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Tumor angiogenesis assessed by three-dimensional power Doppler ultrasound in early, advanced and metastatic ovarian cancer: a preliminary study

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KEYWORDS: 3D ultrasound; ovarian cancer; power Doppler

ABSTRACT

Objective *To evaluate tumor vascularity by three-dimensional power Doppler ultrasound (3D-PDU) in early and advanced stage primary ovarian cancers and in metastatic tumors to the ovary.*

Patients and methods *This was a retrospective analysis of clinical and sonographic data from 49 women with primary ovarian cancers or metastatic tumors to the ovary. All women underwent 3D-PDU prior to surgery. Vascularization index (VI), flow index (FI) and vascularization flow index (VFI) from solid portions or papillary projections in the tumors were calculated using the Virtual Organ Computer-aided AnaLysis (VOCAL™) program. Definitive histological diagnosis was obtained in each case.*

Results *Among the 49 women, 10 had stage I primary cancers (five low-malignant potential tumors and five invasive tumors), 26 had advanced stage primary ovarian cancers and 13 had metastatic tumors to the ovary. Mean VI and VFI were significantly higher in advanced stage tumors and metastatic tumors as compared with early stage tumors. No differences in 3D-PDU indices were found between advanced stage and metastatic cancers.*

Conclusions *Vascular indices derived from 3D-PDU tend to be higher in advanced stage and metastatic ovarian cancers as compared with early stage ovarian tumors.*

INTRODUCTION

Angiogenesis plays an essential role in tumor growth and metastasis[1]. Several studies using immunohistochemical techniques have demonstrated that, in ovarian cancer, angiogenesis may be a prognostic factor[2,3]. Angiogenesis may be assessed *in vivo* using pulsed and color Doppler ultrasonography, the conventional methods for assessing tumor vascularization. In ovarian tumors Doppler ultrasound has been used mainly for discriminating between benign and malignant lesions. Although initial studies on color and spectral pulsed Doppler were encouraging[4], further studies revealed a wide overlap in pulsed Doppler indices between malignant and benign tumors, making this approach impractical from the clinical point of view[5]. Three-dimensional power Doppler ultrasound (3D-PDU) is a relatively new technique that allows tumor vascularization assessment, both quantitatively by means of 3D-PDU-derived vascular indices[6] and qualitatively by depicting three-dimensionally the tumor vascular network[7].

Some studies using immunohistochemical staining have found quantitative differences in angiogenesis in ovarian cancer according to tumor stage[8] and other studies have shown that Doppler assessment of tumor vascularization is related to microvessel density in cervical, prostate and breast cancer[9–11]. The aim of the present study was to evaluate whether differences exist in tumor vascularization as assessed by 3D-PDU in early and advanced stage primary ovarian cancers and in tumors that have metastasized to the ovary.

PATIENTS AND METHODS

In this retrospective study data from 49 women diagnosed as having primary or metastatic ovarian cancer, and who were evaluated and treated at our institution between January 2003 and October 2005, were reviewed. A group of these patients are included in a previous study[12].

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The patients' mean age was 52.9 (range, 17–82) years. Twenty-eight (57.1%) women were postmenopausal and 21 (42.9%) women were premenopausal. Four women had bilateral tumors identifiable at ultrasound but only one tumor per patient was used for analysis in these four women.

All the women had been evaluated by transvaginal ultrasound using a Voluson 730 Pro (GE Healthcare, Zipf, Austria) with a 5–7.5-MHz transvaginal probe and color, power and pulsed Doppler as well as three-dimensional (3D) ultrasound capabilities. Transabdominal ultrasound (3.5–5-MHz) was also performed in the case of large tumors.

The scanning technique has been described in detail elsewhere[12]. Following B-mode evaluation, the two-dimensional power Doppler gate was activated to assess tumor vascularization. Power Doppler settings were set to achieve maximum sensitivity to detect low velocity flow without artifacts (frequency, 5 MHz; power Doppler gain, 20 (range, 1–30); dynamic range, 20–40 dB; edge, 1; persistence, 2; color map, 1; gate, 2; filter, 3; pulse repetition frequency, 0.6 kHz). Central vessel vascularization was defined as the presence of color spots within papillary projections, solid areas or the central part of solid tumors. Power Doppler imaging was used to identify vessels for subsequent pulsed Doppler interrogation to obtain a flow velocity waveform and to confirm the arterial nature of the vessel. Pulsatility index (PI), resistance index (RI) and peak systolic velocity (PSV, cm/s) were recorded automatically. The lowest PI and RI and the highest PSV found in a given tumor, independently from the vessel in which they were obtained, were used for analysis. In cases in which no arterial flow was found ($n = 2$) pulsed Doppler data were not calculated.

