Constitutively enhanced lymphatic pumping in the arms of women who later develop breast cancer-related lymphoedema

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Short title: Enhanced lymphatic pumping before the onset of breast cancer-related lymphoedema

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Abstract

Lymph drainage rate in both arms is greater in women destined to develop breast cancer-related lymphoedema (BCRL) than in those who do not develop BCRL, indicating a constitutive predisposition. We explored constitutive differences here, measuring the maximum pressure ($P_{\text{pump}}$) and rate of $^{99m}$Tc-Nanocoll transport generated by the contractile arm lymphatics before breast cancer surgery in 26 women who were followed for 2 years to determine their eventual BCRL/non-BCRL status. $P_{\text{pump}}$ and tracer transport rate in the ipsilateral arm were measured by lymphatic congestion lymphoscintigraphy pre- and post-surgery. BCRL occurred in 10/26 (38.5 %) cases. $P_{\text{pump}}$ in the women who later developed BCRL ($40.0 \pm 8.2$ mmHg) was 1.7-fold higher than in those who did not develop BCRL ($23.1 \pm 10.8$ mmHg, $P = 0.001$). Moreover the rate of lymph tracer transport into the forearm was 2.2-fold greater in the arms of women who later developed BCRL ($P = 0.052$).

Surgery did not significantly reduce $P_{\text{pump}}$ at 21 weeks but impaired forearm tracer transport in pre-BCRLs by 58 % ($P = 0.047$), though not in non-BCRLs. Women destined to develop lymphoedema have higher pumping pressures and lymph transport, indicating harder working lymphatics prior to cancer treatment. Axillary lymphatic damage from surgery appears to compromise lymph drainage in only those women constitutively predisposed but not in those women with constitutively lower lymphatic pressures and transport.

Clinical Perspectives

- Breast cancer-related lymphoedema (BCRL) is a common sequel to breast cancer treatment but recent findings indicate that axillary surgery is not the sole cause.
- A constitutive predisposition to BCRL emerges from the prospective study reported here in which BCRL-destined patients were found to generate higher lymphatic pumping pressures and had faster lymph transport than non-BCRL patients before surgery.
• The higher apparent lymphatic work rate in the pre-BCRL patients may make this population more susceptible to (even minor) axillary trauma, tipping them into chronic lymphatic failure. The pathophysiology of BCRL is analogous to high preload and high afterload cardiac failure in systemic hypertension. Pharmacological interventions aimed at reducing lymphatic pre- and/or afterload should, in theory, prevent BCRL if introduced earlier enough in at risk patients.

• This suggests, in theory, that women with higher lymphatic preloads could be identified as an ‘at risk’ group prior to cancer treatment.

Summary statement

The risk of developing lymphoedema after breast cancer treatment is not attributable solely to axillary lymphatic obstruction, but also depends on a constitutive predisposition through higher lymphatic preload, which makes some women more vulnerable to the axillary interventions.
Introduction

Breast cancer-related lymphoedema (BCRL) is a common sequel to breast cancer treatment, the risk of which is increased by obesity, extensive axillary lymph node dissection and radiotherapy to the regional lymph nodes (DiSipio et al. 2013; Basta et al. 2015; Whelan et al. 2015). The pathophysiological basis of BCRL is complex and the former assumption of regional lymphatic obstruction to the ipsilateral arm following axillary trauma (stopcock hypothesis) is an incomplete explanation. Features of BCRL not readily explained by a simple stopcock hypothesis include sparing of many breast cancer patients despite extensive axillary surgery; the occurrence of BCRL in patients after only sentinel lymph node biopsy; delayed onset of swelling; and non-uniformity of the swelling along the arm. Moreover the contralateral arm does not appear to be normal in women with unilateral BCRL; it exhibits lymphatic capillary dilatation in the dermis and greater contralateral hand lymph drainage when there is ipsilateral hand swelling (Mellor et al. 2000; Stanton et al. 2006b).

A striking recent finding was that women who later develop BCRL have higher lymph drainage rates in both arms, in both the muscle (+22–29 %) and subcutis (+22–50 %), before any swelling is evident, compared with women who do not later develop BCRL (Stanton et al. 2009a). The raised lymph drainage rates were observed at 7 months post-surgery, so they could have been either a systemic effect of the cancer treatment or an inherent, constitutive property. To resolve this point a recent prospective study examined breast cancer patients before the cancer surgery. Quantitative lymphoscintigraphy was performed before surgery and the patients followed for 13 months post-surgery. In the 7 out of 38 patients (18 %) who developed BCRL, the pre-operative lymph removal rate constant \( k \) was 16 % higher than in patients who did not later develop BCRL (Bains et al. 2015). These findings, along with those of Stanton et al. (2006b, 2009a) and Mellor et al. (2000), indicate that there are constitutive, pre-operative, bilateral differences in lymphatic physiology in BCRL-destined patients. Lymphatic drainage involves active contractile pumping, both in animal models (McHale & Meharg 1992; Li et al. 1998; McCloskey et al. 2002; von der Weid et al.
2014) and human limbs (Olzewski & Engeset 1980). To assess lymphatic collector vessel pump function in the arms of women with established BCRL, pump function was quantified as the maximum pressure ($P_{pump}$) generated in the collector lymphatics when lymph flow was blocked by inflating a cuff around the upper arm (lymphatic congestion lymphoscintigraphy, Modi et al. 2007). $P_{pump}$ was found to be significantly impaired in the lymphoedematous arm, by 38 % relative to healthy control subjects. Moreover there was a strong negative correlation between $P_{pump}$ and the magnitude of the swelling, i.e. the weaker the pump, the greater the swelling.

