REVIEW ARTICLE

Screening for Ovarian Cancer: The Possible Improvement by 3D Ultrasound and 3D Power Doppler

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Abstract

In developed countries more women die annually from ovarian cancer than from all other gynecologic malignancies combined. The fact that the ovaries are deep within the pelvic cavity and difficult to palpate is an obstacle to early diagnosis, especially in peri-post menopausal women, the group with the highest incidence of the disease. Seventy percent of patients are not diagnosed with the disease until the cancer has metastasized beyond the ovaries and is at stage 3 or 4. Patients with stage 3 or 4 have a 5-year survival rate of only 20-30%. Given the burden of suffering associated with ovarian cancer and the clear survival gradient related to the stage of disease at diagnosis, there is great need for development of effective screening methods for early detection of epithelial ovarian cancer. Better understanding of ovarian cancer etiology and increasing knowledge of tumor biology have both contributed to identify efficient Serum Tumor Markers, to screen high-risk populations. Technical advances in the field of ultrasound made transvaginal sonography (TVS) become the most important diagnostic tool, and multimodal (Serum markers plus TVS) screening appears to be a diagnostic break-through in fighting ovarian cancer. Five case reports illustrate that new ultrasound technologies such as 3D volume acquisition and 3D power Doppler imaging promise more reliable identification of an abnormal ovarian tumor vascularity and tumor-typical vascular architecture, thus facilitating early stage 1 – detection of the disease.

Keywords: Screening methods, Early detection, Transvaginal sonography, Multimodal screening.

WHY TO SCREEN FOR OVARIAN CANCER?

In developed countries more women die annually from ovarian cancer than from all other gynecologic malignancies combined. For example, in the United States approximately 22,000 new cases are diagnosed each year, and 15,000 of these women will die of the disease.[1]

Nondescript signs and symptoms make it the seventh leading cause of cancer related deaths in women.[2] In 2008, there were 21,650 cases reported which resulted in the deaths of 15,520 women in the United States.[3]

Symptoms usually do not become apparent until the tumor compresses or invades adjacent structures, ascites develops, or metastases become clinically evident. However, studies surveying ovarian cancer patients demonstrate that over 95% of EOC patients had abdominal complaints for many months before their diagnosis.[4] The fact that the ovaries are deep within the pelvic cavity and difficult to palpate is an obstacle to early diagnosis, especially in peri-post menopausal women, the group with the highest incidence of the disease. 70% of patients are not diagnosed with the disease until the cancer has metastasized beyond the ovaries and is at stage III or IV because of these reasons.[5]

Patients with stage 3 or 4 have a 5-year survival rate of only 20-30%, compared with the 5-year survival of over 90% in patients with stage IA ovarian cancer, when disease is confined to the ovary.[6] Given the burden of suffering associated with the development of ovarian cancer and the clear survival gradient related to the stage of disease at diagnosis,[7] there has always been much enthusiasm for the development of effective screening methods/assays for the early detection of epithelial ovarian cancer.

There are several different types of ovarian cancers depending upon the cell type of origin. Epithelial cell ovarian cancer (EOC) constitutes 90% of ovarian cancers, while gonadal-stromal (6% occurrence), and germ cell (4% occurrence) tumors make up the rest of the incidence of ovarian cancer patients.[8]

The stages (I-IV) of ovarian cancer are determined by the extent of metastasis. Stage I EOC is confined to the ovaries, stage II involves other pelvic structures. In stage III, the disease has spread beyond the pelvis into the upper abdominal cavity or into the draining nodal beds. Stage IV is defined as disease outside of the peritoneal cavity and often includes parenchymal liver lesions or malignant pleural

effusions. Patients with stage I disease most commonly undergo bilateral oophorectomy, hysterectomy, and surgical staging including peritoneal biopsies, omentectomy, and pelvic and aortic lymph node dissection. In select cases of younger patients who wish to preserve fertility, only the affected ovary may be removed and a hysterectomy would not be performed.[9]

Each cancer type typically *metastasizes* to different areas in the body. This phenomenon is called the "seed *vs* soil" hypothesis which was first observed by Stephen Paget in 1889.[10]

The "seed *vs* soil" observation applies in ovarian cancer: the most common sites of metastasis are within the peritoneal cavity. This is explained by the fact that mesothelial cells that express *mesothelin*, line the walls of the peritoneal cavity as well as the organs within it. Gubbels et al have shown that MUC16 (CA 125), present on the surface of cancer cells, binds readily to mesothelin.[11] The peritoneal dissemination of metastasis is facilitated by the clockwise flow of peritoneal fluid (PF).

DIFFICULTIES IN OVARIAN CANCER SCREENING

The ability to detect early-stage epithelial ovarian cancer by a simple test has long been desired, yet never achieved. Several aspects of ovarian cancer have led to the frustrations that have been encountered in attempts to screen for the disease.[12]

The time required for localized disease to progress to dissemination depends on the tumor type; therefore the appropriate interval at which to pursue screening is at this point chosen arbitrarily. Other impediments to screening relate to the low prevalence of ovarian cancer in the general population. Therefore, a screening method should have a specificity of 99.6% to achieve a positive predictive value of 10%, i.e. to limit the number of unnecessary surgical procedures to 10 for each case of cancer detected.[13] A specificity lower than this is likely to be unacceptable in the general population, although it may be acceptable to those with a positive family history of breast or ovarian cancer.

As ovarian cancer of epithelial cell origin (EOC) is the most common type, screening methods have to take into account the specific morphological and biochemical characteristics of this tumor group. The majority of EOC cases are sporadic in nature and occur in women with no known predisposing factors. Thus, in the general population, the overall risk of EOC is low (2-5%). Only a small percentage (5-10%) of EOC patients have a genetic predisposition to the disease. Ninety percent of these patients are carriers of mutated BRCA1 and/or BRCA2 genes, which are also implicated in hereditary breast cancer. These genes normally act as tumor suppressors and regulate cellular proliferation and DNA repair by maintaining chromosomal integrity. Mutations in these genes render the proteins unable to perform their intended functions. The lifetime risk of ovarian cancer for patients with BRCA1 mutations is 20% to 60%, and the risk for BRCA2 mutation carriers is 10% to 35%.[1,14]

The normal ovarian surface epithelium (OSE) covers the surface of the ovary. OSE is a monolayered squamous-to-cuboidal epithelium which functions to shuttle molecules in and out of the peritoneal cavity, as well as participates in the rupture and repair that accompanies every ovulation.[15] The OSE derive from the embryonic celomic epithelial cells which are a part of the mesoderm. The fallopian tube, uterus, and endocervix are derived from the Mullerian duct which is an invagination of the celomic epithelium. It is hypothesized that OSE cells retain the ability to differentiate into four major histological subtypes, which could explain the distinct histological EOC subtypes. There are four common sub-types of ovarian cancer of epithelial cell origin (EOC), including serous (fallopian tube-like), endometrioid (endometrium-like), mucinous (endocervical-like), and clear cell carcinoma (mesonephros-like).[2,15]

The differentiation of OSE cells from cuboidal epithelial cells to a mesenchymal phenotype that is characteristic of Mullerian duct derived tissues, is called epithelial-mesenchymal transition (EMT). The occurrence of EMT serves the purpose to aid cells in movement during embryo tissue generation, tissue regeneration after wounding, and obviously plays a role in the development of cancer.[16] OSE cells normally undergo EMT to heal the wound that forms following ovulation.

OSE cells express low levels of the mucin MUC16 (CA125). Mullerian duct derived tissues express high levels of MUC16 (CA125), as do ovarian tumors.[17] MUC16 (CA125) over- expression in ovarian tumors is an important marker for progression and regression of EOC.

The expression of markers that are associated with those of Mullerian duct derived tissue are found in ovarian inclusion cysts. Inclusion cysts are known to be the site of many neoplasms. The OSE lining in inclusion cysts expresses high levels of EOC markers MUC16 (CA125) and CA19-9. The hypothesis that epithelial ovarian cancer may derive from inclusion cysts is based upon the Incessant Ovulation Theory, first proposed by Fathalla in 1971.[18]

Higher ovulatory activity is associated with an increased accumulation of inclusion cysts and invaginations of the OSE, which provide a hospitable environment for tumor cell growth.[19]

This theory is supported by epidemiological data demonstrating that women who have been on oral contraceptives, or who have been pregnant and/or breastfeeding, have a decreased risk of ovarian cancer.

High gonadotropine levels typical for ovulation and menopause and known to induce changes of OSE, and oxidants, causing DNA-alterations in the OSE at the site of ovulation, lay the foundations for two more hypothesis regarding the origin of OEC–origin, however both are linked to the Incessant Ovulation Theory.[19,20]

Dubeau in 1999 first proposed the hypothesis that the fimbriae of the fallopian tubes, which are in close contact with the surface of the ovary during oocyte collection, and sometimes adhere to the surface of the ovary due to inflammation, are a prime site for the development of metaplasia.[21]

ATTEMPTS TO SCREEN—SOME LESSONS LEARNED

During the last 15 years, large prospective studies of screening for ovarian cancer have been performed.[22] Two distinct strategies have emerged, one based on ultrasound as the primary test, and the other involving the serum tumor marker CA 125 screening with ultrasound as the secondary test (multimodal screening). Tables 1 and 2 summarize the prospective ovarian cancer screening studies in the general population.[23-38] If we exclude those which used trans-abdominal ultrasound, an abandoned screening strategy due to unacceptably high rate of false positive results, several important lessons could be learned.

As seen in the Tables 1 and 2, the data suggest that sequential multimodal screening has greater specificity and positive predictive value compared to strategies based on transvaginal ultrasound alone. For each case of ovarian cancer detected, five women underwent surgery in the multimodal studies compared to 24 women in the studies using ultrasound alone. However, transvaginal ultrasound as a first line test may offer higher sensitivity for early stage disease given that 23/37 (62.2%) cancers detected using ultrasound alone were stage I, compared to 8/19 (42.1%) cancers detected by the multimodal strategy. An ultrasound-based strategy may have a greater impact on ovarian cancer mortality, albeit at a higher price in terms of surgical intervention for false positive results.

The Tables 1 and 2 address most relevant studies published until 2003. Our own ovarian cancer screening trial, which started in January 2001 will be described at the end of this chapter. The developments that followed since 2003 are best summarized in reference to the screening tests, target populations and newly published trials. The possible role of 3D ultrasound technology, especially 3D power Doppler imaging, in early and accurate detection of ovarian malignancy will be discussed as well.

SCREENING TESTS

Screening for ovarian cancer has been based on strategies using serum tumor markers or transvaginal ultrasound images of the ovaries, or a combination of both.

