

# Doppler Ultrasonography of the Uterine Artery and the Response to Chemotherapy in Patients with Gestational Trophoblastic Tumors<sup>1</sup>

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## ABSTRACT

**Purpose:** Increasing new blood vessel formation (neovascularization) within tumors is an adverse prognostic factor for survival in several cancers. Neovascularization is usually determined histopathologically and not *in vivo*. To assess neovascularization *in vivo*, we have used Doppler ultrasonography (US) to measure the uterine artery pulsatility index (UAPI) in patients with gestational trophoblastic tumors (GTTs). Here, we assess whether the UAPI can provide independent prognostic information predictive of methotrexate resistance (MTX-R), a drug central to the management of GTT.

**Experimental Design:** All patients treated for GTTs between March 1994 and January 1999 had their records reviewed to determine their pretreatment Charing Cross Hospital (CXH) prognostic score, uterine volume, the lowest UAPI of either uterine artery, number of metastases, and human chorionic gonadotropin (hCG) concentration. Of the 164 patients for whom all data were available, 47 subsequently developed MTX-R, defined as a plateaued or rising hCG in two consecutive samples.

**Results:** UAPI, hCG, uterine volume, presence of metastases, and the overall CXH prognostic score were all predictive of MTX-R on univariate analysis. Moreover, the UAPI remained a significant independent predictor of MTX-R on multiple logistic regression analysis. After adjustment for the CXH prognostic score, the odds ratio for the risk of MTX-R in patients with a UAPI  $\leq 1$  compared with those with a UAPI  $> 1$  was 2.68 (95% confidence interval, 1.25–5.74;  $P = 0.01$ ). The unadjusted odds ratio for the

above comparison was 2.32 (95% confidence interval, 1.14–4.7;  $P = 0.02$ ).

**Conclusions:** The UAPI, as an indirect *in vivo* measure of functional tumor vascularity, independently predicts the response to chemotherapy in GTTs.

## INTRODUCTION

New blood vessel formation (neovascularization) is a common feature of malignancy. An increasing number of immunohistochemistry studies have shown that neovascularization is an independent adverse prognostic factor for many tumors (1–3). These studies are *ex vivo*, requiring biopsy material that may not always be representative of the whole tumor and do not provide a functional assessment of neovascularization. Consequently, there is considerable interest in developing *in vivo* techniques to determine tumor vascularity. Doppler US<sup>3</sup> is a widely available, cheap technique to assess changes in larger vessels supplying tumors. More expensive *in vivo* approaches usually confined to research centers include magnetic resonance imaging and positron emission tomography (4–7). However, previous studies using all of these *in vivo* methods have thus far failed to predict response to chemotherapy independently of known prognostic factors (4–6, 8–15).

GTTs, including invasive hydatidiform mole and choriocarcinoma, originate in the uterus and provide an excellent example of a richly vascularized neoplasm (16). As part of the routine staging of this disease, all patients undergo Doppler US of the pelvis to assess uterine volume and blood flow through the uterine arteries. The latter is used to calculate the UAPI. The uterine volume is known to correlate with tumor burden and is used together with several other variables to determine the risk of GTT becoming resistant to chemotherapy with single-agent MTX (17). In contrast, the UAPI has not been shown previously to independently predict drug resistance in women with GTT. The UAPI reflects impedance to blood flow within the uterus/tumor. A low UAPI indicates increased arteriovenous shunting, probably associated with neovascularization found in GTT (16) and might therefore be expected to be an adverse prognostic factor.

Consequently, in this study we have examined whether the UAPI, as an indirect *in vivo* measure of neovascularization, is independently predictive of the risk of MTX-R in GTTs.

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<sup>3</sup> The abbreviations used are: US, ultrasonography; GTT, gestational trophoblastic tumor; UAPI, uterine artery pulsatility index; MTX, methotrexate; MTX-R, MTX resistance; CXH, Charing Cross Hospital; hCG, human chorionic gonadotropin; MVD, mean vascular density; CI, confidence interval.

