REVIEW ARTICLE

Ultrasonic Characterization of Adnexal Masses

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Abstract

Adnexal masses have an etiological spectrum ranging from gynecologic to non-gynecologic causes. Because they can be benign or malignant, their evaluation has to include a careful analysis of the patients history, a physical examination, and laboratory and imaging tests. Transvaginal ultrasonography remains the standard for evaluation of adnexal masses. Findings suggestive of malignancy in an adnexal mass include a solid component or intracystic proliferations, thick septations (greater than 2 to 3 mm), bilateral occurrence, blood flow within the solid component of the mass, and presence of ascites. Tumor-neoangiogenesis has typical features of flow pattern and vascular architecture, indicative of malignancy, which can be visualized by Doppler ultrasound. Power Doppler with its increased sensitivity for slow flow and small vessels is ideal for this purpose and, in combination with acquisition of a volume data set of the region of interest (RoI), gives new insights in tumor angiology and appears to be an additional diagnostic tool. An important predictor of malignancy is a resistance index (RI) below 0.42 in arterial tumor vessels. 3D rendering modes like magic cut, NICHE mode, power Doppler glass body rendering, can make valuable contributions to differential diagnose of adnexal masses. A variety of adnexal masses is illustrated in their specific sonographic appearance, with special regard to ovarian carcinoma.

Keywords: Adnexal mass, 3D power Doppler, Tumor neoangiogenesis, 3D rendering, Ovarian cancer.

INTRODUCTION

Ultrasonography is accepted as the primary imaging modality in the evaluation of adnexal masses.[1] The use of ultrasound in the detection of a suspected adnexal mass and its differentiation from a uterine mass has been well established. Because ultrasound depicts the mass, characterization of the mass is typically performed during the same examination. Thus, *de facto*, ultrasound becomes the main triage method before treatment. The majority of adnexal masses are benign cysts. However, 25% of ovarian neoplasms are malignant.[2] For this reason, surgical removal of a suspected ovarian neoplasm is the gold standard. In most institutions, the type of surgery performed (laparoscopy *vs* laparotomy) depends on the probability of malignancy.[3] The optimal ultrasound techniques and diagnostic criteria to be used when characterizing a suspicious adnexal neoplasm remain controversial. However, several meta-analysis revealed significantly higher performance for combined techniques than for morphologic information Doppler ultrasound indices, or color Doppler imaging alone.[4] In a multicenter European study, color Doppler evaluation was more accurate in the diagnosis of adnexal malignancies compared to gray scale sonography (kappa = 0.82 and 0.65, respectively) because of significantly higher specificity (0.94 *vs* 0.84; $P < 0.001$). The evaluation of the cancer antigen 125 serum concentration did not seem to increase the accuracy of either method.[5] Color and pulsed Doppler sonography depicts the vascularity of the pelvic organs and can be used for assessment of angiogenesis in tumor masses, producing insights into tumor histology and metabolism. It has a primary role in detection of vasculature and assessment of blood flow features of malignant pelvic lesions.[6] Technological advances such as three-dimensional volume acquisition and three-dimensional power Doppler may have clinical utility in the identification of abnormal ovarian architecture, as well as vascularity. The addition of three-dimensional power Doppler provides a new tool for measuring the quality of tumoral vascularity, and its clinical value is being evaluated.

The Basis of Angiogenesis

All organs of human body have a physiological duty to form certain compounds and molecules while disintegrating others, with the aim of maintaining the frail molecular equilibrium. In order to perform their task, all the organs and body parts must be connected by a single vascular network. Like any other vital tissue, vascular endothelium has the ability to regenerate and to spread through other

tissues in order to perfuse them. The formation of new blood vessels is called angiogenesis and it results in neovascularization. More than 100 years ago, Coman and Sheldon discovered that tumor angiogenesis differs from vascularity in normal tissues.[7] It was long believed that simple dilatation of existing host blood vessels was responsible for tumor hyperemia.[8] Vasodilatation was generally thought to be a side effect of tumor metabolites released from necrotic parts of the tumor. However, some authors suggested that tumor hyperemia might be related to new blood vessel growth, i.e. neovascularization, rather than to dilatation of pre-existing vessels. A report published in 1945 revealed that new vessels in the neighborhood of a tumor implant arose from host vessels and not from the tumor itself.[9] Further experiments during 1960s with isolated perfused organs brought the new and exciting concept – that tumor growth is restricted in the absence of a vascular response of the surrounding host tissue.[10,11] In the following decades, scientists showed that tumors implanted into animals consistently induced the growth of new capillaries. Viable tumor cells were found to release diffusible angiogenic factors that stimulated new capillary growth and endothelial mitosis.[12,13] On the basis of these observations, Judah Folkman proposed a hypothesis that, once a tumor had occurred, every further increase in the tumor cell population had to be preceded by an increase in new capillaries, which sprouted towards the early growth of the tumor.[14] Since Folkman's hypothesis, for over 25 years, it has been clear that the development of new blood vessels—called angiogenesis—is crucial for sustaining tumor growth, as it allows oxygenation and nutrient perfusion of the tumor and removal of waste products.[15,16] Moreover, increased angiogenesis coincides with increased tumor cell entry into the circulation, and thus facilitates metastasis.[17,18]

Cancer cells activate quiescent vasculature to produce new blood vessels via an "angiogenetic switch", often during the premalignant stages of tumor development.

The concept of an "angiogenic switch" means that as the tumor grows and cells in the center of the tumor mass become hypoxic, the tumor initiates recruitment of its own blood supply, by shifting the balance between angiogenesis inhibitors and stimulators towards angioneogenesis. Thus, neovascularization precedes and promotes tumor progression and metastasis.

The density of the capillary network seems to be one of the factors determining the malignancy of a tumor. Metastases of highly vascularized tumors appear earlier than those of poorly vascularized tumors.[19,20] The reason for this is not just the capillary permeability that enables shedding of tumor cells into the bloodstream, but also a current hypothesis that both the primary tumor and the distant metastases are involved in complex regulation by angiogenic and antiangiogenic factors.[21]

The first indication of its importance in tumor angiogenesis came in the early 80s from observations that tumors secreted soluble factors which could stimulate vascular endothelial growth.[22] The basic and acidic fibroblast growth factors were among the first to be identified and were soon followed by many others such as vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and transforming growth factors (TGF-alpha- and beta), to mention some of the most important.[23]

Investigation of these factors, powerful molecules that control the formation of new blood vessels, are still in progress.[24,25] One study reported on the expression of VEGF in ovarian epithelial tumors (OETs) in both epithelial and stromal compartments.[26] The study showed that VEGF is significantly increased in malignant OETs compared to benign and borderline tumors, and concluded that this factor might play a role in the development of ovarian cancer.

Expression of VEGF depends on tissue hypoxia, generating a feedback mechanism to reduce hypoxia by means of neoangiogenesis. The regulation of VEGF expression by hypoxia is mediated by a family of hypoxia-inducible transcription factors (HIF), which increase transcription of the VEGF gene.[27]

The ability of growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), the insulin-like growth factor (IGF) system, and HIF-1 alpha to increase the rate of endothelial cell proliferation has been demonstrated in several animal models; and it is important for understanding tumor biology to appreciate the interesting fact that proliferation is largely limited to ischemic zones, even following systemic administration of these factors.[28]

The role of the IGF system in malignancy and its oncogenic potential was reviewed in 2006 by Samani and coworkers: they found that IGF-I receptor (IGF-IR) signaling and functions are mediated through the activities of a complex molecular network of positive (e.g. type I IGF) and negative (e.g. the type II IGF receptor, IGF-IIR) effectors. Under normal physiological conditions, the balance between the expression and activities of these molecules is tightly controlled. Changes in this delicate balance (e.g. overexpression of one effector) may trigger a cascade of molecular events that can ultimately lead to malignancy. In recent years, evidence has been mounting that the IGF axis may be involved in human cancer progression and can be targeted for therapeutic intervention. Because IGF targeting for anticancer therapy is rapidly

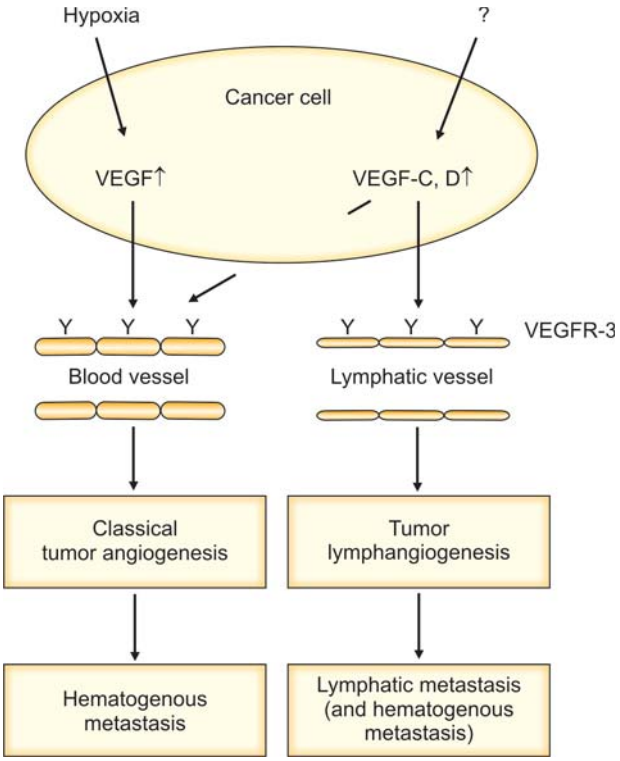


Fig. 1: Dissecting tumor angiogenesis. Hypoxic tumor cells produce VEGF, which binds to and activates VEGFR-2 on vascular endothelial cells, leading to classical tumor angiogenesis. Tumor that secrete VEGF-C or VEGF-D may induce lymphangiogenesis by activating VEGFR-3 on lymphatic vessels, a process known as tumor lymphangiogenesis. Classical tumor angiogenesis has been shown to correlate with hematogenous metastasis. In the animal models, induction of lymphangiogenesis by VEGF-C or VEGF-D led to an increase in tumor metastasis via the lymphatic system

becoming a clinical reality, Samani et alia stressed that understanding of this complexity is timely because it is likely to have an impact on the design, mode of action, and clinical outcomes of newly developed drugs.[29]

Besides hemangiogenesis, pathways for lymphangiogenesis have also been proposed (Fig. 1).[16] Electron microscopy, *in vitro* cultures and experiments on animal models have enabled us to understand the visible part of the angiogenic development of tumor vasculature.

Tumor Neovascularization—Current Concepts

In general, tumor vasculature consists of the vessels recruited from the pre-existing network of the host vasculature, and the vessels grown from the host vessels under the influence of the angiogenic factors of cancer cells.[30-32] Although the tumor vasculature originates from the host vasculature, its organization may be completely different, depending upon the tumor type, its growth rate and its location. Macroscopically, tumor vasculature can be studied in terms of two

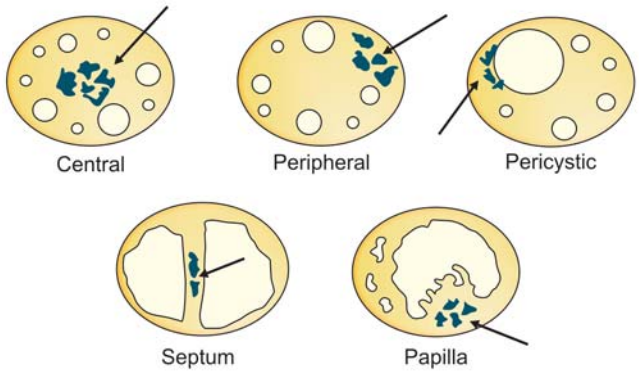


Fig. 2: Schematic presentation of different types of vascularization within abnormal ovarian tissue

ideal categories: peripheral and central. In tumors with peripheral vascularization, the centers are usually poorly perfused. In those with central vascularization, the opposite is expected. However, a tumor may consist of many territories, each exhibiting one or the other type of the ideal vascular pattern (Fig. 2). Microscopically, tumor vasculature is highly heterogeneous and does not conform to the standards of normal vascular organization. Tumor neovascularity can be differentiated from normal vascular beds by the several main characteristics.[33]

1. A single branch, varied in caliber, formed from narrow and dilated segments;
2. Elongation and coiling;
3. Nonhierarchical vascular network, vascular rings and sinusoids;
4. No normal precapillary architecture with dichotomous branching, and no decrease in diameter of the higher-order branches;
5. Incomplete vascular wall: various gaps in the endothelium, discontinuity of the basal membrane and no muscular layer except in pre-existing vessels encased by the tumor.

A key difference between normal and tumor vessels is that the latter are dilated, saccular and tortuous, and may contain tumor cells within the endothelial lining of the vessel wall.[34] In addition, unlike normal tissue with a relatively fixed route between the arterial and venous circulation, a tumor may have blood flowing from one venule to another via a series of vessels, or directly via an arterio-venous shunt.

However, the morphologic appearance of the tumor vascular bed may not necessarily allow direct assessment of the function of the tumor microcirculation. This is because only 20-80% of tumor vessels are perfused within any given tumor at a particular time. Within a particular tumor, one investigator has noted variations in flow that can be as high as tenfold.[35] Another important aspect to consider is that the microvascular permeability of vessels within tumors is

very heterogeneous, and tumors have been shown to be up to eightfold more permeable than normal tissues. Vascular endothelial growth factor (VEGF) has been shown to increase vascular permeability.[20]

VEGF is 50,000 times more potent in inducing vascular leakage than histamine.[36]

The mechanism of this effect appears to be fenestration of the endothelium of small venules and capillaries through a Src kinase-dependent mechanism.[37]

One of the main characteristics of the tumor interstitial space is an expansion of its volume by three to five times compared to most host tissues. This results in a high interstitial fluid pressure of up to 50 mm Hg, when compared to normal tissues, in which the interstitial pressures are slightly subatmospheric. The major pathophysiologic mechanisms attributed to interstitial hypertension are absence of functioning lymphatic vessels, the high permeability of the vascular wall, and the rapid proliferation of tumor cells in confined spaces.[32] High interstitial pressure leads to compression of vessels inside the tumor, and this may even lead to local stasis. The relative perfusion of tumors resulting from these factors varies according to their growth. Initially, tumors have a hyperemic periphery with a relatively well perfused rim of tissue and later a relatively ischemic area centrally. As the tumor enlarges, areas of central necrosis develop. Accordingly, four regions with different perfusion rates can be recognized in a tumor: an avascular (necrotic) region; a semi-necrotic (ischemic) region; a stabilized microcirculation; and an advancing front as a region of tumor hyperemia.[30,38] Depiction of these areas by imaging has practical importance because spatial distribution of chemotherapeutic agents varies according to the degree of tumor vascularity within different tumor regions. The multitude of data indicating that the control of angiogenesis is separate from the control of cancer cell proliferation suggests the possibility that drugs inhibiting angiogenesis could offer a treatment complementary to traditional chemotherapy, which directly targets tumor cells.[12,15,39] This exciting possibility has stimulated research on tumor angiogenesis and introduction of new three-dimensional power Doppler evaluation of tumor vessels architecture.[40-44]

EVOLUTION OF 2D CONVENTIONAL AND COLOR DOPPLER ULTRASOUND IN IMAGING OF ADNEXAL TUMOR ANGIOGENESIS

It is clear that conflicting attitudes towards Doppler ultrasound in the evaluation of the vascular characteristics of malignant adnexal masses arise from the different results obtained from a number of studies published in the past several years. It is also important to stress that pulsed Doppler analysis and vascular resistance to blood flow were, and still are, one of the main features in the assessment of tumor vascular characteristics. All these studies have concentrated on differences of vascular resistance to blood flow between benign and malignant adnexal masses (Figs 3 and 4). It is a fact that a difference in vascularity exists, and that blood vessels in malignant adnexal lesions show lower resistance to blood flow than those in benign adnexal masses (Figs 5 and 6).