To bring together many of the findings reviewed above, we proposed the following working hypothesis (Stanton et al. 2009b). In pre-BCRL patients the work of lymphatics is already high due to their raised fluid load. After axillary surgery, lymphatic pump failure may then develop in a manner analogous to cardiac failure following a chronically raised afterload (hypertension). Lymphatic afterload is probably increased chronically by axillary node damage during surgery and radiotherapy, since nodal excision raises lymph outflow resistance in sheep (Kim et al. 2003). The chronically increased work by the lymphatic muscle fibres leads eventually to reduced lymphangion contractility, as in hypertensive heart failure; animal studies show lymphatic failure at increased distending pressures (Li et al. 1998; Levick & McHale 2003; Davis et al. 2012). The resulting decline in lymph drainage rate leads to oedema in the drainage territory of the failing lymphatics. The chronic pump failure hypothesis offers a rational explanation for the variable delay in onset of BCRL, since a variable period is likely to be required for overload failure to reach a critical point. Pump failure can also offer a rational explanation for the regionality paradox (Modi et al. 2005; Stanton et al. 2009b); if the constitutionally weakest lymphatic collector vessels fail first, swelling will be localised to their drainage territory, i.e. hand, forearm or upper arm.

In light of the constitutive, pre-operative differences in lymph flow in lymphoedema-destined breast cancer patients, the question arose as to whether these patients might also have constitutive differences in lymphatic pump function prior to pump failure. The present study
evaluates this possibility by measuring maximal lymphatic pump pressure $P_{\text{pump}}$, plus several secondary measures of lymphatic transport rate, in the ipsilateral arm of women recently diagnosed with breast cancer. The study was carried out on the ipsilateral arm before and after axillary surgery, and the patients were then followed for 2 years to see who did and who did not develop BCRL.

Patients and Methods

Patients

The study was approved by the National Research Ethics Service (reference 09/H0701/112) and the Administration of Radioactive Substances Advisory Committee (ARSAC) (certificate number 295/3230/25986). Procedures were carried out in accordance with the Declaration of Helsinki (2013) of the World Medical Association. All patients gave written informed consent. Twenty-six women, aged 53 ± 12 (S.D.) years and recently diagnosed with breast cancer, were recruited from the Breast Clinics at St George’s Hospital, London; The Royal Marsden Hospital, Sutton, Surrey; and Croydon University Hospital, Croydon, Surrey. Details of the breast cancer and its treatment are summarised in Table 1. Patients underwent mastectomy or wide local excision, and axillary lymph node clearance surgery (ANC) or sentinel lymph node biopsy (SLNB), as recommended by the multidisciplinary team. All patients received radiotherapy.

Baseline arm assessment and lymphatic congestion lymphoscintigraphy (LCL) were performed before axillary surgery (the pre-surgical visit) and repeated 21 ± 15 (S.D.) weeks after surgery (the post-surgical visit). Diagnostic arm assessment for lymphoedema (without LCL) was performed at 13.0 ± 2.8 months and 25.0 ± 4.7 months post-surgery, and also shortly after BCRL onset in the BCRL patients. In 6 patients the post-surgical LCL was not performed because the patients were unavailable or declined, but these patients were nevertheless followed to see whether or not they developed BCRL. Height was measured pre-surgery and weight was recorded on all visits to calculate body mass index (BMI). The breast cancer patients who later developed BCRL will be
referred to as ‘pre-BCRL’ patients, and the patients who did not develop BCRL as ‘non-BCRL’ patients. The ipsilateral arm of the pre-BCRL patients was the dominant side in 40 % of cases and the non-dominant arm in 60 %; in the non-BCRL patients the ipsilateral arm was the dominant side in 44 % of cases and the non-dominant side in 56 %.

**Assessment of the arm for lymphoedema**

Arms were assessed both by clinical criteria and by volume measurement. The clinical features of lymphoedema are evident at an early, minor stage that is not readily demonstrated by whole-arm volume measurement, because arm volumes can change with change in body mass after cancer treatment (see Results). For this reason the diagnosis of BCRL was made clinically, rather than on arm volumes per se. The diagnosis of BCRL was always confirmed independently by a Lymphoedema Practitioner. Clinical assessment was performed as described by Stanton et al. (2006a). Briefly, lymphoedema was considered to be present if any of the following were detectable: (i) decreased visibility of subcutaneous veins on the ventral forearm and dorsal hand; (ii) smoothing or fullness of the medial elbow and distal upper-arm contours; (iii) increased skin and subcutis thickness if the tissues are pinched between finger and thumb; (iv) pitting oedema upon application of thumb pressure for 60 s. In addition, the thickness of the posterior axillary fold (PAF) was assessed by the ‘pinch test’ (Roberts et al. 1995). Patients with ipsilateral PAF thickening also had arm oedema. In addition to the clinical assessment, the volume of each arm was measured between the ulnar styloid process (wrist) and anterior axillary fold (upper arm) using an opto-electronic limb volumeter (Perometer 350S, Pero-System Messgeräte GmbH, Wuppertal, Germany), as evaluated by Stanton et al. (1997).

**Lymphatic congestion lymphoscintigraphy (LCL)**

LCL was performed as described by Modi et al. (2007) with minor changes (below). To check the efficacy of tissue compression by the upper arm congestion cuff, preliminary experiments were
performed in which antecubital venous pressure ($P_v$) was compared directly with the applied cuff pressure ($P_{cuff}$) in the arms of 6 healthy participants. The participant reclined on a bed with the forearm heart level. A cannula (Venflon 18G) was inserted into the antecubital vein and connected via fine bore tubing containing heparinised saline to a calibrated pressure transducer (SensoNor dome transducer and BPM-832 pressure amplifier; Linton Instrumentation, Diss, Norfolk). $P_v$ was displayed on a computer based data recording system (PowerLab 4/30 and LabChart, AD Instruments; PMS Ltd, Maidenhead, Berkshire). A standard blood pressure cuff around the upper arm was inflated in a series of steps over the range 22–46 mmHg. $P_v$ increased in parallel with the increases in $P_{cuff}$, albeit with absolute values 2.9–3.1 mmHg less than $P_{cuff}$. These results showed that the congesting cuff pressures were transmitted to the deeper tissues with minor attenuation, in accordance with the routine use of congesting cuffs to measure arterial blood pressure.

