ORIGINAL ARTICLE

Visual Grading System, Blood Flow Index, and Tumor Marker SCC Antigen as Prognostic Factors in Invasive Cervical Carcinoma

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Background: The purpose of this study was to develop a new visual grading system for the vascular ratio, to assess the correlation of the prognostic significance of the visual grading system, tumoral blood flow velocity and squamous cell carcinoma (SCC) antigen serum levels in invasive SCC of the cervix.

Patients and Methods: A total of 117 patients with invasive cervical carcinoma were enrolled for the assessment of vascular grading and tumor flow using transvaginal ultrasound with power Doppler angiography (TV-PDA) before treatment. The modified visual grading system of the vascular ratio (grades 1 to 3) and six blood flow characteristics of tumor vessels (pulsatility index, resistance index, peak systolic velocity, end-diastolic velocity, time-averaged maximum velocity, vascular index) were measured by TV-PDA. In addition, serum levels of SCC antigen were randomly obtained in 74 patients with invasive cervical SCC (stages Ia to IIIb) before treatment.

Results: The modified visual grading system of the vascular ratio significantly correlated with pretreatment SCC antigen serum levels ($p < 0.05$) and intratumoral resistance index values ($p < 0.05$, $r^2 = 0.49$) from multiple regression analysis in 71 patients with invasive SCC of the cervix. Modified visual grading of the vascular ratio negatively correlated with intratumoral resistance index values (Pearson's correlation coefficient, $r = -0.571$, $p = 0.001$), but positively correlated with pretreatment serum levels of SCC antigen ($r = 0.296$, $p = 0.012$). In addition, this modified vascularity grading positively correlated with FIGO stage ($r = 0.336$, $p = 0.004$) by correlation analysis.

Conclusion: The modified visual grading system of the vascular ratio is a valuable sonographic marker in assessing invasive cervical carcinoma. The three major advantages of this semi-quantitative analysis are ease of discrimination, immediate results, and avoiding wasting time. This semi-quantitative analysis with pretreatment SCC antigen serum levels are two effective markers for assessing invasive cervical carcinoma.

KEY WORDS — blood flow velocity, cervical carcinoma, color Doppler ultrasound, serum SCC antigen

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Introduction

The production of new vessels within a defined area is called angiogenesis. This event is also indispensable for tumor growth [1]. Using the advanced technique of Doppler ultrasound, angiogenesis can be assessed *in vivo* [2]. Neovascularized vessels can be differentiated from normal vessels using color Doppler ultrasound [3]. Recently, with regard to invasive cervical carcinoma, several studies have demonstrated that angiogenesis is an independent factor in predicting disease prognosis [4–7].

Our previous study demonstrated the application of transvaginal ultrasound with power Doppler angiography (TV-PDA) in the grading of the vascular ratio within a cervical mass [8]. Due to the difficulty in discriminating between grades 3 and 4 using our defined grading of the vascular ratio, we developed a new modified grading system and conducted this study to assess the association between this modified visual grading of the vascular ratio, blood flow velocity within a tumor, and pretreatment squamous cell carcinoma (SCC) antigen serum levels in invasive SCC of the cervix.

Table 1. Characteristics of the 117 women with invasive cervical carcinoma included in this study

Mean age, *yr* (range)	54 (25–85)
Premenopausal, *n*	66
Postmenopausal, *n*	51
Clinical staging (FIGO), *n*	
Ia	8
Ib1	55
Ib2	14
IIa	22
IIb	12
IIIa	2
IIIb	4
IV	0
Histology, *n*	
SCC type	93
Adeno type	19
Adenosquamous type	1
Papillary adeno type	1
SCC + adeno type	3
Pretreatment tumor marker (SCC-Ag), *n*	
Available	74
Unavailable	43

Patients and Methods

Patient population

The study population consisted of 117 patients selected from women admitted to the Department of Obstetrics and Gynecology of Taipei Veterans General Hospital with the diagnosis of invasive cervical carcinoma between March 1998 and October 2001. The general characteristics of the patients are shown in Table 1.