For 3D imaging the volume was activated to obtain a 3D box from either papillary projections, solid areas or, in the case of mostly solid tumors, the whole tumor. Once a 3D volume had been obtained, it was stored on a hard disk in the ultrasound machine. The stored volumes were further analyzed using the Virtual Organ Computer-aided AnaLysis (VOCAL) program (Sonoview™, Kretztechnik, Zipf, Austria). Volume acquisition time lasted from 15 to 20 s depending on the size of the volume box. Using the VOCAL program, three vascular indices were calculated from every solid area or papillary projection with color signals within it, excluding cystic (fluid-filled) areas in which blood vessels would not be found (Figures 1 and 2).

The three-dimensional volume consists of 'voxels' (smallest unit of volume). Voxels contain information about gray-scale and color according to an intensity scale ranging from 0 to 100. The vascular indices calculated were the vascularization index (VI), which is expressed as a percentage and represents the relative proportion of power Doppler data within the defined volume; the flow index (FI), which is the mean signal intensity of this power Doppler information; and the vascular flow index (VFI), which is a combination of both[6].

Measurements were undertaken using the manual mode, in plane 'A' and a 9°-rotation step. If there was more than one non-contiguous solid area, each area was evaluated separately and the highest VI, FI and VFI values (which could have been obtained from different areas) were used for analysis. If the tumor showed areas

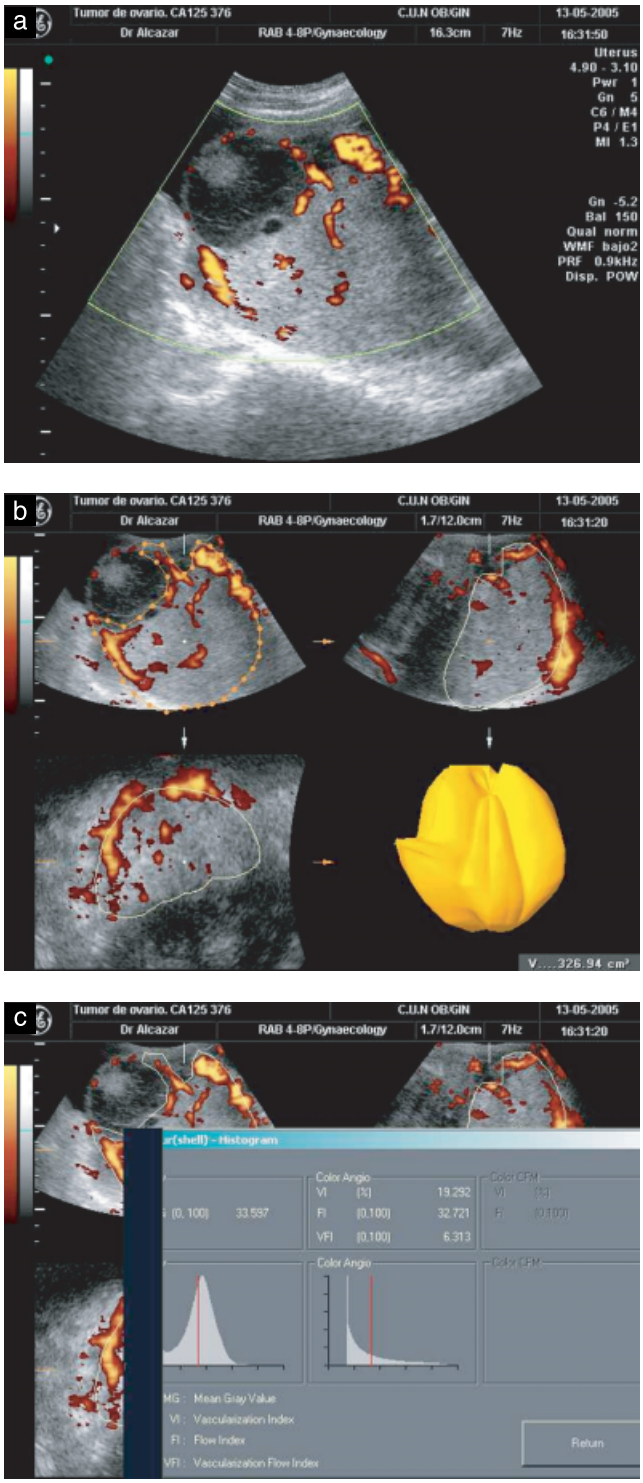

Figure 1 (a) Transvaginal ultrasound scan showing a solid cystic vascularized adnexal mass. (b) Tumor volume estimation using the Virtual Organ Computer-aided AnaLysis (VOCAL) program. In this case only the solid portion of the tumor is included in the analysis. (c) Three-dimensional power Doppler-derived indices obtained using the VOCAL program. This case was a stage IIIc serous–papillary adenocarcinoma of the ovary.