For the LCL study the patient lay supine on a bed and the brachial artery blood pressure (BP) was measured by auscultation from the contralateral arm using a mercury sphygmomanometer and a Riva-Rocci congestion cuff (AC Cossor & Son Ltd, London). For 2 patients with particularly large arms the larger, alternative cuff size was used, and the standard size for all others. The cuff was secured around the ipsilateral upper arm with the tubing at the top and the cuff bladder centred anteriorly. The cuff was wrapped closely and evenly around the arm and the overlap fastened down with adhesive tape. The sphygmomanometer was then reattached to the cuff. The arm was supported so that the forearm was horizontal and at heart level. The gamma camera (Argus Epic; MIC Ltd, Fleet, Hampshire) (128 x 128 matrix, low-energy general purpose collimator) was positioned above the ipsilateral arm for ventral viewing, with the forearm, upper arm, axilla, and adjacent part of the trunk within the field of view. Patients acclimatised to their surroundings for 45 min, including 20 min whilst lying down before the tracer injection and scan. The ambient laboratory temperature was 24.0 ± 0.6 (S.D.) °C ($n$ = 26) on the pre-surgical visit and 23.8 ± 0.6 °C ($n$ = 20) post-surgery. Skin temperature, recorded from the ipsilateral forearm (YSI 4600 digital thermometer; Henleys Medical Supplies Ltd, Welwyn Garden City, Hertfordshire), was 29.6 ± 1.3 °C pre-surgery and
29.5 ± 1.5 °C post-surgery. The cuff was inflated to 60 mmHg (or 50 mmHg if the diastolic BP was < 60 mmHg), using the sphygmomanometer. After 2 min of congestion, 50 µl of \textsuperscript{99m}Tc-Nanocoll (GE Healthcare, Hatfield, Hertfordshire) of activity 8.4 ± 1.6 MBq (n = 26) was injected intradermally between the 2\textsuperscript{nd} and 3\textsuperscript{rd} metacarpal heads of the ipsilateral hand, using a microneedle of outer diameter 0.2 mm (Unimed SA, Lausanne, Switzerland). The equivalent radiation dose was 0.03 mSv. The injections were performed consistently and by the same operator throughout, taking on average 52 ± 13 s (n = 26) to complete. The injected activity was 8.1 ± 1.5 MBq and the duration of the injection was 57 ± 16 s for the pre-BCRL group, and 8.6 ± 1.6 MBq and 49 ± 11 s for the non-BCRL group (n = 10 and 16, \( P = 0.41 \) and \( 0.18 \), unpaired t tests). Lymphatic density is higher in the dermis than subcutis, so dermal injections provided rapid access of the tracer to the hand and arm collector lymphatics for gamma-camera imaging (Stanton \textit{et al.} 1999b; O’Mahony \textit{et al.} 2004; Mellor \textit{et al.} 2010). Lymphatic vessels are not readily imaged following intramuscular administration of radiotracer in the arm (Stanton \textit{et al.} 2003). \textsuperscript{99m}Tc-Nanocoll was used as the lymphatic tracer instead of Technescan HIG (human IgG), used formerly (Modi \textit{et al.} 2007), because Technescan HIG had been withdrawn by the sole manufacturer. Radiochemical purity of the \textsuperscript{99m}Tc-Nanocoll was 95–100 %.

As soon as the injection was complete, a dynamic sequence of 40 x 2.5-min acquisitions was commenced, total duration 70 min. The cuff pressure \( (P_{\text{cuff}}) \) was held at 60 mmHg for 10 min, a period long enough to demonstrate trapped tracer unable to pass beyond the lower border of the cuff. \( P_{\text{cuff}} \) was then deflated in 10 mmHg steps every 10 min until \( P_{\text{cuff}} = 0 \) mmHg. A further static image was acquired after completion of the dynamic sequence but while the patient remained in position, namely an outline of the forearm, upper arm, cuff and shoulder region drawn on the camera face using a \textsuperscript{57}Co pen marker (High Technology Sources Ltd, Didcot, Oxfordshire) to create a template for the region of interest analysis (see below). The syringe activity was measured before and after injection using the gamma camera, to calculate the injected activity. No blood samples were collected.
Measurement of lymphatic function

Three regions of interest (ROI) were analysed, namely the forearm (ROI1), the subcuff region of the upper arm (ROI2), and the axillary-supraclavicular region (ROI3) (Figure 1). The counts from each 2.5 min acquisition were plotted against time for each ROI. At high cuff pressures the tracer was transported into the forearm and on towards the lower border of the cuff, but was unable to move more proximally because the lymphatic pump was unable to generate sufficient pressure to overcome the cuff pressure. As cuff pressure was reduced, there came a point at which lymphatic pressure was high enough to force tracer under the cuff and into the axilla.

**Primary measure of lymphatic function, \( P_{\text{pump}} \) \( (\text{mmHg}) \)** \( P_{\text{pump}} \) was defined as the \( P_{\text{cuff}} \) at which radioactivity in ROI3 (axilla) first exceeds the background level (Modi et al. 2007).

**Secondary indices of lymphatic transport** The secondary indices of lymphatic transport, derived using fractional counts (local counts divided by injected activity), were (1) the rate of rise of \(^{99m}\text{Tc}\)-Nanocoll activity in ROI1 during the initial 2.5–10.0 min period of trapped lymph (\( dA/dt_{\text{ROI1}} \), min\(^{-1}\)); (2) the maximum \(^{99m}\text{Tc}\)-Nanocoll count in ROI1; and (3) the rate of rise of \(^{99m}\text{Tc}\)-Nanocoll activity in ROI3 over the virtually linear, 20 min segment of the counts-versus-time plot after \( P_{\text{pump}} \) exceeded \( P_{\text{cuff}} \). Linear transport occurred at 35–55 min for the pre-BCRL group and 42.5–62.5 min for the non-BCRL group (\( dA/dt_{\text{ROI3}} \), min\(^{-1}\)). All counts were decay corrected with standardisation to the injection time.

**Statistical analysis**

Results are presented as the mean ± standard deviation (S.D.) in the text and as the mean ± standard error of the mean (S.E.M.) in the tables and figures. The normality of data sets was tested using the D’Agostino-Pearson Omnibus test. Student’s paired or unpaired t test was used to compare differences between groups, or the Mann-Whitney test in the case of non-Gaussian distribution.
(comparison of \( P_{\text{pump}} \) in the pre-BCRL and non-BCRL groups). The foregoing together with linear regression and two-way analysis of variance (ANOVA) were as implemented in GraphPad Prism version 6 (GraphPad Software, La Jolla, CA, U.S.A.). Differences were considered significant if \( P \leq 0.05 \).