SERUM TUMOR MARKERS

In epithelial ovarian cancer, a number of tumor markers have been identified. Serum CA 125 continues to be the tumor marker most extensively used in ovarian cancer screening.[39] CA125 itself is a repeating peptide epitope on the large molecular weight mucin, MUC16.[40]

This mucin is expressed at low levels by normal ovarian surface epithelium and is overexpressed by EOC tumor cells.[41]

Tumor cells secrete mucin (MUC16) into the peritoneal fluid (PF) and from the abdominal cavity this mucin leaks into the blood stream and can then be detected via the CA125 serum assay.

Although CA 125 is elevated (>35 U/ml) in more than 80% of patients with epithelial ovarian cancer, it is only in 25% sensitive for early stage disease.[42] Indeed, its value as an initial screening tool is limited since picking up stage III disease at an earlier time may not alter outcome. To improve further the performance of CA 125 as a screening tool, an algorithm incorporating age, rate of change of CA 125 and absolute levels to calculate an individual's risk of ovarian cancer has been described.[43] This increases the sensitivity of CA 125 in comparison with a single cutoff value, because women with normal but rising levels are identified as being at increased risk. This approach was an integral part of the multimodal screening strategy adopted in the St Bartholomew's Hospital randomized control trial, published in the year 2000.[44]

Because CA125 levels are elevated in less than half of the cases in early-stage ovarian cancers, underscoring the lack of sensitivity to diagnose curable disease, CA125 appears not suitable to be used as a screening test, but mainly as a measure of disease progression, regression, and predictor of recurrence during treatment for EOC.

Another limitation of serum CA 125 represents that it is not specific for ovarian carcinoma because it can be elevated in many benign conditions such as endometriosis, uterine fibroids, pelvic inflammatory disease, ascites or pleural effusion.[45] It is now known that the CA 125 antigen carries two major antigenic domains classified as A (the domain binding monoclonal antibody OC125) and B (the domain

Table 1: Prospective ovarian cancer screening studies using ultrasound as the primary test in the general population

Study	*Inclusion criteria*	*Screening strategy*	*No. screened*	*No. of invasive epithelial ovarian cancers detected*[a]	*No. of positive screens*	*No. of positive screens/cancer detected*[b]
ULTRASOUND (US) APPROACH						
GREY-SCALE US (LEVEL 1 SCREEN), then repeat GRAY-SCALE US (LEVEL 2 SCREEN)						
van Nagell et al.[7]	Age > 50 years and postmenopausal or > 30 with positive family history	TVS Annual screens Mean 4 screens/women	14469	11 (6) 5 stage I	180	16.4
Hayashi et al.[8]	Age > 50 years	TVS	23451	3 (3)	258	[c]
Tabor et al.[9]	Aged 46-65 years	TVS	435	0	9	—
Campbell et al.[10]	Age > 45 years or with positive family history (4%)	TAS 3 screens at 18 monthly intervals	5479 2 stage I	2 (3)	326	163
Goswamy et al.[11]	Age 39-78 Postmenopausal	TAS	1084	1 1 stage I	Not precised	-
GREY-SCALE US and CDI (LEVEL 1 SCREEN)						
Vuento et al.[12]	Aged 56-61 years	TVS and CDI	1364	1	5	-
Kurjak et al.[13]	Aged 40-71 years	TVS and CDI	5013	4 4 stage I	38	9.5
Schulman et al.[14]	Age > 40 years or > 30 with positive family history	TVS and CDI	2117	1	18	18
GREY-SCALE US (LEVEL 1 SCREEN) and other tests (LEVEL 2 SCREEN)						
Sato et al.[15]	Age > 30 years	TVS then tumor markers if TVS +, CT and MRI if all previous +	51550	16 (6) 12 stage I	324	20.3
Parkes et al.[16]	Aged 50-64	TVS then CDI if TVS +	2953	1 1 stage I	15	15
Holbert et al.[17]	Postmenopausal Aged 30-89 years	TVS then CA 125 if TVS +	478	1 1 stage I	33[d]	-
TOTAL[e]				37 (16) 23 stage I	880	23.8

TAS = transabdominal ultrasound; TVS = transvaginal ultrasound; CDI = Color Doppler imaging

[a]The borderline/granulosa tumors detected are shown in parenthesis.

[b]Only invasive epithelial ovarian cancers included.

[c]Only 95 women consented to surgery and there are no follow-up details on the remaining.

[d]Only 11 of these women underwent surgery.

[e]Studies used TAS are excluded.

Table 2: Prospective ovarian cancer screening studies using serum CA 125 as the primary test in the general population

Study	*Inclusion criteria*	*Screening strategy*	*No. screened*	*No. of invasive epithelial ovarian cancers detected*	*No. of positive screens*	*No. of positive screens/cancer detected*
CA 125 ONLY						
Einhorn et al.[18]	Age > 40 years	Serum CA 125	5550	6 2 stage I	175	29.2
MULTIMODAL APPROACH						
CA 125 (LEVEL 1 SCREEN), then GREY-SCALE US (LEVEL 2 SCREEN)						
Jacobs et al.[19]	Age > 45 years Postmenopausal	RCT Serum CA 125 TAS/TVS, if CA 125 3 annual screens	10958	6 3 stage I	29	4.8
Jacobs et al.[20]	Age > 45 years Postmenopausal	Serum CA 125 TAS, if CA 125	22000	11 4 stage I	41	3.7
Adonakis et al.[21]	Age > 45 years	Serum CA 125 TVS, if CA 125	2000	1 (1) 1 stage I	15	15
Grover et al.[22]	Age > 40 years or with positive family history (3%)	Serum CA 125 TAS/TVS, if CA 125 3 screens	2550	1	16	16
TOTAL[a]				19 (1) 8 stage I	101	5.3

RCT = randomized controlled trial

[a]Only multimodal approach studies included.

binding monoclonal antibody M.[11] New generation assays, combining monoclonal antibodies to the two distinct regions of the molecule, have been shown to have improved specificity for the detection of early ovarian cancer.[46]

Lysophosphatidic acid (LPA), a bioactive phospholipide with mitogenic and growth factor-like activities,[47] is a tumor marker which was considered promising in ovarian cancer screening. In a small pilot series plasma LPA levels were elevated in 9 out of 10 patients with stage I ovarian cancer, 24 of 24 patients with stage II, III and IV ovarian cancer, and all 14 patients with recurrent ovarian cancer.[48]

In comparison, among a subset of patients with ovarian cancer, only 28 out of 47 had elevated CA 125 levels, including 2 of 9 patients with stage I disease. Larger studies on the use of LPA in primary screening—perhaps in combination with other procedures, such as transvaginal ultrasound—for earlier detection and improved outcome for patients with ovarian cancer are in demand.[49]

"The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass" was the title of study produced by Moore et al in 2008: Serum and urine samples were obtained preoperatively from women undergoing surgery for an adnexal mass. The samples were analyzed for levels of CA 125, SMRP, HE4, CA72-4, activin, inhibin, osteopontin, epidermal growth factor (EGFR), and ERBB2 (Her2) and were compared to final pathology results. Two hundred and fifty-nine patients with adnexal masses were enrolled. Of these, 233 patients were eligible for analysis with 67 invasive epithelial ovarian cancers and 166 benign ovarian neoplasms. In the analysis, HE4 had the highest sensitivity for detecting ovarian cancer as a single tumor marker, especially for Stage I disease. Combined CA 125 and HE4 were a more accurate predictor of malignancy than either alone.[50]

Anderson et al, in January 2010, published a nested case-control study, assessing the *lead time* of selected ovarian cancer biomarkers which are identified as potential ovarian cancer biomarkers: CA 125, human epididymis protein 4 (HE4), mesothelin, B7-H4, decoy receptor 3 (DcR3), and spondin-2 have. Except for CA 125, their behavior in the prediagnostic period had not been evaluated. As per their results, smoothed mean concentrations of CA125, HE4, and mesothelin (but not of B7-H4, DcR3, and spondin-2) began to increase (visually) in cancer patients relative to control subjects approximately 3 years before diagnosis but reached detectable elevations only within the final year before diagnosis. The authors concluded that serum concentrations of CA125, HE4, and mesothelin may provide evidence of ovarian cancer 3 years before clinical diagnosis, but the likely lead time associated with these markers appears to be less than 1 year.[51]

Transvaginal ultrasound is used in most screening strategies either as the sole screening modality or as a secondary test after primary screening with serum CA 125 (multimodal screening). As data regarding outcome accumulate with long-term follow-up of the participants of the early screening trials, it has been possible to define further risk of ovarian cancer associated with various ultrasound findings.

Particular results of the largest ultrasound-based ovarian cancer screening project from University of Kentucky might have a definitive impact on design of future ovarian cancer screening in the general population.[52] Van Nagell et al established that unilocular ovarian cysts less than 10 cm in diameter, found in 256 out of 7705 (3.3%) asymptomatic women aged more than 50 years, were associated with a minimal risk for ovarian cancer because there were no cases of ovarian carcinoma during a 5-year follow-up period.[53] In contrast, 7 out of the 250 women in the same study with complex cystic ovarian tumors, including wall abnormalities or solid areas, had ovarian carcinoma suggesting that these morphologic appearances are associated with a significant risk for malignancy.

In many screening algorithms, volume cut-offs are used in addition to morphology characteristics to identify women for intensive surveillance. Based on the data of 58,673 observations of ovarian volume, authors from Kentucky concluded that the upper limit of normal for ovarian volume is 20 cm^3 in premenopausal women and 10 cm^3 in postmenopausal women.[54] Such data are very valuable in determining optimal strategies for operative intervention.