TV-PDA

All of the women were scanned using color Doppler ultrasound (Diasonics Gateway; Diasonics, Milpitas, CA, USA) equipped for color Doppler imaging and color Doppler angiography. All women were scanned while in the semi-lithotomy position with an empty urinary bladder within 2 days of undergoing treatment. A 7.0 MHz curved-array endovaginal probe was used, and the field of view was set within 112 degrees. The uterine cervix was scanned and the tumor mass was carefully identified in grayscale mode. Vascularity within the tumor was detected after shifting to angio mode. The color gain was adjusted from 105 to 115, and the scale was adjusted using pulse repetition frequency (PRF) and set at 800–900 Hz to filter out the low strength signals. The temporal filter was adjusted and set at 1.0 to eliminate the blue color within the region of interest (box).

To estimate vascularity within the tumor, the vascular ratio of the visual grading system was defined as the number of colored pixels of intratumoral vessels divided by the number of total pixels of intratumoral vessels in the defined tumor section. The most abundant vascularity in single plane was selected as the defined tumor section for grading analysis. Vascularity within the tumor was classified

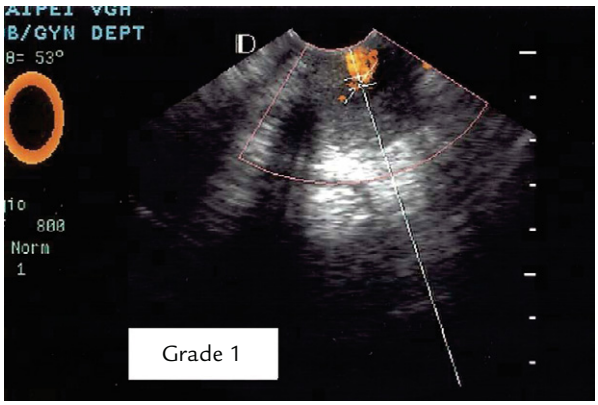

Fig. 1. *Power Doppler ultrasound shows grade 1 vascularity (0 < vascular ratio < 25%) within the tumor.*

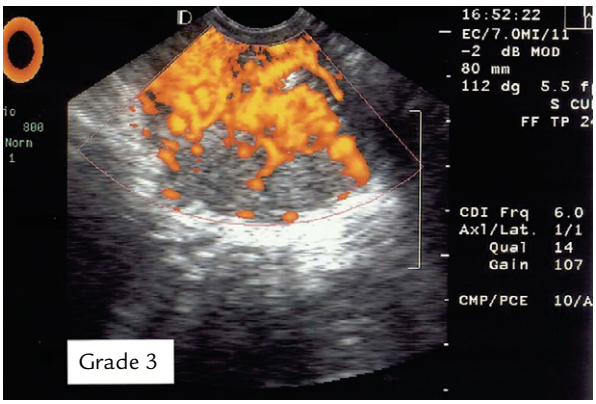

Fig. 3. *Power Doppler ultrasound shows grade 3 vascularity (50% < vascular ratio < 100%) within the tumor.*

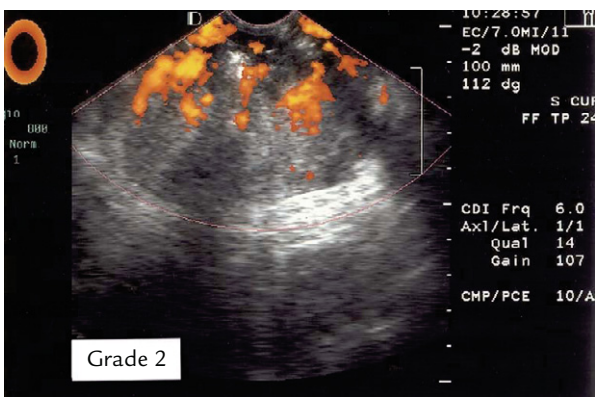

Fig. 2. *Power Doppler ultrasound shows grade 2 vascularity (25% < vascular ratio < 50%) within the tumor.*

into four grades. Grade 0 was defined as absence of vascularity, grade 1 as 0 < vascular ratio < 25%, grade 2 as 25% < vascular ratio < 50%, and grade 3 as 50% < vascular ratio < 100% (Figs. 1–3).