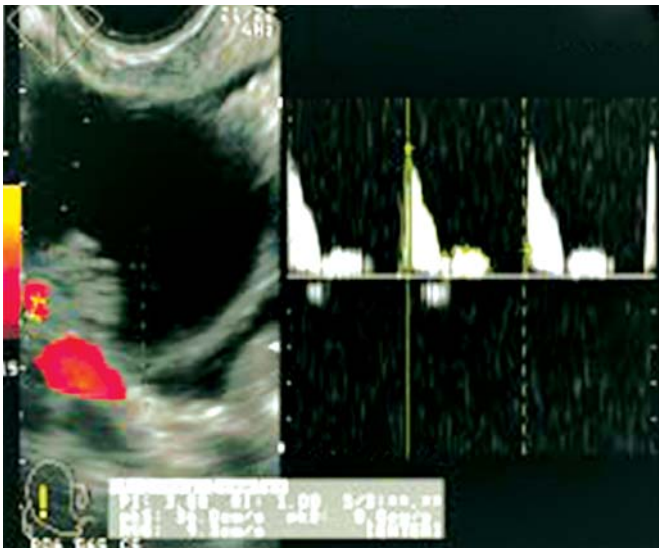


Fig. 3: A transvaginal sonogram of a ovarian tumor with small echogenic formation (papilla) protruding into the lumen. Pulsed Doppler imaging demonstrates high resistance to blood flow. Benign ovarian tumor was confirmed by histopathology

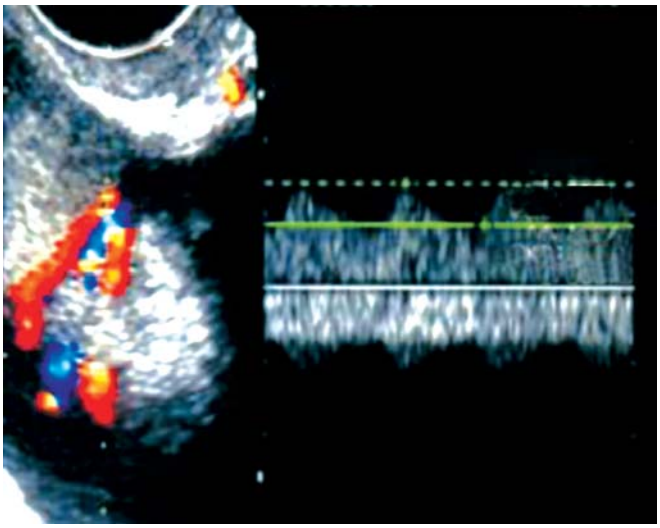


Fig. 4: A malignant ovarian tumor with its characteristic image created by a solid counterpart. Pulsed Doppler waveform analysis shows low-resistance to blood flow

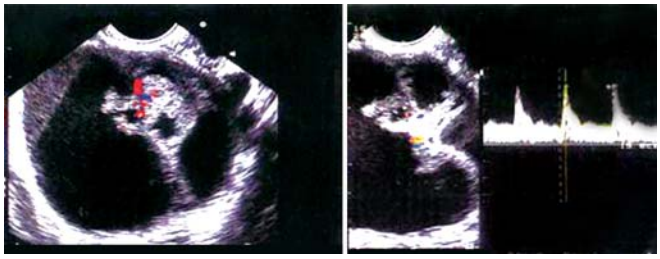


Fig. 5: Complex ovarian tumor, morphology suspicious for malignancy. Pulsed Doppler signals revealed moderate to high vascular resistance. Histopathology confirmed benign adnexal lesion

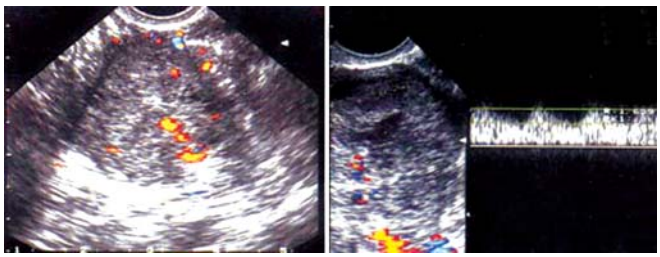


Fig. 6: The advanced stage of ovarian carcinoma. Randomly dispersed vessels are produced in central and peripheral parts of the tumor creating the potential for its proliferation and growth. Tumoral vessels are deficient in their muscular elements and present diminished resistance to blood flow

Before using any diagnostic technique related to sonographic depiction of ovarian vascularity and flow, it is important that the investigator distinguishes the concepts of "tumor vascularity" and "tumor blood flow". Tumor vascularity refers to the number of vessels per unit volume, whereas tumor blood flow is a measure of the number of flowing blood elements over a certain period of time in a selected area of interest.[33]

Frequency-based color Doppler imaging provides information about blood flow by analyzing the changes in Doppler shift which are proportional to velocity changes. Since, the introduction of this diagnostic technique for the assessment of ovarian vascularity, opinions concerning its usefulness in the detection of adnexal malignancies have been equally divided.[45-52] Tumor blood vessels have a paucity or lack of a media muscularis which is normally part of the vessel wall, and hence are more distensible. This combined with arteriovenous shunts seen in the tumor vascular network results in low impedance to flow. However, because of focal areas of narrowing and dilatation within tumor vessels, focal areas of high systolic velocity can also be found.[53] Another factor that confounds this is the fact that most tumors have areas of variable perfusion. Our study reported on pre-existing vessels with normal wall structure in 60% of malignant ovarian tumors.[54]

This contributes to uneven tumor blood flow that makes it difficult to generalize a "characteristic" flow of ovarian tumors. [55] Diagnostic accuracy of values of flow indices in differentiating benign from malignant lesions has varied considerably, from over 96% to less than 40%.[56,57] More than 15 years of experience in multiple centers have shown that the overlap in the specific impedance values obtained by frequency-based transvaginal color Doppler imaging from vessels surrounding the ovary does not allow differentiation of benign *vs* malignant ovarian masses on the basis of impedance values alone.[58] Other limiting factors for this type of imaging represent slow flow and vessels of small diameter which are barely detectable.[59] Another problem of this technique is that only those vessels which are depicted can be adequately studied. More precisely, it seems more important to provide information regarding the vascular network rather than particular vessels.[60] A solution to this problem has been offered by the introduction of amplitude-based transvaginal color Doppler imaging.[61] This imaging modality, known also as power Doppler ultrasound or color Doppler Angio® takes into account the area under the curve of a spectral waveform and is related to the number of blood elements flowing over time. Power Doppler sonography has been found to be superior to frequency-based color Doppler sonography, especially in situations of low blood flow (low velocities), with the potential to detect alterations in blood flow.[62] Power Doppler ultrasound has the advantage that it is more sensitive, less angle- dependent and not susceptible to aliasing.[63,64] In this technique, the hue and brightness of the color signal represent the total energy of the Doppler signal. It displays the total flow in a confined area, giving an impression similar to that of angiography. The sensitivity of power Doppler imaging is 14 dB greater than the standard Doppler imaging.[65] Because of this greater sensitivity in displaying smaller vessels, the vascularity is shown more completely. Several studies demonstrated the correlation of tumor vascularity as depicted on power Doppler imaging to an estimation of vascularity seen histologically. There was good correlation (r = 0.82) of vessels greater than 50 micron in size but poor correlation with the actual microvessel count, which typically includes vessels less than 15 microm.[66,67] Power Doppler imaging has serious limitations in assessing temporal changes in flow and vessel size due to blooming of the color signal. This imaging modality permits the depiction of even smaller vessels, but paradoxically, small intraparenchymal arterioles in benign and normal tissues may show a low impedance and a low-velocity blood flow pattern, giving rise to false-positive results.

It is clear that there is 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.

THREE-DIMENSIONAL ULTRASOUND AND POWER DOPPLER IMAGING OF BENIGN AND MALIGNANT ADNEXAL MASSES

Three-dimensional ultrasound (3D US) is a new, emerging technology that provides additional information to the evaluation of adnexal masses.[68] Multiplanar and volume rendering display methods combined with the ability to rotate volume data into standard orientations are essential components of 3D US's current and future success.[69]

Patient characteristics, CA125 level and two-dimensional (2D) ultrasonography are commonly used to predict the probability of malignancy of an adnexal/ovarian mass. To evaluate three-dimensional (3D) ultrasonography in its contribution to the prediction of malignancy, Geomini and coworkers in 2007 investigated in 181 patients whether addition of 3D features to a diagnostic model could improve the discriminative capacity of the model. Women with an adnexal mass scheduled for surgery underwent 2D and 3D ultrasonographic examination in the week prior to surgery. Stepwise logistic regression was used to construct two models for the prediction of malignancy: a model based on patient characteristics, level of CA125 and 2D ultrasonography and a second model based on patient characteristics, level of CA125, 2D and 3D ultrasonography. Out of 181 women with an adnexal mass, 144 were benign and 37 showed malignancy on histopathology. The 3D model discriminated better between benign and malignant adnexal masses than the 2D model (areas under the ROC curve of 0.92 and 0.82, respectively, p = 0.02). The calibration of both models was good. The authors concluded that in the assessment of the ovarian mass, the use of 3D ultrasonography significantly improved the prediction of malignancy as compared to patient characteristics and 2D ultrasonography.[70]

Mansour and coworhers published in 2009 the results of a study where they had looked at 400 patients with adnexal masses, using a Risk of Malignancy Index (RMI) containing menopausal status, Ca 125 serum levels, and ultrasonographic morphology criteria. To this RMI, they added 3-dimensional power Doppler (3D PD)for the assessment of tumor vascularity, classifying patterns like avascular, parallel, and chaotic, to evaluate possible contribution of 3D PD to the malignancy risk prediction. Sensitivity of RMI for prediction of malignancy was 88%, sensitivity of 3D PD for prediction of malignancy was 75%, and adding 3D PD to RMI increased its sensitivity to 99%.[71]

Two months earlier Chase and Crade published in the same journal a series of 66 cases of preoperative assessment of adnexal masses. They found that the positive predictive value (PPV) and the negative predictive value (NPV) of 3D vascular ultrasound were 100% and 95%, respectively. The PPV and the NPV of 2-dimensional ultrasound in predicting malignancy were 37% and 100%, respectively. An abnormal level of Ca 125 had a PPV and NPV of 73% and 83%, respectively, in this population. According to the authors, 3D ultrasound examination of vascular architecture is discriminatory in distinguishing benign ovarian masses from malignancy.[72]

Multiple sections of the tumor, rotation, translation, and reconstruction of 3D plastic images allow more precise evaluation of the tumor without increasing scanning time and patient discomfort.[73,74] 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.[43,75,76] The surface mode is used in the assessment of superficial structures. If a cystic ovarian mass is found, three-dimensional surface-rendered image offers new possibilities for evaluation and the differentiation between benign and malignant disease[77] (Table 1) (Figs 7A to 8C).

