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Transvaginal colour flow imaging: a possible new screening technique for ovarian cancer

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Abstract

***Objective*—To assess whether changes in the intraovarian vasculature or blood flow impedance can be used to identify potentially malignant masses.**

***Design*—Open, non-comparative prospective study.**

***Setting*—Ovarian screening clinics at King's College Hospital and the Hallam Medical Centre.**

***Subjects*—50 Women selected on the basis of their medical history and the result of a previous transvaginal ultrasound scan. Thirty women (10 premenopausal (scan taken on days 1 to 8 of the menstrual cycle) and 20 postmenopausal) had normal ovaries, and 20 had at least one ovary with an abnormal morphology or volume, or both.**

***Interventions*—Women with a positive result on screening were referred for laparotomy.**

***Main outcome measures*—Presence or absence of coloured areas (neovascularisation) and the pulsatility index within each ovary. The pulsatility index is a measure of the impedance to blood flow, a low value indicating decreased impedance and a high value increased impedance to blood flow.**

***Results*—Two women with a positive result on screening had hydrosalpinges, 10 a benign tumour or a tumour-like condition, and eight primary ovarian cancers. No areas of neovascularisation were seen in the 30 women with morphologically normal ovaries and the two patients with hydrosalpinges; the pulsatility index ranged from 3·1 to 9·4. Similarly, nine patients (10 affected ovaries) with a non-malignant mass had no signs of neovascularisation and the pulsatility index varied from 3·2 to 7·0. One patient with bilateral dermoid cysts containing nests of thyroid-like cells had vascular changes and pulsatility index values of 0·4 and 0·8. Seven patients (eight ovaries) with primary ovarian cancer (one stage IV, four stage III, and two stage Ia) showed clear evidence of neovascularisation and pulsatility index values were from 0·3 to 1·0. One patient with an intraepithelial serous cystadenocarcinoma in a small ovary (<5 ml volume) had no signs of any vascular change and the pulsatility index was 5·5.**

***Conclusion*—Transvaginal colour flow imaging may be used to identify potentially malignant ovarian masses and help elucidate the early stages of tumorigenesis. The routine application of this technique may reduce the rate of false positive results of an ultrasonography based screening procedure.**

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Introduction

Primary ovarian cancer affects about 5000 women in the United Kingdom each year. The five year survival rate depends on the stage of the disease at the time of diagnosis and ranges from less than 5% at stage IV to more than 80% at stage Ia or Ib. The overall mortality is more than 80% and a late diagnosis by traditional techniques is probably the main reason for the poor prognosis.[1,2] The outcome may be improved by the introduction of new treatment regimens, but the development of procedures for the early detection of the disease is probably the best approach to achieve a reduction in the mortality.

We are investigating the use of pelvic ultrasonography as a screening procedure for early ovarian neoplasms. Initially Campbell *et al* showed that ovarian size and morphology as assessed by transabdominal ultrasound examination agreed well with results obtained by direct measurement and observation at laparotomy.[3] Recently the results of a prospective study of 5479 self referred women without symptoms have been described in terms of the ovarian masses detected,[4] the value of the screening procedure over time (see previous paper[5]), and the development of new screening strategies entailing the use of defined changes in ovarian volume (S Campbell *et al*, unpublished data). Five primary ovarian cancers were detected at stage Ia or Ib, and evidence from a follow up study at least one year after the last screening showed that the detection rate was 100% within the limitation of the study design. A screening procedure based on the presence of abnormal ovarian morphology at the first scan and a defined volume change on rescanning would have given a false positive rate of 1·6% and a positive predictive value of 2·0%—that is, the odds against a positive screen result indicating the presence of primary ovarian cancer were 1:50. This odds ratio is mainly due to the difficulty of distinguishing malignant tumours from benign masses, tumour-like conditions, or hydrosalpinges. We report the use of transvaginal ultrasonography with colour flow imaging for the detection of intraovarian

