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Editorial

Color Doppler imaging and ovarian tumor angiogenesis: the Janus approach

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The detection and assessment of ovarian lesions are an important part of gynecological practice. In particular, there is good evidence for the need to detect and treat malignant disease in asymptomatic women before the capsule has ruptured (i.e. at FIGO stage I)[1]. Moreover, advanced knowledge of whether an overt ovarian mass is malignant might be useful for improving the effectiveness of surgical treatment. Pelvic ultrasonography has been developed continuously and (in conjunction with biochemical and genetic markers) is being used to achieve these objectives with increasing accuracy. At the same time, knowledge is being accrued about the relationship between ultrasound-derived indices of ovarian tumor vascularity and molecular aspects of angiogenesis. There is now the exciting prospect that color Doppler imaging with pulsed Doppler spectral analysis might also be used to monitor the development and intermediate effectiveness of novel anticancer treatments.

Looking backwards

Transabdominal ultrasonography was initially used for the investigation of overt abdominal swellings[2] and much later for the detection of early ovarian cancer in asymptomatic women[3]. The technique was undoubtedly of value for detecting transient, persistent and overt ovarian cysts. However, the anticipation of ultrasound-derived end-points which could be used to distinguish malignant from benign cysts proved to be premature[4]. Subsequently, however, the resolving power of pelvic ultrasonography was increased substantially by the advent of transvaginal probes. This revolutionary development was first applied to systematic studies of overt ovarian masses in patients awaiting surgery. The findings included the identification of morphological criteria, which could be used to classify a tumor as benign (i.e. the cyst must be unilocular and thin-walled with no irregularities in outline or expanding processes or septa, or echogenicity) or malignant (i.e. the ovary must be multicystic, or a single cyst must be multilocular with dense or irregular septa, have a poorly defined border with papillary formation on the inner surface, or increased fluid in the peritoneal cavity)[5,6]. A similar analysis was used for the retrospective evaluation of data from a screening program for asymptomatic women, who had at least one first-degree relative affected with ovarian cancer[7]. Undoubtedly, the use of transvaginal B-mode imaging has been successful in revealing a variety of form – both within and between different types of tumor and in improving the specificity (and hence reducing the false-positive rate) of screening and diagnostic procedures for ovarian cancer. In our opinion, however, the principal aim when using this approach alone must be to use only those end-points which are most reproducible and produce the minimum number of false-negative results.

The introduction of transvaginal color Doppler imaging with pulsed Doppler spectral analysis represented a quantum leap in technical development. This additional facility enabled studies to be undertaken on the vascularity and blood flow within simple and complex forms of ovarian cysts[8]. The presence or absence of color and the detection or non-detection of flow velocity waveforms could be used as categoric end-points (which depend on the presence of high blood velocity). The resistance index (RI) or pulsatility index (PI) were used as continuous variables; these values are highly dependent upon diastolic velocity and are

Janus was a Roman God of New Beginnings and was depicted with two faces (one looking backwards and the other forwards) on silver coins minted between 225 and 212 BCE.

thought to reflect impedance distal to the point of sampling. The use of these end-points in relation to derived cut-off values gave encouraging results for distinguishing between benign and malignant tumors[9–12]. However, when ultrasound equipment became more sensitive (in terms of detecting lower blood velocity), more areas of color, flow velocity waveforms and vessels with low RI and PI were found in benign tumors[13], and the technique did not appear to offer significant advantages over B-mode imaging[14,15]. Paradoxically, the advance in technology was a contributory factor in momentarily reducing the usefulness of the technique for detecting ovarian cancer. There was renewed interest in the potential use of color Doppler imaging for this purpose, however, when it was found that reproducible measurements could be made of the peak systolic velocity in ovarian follicles and corpora lutea[16], and a variety of overt tumors[17].

The accurate measurement of blood velocity is dependent upon the angle of the ultrasound beam to the vessel. Unfortunately, it is not possible to determine the angle of insonation to small intratumoral vessels. However, the volume of tissue under the range gate is large enough to include many small vessels, which may be arranged in a tortuous manner. It is now generally accepted that one or more of these small vessels will be at a low or zero angle to the pulsed Doppler ultrasound beam because reproducible measurements can be made. Recent work from Hata and colleagues[18] has shown that the intratumoral peak systolic velocity is the most useful index for discriminating between overt benign and malignant tumors. This conclusion was reinforced by the findings of Tailor and colleagues[17] that the intratumoral peak systolic velocity and time-averaged maximum velocity were better indices than either the RI or PI and that the combined use of one index for velocity and another for impedance was more useful than using either alone. Most malignant tumors have ultrasound indices indicative of increased vascularity, and it is tempting to speculate that the false-positive results may have arisen from benign tumors with partially transformed cells.