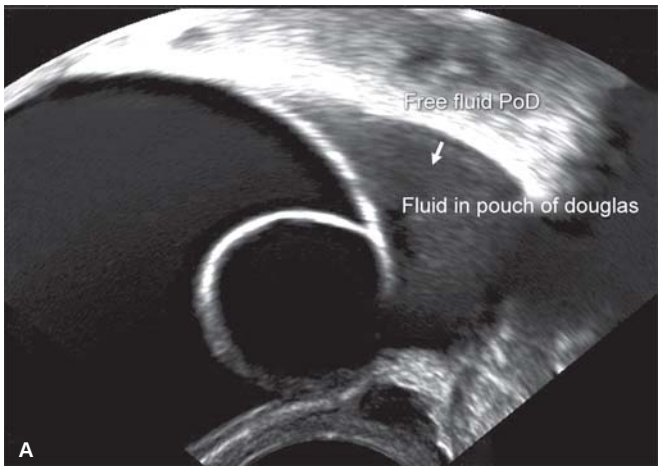


Fig. 7A

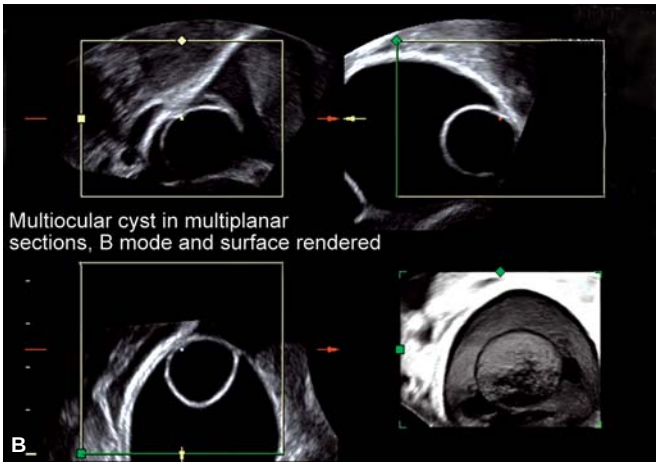
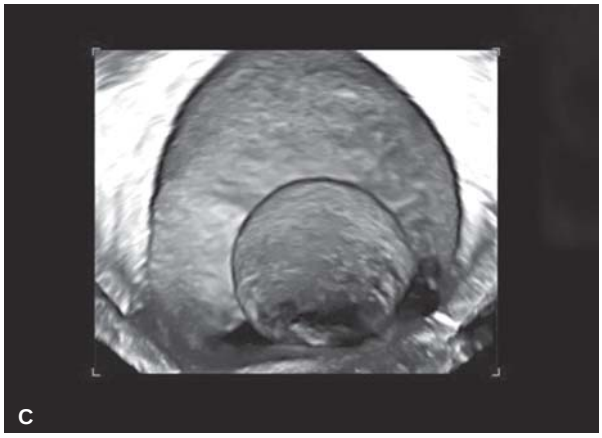
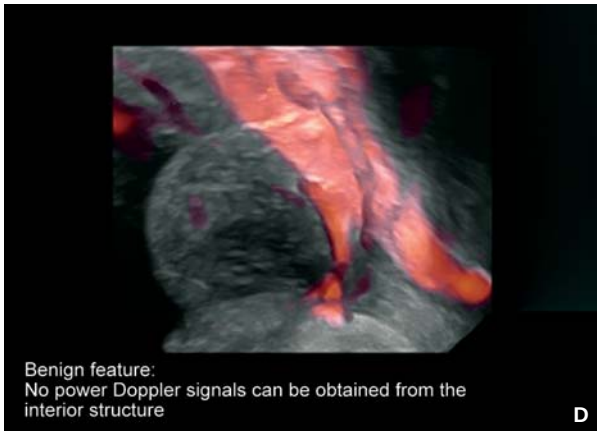
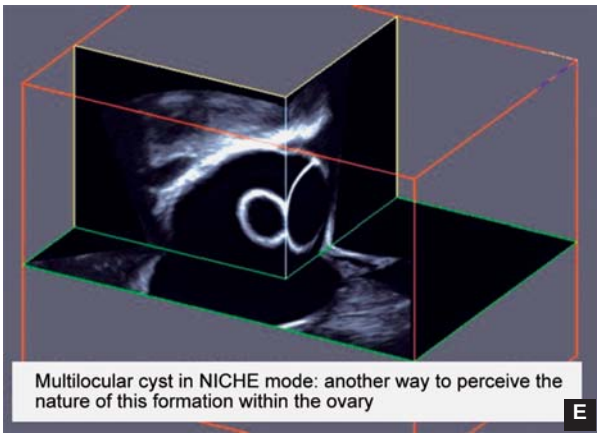
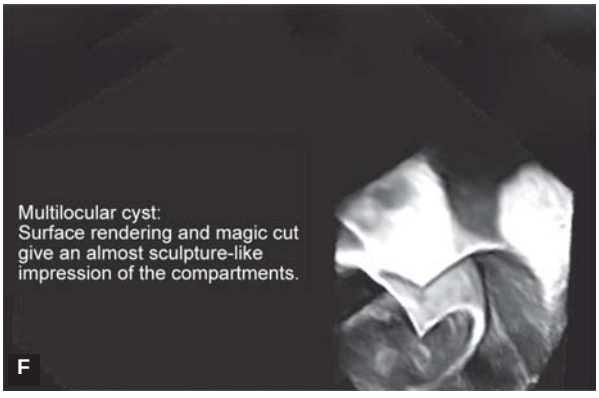


Fig. 7B

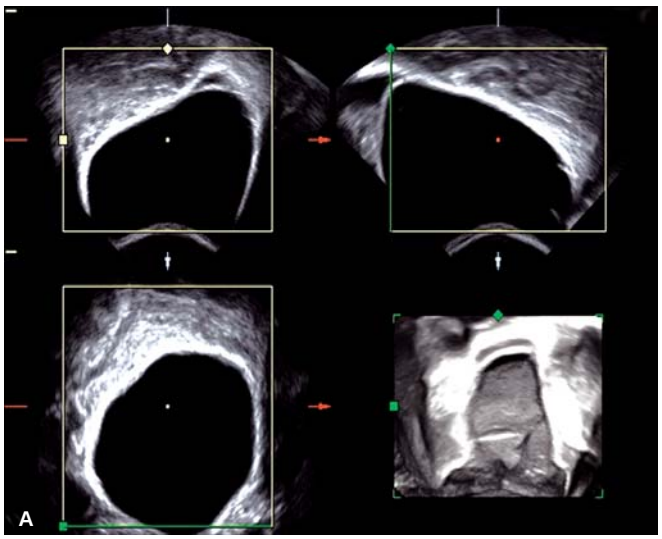


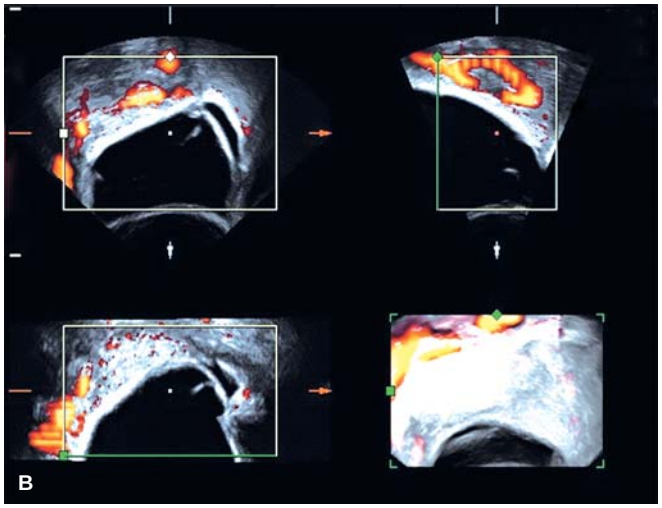


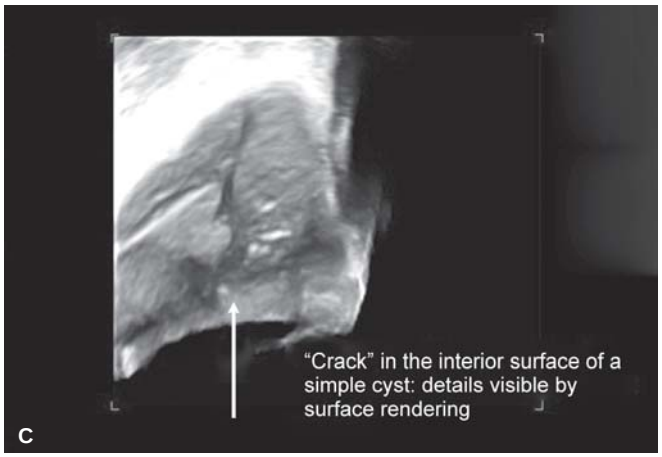




Figs 7A to F: Ovarian multilocular cyst featured in B mode, multiplanar, 3D PD mode, NICHE mode, and "magic cut"







Figs 8A to C: Ovarian simple cyst, multimodal assessment. Details of the interior surface become visible

Application of "transparent maximum/minimum" mode enables visualization of the intratumoral calcification or identification of the bone structures in dermoid tumors[78] (Figs 9 and 10).

Table 1: Advantages of three-dimensional ultrasound observed by different authors

Authors	*No. of patients analyzed*	*Advantages of 3D US*
Chan et al 1997[70]	8	- Enhanced morphologic evaluation of adnexal masses - Additional views - High-speed image acquisition - Real time analysis of the acquired image data at a later time
Weber et al 1997[71]	50	- Multiplanar view - Rotation of the stored volume - Reconstruction of the inner surface of a cystic ovarian tumor
Bonilla-Musoles et al 1995[65]	67	- Calculation of ovarian volume - Observation of papillary projections - Extent of capsular infiltration
Geomini et al 2007[67]	181	- Significant improvement of prediction of malignancy
Mansour et al 2009[68]	400	- Sensitivity of risk of malignancy index (RMI) was increased from 88 to 99% by adding 3D power Doppler
Chase et al 2009[69]	66	- 3D ultrasound examination of vascular architecture is discriminatory in distinguishing benign ovarian masses from malignancy.

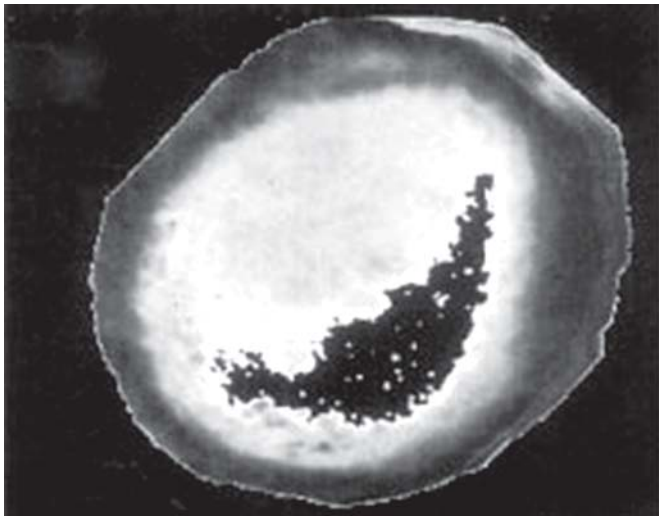


Fig. 9: Three-dimensional scan of a bizarre ovarian tumor containing echogenic fluid and intracystic echoes. Histopathology revealed dermoid cyst

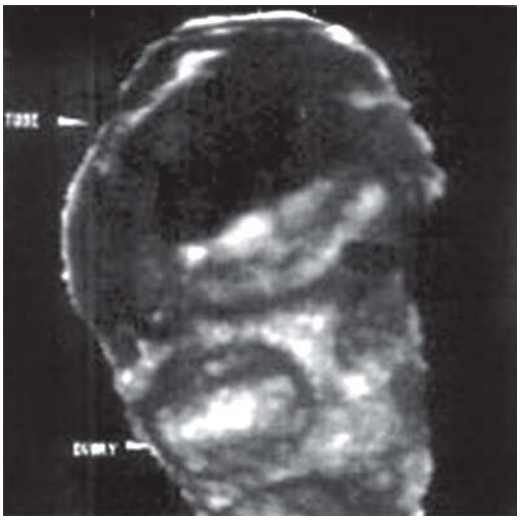


Fig. 11: Three-dimensional view of a dilated tube in a patient with hydrosalpinx. Ipsilateral ovary containing follicles is clearly visualized close to the affected tube. This is an illustrative example of how surface rendering defines spatial relations of a tubal lesion with a nearby ovary

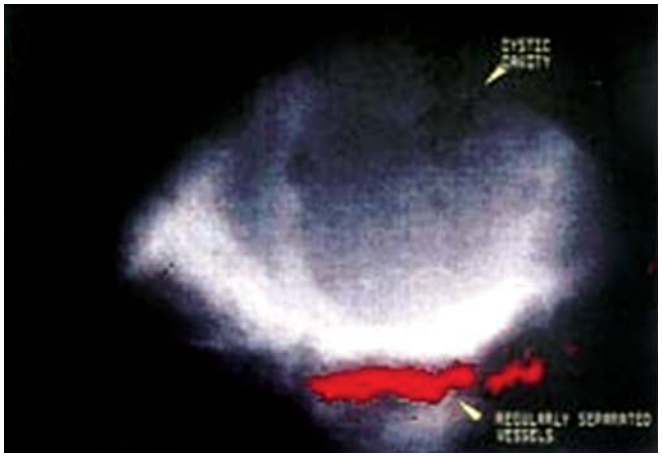


Fig. 10: Regularly separated vessels are detected at the periphery of the dermoid cyst by three-dimensional power Doppler imaging

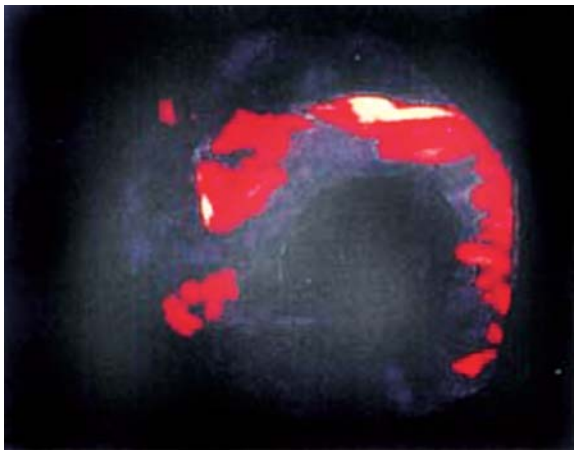
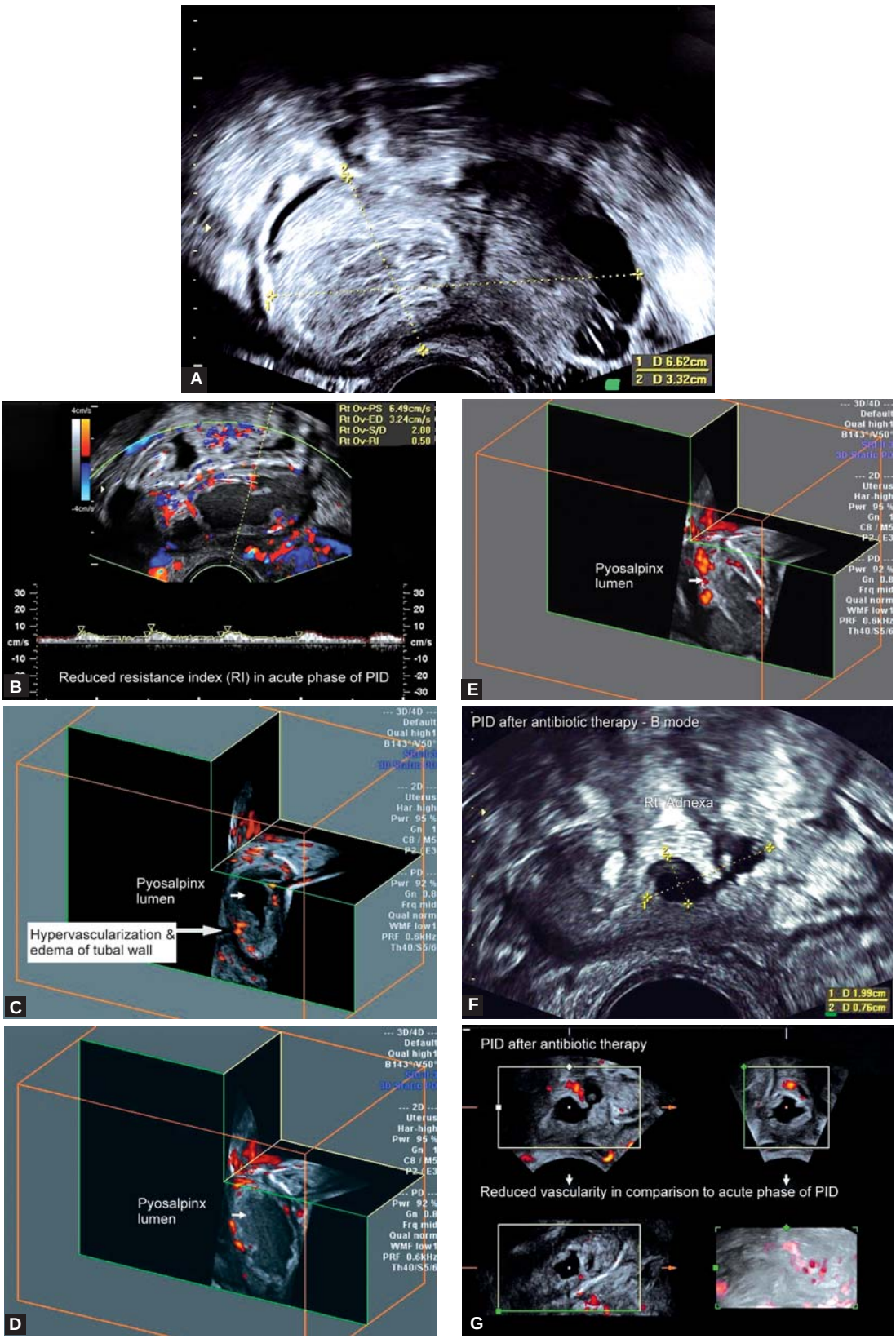