Recently, Kurman et al suggested an approach to early detection of ovarian cancer focussing on low volume rather than low stage of disease, to intercept the more aggressive tumors like high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcomas), and undifferentiated carcinomas, which account for most ovarian cancers. According to this research group, a more rational approach to early detection of ovarian cancer should focus on low volume rather than low stage of disease.[55]

Postmenopausal women from the general population with an elevated serum CA 125 level but normal ovarian morphology on ultrasound were found to have a cumulative risk of ovarian cancer during a median follow up of 6.8 years, of 0.15%, which was similar to 0.22% of the entire population of 22,000 women.[56] In contrast, those with an elevated serum CA 125 level and abnormal ovarian morphology on ultrasound had a significantly increased cumulative risk of 24%. Using ovarian morphology to

interpret pelvic ultrasound images has been shown to increase sensitivity, and use of morphology scoring index for complex ovarian tumors may improve the positive predictive value of a multimodal screening strategy.[57]

In a 2008 published Korean study, 202 patients who underwent surgery for ovarian tumors were reviewed retrospectively from September 2000 to July 2006. In all patients, the morphology index (MI) score and serum CA-125 level were measured preoperatively. The association of the final pathologic diagnosis with the MI score and serum CA 125 level were examined. The sonographic MI system was an accurate and simple method to differentiate a malignant tumor from a benign ovarian tumor. The accuracy of the sonographic MI system improved when the serum CA 125 level was considered and ovarian teratomas were excluded.[58]

In the United Kingdom, a trial called UKCTOCS is looking at ovarian cancer screening in women in the general population. Between 2001 and 2005, a total of 202 638 postmenopausal women aged 50-74 years were randomly assigned to no treatment (control; n = 101 359); annual CA 125 screening (interpreted using a risk of ovarian cancer algorithm) with transvaginal ultrasound scan as a second-line test [multimodal screening (MMS); n = 50 640]; or annual screening with transvaginal ultrasound (USS; n = 50 639) alone in a 2:1:1 ratio using a computer-generated random number algorithm. In interpretation of the results, the authors consider the sensitivity of the MMS and USS screening strategies as encouraging. Specificity was higher in the MMS than in the USS group, resulting in lower rates of repeat testing and surgery. This in part reflects the high prevalence of benign adnexal abnormalities and the more frequent detection of borderline tumours in the USS group. The prevalence screen could establish that the screening strategies are feasible. The results of ongoing screening are awaited so that the effect of screening on mortality can be determined.[59]

The first prospective randomized report of a multimodal ovarian cancer screening originates from a Japanese research group: Asymptomatic postmenopausal women were randomly assigned between 1985 and 1999 to either an intervention group (n = 41,688) or a control group (n = 40,799) in a ratio of 1:1, with follow-up of mean 9.2 years, in Shizuoka district, Japan. The original intention was to offer women in the intervention group annual screens by gynecological examination [sequential pelvic ultrasound (US) and serum CA125 test]. Women with abnormal US findings and/or raised CA125 values were referred for surgical investigation by a gynecological oncologist. The proportion of stage I ovarian cancer was higher in the screened group (63%) than in the control group (38%), which did not reach statistical significance (P = 0.2285).[60]

TARGET POPULATIONS

Participants for ovarian cancer screening trials are recruited from general and high-risk populations on the basis of risk factors for the disease.

GENERAL POPULATION

Age and Menopausal Status

The majority of ovarian cancers occur in the general population, and age greater than 50 years and postmenopausal status have been used to define those eligible for screening. According to the FIGO report 2001,[6] appearance of ovarian cancer was most common among women in early postmenopause, at average age of 54 years. Law et al.[61] used national statistics to determine the number of years of life lost through deaths from a particular cancer at each age. They concluded that screening would be most effective (i.e. associated with the largest number of years of life saved per person screened) if done 5 years before loss of life peak. The peak occurred in ovarian cancer during the age range 55-59 years, and the authors' argument provides further justification for using 50 years as the cutoff to commence population screening.

HIGH-RISK POPULATION

Family History and/or Genetic Predisposition

Approximately 5-10% of ovarian cancers are inherited. Mutations in BRCA1 and BRCA2 genes account for about 75% of families with a highly penetrant, dominantly inherited breast or ovarian cancer family history. Recent estimates of the lifetime risk for ovarian cancer in women harboring a BRCA1 mutation are 40-60%.[62] Various studies have put forward schemes for stratifying women into different risk categories of risk for breast and ovarian cancer by virtue of a family history, genetic predisposition or both. Pharoah et al.[63] reviewed the relevance of family history in defining the target population for familial ovarian cancer screening, and proposed the adoption of a unified management strategy based on eligibility criteria from UK National Familial Ovarian Cancer Screening Study (Table 3). A survey by Vasen et al.[64] of the European Familial Breast Cancer Collaborative Group found that the following high-risk populations were offered ovarian cancer screening: BRCA1 and BRCA2 mutation carriers; members of breast/ovarian cancer families; and, in some centers, members of «breast cancer only» families with an early onset of breast cancer.

Table 3: Eligibility criteria for the UKCCCR National Familial Ovarian Cancer Screening Study[39]

An eligible woman must be over 25 years of age and a first degree relative of an affected member of an "at risk" family. At risk families are defined by the following criteria:

1. Two or more first degree relatives[a] with ovarian cancer.
2. One first degree relative with ovarian cancer and one first degree relative with breast cancer diagnosed under 50 years of age.
3. One first degree relative with ovarian cancer and two first or second degree relatives[b] with breast cancer diagnosed under 60 years of age.
4. An affected individual with one of the known ovarian cancer predisposing genes.
5. Three first degree relatives with colorectal cancer with at least one diagnosed before the age of 50 years and at least one first degree relative with ovarian cancer.

[a]A first degree female relative is mother, sister or daughter.
[b]A second degree female relative is grandmother, grand-daughter, aunt or niece.

OVARIAN CANCER SCREENING TRIALS

Clinical trials of ovarian cancer screening have involved strategies using ultrasound alone, and a multimodal approach with CA 125 as a primary test and ultrasound as a secondary test. Prospective studies have involved both the general and high-risk populations.

GENERAL POPULATION

Ultrasound Screening

In the evaluation of data from the 2000 University of Kentucky trial, the results of annual transvaginal ultrasound screening performed on 14,469 asymptomatic women aged 50 years or more and women aged 25 years or more with a family history of ovarian cancer were reported.[23] Hundred and eighty patients with persisting transvaginal abnormalities were subjected to a surgical intervention. 17 primary ovarian cancers were detected of which 11 were epithelial ovarian cancers (EOC), three were granulosa cell tumors, and three were borderline tumors. Of the EOC, 5 were stage I, 3 were stage II and 3 were stage III. In this study, transvaginal ultrasound (TV US) as a screening modality was associated with sensitivity of 81%, specificity of 98.9%, positive predictive value of 9.4%, and negative predictive value of 99.97% for detection of all primary ovarian cancers. The survival of patients with EOC in the annually screened population was 92.9% at 2 years, and 83.6% at 5 years. What is encouraging about these results is that annual TV US screening appeared to achieve the primary goal of earlier detection of the disease, which translates into a reduction in mortality associated with ovarian carcinoma. On the other hand, data from this study suggested that in certain cases, length of time required for ovarian cancer to progress from a localized sonographically detectable tumor to widespread regional disease is quite short. In four patients in the false-negative group, disease progression from sonographically normal ovaries to stage II or III ovarian cancer occurred in less than 12 months. Authors stated that for future screening algorithms, a screening interval of 6 months should be taken into consideration. In 2000, the Japanese ovarian cancer screening trial was published: 51,550 women aged 30 years or more attending for annual cervical screening underwent TV US screening for ovarian cancer.[31] Three hundred and twenty-four women with masses of more than 60 mm in diameter or with a mixed echo pattern or persistently raised tumor markers underwent laparotomy. Twenty-two primary ovarian tumors and two metastatic tumors were detected. Of the 22 primary tumors, 16 were EOCs, four were borderline malignancies and two were germ cell tumors. 11 (68.7%) of the EOCs were stage I, with tumor markers positive in 5 (45.4%) of the 11 cases. The positive predictive value of the screening strategy was 4.9%; in other words, 20 operations were undertaken for each detected case of ovarian cancer. As no follow-up data were reported on any of the trial participants, it is difficult to assess the sensitivity of the screening strategy. Before the onset of the screening, the authors noted that only 29.7% out of 35 cancers diagnosed in the department, were stage I, while after the trial was initiated, 58.8% of 85 ovarian cancers treated were stage I.

Multimodal Screening

One of the most active groups in screening for ovarian malignancy led by Jacobs, reported the results of the first completed randomized trial of ovarian cancer screening.[35] This study which was published in 1999, randomized asymptomatic postmenopausal women aged 45 years or older to no screening (n = 10,977) or to annual multimodal screening for 3 years (n = 10,958). In the screening group 29 women with elevated CA 125 values and abnormal ultrasound findings were referred for surgical investigation. All 6 ovarian cancers detected were EOCs; 3 were stage I and 3 were stage III. The authors found a high positive predictive value of 20.7% with this schema and were encouraged by a longer median survival (72,9 months) in women with ovarian cancer in the screened group when compared to the control group (41,8 months). The mortality rates, however, were not significantly different between the groups. The authors concluded that the results do not justify ovarian cancer screening in the general population but do support the need for a larger randomized trial that is powered to assess the impact of screening on mortality.

The Kentucky Ovarian Cancer Screening Trial confirmed that screening can detect ovarian cancer at an earlier stage than it is normally detected without screening. It also established the fact that the combination of serum CA 125 with transvaginal sonography (TVS) is probably more effective than TVS alone. The study design suggested that if the ultrasound was abnormal, a repeat TVS in four weeks was performed. If the repeat ultrasound was abnormal, CA 125 blood test and morphology indexing (MI) of form and structure of the ovarian mass was done. To improve accuracy in differentiating a benign ovarian tumor from ovarian cancer, MI was used to identify certain patterns that are associated with benign or noncancerous tumors. If the patient's CA 125 was normal and the morphology index indicated a benign tumor, the patient was considered not to need surgery and was followed periodically with repeat ultrasound.[65]

Both the Kentucky trial and the trial from the United Kingdom (UKCTOCS) detected ovarian cancer at a significantly earlier stage than when women did not have screening. The University of Kentucky Ovarian Cancer Screening Program is an ongoing trial; results from this trial were published in 2007 in the journal Cancer. Of women whose ovarian cancer was detected by screening, 82 percent had stage I or II disease compared to 30 percent of women in the unscreened population. Without screening, about 70 percent of women presented with stage III or IV disease. This is important to note because only 30 percent of women with advanced ovarian cancer will be alive in five years after treatment and two-thirds of them will still have disease that cannot be cured. Therefore, the ultimate cure rate for a woman with advanced ovarian cancer is only about 10 percent. So clearly, something needs to be done to increase early detection.

HIGH-RISK POPULATION

For women with a known germ line BRCA 1,2 mutation or with a family history suggesting a significant possibility of a genetic predisposition to ovarian cancer, the appropriate screening strategy remains undefined. In several studies, most authors advocate screening using TV US and serum CA 125 in patients who elect to delay or decline prophylactic oophorectomy. However, there is no consensus concerning the appropriate interval for screening.

Karlan et al reported the results of an ovarian cancer screening program launched in 1991, involving 1261 women aged over 35 years with a family history of ovarian, breast, colon or endometrial carcinoma, or a personal history of breast cancer.[66] Screening with TV US, color Doppler imaging and CA 125 was initially performed biannually until 1995, and annually thereafter. Two tumors of low malignant potential, on stage I EOC and 7 cases of primary peritoneal serous papillary carcinoma were diagnosed. Ultrasound abnormalities triggered surgical exploration in all three cases of ovarian disease. In 2 out of 7 cases, elevated levels of CA 125 were the harbinger of peritoneal serous papillary carcinoma, in two, abnormal ultrasound findings prompted diagnosis, and three developed interval cancers 5, 6 and 16 months after screening. At least three of the patients with primary peritoneal cancer carried mutations of the BRCA1 gene. Multifocal peritoneal serous papillary carcinoma may be a phenotypic variant of familial ovarian cancer, and screening strategies for these women cannot rely on ultrasound and CA 125 testing to detect early disease.

