

Manuscript title:

Prevalence and prognostic significance of secondary lymphedema following breast cancer

Running head:

Prognostic significance of lymphedema following breast cancer

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Abstract

Background: The adverse consequences of lymphedema following breast cancer in relation to physical function and quality of life are clear; however, its potential relationship with survival has not been investigated. The purposes were to determine the prevalence of lymphedema and associated upper-body symptoms at six years following breast cancer and to examine the prognostic significance of lymphedema with respect to overall 6-year survival (OS).

Methods and Results: A population-based sample of Australian women (n=287) diagnosed with invasive, unilateral breast cancer was followed for a median of 6.6 years and prospectively assessed for lymphedema (using bioimpedance spectroscopy [BIS], sum of arm circumferences [SOAC] and self-reported arm swelling), a range of upper-body symptoms and vital status. OS was measured from date of diagnosis to date of death or last follow-up. Kaplan-Meier methods were used to calculate OS and Cox proportional hazards models quantified the risk associated with lymphedema. Approximately 45% of women had reported at least one moderate to extreme symptom at 6.6 years post-diagnosis, while 34% had shown clinical evidence of lymphedema and 48% reported arm swelling at least once since baseline assessment. A total of 27 (9.4%) women died during the follow-up period, and lymphedema, diagnosed by BIS or SOAC between 6-18 months post-diagnosis, predicted mortality (BIS: HR=2.5; 95% CI: 0.9, 6.8, p=0.08; SOAC: 3.0; 95% CI: 1.1, 8.7, p=0.04). There was no association (HR=1.2; 95% CI: 0.5, 2.6, p=0.68) between self-reported arm swelling and OS.

Conclusions: These findings suggest that lymphedema may influence survival following breast cancer treatment and warrant further investigation in other cancer cohorts and explication of a potential underlying biology.

Condensed abstract:

A population-based sample of women diagnosed with invasive, unilateral breast cancer was prospectively assessed for lymphedema, upper-body symptoms and vital status for a median of 6.6 years, to evaluate the relationship between lymphedema and survival. Approximately 45% of women had at least one upper-body symptom at 6.6 years post-diagnosis, while 34% had clinical evidence of lymphedema. A total of 27 (9.4%) women died during the follow-up period, and clinically evident lymphedema predicted mortality (HR=3.0; 95% CI: 1.1, 8.7, $p=0.04$). These findings suggest that lymphedema may influence breast cancer survival and warrant further investigation and explication of a potential underlying biology.

Introduction

In Westernised countries, secondary lymphedema (characterised by regional swelling, typically of an extremity) presents mostly following treatment for cancer¹, in particular breast, genitourinary and gynaecological cancers and melanoma. While 5-year overall survival rates following such cancers generally exceed 80%², treatment-associated morbidity is common and persists well beyond the active treatment period³⁻⁴. Specifically, lymphedema develops in approximately one-fifth of these cancer survivors⁵, with incidence increasing over time.

Lymphedema presents when the demand for lymphatic drainage exceeds the capacity of lymphatic circulation⁶. This may occur as a consequence of tumor-induced neo-lymphangiogenesis, altering the original flow architecture of draining lymph nodes⁷. Such changes in lymph flow also may be responsible for changes in the immunological profile observed in draining lymph nodes of women with breast cancer, which have been linked to survival⁸. Moreover, areas of lymph congestion (i.e., manifesting lymphedema) may be associated with altered immune function from 'sluggish' migration of immune cells

(including lymphocytes, monocytes, and dendritic cells), limiting the capacity of the immune system to eliminate cancer antigens. In particular, defects on dendritic cells, key cells in the presentation of antigen, have been well characterised in patients with breast cancer and may be responsible for the late appearance of distal disease following mastectomy⁹. Indeed, local immune deficiency derived from lymphedema may explain the increased risk of infection observed among those with lymphedema¹⁰. Furthermore, metastases (and by inference, survival) may then be a further consequence of immune deficiency and are clearly a prime concern for cancer patients and their clinicians. Yet, with the exception of the work regarding Stewart-Treves syndrome¹¹, the relationship between lymphedema following cancer and survival has, to date, not been evaluated.

As a follow-up to the Pulling Through Study, our longitudinal, population-based, cohort study designed to track the physical and psychosocial concerns, including lymphedema, of women six to 18 months after breast cancer diagnosis⁴, we recontacted women approximately five years later. The purposes of this follow-up work were: 1) to determine the prevalence of upper-body symptoms and lymphedema at six years following breast cancer; 2) to estimate the 6-year cumulative burden of lymphedema; and 3) to examine the prognostic significance of lymphedema with respect to overall 6- year survival (OS).

