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Increased Arterial Inflow Demonstrated by Doppler Ultrasound in Arm Swelling Following Breast Cancer Treatment

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Doppler ultrasound was used to estimate the mean arterial flow in the arms of 50 patients with arm oedema following breast cancer treatment (oedema group). They were compared with 26 treated breast cancer patients with no arm swelling (control group). Flows on the treatment side were expressed as a percentage of the flow in the contralateral normal arm. Mean percentage blood flow was 68% higher in the swollen arms than the contralateral normal arms compared to a mean increase of 38% on the breast cancer treatment side in the control group. In both groups there was a significantly higher proportion of patients with increased (> 150%) rather than decreased (< 50%) flow on the treatment side. The ratio of increased to decreased flow was 27:4 in the oedema group and 6:0 in the control group. A neurological deficit on the treatment side was associated with an increased incidence of higher flow on the same side in the oedema group. These findings demonstrate that breast cancer treatment results in a significant increase in arterial flow in the arm on the treatment side, and that this increase is even higher in those patients with swelling. Increased blood flow is likely to contribute to arm swelling. One explanation for increased flow would be neurological deficit with loss of sympathetic vasoconstrictor control.
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INTRODUCTION

CHRONIC ARM swelling is a common complication of breast cancer treatment [1]. Once established, it tends to be permanent and difficult to treat. Lymphatic obstruction has traditionally been considered to be the cause of arm swelling [2–4], but this may be an oversimplification of the pathophysiology which is poorly understood.

Oedema develops when there is an imbalance between the capillary filtration rate and lymph flow. An increase in capillary filtration encourages swelling, particularly when the lymphatic drainage capacity is compromised. Recent studies suggest that capillary filtration is increased rather than reduced in the swollen arm after breast cancer treatment [5]. An increase in limb blood flow would explain this finding. In 1967, Jacobsson, using a variety of techniques, including venous occlusion plethysmography, arteriovenous pO_2 differences and isotope clearance rates, demonstrated increased blood flow in a study of 25 patients [6].

Doppler is a non-invasive method of estimating arterial flow [7, 8]. It was used to measure subclavian artery flow in breast cancer patients with and without arm swelling to determine if limb blood flow was increased.

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PATIENTS AND METHODS

Patients

50 patients with chronic arm swelling (oedema group) following breast cancer treatment were studied using Doppler and grey scale ultrasound to measure the arterial flow in both arms. All patients exhibited more than a 200 ml difference between swollen and unaffected arms (this difference or greater is regarded as indicative of lymphoedema [1]).

A group of 26 breast cancer patients were randomly recruited from the follow-up clinic of the Breast Unit of the Department of Medicine, The Royal Marsden Hospital, and studied for comparison. The criteria for inclusion were similar treatment, age range and range of time from treatment as the study group with no arm swelling and willingness to attend for the ultrasound examination (demographic and treatment details are given in Tables 1 and 2).

The patients' notes were studied for reference to symptoms of motor or sensory deficit in either arm, and the results of clinical neurological examinations were noted. Patients were recorded as having a neurological deficit if the symptoms and signs were restricted to the arm on the side of treatment only and did not antedate treatment.

Methods

Grey scale (7 MHz) and spectral Doppler (5 MHz) ultrasound studies were performed using a linear array transducer on an Acuson 128 scanner. Measurements were made in the region of the axillo-subclavian junction with the patients lying supine and their arms in an identical position by their sides. For each patient, mean velocity measurements were made with the same Doppler gain settings, spectral trace scales using similar beam

Table 1. Patients' characteristics

	Patients with swollen arm (oedema group)	Control group with no arm swelling
Number of patients	50	26
Average age (years)	61.4	61.1
Range	(36–85)	(44–82)
Bilateral breast disease	3 (6%)	1 (4%)
Right side	27 (54%)	19 (73%)
Left side	23 (46%)	7 (27%)
Patients with metastatic disease distant to the region of the primary	13 (26%)	6 (23%)
Average time (years) since disease was first treated	8.2	6.5
Range	(8 months–35 years)	(3 months–28 years)