Results

Clinical, surgical and morphometric data

**Incidence of BCRL and cancer treatment**  BCRL was diagnosed clinically in 10/26 women (38.5 %), with onset at 7 ± 4 months (0–12 months) post-surgery. BCRL developed in 7/14 (50.0 %) patients receiving a mastectomy, 3/12 (25.0 %) patients receiving wide local excision, 9/19 (47.4 %) patients receiving axillary node clearance, and 1/7 (14.3 %) receiving sentinel node biopsy. The number of lymph nodes removed in the pre-BCRL group, 15.8 ± 9.5 per patient, was not significantly different from non-BCRL, 13.2 ± 10.5 (\( P = 0.53 \), unpaired \( t \) test). The number of removed nodes that were positive for cancer was again similar in the two groups (4.3 ± 3.6 pre-BCRL versus 3.3 ± 4.2 non-BCRL, \( P = 0.54 \)).

**Arm volumes**  Before surgery both the ipsilateral and contralateral arm volumes for the pre-BCRL group were larger than for the non-BCRL group, by 22.1 % (\( P = 0.055 \), unpaired \( t \) test; Table 2) and 21.5 % respectively (\( P = 0.054 \)). Surgery had no statistically significant early (i.e. at mean 21 weeks) effect on arm volume in either the pre-BCRL or non-BCRL group (Table 2; \( P = 0.31 \) and 0.48 for pre-BCRL ipsilateral and contralateral arms respectively; \( P = 0.80 \) and 0.64 for non-BCRL, paired \( t \) tests). However the above analysis hides a significant difference (Table 2); after surgery, ipsilateral volumes were significantly greater than contralateral volumes in the pre-BCRL patients (\( P = 0.04 \), due to small, divergent changes; there was a small increase in ipsilateral volume (not itself statistically significant) and a small fall in contralateral volume (not itself statistically significant), resulting in a significant difference between the arms (Figure 2). Over 2 years ipsilateral arm volume tended to increase with time in the pre-BCRL group, as expected, but not in the non-BCRL group or
contralateral arms. In some cases the increase in ipsilateral arm volume was very small, especially at the time of diagnosis, emphasising the importance of using clinical criteria to diagnose early lymphoedema (Stanton et al. 2006).

**Body mass index (BMI)** Prior to surgery the pre-BCRL group’s mean BMI ($29.5 \pm 5.0 \text{ kg/m}^2$) was 20 % higher than that of the non-BCRLs ($24.7 \pm 3.6 \text{ kg/m}^2$), in keeping with their 22 % greater arm volumes. Higher BMI is a known risk factor for BCRL (McLaughlin et al. 2008; DiSipio et al. 2013); our arm volume data indicate that this related parameter is a risk factor. BMI did not change significantly up to 25 months post-surgery ($P = 0.044$ for pre-BCRL BMI versus non-BCRL BMI, $P = 0.98$ for time-points; two-way ANOVA).

**Tracer movement along the lymphatic system of the arm**

Representative images from a dynamic scan of a pre-BCRL, pre-surgical patient are shown in Figure 1. The radiotracer entered the hand and forearm lymphatics rapidly, in some cases while the injection was still in progress (< 1 min). After the injection was complete, the tracer traversed the distance from the hand depot to the distal border of the cuff (mean distance 30.4 cm) at a velocity of $7.2 \pm 4.3 \text{ cm/min}$ ($n = 26$). Up to 3 (mean 1.6) lymph tracks were imaged in the forearm during pre-surgical LCL and 1–4 (mean 1.7) tracks during the post-surgical LCL. Dermal rerouting of lymph drainage was not evident in any patient, either pre- or post-surgery. Tracer accumulated at the distal border of the congestion cuff until the pressure in the cuff was lowered sufficiently to allow tracer to pass under the cuff (ROI2) and into the axilla (ROI3).

**Comparison of lymphatic function in the pre-BCRL and non-BCRL patients before surgery**

$P_{\text{pump before surgery}}$ Maximal lymphatic pump pressure in the pre-BCRL and non-BCRL groups was compared at the pre-surgical time-point, in order to test the hypothesis that there is an early, constitutive difference in lymphatic pumping in pre-BCRL women. Individual $P_{\text{pump}}$ values are plotted in Figure 3. $P_{\text{pump}}$ in the pre-BCRL group ($40.0 \pm 8.2 \text{ mmHg}$) was on average 73 % higher than $P_{\text{pump}}$ in
the non-BCRL group (23.1 ± 10.8 mmHg, n = 10 and 16). The marked difference in pump pressure was highly significant ($P = 0.0007$, Mann-Whitney test). A bimodal distribution of $P_{\text{pump}}$ has been reported in healthy individuals previously (Modi et al. 2007). It is interesting to note that when the $P_{\text{pump}}$ values of both pre- and non-BCRL groups are pooled, a bimodal distribution is evident (Figure 3, right array). This raises the possibility that two human populations, with high and low $P_{\text{pump}}$ respectively, account for the bimodality in the data of Modi et al. (2007).

**Lymphatic transport dynamics before surgery** There were large differences in the lymphatic transport of $^{99m}$Tc-Nanocoll between the two groups before surgery. These are illustrated in Figure 4A, B, which shows the time-course of activity in each of the three ROIs over the duration of the 70 min scan. Several features distinguished the pre-BCRL from the non-BCRL group. (i) The rate of rise of tracer activity in ROI1 during the initial trapped lymph phase (2.5–10.0 min), $dA/dt_{\text{ROI1}}$, was faster in the pre-BCRL patients that in the non-BCRL patients. (ii) The peak, accumulated activity in ROI1 during the trapped lymph phase was higher in pre-BCRL than non-BCRL patients. (iii) The rate of rise activity in ROI3 after tracer began to be pumped beyond the cuff, $dA/dt_{\text{ROI3}}$, was faster in pre-BCRL than non-BCRL patients. Data analysis (Table 3) showed that $dA/dt_{\text{ROI1}}$ for pre-BCRL patients was approximately double that for non-BCRL patients ($P = 0.052$) and $dA/dt_{\text{ROI3}}$ for pre-BCRL patients was 1.55 times higher than for non-BCRL ($P < 0.0001$). All four measures of lymphatic function were thus consistent with a more active lymphatic pump in pre-BCRL patients compared with non-BCRL patients.