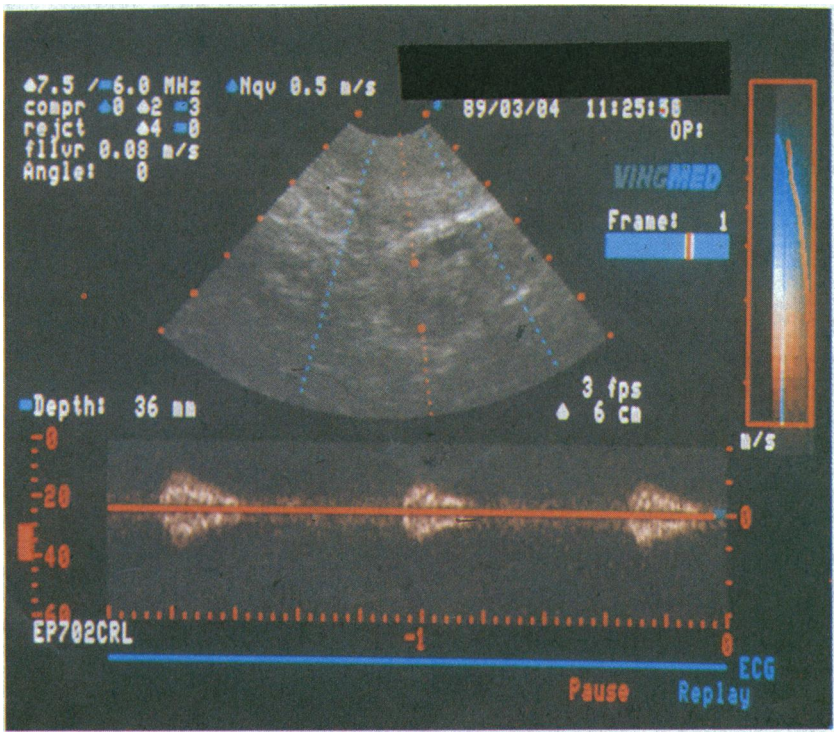

FIG 1—*Flow velocity waveforms showing intraovarian impedance to blood flow in healthy postmenopausal woman aged 56 (pulsatility index 6·1)*

vascular changes (neovascularisation) and the measurement of impedance to blood flow in ovarian masses as potential indices of early oncogenesis.

Subjects and methods

We studied three groups of selected volunteers: (*a*) premenopausal women with normal ovaries on days 1 to 8 of the menstrual cycle, (*b*) postmenopausal women with normal ovaries, and (*c*) patients with a positive result on screening or from a scan. The women were selected on the basis of their medical history and the result of transvaginal ultrasound scans at either the ovarian screening clinic at King's College Hospital (40 women) or the Hallam Medical Centre (10 women). At each screening the result of the scan was regarded as positive if any of three criteria were considered to be abnormal: (*a*) ovarian morphology (areas of hyperechogenicity or hypoechogenicity), (*b*) outline (irregular), or (*c*) volume (>96th centile for age and menopausal state). Women with a positive result at the first scan were rescanned three weeks later to exclude transient changes in ovarian morphology or size. Thirty women (10 premenopausal, 20 postmenopausal) had a negative screen result on screening—that is, both ovaries were ostensibly normal—and 20 had a positive result—that is, at least one ovary was abnormal. Six subjects were referred from the gynaecology clinic at King's College Hospital and had only one ultrasound scan. Five had symptoms suggestive of ovarian cancer and the sixth was having irregular bleeding during hormone replacement treatment. All other women were without symptoms. All subjects with a positive result on screening were informed about the importance of the findings and agreed to be examined by transvaginal colour flow imaging before operation.

