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Three Dimensional Ultrasound and Power Doppler in Assessment of Uterine and Ovarian Angiogenesis: a Prospective Study

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Aim. To determine whether three-dimensional power Doppler can improve the recognition of pelvic tumor morphology and angiogenesis.
Methods. Using this technique we analyzed 180 adnexal masses and 110 uterine lesions. Tumor volume, morphology, and vascularity were evaluated in each patient. Irregular and randomly dispersed vessels with complex branching depicted by comprehensive three dimensional display were suggestive of pelvic malignancy, while linear-like vascular morphology, single vessel arrangement and regular branching were typical for benign structures.
Results. Addition of qualitative analysis of vascular architecture of adnexal tumor to morphological parameters reached 96.15% sensitivity and 98.73% specificity. When endometrial lesions were prospectively analyzed, sensitivity and specificity were 91.67% and 98.49%, respectively. Because the lowest positive predictive value of 16.67% was obtained for myometrial lesions, this method should not be advised for their evaluation.
Conclusion. Good results achieved by three dimensional ultrasound can be explained by improved recognition of the pelvic lesion anatomy, characterization of the surface features, detection of the tumor infiltration, and precise depiction of the size and volume. Three dimensional power Doppler imaging can detect structural abnormalities of the malignant tumor vessels, such as arteriovenous shunts, microaneurysms, tumoral lakes, disproportional calibration, coiling, and dichotomous branching. Therefore it enhances and facilitates the morphologic and functional evaluation of both benign and malignant pelvic tumors.

Key words: adnexa uteri; angiography; angiogenesis, physiologic; neovascularization, physiologic; ovary; vasodilatation; ultrasonography, Doppler, color; ultrasonography, prenatal

It has been over 100 years now since the first observation that tumors had an increased vascu- larity compared to normal tissues (1). It was long believed that simple dilatation of existing host blood vessels accounted for this tumor hyperemia (2). Vasodilatation was generally thought to be a side-effect of tumor metabolites or of necrotic tumor products escaping from the tumor.
The new concept that tumor growth is restricted in the absence of the vascular response was developed in the 1960s. Experiments with isolated perfused organs revealed that tumor growth was severely restricted in these organs because of the absence of neovascularization (3-6). This process was called angiogenesis, a term coined in 1935 by Hertig to describe the proliferation of new vessels in the placenta (7). Viable tumor cells release diffusible angiogenic factors, which stimulate new capillary growth and endothelial mitosis in vivo (8-10), even when tumor cell proliferation has been arrested by irradiation (11). From these observations, Folkman (10) proposed a hypothesis that once tumor take occurs, every further increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor (10).
Since Folkmans's hypothesis, for over 25 years it has been clear that the development of new blood vessels is necessary to sustain the growth, invasion, and metastasis of tumors (12-14). Angiogenesis is crucial for sustaining tumor growth, as it allows oxygenation and nutrient perfusion of the tumor and removal of waste products. Moreover, increased angiogenesis coincides with increased tumor cell entry into the circulation, and thus facilitates metastasis (15,16). Cancer cells activate quiescent vasculature to produce new blood vessels via an ”angiogenetic switch”, often during the premalignant stages of tumor development.
The multitude of data suggesting that the control of angiogenesis is separate from the control of cancer cell proliferation raise the possibility that drugs inhibiting angiogenesis could offer a treatment complementary to traditional chemotherapy, which directly targets tumor cells (12,14,17). This exciting possibility has stimulated research on tumor angiogenesis and introduction of new three dimensional power Doppler evaluation of tumor vessels architecture (18-22).

Methods

Between January 1997 and January 1999, we prospectively evaluated 180 adnexal masses and 110 uterine lesions by three dimensional ultrasound with power Doppler facilities. Hundred and fifty women studied were premenopausal (mean age 35, range 18-49 years), 15 women were perimenopausal (mean age 48, range 46-52 years), while 125 patients were postmenopausal (mean age 62, range 50-78 years).