Fig. 12: Three-dimensional power Doppler scan of regularly separated vessels in a patient with pelvic inflammatory disease



Figs 13A to G: Pelvic Inflammatory Disease (PID) case 2, featured by hyperemia with low resistance index (RI), edema of tubal wall, and purulent exsudation (pyosalpinx). After antibiotic therapy, and with normal C-reactive protein (CRP) serum level, reduced vascularity

Table 2: Histopathology, 3D ultrasound morphology and vascular geometry in patients with Fallopian tube carcinoma

Histopathology	*3D ultrasound morphology*	*Vascular geometry*
Adenocarcinoma	Sausage shaped complex mass 4.0 × 2.8 × 3.2 cm with papillary projections, regular surface, no infiltration through the capsule	Chaotic vessels arrangement, disproportional calibration of the vessels and irregular branching
Adenocarcinoma	Sausage shaped cystic mass 5.8 × 3.6 × 2.8 cm with pseudosepta and papillary projections, regular surface, thickened capsule	Chaotic vessels arrangement, demonstration of the arteriovenous shunts, tumoral lakes and microaneurysms
Adenocarcinoma	Sausage shaped cystic mass 8.4 × 4.5 × 6.0 cm with solid parts and papillary projections, regular surface, thickened capsule	Chaotic vessels arrangement, irregular branching, visualization of numerous blind ends
Adenocarcinoma	Sausage shaped complex mass 7.8 × 4.2 × 3.0 cm with papillary projections, irregular surface, infiltration through the capsule, bilateral tumors	Chaotic vessels arrangement, demonstration of the vascular stenosis, tumoral lakes and irregular branching
Anaplastic carcinoma	Sausage shaped mass 4.0 × 3.0 × 2.6 cm with papillary projections, regular surface, no through the capsule	Chaotic vessels arrangement, visualization of the arteriovenous anastomosis, infiltration microaneurysms and irregular branching

The "niche" aspect of three-dimensional ultrasound reveals intratumoral structures in selected sections which is mandatory for evaluation of the tubal pathology (Figs 13A to G). Our team reported on five cases of the primary Fallopian tube carcinoma in preoperative diagnosis selected from a cohort of 520 patients with a previous scan suggestive of an adnexal tumor.[79] Using two-dimensional ultrasound three Fallopian tube carcinoma were detected preoperatively.

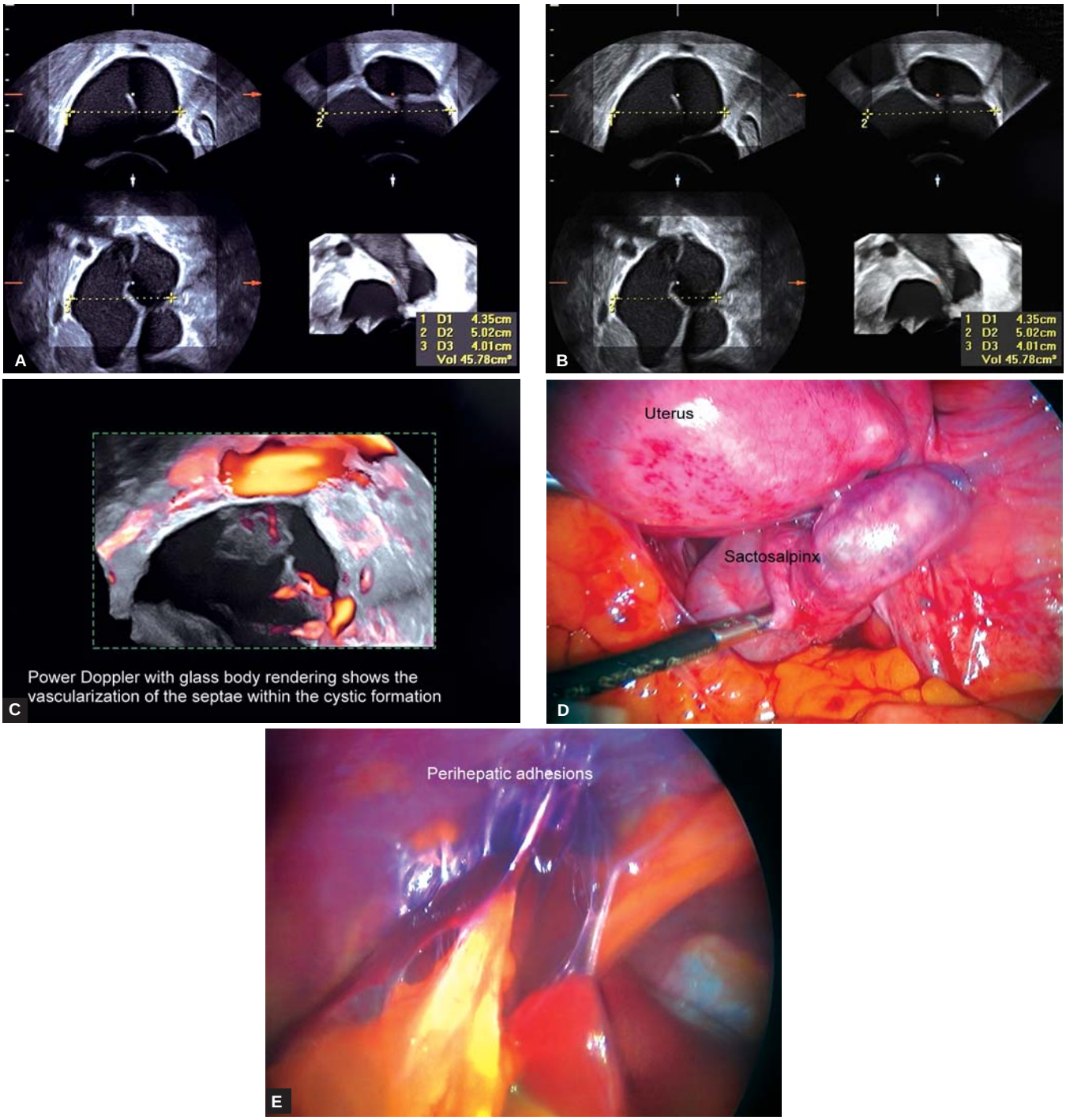
Morphological assessment demonstrated sausage shaped cystic masses with papillary projections in two patients and a complex adnexal mass in one patient. Three-dimensional ultrasound analysis enabled accurate diagnosis of the Fallopian tube carcinoma by detecting intraluminal papillary projections in all five cases of tubal malignancy. Furthermore, 3D ultrasound enabled precise detection of tubal pathology in each of five cases by simultaneous observation of the neighboring structures: uterus and ipsilateral ovary. One patient had bilateral Fallopian tube lesions. Preoperative 3D ultrasound imaging correctly predicted bilaterality, while 2D imaging found only unilateral changes (Table 2).

Furthermore, this technique is especially useful in evaluation of the complex ovarian lesions such as ovarian dermoids, endometriomas, fibromas and inflammatory disease, which may give wrong impression of malignancy when using conventional transvaginal sonography and color Doppler ultrasound (Figs 11, 12, 14A to 16H).

Perifollicular hyperemia (expressing high metabolic activity), with vasorhexis during ovulation caused the extraordinary hemorrhage. Demonstration of perifollicular hyperemia and intrafollicular fibrin strands in 3D PD and NICHE mode. Laparoscopic intervention with coagulation of the rupture site, and lavage.

In our further study, we detected a significant reduction in the rate of false-positive findings between 2D transvaginal and color Doppler ultrasound and combined 3D static and power Doppler ultrasound: 76.92% *vs* 91.67% [80]. It is important to present a case of serous cystadenocarcinoma in a 38-year-old patient measuring only 3 cm, missed by 2D transvaginal color Doppler, but successfully identified by combined 3D static and power Doppler imaging. Transvaginal sonography did not demonstrate small papillary projections (less than 5 mm in maximum diameter) extending the cystic wall. Although pulsed Doppler waveform analysis demonstrated peripheral vascularity, resistance index values were above the cut-off value proposed for the diagnosis of ovarian malignancy and measured 0.46. Three-dimensional ultrasound clearly depicted papillary protrusions and power Doppler enabled detection of tiny irregular vessels within the papillary protrusion.

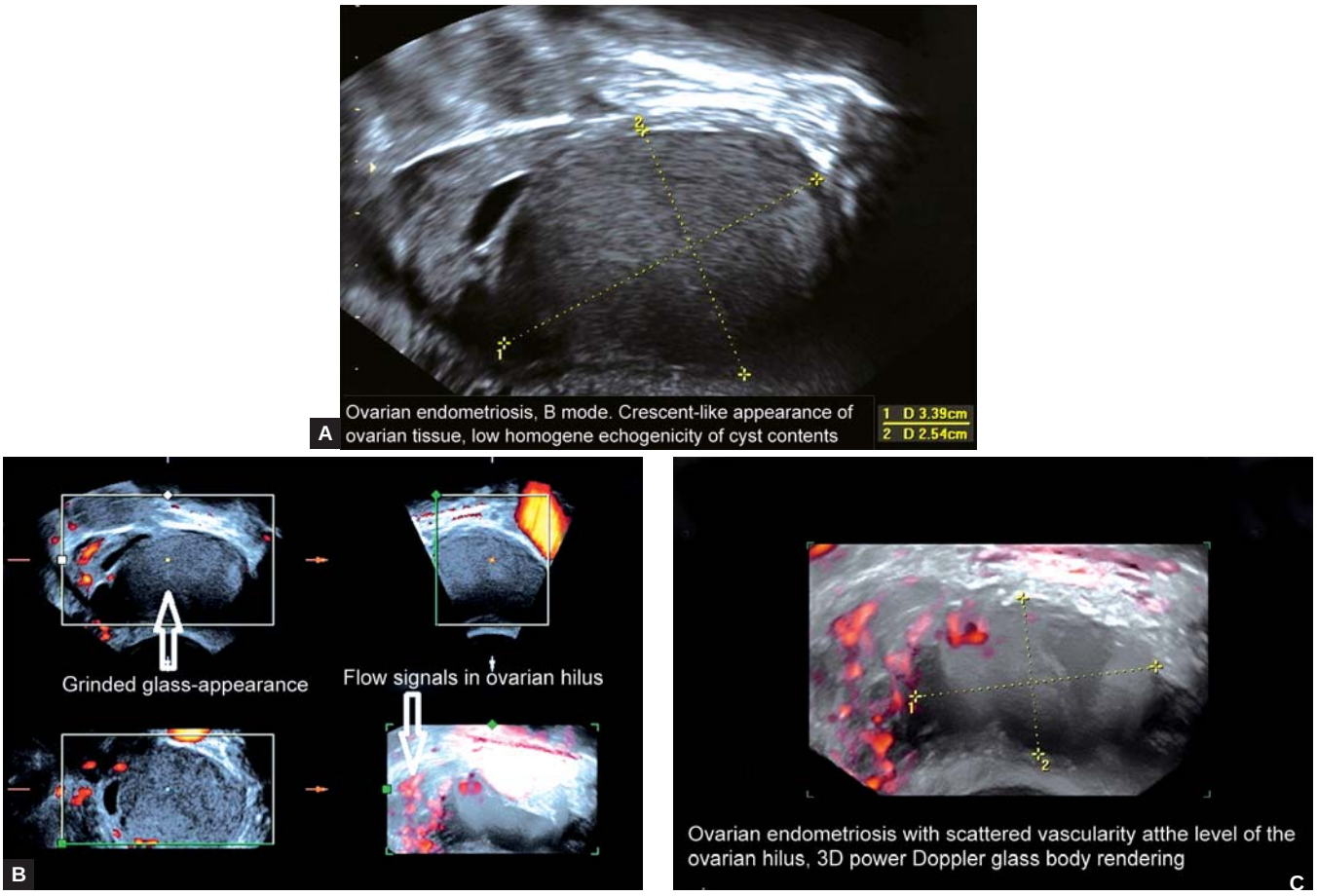
As already mentioned, a new tool for study of angiogenesis is 3D power Doppler.[81] This three-dimensional modality allows the clinician to visualize the many overlapping vessels easily and quickly, and to assess their relationship to other vessels or surrounding tissues. The implementation of the 3D display permits the physician to view structures in three-dimensions interactively, rather than having to assemble the sectional images mentally. The 3D power Doppler system may thus improve the information available on tumor vascularity and speed up the entire patient management process.[82]



Figs 14A to E: Endresult of insufficient PID management: Hydrosalpinx resembling a multicystic adnexal mass with vascularized septae. Laparoscopy reveals hydrosalpinx and perihepatic adhesions, typically left behind by Chlamydia infection

While 2D color Doppler was useful in detecting vascularized structures, 3D power Doppler is excellent in the study of vascular morphology. Morphological analysis of the blood vessel system represents another approach to tumor diagnosis which is extensively evaluated in the last five years.