Measurement of blood flow velocity waveforms within the tumor

By activating the pulse Doppler mode, we adjusted the Doppler gate over the target color area. We then set the distance of the gate appropriately and adjusted the angle of the detectable sample site below 65 degrees. We defined the detectable Doppler signals as a series of reproducible, similar arterial waveforms obtained from at least three separate consecutive cardiac cycles. The satisfactory samples from the detectable Doppler signals were processed and analyzed using on-line spectra. All the measurements were recorded using manual or automatic methods, including peak systolic velocity (PS), end-diastolic velocity (ED), time-averaged maximum velocity (TAMXV), pulsatility index (PI), resistance index (RI), and vascular index (VI). When more than one set of satisfactory samplings were obtained, the lowest set of PI, RI and VI values were recorded as the calculated value. PI, RI and VI were calculated as follows:

$$PI = \frac{PS - ED}{TAMXV}$$

$$RI = \frac{PS - ED}{PS}$$

$$VI = \frac{PS}{ED}$$

Intraobserver reliability was assessed by one observer. All the scans were performed by one of the authors (YCW) to avoid interobserver variation. Intraobserver reliability of the measurements of vascularity and flow were assessed by intraclass correlation coefficients (ICCs) [9].

Pretreatment measurements of serum SCC antigen

The serum levels of SCC antigen were randomly obtained from 74 patients with invasive cervical SCC (stages Ia–IIIb) using a commercially available kit (Abbott SCC-RIA kit; Abbott Japan Co. Ltd., Tokyo, Japan) 2–3 days before treatment. The Abbott

SCC-RIA kit is a solid phase radioimmunoassay based on the "sandwich" principle. Samples were incubated simultaneously with anti-SCC Ag coated beads and ^{125}I-labeled anti-SCC Ag. Both the immobilized antibodies and the radiolabeled antibodies were bound to SCC antigen to form sandwiches. The upper limit of normal for the results of this clinical study was set at 1.5 ng/mL, according to the commercial protocol.

Statistical analysis

Data analysis was performed using SAS, JMP version 6.12 (SAS Institute, Cary, NC, USA); tests included one-way analysis of variance, simple regression analysis, multiple regression analysis, and Pearson's correlation coefficient. Statistical significance was set at $p < 0.05$.

Results

The clinical staging of cancer for each patient was assigned according to FIGO. The characteristics of the case groups are presented in Table 1.

Visual grading of TV-PDA vascular ratio findings

The total pick-up rate was 97.43% (114/117) among the 117 patients with invasive cervical carcinoma using TV-PDA. Among the 74 patients with invasive cervical SCC with available pretreatment serum levels of SCC antigen, vascularity within the tumor was classified as grade 1 in 28 patients, grade 2 in 19 patients, and grade 3 in 24 patients. Three patients had an absence of vascularity (grade 0) within their tumors.

TV-PDA blood flow velocity findings

Sixty-six patients (56.4%) were premenopausal and the other 51 patients (43.6%) were postmenopausal. No significant differences were found in mean PI, RI or VI between the tumors in premenopausal women (PI, 0.70 ± 0.28; RI, 0.45 ± 0.15; VI, 2.02 ± 0.89) and those in postmenopausal women (PI, 0.72 ± 0.03; RI, 0.48 ± 0.12; VI, 2.02 ± 0.45) (PI, $p = 0.097$; RI, $p = 0.261$; VI, $p = 0.580$). Similarly, no significant differences were found in mean PS, ED and TAMXV between tumors in premenopausal patients (PS, 12.18 ± 5.11; ED, 6.64 ± 3.20; TAMXV, 8.84 ± 3.88) and those in postmenopausal patients (PS, 10.18 ± 3.42; ED, 5.35 ± 2.44; TAMXV, 7.28 ± 2.62) (PS, $p = 0.82$; ED, $p = 0.44$; TAMXV, $p = 0.80$).

Visual grading system of the vascular ratio, blood flow velocity, and pretreatment SCC antigen serum levels

The modified visual grading of the vascular ratio significantly correlated with pretreatment SCC antigen serum levels ($p = 0.0389$) and intratumoral RI values ($p = 0.0125$, $r^2 = 0.49$) from multiple regression analysis in 71 patients with invasive SCC of the cervix (3 patients with undetectable intratumoral microvessels were excluded) (Figs. 4 and 5). The mean RI and mean pretreatment SCC antigen serum level for patients in each vascular grade are shown in Table 2. In addition, the modified vascular ratio grading system positively correlated with FIGO staging (Pearson's correlation coefficient, $r = 0.336$, $p = 0.004$) by correlation analysis.