OVARIAN CANCER—THE ROLE OF 3D ULTRASOUND AND 3D POWER DOPPLER IMAGING

Improvements in ultrasound technology such as 3D volume acquisition and 3D power Doppler imaging may have clinical utility in a more reliable identification of an abnormal ovarian vascularity and architecture. 3D volume acquisition allows for careful evaluation of the internal surfaces of cyst walls for excrescences otherwise not appreciated by 2D ultrasound.[67,68] While the addition of 3D power Doppler provides a new tool for measuring the quality of ovarian tumor angiogenesis,[69] improving accurate diagnosis of ovarian malignancies,[70] its clinical value for the early detection of ovarian carcinoma has yet to be determined.

WHAT DOES 3D ULTRASOUND ADD?

In the pioneer work, Bonilla-Musoles et al[67] tried to determine whether 3D ultrasound may offer advantages over 2D ultrasound as a screening tool for the evaluation of ovarian lesions. Seventy-six women with ovarian masses first detected with 2D ultrasound were then evaluated with 3D ultrasound. The 3D sonographic criteria, used for diagnosing ovarian malignancy were based on the morphologic scoring system for 2D transvaginal ultrasound examinations proposed by different authors.[71-74] A score greater than 4 caused suspicion of a malignant ovarian mass.[74] The images were dissected in the three perpendicular planes, and the areas indicative of malignancy, as suggested by 2D ultrasonography, were determined to be either negative or positive and confirmatory. Five lesions observed on 2D ultrasound were suspected to be malignant. 3D sonography identified four of these lesions as malignant. The remaining one suspected to be malignant on 2D ultrasound was diagnosed as endometriosis with 3D

sonography. One additional ovarian carcinoma was diagnosed by 3D scanning. Two of the malignant lesions were FIGO stage IA. The other tumors were FIGO stages IC, IIC, and IIIB, respectively. Authors stated that observation of papillary projections (especially those less than 3 mm), characteristics of cystic walls, and the extent of capsular infiltration was superior with 3D ultrasound in comparison to conventional 2D sonographic measurements, as was the calculation of ovarian tumor volume. They also pointed out that eventually 3D ultrasound imaging will allow diagnosis of ovarian malignancy at an earlier stage than it is possible with currently established diagnostic techniques.

ADVANTAGES OF 3D POWER DOPPLER IMAGING

There are two potential advantages of this new imaging modality: more accurate visualization of ovarian tumor neovascularization and more effective detection of stage I disease.

More Accurate Visualization of Ovarian Tumor Neovascularization

In order to determine whether three-dimensional power Doppler can improve the ability to differentiate benign from malignant ovarian masses, Kurjak et al[75] performed transvaginal color Doppler and 3D power Doppler analysis on 120 patients with ovarian lesions. As a result, in each of 11 ovarian malignancies, preoperative diagnosis by 3D power Doppler was confirmed by histopathology. Transvaginal color Doppler missed one case of serous cystadenocarcinoma, while 3 benign lesions (dermoid cyst, ovarian fibroma, and ovarian cystadenofibroma) were considered false positive. In a case of cystadenofibroma, 3D power Doppler findings were falsely positive. Authors emphasized that irregular and randomly dispersed vessels with complex branching, depicted by 3D power Doppler imaging, were indicative for ovarian malignancy. Such qualitative analysis of the tumor vascularity architecture had a sensitivity, specificity, and positive predictive value (PPV) of 100, 99.08 and 91.67% in detection of ovarian malignancy, respectively. In a study published by Cohen et al,[76] 71 women with a known complex pelvic mass were referred for a preoperative ultrasound evaluation with both two-dimensional gray-scale and 3D power Doppler ultrasound. All the women underwent surgical exploration, and 14 had ovarian cancer. Two-dimensional gray-scale ultrasound identified 40 masses as suspicious for cancer, including all 14 malignancies, yielding a sensitivity, specificity, and PPV of 100, 54 and 35% respectively. However, evaluation with 3D power Doppler identified only 28 cases as suspicious (including all cancers), resulting in a sensitivity, specificity, and PPV of 100, 75, and 50% respectively. Even though all malignancies were correctly identified by both 2D and 3D imaging, the specificity was significantly improved with the addition of 3D power Doppler. This improved diagnostic accuracy, authors stated, may promote improved patient care by separating complex benign masses from ovarian cancer, therefore facilitating appropriate physician referral.

Despite the inability of currently available screening algorithms to achieve the desired positive predictive value (PPV) of 10, there may be an advantage in producing a stage migration to lower stages at the time of diagnoses, thereby resulting in improved survival. Equally important recent studies have demonstrated that women who have their initial surgery performed by gynecologic oncologists, and women who have their surgeries at centers experienced in the treatment of ovarian cancer have higher survival rates. A cost-effectiveness analysis conducted by Bristow et al. revealed that the strategy of expert center referral had an overall cost per patient of $50,652 and had an effectiveness of 5.12 quality-adjusted life years (QALYs). The strategy of referral to a less experienced center carried an overall cost of $39,957 and had an effectiveness of 2.33 QALYs. The expert center strategy was associated with an additional 2.78 QALYs at an incremental cost of $10,695 but was more cost-effective, with a cost-effective ratio of $9893 per QALY compared with $17,149 per QALY for the less experienced center referral strategy.[77]

Kupesic and Kurjak reported already in 2000 on the use of contrast-enhanced, 3D power Doppler ultrasound in the differentiation of benign and malignant adnexal lesions.[78] A total of 45 patients with complex adnexal lesions of uncertain malignancy at transvaginal B mode and/or color Doppler ultrasound were prospectively evaluated with 3D power Doppler before and after injection of contrast agent. There were 12 cases of ovarian malignancy and 33 benign adnexal lesions. Of the 12 ovarian cancers, seven (58.3%) showed vascular distribution suggestive of malignancy at non-enhanced 3D power Doppler imaging. After injection of contrast agent, a penetrating vascular pattern and/or a mixed penetrating and peripheral pattern were detected in all cases of ovarian malignancy. One cystadenofibroma demonstrated penetrating vessels at initial scan, whereas two benign lesions (fibroma and cystadenofibroma) were misdiagnosed as malignant with contrast-enhanced 3D power Doppler. The use of a contrast agent with 3D power Doppler showed diagnostic efficiency (95.6%) that was superior to that of non-enhanced 3D power Doppler ultrasound. The authors concluded that contrast-enhanced

3D power Doppler imaging might, more precisely, discriminate benign from malignant complex adnexal masses.

Methods for *vascular sampling* by three-dimensional power Doppler angiography in solid and cystic-solid adnexal masses were described by Alcazar and Prka in 2009, in a study which analyzed the difference in reproducibility of 3D-PD vascular sampling between manual and 5 ccm sphere sampling. 3D power Doppler angiography has been proposed as a method of predicting malignancy in adnexal masses. This new technique allows the objective assessment of tumor vascularisation by means of power Doppler signals. The rationale of the technique is based on the fact that malignant ovarian tumors have a higher microvascular density than do benign tumors. Vascularity Index (VI) is thought to reflect vascular density, FI is thought to reflect blood flow in those vessels.[79]

"Vascular Sampling"—a terminus created by Alcazar—is based on the manual outlining of solid tumor areas using the VOCAL software (GE Medical Systems) to measure their vascularisation. In conclusion, both manual and 5 ccm sphere sampling of 3D-PD angiography data sets are reproducible methods.[80]

Ultrasound screening—as stated before by several other studies—could be more effective when a morphology indexing system is used. This may be of value especially for less experienced sonographers. Ameye et al, conducted a multicenter study with 1573 patients forming four subgroups of adnexal masses to improve pattern recognition: (1) unilocular cyst, (2) multilocular cyst, (3) tumor with at least one solid component but no papillation,(4) tumor with papillation. In each subgroup the associated likelihood of malignancy was calculated, using all possible combinations of variables ranging from demographic characteristics, gray scale findings, blood flow indices, tumor marker CA 125, family history of breast or/and ovarian cancer, to color score (no flow, minimal flow, moderate, strong flow). The authors concluded that the subgroup system with likelihood calculation may improve characterization of ovarian tumors by nonexperts in gynecological sonography.[81]

Detection of Stage I Disease

Preliminary results of our team in Zagreb showed that 3D power Doppler ultrasound can enhance and facilitate morphologic and functional evaluation of an early stage ovarian cancer.[82] A five-year retrospective analysis was performed on the data from 43 referred patients with suspected stage I ovarian cancer subsequently confirmed by histopathologist. All the patients were preoperatively evaluated by four complementary sonographic methods: 2D transvaginal gray-scale, 2D transvaginal color Doppler, 3D ultrasound and 3D power Doppler, during the week prior to surgery. Our results clearly demonstrated the significant impact of 3D power Doppler imaging on the accurate detection of stage I ovarian cancer. By using combined 3D morphology and vascular score indexing, we reached diagnostic accuracy of 97.7% in preoperative sonographic assessment of the suspected lesions (Table 4). These findings justify implementation of 3D ultrasound with power Doppler facilities in ovarian cancer screening programs, especially as a secondary screening tool.

Table 4: Diagnostic accuracy of four different techniques (2D transvaginal US, 2D transvaginal color Doppler, 3D US, and 3D power Doppler) in preoperative sonographic assessment of 43 patients with suspected stage I ovarian cancer[53]

	Preoperatively	
Technique	*No. of detected cancers (%)*	*No. of missed cancers (%)*
2D US	30 (69.8)	13 (30.2)
Combined 2D US and Doppler score	37 (86.0)	6 (14.0)
3D US	32 (74.4)	11 (25.6)
3DPD US	41 (95.3)	2 (4.7)
Combined 3D US and Doppler score	42 (97.7)	1 (2.3)

ZAGREB OVARIAN CANCER SCREENING TRIAL

Following our first attempt to screen for ovarian cancer,[29] in January 2001 we initiated the new ovarian cancer screening trial at our department, based on new diagnostic tools now used routinely by us.

SUBJECTS AND METHODS

During a five-year period, approximately 10,000 asymptomatic postmenopausal women of 50 years and 25 years of age with a positive family history of ovarian and/or breast cancer in at least one primary or secondary relative were offered to participate in the trial. The screening algorithm is illustrated in Figure 1.

