largest published data base to date.[11] They presented a retrospective review of 61 patients with CVD compared to a matched control group. The patient distribution was as follows: 39 RA, 13 SLE, 4 SSc 4 dermatomyositis (DM) and one with polymyositis (PM). They concluded overall no significant difference between the CVD and control group in terms of acute or late complications. Of this group, three patients suffered fatal complications and they all resided within the CVD group. Of the three fatalities, one had SLE with uterine sarcoma and subsequent radiation induced bowel necrosis.

Another had RA with infiltrating ductal carcinoma of the left breast and died of chronic pericarditis secondary to radiation therapy. There was also one difference in the incidence of late complications compared to none in the control group. They found the highest incidence of normal tissue damage were those in whom CVD was diagnosed after radiation therapy. This occurred in four of six patients. SLE patients had a higher rate of acute reactions while late complications were actually less in this group. They are clear in pointing out that the three fatal complications are not significant and that two of the complications occurred in patients with excessive doses that would cause a high risk of late complications anyway. They found no statistically significant increase of complications in those with CVD diagnosed prior to therapy compared to those diagnosed following therapy. Their conclusion is that although there is no reason to withhold radiation treatment to individuals with CVD, it would be prudent to be cautious in the treatment of these individuals until more studies have been reported.

Strober *et al.* present an uncontrolled feasibility study to support the use of total lymphoid irradiation for the treatment of intractable lupus nephritis.[12] All patients had a diagnosis of SLE, histopathologic evidence of lupus nephritis involving more than 85% of the glomeruli and failure to respond to Prednisone therapy. Ten patients were subsequently treated with 6 MeV photons. The mantle field utilized 2000 cGy in 200 cGy fractions five days per week. The subdiaphragmatic field received 2000 cGy in 150-200 cGy fractions and differing techniques for males and females of child bearing age were utilized. The kidneys were not irradiated in any instance. This study showed that total lymphoid irradiation may be an alternative to cytotoxic drugs in the treatment of lupus nephritis. Extrarenal manifestations of lupus nephritis, including skin rash, headaches, myalgia, arthralgia, ect were present in nine of the ten patients prior to RT and absent or minimal in all at last observation.

Teo *et al.*[13] retrospectively reviewed 1154 patients treated for nasopharyngeal carcinomas (NPC) from 1976-1986. Of this group, 10 patients were found to have dermatomyositis (DM). All but one patient had DM prior to RT. Two of the ten patients developed severe radiotherapy complications (skin necrosis) and all ten exhibited subcutaneous indurated fibrosis affecting both sides of the neck. Because of this response, they recommend avoidance of elective cervical irradiation in node negative NPC with CM.

Conclusion

The purpose of this report was to present potential concerns when SLE is diagnosed during radiation treatment to the breast. The largest study to date by Ross *et al.*, had 61 patients with a matched control group and showed no statistical differences in acute or late effects overall, but side effects were increased in breast cancer patients.[11] In this study there were 13 patients with SLE. The remainder or the literature deals with limited case reports. The patient presented here did indeed have a significant and extreme acute reaction to radiation. We recommend a diligent search for SLE or other CVD if the patient experiences an unusually extreme acute reaction. It is also clear that in this case it was beneficial to repeat tests and to utilize more specific tests based upon clinical suspicion. It is still too early to determine what possible late effects will develop in this patient. The proper dose and fraction size is not known in treating individuals with SLE.[7] There are a very small number of patients treated with radiation therapy who have SLE and it is clear a randomized trial is not feasible. If a patient experiences severe acute side

effects in the first week of radiation, consideration should be given to SLE testing. If SLE is diagnosed, radiation therapy should be discontinued and further surgery recommended. Mastectomy represents an equally efficacious treatment and each patient should be given this alternative and apprised of the possible increased risk of side effects associated with radiation and CVD.

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Phase II study of fluorouracil, leucovorin and interferon alpha-2a in patients with advanced colon cancer

Borut Štabuc, Aleksandra Markovič, Erik Brecelj, Samir Bešlija, Tomaž-Edvard Cizej

Institute of Oncology, Ljubljana, Slovenia

Based on in vitro studies that have demonstrated synergy between fluorouracil (5-FU) and leucovorin (LV) as well as between 5-FU and recombinant alpha-2a interferon (IFN) against colon cancer cell lines a phase II study was carried out to evaluate the toxicity and clinical activity of 5-FU modulated with LV and IFN in patients with metastatic colon cancer. Twenty-two chemotherapy naive patients with measurable metastases of colon cancer have been treated with daily doses of 5-FU 600 mg/m^2 in 6-hr intravenous infusion, and of LV 20 mg/m^2 intravenously and IFN 6 MU subcutaneously, for 5 days every 4 weeks. Median age was 60 years, median PS (ECOG) was 1 (range 0-2). Liver, soft tissue and lung metastases were found in 12, 5 and 8 patients, respectively. Nineteen patients had a single metastatic site, two double, whereas one had more than two metastatic sites. Patients had 2-9 (mean 5) cycles of treatment. Objective response was observed in 7 patients (32%), and stable disease in 7 patients (32 %). Overall median survival was 12.5 months, and for responders 14.4 months. Responses were generally short and median time for progression was 5.5 months. The most frequent adverse reactions were flu-like syndrome (50%), nausea/vomiting (36%), diarrhoea (13%), stomatitis (27%) and leucopenia (13%).

This regimen of 5-FU with LV and IFN administration does not appear to be superior to previously published schedules of 5-FU with IFN or 5-FU with LV.

Key words: colonic neoplasms-drug therapy; clinical trials phase II; fluorouracil; leucovorin; interferon-alpha; neoplasms metastasis

Introduction

Colon cancer is one of the most common malignancies in Slovenia, with more than 400 new cases diagnosed each year. In 1995, the incidence of colon cancer per 100,000 population in Slovenia was 22.2 for male and 21.7 for female.[1]

Despite better surgical treatment and adjuvant chemotherapy, approximately 50% patients develop metastases. Patients with metastatic colon cancer have uniformly poor prognosis, because there is no effective therapy.

For more than 40 years fluorouracil (5-FU) has been the mainstay in the treatment of

Correspondence to: Assist. Prof. Borut Štabuc, MD PhD, Department of Medical Oncology, Institute of Oncology, Zaloška 2, SI-1000 Ljubljana, Slovenia. Tel.: +386 61 302 828; Fax:+386 61 302 828.

colorectal cancer. As a single agent or in combinations with other chemotherapeutic agents, the overall response in advanced colorectal cancer is in the range of 17% to 24%, with median survival time of approximately 11 months.[2] In an attempt to improve these results several approaches have been adopted. The most promising is the adjunction of a modulatory agent, leucovorin (LV), which interferes with the mechanisms of 5-FU cytotoxicity. Experimental studies have demonstrated that LV is metabolised to a compound that stabilised the ternary complex formed between the 5-FU metabolite fluorodeoxyuridilate and enzyme thymidylate synthase, a key enzyme in DNA synthesis thereby inhibiting enzymatic function and increasing cytotoxicity of 5-FU.[3-5] In several randomised trials in metastatic colorectal cancer, 5-FU/LV combination produced statistically significant improvements in response rate compared with 5-FU alone, and a modest improvement in survival.[6-8]

In preclinical models, combining the recombinant interferon alpha-2a (IFN) and 5-FU resulted in the augmentation of the cytotoxic effects of 5-FU. The principal mechanism of IFN modulation of 5-FU is currently uncertain. However, evidence exists that this agent alters the 5-FU plasma pharmacokinetics by increasing the area under the concentration (AUC)[9] and the formation of fluorodeoxiuridine-monophosphate,[10] inhibiting the 5-FU induced upregulation of thymidilate synthase, as well as thymidine kinase,[11] and enhancing incorporation of fluorodeoyiuridine triphosphate into DNA.[12]

In 1989, Wadler published promising results of the treatment of metastatic colorectal carcinoma with a combination of 5-FU and IFN. The reported response rate was 76% in a subgroup of non-pre-treated patients.[13] In 1991, inspired by these results, we started a clinical trial in patients with metastatic colon cancer using double modulation of 5-FU with LV and IFN. The aim of this study was to evaluate the toxicity, response rate and overall survival in the treatment with this drug combination. However, as the recent reports of studies on 5-FU double modulation by LV and IFN are conflicting,[14] we reviewed and discussed available clinical studies using double modulation of 5-FU with LV and IFN.

Patients and methods

Between December 1991 and January 1994, 22 patients with measurable metastatic colon cancer and Eastern Co-operative Oncology Group (ECOG) performances status of 0 to 2 were entered into the study protocol approved by Medical Ethics Committee at the Ministry of Health of the Republic of Slovenia. The patients who had received no prior chemotherapy for metastatic disease had to meet the following additional criteria: metastatic disease not amenable to surgery, prior adjuvant therapy with 5-FU alone or with levamisol had to be completed >8 months before study entry, no evidence of cardiovascular disease, adequate organ function including normal bone marrow function (leukocyte count >3,500/μL, platelet count >100,000/μL), renal function (creatinin <1.5 mg/dL or creatinin clearance >60 ml/min), and normal hepatic function (bilirubin < 38 mmol/L, liver transaminase level < 3 times the normal values, an albumen value>36 g/L, negative hepatitis B surface antigen).

Patients with active cardiac disease, infection, clinically significant pulmonary and neurologic dysfunction, cerebral metastases, any significant intercurrent illness, requirement of systemic steroids, psychiatric history, or those who had undergone surgery, chemo-immuno- or radiation therapy were not eligible.

In the 4 weeks before entering the trials, the size of the metastatic lesions was determined by computed tomography and/or

ultrasound and /or X-ray examinations and the serum levels of the carcinoembryonic antigen (CEA) was measured. The size of the lesions was determined after every second treatment cycle. The patients with disease progression had the best supportive care with no additional second line chemotherapy.

Treatment plan

Patients received LV (Leucovorin Ben Venue Laboratories Bedford) 20 mg/m^2/day intravenously (i.v.) and half-an hour later 5-FU which was administered in 6-hr continuos intravenous infusion at a dose of 600 mg/m^2/day diluted in 1,000 ml of normal saline. 5-FU was obtained commercially. Recombinant human IFN α-2a (Roferon; Hoffman La-Roche) was administered subcoutaneusly (s.c.) in a dose of 6 MIU consecutively after each 5-FU administrations. Patients received all three drugs for 5 consecutive days. Cycles were repeated at 28-day intervals. The dose of 5-FU was modified for myelosupression, diarrhoea and stomatitis. In grade 3 or 4 toxicity the dose of 5-FU was reduced for 20%. Subsequent courses were delayed until haematological recovery to the following values: granulocytes, 1,500 /μL or higher and platelets 100,000 or higher, and resolution of all nonhematologic toxicity to Grade I or baseline. There was no reduction of the doses of LV and IFN.