Crade in 2009 stressed the diagnostic value of "vascular signature"of pelvic masses. Using 3D Doppler and the software of Voluson 730 Expert (Milwaukee, USA), he described a "tissue block" technique, where several volumes of a certain region of interest (ROI) in the adnexal mass, containing vascular patterns picked up by color or power



Figs 15A to C: Ovarian endometriosis with scattered vascularity in the hilus area, " grinded glass"-like low echogenicity of intracystic fluid, and "crescent" appearance of the remaining healthy ovarian tissue featuring benign character of the lesion

Doppler, are stored away and rendered without physical presence of the patient by means of a selection of software features like "Magic Cut", tomographic ultrasound imaging (TUI), or NICHE-mode. With this technique, he developed criteria of vascularity appearance suggesting malignancy:

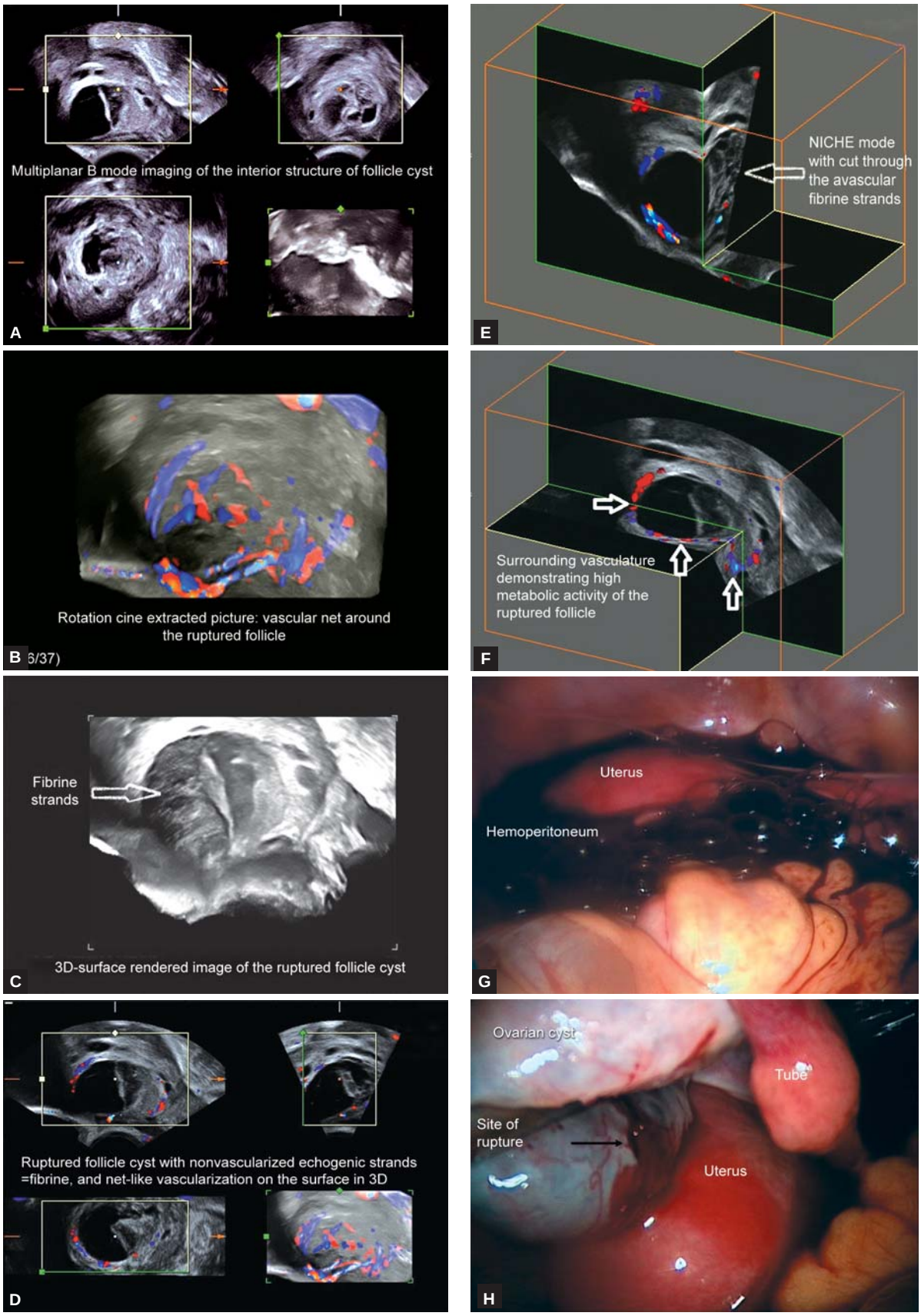
1. Loss of tree-like branching of vessels
2. Sacculation of arteries and veins
3. Focal narrowing of arteries
4. Internal shifts in velocity within arterial lumen
5. "Beach Ball"-finding of increased and disorganized peripheral flow, surrounding the surface of a malignancy, thus forming a ball –like power Doppler image of vascular signals
6. Increased flow to a center of a solid region
7. Crowding of vascularity
8. "Start and Stop" arteries showing arteries that stop abruptly within the tumor, without developing the tree-like branching how it would be normally seen in a benign mass.[83]

There is a distinct impression that the distribution and branching pattern of blood vessels supplying a fast-growing tumor differ from those in a normal blood supply to normal organs. This means that blood vessel distribution seems to carry additional information that is missed in the present diagnostic approaches.

In 2000, we published a study, in which we presented results of 3D power Doppler imaging in interactive analysis of ovarian tumors microcirculation anatomy.[84] Our eighty-one patients had benign ovarian conditions (Fig. 17).

The most common ovarian lesion in premenopausal patients were ovarian endometrioma (26 of 58) (Figs 18 and 19) and dermoid cysts (19 of 58), whereas the most common ovarian tumor during the postmenopausal period was serous cystadenoma (22 of 28). In contrast to the findings in a malignant neoplasm, in benign lesions (endometrioma) the vessels usually present straight regularly branching, coming from a hilar vessel and running along the surface of the tumor. Similar vascular anatomy is detected in dermoid cysts.

From nine cases of malignant ovarian tumors, the most common ovarian malignancy was serous cystadeno-carcinoma detected in six postmenopausal patients (5 of 28),



Figs 16A to H: Ruptured follicle cyst with massive hemoperitoneum (0.9 L)

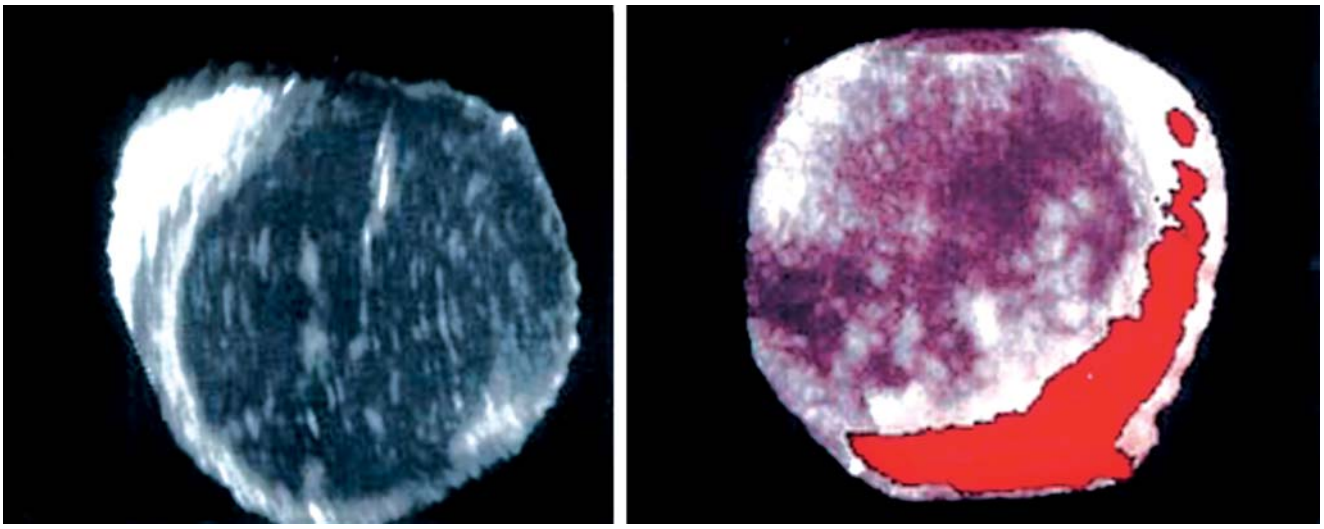


Fig. 17: Three-dimensional scan of an ovarian cyst. Note regular walls of the cyst and echogenic content suggestive of a benign lesion. Corpus luteum cyst was found at the time of laparoscopy. Three-dimensional power Doppler scan of the same corpus luteum cyst. Single vessel arrangement is clearly displayed at the periphery of the cystic lesion

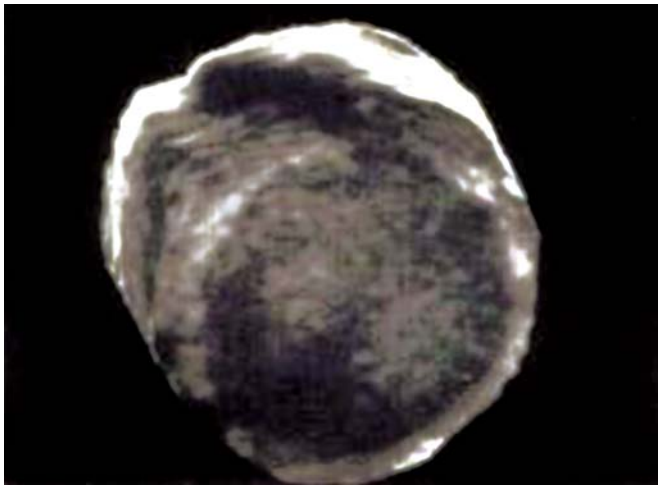


Fig. 18: Three-dimensional scan of an ovarian endometrioma. Homogenous high level internal echoes are visualized by this technique

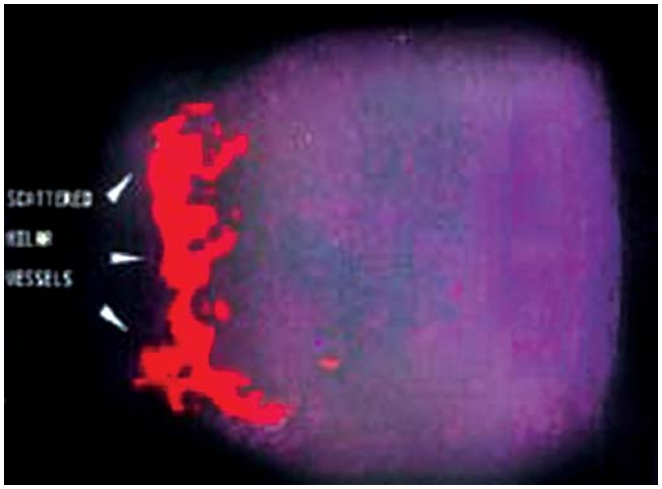


Fig. 19: Scattered vascularity at the level of the ovarian hilus depicted by three-dimensional power Doppler is typical of ovarian endometrioma

one perimenopausal (one of four) and one premenopausal patient (one of 58). Mucinous cystadenocarcinoma was diagnosed in two postmenopausal patients (two of 28). 3D power Doppler ultrasound accurately detected characteristic structural abnormalities of the malignant tumor vessels such as microaneurysms, arterio-venous shunts, tumoral lakes, disproportional calibration, elongation, coiling, and dichotomous branching (Figs 20 and 21).

We observed that the tumor vessels are usually randomly dispersed within the stroma and periphery, and some of them formed several tangles or coils around the surface. The course of the main tumor vessel is usually irregular, with more complicated branching. The diameters of these vessels were felt to be more "uneven" and "thorn-like". Our study demonstrated that a qualitative analysis of the tumor vascularity architecture by 3D power Doppler added to morphological parameters assessed by 3D ultrasound is clinically pertinent, reaching sensitivity and specificity of 100 and 98,76% respectively.

Further Possibilities in the Evaluation of Tumor Angiogenesis

Our group made efforts in describing branching structures of tumor microvasculature like the blood vessel tree which is a mathematically complicated task that requires advanced electronic calculators. The branching pattern is the result of some principle (mathematical law) that acts repeatedly upon the blood vessels, so that they branch out in similar ways at different scale factors. Such objects that are self-similar at different scales are called fractals. A "fractal" is a

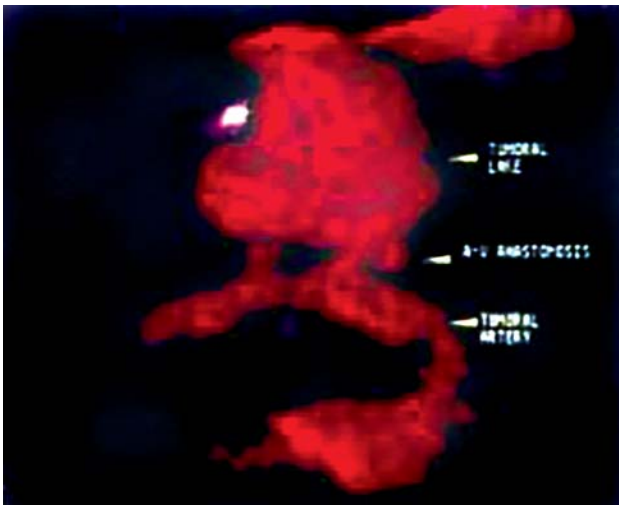


Fig. 20: Malignant tumor neovascularization is characterized by arterio-venous (A-V) shunts, stenosis and blind-ending lakes. All these features can be assessed using three-dimensional power Doppler imaging

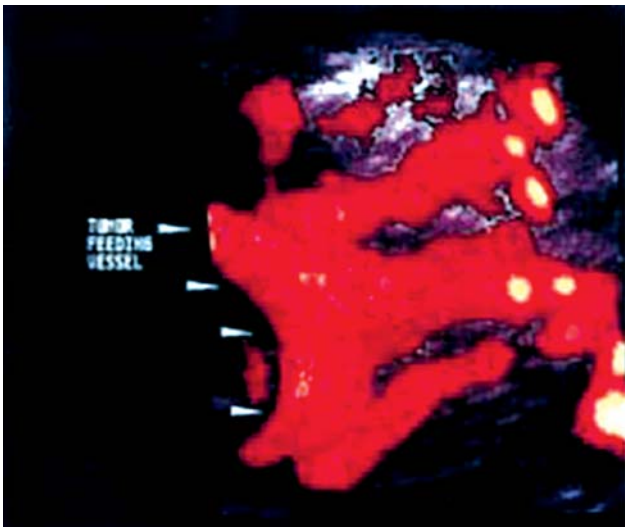


Fig. 21: Three-dimensional power Doppler scan of a malignant tumor neovascularization. Note the three generations of the vessels, areas of stenosis, microaneurysms and tumoral lakes

Fig. 22: Fern leaf as example for fractal geometry

shape that yields detail forever, no matter how far one zooms in. It is best compared to the trunk of a tree sprouting branches which in turn split off into smaller branches which themselves yield twigs again. If the underlying rule changes, the branching pattern must change too. We postulate that blood vessel trees are an example of fractal geometry [85] (Fig. 22).

The normal blood vessel trees (arteries and veins) form branched structures with ever-smaller branches and diameters. The underlying proposition of our hypothesis is that there is a change in fractal dimension when the branching becomes unregulated by abnormal processes, i.e. when the ordered growth is replaced by disordered growth.

Currently, the application of 3D power Doppler in the evaluation of neoplasms is mainly quantitative or semi quantitative for example in detecting whether the vascularity is present or not. Further development of the technology and the introduction of 3D quantification of blood flow, called the 3D color histogram, may improve our ability to differentiate between benign and malignant tumors and predict tumor prognosis.[86-88] The 3D color histogram measures the color percentage and flow amplitudes in the volume of interest. The histogram enables the vascularization and blood flow within a tissue block to be quantified, in contrast to 2D color histograms in which only single planes can be investigated.