Primary screening include annual transvaginal ultrasound (TV US) and transvaginal color Doppler (TVCD) examination/scoring according to the sonographic and color Doppler criteria established previously from our team).[83] Women with an abnormal first level screen underwent a repeat TV US and TVCD sonogram depending on morphologic appearance: in the case of simple ovarian cyst after 4-6 weeks, while if complex ovarian cyst persisted, within 2 weeks. In patients with a persistently abnormal screen, secondary screening will be considered necessary, including 3D, 3D power Doppler and contrast-enhanced 3D

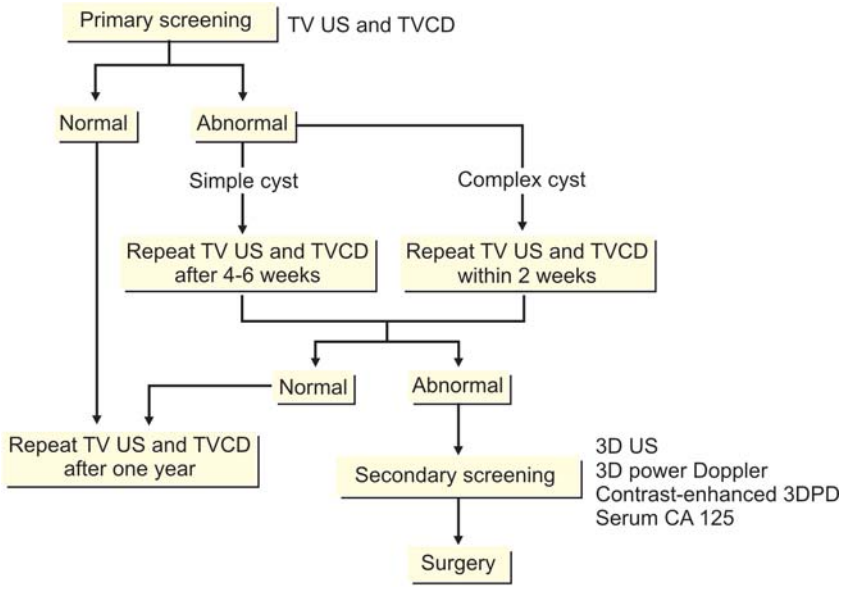


Fig. 1: Screening algorithm of the Zagreb ovarian cancer screening

power Doppler ultrasound evaluation, with a serum CA 125 determination. For an examination/scoring, three-dimensional sonographic and power Doppler criteria established in our previous study were used.[83] In the case of an abnormal second level screen, surgical removal of the tumor and pathological examination was recommended.

ILLUSTRATIVE CASE NO. 1—MORE ACCURATE DIAGNOSIS OF OVARIAN CANCER

In an asymptomatic, 51-year-old postmenopausal patient, on her first annual screen at our department, complex ovarian tumor suspicious of an early stage ovarian cancer was detected. Asking the patient for family history, we found that her aunt and uncle (mother's brother) died from colorectal cancer. In the first step, transvaginal gray scale ultrasound was performed, which revealed a complex cystic-solid tumor of the left ovary, measuring 4.5 cm in the largest diameter, with detectable several high-level echo foci within the solid component of the lesion (Fig. 2). According to our sonographic criteria, morphology score of 6 (volume > 10 cm^3, solid area > 1 cm, and mixed/high-level echo pattern) was suggestive of ovarian malignancy. Another step in our primary screening represented transvaginal color Doppler analysis of tumoral blood flow within the solid part of the tumor. It revealed RI of 0.36 (Fig. 3A) to 0.40 (Fig. 3B) as the lowest values.

According to our color Doppler criteria for ovarian malignancy, this finding was indicative for a malignant ovarian lesion. Three-dimensional ultrasound scan, as a part of our secondary screening process, clearly depicted a

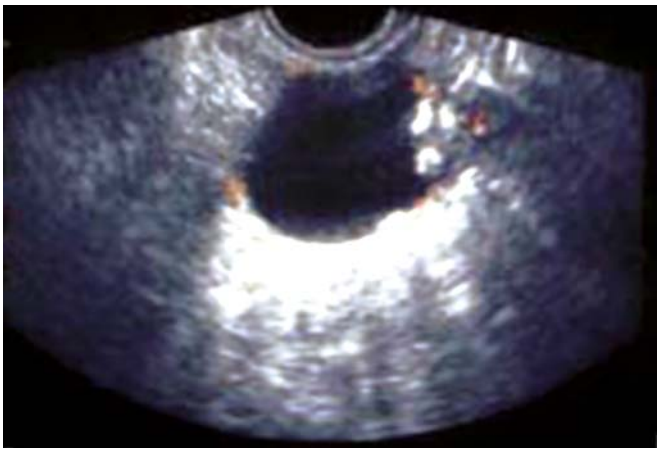
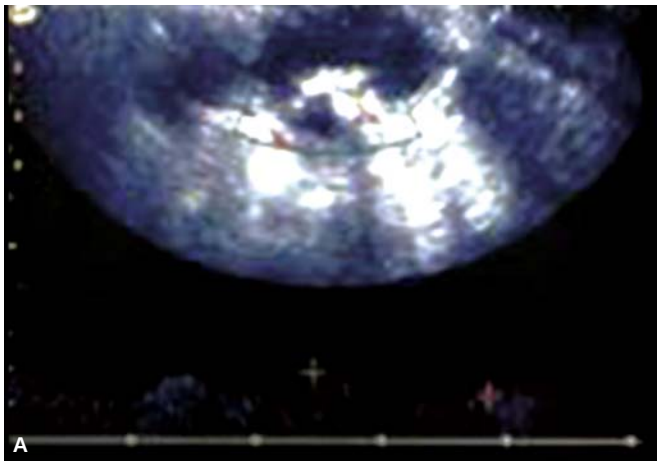
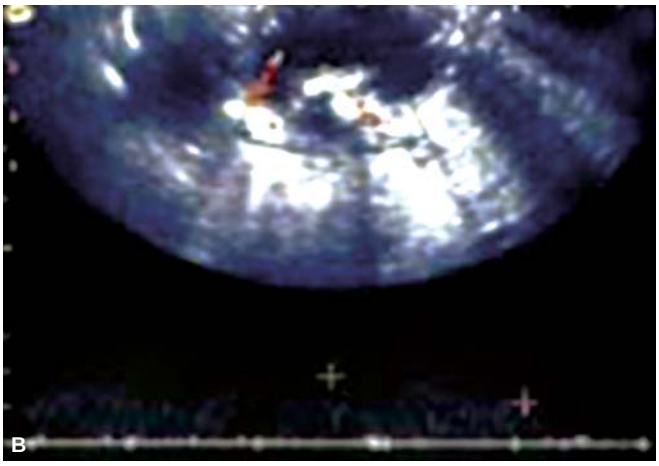


Fig. 2: Transvaginal ultrasound scan of a complex cystic-solid ovarian tumor in a 51-year-old postmenopausal patient. Note several high-level echo foci within the solid component of the lesion

hyperechoic area within the solid part of a complex ovarian tumor (Fig. 4) 3D ultrasound did not add any significant morphological findings in comparison to 2D transvaginal gray scale US, besides more precise volume calculation.

However, the vascular pattern obtained by further analysis with 3D power Doppler imaging revealed single-vessel arrangement and regularly separated vessels within the solid part of the tumor (Fig. 5), indicative of a benign ovarian lesion. As a result, 3D US combined index score of 6, and especially data on tumor vessels architecture enabled us to presume benign character of the described complex ovarian tumor. Also, CA 125 serum level of 10.5 U/ml was in normal ranges. Unilateral adnexectomy was initially performed via laparotomy, and «ex tempore» biopsy of the





Figs 3A and 3B: Further analysis of tumoral blood flow within the solid part of the tumor using transvaginal color Doppler ultrasound revealed RI of 0.36 (A) to 0.40 (B), suggestive of ovarian malignancy

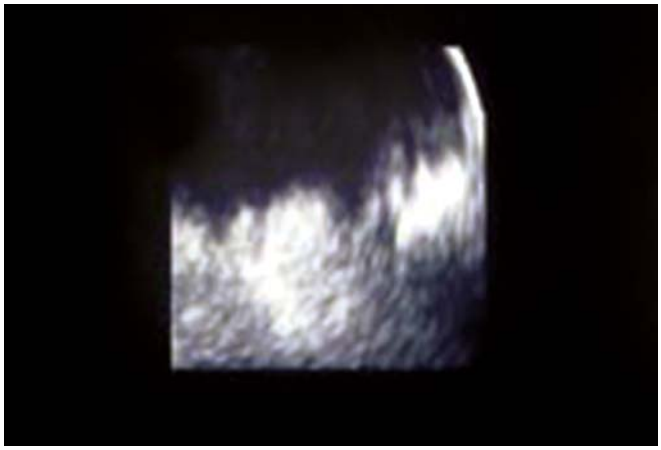


Fig. 4: The same patient as in Figures 2 and 3. Three-dimensional ultrasound scan of a hyperechoic areas within the solid part of a complex ovarian tumor

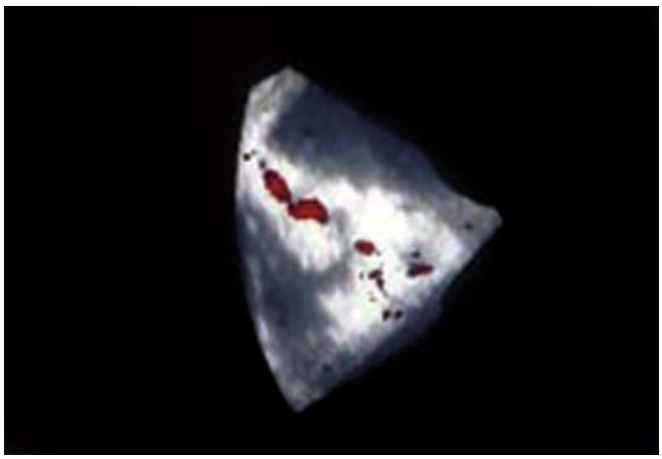


Fig. 5: Transvaginal 3D power Doppler imaging in the same patient revealed single-vessel arrangement and regularly separated vessels within the solid part of the tumor, indicative of a benign ovarian lesion. Histopathology revealed benign cystic teratoma

left ovary reported benign cystic teratoma. This surgical procedure was considered adequate. Final pathology confirmed previous finding.

From the case described above, we will try to emphasize several outstanding details in the application of multimodal ovarian cancer screening:

1. False-positive findings on transvaginal color Doppler analysis tended to involve non-neoplastic lesions that contained dilated vessels because of possible local metabolic imbalances caused by underlying inflammatory process or necrosis;
2. With the addition of 3D power Doppler (to study tumor vessels architecture) as a secondary screening tool, the specificity of a screening test could be significantly improved. This imaging modality might accurately discriminate benign from malignant complex ovarian lesions on the basis of qualitative analysis of tumoral microcirculation;
3. This improved diagnostic accuracy may promote improved patient care in terms of different surgical approaches to benign ovarian tumors (laparoscopy) and ovarian cancer (laparotomy, laparoscopy in the future for the early stage disease),[84] therefore facilitating appropriate physician referral.