Response criteria

Treatment response was evaluated after each 2nd cycle. The responders and those with stable disease were subjected to regular clinical and radiological follow up every two months until progression, and then after 3 months until deaths. Tumor response was defined according to World Health Organisation (WHO) criteria.[15] A complete response (CR) was defined as the disappearance of all known disease symptoms on two separate measurements performed in at least 4 week interval; partial response (PR) was defined as a ≥ 50% decrease in the sum of products of the largest perpendicular diameters of all measurable lesions for a minimum of 4 weeks, without the appearance of new lesions; stable disease (SD) was defined as a decrease by <50% or increase by <25%, with no new lesions; progressing disease (PD) was defined as a 25% or greater increase in measurable disease or appearance of new lesions. The duration of response was measured for patients with objective response from the onset of objective response to the time of progression. The duration of survival was measured from the first day of treatment until the date of death.

Statistical Analysis

The survival, duration of response and the time to progression were calculated using log-rank test,[16,17] while the calculations of the 95% confidence interval for response rate were made by Brookmeyer and Crowley's method.[18]

Results

The characteristics of the 22 patients entered into this study are listed in Table 1. Seven patients received prior adjuvant therapy consisting of 5-FU and levamisol. The majority of patients had minimal cancer related symptoms. Their ECOG PS was 0-1 except in 1 patient. Patients received 2 to 9 cycles of chemoimmunotherapy (mean 5 cycles). All patients were eligible and evaluable.

Tumour response

The overall response rate was 32% (95% confidence interval (CI) 14% to 52%) (Table 2). All

Table 1. Patients' characteristic

Characteristic	No. of patients
Age, years	
Median	60
Range	31-78
Male/Female	11/11
ECOG performace status	
0/1/2	13/8/1
Prior adjuvant therapy	7
Sites of disease:	
- Liver	12
- Lung	8
- Peritoneum	2
- Lymph nodes	3
No. of metastatic sites	
1	19
2	2
3	1

responses were partial. There was no complete response. The median time to achieve initial response was 2.5 months (range 1.5 to 4.0). Most responding patients showed continued improvement in their disease status, and the median time to achieve best clinical response was 4.0 months (range 1.5 to 5.0). In addition, 32% of patients had stable disease (range 3 to 14 months). Two of twelve patients with metastases involving liver only, two of eight patients involving lung only and two patients with the involvement of both, lung and an extrahepatic site responded. In two patients with lung and soft tissue metastases, CR was observed in one metastatic site, in the lung of one patient and in the soft tissue of the other. In one patient who had liver, lung and soft tissue metastases CR was observed in the lung and soft tissue whereas in the liver only PR was noticed. The patient who had experienced daily hectic fever to 38.5°C caused by liver metastases, completely resolved the fever within 2 weeks of the initiation of therapy. In the group of seven patients, treated with adjuvant chemotherapy, we did not see any response to chemoimmunotherapy.

Survival

Overall median survival time was 12.5 months and of responders 14.4 months (Figure 1). Median follow up was 12 months. Among 22 patients, 20 patients died. Their survival ranged from 4 to 30 months. Two patients are still alive 26 and 36 months after beginning the study. The median time to progression was 5.5 months in 20 patients. In the patients with objective response the median time to progression was 8.7 months. The two survivors have been without any

Table 2. Tumor response

Response	No. of patients	Response rate (%)
Objective response	7	32 (95% C.I. 14-52%)
CR	0	0
PR	7	32
Stable disease	7	32
Progressive disease	8	36

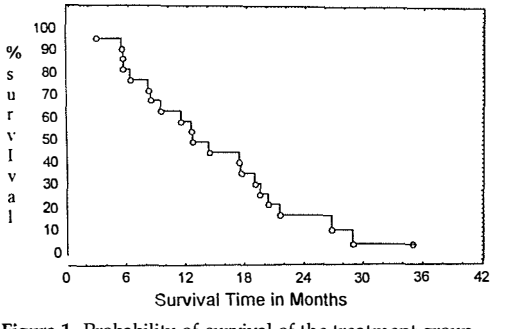

Figure 1. Probability of survival of the treatment group.

sign of progression in the last 22 and 30 months, respectively.

Toxicity

As shown in Table 3, the side effects of chemotherapy were generally mild and not exceeding the toxicity grade of 3 to 4, neither causing toxicity related deaths nor requiring discontinuation of chemotherapy due to toxicity. In one patient chemotherapy was postponed for 14 days due to grade 2 leucopenia. One patient requested a dose reduction of 5-FU due to anorexia and plantar-palmar erytroderma. The most common toxicity was flu-like syndrome which occurred in 11 patients. After the 3rd cycle, the majority of the patients did not complain anymore of any of these types of toxicity. Nausea and vomiting grade 1 and 2, mild diarrhoea, leucopenia and plantar-palmar erythroderma occured in 8,3,3, and 6 patients, respectively.

Table 3. Toxicity: The worst grade per patient across all cycles

	Toxcity grade per patient			
	1	2	3	4
Flu-like	7	4		
Nausea/vomiting	6	2		
Stomatitis	3	2		
Diarrhoea	3	2		
Anorexia	3	3		
Leukopenia	2	3		
Plantar-palmar Erythrodema	5	1		

Discussion

In an effort to improve its antitumor activity, a number of strategies to modulate the cytotoxicity of 5-FU have been investigated. One approach has focused on the use of the LV in combination with 5-FU to stabilise the binding of the active metabolite fluorodeoxyuridine monophosphate to its target enzyme, thymidylate sinthase. The 5-FU/LV combination produced statistically significant improvements in response rates compared with 5-FU alone in several randomised trials in metastatic colorectal cancer,[19-21] although improved survival was noted in two trials only.[7,8] Inhibition of thymidine incorporation into DNA is likely to be enhanced by IFN as a result of its inhibitory effect on thymidine kinase.[11] Based on this biochemical rationale of inhibiting two key enzymes of 5-FU metabolism, a number of trials have been performed in order to investigate the possibility of biochemical double modulation and to assess the feasibility of this combination (Table 4).

We have demonstrated the feasibility of administering 6 MIU IFN with 5-FU and low dose LV. Our combination of 5-FU/LV/IFN has activity in previously untreated patients with colon cancer. We have observed a 32% overall response rate. The reason for such a response rate and low toxicity may lie in a relatively high dose of 5-FU and the administration of 5-FU in prolonged infusion. A dose - response relationship has been suggested for 5-FU in colon cancer. 5-FU is a drug with very short plasma half-life of approximately 12-18 minutes. The drug has cytotoxic activity against cells in S phase. With bolus administration, a small proportion of cancer cells would be susceptible.[22,23] Thus, there exists a sound rationale for the preference of continu-

Table 4. Results of trials of 5-fluorouracil (5-FU), leucovorin (LV) and interferon alpha (IFN) for treatment of meatastatic colorectal cancer

Reference	5 - FU/LV/ IFN regimen	No. of patients	Objective response (%)
Yalavarthi[27]	5-FU 370 mg/m2 i.v. d 2 - 6q 21 d LV 500 mg/m2 i.v. d 2 - 6 q 21 5 MIU INF s.c. d 1 -7 q 21 d	31	23
Seymour[28]	5FU 400 mg/m2 bolus, then 5FU 400 mg/m2 by i.v. infusion over 22 hours d 1.2 q 14 d LV 200 mg/m2 i.v. 6 MIU IFN s.c. every 48 hours troughout	128	31
Recchia[29]	5-FU370mg/m2 i.v. d 2 -6 q 21 d LV 500 mg/m2 i.v. d 2 - 6 q 21 d 3 MIU IFN x 3 s.c. d q 28 d	32	22
Grem[30]	5-FU 370 mg/m2 i.v. d 2- 6q 21 d LV 500 mg/m2 i.v. d 2 - 6 q 21 d 5 MIU IFN s.c. d 1 - 7 q 21 d	54	44
Labianca[31]	5-FU 400 mg/m2 i.v. x 5 d q 28 d LV 200 mg/m2 i.v. x 5 d q 28 d 3 MIU IFN - α2b s.c. x 5 d q 21 d	36	22
Kreuser[32]	5-FU 500 mg/m2 i.v. x 7 d q 21 d LV 200 mg/m2 i.v. x 7 d q 21 d 5 MIU IFN - α2b s.c. x 7 d q 21 d	62	29
Cascinu[33]	5-FU 370 mg/m2 i.v. d 2 - 6 q 21 d LV 200 mg/m2 i.v. d 2 - 6 q 21 d 3 MIU IFN s.c. d1 -7 q 21 d	45	51

ous infusion 5-FU to bolus injections. In many trials, objective response rate was higher and toxicity much lower in the patients who received continuous infusion of 5-FU than in the patients treated by bolus injections.[24,25]

Numerous trials have been performed with different doses of LV (15 to 500 mg/m^2). In general, in clinical trials no difference was indicated in the activity between low (20 mg/m^2) and higher doses of LV (250 mg/m^2).[19,26]

The first clinical results of the 5-FU/LV/IFN combination were published in 1990.[27] The treatment schedule consisted of 1-20 MIU IFN/day for 5 days and 370-425 mg/m^2/day 5-FU, and 500 mg/m^2/day LV, given in 5 consecutive days. Only 7 of 31 patients (23%) demonstrated PR, which was disappointing. In the next three years various modes of drug administration were studied in order to define an optimal therapeutic approach (Table 4). Although the majority of phase II studies with 5-FU, LV and IFN have

demonstrated objective response in approximately 30% of patients with advanced colorectal cancer, their relevance seems hampered by small numbers of patients.