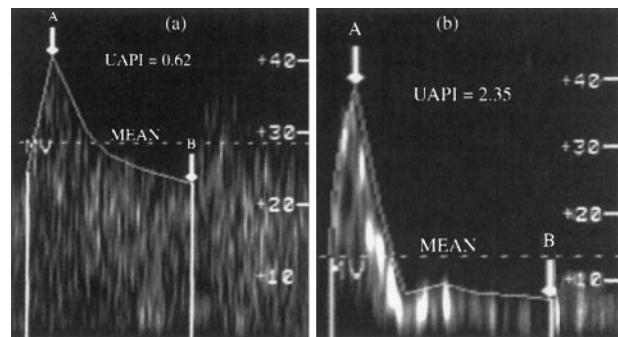
## PATIENTS AND METHODS

**Study Population.** A screen of the CXH Trophoblastic Tumor Database revealed that 282 patients had been treated for GTT with MTX between March 1994 and January 1999. The decision to treat patients with MTX was based on the CXH prognostic scoring system, which closely resembles the WHO scoring system. Both systems include variables identified previously to correlate with the risk of developing MTX-R (17). These variables are patient's age, the type of and interval from the end of the antecedent pregnancy to chemotherapy, patient and partner's blood groups, history of prior chemotherapy, the serum hCG concentration, the presence or absence of metastases and the size of the largest tumor mass based on a chest X-ray and pelvic US. The CXH prognostic scoring system assigns a risk score to each of these variables. The overall risk is obtained by adding these individual scores. A patient with a score between 0 and 5 is at low risk of MTX-R. Patients scoring 6 to 8 and >8 are at medium- and high-risk of MTX-R, respectively. Only patients scoring in the low- or medium-risk categories are initially treated with MTX (18) and were therefore included in this study (all high-risk patients were excluded). The details of the individual prognostic factors, the total CXH prognostic score, and treatment details were obtained for all patients in the study, from the Trophoblastic Tumor Database.

Patients at CXH undergo a single pelvic ultrasonographic examination before chemotherapy for GTT. The results of Doppler assessments are not routinely entered into the database and were therefore obtained from the original US reports. These reports were missing for 20 patients, and in an additional 98 patients, Doppler assessments of the uterine arteries had only been qualitatively documented as high, low, or normal. Because our intention in this study was to quantitate the relationship between tumor vascularity measured by Doppler US and MTX-R, these patients were excluded from the subsequent analysis. Therefore, the final study population comprised a total of 164 patients.

**US.** The total uterine volume and the UAPI were measured as described previously (8). US took an average of 15–20 min to complete per patient. Doppler assessments were performed using a HDI 3000 US unit (Advanced Technology Laboratories, Bothell, WA) with a 2–4 MHz curvilinear array probe. Uterine volume was calculated using the prolate ellipsoid formula: uterine volume ( $\text{cm}^3$ ) =  $L$  (cm)  $\times$   $AP$  (cm)  $\times$   $W$  (cm)  $\times$  0.523, where  $L$  is length,  $AP$  is maximum antero-posterior diameter, and  $W$  is maximum width ( $1 \text{ cm}^3 = 1 \text{ ml}$ ; Ref. 8).

UAPI was chosen to assess blood flow in this study, because it is independent of the angle of insonation (19), and this angle cannot reliably be estimated for uterine arteries because of their small diameter and tortuosity. The UAPI is given by the formula:  $\text{UAPI} = (A - B)/\text{mean}$ , where  $A$ ,  $B$ , and the mean are the maximum, minimum, and time averaged Doppler frequency shift of the ultrasound beam after reflection from the moving column of blood in the uterine artery (Fig. 1). The UAPI was calculated by averaging the values from a minimum of three cardiac cycles using the scanner software. The UAPI reflects the impedance to flow distal to the point of sampling; an increase in impedance will result in an increase in the UAPI and *vice versa*.



**Fig. 1** Doppler traces of the uterine artery in patients with GTT. *a*, a patient with a UAPI of 0.62 who developed MTX-R. *b*, a patient with a UAPI of 2.35 who did not develop MTX-R. *A*, *B*, and the *MEAN* are the maximum, minimum, and average Doppler changes in frequency over a cardiac cycle, respectively.