Three dimensional studies of the adnexa and uterus were performed using a B mode scanner, which monitors spatial orientation of the images and stores these as a volume set in the memory of the computer (Voluson 530, Zipf, Austria). Once the region of interest was identified, volume box was superimposed, ultrasound probe was kept steady, and the patient was asked to lie still on the examination bed. The volume mode was switched on, and three dimensional ultrasound volume was generated by the automatic rotation of the mechanical transducer through 360º. The acquisition time ranged between 2 and 11 seconds, depending on the size of the volume box. Three perpendicular planes were displayed simultaneously, thus allowing better understanding of the pelvic lesion morphology. Evaluation of the stored volumes took between 15 and 25 minutes, depending on the number of slices, rotation angle and rendering modes used. Since the number and orientation of reformatted planes are not limited, meticulous evaluation of numerous sections through the studied structure becomes possible. When three perpendicular reformatted sections are displayed on the screen, longitudinal plane is chosen for volume measurements, while the other two planes are used to ensure that the entire tumor is included in the measurement. The actual measurement is performed by delineating the lesion in a number of parallel longitudinal sections 1-2 mm apart. The volume of the lesion is then automatically calculated by the in-built computer software program. From the stored image, in each case we obtained a plastic image of the lesion as well as surface rendering. Surface rendering mode allowed exploration of the outer and/or inner wall of the tumor (Fig. 1), whereas in patients with certain amount of free or intracavitary fluid we were able to evaluate the relationship with surrounding structures. Niche aspect allowed detection and analysis of the selected sections of the lesion such as papillary projections (Fig. 2), septa and solid parts. Application of ”transparent maximum/minimum” mode allowed visualization of the intratumoral calcification or identification of the bone structures in dermoid tumors.

Figure 1. Three-dimensional view of an unilocular ovarian cyst. Note regular borders and inner walls of the lesion.

Figure 2. Three-dimensional scan of an early stage of ovarian carcinoma. A papillary projection protruding into the cystic cavity is clearly demonstrated.

Figure 3. The same patient as in Figure 2. Randomly dispersed vessels with irregular course and branching are shown within the papilla, indicating the malignant nature of the ovarian tumor. Serous cystadenocarcinoma was confirmed by histopathology.

Figure 4. A three-dimensional power Doppler image of a simple ovarian cyst angiogenesis. Note regularly separated vessels at the periphery of the cystic lesion.

Figure 5. Chaotic vessel arrangement in a malignant ovarian neoplasm. At this stage of technological development the estimation of the vessel density by three-dimensional power Doppler is rather subjective. It is expected that the integrated software for calculation of the total tumor perfusion in three-dimensional perspective will overcome this problem. In addition, the use of contrast agents may facilitate the detection rate of tiny tumoral vessels and thus increase the sensitivity and specificity of the procedure.

At the end of each examination, combined angio- and rendering mode was used, allowing simultaneous analysis of the morphology, texture and vascularization.

Comprehensive 3-D display allowed interactive analysis of the tumor microcirculation architecture: irregular and randomly dispersed vessels with complex branching were suggestive of ovarian malignancy (Fig. 3), whereas linear-like vascular morphology and single vessel arrangement were typical for benign structures (Fig. 4).

Morphological and vascular criteria analyzed by three dimensional ultrasound and power Doppler for diagnosing adnexal and uterine malignancy are listed in Tables 1 and 2.

Table 1. Three-dimensional sonographic and power Doppler criteria for the diagnosis of ovarian malignancy

Table 2. Three dimensional sonographic and power Doppler criteria for the diagnosis of endometrial malignancy

Table 3. Histopathological diagnoses in 180 adnexal lesions

All the cases were operated on by the same surgical team (laparotomy, laparoscopy, or hysteroscopy) and histo- pathological diagnosis was considered final. Malignant tumors were classified according to the International Federation of Gynecology and Obstetrics (FIGO) system. The study protocol was approved by the hospital's ethical committee, and all the patients consented to participate in the study.