Despite some clinical trials which showed that 5-FU plus IFN is more effective than 5-FU alone in terms of response rate and event free survival, but not of overall survival,[34] in three recent randomised clinical trials using double modulation of 5-FU, the investigators found that no benefit could be achieved with the addition of IFN. An Italian trial was aimed to clarify whether IFN could further enhance the therapeutic potential of 5-FU in combination with LV. Eighty-three patients were entered into this trial. The therapy in the first treatment arm consisted of 200 mg/m^2/day LV, followed by 370 mg/m^2/day 5-FU on days 1-5. In the second treatment arm, 3MIU IFN was administered three times a week subcoutaneusly. The response rates in the 5-FU/LV and IFN arms was 45% and 22%, respectively.[29] Pensel randomised 55 patients between treatment with 5-FU/LV with or without IFN administered at a daily dose of 5MIU. Preliminary results did not suggest any statistically significant difference in terms of treatment outcome between the two arms.[35] Disappointing results were also reported by the Hellenic Co-operative Oncology Group. Among 95 patients enrolled in trial the response rates in the arm without IFN and in the arm with IFN were 19% and 6%.[36]

According to phase II studies and preliminary results of three randomised trials, the concept of 5-FU double modulation using IFN and LV does not seem to fulfil its promise either. Therefore, for cost considerations and because combined treatment with 5-FU, LV and IFN seems to result in enhanced toxicity, currently used dose regimens of this approach cannot be recommended for routine use. As the modulatory effects of IFN on chemotherapy in experimental models are highly dependent on the timing and the dose of IFN, alternative schedules of 5-FU and IFN combination are worth exploring in clinical trials.

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Thyroid doses due to stereotactic radiosurgery of the brain

Claudio H. Sibata, Carlos E. de Almeida, Ku Shin

Roswell Park Cancer Institute, Department of Radiation Medicine Buffalo, N.Y.

Background and purpose: Radiosurgery is an irradiation technique in which one high dose fraction or more is applied to a small intracranial volume stereotactically located. The technique is presently in use at the Roswell Park Cancer Institute and it uses 6 non-coplanar arcs, delivered by a 6 MV photon beam from a linear accelerator. One of the issues discussed in the most recent BEIR Report V is the radiogenic aspects of solid tumours and the risks related to external radiation. Thyroid carcinoma has been observed to increase consistently in a number of irradiated populations. The thyroid gland in children under 15 years of age, has one of the highest risk coefficients of any organ and is the only tissue with convincing evidence of risk at about 10 cGy. Since children of different ages may also present clinical situations that requires radiosurgery, an investigation was conducted to assess the doses to the thyroid and other nearby organs.

Materials and methods: In-vivo patient measurements were conducted, using TLDs and diodes at the surface of the patient neck corresponding to the thyroid plane as well as with the Alderson phantom for similar geometry and at 0,6 cm of depth in the same plane.

Results: The measured thyroid doses in the order of 10 cGy are essentially independent of cone size since the typical doses used in radiosurgery increases considerably as the cone size decreases.

Conclusions: A recommendation is made to eliminate or to displace the treatment arc which projection passes through the thyroid plane. This procedure must be adopted for children under 5 years of age and strongly recommended for children up to 15 years of age.

Key words: radiosurgery; thyroid gland - radiation effects; radiation dosage; thyroid neoplasm; arteriovenous malformations

Introduction

Stereotactic radiotherapy is an irradiation technique in which one high dose fraction or more is delivered to a small intracranial volume stereotactically located.[1-3]

It is used mainly for treating surgically inoperable arterio-venous malformations (AVM), brain metastasis and other small brain tumours. Radiosurgery can be generally performed in two ways:

Correspondence to: Prof. Carlos Eduardo de Almeida, PhD, Chairman, Laboratorio de Ciencias Radiologicas - LCR, Instituto Nacional de Cancer and Universidade do Estado do Rio de Janeiro, Rua Sao Francisco Xavier 524, Pav. Haroldo Lisboa, Rio de Janeiro, Brasil, CEP 205050-013

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1. Using the gamma-knife, a commercial unit that uses 201 focused beams from 201 Cobalt-60 sources, each with a nominal activity of 30 Ci, resulting in a dose rate of about 200 cGy/min at the isocenter.[4]
2. Using a linear accelerator with single plane full rotation or arcs,[5,6] multiple non-coplanar converging arcs[7,8] and dynamic radiosurgery,[9] all with small field sizes (1-3 cm).

The technique is presently in use at the Roswell Park Cancer Institute and it uses 6 non-coplanar arcs delivered by a 6 MV photon beam from a Varian linear accelerator model Clinac 2100 C and a stereotactic Fisher system. The stereotactic frame is fixed to the patient's skull by four screws and the localising rods attached to the base.

A CT image enables the localisation of the tumor and the internal structures of the brain in relation to the fidutial points in the stereotactic frame. The same mental assembly used during the CT examination is attached to the linac treatment couch in order to reproduce the patient's position during the CT images. The dose distribution is calculated using a treatment planing system also elaborated by Fisher.

Statement of the problem: Radiogenic cancer of the thyroid

One of the issues discussed in the most recent BEIR Report V[10] is the radiogenic aspects of the solid tumours and the risks related to external radiation. Thyroid carcinoma has been observed to increase consistently in a number of irradiated populations. Indeed, it was the first solid tumor to increase in frequency in the Japanese atomic bomb survivors, among persons exposed to therapeutic doses of X-rays as infants[10] and, on the Marshall Islands inhabitants exposed to radioactive fallout.[10]

Based on the on-going studies, the BEIR V Report suggest that:

1. The susceptibility to radiation-induced thyroid cancer is greater in early childhood than at any time later in life. Moreover the tumours usually become apparent after sexual maturation in those exposed before puberty.
2. Females are two to three times more susceptible than males to radiogenic as well as spontaneous thyroid cancer.
3. Radiogenic cancer of the thyroid is frequently preceded or accompanied by benign nodules, being generally a papillary growth.

The incidence of thyroid cancer as result of low LET radiation therapy of benign diseases in children is reported in five cohort studies; the Israel Tinea Capitis Study[10] the Rochester Thymus Study,[10] studies of children irradiated for enlarged tonsils in addition to two case control studies (patients with cervical cancer and childhood cancer) enhances the correlation between solid tumours and exposure to external radiation. The combined studies include almost 120 000 people (58 000 exposed and 61 000 unexposed). The thyroid gland in children under 15 years of age, has one of the highest risk coefficients of any organ and is the only tissue that shows convincing evidence of risk at about 10 cGy.[11]

During the radiosurgery treatment planing procedure, special attention is given to the potential doses to the brain stem or to the optical chiasm but the thyroid dose is normally overlooked since its plane is not imaged in the CT scans. Since children of different ages may also present clinical situations that require radiosurgery, an investigation was conducted in order to assess the doses to the thyroid and other nearby organs.

As a result of this study recommendations are proposed in order to minimise the potential risks involved with this procedure.

Materials and methods

This study was done using as a reference, the technique in use at the Roswell Park Cancer Institute which uses 6 non-coplanar arcs of a 6 MV photon beam from a Varian linear accelerator model Clinac 2100 C. In addition, the 18 MV photon beam from the same machine was used to verify the dose level in case this beam is used for radiosurgery.[12]

A complete treatment simulation of several clinical cases was done using an Alderson phantom and placing the TLDs at 0,6 cm of depth of a phantom slab corresponding to the thyroid plane and the diode at the surface in order to verify the adequacy of the two measuring techniques. *In-vivo* patient measurements were conducted, using a pair of TLDs and one diode per each irradiation (both systems with full build-up material for 6 MV photon) placed at the surface of the patient neck in a region located between the thyroid cartilage and the furcula.

The general specifications of the dosimetric systems used are:

1. Thermoluminescent dosimeters. Lithium fluoride LIF-100 conventional chips were selected from a large batch of the existing chips with a reproducibility better than 2% and sensitivity high enough for the doses used in this study. The annealing routine for pre-irradiation was 400 °C for 1 hour and 100 °C for 2 hours and a pre-reading treatment of 100 °C for 15 min.
2. Energy compensated diodes. The photon diodes model Isorad used were manufactured by Nuclear Associates. They have cylindrical shape, diameter of 7.1 mm, sensitive volume of 0.25 cc, wall thickness equivalent to 600 mg. cm^{-2} and dose linearity better than 0.5%. The angular dependence was not considered once the diodes were always placed with its main axis perpendicular to the beam direction, geometry similar to the one used for its calibration.

Both systems were calibrated at 5 cm of depth in a lucite phantom, using a 6 MV photon beam and a calibrated 0,6 cc Farmer type ionisation chamber.

An individual calibration factor for 6 MV was assigned to each individual diode and the TLD as well.

In order to improve the signal measured the exposure time of each arc was increased by 3 times and the doses measured were normalised for the typical prescribed dose of 1500 cGy.

The combined uncertainties of Type A and Type B for 1 sigma, involving all steps of the calibration and measurement procedures are smaller than 3%, largely due to the intrinsic characteristics of the detectors such as 2,0 % for the TLDs, 1,5 % for the diodes and 1,0% for the beam calibration with the ion chamber.

Results

The results of the measurements made at the phantom and patient surface with TLDs and diodes were identical to the ones made with TLDs at 0,6 cm of depth in the phantom.

The measurement results of the added doses due to the complete treatment simulation using the 6 arcs as well as of each individual arc clearly indicates that the thyroid doses are essentially due to the contribution of the beam when it passes through the midline of the brain with negligible contributions from room scatter and machine leakage.

The magnitude of the thyroid doses is essentially the same, in the order of 10 cGy, as indicated in Figure 1 curve D, being slightly independent of the cone diameter since the prescribed tumour doses may vary significantly according to cone diameter. The typical maximum doses for a treatment of an AVM at the 80% isodose line may range from 1200 cGy for a 35.2 mm cone diameter at the isocenter to 4000 cGy for a 10 mm cone diameter depending on each clinical situation.[13,14]

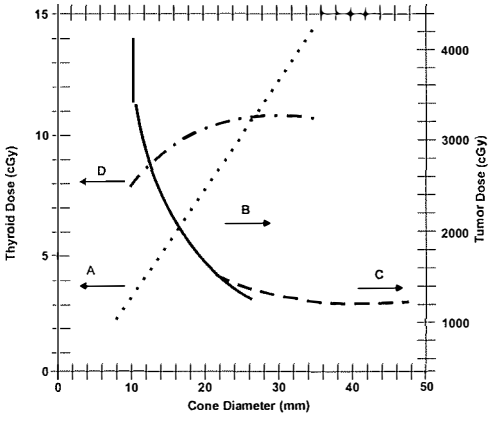

Figure 1. Thyroid doses to radiosurgery of the brain.

Curve A - Thyroid doses measured in the Aldreson Phanton for different cone sizes and a fixed tumor dose of 1500 cGy.
Curve B - Typical tumor doses used by Flickinger.
Curve C - Typical tumor doses used by Kjellberg.
Curve D - Thyroid doses as described in Curve A, normalized for the typical doses shown in Curves B and C, as function of cone size.