TRANSVAGINAL COLOUR FLOW IMAGING

All women had an empty bladder and were scanned in the lithotomy position with a slight reverse Trendelenburg tilt to localise free fluid in the pouch of Douglas. Premenopausal women were scanned during days 1 to 8 of the menstrual cycle to exclude changes in intraovarian blood flow that are known to occur during the formation of a corpus luteum.[6] A Sonotron Vingmed CFM 700 colour Doppler scanner was used (Diasonics Sonotron, Bedford). The 7·5 MHz transducer produced a sector angle of 110° that was offset 15° to allow a good view of the reproductive organs. The spatial peak temporal average intensity was about 65 mW/cm^2, which is well within the highest limit recommended by the Food and Drug Administration of America for use in fetal medicine. Before it was used the probe was covered with a coupling gel and inserted into a condom, which was coated with gel and inserted into the vagina. The ovaries were assessed after a systematic evaluation of the uterus[7] and were usually located above the internal iliac vessels, which appeared as anechoic tubular structures with echogenic walls. The ovaries were often easier to identify in premenopausal women owing to the presence of follicles. After the menopause the ovaries gradually become smaller and more uniformly echogenic but their position may invariably be found or confirmed by reference to the uterus and iliac vasculature.

A range gate was placed across an appropriate vessel or vessels. Flow velocity waveforms, which illustrate the distribution and intensity of the shifted Doppler frequencies with time, were displayed on line. The angle of the transducer was moved to obtain the maximum waveform amplitude and clarity. The pulsatility index was calculated electronically, from a smooth curve fitted to the average waveform over three cardiac cycles, according to the formula: pulsatility index=A−B/mean, where A is the peak systolic Doppler shift frequency, B the maximum end diastolic Doppler shift frequency, and mean the mean maximum Doppler shift frequency over the cardiac cycle.[8] The lowest pulsatility index was recorded from ovaries in which an appropriate blood vessel could not be identified. All data on scans were recorded on videotape or Polaroid film for subsequent appraisal. Numerical data were entered and stored on a microcomputer for statistical analysis.

Ovarian morphology and size were reassessed. Subsequently both ovaries were examined for prominent areas of vascularisation (probably reflecting neovascularisation). These vessels usually appeared as continuously fluctuating colour rather than the pulsatile colour seen with normal arteries.

PULSATILITY INDEX

The pulsatility index is a useful way of expressing blood flow impedance distal to the point of sampling—particularly when the diastolic frequency shift is below the level of the filter or reversed. The formula shows that the index will increase if the proximal conditions remain constant and the distal vascular bed constricts. Conversely, a low value for the pulsatility index will indicate decreased impedance to blood flow in the distal vasculature. Errors in the determination of the pulsatility index increase as the value decreases. For example, six replicate determinations of the index in four women with values between 0·6 and 0·9 gave an average coefficient of variation of 9·8%. A corresponding value of 6·3% was obtained from the same number of replicate determinations on four women with values from 3·5 to 5·5.

HISTOPATHOLOGY AND CLASSIFICATION

Women with a positive result on screening were referred for laparotomy. The extent of malignant disease was estimated by the surgeon, and the final diagnosis was based on the histology report. All abnormal ovarian tissues were classified according to criteria recommended by the World Health Organisation.[9] The stage of each primary ovarian cancer was determined from the operation records according to the revised recommendations of the International Federation of Gynaecologists and Obstetricians.[10]

Results

Eight of the 20 women with a positive result on a scan or screening had primary ovarian cancer. The six women with symptoms had the disease at stage III or IV. The patient with irregular uterine bleeding and two women without symptoms had stage Ia disease. Ten patients had a benign tumour or a tumour-like condition, and the remaining two who were premenopausal (aged 45 and 47 years) had a hydrosalpinx.

TABLE I—*Intraovarian impedance to blood flow in healthy premenopausal women*

Menopausal state	No of women	Age (years) Mean	Age (years) Range	Pulsatility index* Mean	Pulsatility index* Range
Premenopausal†	10	32	28-36	4·9	3·1-6·6
Postmenopausal	20	56	47-64	5·5	4·0-9·4
Total	30	48	28-64	5·1	3·5-9·4

*Average reading from right and left ovaries.
†Days 1 to 8 of menstrual cycle.