Methods and Materials

Study design and sample recruitment of the original 'Pulling Through Study'

Following ethical approval, 511 women diagnosed in 2002 with a first, primary, invasive, unilateral breast cancer, aged 74 years or younger, and residing within 100 kilometres (*i.e.*, 62 miles) of Brisbane, Australia, were randomly selected from the Queensland Cancer Registry. Younger women (<50 years) were over-sampled 1.3-fold to ensure adequate numbers for specific age-group analyses. Thirty-five women were subsequently deemed ineligible. Doctor

consent was obtained for 88% (n=417), and of these, informed consent was obtained for 68% (287 women or 60% of those potentially eligible). Participation involved a clinical assessment and/or completion of a self-administered questionnaire every three months between six and 18 months post-diagnosis.

At baseline assessment (6 months post-diagnosis), study participants completed a mailed, self-administered questionnaire on a range of demographic, treatment and general health characteristics. Lymphedema status was evaluated in the clinic using two objective measures, bioimpedance spectroscopy (BIS) and sum of arm circumferences (SOAC), and in the questionnaire by subjective account of self-reported arm swelling. Tumour characteristics were abstracted from histopathology reports at the Queensland Cancer Registry. Full details of the study design, outcome measures and subsequent results have been published previously⁴. Of relevance to this paper, approximately one-third of women participated on a questionnaire-only basis; hence they lack objective assessments of lymphedema and are omitted from survival analyses related to those outcomes.

Design and sample recruitment of the 'Pulling Through Study: A follow up'

The follow-up study reported here commenced approximately six years following the date of breast cancer diagnosis for those in the original 'Pulling Through Study'. Of the 287 original participants, 11 withdrew from the study and were therefore not recontacted. The records of the remaining 276 women were cross-referenced with the mortality database at the Queensland Cancer Registry in August, 2008, to determine vital status, including date and cause of death. The search indicated 16 women were deceased, leaving 260 women to be recontacted. Address details were confirmed through a search of the electronic White Pages, and a change of address search was carried out through Australia Post. When an address could not be confirmed from these sources, the last postal address recorded in our files was

used.

Institutional ethical approval was sought and approved for all aspects of recruitment and study implementation. Participants were followed until 15 April 2009 when a second search of the mortality registry was undertaken to determine vital status of all 287 original participants of the Pulling Through Study; therefore, irrespective of participation in the 6-year follow-up study, all observations not previously ended were censored at this date.

Data collection

The questionnaire was designed to collect information on the presence of upper-body symptoms and self-reported arm swelling, using the same questions as in the original study described elsewhere^{4,12}. In summary, using items from the Functional Assessment of Cancer Therapy – Breast (FACT-B+4) questionnaire¹³, as well as the Disability of the Arm, Shoulder and Hand (DASH) questionnaire¹⁴, information was collected on the presence of stiffness, pain, weakness, poor range of motion, tingling and numbness on the affected side, and severity was rated using a 5-point Likert scale. Women were also asked whether they had experienced arm swelling in the past 12 months.

For our objective assessment of lymphedema, BIS measurements were taken on each arm using a SEAC SFB7 monitor (SEAC Australia, Impedimed, Brisbane, Australia). The impedance of the extracellular fluid for each limb was measured using the manufacturer's software, to compute the ratio of impedance values, comparing the treated and untreated sides. A participant was classified as having lymphedema when the impedance ratio was more than three standard deviations above normative data, with the side of dominance taken into account (also coincides with an L-DEX score of greater than 10)¹⁵⁻¹⁶. During the original Pulling Through Study, lymphedema also was measured using the sum of arm circumference

method and lymphedema was diagnosed when the difference of the sums was greater than 5 cm¹⁷.

Statistical analysis

Proportions of those reporting moderate to extreme upper-body symptoms at six years post-diagnosis were calculated and compared with 6-month post-diagnosis data. Point prevalence of lymphedema according to BIS and self-report were also calculated at six years post-diagnosis. Cumulative burden, representing the proportion of the sample that experienced lymphedema at any stage from six months to six years after diagnosis, was calculated using data collected via self-report and BIS; since circumferences were not measured at the 6-year follow-up, lymphedema status based on SOAC is not available.