Additional measurements have shown an appreciable thyroid dose reduction (50-70 %), for all cone diameters when the treatment target is located 5 to 20 mm laterally from the midline. On the other hand, the displacement of the isocenter depth only 2.5 cm towards the thyroid, is sufficient to increase the thyroid doses by 30 %.

However, when the treatment target is laterally displaced 5 to 20 mm from the midline, the eye doses may increase by as much as 80%.

Finally, the doses measured for the same sites and geometric situations where a 18 MV photon beam is used, are 30-40% higher than the ones measured with a 6 MV photon beams one would expect.

Conclusions and recommendations

The results of the present work clearly indicates that the thyroid doses due to radiosurgery may be easily reaching the current acceptable risk rate for this organ.

In order to reduce the thyroid doses to a minimum, it is recommended to eliminate the arc or plane of rotation that passes through the midline of the brain or whenever its projection is close to the thyroid. This procedure must be used for treating children under 5 years of age and strongly recommended to children up to 15 years of age.

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The assessment of telemedical procedures in countries of transition

Haris Boko

Medical School, University of Zagreb, Croatia

Different applications of the initial idea to overcome time and distance barriers in order to achieve satisfactory level of physical diagnosis and medical prognosis, are expanding so rapidly that telemedicine related procedures have became the utmost growing segment of health related markets. Telemedicine is not only use of telecommunications to assist in the delivery of health care but has significant social role in order to overcome various problems associated to demands of equal and adequate provision of medical services. Successful health policy leads to increased welfare through better health outcomes, greater equity, more consumer satisfaction, and lower total costs than would occur in the absence of public action. Managerial skills, technology assessment know-how and efficient transfer of technology is of fundamental value for better performance of health services, while such skills are in the shortest supply in all developing countries. Practical implementations of certain telemedical procedures are significantly biased by specific level of social development. These applications in countries in transition should be assessed in order to accelerate the process of training, diversify health providers, raise significance of cost-benefit approach, and lead toward more efficient and less expensive health care system, while maintaining potentials to preserve the main virtue of the former health system, namely the widespread coverage of population.

Key words: telemedicine; health planning; health planning technical assistance

Health in transition

Transition to an institutionalized, consolidated democracy requires to set bureaucracy capable of discharging their duties with reasonable efficiency, to provide effectiveness of its laws over a given territory and to orient state institutions towards decisions of the public benefit. The government's responsibility is therefore to ensure that everyone can exercise fundamental rights, at least to the extent that society can afford. In health by means of constituting essential medical care package that might justifiably be financed by general revenues, sometimes with some contribution from user fees. These should be highly cost-effective services that would greatly improve the health, especially of the poor satisfying the argument that the basic health care is fundamental right. Beyond the well-defined package of essential services, the government's role should be limited to improving the capacity of insurance and

Correspondence to: Haris Boko PhD, Medical School, University of Zagreb, Croatia.

health care markets to provide discretionary care.[1] Higher developmental level will enable more comprehensive set of services above the minimum. However, on the account of gradually lower cost-effectiveness.

Governments mostly build facilities, buy equipment and supplies, hire and train people, set fees or other service conditions, regulate providers and insurers, disseminate information, determine overall policy and maintain surveillance of disease conditions. Most of failures to achieve better health outcomes derive, however, not from the wrong choice of objectives but wrong choice of policy instruments. Particularly from too much reliance on direct provision of care and central control of health facilities and too little use of the financial, informational and regulatory instruments of the government. Governmental task is to allocate resources so as to obtain the most improvement in health taking into account the private market's response to public sector spending. The responsibility is to shift the role from providing care to financing and stimulating competition among providers in order to enable clear distinction between essential and discretionary spending in the most cost-effective way. In the same time, however, to provide information and incentives to improve the allocation of resources by the private sectors.

In transitional countries of Europe governments were responsible for both the finance and delivery of health care while presently, health system is in the deep crisis.[2] Over employment, over capacitating, leaving of experienced professionals to practice fee-for-service medicine. Dramatic drop in spending is the reason of significant shortages of pharmaceuticals and equipment. The main strategic presumption of the public policy should be the necessity to preserve the main virtue of the previous system: widespread coverage of the population. Potentially, the most important force is decentralization of health services. That might be successful only if local agencies have sound financial resources, administrative capacity and responsibility toward local population, while clinical and managerial skills of health professionals need to be substantially upgraded.

Technology assessment

One of the main developmental tendencies is that biomedical spending made of health the dominant financial sector. World health spending exceeded total of $1700 billion and equals roughly to 8% of total World Gross National Product. Nearly 60% of the sum ($1000 billion) is government spending. Established market economies (22 countries with population of 800 million, or 15% of World total) spend $1500 billion, or almost 90% of World total.[3] The industry is flourishing while technologies are becoming increasingly sophisticated. Within the last few decades amount of physicians has increased by five times worldwide, while hospital beds increased by four times.

To reduce both capital and recurrent costs without deteriorating the quality of care, governments should reallocate public policy spending toward the equipment that provide essential services, improve efficiency by rearranging the clinical protocols, and ensure control of specialized equipment. WHO estimates that the half of all the medical equipment in developing countries is not utilizable, due to lack of undertaken technology assessment procedures. Information on costs does promote allocate efficiency and therefore the most justified public measure will combine a strong rationale for public action with a cost-effective health interventions. Cost-effectiveness analysis requires data on expenditures and on health outcomes that are seldom quantified, and takes considerable time and efforts for public systems in developing countries to learn how to gather and use it.

The transition should facilitate involvement by the private sector by means of encouraging private finance and provision of insurance (with incentives to contain costs) for all discretionary clinical services, and delivery of services by the private sector, including those publicly financed. Governmental instruments should improve managerial skills of public service, ensure the delivery of the essential package and reduce expenditures for discretionary services.

According to the World Bank survey,[4] the principal factor affecting health policy changes in transitional countries of Europe is to improve the management of public health services. The believe is that promising professionals should demonstrate not only the excellence in managerial skills, but also to posses analytical capabilities, political sensibility and ethical sensitivity. Consequently, transfer of technology and managerial know-how is becoming critical. The appropriate planning in health and well-trained management is therefore of fundamental value for better performance of health services, while such skills are in the shortest supply in all developing countries.

In its strategy for the forthcoming century, World Health Organization demands that "all member states should have established formal mechanism for the systematic assessment of the appropriate use of health technologies and of their effectiveness, efficiency, safety and acceptability, as well as reflecting national health policies and economic restraints". Technology does not refer solely to the instrumentation and people operating it,[5] and accordingly, The Office of Technology Assessment of the US Congress defines medical technology as "drugs, devices and medical and surgical procedures used in medical care and the organizational and supporting system within which such care is provided".

Telemedicine

The task of telemedicine is to overcome time and distance barriers in order to focus to physical diagnosis and prognosis. It is most commonly defined as "instrumentation, monitoring and management of patients and the education of patients and staff using systems which allow ready access to expert advice and patient information no matter where the patient or relevant information is located".[6] Telemedicine is not only use of telecommunications to assist in the delivery of health care but has significant social role in order to overcome various problems associated to demands of equal and adequate provision of medical services and health care. That include funds, expertise and resources which relate to the lack of facilities and systems due to the need to reduce the costs of health professionals and to ensure satisfactory level of health care in rural and remote areas.

Besides activities in improving health care, significant interest in telemedicine are of telecom operators since they can generate additional traffic over existing networks, have opportunities to extend telecom networks and of equipment providers that can achieve new sources of revenues. These advantages made of telemedicine related procedures the most rapidly growing segment of health related markets.

In undeveloped countries there is an urgent need in low-cost solutions in delivery of health services and access to appropriate expertise, especially in emergencies.

Factors to consider implementation of telemedicine are primarily to identify the types of medical services where telemedicine could be useful. To ensure efficient application, the available telecom infrastructure should be investigated, the diversity of engaged players has to be achieved, telemedical needs identified and the cost-benefit analysis evaluated.

Since the very beginning of telemedicine, in 1967 at Boston Airport, when collected X-rays were illuminated by ordinary light box, scanned by black and white television and transferred to video monitor at radiology department of Massachusetts General hospital, teleradiology is the leading telemedical application with approximately 70% of sales in telemedicine. It is defined as "the electronic transmission of images of the patients' anatomy and/or pathology from one location to another for interpretation or consultation".

Typical teleradiology equipment includes digital radiographic unit, software expenses and telecommunication lines.[7] Digital radiographic unit employs X-ray detector of up to 41x41 cm in size and pixel pitch of less than 0.1mm with 16 bits of dynamic range (contrast). Photolitographic techniques are employed to create photodiodes using rectangular glass-panel substrate with successive thin-film layers of silicon, metals and insulators. Over the photodiode array is applied scintillation material (cesium iodide) which converts X-ray photons to visible light while amorphous silicon converts the light into electricity.[8] Besides telemedical application substantial benefit of digital radiography is electronic storage when no darkroom, chemicals or hazardous waste to dispose is necessary. Such system allows computer-aided diagnosis and fewer numbers of retakes with wider dynamic range of images.

The predominant software standard is DICOM (digital imaging and communications in medicine) that was set up to define network interface and data model for imaging devices that can facilitate information systems integration. Contemporary version is DICOM 3.0 that employs V42bis error correction and compression protocol, blitz string encoding algorithm and MPEG (motion picture expert group) and JPEG (joint photograph expert group) standards for moving and still image compression.

Main fields of applications besides radiology is surgery, dentistry, ophthalmology, home health care, rehabilitation, psychiatry, nursing and dermatology. Two basic techniques are employed: IATV (interactive live tele-video remote transmission of images across computer/television lines in which participants can interact through visual imaging information) and S&F (store and forward imaging that provides the ability to store and transmit diagnostic images to remote locations for interpretation).

Main obstacles of contemporary telemedical applications consist of usual hesitation to application of new technology and doubt of insurance providers to cover risks of remote consultation. Controversial are the issues of unauthorized access to confidential personal medical records and physicians licensing and the lack of generally accepted standard and compatible hardware on both ends of telecommunication link that can deter cost-effective implementation. General shift from rural to managed care is an obvious tendency.