Table 2. Treatment received by each group of patients

	Oedema group (Swollen arm) $n = 50$		Control group (No arm swelling) $n = 26$	
Surgery	No RT	RT	No RT	RT
None	0	2 (4%)	0	1 (4%)
Wide local excision	1 (2%)	24 (48%)	2 (8%)	10 (38%)
Simple mastectomy	2 (4%)	12 (24%)	3 (11.5%)	7 (27%)
Radical or modified radical mastectomy	6 (12%)	3 (6%)	1 (4%)	2 (8%)

RT, Radiotherapy.

to vessel angles on the two sides. The sample gate was set slightly wider than the artery with a beam to vessel angle of less than 60°. The maximum Doppler shift was sought with the transducer aligned so that the artery ran within the plane of the image. The angle corrected, time-averaged mean flow velocity was estimated for at least one complete cardiac cycle from a spectral trace of similar cycles using the built-in analysis software.

The cross sectional area of the artery was obtained using the grey scale image at the same site as the Doppler measurements. The transducer was kept perpendicular to the vessel to ensure that the true diameter was measured and the antero-posterior diameter in systole was used to calculate the cross sectional area of the equivalent circle.

Analysis

The mean blood flow (ml/min) was calculated as the product of the cross sectional area and the time averaged mean velocity. The flows on the treatment side were expressed as a percentage of the contralateral normal arm to allow comparison. Differences of less than 50% between the two arms were regarded as being within normal limits. Differences of over 50% were regarded as abnormal.

Statistics

The χ^2 test with Yates correction was used to compare frequencies; when only small samples were available, Fisher's exact test was used.

Ethics

Ethical approval was received from the Royal Marsden Hospital ethics committee.

RESULTS

Doppler blood flow measurements were easily obtained at the axillo-subclavian artery junction in both arms in all patients.

The mean blood flow in the normal (contralateral) arms was 298 ml/min (range 21–1560) in the oedema group and 280 ml/min (range 76–816) in the control group. The mean blood flow was increased on the treated side in both groups, 460 ml/min (range 22–3180) in the side of the swollen arms of the oedema group, and 340 ml/min (range 50–675) in the arms of the treated side in the control group without arm swelling.

To allow comparision between patients, all flows on the side of treatment were expressed as a percentage of the contralateral normal control arm.

In the oedema group, the mean of the percentage flow was 68% greater than the contralateral normal arm. In the control group, the mean of the percentage flow was increased by 38% on the side of the breast cancer treatment.

27 (54%) patients in the oedema group and only 6 (23%) of the control group had a greater than 50% increase of flow on the side of treatment ($P = 0.02$). The proportion of patients with a greater than 50% increase in flow compared with those of normal or decreased flow was highly significant in both the oedema ($P < 0.0001$) and control group ($P < 0.017$) (Table 3).

5 patients of the oedema group and 2 of the control group had increases in flow of over 300%. Flow in four swollen arms was decreased (30% of the contralateral normal arm in two, and just under 50% in two others). The lowest flow on the treatment side in the control group was 64% of the contralateral normal side.

In the oedema group, the presence of a neurological deficit in the treated arm was associated with an increased incidence of higher flow (21:5) on the side of swelling, compared with an equal distribution of higher and lower flow (12:12) in the absence of any neurological deficit ($P < 0.046$). There was no association in the control group ($P = 0.39$) (Table 4).

DISCUSSION

This is the first study to measure arterial blood flow using Doppler ultrasonography in chronically swollen arms following breast cancer treatment.

Absolute measurements of arterial blood flow are operator, patient and machine dependent; this complicates measurements and comparisons between individuals of different body habitus.