The absolute difference in mortality between the two groups was calculated ($[\text{number of deaths in lymphedema group}/\text{number in lymphedema group}] - [\text{number of deaths in no lymphedema group}/\text{number in no lymphedema group}]$). Mean survival times were calculated using the Kaplan-Meier method and differences between groups (lymphedema status: no/yes) were assessed using the Log-rank test. Cox proportional hazards regression models were utilised to estimate the hazard ratio (HR) and 95% confidence intervals (CIs) for the association between lymphedema status (as defined by cumulative burden between 6 and 18 months post-diagnosis) and overall 6-year survival (OS). Due to limited statistical power, bivariate model estimates were adjusted, one at a time, for known markers of breast cancer prognosis. All analyses were performed using SPSS version 16.

Results

Characteristics of the study participants

As indicated earlier, our first death search had shown that sixteen of the original cohort had

died prior to this follow-up study, leaving 260 potential participants in the follow-up study. Of these, 22 (8.5%) were lost to follow-up, 36 (13.8%) refused to participate and 7 women had passed away. The remaining 195 (75%) women provided consent, and of these, 94% (n=183) returned the questionnaires and 85% (n=166) had BIS measures taken. Four additional women died between time of the 6-year follow-up assessment (two participants and two previously lost-to-follow-up) and the final death search, making a total of 27 deaths from the original cohort of 287.

Demographic and disease characteristics of the 158 women with complete outcome data (BIS and self-reported assessment of lymphedema) from the follow-up study were similar to the original Pulling Through Study cohort (n=287) (Table 1). The additional 25 women with questionnaire information only (n=183) did not alter this profile (data not shown). Similarly, the characteristics of the 190 women with sufficient data to determine cumulative burden of lymphedema between 6-18 months post-diagnosis, and therefore included in the survival analyses, were comparable to the initial research sample (Table 1). Of note, the original cohort was shown to be representative of the wider Queensland breast cancer population⁴.

Median follow-up of the participants was 80 months (range 10-87 months) or 6.6 years. There were a total of 27 deaths; metastatic breast cancer was the recorded cause of death for 23 of the women, one death was due to cancer at a site other than the breast, and three women died due to non-cancer causes.

Upper-body symptoms and lymphedema

Prevalence of various upper-body symptoms at the 6-year follow-up ranged from 7.4-15.6%, with 19% of women reporting two or more moderate to severe symptoms (Table 2).

Generally, the prevalence of these symptoms declined between the baseline assessment at 6

months following breast cancer diagnosis and the follow-up study. This was, in part, due to somewhat lower prevalences reported at baseline by the participants in the follow-up study and, in part, due to modest but real declines in the proportions of women reporting symptoms. Numbness was the single symptom reported most frequently at both time points (29.2% at 6 months and 15.6% at 6 years post-diagnosis), despite a reduction of almost 50% in prevalence.

Using BIS, point prevalence of lymphedema at the 6-year follow-up was 6.5% (95% CI: 3.6, 10.6), by which time 34% (95% CI: 28.3, 40.2) of women showed evidence of the condition at one or more testing phases. Two new cases of lymphedema were identified for the first time at the last assessment, for an incidence rate of 1.2% (95% CI: 0.3, 4.3) between 18 months and 6 years following breast cancer diagnosis.

The prevalence of self-reported arm swelling at the 6-year follow-up was 22% (95% CI: 16.0, 29.6), and the incidence between 18 months and 6 years post-diagnosis was 6% (95% CI: 3.4, 10.4) based on 11 women not previously reporting arm swelling during the original study. Consequently, 48.2% (95% CI: 43.0, 53.5) of the sample experienced arm swelling at some point during the 6-year follow-up period.

Lymphedema and overall survival

The 6-year absolute mortality was 12.9% and 14.6% among those with lymphedema diagnosed by BIS or SOAC, respectively, compared to 5.5% and 5.2% for those without lymphedema based on BIS or SOAC. Absolute differences between the two groups therefore were 7-9%, depending on the measure used. Those with evidence of lymphedema between 6-18 months following diagnosis survived for a mean of 79-82 months compared to 85 months among those without clinical evidence of the condition (BIS, $p=0.07$; SOAC, $p=0.03$).

Kaplan-Meier curves indicate that survival diverged early on between the two groups; those with lymphedema showed poorer survival throughout the follow-up period, irrespective of BIS or SOAC as the mode of diagnosis (Figure 1).