Telemedicine in countries of transition

Telemedical applications in countries of transition are confronted with different problems than in both highly industrialized societies and in undeveloped countries. These countries might become biggest beneficiaries of the new technology since these health systems are not characterized with insufficient access to health services, with expenses confronted with vast rural distances or absence of medical facilities and telecom infrastructure. On the other side, provision of telemedical services is less for-profit influenced and controversial legal issues are still no significant obstacle in larger implementation of the new technology. The cost-benefit approach show great potentials since for the relatively low-cost additional specialized equipment used on existent telecom network, medical

professionals can be in touch with high-tech medical techniques and managerial skills. A great potential for on-line training and emergency advisory instructions is obvious. Internal health structure is characterized by significant difference in quality and quantity of health services between central and provincial regions.[9] Telemedicine can improve socially unacceptable health related disadvantages of regional-level health provision as well as assist in solving the problem of over employment in central regions on the account of underemployment of skilled health professional in provinces. It can be a powerful tool in assembling of medical record simultaneously editable by multiple physicians enabling interface linking multiple distributed data repositories while maintaining full data integrity.[10] Stronger implementation of telemedical services will allow better regulatory mechanism rather than state provision of health care. It allows potentials that the main advantage of the former health system, namely widespread coverage of population be maintained and essential package of health services be provided in its whole on every location and by well-trained professionals. Telemedical applications will improve the number of skilled professionals and accelerate the process of training, diversify health providers, raise significance of cost-benefit approach, and lead toward more efficient and less expensive health care system.

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Ultrazvok z visoko ločljivostjo in energijski Doppler - prednosti preinvazivnega diagnosticiranja solidnih lezij v dojki, izkušnje enoletnega dela.

Brnić Z, Drinković I, Hebrang A

Raziskavo smo izvedli z namenom, da bi ocenili zmogljivosti ultrazvoka (UZ) z visoko ločljivostjo in energijskega Dopplerja pri razlikovanju med malignimi in benignimi solidnimi lezijami v dojki.
Bolniki in metode: Z UZ z visoko ločljivostjo in energijskim Dopplerjem smo pregledali 25 žensk. Pri pregledu smo upoštevali Grayevo lestvico za določanje malignosti ali benignosti. Žilje v lezijah smo razvrstili v naslednje tri skupine: penetrantno žilje, periferno žilje in žilje, nedoločjivo z energijskim Dopplerjem. Diagnoza je bila dokončno potrjena s citološko preiskavo in/ali z biopsijo.
Rezultati: Z UZ z visoko ločljivostjo smo bolj pogosto odkrili nepravilne oblike, nehomogenosti in razredčitve v ozadju kot s klasičnim UZ. V razmeroma velikem številu primerov smo odkrili zasenčenje ob straneh, vendar takšna odkritja niso imela nobenega vpliva na natančnost diagnoze. Z UZ z visoko ločljivostjo se je izboljšala vidljivost manjših karcinomov, intraduktalnih kalcinacij in papilomov. Z energijskim Dopplerjem pa smo v 15/25 lezijah, od katerih jih je bilo kar 8 malignih, zasledili pretok. Penetrantno žilje smo opazili v 6/8 karcinomih in v samo 2/17 benignih spremembah. Od 11 fibroadenomov je bilo 6 avaskualrnih, 4 od 11 pa je imelo periferno žilje. V 3/6 drugih benignih lezijah smo z energijskim Dopplerjem odkrili pretok.
Zaključek: UZ z visoko ločljivostjo in energijski Doppler lahko uspešno prispevata k boljšemu razlikovanju med bengnimi in malgnimi solidnimi lezijami v dojki in sta koristen dodatek k mamografski preiskavi in kliničnemu pregledu, preden se odločimo za bolj invazivne diagnostične postopke.

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Hipertrofična pilorična stenoza: ultrazvočna diagnoza

Goran Roić, Slavko Čop,Vesna Posarić, Ercegović S

V raziskavi smo analizirali izvide ultrazvočne preiskave 68 bolnikov, pri katerih smo sumili, da so oboleli za hipertrofično piloričnо stenozo (HPS). Od 45 bolnikov s pozitivnim ultrazvočnim izvidom je bila HPS tudi po kirurškem zdravljenju potrjena. Dolžina pilorične mišice je bila pri 82,6% bolnikov (38 bolnikov) 5-7 mm, pri 10,9% bolnikov (5 bolnikov) pa je bila dolžina te mišice 3-5 mm in pri samo 6,4% bolnikov (3 bolniki) je bilo potrebno tudi rentgensko slikanje z barijem zaradi z ultrazvočno preiskavo ugotovljene sumljive debeline pilorične mišice (3,0-3,4 mm). Rentegensko slikanje z barijem je potrdilo diganozo HPS pri enem bolniku, pri dveh pa je to diagnozo ovrglo. Debelina pilorične mišice je bila pri vseh bolnikih z ugotovljeno HPS 1,5 krat večja kot debelina antralne mišice. Občutljivost ultrazvoka je bia 98%, specifičnost pa 92%. Na osnovi rezultatov naše raziskave ugotavljamo, da sodi ultrazvočna preiskava pilorusa med najbolj priporočljive diagnostične metode za ugotavljanje HPS.

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Paliativno zdravljenje bolečih kostnih metastaz pri bolnikih s karcinomom prostate z uporabo Re-186-HEDP

Klutmann S, Bohuslavizki KH, Schulte U, Kröger S, Bleckmann Ch, Mester J, Clausen M

Skelet je drugo najbolj pogosto mesto metastaziranja karcinoma prostate. Čeprav so ozdravitve pri bolnikih s tako razširjeno boleznijo silno redke, pa je pričakovana življenska doba bolnikov vendarle nekaj let. Zato je pomembna njihova kakovost življenja. Ustrezno bolečinsko zdravljenje je še vedno najpomembnejši cilj pri obravnavi bolnikov z napredovalo boleznijo karcinoma prostate. Bolečine skušamo najprej umiriti s perifernimi in centralnimi analgetiki. Ob bolečinah zaradi solitarne kostne metastaze znatnemu številu bolnikov pomaga teleradioterapevtično obsevanje. V primeru bolečih multiplih kostnih metastaz, pa je učinkovito obsevanje le-teh z intravensko aplikaciranimi radiofarmacevtiki, ki so sevalci žarkov β, zdrava tkiva pa prejmejo zanemarljivo obsevalno dozo. Zaradi svojih fizikalnih lastnosti (kratek razpolovni čas in možnost spremljanja kopičenja radiofarmaka z gama kamero) sta za takšno zdravljenje primerna Re-153 in Sm-153. Odgovor na zdravljenje dosežemo v 70-80 %. Analgetski učinek se pokaže 1-6 mesecev po zdravljenju. Zaradi blagih stranskih učinkov, ki se kažejo predvsem kot prehodna trombopenija, lahko zdravljenje ponovimo. Do sedaj pa niso dokazali, da bi zdravljenje z Re-186-HEDP lahko vplivalo na dolžino preživetja.

Radiol Oncol 1998; 32(4): 373-83.

Tumorski supresor gen p53

Jezeršek B in Novaković S

Spremembe tumor supresorskega gena p53 so najpogostejše genetske spremembe, ki so jih doslej odkrili pri bolnikih z malignimi boleznimi. Proizvod omenjenega gena - p53 protein je namreč pomemben element različnih biokemičnih poti, ki so ključne v procesu karcinogeneze: vključno s procesom prepisovanja DNA, ohranjanja stabilnosti genoma, popravljanja poškodb DNA, ter kontrolo celičnega ciklusa. Analiza mutacij p53 gena in spoznavanje biokemičnih poti programirane celične smrti ter zaustavitve celičnega ciklusa, ki ju sproži p53 protein, sta prispevala k razumevanju patogeneze raka na molekularnem nivoju, kot tudi mehanizmov, preko katerih p53 deluje kot "zaviralec" tumorjev. Namen tega preglednega članka je strniti dosedanja dognaja o p53, saj naj bi razumevanje navedenih procesov v bodoče olajšalo iskanje novih racionalnih pristopov k zdravljenju malignih bolezni na molekularnem nivoju.

Radiol Oncol 1998; 32(4): 385-92.

Mikronukleus test pri osebah s poklicno izpostavljenostjo citostatikom

Garaj-Vrhovac V in Kopjar N

Avtorji so s pomočjo mikronukleus testa v kombinaciji z različnimi tehnikami barvanja (Giemsa, DAPI in srebro) proučevali možne poškodbe DNK zaradi delovanja citostatikov. Krvne vzorce za preiskave so jemali medicinskim sestram, ki so na onkoločkih oddelkih brez ustrezne zaščite opravljale delo v zvezi s pripravo in aplikacijo različnih citostatikov. Limfocite so gojili in vitro pri temperaturi 37°C. Za preprečitev citokineze so po 44 urah dodali citohalazin B v končni koncentraciji 3 μg/ml. Dobljeni rezultati kažejo statistično značilen porast skupnega števila mikronukleusov pri izpostavljeni skupini v primerjavi s kontrolno skupino. Z DAPI tehniko so prikazali signal-pozitivne in signal-negativne mikronukleuse, medtem ko je tehnika barvanja s srebrom razkrila Ag-NOR-pozitivne in Ag-NOR-negativne mikronukleuse. V primerjavi s kontrolno skupino je bilo število signal-pozitivnih in Ag-NOR- pozitivnih nukleusov pri izpostavljenih osebah zvišano, kar kaže, da so nekateri kromosomi bolj dovzetni za poškodbe, ki jih povzročajo citostatiki.

Radiol Oncol 1998; 32(4): 393-9.

Vloga tiroksina pri karcenogenezi pri obsevanju ščitnice

Koritnik K, Cör A

Namen študije je bil preveriti hipotezo o zaščitni vlogi.dajanja tiroksina podganam pred obsevanjem in po njem, na pojavljanje ščitničnega karcinoma. Dajanje tiroksina pred in med obsevanjem naj bi preko povratne zveze s hipofizo preprečilo povečano.izločanje tirotropina, povzročeno z radiacijsko okvaro ščitničnega tkiva. Na takšen način stabilizirane celice naj bi bile med obsevanjem manj radiosenzibilne.

V poskusu smo 81 podgan seva Wistar obeh spolov, starih 3 do 4 tedne najprej razdelili v dve skupini, v tiroksinsko (T_4) in vodno (H_2O). Tiroksinski skupini smo 15 dni dvakrat dnevno intraperitonealno injicirali enoodstotno raztopino tiroksina (0,01 mg/100 g telesne teže), vodna skupina pa je na enak način dobivala sterilno fiziološko raztopino. Po 10 dneh smo vsako od osnovnih skupin razdelili v dve podskupini; podgane iz obeh obsevanih podskupin (T_4/X in H_2O/X) so prejele 10 Gy na področje vratu. Na telekobaltovem aparatu smo jih 5 dni zapored obsevali z direktnim poljem. Med dveletnim opazovanjem smo sproti žrtvovali vse živali v slabem stanju in jim odvzeli ščitnice. Preostale ščitnice smo odvzeli ob koncu poskusa. Vse žleze smo patohistološko analizirali. Poleg tega smo pregledali vse sumljive in povečane esktratiroidne organe in tkiva ter zabeležili vsa pojavljanja tumorjev.