Using power Doppler, the uterine arteries were located prior to spectral Doppler analysis, and both uterine arteries were examined. The lowest UAPI from either uterine artery was used for analysis, because it is a reflection of the maximal deviation from the normal impedance.

**Chemotherapy and Response Evaluation.** Patients were initially treated with fortnightly cycles of 50 mg of methotrexate i.m. on days 1, 3, 5, and 7, with 7.5 mg of oral folinic acid rescue on days 2, 4, 6, and 8 (the MTX regimen; Ref. 18). Response to chemotherapy was monitored by twice weekly measurements of hCG concentrations. The development of MTX-R was defined by a plateau or a rise in two consecutive hCG concentrations (18). Patients with MTX-R were changed to actinomycin D 0.5 mg i.v., daily for 5 days every 2 weeks, if their hCG concentration at resistance was  $\leq 150$  IU/ml (20). Otherwise patients were treated with etoposide, methotrexate, and actinomycin D, and cyclophosphamide and vincristine was given i.v. as a weekly alternating schedule (20). Treatment was continued in all patients for 6 weeks beyond the fall of the hCG to normal ( $\leq 4$  IU/ml).

**Statistical Analysis.** SPSS V9.0 (SPSS, Chicago, IL) was used for the statistical analyses. Univariate analysis was performed using the Mann-Whitney  $U$  test or  $\chi^2$  test, and the correlation between prognostic variables was tested using Spearman's correlation coefficient. UAPI was subdivided into high- and low-risk categories for MTX-R using a ROCs curve to maximize sensitivity and specificity. These UAPI subgroups were used in the subsequent multivariate analyses of predictors of MTX-R, using binary logistic regression with forward stepwise selection. All significant prognostic factors on univariate analysis were initially included.

## RESULTS

**Patient Characteristics.** Overall, 164 patients were eligible for inclusion in the study. They ranged in age from 16 to 50 years (median, 28 years), and GTT followed a molar pregnancy in 150 patients (91%). The hCG concentration at the start of chemotherapy ranged from 13 to 27,600 IU/ml (median, 6,489), the uterine volume from 20 to 845 ml (median, 140 ml), and the UAPI from 0.16 to 4.8 (median, 1.0). Eleven patients

Table 1 Factors prognostic of MTX-R by univariate analysis<sup>a</sup>

Prognostic factor	All patients n = 164	MTX-sensitive patients n = 117	MTX-R patients n = 47	P
<b>hCG (IU/ml)</b>				
Median	6489	3476	27450	<0.0001 <sup>b</sup>
<1000	47	43	4	<0.0001 <sup>c</sup>
1000–10,000	42	32	10	
10,001–100,000	68	40	28	
>100,000	7	2	5	
<b>Uterine volume (ml)</b>				
Median	140	130	183	0.0039 <sup>b</sup>
<120	66	54	12	0.0088 <sup>c</sup>
120–240	72	51	21	
>240	26	12	14	
<b>UAPI</b>				
Median	1.0	1.2	0.8	0.0002 <sup>b</sup>
<1	75	44	31	0.0016 <sup>c</sup>
1 to <2	50	38	12	
≥2	39	35	4	
<b>No. of metastases</b>				
0	153	113	40	0.0345 <sup>c</sup>
1–4	9	4	5	
5–8	2	0	2	

<sup>a</sup> Factors found to be nonprognostic of MTX-R on univariate analysis included age, type of antecedent pregnancy, the interval from the antecedent pregnancy to starting chemotherapy, blood group, site of metastases, and previous treatment with chemotherapy.

<sup>b</sup> For the comparison of MTX-sensitive and MTX-R patients using the Mann-Whitney *U* test.

<sup>c</sup> For the comparison of MTX-sensitive and MTX-R patients using the  $\chi^2$  test for the prognostic factor overall.

(6.7%) had metastases, all of which were either to the lung or vagina. None of the patients had received any prior chemotherapy for GTTs.