Unadjusted Cox proportional hazards models estimated that the risk of death was increased 2-3 fold among women with objective evidence of lymphedema between 6-18 months post-diagnosis (Table 3; BIS HR=2.5, 95% CI: 0.9, 6.8; p=0.08; SOAC HR=3.0, 95% CI: 1.1, 8.7; p=0.04). The magnitude of these associations remained similar when adjusted separately for established breast cancer prognostic factors, including age, socioeconomic status, body mass index, presence of co-morbidities, tumour size, tumour grade, extent of chest wall surgery, extent of axillary surgery, number of positive nodes, estrogen-receptor status, progesterone-receptor status, and receipt of hormone therapy, chemotherapy or radiotherapy. With these separate adjustments, the HR for lymphedema diagnosed by BIS ranged between 2.1 to 3.1 (p-values ranged from 0.03-0.10). When lymphedema status was diagnosed by SOAC, adjustment for other prognostic factors led to HRs between 3.0 and 3.7 (p-values<0.05 for all). Despite small sample size and limited statistical power, two further, multivariable models were conducted, adjusting concurrently for age, socioeconomic status, type of surgery, receipt of radiotherapy, chemotherapy and/or hormone therapy and number of positive nodes. Results remained similar to those observed in the bivariate analysis (BIS HR=2.8, 95% CI: 0.9, 8.5; p=0.06; SOAC HR=5.1, 95% CI: 1.5, 17.8; p=0.01), although as expected, confidence intervals widened. The average survival times for women who did or did not self-report arm swelling between 6-18 months were similar, and the HR was not significantly different than 1.0 (Table 3).

Conclusions

Upper-body morbidity following breast cancer is common, may persist well beyond the active

treatment period and can present years following breast cancer. Lymphedema is considered the most feared upper-body concern. Results from this prospective, longitudinal, population-based study demonstrate that despite declines in the number of women experiencing upper-body symptoms, nearly one in two women still report at least one moderate to extreme upper-body symptom at 6 years following breast cancer diagnosis, and of these women, roughly 40% report multiple upper-body symptoms. Clinical evidence of lymphedema was observed in fewer women (6.5%) at the 6-year follow-up, but still 2 new cases were identified at this time (not previously diagnosed during our 6-18 month surveillance period). Recognizing the intermittent nature of lymphedema in many women, the cumulative incidence of 34% remains noteworthy. These results are in line with those reported by other prospective¹⁸ and retrospective¹⁹ studies, and demonstrate that despite advances in breast cancer detection and treatment over the past decade, such as sentinel node biopsy, upper-body morbidity during the survivorship period remains a concern.

The adverse consequences of having lymphedema are well-documented, with presence of the condition having a profound effect on all aspects of quality of life^{3,20}. However, this study is the first, to our knowledge, to evaluate the potential impact of lymphedema on survival. Results provide positive, albeit preliminary, evidence that lymphedema may be an important prognostic factor, associated with a 2-3-fold increased risk of death during the 6 years following breast cancer diagnosis. Further, the observed relationship between lymphedema and poorer survival may be independent of other recognized indicators of disease severity and outcome, because it was not explained away by adjustment for factors such as original disease status, extensiveness of treatment, or other personal characteristics. Whether or not the relationship reflects a direct effect of lymphedema on mortality among women with breast cancer, the possibility of lymphedema as a marker of undetected malignant disease, and/or that women predisposed to develop lymphedema are more susceptible to further immune-

related changes that adversely impact their chances of survival, cannot be determined from these results.

Lack of prior investigation into the relationship between lymphedema and survival is somewhat surprising given lymphedema's underlying pathology as a lymphostatic disease associated with immune deficiency. A timely immune response is necessary for effective defence against antigens, including tumour cells, which if left unchecked, may lead to metastasis of the existing cancer, or over time, subsequent development of a new primary. Critical to this process, dendritic cells come into contact with a tumour antigen, migrate to the lymph node where they act as antigen-presenting cells, and activate lymphocytes which, in turn, can destroy the tumour cells²¹. However, antigen-loaded dendritic cells have limited lifespans and need to encounter tumour-specific T-cells before they undergo apoptosis. Consequently, a lymphostatic disease, such as lymphedema, may disrupt the speed with which a dendritic cell can reach a lymph node and activate T lymphocytes. This simplistic description of one aspect of the immune response provides a biological mechanism by which the presence of lymphedema could independently impair survival. However, this scenario requires that the immune response is maintained by the presence of residual tumour following surgery, which is presumably not the case for most patients, particularly following chemotherapy and/or radiation. Alternatively, this model may explain the failure of immunosurveillance in the case of new primary lesions appearing in the surgically intervened breast.