TABLE II—*Intraovarian impedance to blood flow in patients with tumour-like condition or benign tumour*

Case No	Age (years)	Years since menopause	Histopathology	Pulsatility index (abnormal ovary)
1	53	2	Serous cystadenoma	4·4
2	53	3	Simple serous cyst	5·0
3	35	NA*	Bilateral dermoid cysts†	0·4, 0·8
4	57	4	Simple serous cyst	7·0
5	50	NA*	Simple serous cyst	3·2
6	51	NA*	Serous cystadenoma	4·9
7	63	12	Serous cystadenoma	5·8
8	49	NA*	Endometriotic cyst	5·4
9	49	NA*	Endometriotic cyst	4·8
10	48	Artificially menopausal	Haemorrhagic follicular cyst	5·2

*Premenopausal; scan on days 1 to 8 of menstrual cycle.
†With nests of thyroid-like cells.

TABLE III—*Intraovarian impedance to blood flow in patients with borderline or malignant ovarian tumours*

Case No	Age (years)	Years since menopause	Histopathology	Stage of cancer[9]	Pulsatility index (abnormal ovary)
1	72	32	Endometroid adenocarcinoma	IV	0·9
2	25	NA*	Bilateral, papillary cystadenocarcinoma	III	0·6, 0·3
3	56	6	Poorly differentiated serous cystadenocarcinoma	III	0·3
4	72	20	Mucinous cystadenocarcinoma	III	0·9
5	77	30	Papillary, serous, cystadenocarcinoma	III	0·8
6	52	3	Serous cystadenocarcinoma	Ia	0·4
7	63	14	Intraepithelial serous cystadenocarcinoma	Ia	5·5
8	55	1	Endometrioid cystadenocarcinoma	Ia	1·0

*Premenopausal; scan on day 6 of menstrual cycle.

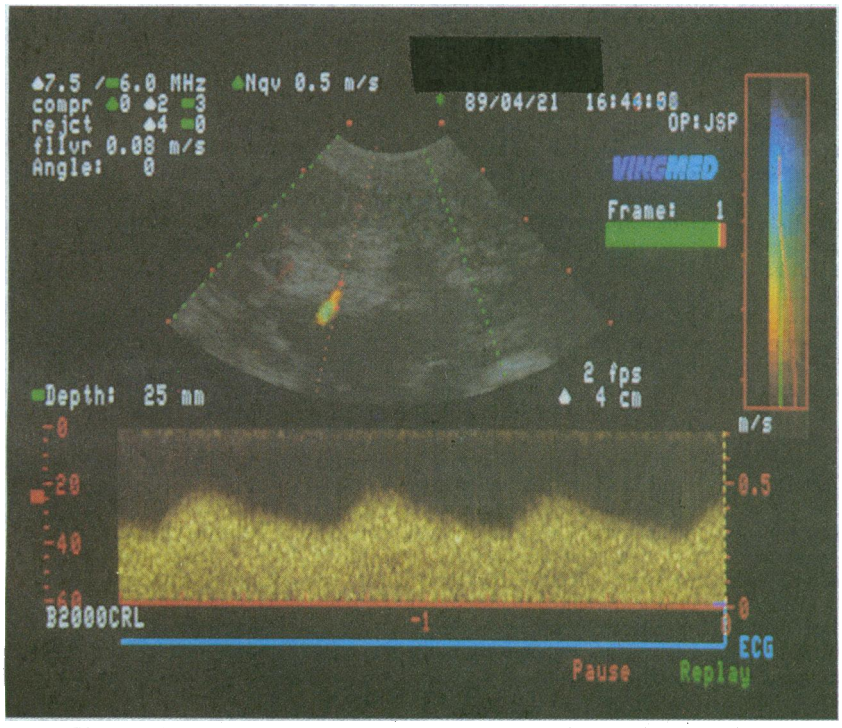

FIG 2—*Flow velocity waveforms showing intraovarian impedance in menopausal woman aged 52 with serous cystadenocarcinoma (case 6; pulsatile index 0·4 (table III)). Note coloured area indicative of neovascularisation*