Table 3. Arterial flow in swollen arms of the oedema group and the treated side of the control group (expressed as a percentage of normal arm)

	Study group $n = 50$	Control group $n = 26$	
Increased flow > 150% of normal arm	27 (54%)	6 (23%)	($P = 0.02$)
Flow within normal limits 150–50% of normal arm	19 (38%)	20 (46%)	
Decreased flow < 50% of normal arm	4 (8%)	0	
	I ($P = 0.0001$)	I ($P = 0.017$)	

Table 4. Incidence of higher and lower flows on side of treatment related to local neurological deficit

	Motor or sensory deficit in the arm on the side of treatment	No motor or sensory deficit
Oedema group	(P = 0.046)	
Higher flow	21(42%)	12(24%)
Lower flow	5(10%)	12(24%)
Control group	(P = 0.39)	
Higher flow	5(19%)	9(35%)
Lower flow	2(8%)	10(38%)

Limb flow is affected by ambient temperature and recent exercise. Variations in flow of 8 to 440 ml/min in enclosed segments of arm during venous plesythmography were noted by Jacobsson [6]. The range of differences were related to temperature of the arm and postocclusive hyperaemia as well as inter-patient differences. This 55-fold difference is partly reflected in our results.

Errors due to inter-patient variability do not occur when comparisons are made between the right and left sides, the patient acts as her own control at the one examination with the same machine settings for each side. The marked variations which can occur, due to ambient temperature, exercise and other factors affecting distribution of blood flow within the body, are also prevented by comparing the two sides at the one examination. Differences in flow in each patient were expressed as a percentage of the contralateral, normal arm to allow comparison between patients. Problems remain with errors due to beam to vessel angle measurement (± 5%) and cross sectional area measurements (± 10%). Errors introduced by differences in depth of the artery tend to underestimate flow in the swollen arms [7, 9, 10]. We assumed a potential for 50% error, and differences of flow of less than 50% between the two arms were regarded to be within normal limits.

A significantly higher proportion of patients in the oedema group had increased flow on the side of treatment compared with the control group, and in both groups significantly more patients had increased rather than decreased flows on the side of treatment. Jacobsson, in the only previous study to measure blood flow in postmastectomy lymphoedema, demonstrated an overall increase in skin and subcutaneous blood flow in swollen arms [6]. 25 patients were examined with a variety of techniques. Venous occlusion plethysmography showed that the blood flow in the lymphoedematous limb was increased both at 35°C and at 43°C, and there was a greater increase in flow at the higher temperature in the swollen limb. No difference in muscle blood flow, measured using ^{85}Xe, was recorded between the swollen and the contralateral normal arm, but higher flows were demonstrated in both the skin and subcutaneous tissues of the swollen arm using [^{125}I] 4-iodo-antipyrine. The clearance of ^{85}Kr injected intra-arterially was more rapid from the skin of the oedematous arm when vasodilation was induced by body heating. These differences from the normal arm were attributed to higher flow in the skin and subcutaneous vascular beds.

The average increase in flow in the swollen arms of 68% measured by Doppler ultrasonography is of similar magnitude to the 42% increase recorded by Jacobsson using plethysmography. He also reported a 3–5-fold increase in the flow in the swollen arms in a few cases, similar to the marked increase found in 7 of our cases. Venous occlusion plethysmography can be difficult to use when oedema is present since the measurement of blood flow depends on evaluating the rate of swelling. Not only may swelling itself be exacerbated, but pitting of the tissues by the strain gauge can occur, although this is more likely to cause an underestimation of blood flow rates. Isotope clearance methods are semi-invasive and employ radiation, albeit in small doses. Interpretation of clearance depends on knowing the volume of distribution of the tracer. Doppler sonography has the advantages of simplicity, non-invasiveness and repeatability, and it does not impede blood flow.