Results

Hundred and fifty five patients had benign adnexal conditions, while 25 patients had malignant adnexal tumors. Table 3 reviews histopathological diagnosis of the adnexal conditions.

The most common ovarian lesion during the premenopausal period was endometrioma (41/ 150), whereas the most common ovarian tumor during the postmenopausal period was serous cystadenoma (33/125). The most common ovarian malignancy was serous cystadenocarcinoma detected in 15 postmenopausal patients, 1 perimenopausal, and 1 premenopausal patient. There was no significant difference of ovarian tumor volume between benign and malignant adnexal lesions (82.8±65.2 vs. 85.8±64.5 SD; $p>0.05$).

Three dimensional ultrasound allowed clear depiction of the cystic walls, and detection of wall proliferations. In 21 out of 25 adnexal malignancies (84.0%) we were able to obtain papillary protrusions, both in virtual images and surface rendering.

Power Doppler allowed identification of tiny irregular vessels within the wall proliferations (21/21), which was mandatory for differentiation between malignant and benign lesions (Fig. 5).

In 14 out of 25 adnexal malignancies (56.0%) we detected thick septa, whereas in 11 patients (44.0%) solid contenparts with mixed echogenicity were identified. In each of these abnormal wall structures we found signs of neovascularization, characterized by irregular vessels with dichotomous branching.

In patients with advanced malignancy (N=11) we were able to analyze the surface of the tumors. Free fluid in cavum Douglasi permitted effective targeting and rendering of abnormal outer walls in the surface mode thus providing sonomorphologic evaluation of the capsule infiltration and tumor spread.

Table 4 shows sensitivity, specificity, positive and negative predictive values, as well as efficiency of 3-D ultrasound and power Doppler in differentiation of the ovarian lesions.

Table 4. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and efficiency (percent) of 3-D power Doppler ultrasound in the detection of ovarian, endometrial, and myometrial malignancy

Table 5. Volume and vascularity of the endometrial lesions (N=57) obtained by 3-D power Doppler

In most of the patients with benign lesions (119/121), we detected vessels with regular branching, usually encircling the cyst or demonstrating single vessel arrangement. In only two patients with benign lesions (one with serous cystadenoma and one with fibrocystadenoma) the course of tumor vessels was irregular, giving an impression of a malignant tumor. However, in 24 patients with malignant adnexal growths we identified irregular vessels with complicated branching. In only one patient with early stage of serous cystadenocarcinoma we found regularly separated intraseptal vessels, indicative of a benign lesion.

The endometrial volume in patients with normal and/or atrophic endometrium, hyperplasia, polyps, and carcinoma is demonstrated in Table 5. In patients with endometrial carcinoma, mean endometrial volume was 37.0±31.8 mL SD. The endometrial volume in hyperplasia had the mean value of 7.82±7.60 mL SD and was significantly higher than the volume in patients with polyps (mean 2.63±2.12 mL). In patients with atrophic or normal endometrium the mean volume was 0.8±1.51 mL).

Subendometrial hallo was regular in all patients with benign endometrial lesions, whereas 8 out of 12 patients with endometrial carcinoma had irregular endometrial-myometrial border. Intracavitary fluid was present in 4 patients with benign endometrial lesions and in 5 patients with endometrial malignancy. Dichotomous branching and randomly dispersed vessels were detected in 91.67% of the

patients with endometrial carcinoma, whereas only one patient with necrotic polyp presented similar vascular geometry. Remaining patients with benign endometrial pathology presented single vessel arrangement and regular branching. Diagnostic reliability of 3-D power Doppler ultrasound in detection of endometrial malignancy is presented in Table 4.