It is also plausible that genetic predisposition and/or undetected primary tumours or disease progression could alter the architecture, immune composition and function of existing lymph nodes, which may contribute to the development of lymphoedema as well as influence survival. Indeed, altered immune profiles, such as the increased presence of CD4 helper lymphocytes, CD1 dendritic cells and T-regulatory lymphocytes, have been identified in

draining lymph nodes of patients with breast cancer^{8, 22}, and a critical role for the immune function of sentinel lymph node(s) has been established in breast cancer²³. Such changes in cell composition would have radical consequences for the capacity of the immune system to control disease progression and hence survival. Additionally, other variations in the architecture of the lymph nodes, such as substantial changes to the channels of lymph flow inside the lymph node, whether inherited or tumour-instigated, could make selected women more susceptible to lymphedema and subsequently cancer-related death. At least some of these are likely the result of major lymphangiogenesis regulation driven by processes still poorly understood and that probably involve molecules such as VEGFR⁷. Setiadi et al have recently demonstrated substantial architectural and functional differences between axillary tumour-draining lymph nodes compared with healthy lymph nodes²⁴, providing further evidence for a potential explanation of our findings. Clearly more research into the biology potentially underlying a relationship between lymphedema and breast cancer mortality is needed and now can be more readily studied with recently developed techniques²⁴.

In contrast to findings derived from clinical assessment and objective measurement of lymphedema, there was no relationship between lymphedema based on self-reported arm swelling and survival. This may be because there is, in fact, no relationship. Alternatively, it may be that self-reported arm swelling is not an accurate measure of lymphedema. Previous work using this same dataset demonstrated that self-report of upper-body symptoms, including swelling, is common among those with and those without objective evidence of lymphedema³. Further, while presence of self-reported symptoms is associated with lymphedema, its potential as a diagnostic tool for lymphedema is limited¹².

The strengths of this work come from the study's longitudinal and prospective design as well as its assessment of a population-based sample of women generally representative of the

wider breast cancer population. Additionally, objective as well as self-reported measures were used to determine lymphedema status, including traditional methods as well as one novel approach. The objective assessment by BIS is non-invasive, relatively quick and easy, and directly measures changes in extracellular fluid levels, potentially providing a more accurate and sensitive detection method for diagnosing early lymphedema in both research and clinical settings.

The main limitation of this work relates to the survival analyses, for which statistical power is limited. Moreover, although results persisted following adjustment for a range of potential confounders, they are based only on the two-thirds of women in the original study who participated in the clinical examination. We have shown here that these 190 women were generally comparable for age and a number of disease-related characteristics to the larger, representative sample of 287 women. Still, those who originally participated in the Pulling Through Study on a questionnaire-only basis were subsequently more likely to die during the follow-up period (12-14%) compared to those with clinical measurements (7-8%). We therefore conducted sensitivity analyses to see how their inclusion would influence outcomes of the survival analyses. When all those missing clinical measures were included as not having lymphedema, the results were attenuated. Yet even in this highly unlikely scenario, HRs of 1.56-1.81 were observed, similar in magnitude to elevated mortality risks associated with other acknowledged prognostic factors for breast cancer, such as tumour size, tumour grade, stage and estrogen-receptor status, which range from 1.5-1.9²⁵. Hence, we believe the results observed in this study are sufficiently robust to support a continued concern regarding a role for lymphedema in survival following breast cancer.

As a first report of an association between lymphedema and subsequent mortality among women with breast cancer, these results must be interpreted with caution. Women treated for

breast cancer fear the development of lymphedema²⁶, and those with secondary lymphedema fear its progression²⁷. The findings reported here could exacerbate such fear and are in need of replication in other breast cancer cohorts as well as other cancers before being disseminated in the clinical or public health setting. Nonetheless, the findings are compelling and provocative, suggesting that lymphedema may influence quantity as well as quality of life, either because it represents a subset of women with inherited genetic susceptibility to impaired immune response, it is a surrogate measure of undetected disease, and/or it is an independent prognostic factor. Irrespective, the findings emphasize the importance of routine monitoring for lymphedema following treatment for breast cancer, which may optimise early detection and treatment of lymphedema as well as possible subsequent disease.

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Author Disclosure Statement

No competing financial interests exist for any author.