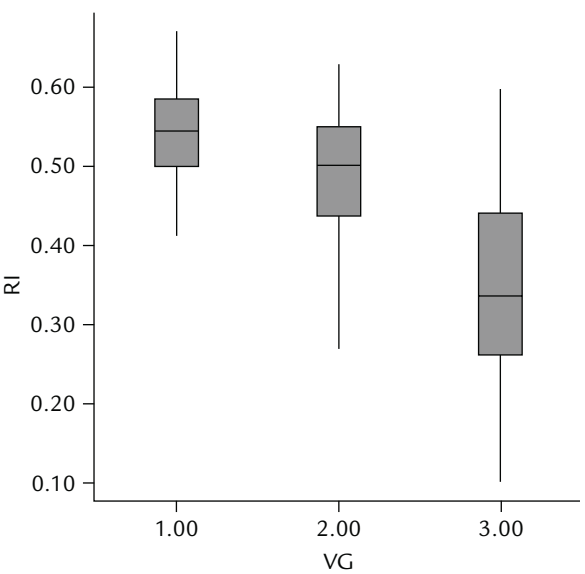

Fig. 4. *Box plot showing the correlation between the modified visual grading system of vascular ratio (VG) and intratumoral resistance index (RI) in 71 patients with invasive cervical carcinoma.*

Discussion

Several reports in the literature have addressed the reasons why power Doppler ultrasound is able to detect blood flow within microvessels [10,11]. We found a new visual grading system of the vascular ratio using TV-PDA that provided more sonographic characteristics than color Doppler imaging in different subclassifications of cervical carcinoma [8]. The advantages of this visual grading system include ease of discrimination. It is convenient to check intratumoral vascularity of invasive cervical carcinoma using the power mode of a color Doppler ultrasound. Immediate results are available to avoid wasting time. This semi-quantitative analysis from our study did not need any additional software to analyze the number of colored pixels and total pixels in a defined tumor section. Two-dimensional (2-D) ultrasound of a tumor does not generally demonstrate an accurate representation of total vascularity. However, in our study, we were able to demonstrate the greatest intratumoral vascularity from a defined plane of 2-D ultrasound after rotating the vaginal probe.

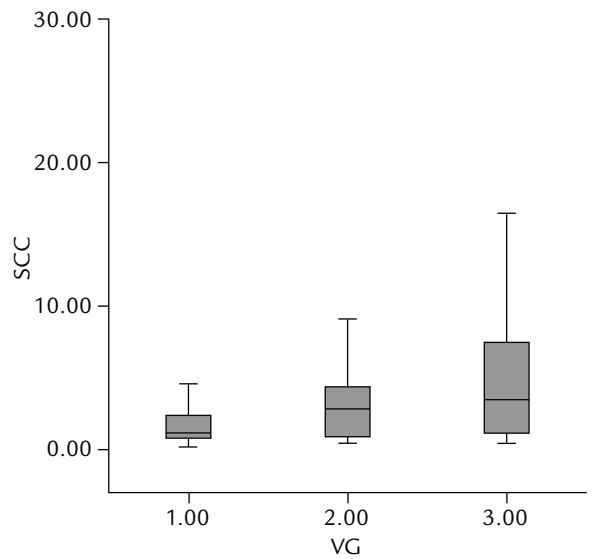

Fig. 5. *Box plot showing the correlation between the modified visual grading system of vascular ratio (VG) and squamous cell carcinoma antigen (SCC) in 71 patients with invasive cervical carcinoma.*