**Comparison of lymphatic function before and soon after surgery**

**Pre-BCRL patients, before versus soon after surgery** The marginal decline in $P_{\text{pump}}$ from 40.0 ± 8.2 mmHg prior to surgery to 36.7 ± 10.0 mmHg at 21 weeks after surgery was not statistically significant ($n = 9$ pairs, $P = 0.50$, paired $t$ test). By contrast, the indices of lymphatic transport in pre-BCRL patients were all significantly lower at 21 weeks post-surgery (Figure 5). During the trapped-lymph phase, $dA/dt_{\text{ROI1}}$ post-surgery fell to 42% of its pre-surgery level ($P = 0.047$, comparison of
slopes) and the maximum count in ROI1 fell to 51 % of the pre-surgery level ($P = 0.025, n = 9$, paired t test). When cuff pressure was lower than lymph pressure, $dA/dt_{ROI3}$ post-surgery fell to 27 % of the pre-surgery level ($P < 0.0001$, comparison of slopes).

**Non-BCRL patients, before versus soon after surgery**  Surgery had much less effect on the lymphatic system in non-BCRL patients than in pre-BCRL patients. $P_{pump}$ was well maintained at 21 weeks after surgery in the non-BCRL patients (pre-surgery 23.1 ± 10.8 mmHg, post-surgery 24.5 ± 11.3 mmHg; $n = 11$ pairs, $P = 0.78$, paired t test). The two lymphatic transport indices for ROI1 were likewise little changed post-surgery (Figure 5); $dA/dt_{ROI1}$ was not reduced (21 % increase not significant, $P = 0.56$) and the maximum count in ROI1 was not reduced (20 % increase not significant, $P = 0.32, n = 11$). $dA/dt_{ROI3}$ declined a little post-surgery, by 28 % ($P = 0.0008$), but this was a relatively small change compared with the 73 % fall in $dA/dt_{ROI3}$ in the pre-BCRL patients (Figure 5).

**Comparison of pre-BCRL versus non-BCRL patients soon after surgery**  After surgery $P_{pump}$ for the pre-BCRL group (36.7 ± 10.0 mmHg, $n = 9$) still exceeded that of the non-BCRLs (24.5 ± 11.3 mmHg, $n = 11$) but the difference was smaller than before surgery (12.1 mmHg; $P = 0.022$, unpaired t test). Because $dA/dt_{ROI1}$ in the pre-BCRL group had been greatly reduced by surgery, it was no longer higher than in the non-BCRL group ($P = 0.45$). The same was true for peak activity in ROI ($P = 0.18$). In the case of $dA/dt_{ROI3}$, the decrease caused by surgery in the pre-BCRL group was so large that the pre-BCRL value after surgery was significantly lower than in the non-BCRL group ($P = 0.0003$).

**Discussion**

The primary aim of this study was to test the hypothesis that women destined to develop arm lymphoedema after breast cancer treatment have constitutive differences in arm lymphatic physiology. The previous evidence underlying the hypothesis was summarised in the Introduction and included raised lymph flows in pre-BCRL women (Stanton et al. 2009a, Bains et al. 2015). The present data, obtained before axillary surgery or radiotherapy, support the hypothesis; the results showed highly significant differences in lymphatic pump pressure and lymph tracer transport.
between those who later developed BCRL and those who did not. Moreover the data indicate a more active, not weaker lymphatic system in the pre-BCRL women. By contrast, once long-standing lymphoedema is present (mean 7.4 years), $P_{\text{pump}}$ is reduced, indicating a weakened lymphatic pump (Modi et al. 2007). The second main finding was that although the breast cancer treatment did not cause a significant, early (21 week) fall in lymphatic pump pressure, it did cause striking reductions in forearm lymph transport rates in the pre-BCRL patients, with relatively little effect in non-BCRL patients. Thus, whereas a marked fall in pump pressure is a later event in lymphoedema development (Modi et al. 2007), our new results reveal evidence of impaired lymphatic transport rates within 5 months of treatment.

**Arm volumes, incidence of BCRL and risk factors**

We applied strict clinical criteria, developed previously, for the diagnosis of BCRL (Stanton et al. 2006). The diagnosis of early, mild BCRL based purely on arm volume measurement can be confounded by other factors, such as weight change, that affect arm size (Miller et al. 2013; Vagenas et al. 2015). The incidence of BCRL (38.5 %) is within the range reported in recent studies; meta-analysis of nine prospective cohort studies gives an incidence of BCRL diagnosed by more than one method of 28.2 % (mean; range 11.8–53.5 %) (DiSipio et al. 2013). Nevertheless the question arises as to whether 2 years is sufficient follow-up; DiSipio et al. reported that the incidence of BCRL increases up to 2 years after diagnosis or surgery (24 studies), whereas two questionnaire studies reported an increase beyond 2 years (Paskett et al. 2007; Norman et al. 2009). Paskett et al. (2007) reported that whereas the estimated prevalence (repeated episodes or continuous swelling) was 23–29 % for any assessment interval, the incidence of episodic swelling increased beyond 2 years (48 % at 2 years, 54 % at 3 years). Norman et al. (2009) reported an increase in cumulative incidence from 30 % at 2 years to 41 % at 5 years. Data from objective and subjective measures of lymphoedema rates can show considerable discordance (McLaughlin et al. 2008). In the present study all cases of BCRL developed within 12 months of surgery and were diagnosed according to
strict clinical criteria with confirmation by a Lymphoedema Practitioner; no cases arose in the second year.