Thirty-eight (26%) of the 148 low-risk patients and 9 (56%) of 16 medium-risk patients developed MTX-R. All patients with MTX-R, however, achieved a complete remission with second-line chemotherapy.

**Univariate Analysis.** Variables predictive for MTX-R before chemotherapy on univariate analysis (median, MTX-R versus MTX sensitive) were hCG (27,450 versus 3,476 IU/ml;  $P < 0.0001$ ), uterine volume (183 versus 130 ml;  $P = 0.0039$ ), and UAPI (0.8 versus 1.2;  $P = 0.0002$ ; Table 1; Fig. 2). The number of metastases present before chemotherapy also predicted the risk of MTX-R ( $P = 0.0345$ ; Table 1). In agreement with our previous experience, an increasing CXH prognostic score correlated positively with an increased risk of MTX-R (Table 2 and Fig. 3; Ref. 18).

The risks of MTX-R for subgroups of UAPI are summarized in Table 3 and show a significant and progressive increase in the incidence of MTX-R in inverse relation to UAPI ( $P = 0.001$ ). Patients with a UAPI <1 had a 41.3% (95% CI, 30.1–53.3) risk of MTX-R compared with a 10.3% (95% CI, 2.9–24.2) risk in patients with a UAPI ≥2.

To determine the optimum cutoff values for UAPI to predict MTX-R, a receiver operating characteristics curve was constructed. A UAPI ≤1 provided maximal sensitivity and specificity for predicting MTX-R of 68 and 62%, respectively. Therefore, in subsequent analyses two groups were used, UAPI

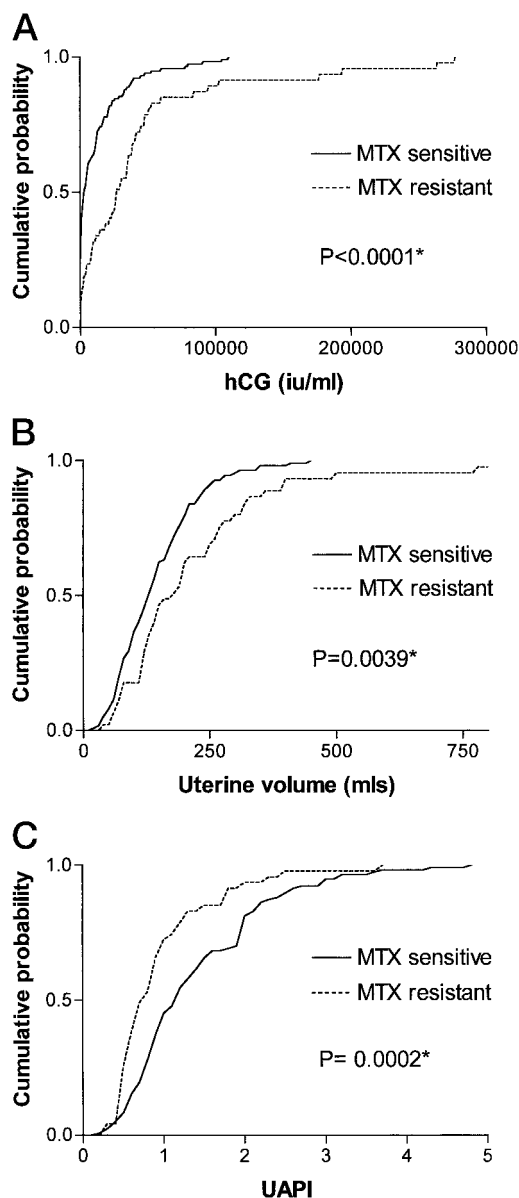


Fig. 2 Cumulative probability distributions of hCG (A), uterine volume (B), and UAPI (C) in MTX-R ( $n = 47$ ) and MTX-sensitive ( $n = 117$ ) patients. \*, for the difference between the two curves for MTX-R and MTX-sensitive patients, for hCG, uterine volume, and UAPI, using the Mann-Whitney *U* test.