Based on our previous study [8], preoperative SCC antigen serum levels are independent of intratumoral blood flow analysis (six basic parameters: PI, RI, TAMXV, PS, ED, and VI [PS/ED]) in SCC. As we know, SCC associated antigen (SCC-Ag) is a subfraction of TA-4, which is obtained from squamous cell cancer tissue of the uterine cervix and has been characterized as a glycoprotein. Some researchers have indicated that serum TA-4 levels may reflect the extent of disease in women with cervical SCC, and it is a useful adjuvant marker in predicting prognosis, detecting recurrence, and monitoring disease status [12–15]. So far, the relationship between imaging findings of tumor morphology and serum SCC-Ag levels is unknown. Here, we found that the visual grading system of the vascular ratio positively correlated with preoperative SCC antigen serum levels (Table 2). Based on our previous vascular grading [8], pretreatment SCC antigen serum levels were obvious from grade 1 to grade 3, but it was difficult to differentiate grade 3 from grade 4. The possible causes may be due to the limitation of the visual grading system of the vascular ratio from 2-D sonographic images or the variable changes in circulating serum SCC-Ag. In this study, we revised the definition of the previous grading system, and these changes presented a better discriminative grading system in assessing invasive

Table 2. Modified visual grading system of the vascular ratio, intratumoral resistance index (RI), and pretreatment squamous cell carcinoma (SCC) antigen serum level in 74 patients with invasive cervical carcinoma

Vascular grade	Definition	*n*	RI	Pretreatment serum SCC antigen
0	Absence of vascularity	3	Non-detectable	0.447 ± 0.333
1	0 < vascular ratio < 25%	28	0.526 ± 0.018	3.175 ± 0.748
2	25% < vascular ratio < 50%	19	0.468 ± 0.026	6.022 ± 2.354
3	50% < vascular ratio < 100%	24	0.345 ± 0.131	15.204 ± 7.115

cervical carcinoma before treatment. However, the effect of active bleeding of tumor masses or passive tumor bleeding due to transvaginal probe contact with the surface of the tumor mass was magnified. To lessen the active and passive bleeding effects, we avoided transvaginal ultrasound examinations immediately after colposcopic biopsy, and contact with cervical masses was as slight as possible when the transvaginal probe was inserted into the vagina.

The intratumoral RI value is a significant index of intratumoral angiogenesis and several reports in the literature have demonstrated its use in differentiating benign and malignant pelvic masses [16–19]. Intratumoral RI value assessment of angiogenesis may represent the variation in targeting intratumoral vessels. The lowest RI value of target vessels detected after at least three examinations could be considered as the target vessel with the largest diameter or most likely confluent vessel area. Either large intratumoral vessels or confluent vessels within tumors will contribute to the grade of vascularity within tumors under 2-D power Doppler mode. It is reasonable that the lower RI value from spectral Doppler analysis, the higher grading of the vascular ratio within tumors was demonstrated from this study. In our present study, the intratumoral RI value negatively correlated with clinical staging (FIGO) from Ia to IIIb (r=–0.301, p=0.011). Our results were different from the results of a previous study by Cheng et al [20].

In this study, no significant correlations were found between vascular grading (r=0.207, p=0.623) and mean intratumoral RI value (r=–0.295, p=0.477) from stage Ib1 to Ib2. That is, in stage Ib of cervical SCC, the vascular grading or intratumoral RI value was not the significant factor that contributed to the differentiation of stage Ib1 from Ib2. The above results may be explained as follows. First, there was a limited sample size. Second, the effects of central necrosis within the tumors, especially the target vessels near the central part of the tumor, contributed to the variations in intratumoral RI values and vascular grading. Third, the 2-D image including all the sonographic parameters could not be used to fully describe the 3-D stereograph of the polymorphous shape of cervical SCC. Recently, 3-D Doppler ultrasound provided a quantitative assessment in cervical cancer, but intratumoral vascularity patterns revealed no significant difference in 3-D color Doppler histogram analysis including VI (vascularization index), FI (flow index) and VFI (vascularization flow index) [21].

In conclusion, the modified visual grading system of the vascular ratio is a better sonographic marker than the original vascular grading system [8] for assessing tumor vascularity in patients with invasive cervical carcinoma. It is also better than intratumoral RI for predicting the clinical stage of invasive cervical carcinoma. The three major advantages of this semiquantitative analysis include ease of discrimination, immediate results, and avoiding wasting time. In our study, this noninvasive sonographic diagnostic tool was used to predict pretreatment SCC antigen serum levels according to our modified vascular grading system. The *in vivo* imaging of intratumoral vascularity may be a significant factor in predicting pathologic findings, survival rate, metastasis, and recurrence of invasive cervical carcinoma.

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