The study was not designed to identify risk factors for BCRL but nevertheless showed that BCRL incidence was 3.3-fold higher in the patients receiving axillary clearance than SLNB. Axillary clearance surgery is a known risk factor for BCRL (McLaughlin et al. 2008). The number of lymph nodes removed and the number positive for cancer were very similar for each group. Higher BMI is an established risk factor for BCRL (McLaughlin et al. 2008; DiSipio et al. 2013), and was significantly higher in the pre-BCRL than the non-BCRL group, by 20%; consistent with this was the 22% greater ipsilateral and contralateral arm volumes in the pre-BCRL group, indicating that arm volume is a related risk factor. It is currently unknown whether pre-surgical arm volume is an independent risk factor for BCRL.

Constitutively raised lymphatic function in the BCRL-destined group

The results in Figure 3 showed that collector lymphatics distal to the cuff in pre-BCRL patients were able to pump lymph to a higher maximal pressure than those in non-BCRL patients. Moreover the faster $dA/dt_{ROI1}$ and higher maximum activity in ROI1 of pre-BCRL patients indicated a more rapid transport of lymph from the hand depot into the collector lymphatics of the forearm; and the faster $dA/dt_{ROI3}$ indicated a more rapid transport of lymph into the axilla after $P_{pump}$ exceeded $P_{cuff}$. These findings combine to support the hypothesis of constitutively enhanced lymphatic function in lymphoedema-destined women.

LaPlace’s law and the raised $P_{pump}$

What mechanism(s) might underlie the pre-operative difference in $P_{pump}$ between the two groups? LaPlace’s law states that the pressure $P$ generated by tension $T$ in a thin-walled tube of radius $R$ equals $T/R$. This raises the question of whether the lymphatic smooth muscle contractile force $T$ is
greater in pre-BCRL than non-BCRL patients, or whether the collector vessel radius R is smaller. Although we have no direct evidence on this point, increased lymphatic smooth muscle activity (force and/or frequency of contraction) seems the more likely explanation, because the raised $dA/dt_{ROI1}$ and maximum ROI1 activity indicate an increased rate of lymph transport (flow) - a finding broadly in keeping with the raised lymphatic drainage rate constant $k$ in pre-BCRL patients reported by Bains et al. (2015). Limited evidence against a smaller vessel radius comes from the observation of wider initial lymphatics in the contralateral arm of women with BCRL when compared with the arms of non-BCRL breast cancer patients (Mellor et al. 2000); dilated lymphatic collectors imaged in BCRL arms (median duration 24 months) by magnetic resonance lymphangiography (Liu & Wang 2014), and (in early lymphoedema) collector vessel ectasia observed in histological sections from secondary lymphoedema of the leg (Mihara et al. 2012). Studies employing X-ray lymphangiography have demonstrated dilated lymphatic collectors in BCRL and also following axillary dissection but in the absence of arm swelling (Abe 1976; Clodius 1977; Mortimer 2003).

**Possible causes of raised $P_{pump}$**

Assuming that the high $P_{pump}$, along with the other indices, indicates enhanced lymphatic contractile force in pre-BCRL patients, what might cause this? A possible factor is the physiological adaptation of lymphatic smooth muscle, either by hypertrophy or raised contractility, to the chronically high fluid load (preload) in pre-BCRL patients. A high fluid load is indicated by the raised lymphatic removal rate constant $k$ in pre-BCRL patients (Stanton et al. 2009a; Bains et al. 2015). This is supported by the recent report of raised capillary filtration capacity in both forearms of BCRL patients relative to the arms of matched breast cancer patients without BCRL (Jensen et al. 2015), although equivalent data from pre-BCRL patients is lacking. Also, the findings were based on short (3–4 min) congestions and are contrary to results based on the classic, longer congestion method (Stanton et al. 1999a)

Physiological adaptation of lymphatic smooth muscle to a constitutively high resistance axillary node pathway (afterload) is an additional, speculative possibility. In single lymphangions from the rat...
mesentery, in which input and output pressures were controlled, elevated afterload triggered a
time-dependent increase in lymphatic contractility, modulated by change in preload (Davis et al.
2012; Scallan et al. 2012). Genetic factors have also been related to BCRL susceptibility (Finegold et

Hypothesis linking raised $P_{\text{pump}}$ in pre-BCRL and subnormal $P_{\text{pump}}$ in established BCRL;
potential key role of lymphatic collector vessel smooth muscle work

Not only is $P_{\text{pump}}$ in established BCRL lower than the $P_{\text{pump}}$ of normal, healthy subjects but also the
severity of the swelling correlates negatively with $P_{\text{pump}}$ indicating that partial failure (weakening) of
the collector lymphatic pump over the years contributes to the pathogenesis of BCRL (Modi et al.
2007). The new findings here raise the question ‘why would women with intrinsically stronger
lymphatic pumps than others be more prone to pump failure after surgery?’. The high transport
rates in the pre-BCRL patients (Figure 4A), along with their high lymph flows (Stanton et al. 2009a;
Bains et al. 2015) offers a rational, albeit speculative explanation, as follows. The stroke work of a
contracting lymphangion equals the volume of lymph ejected ($\Delta V$) x active pressure increase ($\Delta P$).
Lymphangion work rate = ($\Delta V/t$) x $\Delta P$, i.e. lymph flow x $\Delta P$. The high lymph flow in the pre-BCRL
population thus imposes a chronically high work rate on the lymphatic smooth muscle. We propose
that this may be close to the maximum chronically sustainable work rate. Surgical removal of lymph
nodes raises the outflow resistance to lymph in sheep (Kim et al. 2003). If the same is true in humans
(perhaps exacerbated by the radiotherapy), then the previous level of lymph flow can only be
maintained by a rise in $\Delta P$ (afterload), further increasing the work rate ($\Delta V/t$) x $\Delta P$. Over a long
period the increased work may cause a partial failure of lymphangion force generation (intrinsic
smooth muscle failure) and/or vessel dilatation; the latter reduces the conversion of wall tension
into pressure (LaPlace’s law) and may impair lymphatic valve competence. The ‘lymphatic work’
hypothesis has as its analogue in the chronic failure of a dilated heart resulting from a high preload
and raised afterload (hypertension). The majority subset of women that avoid BCRL may do so partly
because their lymphatic system normally operates at a lower preload, leaving them with a greater reserve pump capacity.