≤1 and UAPI >1. The odds ratio for MTX-R was 2.32 (95% CI, 1.14–4.7;  $P = 0.02$ ) for patients with a UAPI ≤1, relative to patients with a UAPI >1, on univariate analysis.

Factors in the CXH scoring system, which were not associated with MTX-R on univariate analysis, included the patients' age, type of antecedent pregnancy, interval to chemotherapy, and blood group ( $P = 0.44, 0.9, \text{ and } 0.48$ , respectively;  $\chi^2$ ). This finding was not surprising because patients requiring high-risk combination chemotherapy were excluded from the current study (17). Patients who developed MTX-R required a significantly longer overall duration of treatment (median, 130 versus

Table 2 The risk of MTX-R stratified by CXH prognostic score and UAPI

Prognostic score	All patients				Patients with UAPI ≤1				Patients with UAPI >1			
	Total	MTX-R patients		95% CI <sup>a</sup>	Total	MTX-R patients		95% CI <sup>a</sup>	Total	MTX-R patients		95% CI <sup>a</sup>
		n	%			n	%			n	%	
0–1	23	3	13	(2.8–33.6)	10	1	10	(0.2–33.6)	13	2	15.4	(1.9–45.4)
2–3	69	13	18.8	(10.4–30.1)	31	8	25.8	(12–44.6)	38	5	13.2	(4.4–28.1)
4–5	56	22	39.3	(26.5–53.2)	35	17	48.6	(31.4–66)	21	5	23.8	(8.2–47.2)
6–8	16	9	56.3	(29.9–80.2)	11	8	72.7	(39–94)	5	1	20	(0.5–71.6)
Low risk (0–5) <sup>b</sup>	148	38	25.7	(18.9–33.5)	76	26	34.2	(23.7–47)	72	12	16.7	(8.9–27.3)
Medium risk (6–8) <sup>c</sup>	16	9	56.3	(29.9–80.2)	11	8	72.7	(39–94)	5	1	20	(0.5–71.6)

<sup>a</sup> Confidence interval for percentage of patients with MTX-R.

<sup>b</sup>  $\chi^2 = 5.96$ ,  $df = 1$ ,  $P = 0.015$  for comparison of risk of MTX-R between patients with UAPI ≤1 and UAPI >1.

<sup>c</sup>  $\chi^2 = 3.88$ ,  $df = 1$ ,  $P = 0.049$  for comparison of risk of MTX-R between patients with UAPI ≤1 and UAPI >1.

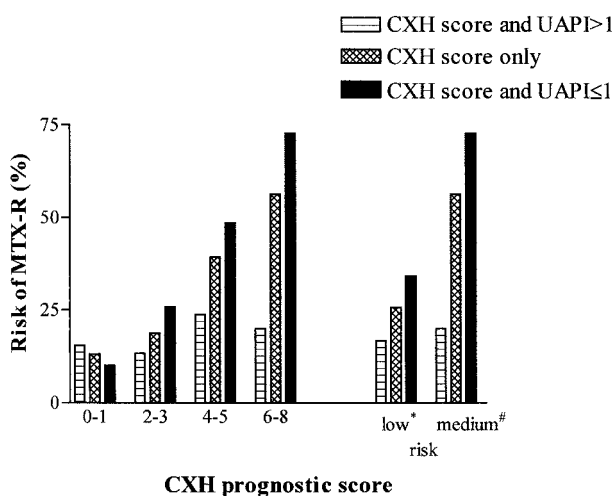


Fig. 3 Risk of MTX-R stratified by CXH prognostic score and UAPI.  $\chi^2$ , 5.96,  $df = 1$ ,  $P = 0.015$  for comparison of risk of MTX-R between patients with UAPI ≤1 and UAPI >1. #,  $\chi^2 = 3.88$ ,  $df = 1$ ,  $P = 0.049$  for comparison of risk of MTX-R between patients with UAPI ≤1 and UAPI >1.

91 days;  $P < 0.0001$ ; Mann-Whitney  $U$  test) than patients without MTX-R.