ILLUSTRATIVE CASE NO. 2—THE DETECTION OF STAGE I DISEASE

Here we present an illustrative case of successfully detected stage IA ovarian cancer in an asymptomatic, 57-year-old postmenopausal patient. She was well-educated and concerned about family history of cancer, because her mother and mother's sister had breast cancer. Besides regular mammography and gynecological check-ups, patient decided to perform gynecological ultrasound in an outpatient clinic, for the first time in her life.

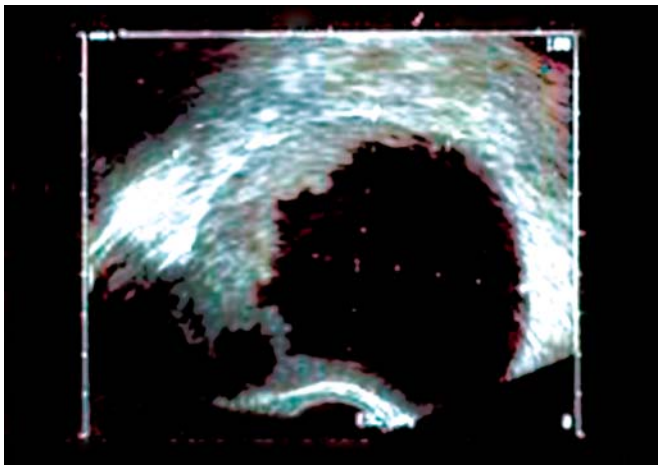


Fig. 6: Complex ovarian tumor in a 57-year-old postmenopausal patient, with noticeable solid component protruding into the cystic cavity. Note thick, irregular septa on the basis of the lesion

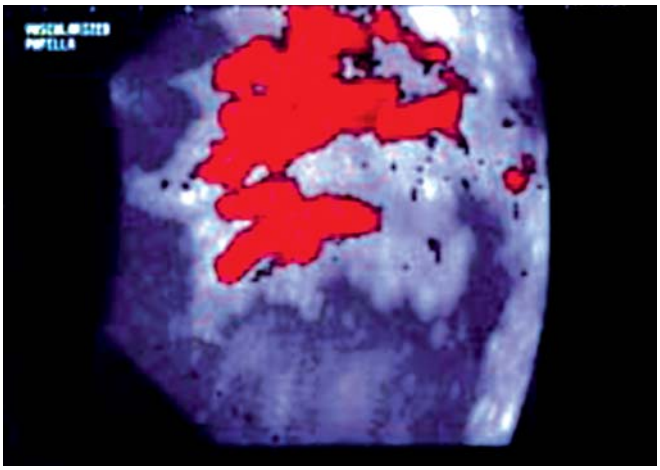


Fig. 8: The same patient as in Figures 6 and 7. 3D power Doppler imaging added important information on tumor microcirculation architecture. Numerous randomly dispersed vessels are shown within the papilla, indicating the malignant nature of the ovarian tumor

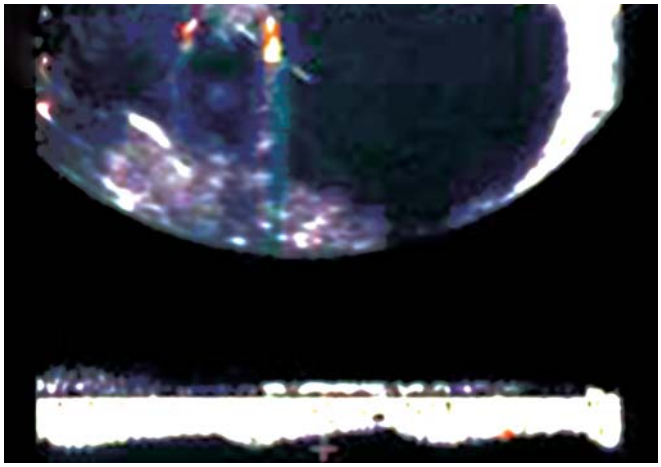


Fig. 7: Further analysis of tumoral blood flow with transvaginal color Doppler ultrasound in the same patient revealed RI of 0.40 as the lowest value. According to 2D color Doppler criteria, this finding was indicative of ovarian malignancy

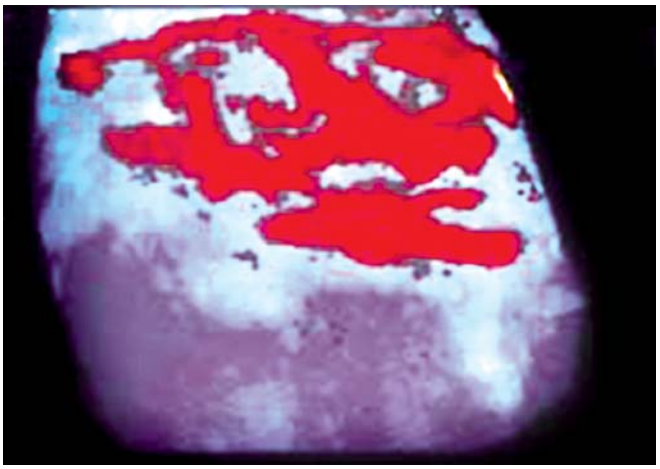


Fig. 9: Further 3D power Doppler analysis in the same patient revealed typical signs of malignant neovascularization within the solid part of the lesion, characterized by irregular course of the tumoral vessels and complicated branching. Histopathological finding was stage IA ovarian endometroid adenocarcinoma

Transvaginal gray scale sonography, performed by her primary care gynecologist, revealed a complex cystic-solid tumor of the right ovary, measuring 8 cm in diameter, with detectable papillary protrusions and thick, irregular septa (Fig. 6). Regarding ovarian morphology indicative for malignancy, she was immediately directed to our department for further ultrasound evaluation. We confirmed previous TV US finding, and morphology score of 8 was highly suspicious for ovarian malignancy. Another step represented transvaginal color Doppler analysis of tumoral blood flow which revealed RI of 0.40 as the lowest value (Fig. 7). According to our color Doppler criteria, this finding was indicative for a malignant ovarian lesion.

The vascular pattern obtained by further analysis with 3D power Doppler imaging clearly depicted disorganized, randomly dispersed vessels with irregular branching in the papilla (Fig. 8) and solid parts (Fig. 9) of the tumor, strongly associated with ovarian malignancy.

As a result, 3D US combined index score of 12, using data on tumor vessels architecture enabled us to make a correct preoperative sonographic diagnosis of an early stage ovarian cancer. On the other hand, CA 125 serum level of 16.3 U/ml was in normal range, giving us a false negative impression of a benign ovarian tumor. Standard oncological surgical procedure was performed, and histopathology reported stage IA endometroid adenocarcinoma of the ovary.

ILLUSTRATIVE CASE NO. 3—THE DETECTION OF STAGE I DISEASE

A West-European lady, 51 years, came to the hospitals first-aid because of lower back pain since 5 weeks. Still regular menstruations, no children. Ultrasound showed a complex ovarian cyst on the right side, and she was referred to the primary care gynecologist. Vaginal examination revealed a fixed mass in the right small pelvis, retroparauterine. Transvaginal ultrasound showed a complex ovarian cyst on the right side, with intracystic fluid of low echogenecity, and intracystic proliferations, which had not been visible by transabdominal ultrasound (Fig. 10), max. diameter 9.2 cm, volume 256 ccm (Fig. 11).

In 3D color and power Doppler mode—TVS vascularisation of the papillary intracystic projections, with irregular branching, stenosis, microaneurysms, and lacunae (Fig. 12). Pulsed wave Doppler shows continuous flow of venous type (Fig. 13). Using combined 3D morphology and vascular score indexing, these sonographic findings were highly suspicious of ovarian melanoma (Figs 14A to D). Serum levels of CA 125 were not increased. MRI did not show any signs of regional or systemic metastasis. The patient was scheduled for laparoscopy/laparotomy with frozen section and bilateral ureter stenting. Laparoscopy showed us an immobile cystic ovarian tumor with smooth surface, of which only 1/5 was visible (Fig. 15). Under these circumstances we continued with laparotomy via midline incision. An immobile right ovarian tumor was released after spilling-free removal of the intracystic fluid (Fig. 14E). Frozen section diagnosis was malignant neoplasm with features of adenocarcinoma. Preliminary diagnosis was followed immediately by staging with hysterectomy, left

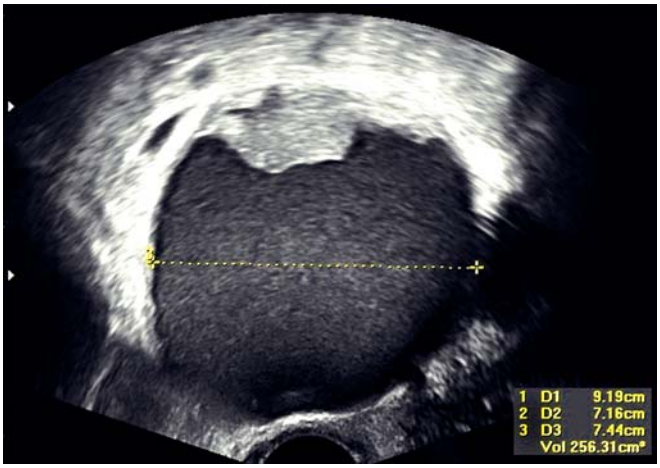


Fig. 11: Ovarian Ca 1A B-mode, TVS. Tumor volume 256 ccm, echogenic fluid, papillary intracystic projections

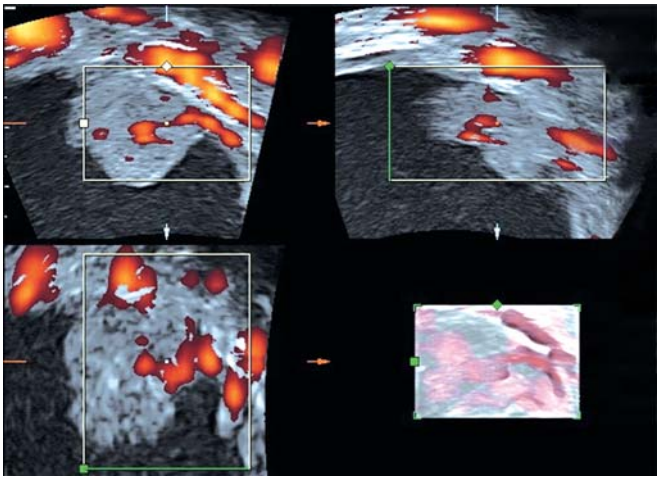


Fig. 12: Ovarian Ca 1A 3D power Doppler. Now, in close range of the tumor, TVS can pick up randomly dispersed vascular signals in the tumor papilla