These developments towards a minimally invasive procedure for assessing intratumoral blood flow were contemporary with reports about the potential role of angiogenesis (i.e. the formation of new blood vessels from the existing network) in tumor progression from hyperplasia to neoplasia[19], and the formation of metastases[20]. Simultaneously, more information was becoming available about the biochemical mechanisms of angiogenesis. In particular, there was a seminal paper by Reynolds and colleagues[21] on the expression of RNA for putative angiogenic factors in ovarian cancer. These authors reported a striking increase in the production of RNA for platelet-derived endothelial cell growth factor in tumors with high blood velocity as determined by color Doppler imaging when compared with the amount from benign tumors and the normal ovary. Previously, platelet-derived endothelial cell growth factor had been shown to have substantial structural homology with thymidine phosphorylase[22] and the enzyme activity was reported to be necessary for promoting angiogenesis[23]. More recently, data from our group[24] have shown that there is a positive statistical correlation between the intratumoral concentration of thymidine phosphorylase (expressed as units/mg protein) in homogenates of benign and malignant ovarian masses and the peak systolic velocity recorded immediately before surgery.

Looking forwards

An analysis of the history of color Doppler suggests that the technology will continue to be developed (in particular, we envisage the introduction of three-dimensional color Doppler imaging). Concurrent developments in data processing suggest that it will soon be possible to calculate individual risks of ovarian cancer from all the available evidence (i.e. from medical, genetic and reproductive histories, and from ultrasound-derived indices and the measurement of serum tumor antigens), and we will probably cease to compare the relative merits of using single variables for this purpose. New clinical applications of research on color Doppler imaging and angiogenesis in ovarian cancer will continue in two directions: first, the development of more practical and effective procedures for the quantitation of angiogenesis for possible use in diagnosis, prognosis or screening, and, second, the study of treatments which might directly or indirectly inhibit localized angiogenesis or destroy established vessels within tumor deposits.

The development of more effective methods for treating (or preventing) ovarian cancer would obviate the need for screening. Some drugs with angiostatic activity have been identified and are already being evaluated in clinical trials[25]. The finding that thymidine phosphorylase plays a major role in ovarian cancer angiogenesis suggests new possible treatments: first, the development of compounds which effectively inhibit thymidine phosphorylase activity; second, the development of prodrugs where the active moiety is mainly released within the tumor by thymidine phosphorylase ; and third, the development of gene therapy whereby cancer cells are transfected with thymidine phosphorylase before the administration of an appropriate prodrug. Thymidine phosphorylase catalyzes two reactions: the reversible phosphorylation of thymidine and other 2′ doxyribosides, and deoxyribosyl transfer between pyrimidines. In particular, it has been shown that 5-fluorouracil (5-FU) is released by thymidine phosphorylase (also called pyrimidine phosphorylase) from 5′-deoxy-5-fluorouridine (5′-DFUR)[26]. Furthermore, thymidine phosphorylase may enhance the toxicity of the active drug 5-FU, by the transfer of 2′-deoxyribose 1-phosphate, so producing 5-fluoro-2′-deoxyuridine. This product can form 5-fluoro-2′-deoxyuridine 5′-monophosphate (5-FdUMP) through the action of thymidine kinase, 5-FdUMP in turn can inhibit thymidylate synthase, restricting *de novo* synthesis of thymidine monophosphate, and can ultimately inhibit the synthesis of DNA. It has also been reported that 5-FU can induce apoptosis in some cancer cells, which in turn might stimulate the immune system. Consequently, it is possible that treatment with 5′-DFUR might be highly selective for the inhibition of angiogenesis in ovarian tumors with high

thymidine phosphorylase expression (before or after gene therapy).

Other possible new treatments involve the family of vasoactive endothelial cell growth factors/vascular permeability factors and their receptors[27] and immune gene therapy with co-stimulatory molecules and/or tumor antigens[28]. We believe that color Doppler imaging (in conjunction with other minimally invasive tests) will be used increasingly to evaluate new treatments before surgery. The past 10 years have produced many exciting technical developments in ultrasonography and the technique has become indispensable in the practice of gynecology – but undoubtedly the best is yet to come.

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