Thirty three patients with myometrial lesions were enrolled in this prospective evaluation: 32 patients with uterine leiomyoma and a single patient with uterine leiomyosarcoma. The mean volume of the leiomyomas undergoing surgery was 78.52± 51.8 mL). In the majority of these lesions (27/32; 84.38%), 3-D power Doppler revealed regular vascular network at the periphery, while findings suggestive of neovascularity due to irregular branching and chaotic vascular arrangement were detected in five secondary degenerative lesions. In the patient with uterine leiomyosarcoma we were able to detect enlarged volume of the tumor (97.2 mL). Tumoral perfusion was characterized by irregular vessels that were randomly dispersed both in the central and peripheral parts of the tumor. The diameters of these vessels were ”uneven”, with numerous microaneurysms and stenosis. Diagnostic reliability of 3-D power Doppler ultrasound in detection of the myometrial growths is shown in Table 4.

Discussion

Angiogenesis is the process of generation of capillary blood vessels, which leads to neovas- cularization. It occurs during embryonic development and during several physiological and pathological conditions in adult life. For example, ovulation and wound healing could not take place without angiogenesis. Angiogenesis is also associated with chronic inflammation and with certain immune reactions. Many non-malignant diseases of unknown cause are dominated by angiogenesis. For example, neovascularization associated with retro- lental fibroplasia or diabetic retinopathy may lead to blindness, and new capillaries may invade joints in arthritis. Solid tumors induce angiogenesis, but tumor angiogenesis differs at least in its temporal manner from the other types of angiogenesis described. In physiological situations, such as during the development of the corpus luteum or during ovulation, angiogenesis subsides or is turned off once the process is completed. In certain non-malignant processes, angiogenesis is abnormally prolonged, although still self-limiting, such as in pyogenic granuloma or keloid formation. In contrast, tumor angiogenesis is not self-limiting. Once tumor-induced angiogenesis starts, it continues indefinitely until the host dies or the tumor is eradicated (3-6).

Recent evidence suggests that the development of metastasis also depends on angiogenesis. Before vascularization, tumors are generally unable to shed cells into the circulation. Tumor cells must gain access to the vasculature in the primary tumor; survive the circulation; stop in the microvasculature of the target organ (23,24); exit from this vasculature (25); grow in the target organ; and induce angiogenesis (26). Angiogenesis is necessary at the beginning as well as at the end of this cascade of events. Tumor cells can enter the circulation by penetrating through the proliferating capillaries. Growing capillaries have fragmented basement membranes and are leaky (27).

Angiogenesis is necessary but not sufficient for continued tumor growth (8). Whereas the absence of angiogenesis will severely limit tumor growth, the onset of angiogenic activity in a tumor permits, but does not guarantee, continued expansion of the tumor population (28). Leukemic cells and tumor cells that grow in the ascites are not dependent upon angiogenesis, because they neither form a ”solid” tumor nor grow in a three dimensional tightly packed cell population. Angiogenesis may not be necessary for certain tumor cells capable of growth as a flat sheet between membranes, i.e., gliomatosis in the meninges (8).

Tumor-induced vessels are often dilated and saccular, and may even contain tumor cells within the endothelial lining (29). Tumor microvas- culature does not conform to the structure of the vasculature of normal tissues (e.g., artery to arteriole to capillary to postcapillary venule to venule to vein) (29). Tumors may contain giant capillaries and arteriovenous shunts without intervening capillaries. Newly formed vessels contain no smooth muscle in their walls, but instead contain only a small amount of fibrous connective tissue (30). Quantitative morphometric studies in induced animal tumors show that vascular volume, length and surface area increase during the early stages of tumor growth, and decrease after the onset of necrosis. The number of vessels of large diameter increases in the later stages of growth (31).

Indeed, the evidence for the regulatory role of angiogenesis in tumor growth is strong but it is still not clear what part the phenomenon does play in the process of cancer metastasis (32-34).