**Multivariate Logistic Regression Analysis.** On multivariate analysis (Table 4) incorporating all univariately significant prognostic factors (hCG, uterine volume, UAPI, and number of metastases), UAPI was found to be independently and inversely related to the risk of MTX-R ( $P = 0.04$ ). The adjusted odds ratio of MTX-R for patients with a UAPI ≤1 was 2.27 (95% CI, 1.02–5.06;  $P = 0.04$ ; Table 4) relative to patients with a UAPI >1. The presence of metastases and hCG concentration were also independently and directly related to MTX-R ( $P = 0.0028$  and  $0.0209$ , respectively; Table 4). In contrast, uterine volume showed no independent relationship to MTX-R.

UAPI was also independent of the total CXH prognostic score for predicting MTX-R in multivariate analysis. The odds ratio of MTX-R for UAPI ≤1 was 2.68 (95% CI, 1.25–5.74;  $P = 0.0115$ ). The risk of MTX-R combining UAPI with the CXH prognostic score is shown in Table 2 and Fig. 3. Patients with a prognostic score of 6–8 and a UAPI ≤1 had a 72.7% risk

Table 3 The relationship between UAPI and the risk of MTX-R

UAPI	All patients	MTX-R patients		95% CI <sup>b</sup>
		n <sup>a</sup>	%	
<1	75	31	41.3	(30.1–53.3)
1 to <2	50	12	24	(13.1–38.2)
≥2	39	4	10.3	(2.9–24.2)

<sup>a</sup>  $\chi^2$  for trend 12.88,  $df = 2$ ,  $P = 0.0016$ .

<sup>b</sup> CI for percentage of patients with MTX-R for each subgroup of UAPI.

of MTX-R. In marked contrast, patients with a prognostic score of 6–8 and a UAPI >1, the risk of MTX-R was only 20% ( $P = 0.049$ ).

## DISCUSSION

There is a clear need to develop *in vivo* methods of assessing tumor vascularity because this has the potential to predict prognosis and to determine the most beneficial type of therapeutic strategy in patients with cancer. Doppler US may provide a useful method for *in vivo* functional assessment of tumor vasculature by assessing hemodynamic changes in the macrovasculature as an indirect reflection of the microvasculature (vessel diameter, <15  $\mu\text{m}$ ) used to define neoangiogenesis (4).

UAPI measures impedance to blood flow in the main arteries supplying the uterus/GTT. A low UAPI (low impedance) would therefore be expected with arteriovenous shunting that is a common feature of neoangiogenesis (16). It therefore follows that a falling UAPI would correlate with increasing neoangiogenesis associated with an enlarging uterus/tumor volume and rising hCG concentration. However, previous work using the UAPI has failed to show this association in GTT, probably because of the small numbers of patients (8, 9, 11, 21). The results of this larger study demonstrate, for the first time, that the UAPI predicts the risk of MTX-R independently of both individual prognostic factors and the total CXH prognostic score in women with GTT.

The ability to independently predict drug resistance should translate into the development of more appropriate therapeutic strategies. In the case of GTT where nearly all patients are cured with existing therapies, the incorporation of UAPI into the

Table 4 The odds ratios and CIs of the significant prognostic factors on multivariate binary logistic regression for MTX-R<sup>a</sup>

Prognostic factor <sup>b</sup>	Odds ratio	95% CI	P <sup>c</sup>
UAPI			0.0448
>1	1.0	<sup>d</sup>	
≤1	2.27	1.02–5.06	
hCG (IU/ml)			0.0028
<1000	1.0	<sup>d</sup>	
1000–<10,000	2.71	0.75–9.79	
10,000–<100,000	5.83	1.81–18.83	
>100,000	23.74	3.29–171.04	
Metastases			0.0209
Absent	1.0	<sup>d</sup>	
Present	5.38	1.29–22.47	

<sup>a</sup> All significant prognostic factors for MTX-R on univariate analysis (Table 1) were included in the analysis.

<sup>b</sup> Uterine volume did not independently predict MTX-R on multivariate analysis.