Ugotovili smo, da se je v skupini H_2O/X pojavilo 8 ščitničnih karcinomov in 7 adenomov, v skupini T_4/X pa trije ščitnični adenomi. V skupini obsevanih podgan brez tiroksina je bila incidenca karcinomov ščitnice statistično signifikantno (P = 0,01) večja kot v obsevani skupini s tiroksinom.

Z opisanim poskusom je bila potrjena hipoteza o zaščitni vlogi dajanja tiroksina pred in med obsevanjem pri postiradiacijski ščitnični karcinogenezi pri podganah.

Radiol Oncol 1998; 32(4): 401-6.

Sistemski lupus eritematodes z izrazito kožno reakcijo med radioterapijo: prikaz primera

Lee RJ, Proulx GM, Donaldson CW, Orner JB

Še vedno si nasprotujejo mnenja o konzervirajočem zdravljenju bolnic s karcinomom dojke, ki imajo tudi kolagensko vaskulatorno bolezen. Poročali so že o resnih kožnih reakcijah pri bolnicah, ki so jih obsevali in so imele sistemski lupus eritematodes ali sklerodermo. Novejša študija primerov s kontrolami pa pri teh bolnicah ne kaže večjega števila komlikacij kot pri tistih brez kolagenske vaskulatorne bolezni. Prikazujemo primer bolnice, ki smo ji diagnosticirali sistemski lupus eritematodes kmalu po začetku obsevanja dojke, ker je razvila resno kožno reakcijo. Opisujemo klinični potek zdravljenja, ob njem pa tudi nasprotujoče dejavnike ter klinične dileme.

Radiol Oncol 1998; 32(4): 407-15.

Zdravljenje bolnikov z metastatskim rakom širokega črevesa s fluorouracilom, levkovorinom in interferonom alfa-2a

Štabuc B, Markovič A, Brecelj E, Bešlija S, Cizej T-E

Predklinične raziskave so pokazale sinergistični protitumorski učinek fluorouracila (5-FU), levkovorina (LV) ter 5-FU in rekombinantnega interferona alfa-2a (IFN). V II. fazi klinične raziskave smo želeli pri bolnikih z metastatskim rakom širokega črevesa ugotoviti toksičnost in učinkovitost zdravljenja s 5-FU, LV in IFN. V raziskavo smo vključili 22 bolnikov z merljivimi zasevki metastaskega raka širokega črevesa. 5-FU smo dajali v 6-urni intravenski infuziji v dnevnem odmerku 600 mg/m^2, LV intravensko v dnevnem odmerku 20mg/m^2 in IFN podkožno v dnevnem odmerku 6 MIE. U_inkovine smo dajali 5 dni zapored vsake 4 tedne. Srednja starost bolnikov je bila 60 let. Srednje stanje zmogljivosti (ECOG) je bilo ocenjeno z 1 (rang 0-2). Dvanajst bolnikov je imelo zasevke v jetrih, 5 v mehkih tkivih in 8 v pljučih. Zasevke v enem organu je imelo 19 bolnikov, v dveh organih 2 bolnika in v 3 organih eden. Bolniki so imeli 2 do 9 ciklov zdravljenja, v povprečju 5 ciklov. Objektivni odgovor na zdravljenje smo ugotovili pri 7 bolnikih (32%), mirovanje bolezni pri 7 bolnikih (32%). Srednje preživetje je bilo 12,5 meseca, pri bolnikih, ki so odgovorili na zdravljenje pa 14,4 meseca. Odgovori na zdravljenje so bili kratkotrajni in so v povprečju trajali 5,5 meseca. Med neželenimi učinki zdravljenja smo gripozni sindrom ugotovili pri 50%, slabost in bruhanje pri 36%, drisko pri 13%, stomatitis pri 27% in levkopenijo pri 13% bolnikov. Kombinirano zdravljenje s 5-FU, LV in IFN glede na rezultate dosedanjih kliničnih raziskav s 5-FU in LV ali 5-FU in IFN ni bolj učinkovit način zdravljenja.

Radiol Oncol 1998; 32(4): 417-21.

Obsevalne doze na ščitnico pri stereotaksični radiokirurgiji možgan

Sibata CH, de Almeida CE, Shin K

Izhodišča in namen. Radiokirurgija je obsevalna tehnika, kjer majhen, stereotaktično lokaliziran volumen obsevamo z eno ali več visokodoznimi frakcijami. Roswell park Cancer Institute trenutno uporablja tehniko s 6 nekoplanarnimi loki ter 6 MeV fotonskim linearnim pospeševalnikom. Ena izmed tem, ki so bile nedavno obravnavane v BEIR Report V, je radiogenski nastanek solidnih tumorjev in tveganje, povezano z zunanjim obsevanjem. Opisujejo stalno povečevanje ščitničnega raka v številnih obsevanih populacijah. Ščitnica ima pri otrocih mlajših od 15 let enega najvišjih koeficientov tveganja med vsemi organi in je edino tkivo s prepričljivimi dokazi za tveganje pri dozi 10 cGy. Ker so pri otrocih različnih starosti lahko prisotna tudi klinična stanja, ki zahtevajo radiokirurgijo, smo opravili raziskavo, da bi določili dozo na ščitnico in bližnje organe.
Material in metode. Opravili smo meritve na pacientih *in vivo*, pri čemer smo uporabljali termoluminiscenčni dozimeter (TLD) in diode na površini bolnikovega vratu, ki je ustrezala legi ščitnice, ravno tako pa tudi meritve na Aldersonovem fantomu s podobno geometrijo na globini 0,6 cm v enaki legi.
Rezultati: Izmerjene ščitnične doze v velikosti 10 cGy so v osnovi neodvisne od velikosti konusa, ker se značilne doze za zdravljenje arteriovenoznih malformacij povečajo z zmanjševanjem velikosti konusa.
Zaključki. Svetujemo eliminiranje ali premik terapevtskega loka, katerega projekcija gre skozi ščitnico. Ta postopek moramo uporabljati pri otrocih do starosi 5 let in jo zelo priporočamo do starosti 15 let.

Radiol Oncol 1998; 32(4): 423-7.

Ocena izvajanja telemedicinskih postopkov v državah na prehodu

Boko H

Različni načini uporabe prvotne zamisli, kako premostiti časovne in prosotorske prepreke in tako zagotoviti zadovoljivo raven fizične diagnoze in medicinske prognoze, se tako naglo množijo, da s telemedicino povezani postopki sodijo v najhitreje razvijajoče se področje na tržišču, ki pokriva zdravtsvu spremljajoče dejavnosti. Telemedicina ne pomeni samo uporabe telekomunikacijskih sredstev, ki so koristen pripomoček pri nudenju zdravničke pomoči, temveč odigrava pomembno družbeno vlogo pri premagovanju raznih problemov, ki se porajajo ob povpraševanju po za vse enako dobrih in primernih zdravstvenih uslugah. Uspešna zdravstvena politika omogoča višje blagostanje družbe zaradi boljših uspehov zdravljenja, večjo pravičnost, večje zadovoljstvo uporabnikov zdravstvenih uslug in na sploh manšje stroške, kot bi bili sicer brez sodelovanja javnega sektorja. Poslovna sposobnost, tehnološki know-how ter učinkovit prenos tehnologij so osnovne zahteve, ki jih je treba izpolniti za uspešno izvajanje zdravstvenih uslug. Na žalost prav v deželah v razvoju takšnih usposobljenosti najbolj primankuje. Na izvajanje nekaterih telemedicinskih postopkov v praksi močno vpliva raven družbene razvitosti. Zato bi bilo potrebno oceniti izvajanje teh postopkov v državah na prehodu, da bi lažje pospešili usposabljanje, različno opredelili ponudnike zdravstvenih uslug, posvetili večjo pozornost razmerju med stroški in koristmi, in se preusmerili k učinkovitejšemu in cenejšemu zdravstvu ter obenem še naprej gojili potencijale za ohranitev učinkovitih vrednot prejšnjega zdravstvenga sistema, to je pokrivanje širokega obsega prebivalstva.

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

Radiosurgery

February 24-27, 1999.

The 4th Congress of International Radiosurgery Society will be held in Sydney, Australia.

Contact Congress Secretariat, Conference Action Pty Ltd., PO Box 1231, North Sydney NSW 2059, Australia; or call +61 2 9956 8333; fax +61 2 9956 5154; E-mail: confact@real.com.au

Gynecological oncology

May 8-13, 1999

The 11th International Meeting of Gynecological Oncology will take place in Budapest, Hungary

Contact Prof. Dr. Péter Bõsze, 1301 Budapest, PO. Box 46, Hungary, fax + (36 1) 275 2172

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The UK's Premier Radiological Meeting will be held in Birmingham, United Kingdom

Contact Radiology 1999 Secretariat, 36 Portland Place, London, WIN 4AT, UK, tel + 44 (0) 171 307 1410; or fax + 44 (0) 171 307 1414

Lymphoma

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Contact the ESMO Head office, Via Soldino 22, 6900 Lugano, Switzerland, or call +41 91 950 07 86; or fax + 41 91 959 07 87; E-mail: esmosecr@dial.eunet.ch

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

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Oncology

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Haematology and oncology

October 9-13, 1999.

The Annual Meeting of German and Austrian Association of Haematology and Oncology will be offered in Jena, Germany.

Contact Dr.G.H. Sayer, Klinikum der Friedrich-Schiller-Universitaet Jena, Klinik fuer Innere Medizin II, 07747 Jena, Germany; or call +49 3641 639100; or fax +49 3641 639219; e-mail: HSAY@polkim.med.uni-jena.de; internet: http/www.weimar-cs.de/kuk/dgho.htm

Lung cancer

September 11-15, 2000.

The "9th World Conference on Lung Cancer" will be offered in Tokyo, Japan.

Contact Dr. Yoshihiro Hayata, The 9th World Conference on Lung Cancer, Tokyo Medical College Cancer Center, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan; or fax +81 3 3342 0893

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Editors greatly appereciate the work of the reviewers who siginificantly contributed to the improved quality of our journal.

Radiol Oncol 1998; 32(4): 435-7.