Pairleitner et al reported on the use of cube method for measurement of blood flow and vascularization in 3D perspective.[87] The vascularization index (VI) represents the vessels in the tissue and is important for situation of both high and low vascularization. The flow index (FI), a mean color value, is important for characterizing high flow intensities (presumably tumors). The vascularization flow index (VFI) is a combination of VI and FI, and identifies the extremes between low vascularization and low blood flow on one side, and high vascularization and high blood flow, on the other. Although both VI and FI showed excellent reproducibility, VFI did not achieve accurate estimation between two observations, which may lead to unreliable measurement. It is expected that VI and FI may become good predictors for tumoral neovascularization, that can replace qualitative or semiquantitative 3D power Doppler evaluation [87].

Kudla and Alcazar in 2009 gave a short comprehensive surview of 3D PD Imaging of Ovarian Pathology, explaining 3D vascularization indices like Vascularization Index (VI), Flow Index (FI), and Vascularization-Flow Index (VFI), and their use in diagnosing advanced, metastatic, and early stage ovarian tumors captured in a defined Region of Interest (ROI). In an attempt to standardize this new technique, they

suggested standard settings of power Doppler gain control to avoid interpretation errors caused for instance by extensive color "leaking": for Voluson 730 Expert (Beta 05 version, RIC 5-9 H TVS probe) which they were using, they recommended PRF = 0.6 kHz and gain 0.8.[89]

Intravenous contrast agents for ultrasound studies are since several years commercially available.[90,91] With the utilization of ultrasound contrast agents perfusion imaging of the tumors has become more clear and provides meaningful physiological and pathological information for clinical decision making. They are of particular importance in cases of "slow flow, low flow and no flow". One of the most exciting areas is the potential for using 3D power circulation which enhance the ability to visualize vessel continuity more completely (in three orthogonal projections), and demonstrate vessel branching (3D vascular reconstruction) more clearly. The 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.[77]

Our group reported on the use of contrast-enhanced 3D power Doppler sonography for the differentiation of adnexal lesions.[92] We found that contrast enhanced, 3D power Doppler sonography provided better visualization of tumor vascularity in suspicious adnexal lesions than that obtained with noncontrast enhanced, 3D power Doppler sonography, and this led to a more exact differential diagnosis (Figs 23 to 25).

With respect to differential diagnosis between malignant and benign ovarian lesions, contrast enhanced, 3D power Doppler sonography reached diagnostic sensitivity and specificity of 100% and 93.9%, respectively. The positive and negative predictive value of this method were 85.7% and 100%, respectively. Therefore, the diagnostic efficiency was improved with the use of sonographic contrast agent from 86.7 to 95.6%. Furthermore, our results show that the pattern of irregularly branching penetrating vessels in suspicious adnexal lesions demonstrated on 3D power Doppler ultrasound with or without contrast enhancement is an important feature that should be considered with other sonographic criteria to predict the likelihood of malignancy.If used together with 3D morphologic ultrasound assessment, enhanced 3D power Doppler imaging might precisely discriminate benign from malignant adnexal lesions (Fig. 26).

Zagreb Experience

The question which ultrasound technique and diagnostic criteria provides the best adnexal lesion characterization has not been answered sufficiently. However, successful adnexal

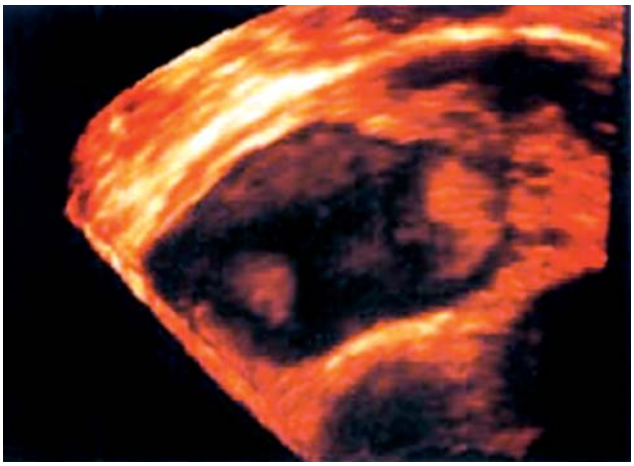


Fig. 23: Three-dimensional scan of a cystic solid tumor containing papillary projections

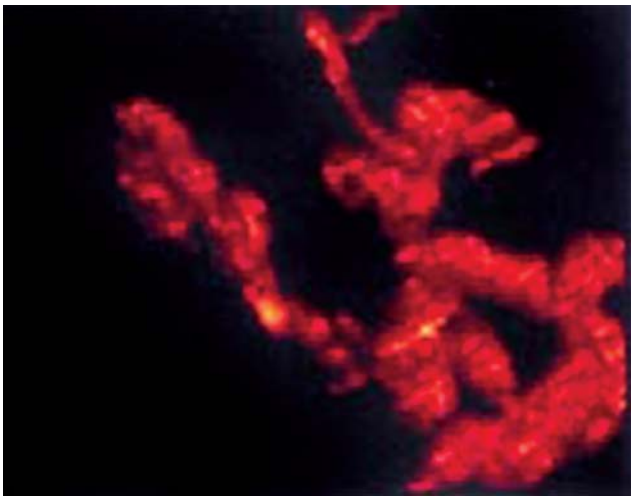


Fig. 24: Non-enhanced three-dimensional power Doppler image of the same tumor. Less than three generations of penetrating intratumoral vessels are displayed within the solid part of the lesion

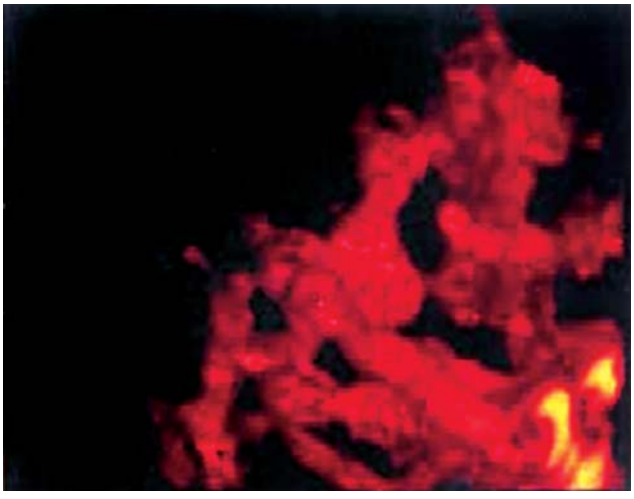


Fig. 25: Enhanced three-dimensional power Doppler scan depicts more than four generations of penetrating intratumoral vessels with irregular course and numerous shunts. Histopathology revealed ovarian malignancy

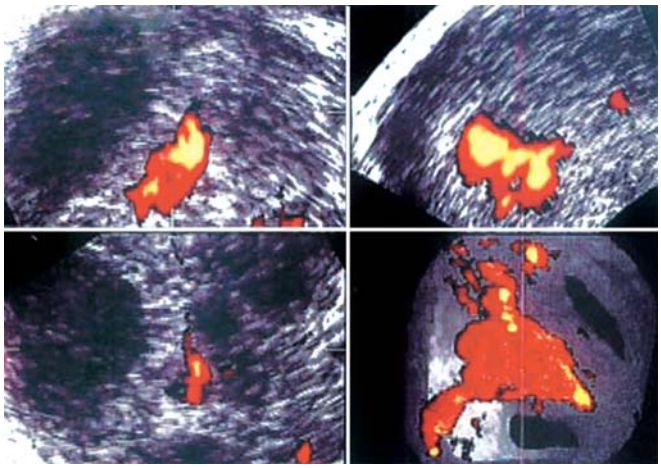


Fig. 26: Simultaneous rendering of all three orthogonal sectional planes, demonstrating a vasculature of a complex adnexal tumor after instillation of the echo-enhancing contrast. Note the three-dimensional view of the tumoral microcirculation on the lower right figure

mass pattern recognition obviously also depends on the performance of the sonographer.[93]

The first multicenter study evaluating the ability of ultrasound examiners using subjective evaluation of gray scale and Doppler ultrasound findings (pattern recognition) to make a specific diagnosis of an adnexal mass, was conducted in 2009 by Sokalska et al. According to their results, it currently seems possible to make an almost conclusive diagnose of dermoid cyst, endometrioma, hydrosalpinx and peritoneal pseudocyst, but beyond that any other histological diagnosis cannot be made conclusively.[94]

Alcazar et al in 2009 examined 173 patients (medium age 52.4 years) with adnexal masses out of which 117 were malignant and 56 benign. After logistic regression analysis only central blood flow in solid tumor areas, and presence of ascites were identified as independent predictors of malignancy, both features correlating to malignancy in 98.6%. The absence of both was found in 82.1% of benign tumors.[95]

Our study [96] summarized a four years experience in classifying an ultrasound examination as suggestive or indicative for adnexal and endometrial malignancy. Subjects for this investigation were 292 patients who were evaluated by five complementary methods in the preoperative sonographic assessment at our unit from January1997 to September 2000. The inclusion criteria were clinical and/or ultrasound d diagnosis of a pelvic mass of probable intra- or extra-adnexal origin, and/or postmenopausal bleeding. Adnexal and/or endometrial morphology and thickness/volume by two-dimensional transvaginal and three-dimensional ultrasound, as well as blood flow analysis by transvaginal color and pulsed Doppler and three-dimensional power Doppler examination were performed in all the examined patients. Two hundred and fifty-one patient with adnexal masses and 41 patients with endometrial lesions were evaluated in respect of gray scale ultrasonography, adnexal/endometrial volume measurement and vascularity assessment. There were 30 histologically confirmed adnexal malignant tumors and 221 benign adnexal masses. Furthermore, histopathology revealed 9 cases of endometrial carcinoma, and 32 benign endometrial lesions (18 cases of endometrial hyperplasia, 8 cases of endometrial polyps and 6 patients with atrophic or normal endometrium). Morphologic assessment by three-dimensional ultrasound yielded additional information in 58% of cases of adnexal malignancy especially considering small papillary projections (< 3 mm), thick septa within the inner surface of wall structure and relationship with surrounding structures in comparison with two-dimensional ultrasound (Tables 3 and 4) (Figs 27 and 28).

Furthermore, this modality was superior to two-dimensional ultrasound in accurate depiction and diagnosis of two cases of Fallopian tube carcinoma, by detecting intraluminal papillary projections and pseudosepta. Three-dimensional ultrasound enabled precise detection of tubal pathology in both cases by simultaneous observation of the neighboring structures: uterus and ipsilateral ovary. With respect to differential diagnosis between malignant and benign adnexal lesions, better morphological assessment by three-dimensional ultrasound slightly improved sensitivity, specificity, positive and negative predictive value in comparison with two-dimensional sonography morphology indexing (Table 5).

The application of three-dimensional power Doppler significantly improved results of morphology indexing. Combined morphology and vascular index ing revealed only one false positive (retroperitoneal fibromatosis) and one false negative finding (serous cystadenocarcinoma). Demonstration of the chaotic, randomly dispersed vessels with irregular branching was suggestive of adnexal malignancy. Other structural abnormalities of the malignant tumoral vessels were microaneurysms, arteriovenous shunts, tumoral lakes, disproportional calibration, coiling and dichotomous branching (Figs 29 and 30).

It seems to us that this technique has brought us a little closer to better understanding of malignant angiogenesis. Our study demonstrated that the qualitative analysis of the tumor vascularity architecture, when added to morphological parameters, is clinically pertinent , reaching a sensitivity and specificity of 97% and 99%, respectively (Table 6).

Table 3: Two-dimensional sonographic and color Doppler criteria for the diagnosis of adnexal malignancy

Volume	< 10 cm^3	0
	> 10 cm^3	2
Cyst wall	smooth < 3 mm	0
thickness/structure	smooth > 3 mm	1
	papillarities < 3 mm	1
	papillarities > 3 mm	2
Septa	no septa	0
	thin septa < 3 mm	1
	thick septa > 3 mm	2
Solid parts	solid area < 1 cm	1
	solid area > 1 cm	2
Echogenicity	sonolucency/low level echo	0
	mixed/high level echo	2
Tumoral blood flow	RI > 0.42	0
	RI 0.42	2

Total score=sum of individual scores. Cut-off score greater or equal of 4 for morphology index and greater or equal to 6 for combined (morphology and vascular) index was associated with high-risk of adnexal malignancy

Table 4: Three-dimensional sonographic and power Doppler criteria for the diagnosis of adnexal malignancy

Volume	< 10 cm^3	0
	> 10 cm^3	2
Cyst wall	smooth < 3 mm	0
thickness/structure	smooth > 3 mm	1
	papillarities < 3 mm	1
	papillarities > 3 mm	2
Septa	no septa	0
	thin septa < 3 mm	1
	thick septa > 3 mm	2
Solid parts	solid area < 1 cm	1
	solid area > 1 cm	2
Echogenicity	sonolucency/low level echo	0
	mixed/high level echo	2
Relationship with	normal	0
surrounding structures	disturbed	1
Vessels' architecture	linear	0
	chaotic	2
Branching pattern	simple	0
	complex	2

Total score: sum of individual scores. Cut-off score greater or equal of 5 for morphology index and greater or equal to 7 for combined index was associated with high-risk of adnexal malignancy

Table 5: Sensitivity, specificity, positive (PPV), and negative predictive values (NPV) of 2D US and 3D US in detection of adnexal malignancy

Technique	*Sensitivity%*	*Specificity%*	*PPV%*	*NPV%*
2D US	80	95	71	97
3D US	87	96	74	98

Table 6: Sensitivity, specificity, positive (PPV), and negative predictive values (NPV) of 2D US/TVCD, 3D US/3D PD and contrast enhanced 3D PD in detection of adnexal malignancy

Technique	*Sensitivity%*	*Specificity%*	*PPV%*	*NPV%*
2D US/TVCD	89	98	86	99
3D US/3D PD	97	99	97	99
enhanced 3D PD	100	99	93	100

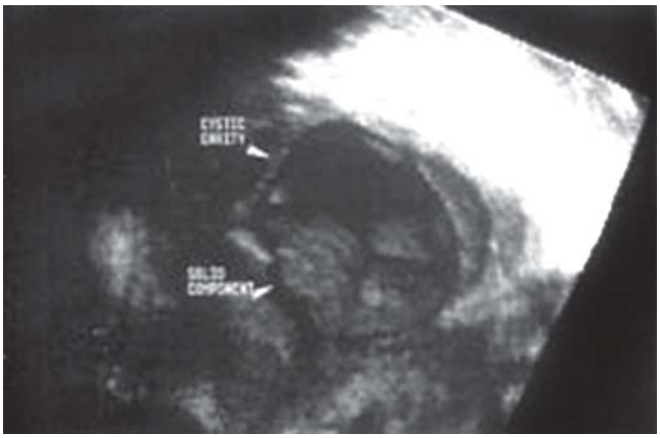


Fig. 27: Complex ovarian tumor as seen by three-dimensional ultrasound. Surface view demonstrates a solid part within cystic cavity. The morphology was suggestive of ovarian malignancy, which was confirmed by histopathology