Additional pathogenic factors may also come into play after pump failure, for instance degenerative histopathological processes. A recent study described progressive histopathological changes in collector lymphatic vessels harvested from patients affected by lower limb lymphoedema after the surgical removal of lymph nodes for gynaecological cancers (Mihara et al. 2012). With increasing disease progression and severity the characteristics of the normal collector lymphatics were lost. In early stage lymphoedema most lymphatic vessels were normal or showed ectasia, which by LaPlace mechanics will tend to reduce pump pressure, even if contractile force were unimpaired. A minority of vessels had a thickened wall and narrower lumen. Sclerosis was commoner and ectasia less common with increasing severity of lymphoedema. Sclerosis of arm collector lymphatics would further impair lymphatic pumping and worsen the swelling. Severe sclerosis was observed in superficial lymphatics in the arm of a patient with refractory BCRL of 18 years’ duration (Yamamoto et al. 2015). Inflammatory triggers for the above might be aggravated by the association between obesity and an abnormal inflammatory response, perhaps involving macrophage migration (Mehrara & Greene 2014; Ghanta et al. 2015).

Impaired lymphatic transport dynamics in pre-BCRL patients following axillary surgery

Transport into forearm from hand depot  The slight fall in pre-BCRL $P_{pump}$ at 4.8 months post-surgery was not statistically significant, yet at the same time point the lymphatic transport into the pre-BCRL forearm was roughly halved; $dA/dt_{ROI1}$ fell by 58 %, maximum ROI1 activity by 48 %. The contrast between the large fall in hand-to-forearm transport rate and relatively unchanged $P_{pump}$ could be explained if the more distal contractile lymphatics (i.e. between hand depot and forearm) are weaker than proximal ones close to the cuff, which were still capable of generating a high $P_{pump}$. The data in Figure 4A versus C may thus be an early clue that distal lymphatics are the weakest link in the chain and begin to fail before more proximal lymphatics.
Transport into the axillary-supraclavicular region  The fall in dA/dtROI3 after surgery was much more pronounced in pre-BCRL (73 % reduction) than non-BCRL patients (28 % reduction), despite the relatively well maintained PPump in the pre-BCRL group. The interpretation of dA/dtROI3 after surgery is complicated by the fact that some lymph nodes have been removed from this ROI. The reduced ROI3 transport in both pre-BCRL and non-BCRL groups may be caused partly by increased lymph drainage resistance after axillary surgery; and the greater reduction in the pre-BCRL group may be caused by the slowed delivery of 99mTc-Nanocoll by the markedly impaired forearm transport vessels (transport in series). Other possibilities include more extensive axillary lymphatic trauma from the cancer surgery or radiotherapy (Whelan et al. 2015), which the study was not designed to explore.

Limitations of present study

To test further the hypothesis of a constitutively different PPump in pre-BRL patients, measurements of PPump and transport dynamics in both arms would have been valuable. This was not feasible in practice because of the demands it would have placed on the patients shortly before their cancer surgery. PPump data at 2 years, after lymphoedema was established, would be useful, to check on the decline in function known to occur over several years (Modi et al. 2007). Many aspects of the proposed working hypothesis remain speculative and call for further work. For example, we are not aware of any comparative study of lymphatic smooth muscle structure and function in the distal collectors versus proximal collectors of the human arm; and apparently only one study (in sheep) has examined the effect of node removal on the hydraulic resistance to lymph transport out of a limb.

Conclusions

A disease model for BCRL emerges from the body of evidence presented here. BCRL-destined women have constitutively higher lymph loads and higher lymphatic pumping pressures. Breast cancer treatment per se has relatively little effect on maximum lymphatic pressure generation initially but nevertheless impairs lymph transport in those subsequently developing BCRL. Therefore
axillary lymphatic obstruction is not the sole explanation for BCRL. In BCRL-destined women the lymphatic contractile work rate is already high and if this is raised further by increased resistance to axillary drainage following cancer treatment and/or further increase in lymph load, the lymphatics are gradually tipped into chronic failure. This is analogous to high preload and high afterload cardiac failure in systemic hypertension. Reduced lymphatic pump activity then leads to overt clinical oedema. Pharmacological interventions aimed at reducing lymphatic pre- and/or afterload should, in theory, prevent BCRL if introduced at an earlier enough stage in patients identified as being at risk. Axillary lymphatic damage from surgery appears to compromise lymph drainage in those women constitutively predisposed but not in those women with constitutively lower lymphatic pressures and transport. This suggests, in theory, that women with higher lymphatic preloads could be identified as an ‘at risk’ group prior to cancer treatment.

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References


### Table 1. Surgical and pathological information.

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Pre-BCRL group - patients who later developed breast cancer related lymphoedema; non-BCRL group - patients who did not develop BCRL. ANC, axillary clearance surgery; DCIS, ductal carcinoma in situ; ER, oestrogen receptor status (positive/negative); ICC, invasive cribriform carcinoma; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LN, lymph node, with the number positive for cancer in brackets; M, mastectomy; NR, not recorded; SLNB, sentinel lymph node biopsy; WLE, wide local excision; †died; *high-grade DCIS. All patients received radiotherapy.
Table 2. Ipsilateral and contralateral arm volumes in the pre-breast cancer related lymphoedema (pre-BCRL) patients and the non-BCRL patients, before and soon after axillary surgery (number of patients in brackets; means ± S.E.M.).

|                     | Pre-surgical arm volumes (ml) | Post-surgical arm volumes (ml) |  |  |
|---------------------|-------------------------------|-------------------------------|  |  |
|                     | Pre-BCRL (10)                 | Non-BCRL (16)                | $P^1$ | Pre-BCRL (10) | Non-BCRL (11) | $P^1$ |
| Ipsilateral         | 2312 ± 205                    | 1894 ± 103                   | 0.055 | 2369 ± 226    | 2007 ± 142    | 0.18 |
| Contralateral       | 2321 ± 192                    | 1910 ± 107                   | 0.054 | 2227 ± 202    | 2049 ± 150    | 0.48 |
| $P^2$               | 0.88                          | 0.59                         | 0.040 | 0.34          |              |      |

$P^1$, comparison of the pre-BCRL and non-BCRL groups (unpaired t test); $P^2$, comparison of the ipsilateral and contralateral arms (paired t test). In both groups, neither the ipsilateral nor the contralateral arm changed significantly in volume after surgery.
Table 3. Summary of measurements of lymphatic function in the pre-breast cancer related lymphoedema (pre-BCRL) patients and the non-BCRL patients, before and after axillary surgery (number of patients in brackets; means ± S.E.M.).

