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Investigation of *In Vivo* skin stiffness anisotropy in breast cancer related lymphoedema

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ABSTRACT

There is a limited range of suitable measurement techniques for detecting and assessing breast cancer related lymphoedema (BCRL). This study investigated the suitability of using skin stiffness measurements, with a particular focus on the variation in stiffness with measurement direction (known as anisotropy). In addition to comparing affected tissue with the unaffected tissue on the corresponding site on the opposite limb, volunteers without BCRL were tested to establish the normal variability in stiffness anisotropy between these two corresponding regions of skin on each opposite limb. Multi-directional stiffness was measured with an Extensometer, within the higher stiffness region that skin typically displays at high applied strains, using a previously established protocol developed by the authors. Healthy volunteers showed no significant difference in anisotropy between regions of skin on opposite limbs (mean decrease of 4.7 +/−2.5% between non-dominant and dominant arms), whereas BCRL sufferers showed a significant difference between limbs (mean decrease of 51.0+/−16.3% between unaffected and affected arms). A large difference in anisotropy was apparent even for those with recent onset of the condition, indicating that the technique may have potential to be useful for early detection. This difference also appeared to increase with duration since onset. Therefore, measurement of stiffness anisotropy has potential value for the clinical assessment and diagnosis of skin conditions such as BCRL. The promising results justify a larger study with a larger number of participants.

1. Introduction

Lymphoedema occurs where the lymphatic system is no longer working efficiently, causing a build-up of fluid in the skin and subcutaneous fat of the affected region. Breast cancer related lymphoedema (BCRL) often occurs when lymph nodes become damaged or removed during breast cancer treatment by radiotherapy or surgery (Halstead, 1921). BCRL normally occurs unilaterally on the side of the patient's treated area. Around a quarter of treated breast cancer patients go on to develop lymphoedema (Mortimer, 1998), but at present there is no method to determine which patients will be affected. Breast cancer patients continue to develop BCRL even though more conservative cancer treatments have been used recently. BCRL can appear years after breast cancer treatment, possibly as a result of the disease being a degenerative process, which begins proximally to the affected site and spreads distally over time (Mortimer, 1998). The rate of spread will determine how quickly a patient notices swelling. After an initial rapid expansion, the swelling may then either persist or increase. After onset of BCRL, there is no cure, although the disease can be treated by compression garments. It is therefore important to begin treatment at an early stage before the disease gets too advanced to allow effective treatment.

Currently there are few tools available to diagnose early stage lymphoedema, or to monitor change in response to therapeutic intervention, although recent research has begun to address this problem (Berry et al., 2008 and Righetti et al., 2007). The gold standard is measurement of volume, which can be performed either by measuring the circumference with a tape measure and calculating the volume using water displacement volumetry or by using optical methods such as the Perometer (Stanton et al., 1997). Normally the site of lymphoedema is compared to the corresponding site on the opposite limb, as this is considered a healthy normal region, which can be assumed to approximate the volume of the affected area before the onset of lymphoedema. However, at various stages, different tissue types within the limb can expand or contract, so an overall volume measurement is not particularly informative. For example, muscle atrophy can occur due to the patient's reduced usage of the arm and this would result in a

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decrease in volume. This makes it difficult to use the technique to monitor response to treatment, as it cannot distinguish between changes in different tissues.

In addition to the confounding factor of changes in each layer of tissue, the volume measurement technique suffers from the disadvantage that it can only detect lymphoedema when the variation between arms is bigger than the normal variation of 4% between a healthy person's dominant and non-dominant arms (Stanton et al., 1999). Therefore the technique is not ideal for picking up changes in limb volume in early stage lymphoedema. In addition, the site of swelling can vary from patient to patient, and not all patients suffer from swelling over the whole limb. Changes in volume cannot easily be measured on hands and the adjoining quadrant of the trunk. The volume technique is therefore only useful on the limbs themselves. Recent changes in breast cancer surgery mean that patients are now undergoing more conservative treatments; lumpectomies are commonly carried out rather than mastectomies, leaving most of the breast post surgery. This has lead to an increase in incidence of lymphoedema in the breast itself, for which standard volume measurements cannot be used. In addition, lymphoedema occurring in other body sites such as the head and neck, also cannot be diagnosed with such techniques, and would therefore benefit from development of novel, localised diagnostic techniques (Nixon et al., 2014).