Sonomorphological evaluation of ovarian tumors using parameters such as presence of papillary protrusions, solid parts, thick septa, and high echogenic reflection patterns is useful in assessing the risk of ovarian malignancy (35). Our results indicate that 3-D ultrasound scanning allows reduction of the false positive findings by detailed investigation of the ovarian lesion. This technique is especially useful in evaluation of the complex ovarian lesions such as ovarian dermoids, endo- metriomas, and

fibromas, which may give wrong impression of malignancy when using conventional transvaginal sonography and color Doppler ultrasound (36,37). Multiple sections of the tumor, rotation, translation, and reconstruction of 3-D plastic images allowed more precise evaluation of the tumor without increasing scanning time and patients' discomfort.

Obvious advantages of three-dimensional ultrasound are improved recognition of the ovarian lesion anatomy, accurate characterization of the surface features, determination of the extent of tumor infiltration through the capsule, and clear depiction of the size and volume of the mass. The volume of the adnexal lesion was not significantly different in benign and malignant adnexal lesions.

Angiogenesis is a common phenomenon in malignant ovarian neoplasms, but the intensity of neovascularization may depend on the characteristics of the individual tumor (38). Therefore, an incremental decrease of the impedance indices in the vessels of adnexal tumors may reflect the increase in angiogenesis and be an indication of the tumor's malignant potential (39). Animal models have shown that angiogenesis can be detected by Doppler ultrasound even in a small volume of malignant tumor (25 mg) (40). This suggested that angiogenesis might be detected with current color Doppler ultrasound equipment even when the carcinoma is confined within the ovarian capsule or when it exhibits low malignant potential. Indeed, the study of several series has shown that color and pulsed Doppler sonography can depict ovarian carcinoma at stage I (41-43). One study detected two of 18 stage-I ovarian carcinomas solely by the presence of an abnormal blood flow pattern in normal-sized ovaries (42), whereas another study found three of 17 stage I cancers on the basis of abnormal blood flow (43). However, in the latter study, two stage I tumors did not demonstrate flow and both were >15 mm in size. It could be argued that these undiscovered malignant tumors had a low potential to induce an angiogenic response, or that the blood vessels were so small that they were impossible to detect with current color and pulsed Doppler equipment.

The newly developed power or energy modes of color Doppler imaging permits the depiction of even smaller vessels, but, paradoxically, small intraparenchymal arterioles in benign and normal tissues may show a low impedance and low-velocity blood flow pattern, giving rise to false-positive results. Nevertheless, the tendency towards progressive decrease in the vascular impedance from benign lesions to borderline, early, and advanced malignancies have been reported (39). This observation was supported circumstantially by an in vivo study which showed that there was a rise in vascularity with tumor progression in the melanocystic system, demonstrated by histopathology (44). This in vivo information is in agreement with the concept that an increased vascular supply may facilitate tumorigenesis and aggressive biological behavior in a neoplastic system.

Since the introduction of transvaginal color Doppler sonography for the assessment of ovarian vascularity (41,45), attitudes concerning its usefulness in the detection of adnexal malignancies have been equally divided. The majority of the published studies on this subject agree that malignant ovarian tumors, in comparison with benign tumors, have characteristic blood flow features. The diagnostic accuracy of impedance values in differentiating benign from malignant lesions has varied considerably, from over 96% to less than 40%. Statistical analyses of the data from various reports are confounded by non-universal selection of Doppler parameters (resistance vs. pulsatility index), methodological errors, operator's experience, and system sensitivity (46). It is clear that there is a need for further improvement in the ultrasonic assessment of pelvic tumor angiogenesis and, to this end, there has been a growing interest in three-dimensional power Doppler ultrasound. This method accurately detects characteristic structural abnormalities of the malignant tumor vessels such as microaneurysms, arteriovenous shunts, tumoral lakes, disproportional calibration, elongation, coiling, and dichotomous branching. Our study demonstrated that a qualitative analysis of the adnexal tumor vascularity architecture added to morphological parameters is clinically pertinent, reaching sensitivity and specificity of 96.15% and 98.73%, respectively.