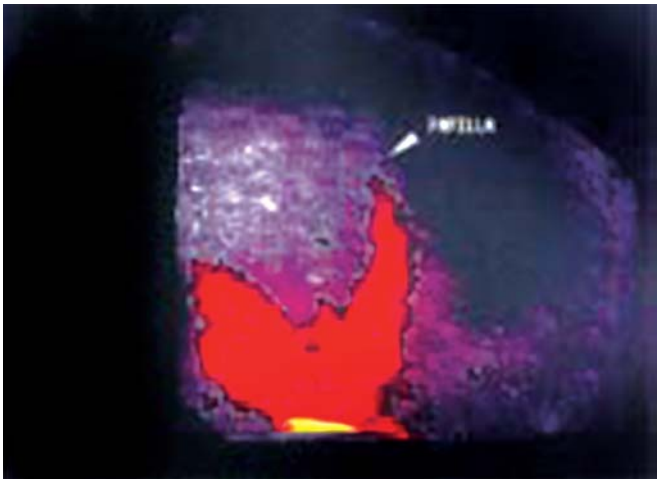


Fig. 28: The volume has been rotated in all three dimensions, allowing visualization of the tumoral vessels with an irregular course and branching

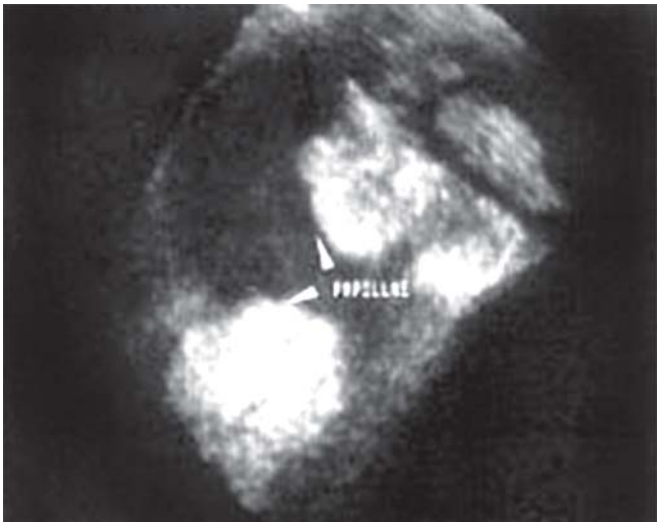


Fig. 29: Complex ovarian tumor as seen by three-dimensional ultrasound. Surface view revealed irregular wall proliferations suggestive of ovarian malignancy

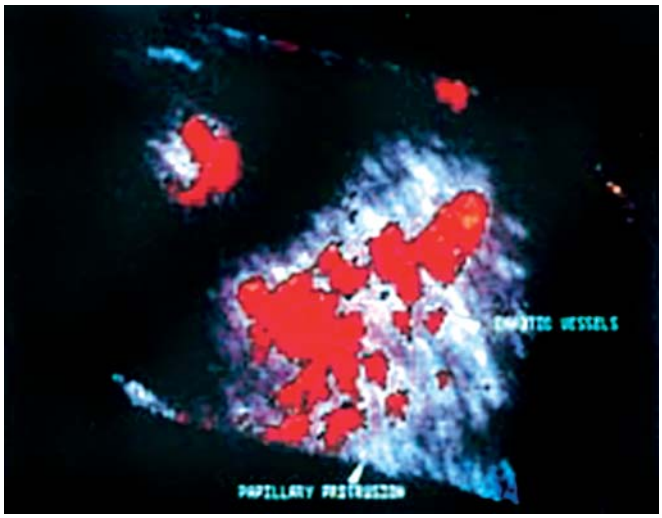


Fig. 30: Three-dimensional power scan of malignant tumor vessels located in a papillary projection. Note their irregular course and branching

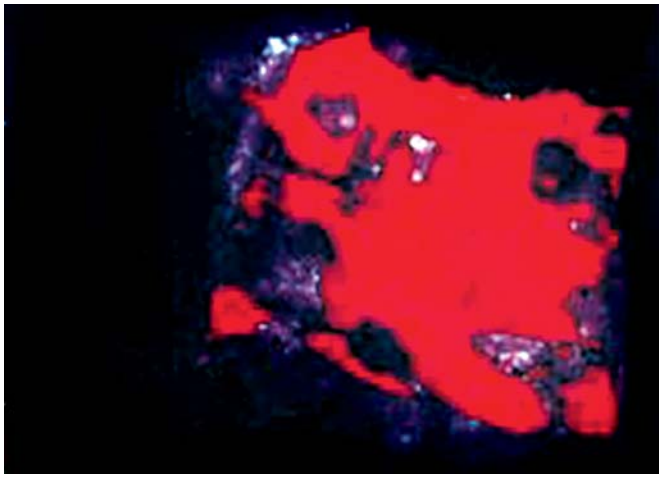


Fig. 31: Three-dimensional power Doppler scan of a stage I ovarian carcinoma in a postmenopausal patient. Randomly dispersed vessels within irregular branching were the only finding indicating ovarian malignancy what was confirmed at the time of surgery

Contrast enhanced three-dimensional power Doppler, performed in 89 patients with complex adnexal masses, improved sensitivity (100%), but regarding the criterion "highly suspicious pattern of tumoral vasculature", two false-positive cases (ovarian fibroma, and fibromatosis retroperitonealis) were diagnosed. In one postmenopausal patient, with a normal size ovary (10.1cm^3), and a solid area of 0.9 cm, three-dimensional power Doppler revealed highly suspicious vascular pattern and the combined score for this ultrasound modality was 7, highly predictive of malignancy. Later, this case was classified as IA stage of ovarian malignancy (Fig. 31).

Furthermore, prior to entering this study, we examined 84 healthy premenopausal patients in early proliferative phase of menstrual cycle, and 63 postmenopausal patients without adnexal or uterine lesions in order to estimate values of ovarian volume. All the examined patients had no history of use of oral contraception, ovarian stimulating drugs and/or hormonal replacement therapy. In premenopausal patients, the mean ovarian volume obtained by two-dimensional ultrasound was 5.8 ± 0.06 cm^3 , and in the postmenopausal group 2.6 ± 0.03 cm^3. Ovarian volume obtained by automatic volume measurement with three-dimensional ultrasound in premenopausal patients was 5.8 ± 0.03 cm^3, while in postmenopausal patients, the mean volume was 2.6 ± 0.01 cm^3. No differences between the volumes of the right and the left ovary were found in pre- and postmenopausal patients using both methods. Similarly, no difference was obtained in terms of ovarian volume measurement by two-dimensional and three-dimensional ultrasound. The overall ovarian volume, of the various neoplasms, obtained in this study, was for each age group significantly above the normal ovarian volume (except one case). Furthermore statistical difference in mean ovarian volume was obtained for benign *vs* malignant ovarian masses (109 cm^3 *vs* 274 cm^3) (Table 7).

Table 7: Ovarian volume obtained by automatic volume measurement by three-dimensional ultrasound

	Mean ovarian volume (cm^3) ± SD
Premenopausal patients	5.8 ± 0.03
Postmenopausal patients	2.6 ± 0.01
Benign ovarian masses	109
Malignant ovarian masses	274

This may suggest: the greater the size of ovarian tumor, the higher the risk of ovarian malignancy, and in this light, ovarian volume measurements may assist in the diagnosis of ovarian neoplasms.

Intracystic proliferations are important predictors of malignancy. Saunders et al in June 2010 assessed1319 patients who had a total of 2870 septated cystic ovarian tumors, without solid areas or papillary projections. There were no cases of ovarian cancer. Patients were followed from 4 to 252 months (mean-77 months).[97]

FUTURE CHALLENGES

Today, most traditional cancer treatments involve some combination of surgery, chemotherapy and radiation. All of these treatment options target the tumor cells directly. In contrast, selectively targeting endothelial cells, the building blocks of blood vessels, could directly inhibit the capillary support system that enables a tumor to grow. The widely held view is that these antiangiogenic therapies should destroy the tumor vasculature, thereby depriving the tumor of oxygen and nutrients.

The dependence of tumor growth on new blood vessel formation makes angiogenesis inhibition a promising treatment option for malignant disease. Encouraging results came out using monoclonal anti-VEGF antibodies or small molecule VEGF inhibitors in different cancer types.

Various additional VEGF-based and non-VEGF-based antiangiogenic therapies will be evaluated in phase II and III studies over the coming years, which will provide more clarity regarding their potential clinical utility.

The recognition of angiogenesis and the potential for antiangiogenic therapies represents a dramatic paradigm shift in medicine and in the evolution of cancer treatment. Boehm and colleagues' have shown that three different mouse tumors, treated with repeated cycles of a newly discovered angiogenesis inhibitor called endostatin, regressed, did not become drug resistant and, after a characteristic number of treatment cycles, became dormant.[98] Such a treatment strategy could help circumvent many of the problems associated with current chemo-therapeutic 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. The results of cyclic endostatin therapy strongly suggests that drugs targeting angiogenesis and the tumor vasculature could become a major new weapon for effectively treating, and indeed preventing, 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 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 meta-state of proliferation balanced apoptosis.[99] Jain in 2005 presented a different hypothesis on antiangiogenic drug efficacy, stating that there is emerging evidence to support that certain antiangiogenic agents can also transiently "normalize" the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery. Drugs that induce vascular normalization can alleviate hypoxia and increase the efficacy of conventional therapies if both are carefully scheduled [100].

Another important question arises: are there tissue-specific differences in the vasculature and consequently in tumor vessel anatomy that affect a tumor's susceptibility to inhibition/disruption? Could 3D power Doppler help in answering these questions? This question is undoubtedly a challenge for all ultrasonographers.

CONCLUSION

Color and pulsed Doppler sonography demonstrates the vascularity of an adnexal mass, facilitating conclusions regarding tumor histology and metabolism. Therefore, blood flow data should be considered to indicate the angiogenic intensity of a tumor, rather than indicating malignancy itself. It seems clear that initial attempts to classify ovarian tumors solely on the basis of their impedance to blood flow have been too simplistic.

The results reported in literature on three-dimensional color Doppler are indeed provocative and, not surprisingly, raise many new questions about the regulation of tumor angiogenesis, the density of tumor vessels and the difference between vessel architecture in benign and malignant growths. Three-dimensional Doppler depiction of tumor angiogenesis has many clinical implication among which most important early detection of ovarian and endometrial cancers. Improved detection and classification of tumor architecture leads to improved diagnostic accuracy , and consequently reduction of false-positive findings and unnecessary invasive procedures. Undoubtedly, techno-logical development and further improvement of real time 3D ultrasound imaging will contribute to more objective evaluation of adnexal tumor morphology and vascularity, which might result in a significant reduction of morbidity and mortality, especially from ovarian cancer.

REFERENCES

1. Givens V, Mitchell GE, Harraway-Smith C, Reddy A, Maness DL. Diagnosis and management of adnexal masses. Am Fam Physician. 2009 Oct 15;80(8):815-20.
2. Koonings PP, Campbell K, Mishell DR (Jr), Grimes DA. Relative frequency of primary ovarian neoplasms: A ten year review. Obstet Gynecol 1989;74:921-26.
3. Le T, Giede C, Salem S, Lefebvre G, Rosen B, Bentley J, Kupets R, Power P, Renaud MC, Bryson P, Davis DB, Lau S, Lotocki R, Senikas V, Morin L, Bly S, Butt K, Cargill YM, Denis N, Gagnon R, Hietala-Coyle MA, Lim KI, Ouellet A, Raciot MH. Initial evaluation and referral guidelines for management of pelvic/ovarian masses. J Obstet Gynaecol Can. 2009;31(7):668-80.
4. Kinkel K, Hricak H, Lu Y, Tsuda K, Filly R. US characterization of ovarian masses: A meta-analysis. Radiology 2000;217: 803-11.
5. Guerriero S, Alcazar JL, Coccia ME, Ajossa S, Scarselli G, Boi M, Gerada M, Melis GB. Complex pelvic mass as a target of evaluation of vessel distribution by color Doppler sonography for the diagnosis of adnexal malignancies: Results of a multicenter European study. Journal Ultrasound Med 2002;21:1105-11.
6. Marret H, Ecochard R, Giraudeau B, Golfier F, Raudrant D, Lansac J. Color Doppler energy prediction of malignancy in adnexal masses using logistic regression models. Ultrasound Obstet Gynecol 2002;20(6):597-604.