References

1. Szuba A, Rockson S.G., Lymphoedema: classification, diagnosis and therapy. *Vasc Med.* 1998; 3(2): 145-56.
2. Australian Institute of Health and Welfare, Cancer in Australia: an overview, 2006. Vol. AIHW cat. no. CAN 32. 2007, Canberra: Australian Institute of Health and Welfare.
3. Hayes S., Rye S., Battistutta D., DiSipio T., Newman B., Upper-body morbidity following breast cancer treatment is common, may persist longer-term and adversely influences quality of life. *Health Qual Life Outcomes.* 2010; 8(92).
4. Hayes S.C., Janda M., Cornish B., Battistutta D., Newman B., Lymphoedema following breast cancer: incidence, risk factors and effect on upper body function. *J Clin Oncol.* 2008; 26(21): 3536-42.
5. Hayes S.C., Review of Research Evidence on Secondary Lymphoedema: Incidence, prevention, risk factors and treatment. 2008, National Breast and Ovarian Cancer Centre.
http://www.nbooc.org.au/bestpractice/resources/SLER247_reviewofresearchevid.pdf.
6. Rymal C., Lymphedema management in patients with lymphoma. *Nurs Clin North Am.* 2001; 36(4): 709-34.
7. Sleeman J., Schmid A., Thiele W., Tumor lymphatics. *Semin Cancer Biol.* 2009; 19(5): 285-97.
8. Kohrt H., Nouri N., Nowels K., Johnson D., Holmes S., Lee P., Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. *PloS Med.* 2005; 2(9): e284.
9. Pinzon-Charry A., Maxwell T., Lopez J., Dendritic cell dysfunction in cancer: a mechanism for immunosuppression. *Immunol Cell Biol.* 2005; 83(5): 451-61.
10. Mallon E., Powell S., Mortimer P., Ryan T., Evidence for altered cell-mediated

- immunity in postmastectomy lymphoedema. *Br J Dermatol.* 1997; 137(6): 928-33.
11. Stewart F, Treves N., Lymphangiosarcoma in postmastectomy lymphedema; a report of six cases in elephantiasis chirurgica. *Cancer.* 1948; 1(1): 64-81.
 12. Hayes S., Rye S., Battistuta D., Newman B., Prevalence of upper-body symptoms following breast cancer and its relationship with upper-body function and lymphoedema. *Lymphology.* 2010; 43: 178-87.
 13. Brady M., Cella D., Mo F., Bonomi A., Tulskey D., Lloyd S., Deasy S., Cobleight M., Shiimoto G., Reliability and validity of the Functional Assessment of Cancer Therapy - Breast (FACT-B) quality of life instrument. *J Clin Oncol.* 1997; 15(3): 974-86.
 14. Solway S., Beaton D., McConnell S., Bombardier C., The DASH Outcome Measure User's Manual, Second Edition. 2 ed. 2002, Toronto: Institute for Work and Health.
 15. Ward L.C., Bioelectrical impedance analysis: Proven utility in lymphoedema risk assessment and therapeutic monitoring. *Lymph Res Biol.* 2006; 4(1): 51-6.
 16. Cornish B., Bioimpedance analysis: Scientific background. *Lymph Res Biol.* 2006; 4(1): 47-50.
 17. Hayes S.C., Janda M., Cornish B., Battistutta D., Newman B., Lymphoedema secondary to breast cancer: how choice of measure influences diagnosis, prevalence and identifiable risk factors. *Lymphology.* 2008; 41(1): 18-28.
 18. Clark B., Sitzia J., Harlow W., Incidence and risk of arm oedema following treatment for breast cancer: a three-year follow-up study. *QJM.* 2005; 98: 343-8.
 19. Petrek J.A., Lymphedema in a cohort of breast carcinoma survivors 20 years after diagnosis. *Cancer.* 2001; 92(6): 1368-77.
 20. Ferrell B.R., Grant M., Funk B., Otis-Green S., Gracia N., Quality of life in breast cancer part 1: physical and social well-being. *Cancer Nur.* 1997; 20: 398-408.
 21. Pinzon-Charry A., Schmidt C., Lopez J., Dendritic cell immunotherapy for breast cancer. *Expert Opin Biol Ther.* 2006; 6(6): 591-604.