Healthy ovaries—Table I shows the intraovarian resistance to blood flow (right and left ovaries) in the 10 premenopausal and 20 postmenopausal women with a negative result on screening. Figure 1 shows an example of ovarian morphology and the flow velocity waveform in a postmenopausal woman. There was no evidence for the presence of any altered or new vasculature within any of the ovaries, and the mean values for the pulsatility index from the cases with a hydrosalpinx were 3·2 and 3·5—that is, within the normal range.

Tumour-like conditions and benign tumours—Table II shows the values for the pulsatility index in 10 women (11 ovaries) with a tumour-like condition or a benign tumour. Only one woman (case 3) with bilateral dermoid cysts containing nests of thyroid-like cells showed evidence of changed vascularity and values for the pulsatility index outside the reference range for healthy ovaries. The values for the pulsatility index in ovarian masses were invariably slightly lower than in the healthy contralateral ovaries (data not shown).

Malignant and preinvasive tumours—Table III shows the values for the pulsatility index in eight women (nine ovaries) with primary ovarian cancer. Figure 2 shows an example of ovarian neovascularisation and the flow velocity waveform in a patient with stage Ia disease. Intratumoral vascular changes were clearly visible in the seven patients (eight ovaries) with low values for the pulsatility index. The seventh subject had abnormal ovarian morphology (not visible using transabdominal ultrasonography) and a small ovarian volume (<5 ml). The pulsatility index in the affected ovary was 5·5 and the abnormal tissue was classified as a very early intraepithelial (or borderline) serous cystadenocarcinoma.

Discussion

To the best of our knowledge this is the first report that transvaginal colour flow imaging can be used to differentiate between primary ovarian cancer and many forms of benign pelvic masses. Previous workers showed the feasibility of combining transabdominal ultrasound imaging with conventional pulsed Doppler (the duplex method) to assess impedance to blood flow in the pelvic vessels of healthy and infertile women.[11] Furthermore, transabdominal colour Doppler has been used to assess intrauterine blood flow in patients with trophoblastic disease.[12] The use of a transvaginal probe, however, facilitates the detection of abnormal morphology in small ovaries, and the combined use of colour Doppler can show the presence or absence of vascular changes (probably neovascularisation) within new tissue growths. The technique is simple to use and the results are displayed clearly. Changes in vascularity can usually be observed as a fluctuating area of colour and the pulsatility index derived from the flow velocity waveform provides a quantitative estimate of blood flow impedance. These results are consistent with the findings that conventional Doppler signals are correlated with neovascular changes in various non-ovarian tumours.[13]

The growth and progression of tumours is dependent on the process of vascularisation (angiogenesis). Recent studies with transgenic mice have shown that for at least one type of cancer angiogenesis occurs during the transition from hyperplasia to neoplasia.[14] Other workers have recently identified and cloned a new platelet derived factor that stimulates endothelial cell growth and chemotaxis in vitro and angiogenesis in

vivo.[15] An inhibitor of angiogenesis, which is produced by cells when they are capable of expressing an active cancer suppressing gene, has also been discovered.[16] The loss of this inhibitor activity occurs concomitantly with expression of both angiogenesis and tumorigenesis. The process of angiogenesis entails protease activity and cell differentiation, proliferation, and migration. Most normal cells do not secrete angiogenic substances except during embryogenesis, growth, wound repair, or formation of corpus luteum.[6] For these last three reasons we screen premenopausal women for early ovarian cancer only during days 1 to 8 of the menstrual cycle or take care to avoid the time of ovulation, the presence of a corpus luteum, and pregnancy.