The reason for the increase in limb blood flow following breast cancer treatment is not clear. One possibility is a loss of sympathetic activity. Our observation that 52% of patients showed a neurological abnormality in the study group is comparable with that of Stoll and Andrews [11], who found between 15 and 73% of their patients, following radiotherapy for breast cancer, had clinically overt peripheral neuropathy. In their cases, the incidence was dose- and energy-dependent, and the onset of neuropathy coincided approximately with the onset of oedema (10–22 and 11–23 months, respectively) as previously noted by Treves [12], Other studies suggest an association of oedema with clinical signs of neural damage [13, 14] or electromyographic changes [15].

The association of the increased arterial flow we have demonstrated with systemic neuropathy suggests that the blood flow changes may be due to autonomic nerve damage which is presumably induced by radiation. The possibility of a neurological cause for arm oedema was raised by Homans in 1940 [16], who postulated that reflex or trophic oedemas could have a neural basis. Sympathetic dystrophy (Sudeck's atrophy, causalgia and oedeme bleu) is associated with autonomic dysfunction, and there is evidence of increased regional blood flow and oedema [17, 18]. In sympathetic dystrophy (stage 1) the arterial flow is increased to 150% (range 112–436) of the control side. In stage 2 dystrophy this decreases to 67% (range 30–87) of the control limb [19]. These changes are of similar magnitude to those we have demonstrated in arms swelling after breast cancer treatment and would be consistent with a similar neurological abnormality.

These results demonstrate that breast cancer treatment results in a significant increase in arterial flow in the arm on the treatment side. This increase is greater in those patients with swelling. One explanation for increased flow would be loss of sympathetic vasoconstrictor control.

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Loss of Heterozygosity of Tumour Suppressor Gene Loci in Human Colorectal Carcinoma

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We used Southern blot analysis and polymerase chain reaction-based techniques to examine deletions of tumour suppressor gene loci in 91 primary colorectal tumours. The tumour suppressor genes studied were MCC and APC on chromosome 5q, p53 on chromosome 17p, DCC on chromosome 18q, and the putative suppressor gene nm23-H1 on chromosome 17q. The most frequent allelic loss observed was in chromosome 17p with 76% (68/89) of informative tumours showing loss of heterozygosity at this locus, followed by 34% (19/55) for DCC, 31% (12/39) for MCC, 17% (9/53) for APC and 16% (3/19) for nm23. No significant differences in the frequency of these suppressor gene allelic losses were observed between Dukes B and C stage adenocarcinomas.

Key words: tumour suppressor genes, colorectal carcinoma, p53, MCC, APC, DCC
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INTRODUCTION

THE MOLECULAR genetic alterations in colorectal cancer are the best understood of all human cancers [1]. It appears this disease arises from the accumulation of mutations during progression from normal epithelial mucosa to adenoma and carcinoma. In addition to the activation of oncogenes such as Ki-*ras*, there is a loss of chromosomal segments at specific loci which may indicate the deletion of tumour suppressor genes. Four such genes associated with colorectal carcinoma have now been identified and cloned: MCC (mutated in colon cancer) and APC (adenomatous polyposis coli) on chromosome 5q [2–4], p53 on chromosome 17p [5], and DCC (deleted in colon cancer) on chromosome 18q [6]. The high frequency of mutations and loss of heterozygosity of these genes in colorectal tumours suggest that their normal gene products have a tumour suppressor function. Loss of heterozygosity of suppressor gene loci has traditionally been studied by Southern blot analysis and more recently using polymerase chain reaction (PCR)-based techniques, while gene mutation has been studied by direct sequencing, RNase mismatch cleavage and single strand conformation polymorphism (SSCP) analysis.

So far, identification of the molecular genetic alterations useful for the clinical diagnosis of colorectal cancer is still in the early stages. Recent work has indicated that deletions within the 17p and 18q chromosomal arms may be of prognostic value [7–9], but not 5q deletions or *ras* oncogene mutations. In the present study we have examined loss of heterozygosity (LOH) of the MCC, APC, p53 and DCC genes in a panel of 91 colorectal tumour specimens, 81 of which were Dukes stage B or C adenocarcinomas. Allelic deletion of the NM23-H1 gene located at chromosome 17q and thought to be involved in suppression

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