In lymphedema, an accumulation of interstitial fluid occurs because the capillary filtration rate into the tissue interstitium exceeds the reduced drainage capacity of the lymphatic system (Mortimer, 1998 and Levick, 2003), causing swelling and an increase in protein concentration and collagen deposition. Swelling occurs primarily in the skin and beneath the skin in the subcutis, which easily swells under internal pressure, due to its low stiffness. As the dermis is stiffer it will initially resist swelling. The increase in resistance to drainage will increase pressure in the lymphatic system, resulting in back flow of lymph particularly towards the skin called “dermal backflow” (Mortimer, 1998). Hence, over time the skin will gradually expand due to its viscoelastic nature and the epidermis will also thicken due to keratin deposition. Since BCRL alters the structural composition of the different layers of the skin, it is hypothesised that mechanical changes will occur. New diagnostic tools that measure mechanical properties may therefore offer information additional to that provided by the measurement devices currently available and may help understanding of the condition. Skin stiffness is known to be dependent on structural proteins, such as collagen fibres. When healthy skin is stretched, differences in collagen fibre orientation give rise to different magnitudes of stiffness in different directions (anisotropy), commonly at least two times stiffer in the stiffest direction than in the softest direction, depending on the test method and test site (Coutts et al., 2006, 2013; Khatyr et al., 2004 and Flynn et al., 2011). With a different protein composition, the directional variation in stiffness in lymphedematous skin is likely to be different to that previously observed in healthy skin. Therefore, in this study, stiffness was also investigated in different directions. Patients were chosen for the study at all different stages of the condition, to allow investigation of correlation between stiffness measure and time since diagnosis.

2. Methods

Seven patients were recruited from the Lymphoedema Clinic at the Royal Marsden NHS Trust, Sutton, Surrey, UK. The inclusion criteria were that the patients were not currently on any medication and that their lymphoedema arose following breast cancer treatment. Five healthy volunteers were also tested as control subjects. The patient and healthy volunteer details are shown in Table 1, with dates of treatment for breast cancer and onset of lymphoedema. Full ethical approval was granted by The Royal Marsden NHS Foundation Trust Local Research Ethics Committee (05/Q0801/54) and informed consent was obtained. Stiffness measurements in both arms were obtained for comparison of normal (contralateral) and affected arms in the BRCL patients, and for assessment of normal differences between arms in healthy volunteers. The limb circumference was also measured for each participant in both arms, using a tape measure, at the site of the stiffness tests.

A skin stretching device called the uni-axial Extensometer (Cardiff-Biometrics Ltd, Cardiff, Wales) was attached to the skin surface on the dorsal forearm, via cyanoacrylate gel on the Extensometer's two brackets (Fig. 1). Whilst stretching the skin by increasing the distance between the two brackets at a constant rate, the device measures the resulting load required to stretch the skin. Skin exhibits two types of mechanical behaviour: the initial phase of a skin's mechanical behaviour consists of a loose slack region, which is low in stiffness and is highly dependent on the degree of slackness and body posture, making it difficult to reproducibly measure stiffness in the low stiffness region (Coutts et al., 2013 and Barbenel, 1995). Once straightened and all slack has been removed, skin is highly resistant to stretching further (the higher stiffness region), and in this region stiffness measurement is more linear and reproducible, where the collagen fibres within the skin are being stretched and measurement is independent of the variable degree of slack or body posture at the start of the test (Coutts et al., 2013). Therefore, in this study, mechanical properties have been quantified using the higher stiffness region alone.

As the Extensometer was controlled by displacement rather than load, and the level of slackness in the skin is variable, initial loading cycles were applied to the skin sample, of increasing displacement, until the displacement required to remove slack and reach and measure the higher stiffness region was known. The skin was then left for five minutes to allow it to return to its original resting condition, as skin is known to exhibit time dependent behaviour. As the current study also involved non-healthy skin, the length of this delay period was determined by doubling that required for healthy skin, where previous investigation by the authors has indicated that the time required to allow healthy skin to recover to its original resting state is approximately two minutes (Coutts et al., 2013). Following this delay, the Extensometer was used to apply the displacement required to measure the stiffness. This was calculated as the gradient of the resulting force–displacement response in the higher stiffness region, as shown in Fig. 2. Where the force required to further stretch the skin had reached 2N, the higher stiffness region was considered to have been reached, therefore the five data points reading closest to a 2N force were used to calculate gradient.