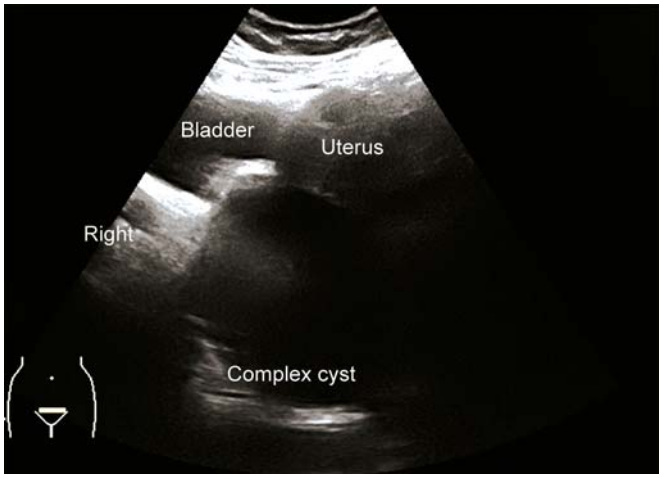


Fig. 10: Ovarian Ca 1A transabdominal ultrasound. Interesting here is only the topographic analysis. Hardly any information about the tumor obtainable because of shadowing and limited penetration/depth

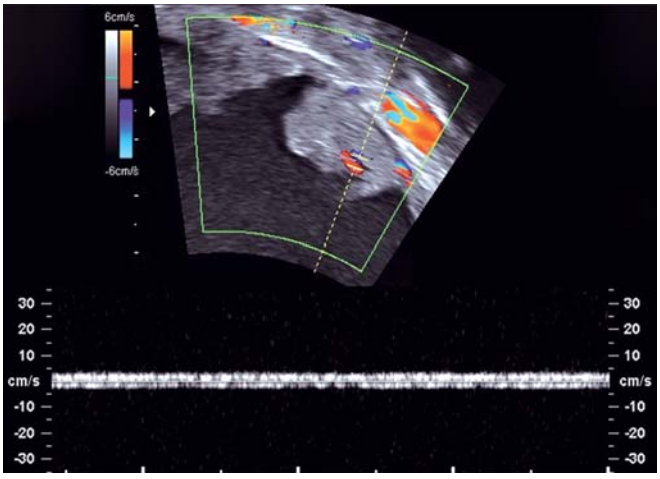


Fig. 13: Ovarian Ca 1A, TVS in CD PW mode: continuous low-resistance flow in the tumor papilla

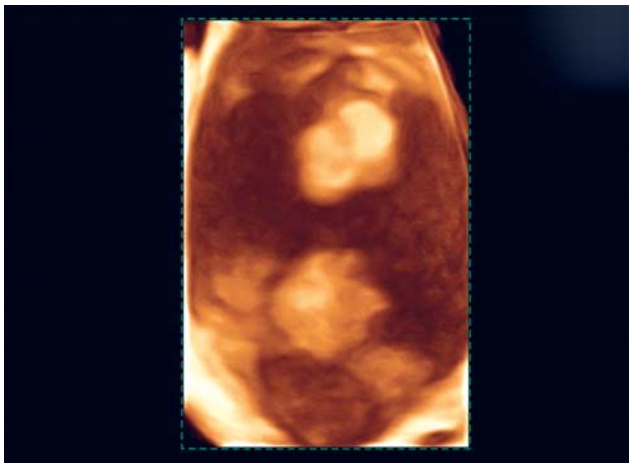


Fig. 14A: 3D Surface rendered papillary protrusions into the cyst. Reduced transparency because of echogenicity (detritus) of fluid contents of the cyst

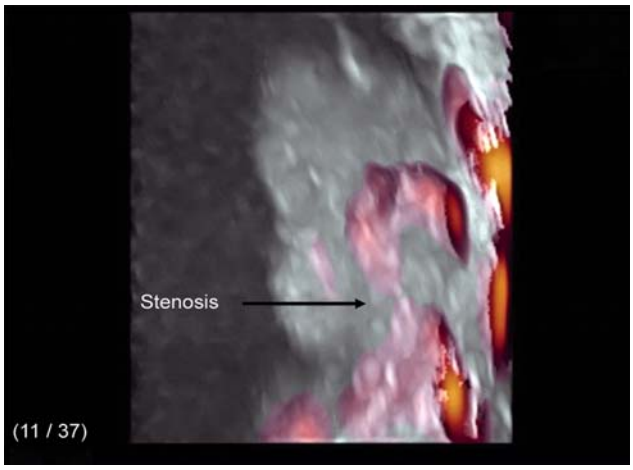


Fig. 14D: Power Doppler in glass body rendering of the tumor papilla: massive changes of vascular caliber within a short distance, depicting stenosis and lacunae due to abnormal chaotic tumor angiogenesis

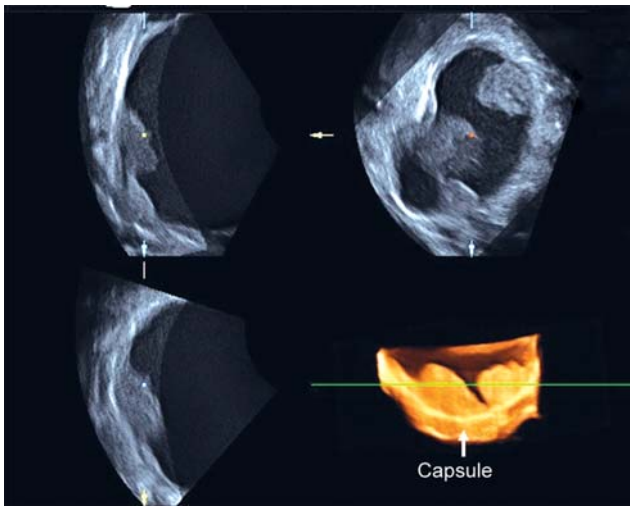


Fig. 14B: 3D Surface rendering and magic cut through the basis of the papillary tumor areas, in an attempt to depict infiltrative lesions of the ovarian capsule

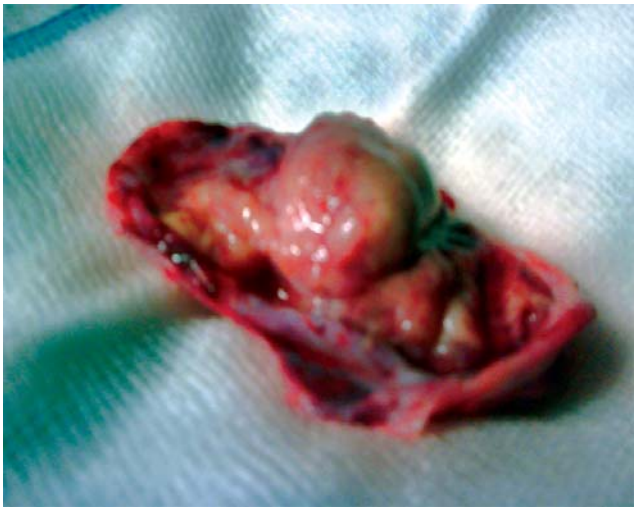


Fig. 14E: Ovarian Ca 1A, macroscopy**:** tumor papillae in the opened (extracorporal) cyst

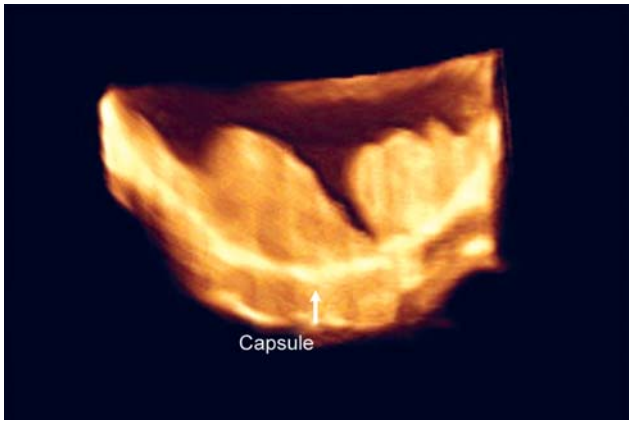


Fig. 14C: 3D Surface rendered, with magic cut through the papillae: the ovarian capsule appears as an echogenic band and shows no interruptions in this section. With tomographic imaging (TI), a systematic macro-"work-up" of the capsule could be possible

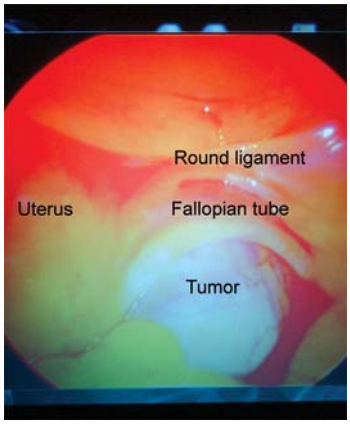


Fig. 15: Ovarian Ca 1A, laparoscopy**:** the tumor is incarcerated in the right small pelvis, immobile due to endometriotic adhesions

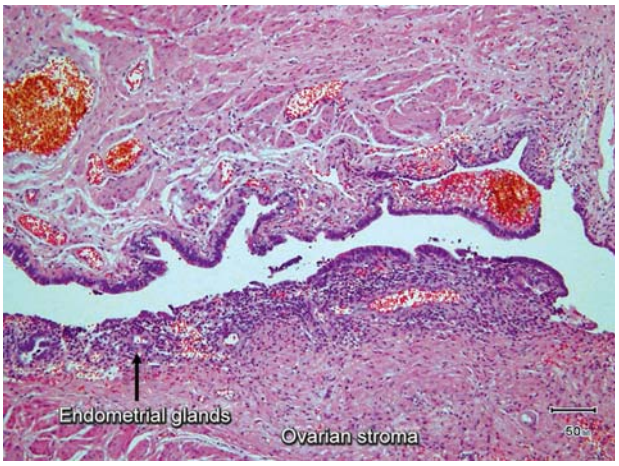


Fig. 16A: Ovarian 1A clear cell carcinoma, histology: endometrial glands, embedded in ovarian stroma (Courtesy of Dr. Hala Abdelaziz)

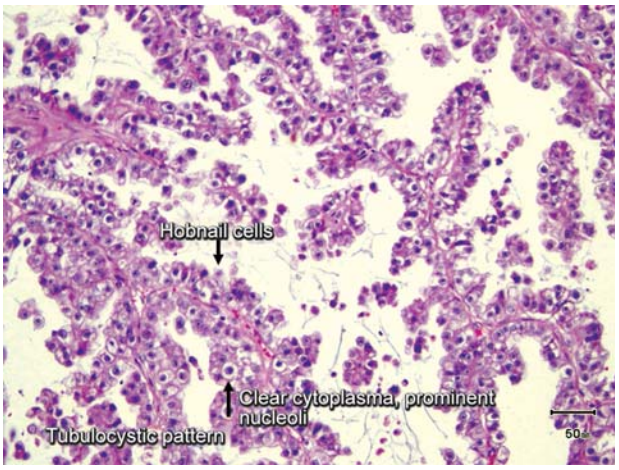


Fig. 16B: Ovarian Ca 1A, tubulo-cystic pattern of the tumor, tubuli lined by hobnail cells with clear cytoplasma and prominent nucleoli (Courtesy of Dr. Hala Abdelaziz)