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<td>$P_{\text{pump}}$ (mmHg)</td>
<td>40.0 ± 2.6</td>
<td>23.1 ± 2.7</td>
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<tr>
<td>$dA/dt_{\text{ROI1}}$ (min$^{-1}$)</td>
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<td>1.07 ± 0.35</td>
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<tr>
<td>$dA/dt_{\text{ROI3}}$ (min$^{-1}$)</td>
<td>0.62 ± 0.02</td>
<td>0.40 ± 0.02</td>
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$P_{\text{pump}}$, lymphatic pump pressure. ROI, region of interest. $dA/dt_{\text{ROI1}}$, rate of rise of fractional count in ROI1 ($x\ 10^3$) determined by linear regression analysis of mean fractional counts over 2.5–10.0 min (both groups) and $dA/dt_{\text{ROI3}}$, rate of rise of fractional count in ROI3 ($x\ 10^3$) by linear regression analysis of mean fractional counts over 35–55 min (pre-BCRL) or 42.5–62.5 min (non-BCRL) (± S.E.).

Peak count$_{\text{ROI1}}$, maximum fractional count in ROI1 ($x\ 10^3$). $P$, comparisons of the pre-BCRL group with the non-BCRL group, before or after surgery - $^1$Mann-Whitney test; comparison of regression slopes for $dA/dt$; unpaired t test for the other comparisons. See text and Figure 5 for pre-surgery versus post-surgery statistical comparisons.
Figure 1. A series of images from lymphatic congestion lymphoscintigraphy performed on the ipsilateral arm of a breast cancer patient who later developed BCRL. The scan was performed prior to axillary surgery. The regions of interest (ROI1: forearm, ROI2: subcuff, ROI3: axilla) are drawn in and the number of minutes elapsed since the injection are shown. The lymphatic tracer traversed the forearm in 2.5 min but is then held back by the congestion cuff at 60 mmHg (0–10 min), and at 50 mmHg (10–20 min, not shown), until the cuff pressure is lowered to 40 mmHg (20–30 min). The pump pressure ($P_{\text{pump}}$, 40 mmHg here) is the cuff pressure at which the intra-lymphatic pressure overcame the surrounding occluding pressure, allowing tracer to pass into the axillary region ROI3.
Figure 2. Change in ipsilateral and contralateral arm volume following axillary surgery for the pre-breast cancer-related lymphoedema (pre-BCRL) and the non-BCRL groups (pre-surgical volume = 100 %, the post-surgical visit was at 21 weeks; means ± S.E.M., n).
Figure 3. Maximum pump pressure ($P_{\text{pump}}$) in collector lymphatics of the ipsilateral arm in breast cancer patients prior to axillary surgery. The 10 pre-breast cancer-related lymphoedema (pre-BCRL) and 16 non-BCRL patients are shown separately (left and centre arrays). $P_{\text{pump}}$ for the pooled group of the 26 patients (right array) shows a bimodal distribution. The horizontal lines indicate the means (40.0, 23.1 and 29.6 mmHg, respectively). $P_{\text{pump}}$ was highly significantly greater in the pre-BCRL patients than the non-BCRL patients ($P = 0.0007$, $n = 10$ and 16, Mann-Whitney test).
Figure 4. Radioactive counts (expressed as a fraction of injected activity) recorded in each region of interest (ROI) in the ipsilateral arm during lymphatic congestion lymphoscintigraphy (means ± S.E.M.) plotted against time since hand depot injection. A. Pre-breast cancer-related lymphoedema (pre-BCRL) patients before surgery. B. Non-BCRL patients before surgery. C. Pre-BCRL patients 21 weeks after surgery. D. Non-BCRL patients 21 weeks after surgery. ROI1 - forearm; ROI2 - subcuff; ROI3 - axilla. The cuff pressure ($P_{cuff}$) in mmHg is shown above each time interval. Before surgery (frame A versus B), the pre-BCRL fractional counts in ROI1 rose faster than non-BCRL counts over 2.5–10 min (greater $dA/dt_{ROI1}$, see text) and reached a higher peak. Also, after $P_{cuff}$ was lowered below $P_{pump}$ the pre-BCRL counts in ROI3 rose faster than non-BCRL counts (greater $dA/dt_{ROI3}$). At 21 weeks after surgery, transport dynamics in the pre-BCRL patients were substantially reduced.
compared with prior to surgery (frame A versus C), whereas transport dynamics in the non-BCRL patients were not reduced after surgery (frame B versus D).
Figure 5. Effect of surgery on $^{99m}$Tc-Nanocoll accumulation in the ipsilateral arm at 21 weeks in pre-breast cancer-related lymphoedema (pre-BCRL) and non-BCRL patients. A. Maximum count in region of interest (ROI1) (forearm; n = 9 pairs for pre-BCRL, 11 pairs for non-BCRL). B. Rate of rise of activity (dA/dt) in ROI1. C. Rate of rise of activity in ROI3 (axillary-supraclavicular region). Mean ± S.E.M., all values x 10$^3$. dA/dt was quantified by linear regression analysis of plots of mean fractional counts versus time. dA/dt$_{ROI1}$ describes transport rate from depot into forearm during the initial trapped lymph phase (2.5–10.0 min). dA/dt$_{ROI3}$ describes transport rate beyond the cuff over 20 min after cuff pressure was reduced below lymphatic pump pressure. P, comparisons of pre-surgery with post-surgery - paired t test (A); comparison of regression slopes (B, C). Surgery greatly reduced arm lymphatic transport in pre-BCRLs, with less effect on non-BCRLs.