This initial loading cycle and delay procedure had to be repeated for both arms for each participant, in each test direction, because in each test direction and for each

Table 1
Participant Information: BCRL patients: 1–7 and healthy volunteers: 8–12.

Ref. No.	Affected Side	Age	Year of Breast Cancer Treatment	Year of BCRL Onset	Left Arm Circumference (cm)	Right Arm Circumference (cm)	Circumference Difference between Arms(cm)
1	Right	64	1995	1996	22.3	26.4	4.1
2	Right	68	2001	2001	23.6	25.2	1.6
3	Left	50	1996	1996	31.5	24.7	6.8
4	Left	56	2005	2005	25.2	23.6	1.6
5	Right	46	2004	2004	28.7	30.1	1.4
6	Right	59	2000	2000	27.5	32.1	4.6
7	Right	28	2005	2005	23.6	24.8	1.2
8	Volunteer 1	48	N/A	N/A	24.4	24.7	0.3
9	Volunteer 2	28	N/A	N/A	23.6	23.2	0.4
10	Volunteer 3	63	N/A	N/A	24.3	23.7	0.6
11	Volunteer 4	60	N/A	N/A	25.3	25.8	0.5
12	Volunteer 5	66	N/A	N/A	25.8	26.5	0.7

skin sample, a different amount of slack was present. It was not feasible to use a high displacement that would suffice for all cases, because once the higher, linear phase is reached, a slight increase in displacement (and hence strain) places the skin under a high tensile force, which could be painful and dangerous to the patient.

The stiffness anisotropy was investigated by repeating this procedure in two directions. The first direction was chosen according to previously published Langer's lines (Langer, 1861), which indicate the stiffest direction of the skin at different body sites. For the site tested on the dorsal forearm, one third of the distance along the radius, proximal to the elbow, the skin is stiffest along the arm. The second and usually least stiff direction at this site, was chosen at 90-degree rotation to the initial direction, across the arm, as shown in Fig. 1. The anisotropy was calculated from stiffness in the stiffer direction (along the arm) divided by stiffness in the softer direction (across the arm). Whilst the data were being collected, an ultrasound probe was positioned between the Extensometer brackets to allow imaging data to be collected, the findings of which will be published separately. The effect of this probe on stiffness measurements was negligible, minimised by use of an acoustic coupling gel, reducing friction, and also by ensuring contact force remained low between the skin and the probe at all times.

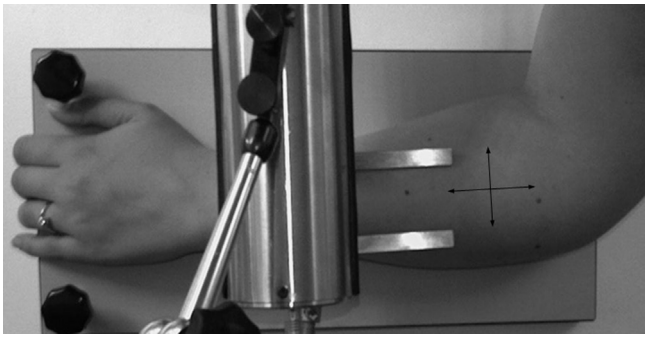

Fig. 1. Extensometer set-up in the 'across' arm test position. Arrows indicate the two test directions (along and across).

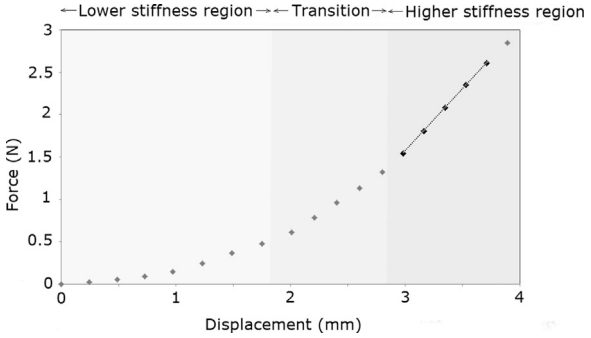

Fig. 2. Sample force–displacement response, showing the region of data used to calculate stiffness in the higher stiffness region. The dotted line represents the least squares linear fit through five data points.