Our results show that the absence of intratumoral neovascularisation and a high pulsatility index can be used to exclude the presence of invasive primary ovarian cancer—thus potentially reducing the rate of false positive results from the screening procedure using conventional ultrasonography while maintaining the detection rate of the disease. The routine application of this new technique will enable us to determine the extent to which women with hydrosalpinges, tumour-like conditions, or benign tumours can be identified and saved unnecessary operations. The pulsatility index in the patient with a very early intraepithelial (or borderline) tumour in a normal size ovary (case 7) suggests that transvaginal colour flow imaging will be used to monitor the progression of very small masses. The bilateral dermoid cysts, which showed neovascularisation, might reflect a non-cancerous response to angiogenic factors produced by nests of thyroid-like cells, or a premalignant condition. The application of this technique to a larger number of cases and the collaboration of pathologists and geneticists will enable the sequence of cellular and molecular events in ovarian tumorigenesis to be defined more precisely.

We have also investigated whether transvaginal colour flow imaging may be used to screen for ovarian and uterine cancer at the same examination. Moreover, the assessment of vascular changes and the resistance to blood flow may reduce the number of scans that are required with conventional ultrasonography to give a definitive result of screening (previous paper[5]). These developments, together with appropriate training and quality control programmes, will facilitate the establishment of screening clinics for gynaecological cancers.

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ONE HUNDRED YEARS AGO

The question which has so long been under the consideration of the Secretary of State for India in Council, on the usefulness of the Netley training to the young medical officers of H.M. Indian Army, has for the present been got rid of by referring it back to the Viceroy of India in Council. It is surely permissible to say that, all things considered, this a "lame and impotent conclusion." Lord Cross and his Council had before them the opinion of those in India who are hostile to the School, and the grounds on which this hostility is based; which, it is notorious, are twofold, namely, the saving to the Government of India of its share of the cost of the School, and the assertion that as good, if not a better, training for the military medical services can be given in the Presidency civil hospitals of Calcutta, Madras, and Bombay. On the same side of the question they had the letter of "Civis," to which so much importance was attached, the authorship of which, it is believed, was no secret to a considerable number of the members of Council, if not to Lord Cross himself; and also the laboured attack on the School and its teaching by a retired Bengal medical officer, to which we have already had occasion to refer as written by a well-known gentleman who, as his life-long service was purely civil, and indeed confined to Calcutta, is not deemed by his old brother officers an authority of great weight on the subject on which he volunteered his opinion. On the other hand, in support of the opinion that the necessity which existed in Lord Herbert's time, and led to the founding of the school, is in existence to this day, Lord Cross in Council had the strongly expressed judgment of some of the leading men in the profession, medical educational experts nearly all of them, as well as that of a number of retired medical officers of the Indian army, representative men, whose names and work are known far beyond the limits of India. It does seem strange with all this before them that the Secretary of State for India, and his twelve advisers, should have felt themselves incapable of coming to a judgment, one way or the other, on this matter, and been driven to "shunt" the responsibility of a decision to the shoulders of the local government of India, who had already given an opinion on the subject, and who, we may suspect, are not likely to stultify themselves by altering it on any amount of fresh evidence and wiser advice. We venture, even at this the eleventh hour, and at the hazard of wearisome iteration, to remind those who have now to decide this question of the consensus of opinion at home on the importance of a real, practical, and specific training in a special school set apart for this work, with experienced teachers of name and reputation and experience, who carry on their work, as it were, under the eye of the whole profession, and the press which represents that profession, and who have at their disposal what the civil presidency hospitals have not—a costly and complete teaching plant that has been accumulating for years. We invite their attention in particular to the statements in the papers before them to the effect that the proposed substitute for the Netley training in the hospitals of India, if that training is to be anything but a mockery and a delusion, would be more costly by far than that at Netley.

Destruction is the easy work of the unwise; construction, in matters educational, is the proper work of men of "light and leading," and such were the men who, acting on the wise advice of Robert Jackson and John Bell, established the Army Medical School for the benefit of the sick and wounded soldiers of Her Majesty's army at home and in the greatest of her possessions. (*British Medical Journal* 1889;i:199)