<sup>c</sup> Overall P for the prognostic factors.

<sup>d</sup> Reference values.

scoring system would help in the more accurate selection of patients for combination chemotherapy as opposed to MTX. This should in turn reduce the total treatment time and potential toxicity.

Intriguingly, assessment of UAPI may have other roles in the management of gestational trophoblastic disease. Thus, after uterine evacuation of hydatidiform moles (pre-malignant gestational trophoblastic disease), only a small proportion of patients actually develop GTT (22). It would clearly be useful to identify these patients at an early time point. Total hCG levels cannot do this, but one small study has suggested that serial UAPI measurements after molar evacuation can identify patients who will develop GTT (23).

Because only 164 (58%) of the 282 treated patients had UAPI values recorded quantitatively, the current study could have been susceptible to selection bias. However, systematic selection and measurement biases are unlikely because the radiologists measuring UAPI were blind to the treatment allocation and subsequent outcome of chemotherapy, and UAPI was recorded prospectively in all of the patients studied. Nevertheless, a prospective study is required to confirm the findings presented here.

The exact mechanism relating tumor vascularity to drug resistance remains incompletely understood. One proposal suggests a reduction in cytotoxic drug exposure in highly vascularized tumors (16). However, this is likely to be more than compensated for by the overall increase in blood flow to the tumor and equilibration of drug concentrations between the plasma and extracellular fluid compartments. Alternative mechanisms for the association of UAPI with MTX-R in GTT may relate to the expression of proteins that are known to promote both angiogenesis and chemoresistance or enhance cell division such as fibroblast growth factor and platelet-derived growth factor/thymidine kinase in other tumor types (4, 24).

It would of course have been interesting to correlate the UAPI findings with changes in the MVD determined immunocytochemically from GTT biopsy samples. However, GTTs are highly vascular, and therefore biopsy is not routinely performed

before chemotherapy because this may precipitate life-threatening hemorrhage. Instead, the diagnosis can usually be made on the basis of the history of molar pregnancy, the serum hCG concentration, and clinical history. We were therefore not able to correlate the UAPI with the MVD. Nevertheless, some studies using noninvasive *in vivo* measures of tumor vascularity have attempted to correlate their findings to MVD in other malignancies (7, 14, 15). Although this correlation has been demonstrated, the concordance is incomplete. This could be because areas of apparent high vascularity on histology may not be functional, and *vice versa*. Indeed, in a recent study assessing the relationship among functional tumor vascularity (measured by Doppler US), MVD, and overall survival in colorectal cancer, only the Doppler findings and not MVD correlated with survival (15).

The failure of MVD in some studies to correlate with prognosis could be because various histological methods of assessing neoangiogenesis are used, all of which are associated with both sampling and measurement error. In contrast, the UAPI is a more objective measure of vascularity than the MVD because it is not based on the subjective selection of regions of interest within a tumor. Moreover, measurement error is minimized with UAPI because it is independent of the vessel diameter and the angle of insonation of the Doppler beam (19). However, although the UAPI would appear to have some advantages, it should be stressed that it may also be “falsely” low in patients with arteriovenous shunting within the uterus attributable to other pathologies including hemangiomas and congenital arteriovenous malformations.

Other *in vivo* measurements of tumor vascularity have been studied such as contrast dynamic enhancement magnetic resonance imaging and intratumoral Doppler pulsatility and resistance indices in non-GTT malignancies (4–7, 14). Some have shown an association with prognosis only on univariate analysis (12–15). This is probably because of the small sample size of these studies and the failure of the *in vivo* techniques used to provide a measure of the total tumor perfusion. Furthermore, similar to MVD and unlike UAPI, these methods also rely on the subjective selection of regions of interest.

In summary, this study provides proof of principle that the UAPI can serve as a noninvasive *in vivo* measure of functional tumor vascularity, which independently predicts the response to chemotherapy. This approach could be extended to other tumors affecting organs with a readily identifiable dominant arterial supply such as renal, testicular, endometrial, and ovarian carcinomas.

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