The endometrial volume in patients with malignant pathology was significantly different from those who had hyperplasia, polyps, or normal endometrium. With combined morphological and power Doppler evaluation, the diagnosis of endometrial carcinoma was made with a sensitivity of 91.67%. There was only one false positive result in a patient with endometrial hyperplasia and one false negative result obtained in a patient with endometrial carcinoma receiving Tamoxifen therapy. In the latter patient, an endometrial volume of 21.02 mL with regularly separated peripheral vessels was identified.

Our results are similar to those of Jurkovic et al (47), who reported that endometrial volume was significantly different in patients with endometrial carcinoma compared to those who had benign lesions. With cut-off value of 13 mL in the diagnosis of endometrial malignancy these authors reached a sensitivity of 100%. There was only one false positive result in a patient with endometrial hyperplasia, which gave a specificity of 98.8% and positive predictive value of 91.7%.

Efficiency of 3-D power Doppler seems to be the lowest in the evaluation of myometrial lesions, since

necrosis, inflammation, and degeneration alter the leiomyoma vasculature. Although we did not detect any false negative finding, we should be aware of small numbers included in this study, which limit the applicability of the sensitivity, specificity, and positive and negative predictive values.

It seems to us that this technique has brought us a little closer to better understanding of malignant tumor angiogenesis. Three-dimensional display allows the physician to visualize many overlapping vessels easily and quickly, as well as to assess their relationship to other vessels or other surrounding tissues. It permits the ultrasonographer to view structures in three dimensions interactively, rather than having to assemble the sectional images in his/her mind. Interactive rotation of power Doppler rendered images provides improved visualization of the tumor vasculature. Malignant tumor vessels are usually randomly dispersed within the stroma and periphery, sometimes forming several tangles or coils around the surface.

Higher detection rate of small vessels after injection of contrast agents may allow application of the mathematical models assessing three-dimensional vascular chaos and fractals. Future 3-D power Doppler units with high resolution three- dimensional gray scale information and simultaneous Doppler shift spectrum analysis will probably increase the usefulness of this method.

The role of color Doppler sonography in the assessment of the architecture of tumor vascularity seems clinically pertinent, justifying expanded research on this topic. This opinion is based on the discovery and molecular characterization of a diverse family of regulators of angiogenesis, both stimulators and inhibitors (17). In the case of solid tumors, a shift in the balance of stimulators and inhibitors can trip the angiogenetic switch, allowing tumors to recruit new blood vessels from the surrounding host vasculature, which provides them with a survival advantage. This process appears to be necessary for tumors to grow beyond microscopic size. It is the ultimate goal of most researchers of tumor angiogenesis to find ways of turning off the angiogenetic switch, as a form of cancer treatment.

Boehm and colleagues (48) have shown that three different mouse tumors regressed after the treatment with repeated cycles of endostatin, a newly discovered angiogenesis inhibitor. The tumors did not become drug resistant and, after a characteristic number of treatment cycles, became dormant. Such a treatment strategy could help circumvent many of the problems associated with current chemotherapeutic regimens, such as acquired drug resistance, attributable to tumor cell genetic instability, or intrinsic resistance, due to poor penetration of certain drugs into the tumor parenchyma (49). The results of cyclic endostatin therapy strongly suggest that drugs targeting angiogenesis and the tumor vasculature will become a major new tool for effective treatment and possibly prevention of human cancer. As with any new treatment procedure, there are a number of important questions for the future. Indeed, a pure angiogenesis inhibitor would be expected to block new blood vessel growth, leaving quiescent blood vessels intact. Such an inhibitor should stop neovascularization in a tumor and effect a static, dormant state. The tumor should neither grow nor regress but should continue to be fed by its established vessels, remaining in a metastable state of proliferation balanced by apoptosis (50).

Another important question is whether there are tissue-specific differences in the vasculature and consequently in tumor vessel anatomy that affect a tumor's susceptibility to inhibition or disruption? In the future, we may use the integrated software for calculating the total extent of the vascularity of an entire mass in three dimensional perspective. Contrast agents are another possibility for enhancing the three-dimensional power Doppler examination by increasing the detection rate of small vessels. This study is now in progress at our Department.

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