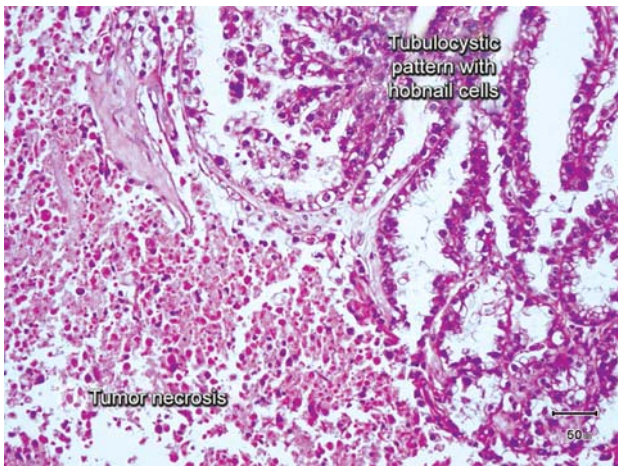


Fig. 16C: Ovarian Ca 1A. On the right side tubulo-papillary pattern with hobnail cells, on the left an area of tumor-necrosis (*Courtesy* of Dr. Hala Abdelaziz)

adnectomy, omentectomy, and para-aortic and iliac lymphonodectomy. The final histological diagnosis was clear cell carcinoma, staging pT1a. Focal endometriosis was found in both ovaries and the right Fallopian tube (Figs 16A to C).

The surprisingly good staging result, looking at a tumor volume of 256 ccm, demands explanations. Kurman et al. suggested a new model which divides ovarian cancer into 2 groups designated type I and type II. Type I tumors are slow growing, generally confined to the ovary at diagnosis and develop from well-established precursor lesions, so-called borderline tumors. Type I tumors include low-grade micropapillary serous carcinoma, mucinous, endometrioid, and clear cell carcinomas. They are genetically stable and are characterized by mutations in a number of different genes including KRAS, BRAF, PTEN, and beta-catenin.[55] Type II tumors are rapidly growing, highly aggressive neoplasms that lack well-defined precursor lesions; most are advanced stage at, or soon after, their inception. These include high-grade serous carcinoma, malignant mixed mesodermal tumors (carcinosarcomas), and undifferentiated carcinomas. The type II tumors are characterized by mutation of TP53 and a high level of genetic instability. Screening tests that focus on stage I disease may detect low-grade type I.[55]

For several decades, endometriosis has been suspected of playing a role in the etiology of ovarian cancer. Epidemiological evidence from large-cohort studies confirms endometriosis as an independent risk factor for ovarian cancer. Further circumstantial evidence for this link was found in the common risk factors for ovarian cancer and endometriosis. These risk factors influence retrograde menstruation and endometriosis in the same positive or negative way. Based on data in the literature, the prevalence of endometriosis in epithelial ovarian cancer has been calculated to be 4.5, 1.4, 35.9, and 19.0% for serous, mucinous, clear-cell and endometrioid ovarian carcinoma, respectively.[85] The risk of malignant transformation in ovarian endometriosis was calculated at 2.5% but this might be an underestimate. In addition, some authors described atypical endometriosis in a spatial and chronological association with ovarian cancer. Finally, molecular studies have detected common alterations in endometriosis and ovarian cancer. These data suggest that some tumours, especially endometrioid and clear-cell carcinomas, can arise from endometriosis. Moreover, endometriosis-associated ovarian cancer represents a distinct clinical entity, with a more favorable biological behaviour, given a lower stage distribution and better survival than nonendometriosis-associated ovarian cancer.[85]

ILLUSTRATIVE CASE NO. 4—THE DETECTION OF STAGE 3 DISEASE

A lady of Middle-East ethnicity, 48 years, with lower abdominal discomfort and bloated feeling in the abdomen since three months, came to see the primary care gynecologist for her annual examination. She had hypertension and diabetes mellitus type 2, was on metformin. Two children with normal vaginal deliveries. One sister suffered from breast cancer, with mastectomy. The patient had a normal gynecological check-up result one year before by a gynecologist, but no ultrasound had been done. Recently she noticed irregular menstruation.

Ultrasound showed bilateral complex adnexal masses of max. diameter of 7 cm , in color Doppler with randomly dispersed vascular pattern in the echogenic components of the mass (Figs 17 and 18).

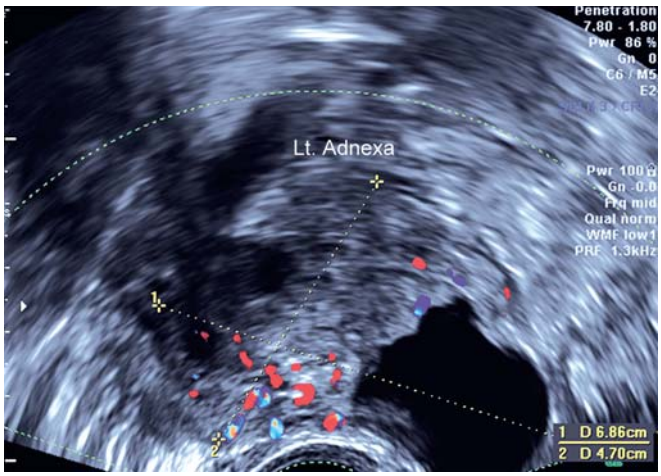


Fig. 17: Ovarian Ca bilateral, stage 3-4, right ovary. Randomly dispersed flow in the solid part of the cystic-solid mass

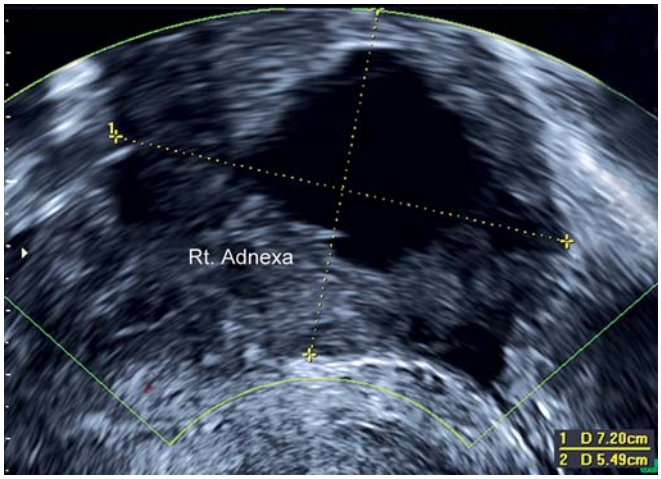


Fig. 18: Ovarian Ca bilateral, stage 3-4, left ovary, bizarre contour of the solid component

Laparotomy was performed after magnetic resonance imaging (MRI). CA 125 preoperative 1404 U per ml, postoperative staging FIGO III. She had metastasis in omentum, para-aortic lympnodes, and uterine infiltration. Cytology of peritoneal fluid (PF) was positive for cancer cells. Histology: high-grade serous-papillary carcinoma, moderately differentiated, of both ovaries. Remission after Carbo-Taxol chemotherapy.

What is important to stress from the previously described cases for ovarian cancer screening?

1. The 3D power Doppler qualitative analysis of tumor angiogenesis allows accurate detection of the earliest appearance of ovarian malignancy, i.e. stage IA ovarian cancer;
2. At the present time, higher equipment costs and more sophisticated operator skills make 3D ultrasound technology ideally available in clinical and university hospital settings as a secondary screening tool;
3. As published by Holbert,[86] and noted in the case above, routine screening for ovarian cancer by standard 2D ultrasound modalities, in terms of primary screening, is a valuable addition to the yearly examination in outpatient clinics and private gynecology office settings;
4. Stage 1 detection of type 2 highly aggressive, fast growing ovarian neoplasma remains a challenge.

AIMS

Application of new 3D ultrasound technologies on patients with «positive» standard ultrasound tests represents an innovation compared with previous ovarian cancer screening trials. In this way, we were able to demonstrate for the first time that a secondary screening based on morphologic and vascular parameters assessed by 3D ultrasound, 3D power Doppler and contrast-enhanced 3D power Doppler may improve early detection of ovarian cancer and accuracy of ultrasound screening strategy in high-risk populations. Regarding this hypothesis, the primary end point of our screening trial was to improve the highest positive predictive value of 20% reached by multimodal screening, resulting in less than five operations for each ovarian cancer found as an excellent surgery to malignancy ratio.

CONCLUSION

Although a critical evaluation of the published screening trials leads to the conclusion that routine screening for ovarian cancer appears to be of advantage, many efforts continue to identify new screening modalities in high-risk populations. It seems that potential balance of benefits, harms and costs of screening may be more favorable in women with an inherited predisposition for developing of

ovarian cancer. In such groups, compared with general population, fewer women need to be screened for each case detected, prevalence of the disease is markedly higher and the ratio of false positives to true positives is lower.

Because most of the ovarian cancers occur in general population, there has been growing interest in the possibility of screening for those with an increased risk, i.e. asymptomatic postmenopausal women. Two main strategies, multimodal and ultrasound based, have emerged, both with some limitations for implementation in a routine screening practice. For the first one, the great challenge is to improve the sensitivity of serum CA 125 as a primary screening tool. The risk of ovarian cancer algorithm (ROCA), an exponential model using data from several prior scans and testing for an exponential rise in the value of the marker, is likely to improve the sensitivity of CA 125 as the first line screening test. More promising ovarian tumor markers appear at the horizon.

In view of the persisting threat especially from ovarian type two cancer, it is comforting to know that 3D ultrasound imaging can emend the ability to differentiate benign from malignant masses, and can significantly increase specificity and positive predictive value (PPV) in ovarian cancer detection. Further analysis with 3D power Doppler (3D-PD) clearly depicts disorganized, randomly dispersed vessels with irregular branching in the solid part of the tumor, strongly associated with ovarian malignancy. 3D power Doppler imaging provides data on tumor vessel architecture for accurate preoperative diagnose of an early stage of an ovarian cancer.[87]

Therefore, the problem of low PPV in 2D ultrasound-only strategies may be solved by introducing the new 3D ultrasound technology, used as a secondary screening procedure. The role of 3D ultrasound, 3D power Doppler and contrast-enhanced 3D power Doppler in early and accurate detection of ovarian was confirmed through the Zagreb Ovarian Cancer Screening Trial.

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