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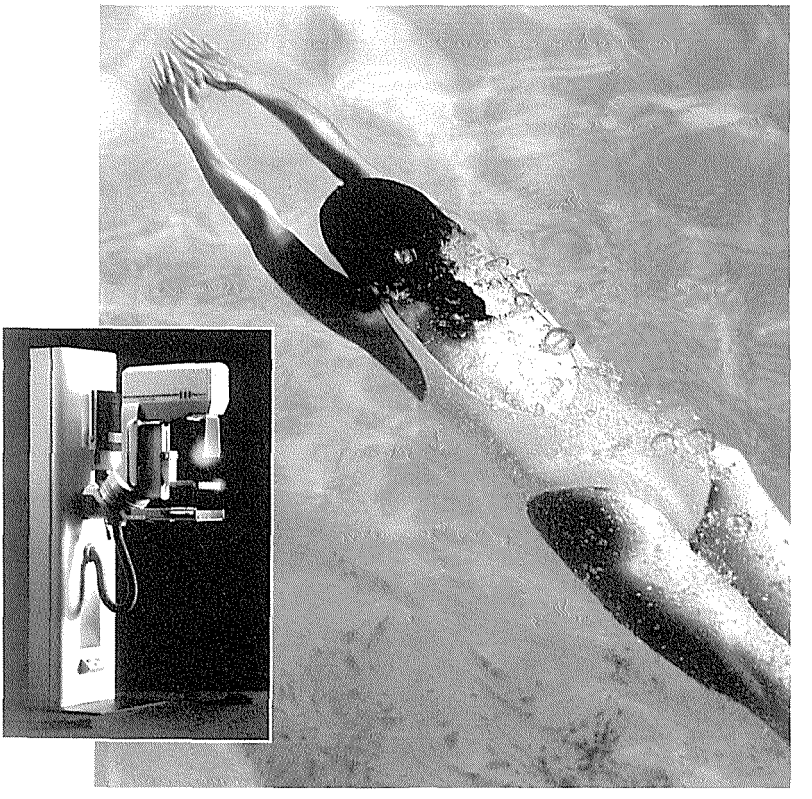

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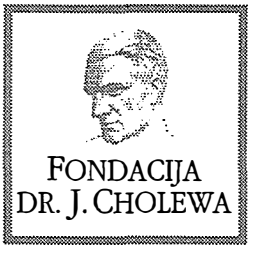

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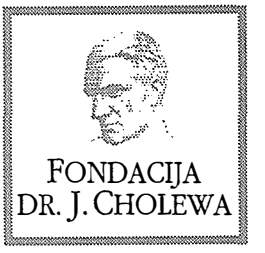

Activity of "Dr. J. Cholewa" foundation for cancer research and education - report for the final quarter of 1998

In the final quarter of 1998 the "Dr. J. Cholewa" foundation for cancer research and education continued with its activities. The Foundation supported the organisation of the already traditional Hepatobiliary School in Ljubljana in the end of June 1998, and the Central European Congress of Oncology, held in Opatija, Croatia, organised jointly by the oncologists from the Republic of Croatia and Republic of Slovenia and their respective professional associations.

The Foundation continues to support regular publication of "Radiology and Oncology" international scientific journal, and the regular publication of the "Challenge ESO Newsletter", the newsletter of the European school of Oncology, both being published and edited in Ljubljana, Slovenia.

For the whole of 1998 the Foundation plans to continue to provide grants for the various European School of Oncology courses, research and educational grants and to provide support for educational and scientific meetings, and to support publishing and editorial activity from the various fields of oncology in Slovenia. New applicants have been invited to submit their proposals, that are being evaluated, with the results expected to be announced shortly.

"Dr. J. Cholewa" Foundation for Cancer Research and Education thus continues to pursue its stated goals, as defined by its statute and meetings of the Board of directors and the Assembly of the Foundation at the end of 1997 and during the 1998. It continues to cooperate with similar institutions in Slovenia and abroad. Several steps will have been taken to further consolidate the Foundation financially. The Foundation will strive to project its positive accomplishments and image in the public.

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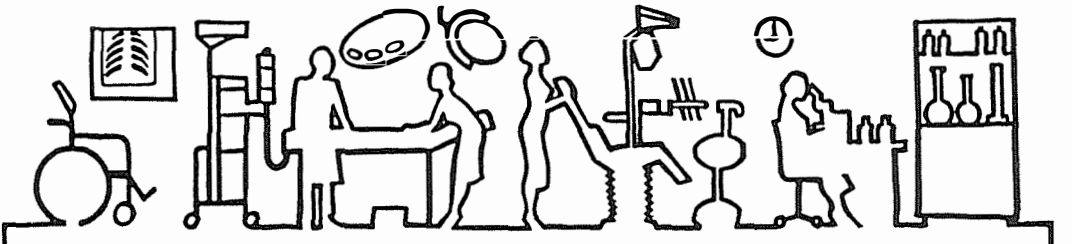

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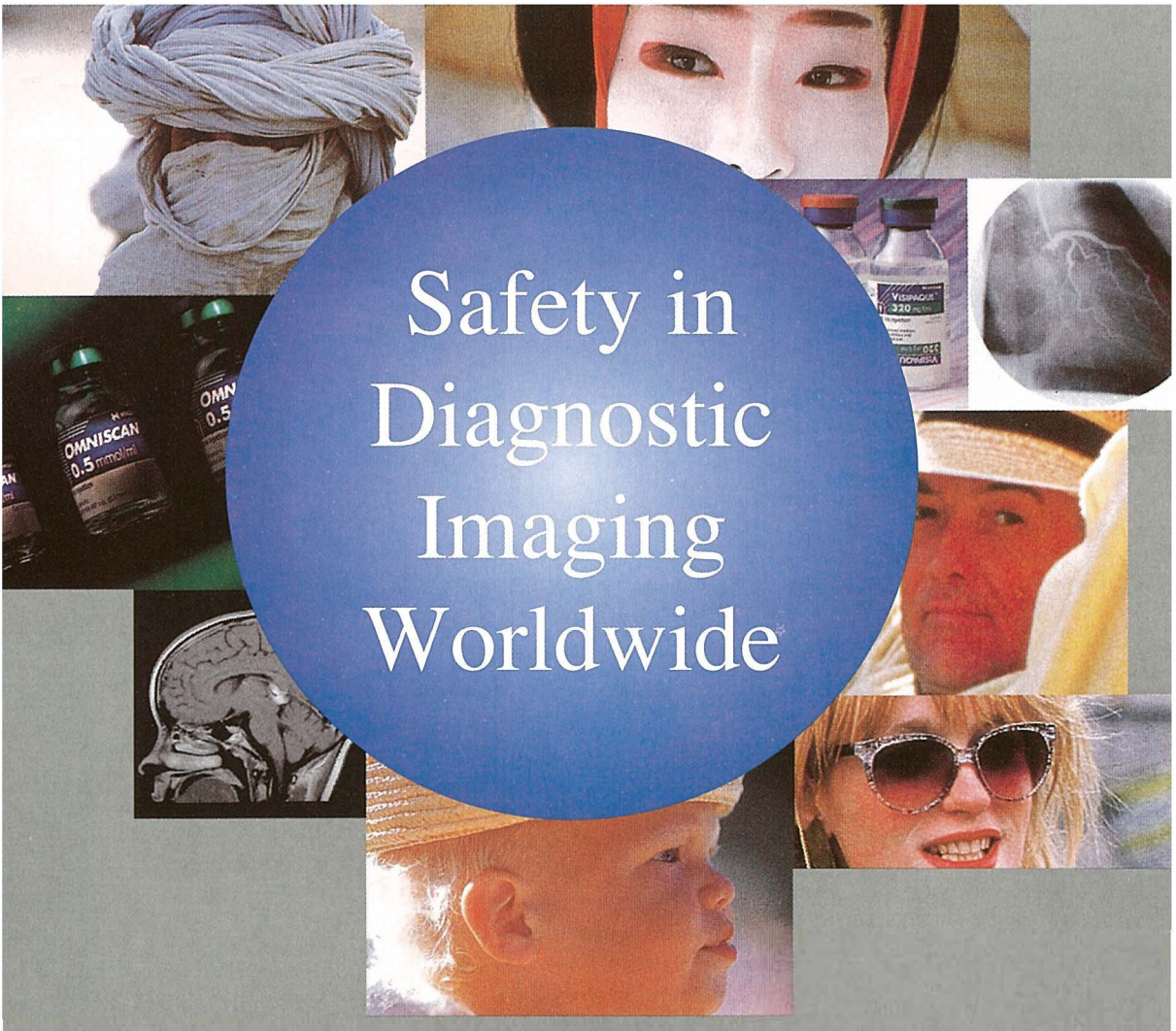

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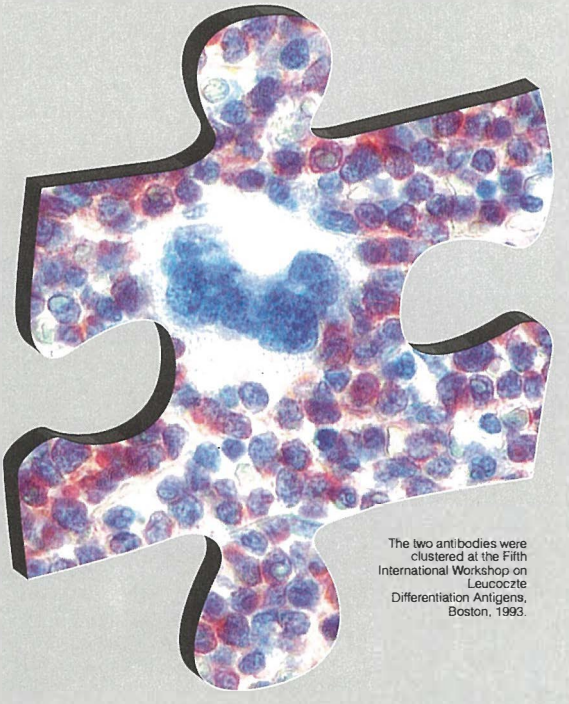

The two antibodies were clustered at the Fifth International Workshop on Leucoczte Differentiation Antigens, Boston, 1993.