22. Nakamura R., Sakakibara M., Nagashima T., Sangai T., Arai M., Fujimori T., Takano S., Shida T., Nakatani Y., Miyazaki M., Accumulation of regulatory T cells in sentinel lymph nodes is a prognostic predictor in patients with node-negative breast cancer. *Eur J Cancer*. 2009; 45(12): 2123-31.
23. Kim R., Emi M., Tanabe K., Arihiro K., Immunobiology of the sentinel lymph node and its potential role for antitumour immunity. *Lancet Oncol*. 2006; 7(12): 1006-16.
24. Setiadi A., Ray N., Kohrt H., Kapelner A., Carcamo-Cavazos V., Levic E., Yadegarynia S., van der Loos C., Schwartz E., Holmes S., Lee P., Quantitative, architectural analysis of immune cell subsets in tumor-draining lymph nodes from breast cancer patients and healthy lymph nodes. *PLoS One*. 2010; 5(8): e12420.
25. Field T., Buist D., Doubeni C., Enger S., Fouayzi H., Hart G., Korner E., Lamerato L., Bachman D., Ellis J., Herrinton L., Hornbrook M., Krajenta R., Liu L., Yao J., Disparities and survival among breast cancer patients. *J Natl Cancer Inst Monogr*. 2005; 35: 88-95.
26. Petrek J.A. Heelan M.C., Incidence of breast carcinoma-related lymphedema. *Cancer*. 1998; 83: 2776-81.
27. Hayes S.C., Reul-Hirche H., Turner J., Exercise and secondary lymphedema: safety, potential benefits, and research issues. *Med Sci Sports Exerc*. 2009; 41(3): 483-9.

Table 1: Demographic and disease characteristics of the study samples.

Characteristic	Original Pulling Through Study cohort (n=287)	Survival analyses participants ^a (n=190)	Follow-up study participants with complete data ^b (n=158)
	n (%) ^c	n (%) ^c	n (%) ^c
Age (years)			
<50	105 (31.4)	72 (32.7)	57 (30.9)
≥50	182 (68.6)	118 (67.3)	101 (69.1)
Most extensive surgery			
CLE ^d	185 (64.9)	126 (66.8)	103 (65.9)
Mastectomy	102 (35.1)	64 (33.2)	55 (34.1)
Largest tumor size			
<16mm	171 (60.3)	117 (62.3)	99 (63.6)
16+mm	116 (39.7)	73 (37.7)	59 (36.4)
Number of nodes positive			
None removed	41 (14.2)	26 (13.6)	19 (12.4)
None positive	158 (55.9)	111 (59.2)	90 (57.9)
1-3	59 (20.1)	39 (19.9)	38 (23.1)
4-9	29 (9.8)	14 (7.3)	11 (6.6)
Overall histologic grade			
One	76 (26.7)	48 (25.4)	44 (28.2)
Two	90 (31.7)	57 (30.1)	47 (29.9)
Three	91 (30.7)	64 (32.9)	49 (30.4)
Unavailable	30 (10.8)	21 (11.6)	18 (11.5)

Histologic type

Infiltrating ductal	210 (72.6)	140 (73.1)	115 (72.5)
Infiltrating lobular	44 (15.6)	28 (15.0)	26 (16.7)
Other	33 (11.7)	22 (11.9)	17 (10.8)

^a those from the original cohort with sufficient data to calculate cumulative burden of lymphedema between 6-18 months post-diagnosis; ^b subset with clinical and questionnaire data at follow-up; ^c results have been appropriately weighted (<50 years, 1.0; ≥50 years, 1.3) for oversampling of younger women; ^d CLE, complete local excision.

Table 2: Count (percent) of women reporting moderate to extreme upper-body symptoms at six months (n=287) and six years (n=183) following breast cancer diagnosis.

	6 months post-diagnosis		6 years post-diagnosis
	Original sample (n=287)	Follow-up sample (n=183)	Follow-up sample (n=183)
Upper-body symptoms ^a	n (%) ^b	n (%) ^b	n (%) ^b
Tingling	40 (13.7)	20 (10.6)	14 (7.4)
Weakness	54 (18.6)	28 (14.8)	25 (13.8)
Pain	41 (14.3)	22 (12.2)	19 (10.1)
Poor range of movement	29 (10.1)	16 (8.9)	20 (11.0)
Numbness	86 (29.2)	53 (28.2)	29 (15.6)
Stiffness	42 (13.9)	23 (11.9)	15 (8.0)
Number of symptoms			
0	148 (52.8)	103 (57.5)	103 (56.2)
1	59 (20.1)	36 (19.2)	45 (24.8)
2	25 (8.6)	16 (8.6)	17 (9.4)
3+	55 (18.5)	28 (14.6)	18 (9.7)

^a Symptoms: tingling and weakness as “moderate to extreme” (items taken from DASH questionnaire); pain, poor range of movement, numbness, stiffness and swelling defined as “somewhat to very much” (items taken from the FACTB+4 questionnaire); ^b Results presented have been appropriately weighted (<50 years: 1.0; ≥50 years: 1.3) for oversampling of younger women.