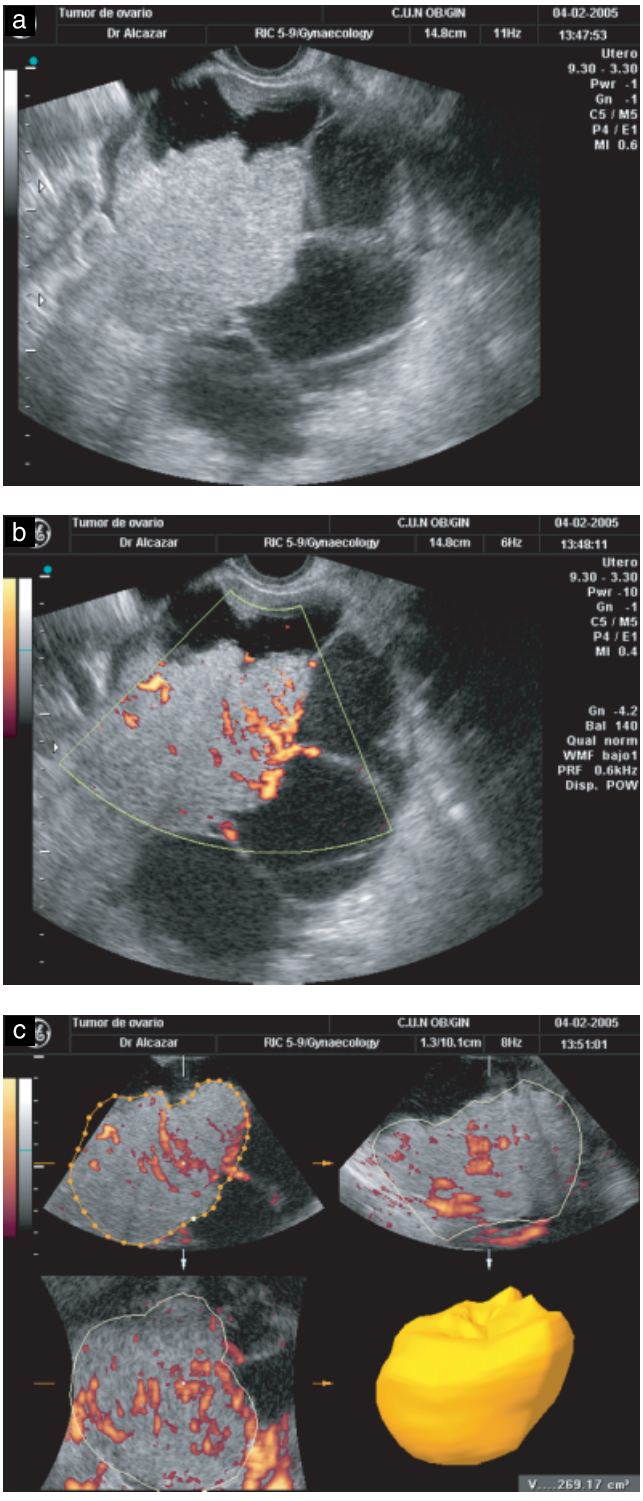

Figure 2 (a) Transvaginal ultrasound scan showing a multilocular cystic lesion with a solid area arising from internal cyst wall. (b) Transvaginal two-dimensional power Doppler ultrasound scan showing color signals within solid area. (c) Solid area volume estimation using the Virtual Organ Computer-aided AnaLysis (VOCAL) program.

of low echogenicity, this area was not included in the VOCAL calculation. All calculations were performed on the ultrasound machine.

All ultrasound examinations and 3D analyses were performed by a single operator. Patients were selected according to availability of the examiner and ultrasound machine. As this was a retrospective study no Institutional Review Board approval was sought.

All patients underwent surgery, and a definitive histological diagnosis was obtained in every case. Tumors were classified according to the World Health Organization (WHO) criteria[13] and ovarian cancers were staged according to International Federation of Gynecology and Obstetrics (FIGO) criteria[14].

The Kolmogorov–Smirnov test was used to assess normal distribution of continuous data. As all continuous data showed a skewed distribution they were compared using the Kruskal–Wallis test and the Mann–Whitney *U*-test with Bonferroni correction. Categorical variables were compared using the Chi-square test. $P \leq 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SPSS 11.0 statistical package (SPSS Inc, Chicago, IL, USA).

RESULTS

Of the 49 patients, five (10.2%) had tumors of low malignant potential, 31 (63.3%) had primary invasive carcinomas and 13 (26.5%) had metastatic tumors to the ovary (source: colon, six cases; endometrium, two cases; breast, two cases and liver, mesothelioma and lung one case each.). Histological diagnoses in primary ovarian cancers, including low-malignant potential tumors, were as follows: epithelial cancer 32 cases, sarcoma one case, immature teratoma one case, limphoma one case, Sertoli–Leydig tumor one case.