7. Warren BA. The vascular morphology of tumors. In Peterson HI (Ed): Tumor Blood Circulation: Angiogenesis, vascular morphology and blood flow of experimental human tumors. Boca Raton, FL: CRC Press, 1979:1-47.
8. Coman DR, Sheldon WF. The significance of hyperemia around tumor implants. Am J Pathol 1946;22:821-26.
9. Algire GH, Chalkley HW, Lagallais FY, Park HD. Vascular reactions of mice to wounds and to normal and neoplastic transplants. J Natl Cancer Inst 1945;6:73-85.
10. Folkman J, Long D, Becker F. Growth and metastasis of tumor in organ culture. Tumor Res 1963;16:453-67.
11. Folkman J, Cole P, Zimmerman S. Tumor behaviour in isolated perfused organs: In vitro growth and metastasis of biopsy material in rabbit thyroid and canine intestinal segment. Ann Surg 1966;164:491-502.
12. Folkman J, Merler E, Abernathy C, Williams G. Isolation of a tumor factor responsible for angiogenesis. J Exp Med 1971;133:275-88.
13. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev Physiol 1991;53:217.
14. Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1989;82:4-6.
15. Auerbach R. Angiogenesis-inducing factors: A review. In: Pick E (Ed): Limphokines, London: Academic Press,1981;4:69-88.
16. Rak JW, St Croix DB, Kerbel RS. Consequences of angiogenesis for tumor progression, metastasis and cancer therapy. Anti-Cancer Drugs 1995;6:3-18.
17. Plate KH. From angiogenesis to lymphangiogenesis. Nature Med 2001;7(2)151-52.
18. Skobe M, Rockwell P, Vosseler S, Fusenig NE. Halting angiogenesis suppresses carcinoma cell invasion. Nature Med 1997;3:1222-27.
19. Gasparini G, Weidner N, Maluta S, et al. Intratumoral microvessel density and p53 correlation with metastasis in head-and-neck squamous-cell carcinoma. Int J Cancer 1993;55: 739-44.
20. Gasparini G, Weidner N, Bevilacqua P, et al. Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. J Clin Oncol 1994;12:454-66.
21. Weidner N. Tumor angiogenesis: Review of current applications in tumor prognostication. Semin Diagn Pathol 1993;10:302-313.
22. Folkman J, Klagsburn M. Angiogenic factors. Science 1987;2345:442-47.
23. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146:1029-039.
24. Plate KH, Breier G, Weich HA, Rissau W. Vascular endothelial growth factor is a potential tumor angiogenesis factor in human gliomas in vivo. Nature 1992;359:845-48.
25. Kim KJ. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo. Nature 1993; 362:841-44.
26. Lu JJ, Stanczyk FZ, Zeng W. Expression of VEGF in ovarian epithelial tumors. J Soc Gynecol Investig 2001; 8(1)(Suppl):766.
27. Pugh CW, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: Role of the HIF system. Nat Med 2003;9(6):677-84.
28. Vincent KA, Shyu KG, Luo Y, Magner M, Tio RA, Jiang C, Goldberg MA, Akita GY, Gregory RJ, Isner JM. Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1 alpha/VP16 hybrid transcription factor. Circulation 2000;102(18):2255-61.
29. Samani AA, Yakar S, LeRoith D, Brodt P. The role of the IGF system in cancer growth and metastasis: Overview and recent insights. Endocr Rev 2007;28(1):20-47.
30. Folkman J. Growth and metastasis of tumor in organ culture. Cancer 1963;16:453-57.
31. Guilino PM. Extracellular compartments of solid tumors. In Becker J (Ed): Cancer. New York: Plenum Press, 1975:327.
32. Jain RK. Determination of tumor blood flow: A review. Cancer Res 988;48:2641-46.
33. Kurjak A, Kupesic S, Breyer B. The assessment of ovarian tumor angiogenesis by three-dimensional power Doppler. In Kurjak A (Ed): Three-dimensional Power Doppler in Obstetrics & Gynecology. The Parthenon Publishing Group, 2000.
34. Jain RK, Ward-Harley K. Tumor blood flow characterization, modifications and role in hyperthermia. Trans Sonics Ultrasonics 1984;31:504-09.
35. Vaupel P, Kallinowski F, Okunieff P. Blood flow oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. Cancer Res 1989;49:6449.
36. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995;146(5):1029-39.
37. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA. Selective requirement for Src kinases during VEGF-induced angiogenesis and vascular permeability. Mol Cell 1999;4(6): 915-24.
38. Fleischer AC. Sonographic depiction of tumor vascularity and flow: From in vivo models to clinical applications. J Ultrasound Med 2000;19:55-61.
39. Hanahan D, Folkman J. Parameters and emerging mechanisms of the angiogenetic switch during tumorigenesis. Cell 1996;86:353-54.
40. Kurjak A, Kupesic S, Ilijas M, Sparac V, Kosuta D. Preoperative diagnosis of primary fallopian tube carcinoma. Gynecol Oncol 1998;68:29-34.
41. Kurjak A, Jukic S, Kupesic S, Babic D. A combined Doppler and morphopathological study of ovarian tumors. Eur J Obstet Gynecol Reprod Biol 1997;71:147-50.
42. Emoto M, Iwasaki H, Mimura K, Kawarabayashi T, Kikuchi M. Differences in the angiogenesis of benign and malignant ovarian tumors, demonstrated by analyses of color Doppler ultrasound, immunohistochemistry, and microvessel density. Cancer 1997;80:899-907.
43. Suren A, Osmers R, Kuhn W. 3D Color Power Angio TM imaging: A new method to assess intracervical vascularization in benign and pathological conditions. Ultrasound Obstet Gynecol 1998;2:133-38.
44. Kurjak A, Kupesic S. Three-dimensional ultrasound and power Doppler in assessment of uterine and ovarian angiogenesis: A prospective study. Croat Med J 1999;40:413-20.
45. Bourne TH, Campbell S, Steers CV, Whitehead MI, Collins WP. Transvaginal colour flow imaging: A possible new screening technique for ovarian cancer. Br Med J 1989;299:1367-70.
46. Kurjak A, Zalud I, Jurkovic D, Alfirevic Z, Miljan M. Transvaginal color Doppler of the assessment of pelvic circulation. 1989;68:131-36.

47. Fleisher AC, Rodgers WH, Rao BJ, Keppler DM, Worrel JA, Williams L, Jones HW III. Assessment of ovarian tumor vascularity with transvaginal color Doppler sonography. J Ultrasound Med 1991;10:563-68.
48. Kurjak A, Zalud I, Alfirevic Z. Evaluation of adnexal masses with transvaginal color ultrasound. J Ultrasound Med 1991;10: 295-97.
49. Weiner Z, Thaler I, Beck D, Rottem S, Deutsch M, Brandes JM. Differentiating malignant from benign ovarian tumors with transvaginal color flow imaging. Obstet Gynecol 1992;79:159-62.
50. Kurjak A, Schulman H, Sosic A, Zalud I, Shalan H. Transvaginal ultrasound, color flow, and Doppler waveform of the postmenopausal adnexal mass. Obstet Gynecol 1992;80:917-21.
51. Hamper UM, Sheth S, Abbas FM, Rosenshein BN, Aronson D, Kurman JR. Transvaginal color Doppler sonography of adnexal masses: Differences in blood flow impedance in benign and malignant lesions. Am J Roentgenol 1993;160:1225-28.
52. Carter J, Saltzman A, Hartenbach E, Fowler J, Carlson L, Twiggs LB. Flow characteristics in benign and malignant gynecologic tumors using transvaginal color flow Doppler. Obstet Gynecol 1994;83:125-130.
53. Hata K, Kata T, Kitao M. Intratumoral peak systolic velocity as a new possible predictor for detection of adnexal malignancy. Am J Obstet Gynecol 1995;172:1496-1500.
54. Kurjak A, Jukic S, Kupesic S, Babic D. A combined Doppler and morphopathological study of ovarian tumors. Eur J Obstet Gynecol Reprod Biol 1997;71:147-150.
55. Levine D, Feldstein VA, Babcook CJ, Filly RA. Sonography of ovarian masses: Poor sensitivity of resistive index for identifying malignant lesions. Am J Roentgenol 1994;162:1355-59.
56. Kurjak A, Kupesic S. Transvaginal color Doppler and pelvic tumor angiogenesis: Lessons learned and future challenges. Ultrasound Obstet Gynecol 1995;6:145-59.
57. Kurjak A, Shalan H, Kupesic S, Predanic M, Zalud I, Breyer B, Jukic S. Transvaginal color Doppler sonography in the assessment of pelvic tumor vascularity. Ultrasound Obstet Gynecol 1993;3:137-54.
58. Brown D, Frates M, Laing F, et al. Ovarian masses: Can benign and malignant lesions be differentiated with color and pulsed Doppler US? Radiology 1994;190:333-36.
59. Stein SM, Laifer-Narin S, Johnson MB, Roman LD, Muderspach LI, Tyszka JM, Ralls PW. Differentiation of benign and malignant adnexal masses: Relative value of gray-scale, color Doppler, and spectral Doppler sonography. Am J Roentgenol 1995;164:381-86.
60. Fleischer AC, Cullinan JA, Peery CV, et al. Early detection of ovarian carcinoma with transvaginal color Doppler ultrasonography. Am J Obstet Gynecol 1996;174:101-06.
61. Rubin JM, Bude RO, Carson PL, Bree Rl, Adler RS. Power Doppler US: A potentially useful alternative to mean frequency-based color Doppler US. Radiology 1994;190:853-56.
62. Rubin JM, Adler RS, Fowlkes JB, Spratt S, Pallister JE, Chen JF, Carson PL. Fractional moving blood volume: Estimation with power Doppler US. Radiology1995;197:183-90.
63. Winsberg F. Power Doppler sonography. J Ultrasound Med 1996;15:164.
64. Papadimitriou A, Kalogirou D, Antonio G, Petridis N, Kalogirou O. Power Doppler ultrasound: A potential useful alternative in diagnosing pelvic pathological conditions. Clin Exp Obstet Gynecol 1996;23:229-32.
65. Burns PN. Harmonic imaging with ultrasound contrast agents. Clin Radiol 1996;51:50-55.
66. Meyerovitz CB, Fleischer AC, Pickens DR, et al. Quantification of tumor vascularity and flow with amplitude color Doppler sonography in an experimental mode: Preliminary results. J Ultrasound Med 1996;15:827.
67. Fleischer A, Wojcicki W, Donnely E, et al. Quantified color Doppler sonography of tumor vascularity in an animal model. J Ultrasound Med 1999;18:547.
68. Bonilla-Musoles F, Raga F, Osborne NG. Three-dimensional ultrasound evaluation of ovarian masses. Gynecol Oncol 1995;59:129-35.
69. Merz E. Three-dimensional transvaginal ultrasound in gynecological diagnosis. Ultrasound Obstet Gynecol 1999; 14:81-86.
70. Geomini PM, Coppus SF, Kluivers KB, Bremer GL, Kruitwagen RF, Mol BW. Is three-dimensional ultrasonography of additional value in the assessment of adnexal masses? Gynecol Oncol. 2007;106(1):153-59.
71. Mansour GM, El-Larnie I, El-Sayed H, Ibrahim A, Laban M, Abou-Louz S, Sobhi K, Abd Allah M, et al. Adnexal mass vascularity assessed by 3-dimensional power Doppler: Does it add to the Risk of Malignancy Index in prediction of ovarian malignancy? International Journal of Gynecological Cancer 2009;19(5):867-72.
72. Chase DM, Crade M, Basu T, Saffari B, Bergman ML. Preoperative diagnosis of ovarian malignancy: Preliminary results of the use of 3-dimensional vascular ultrasound. Int J Gynecol Cancer 2009;19(3):354-60.
73. Chan L, Lin WM, Verpairojkit B, Hartman D, Reece EA. Evaluation of adnexal masses using three-dimensional ultrasonographic technology: Preliminary report. J Ultrasound Med 1997;16:349-54.
74. Weber G, Merz E, Bahlmann F, Macchiella D. Ultrasound assessment of ovarian tumors: Comparison between transvaginal 3D technique and conventional 2 dimensional vaginal ultrasonography. Ultraschall Med 1997;18(1):26-30.
75. Riccabona M, Nelson TR, Pretorius DH. Three-dimensional ultrasound: Accuracy of distance and volume measurements. Ultrasound Obstet Gynecol 1996;7:429-34.
76. Merce LT, Alcazar JL, Barco MJ, et al. Can 3-dimensional Ultrasound change gynecological ultrasonographic examination? Donald School Journal of Ultrasound in Obstet/Gynecol 2009;3(1):6-8.
77. Weber G, Merz E, Bahlman F. Three-dimensional sonography of cystic ovarian tumors. In: Merz E (Ed). 3D ultrasonography and gynecology. Lippincott Williams & Wilkins, Philadelphia 1998.
78. Kurjak A, Kupesic S. Ovarian lesions assessed by three-dimensional ultrasound and power Doppler. In: Kurjak A, Kupesic S (Eds). Clinical application of 3D sonography. The Parthenon Publishing Group 2000.
79. Kurjak A, Kupesic S, Jacobs I. Preoperative diagnosis of the primary Fallopian tube carcinoma by three-dimensional static

and power Doppler sonography. Ultrasound Obstet Gynecol 2000;15:246-51.
80. Kurjak A, Kupesic S, Anic T, Kosuta D. Three-dimensional ultrasound and power Doppler improve the diagnosis of ovarian lesions. Gynecol Oncol 2000;76:28-32.
81. Downey BD, Fenster A. Vascular imaging with a three dimensional power Doppler system. Am J Roentgenol 1995;165:665-68.
82. Kurjak A, Kupesic S, Breyer B, Sparac V, Jukic S. The assessment of ovarian tumor angiogenesis: What does three-dimensional power Doppler add? Ultrasound Obstet Gynecol 1998;12:136-46.
83. Crade M. Tissue block ultrasound and ovarian cancer-a pictorial presentation of findings. Donald School Journal of Ultrasound in Obstetrics and Gynecology 2009;3(1):41-47.
84. Kurjak A, Kupesic S, Sparac V, Kosuta D. Three- dimensional ultrasonographic and power Doppler characterization of ovarian lesions. Ultrasound Obstet Gynecol 2000;16:365-71.
85. Breyer B , Kurjak A. Tumor vascularization Doppler measurements and chaos: What to do? Ultrasound Obstet Gynecol 1995;5:209-10.
86. Fleischer AC, Pairleitner H. Transvaginal Doppler assesses ovarian flow. Diagn Imag 1998;9:47.
87. Fleischer AC, Pairleitner H. 3D transvaginal color Doppler sonography: Current and potential applications. Med Imag Int 1999;9:10-13.
88. Pairleitner H, Steiner H, Hasenoehrl G, Staudach A. Three-dimensional power Doppler sonography: Imaging and quantifying blood flow and vascularization. Ultrasound Obstet Gynecol 1999;14:139-43.
89. Kudla M, Alcazar JL. 3D PD imaging of ovarian pathology-Advantages and limitation of the method: How can we standardize the results? Donald School Journal of Ultrasound in Obstetrics and Gynecology 2009;3(1):48-54.
90. Ophir J, Parker KJ. Contrast agents in diagnostic ultrasound. Ultrasound Med Biol 1989;15:319-33.
91. Goldberg BB, Merton AD, Forsberg F, Liu J, Rawool N. Color amplitude imaging: Preliminary results using vascular sonographic agents. J Ultrasound Med 1990;15:127-34.
92. Kupesic S, Kurjak A. Contrast enhanced three-dimensional power Doppler sonography for the differentiation of adnexal masses. Obstet Gynecol 2000;96:452-58.
93. Van Holsbeke C, Daemen A, Yazbek J, et al. Ultrasound methods to distinguish between malignant and benign adnexal masses in the hands of examiners with different levels of experience. Ultrasound Obstet Gynecol 2009;34:454-61.
94. Sokalska A, Timmerman D, Testa AC, et al. Diagnostic accuracy of transvaginal ultrasound examination for assigning a specific diagnosis to adnexal masses. Ultrasound Obstet Gynecol 2009;34:462-70.
95. Alcazar JL, Royo P, Pineda L, Ruiz-Zambrana, Auba M, Olartecoechea B. Which parameters could be useful for predicting malignancy in solid adnexal masses? Donald School Journal of Ultrasound in Obstetrics and Gynecology 2009;3(1): 1-5.
96. Kurjak A, Kupesic S, Sparac V, Bekavac I. Preoperative evaluation of pelvic tumors by Doppler and three-dimensional sonography. J Ultrasound Med 2001;20:829-40.
97. Saunders BA, Podzielinski I, Ware RA, Goodrich S, Desimone CP, Ueland FR, Seamon L, Ubellacker J, Pavlik EJ, Kryscio RJ, van Nagell JR (Jr). Risk of malignancy in sonographically confirmed septated cystic ovarian tumors. Gynecol Oncol 2010 Jun 14 (Pub Med ahead of publication).
98. Boehm T, Folkman J, Browder T, O'Reilly MS. Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance. Nature (London) 1997;390:404-07.
99. Holmgren L, O'Reilly MS, Folkman J. Dormancy of micro-metastases: Balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nature Med 1995;1:149-53.
100. Jain RK. Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science 2005;7:58-62.