Table 3: Associations between cumulative burden of lymphedema between six and 18 months following breast cancer diagnosis and overall 6-year survival.

	N ^a	Deaths	Bivariate Cox Regression		
			HR ^b	(95% CI)	p-value
BIS ^c					0.08
Never	128	7	1.00	-	
Ever	62	8	2.48	(0.90, 6.83)	
SOAC ^d					0.04
Never	154	8	1.00	-	
Ever	41	6	3.03	(1.05, 8.73)	
Self-report ^e					0.68
Never	154	13	1.00	-	
Ever	125	12	1.18	(0.54, 2.58)	

^a Sample sizes vary based on availability of data to determine lymphedema status; ^b HR = hazard ratio; HR>1 indicates an increased risk of death; ^c BIS, bioimpedance spectroscopy; ^d SOAC, sum of arm circumferences; ^e Self-reported arm swelling

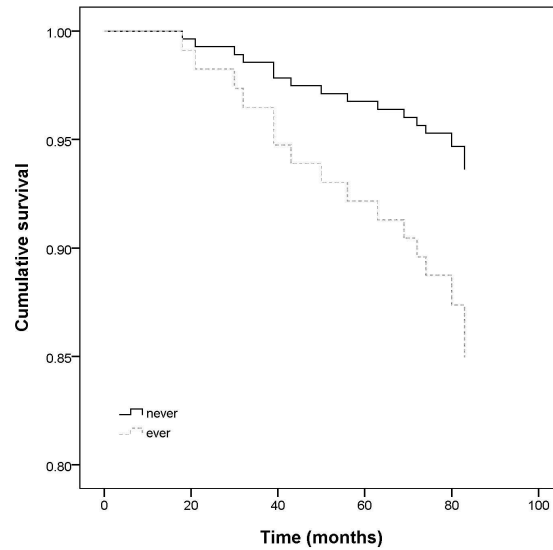
Figure 1. Kaplan-Meier curves for overall 6-year survival and lymphedema status between six and 18 months following breast cancer diagnosis assessed via (A) bioimpedance spectroscopy and (B) sum of arm circumferences.

(A) Lymphedema diagnosed by bioimpedance spectroscopy.

Ever = having any evidence of lymphedema, as defined by bioimpedance spectroscopy, between 6- and 18-months post-diagnosis; never = having no evidence of lymphedema between 6- and 18-months post-diagnosis.

(B) Lymphedema diagnosed by sum of arm circumferences.

Ever = having any evidence of lymphedema, as defined by sum of arm circumference, between 6- and 18-months post-diagnosis; never = having no evidence of lymphedema between 6- and 18-months post-diagnosis.

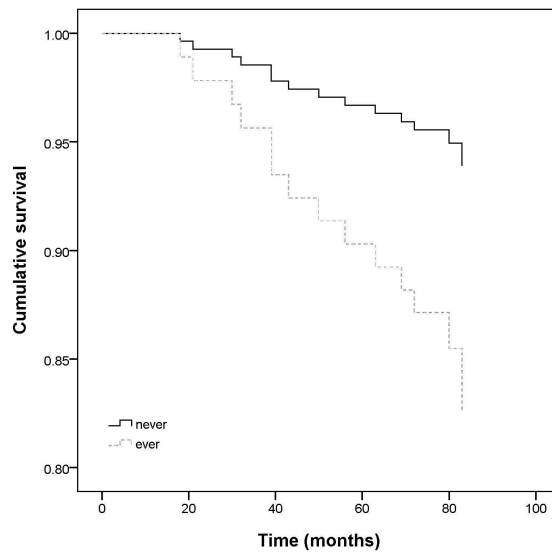


(A) Lymphedema diagnosed by bioimpedance spectroscopy.

Ever = having any evidence of lymphedema, as defined by bioimpedance spectroscopy, between 6- and 18-months post-diagnosis; never = having no evidence of lymphedema between 6- and 18-months post-diagnosis.

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(B) Lymphedema diagnosed by sum of arm circumferences.
Ever = having any evidence of lymphedema, as defined by sum of arm circumference, between 6- and 18-months post-diagnosis; never = having no evidence of lymphedema between 6- and 18-months post-diagnosis.

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