Antibodies available:

Monoclonal Mouse Anti-Human B Cell, CD79a, clone JCB117

Monoclonal Mouse Anti-Human B Cell, CD79a, clone HM57

- **Highly specific for B cells at all stages of maturation**
- **Well documented antibodies**
- **Important new addition to the range of DAKO B and T Cell Markers**
- **Unique products, only from DAKO**
- **Available as two products from the clones JCB117 and HM57**
- **Applicable on DAKO TechMate™ immunostainers**
- **Work well on formalin-fixed, paraffin-embedded material**

DAKO - Your Reference in Immunohistochemistry

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Sumamed®

azitromicin

TADOL® retard

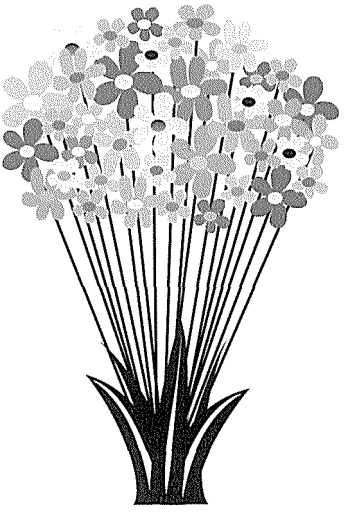
tablete s podaljšanim sproščanjem po 100 mg

tramadol

- *zadoščata samo 2 odmerka na dan*
- *za zdravljenje kronične bolečine in za nadaljevanje zdravljenja akutne bolečine*
- *v sožitju z bolnikom – preprosta uporaba – mirno spanje brez bolečin*

Skrajšano navodilo

Indikacije: Srednje hude do hude bolečine. **Odmerjanje:** Odrasli in otroci, starejši od 14 let, jemljejo 1 do 2 retardni tableti zjutraj in zvečer z malo tekočine. Dnevni odmerek naj ne bo večji od 400 mg (4 retardne tablete). Za lajšanje bolečin pri bolnikih z rakom in hudih bolečin po operacijah lahko odmerke povečamo do 600 mg na dan. **Kontraindikacije:** Preobčutljivost za tramadol, otroci, mlajši od 14 let, akutna zastrupitev z alkoholom, uspavali, analgetiki in drugimi zdravili, ki vplivajo na CŽS, zdravljenje z zaviralci MAO. **Interakcije:** Pri sočasnem jemanju zdravil, ki delujejo na osrednje živčevje, lahko pride do sinergističnega delovanja v obliki sedacije ali do močnejšega analgetičnega delovanja. **Opozorila:** Pri predoziranju lahko pride do dihalne depresije. Previdnost je potrebna pri bolnikih, ki so preobčutljivi za opiate, in pri starejših osebah. Bolnike s krči centralnega izvora moramo skrbno nadzorovati. Pri jetrni in ledvični okvari je treba odmerek zmanjšati oz. presledek med posameznimi odmerki individualno prilagoditi. Bolniki med zdravljenjem ne smejo upravljati strojev in motornih vozil. **Nosečnost in dojenje:** Nosečnicam predpišemo tramadol le pri nujni indikaciji, enako v obdobju laktacije, saj 0,1 % zdravila preide v materino mleko. Zaradi enkratnega odmerka Tadola retard praviloma ni treba prenehati dojiti. **Stranski učinki:** Znojenje, vrtoglavica, slabost, bruhanje, zaprtje, suha usta, utrujenost. Redko pride do palpitacij, ortostatske hipotenzije ali kardiovaskularnega kolapsa. Izjemoma se lahko pojavijo konvulzije. **Oprema in način izdajanja:** 30 retardnih tablet po 100 mg, na zdravniški recept. 10/98.



Podrobnejše informacije so na voljo pri proizvajalcu.

KRKA

Krka, d. d., Novo mesto
Šmarješka cesta 6
8501 Novo mesto

Zdravljenje pomenopavzalnih bolnic z napredovalo ali ponovljeno obliko raka dojk je bolj uspešno z novim selektivnim zaviralcem aromataz :

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anastrozol

- odmerjanje je enostavno, bolnica vzame eno tableto Arimidexa enkrat dnevno
- koncentracije estradiola se hitro zmanjšajo do meje merljivosti
- Arimidex se dobro prenaša, prekinitve zdravljenja so redke
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- nadomestno zdravljenje s steroidi ni potrebno

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Manuscript written in English should be submitted to the Editorial Office in triplicate (the original and two copies), including the illustrations: *Radiology and Oncology*, Institute of Oncology, Vrazov trg 4, SI-1000 Ljubljana, Slovenia; (Phone: +386 61 132 00 68, Tel./Fax: +386 61 133 74 10, E-mail: gsersa@onko-i.si). Authors are also asked to submit their manuscripts on a 3.5" 1.44 Mb formatted diskette. The type of computer and word-processing package should be specified (Word for Windows is preferred).

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General instructions• Radiology and Oncology will consider manuscripts prepared according to the Vancouver Agreement (*N Engl J Med* 1991; **324:** 424-8, *BMJ* 1991; **302:** 6772; *JAMA* 1997; **277:** 927-34.). Type the manuscript double spaced on one side with a 4 cm margin at the top and left hand side of the sheet. Write the paper in grammatically and stylistically correct language. Avoid abbreviations unless previously explained. The technical data should conform to the SI system. The manuscript, including the references may not exceed 15 typewritten pages, and the number of figures and tables is limited to 4. If appropriate, organize the text so that it includes: Introduction, Material and Methods, Results and Discussion. Exceptionally, the results and discussion can be combined in a single section. Start each section on a new page, and number each page consecutively with Arabic numerals.

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Introduction should state the purpose of the article and summarize the rationale for the study or observation, citing only the essential references and stating the aim of the study.

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Results should be presented clearly and concisely without repeating the data in the tables and figures. Emphasis should be on clear and precise presentation of results and their significance in relation to the aim of the investigation.

Discussion should explain the results rather than simply repeating them and interpret their significance and draw conclusions. It should review the results of the study in the light of previously published work.

Illustrations and tables must be numbered and referred to in the text, with appropriate location indicated in the text margin. Illustrations must be labelled on the back with the author's name, figure number and orientation, and should be accompanied by a descriptive legend on a separate page. Line drawings should be supplied in a form suitable for high-quality reproduction. Photographs should be glossy prints of high quality with as much contrast as the subject allows. They should be cropped as close as possible to the area of interest. In photographs mask the identities of the patients. Tables should be typed double spaced, with descriptive title and, if appropriate, units of numerical measurements included in column heading.

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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.

Chapman S, Nakielny R. *A guide to radiological procedures.* London: Bailliere Tindall; 1986.

Evans R, Alexander P. Mechanisms of extracellular killing of nucleated mammalian cells by macrophages. In: Nelson DS, editor. *Immunobiology of macrophage.* New York: Academic Press; 1976. p. 45-74.

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Navoban
tropisteron

- **preprečevanje slabosti in bruhanja pri emetogeni kemoterapiji**
- **učinkovito zdravilo, ki ga odrasli in otroci dobro prenašajo**
- **vedno 1-krat na dan**
- **vedno 5 mg**

Skrajšano navodilo za uporabo: Navoban® kapsule, Navoban® raztopina za injiciranje 2 mg in 5 mg. Serotoninski antagonist. **Oblika in sestava:** 1 trda kapsula vsebuje 5 mg tropisetronovega hidroklorida. 1 ampula po 2 ml vsebuje 2 mg tropisetronovega hidroklorida. 1 ampula po 5 ml vsebuje 5 mg tropisetronovega hidroklorida. **Indikacije:** Preprečevanje slabosti in bruhanja, ki sta posledici zdravljenja s citostatiki. Zdravljenje pooperativne slabosti in bruhanja. Preprečevanje pooperativne slabosti in bruhanja pri bolnicah, pri katerih je načrtovana ginekološka operacija v trebušni votlini. **Odmerjanje in uporaba:** Preprečevanje slabosti in bruhanja, ki sta posledici zdravljenja s citostatiki. **Odmerjanje pri otrocih:** Otroci starejši od 2 let 0,2 mg/kg telesne mase na dan. Največji dnevni odmerek ne sme preseči 5 mg. Prvi dan kot intravenska infuzija ali kot počasna intravenska injekcija. Od 2. do 6. dne naj otrok jemlje zdravilo oralno (raztopino v ampuli razredčimo s pomarančnim sokom ali koka kolo). **Odmerjanje pri odraslih:** 6-dnevna kura po 5 mg na dan. Prvi dan kot intravenska infuzija ali počasna intravenska injekcija. Od 2. do 6. dne 1 kapsula na dan. **Zdravljenje in preprečevanje pooperativne slabosti in bruhanja: Odmerjanje pri odraslih:** 2 mg Navobana z intravensko infuzijo ali kot počasno injekcijo. Glej celotno navodilo! **Kontraindikacije:** Preobčutljivost za tropisetron, druge antagoniste receptorjev 5-HT3 ali katerokoli sestavino zdravila. Navobana ne smemo dajati nosečnicam; izjema je preprečevanje pooperativne slabosti in bruhanja pri kirurških posegih, katerih del je tudi terapevtska prekinitev nosečnosti. **Previdnostni ukrepi:** Bolniki z nenadzorovano hipertenzijo; bolniki s prevodnimi ali drugimi motnjami srčnega ritma; ženske, ki dojijo; bolniki, ki upravljajo s stroji ali vozili. **Medsebojno delovanje zdravil**: Rifampicin ali druga zdravila, ki inducirajo jetrne encime. Glej celotno navodilo! **Stranski učinki:** Glavobol, zaprtje, redkeje omotica, utrujenost in prebavne motnje (bolečine v trebuhu in driska), preobčutljivostne reakcije. Zelo redko kolaps, sinkopa ali zastoj srca, vendar vzročna zveza z Navobanom ni bila dokazana. **Način izdajanja:** *Kapsule:* uporaba samo v bolnišnicah, izjemoma se izdaja na zdravniški recept pri nadaljevanju zdravljenja na domu ob odpustu iz bolnišnice in nadaljnjem zdravljenju. *Ampule:* uporaba samo v bolnišnicah. **Oprema in odločba:** Zložena s 5 kapsulami po 5 mg; številka odločbe 512/B-773/97 z dne 10. 11. 1997. Zložena z 1 ampulo po 2 ml (2 mg/2 ml); številka odločbe 512/B-772/97 z dne 10. 11. 1997. Zloženka z 10 ampulami po 5 ml (5 mg/5 ml); številka odločbe 512/B-771/97 z dne 10. 11. 1997. **Izdelovalec:** NOVARTIS PHARMA AG, Basel, Švica. **Imetnik dovoljenja za promet z zdravilom: NOVARTIS PHARMA SERVICES INC., Podružnica v Sloveniji, Dunajska 22, 1511 Ljubljana, kjer so na voljo informacije in literatura.**
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