All low-malignant potential tumors and five primary invasive carcinomas were stage I, so these 10 cases were grouped as stage I cancers. Twenty-six women with primary invasive cancers had advanced stage tumors (three stage II, 18 stage III and five stage IV). Two women with stage III and two with metastatic cancers had bilateral tumors. B-mode findings according to tumoral stage are shown in Table 1. Metastatic tumors were more frequently solid lesions than were early and advanced stage primary ovarian cancers ($P = 0.009$).

Median VI and VFI were significantly higher in advanced stage tumors as compared with early stage tumors; they were also significantly higher in metastatic tumors as compared with early stage tumors. No differences were found in FI when comparing early stage cancers with advanced stage and metastatic cancers. No differences were found between advanced stage and metastatic cancers in VI, FI and VFI and no differences were found in RI, PI and PSV among all three groups (Table 2).

DISCUSSION

Usually tumor angiogenesis is evaluated by assessing microvessel density following immunohistochemical staining for several endothelial antigens such as CD34, CD31, factor VIII related antigen and VEGF[8,15]. Some

Table 1 B-mode findings according to tumoral stage

Stage	*Unilocular solid,* n *(%)*	*Multilocular solid,* n *(%)*	*Solid,* n *(%)*	*Total,* n *(%)*
Early stage, $n = 10$	4 (40)	5 (50)	1 (10)	10 (100)
Advanced stage, $n = 26$	5 (19.2)	7 (26.9)	14 (53.8)	26 (100)
Metastatic tumors, $n = 13$	1 (7.7)	3 (23.1)	9 (69.2)	13 (100)

Table 2 Three-dimensional power-Doppler and pulsed Doppler indices according to stage of ovarian cancer. Data are expressed as median (interquartile range)

Stage	*VI*	*VFI*	*FI*	*RI*	*PI*	*PSV (cm/s)*
Early stage[a], $n = 10$	9.57 (8.5)	3.06 (3.1)	31.21 (6.1)	0.40 (0.10)	0.59 (0.15)	16.8 (8.5)
Advanced stage[b], $n = 26$	18.02 (10.8)	5.91 (4.1)	33.89 (7.2)	0.45 (0.24)	0.66 (0.45)	14.2 (11.3)
Metastatic tumors[c], $n = 13$	15.10 (11.5)	5.60 (3.2)	34.79 (6.4)	0.42 (0.15)	0.59 (0.31)	14.0 (10.9)
P	a vs. b 0.022	a vs. b 0.005	a vs. b 0.132	a vs. b 0.682	a vs. b 0.501	a vs. b 0.589
	a vs. c 0.014	a vs. c 0.032	a vs. c 0.192	a vs. c 0.740	a vs. c 0.740	a vs. c 0.976
	b vs. c 0.857	b vs. c 0.814	b vs. c 0.879	b vs. c 0.393	b vs. c 0.195	b vs. c 0.816

FI, flow index; PI, pulsatility index; PSV, peak systolic velocity; RI, resistance index; VFI, vascularization flow index; VI, vascularization index.

have found that angiogenesis is a prognostic factor both in early[16] and advanced stage ovarian carcinomas[17]. However, some researchers have challenged these findings[18]. Nonetheless, microvessel density is higher in advanced stage tumors as compared with early stage ones[3,8].

Vascularization can be assessed *in vivo* by Doppler ultrasonography. A correlation seems to exist between the pulsed Doppler indices RI and PI[19] or color blood flow mapping[20] and tumor microvessel density. 3D-PDU, theoretically, provides a more comprehensive means of assessing tumor vascularization than does two-dimensional (2D) color or power Doppler by the use of the vascular indices VI, FI and VFI[6] and three-dimensional depiction of the tumor vascular network[7]. Some studies have shown that 3D-PDU may be useful for differentiating benign from malignant ovarian tumors[21–23].

Since microvessel density tends to be higher in advanced stage ovarian cancers than in early stage tumors, it is logical to assume that vascular indices derived from 3D-PDU would be higher in advanced stage ovarian cancers than in early stage tumors. Apparently, no previous study has used 3D-PDU to assess tumor vascularization according to tumoral stage, and only one previous study has been published that compared PSV (not RI and PI) according to ovarian cancer stage[24]. According to that study PSV did not differ between early and advanced stage ovarian cancer[24]. In another study we found no differences in pulsed Doppler indices between metastatic and primary ovarian cancer[25].

In conclusion, vascularization, as assessed by 3D-PDU indices, is higher in advanced stage and metastatic ovarian cancers than in early stage ovarian cancer. These preliminary results may be of value for future research. It would be worth exploring whether vascular indices derived from 3D-PDU in ovarian cancer could be used as a prognostic factor for this condition.

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