3. Results

A total of 7 lymphoedema patients and 5 healthy volunteers were tested. The circumference of each arm was measured. Lymphoedema subject number 7 was very recently diagnosed with BCRL. Apart from slight swelling, the only sign of lymphoedema was tingling in the patient's fingertips. The difference in overall circumference between the affected and unaffected arms was later found, upon clinical examination, to have reduced to normal levels within six months of the test. Therefore, calculations exclude this patient's data and the figures have been adjusted to either exclude this patient's data, or to show them as a separate case.

From each case, in each direction, and for each arm, stiffness was measured with the Extensometer. To assess the variation in anisotropy for measurements carried out on different occasions, one healthy volunteer was tested three times, with a mean anisotropy of 2.06 and one standard deviation of 0.06.

Fig. 3(a) shows that, for all BCRL patients, the anisotropy in the affected arm was considerably lower than that in the unaffected arm. Similar to the arm circumference in the affected arm being significantly higher than for the contralateral arm (paired t test, $p=0.010$, see Fig. 3b), the anisotropy in the affected arm was significantly lower than in the contralateral arm (paired t test, $p=0.007$).

The first row of Fig. 4 shows the measured skin stiffness for each test direction and each arm, against duration of oedema in years. Stiffness remains constant in the normal (contralateral) arm, but reduces with duration in the along direction in the affected arm, and increases with duration in the across direction in the affected arm. The second row shows the anisotropy for each arm, calculated from the data plotted in the first row, showing that the anisotropy reduces with duration in the affected arm ($r=0.79$, $p=0.06$), but not in the normal arm ($r=0.07$, $p=0.90$). The third

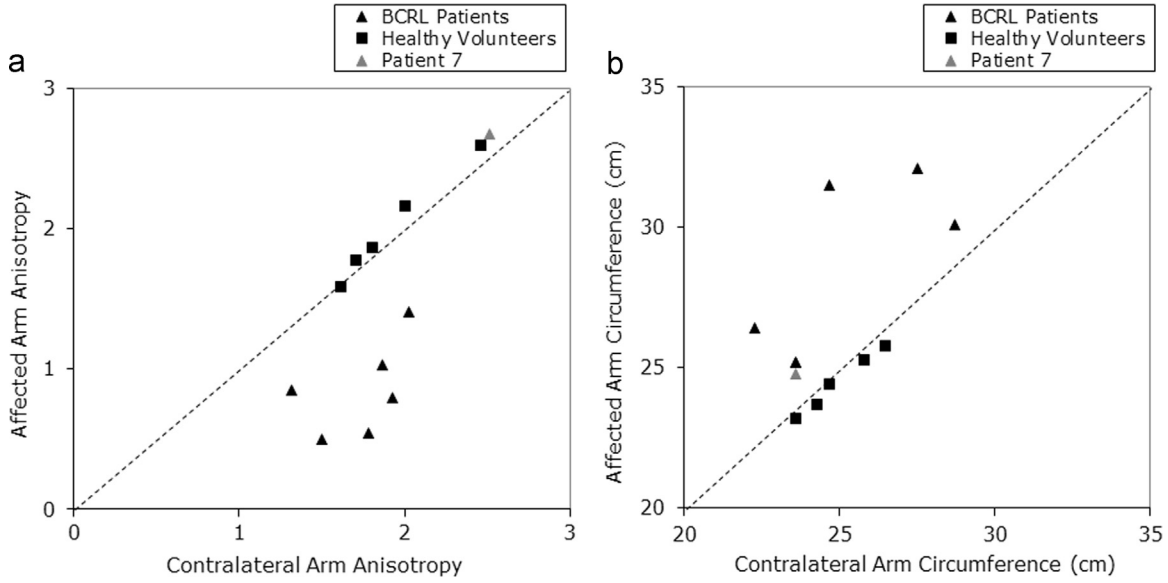

Fig. 3. (a) Skin stiffness anisotropy (unit is dimensionless) in affected and contralateral arms for lymphoedema patients and healthy volunteers (for healthy volunteers: affected = non-dominant arm) and (b) circumference (cm) in affected and contralateral arms for lymphoedema patients and healthy volunteers (for healthy volunteers: affected = non-dominant arm).

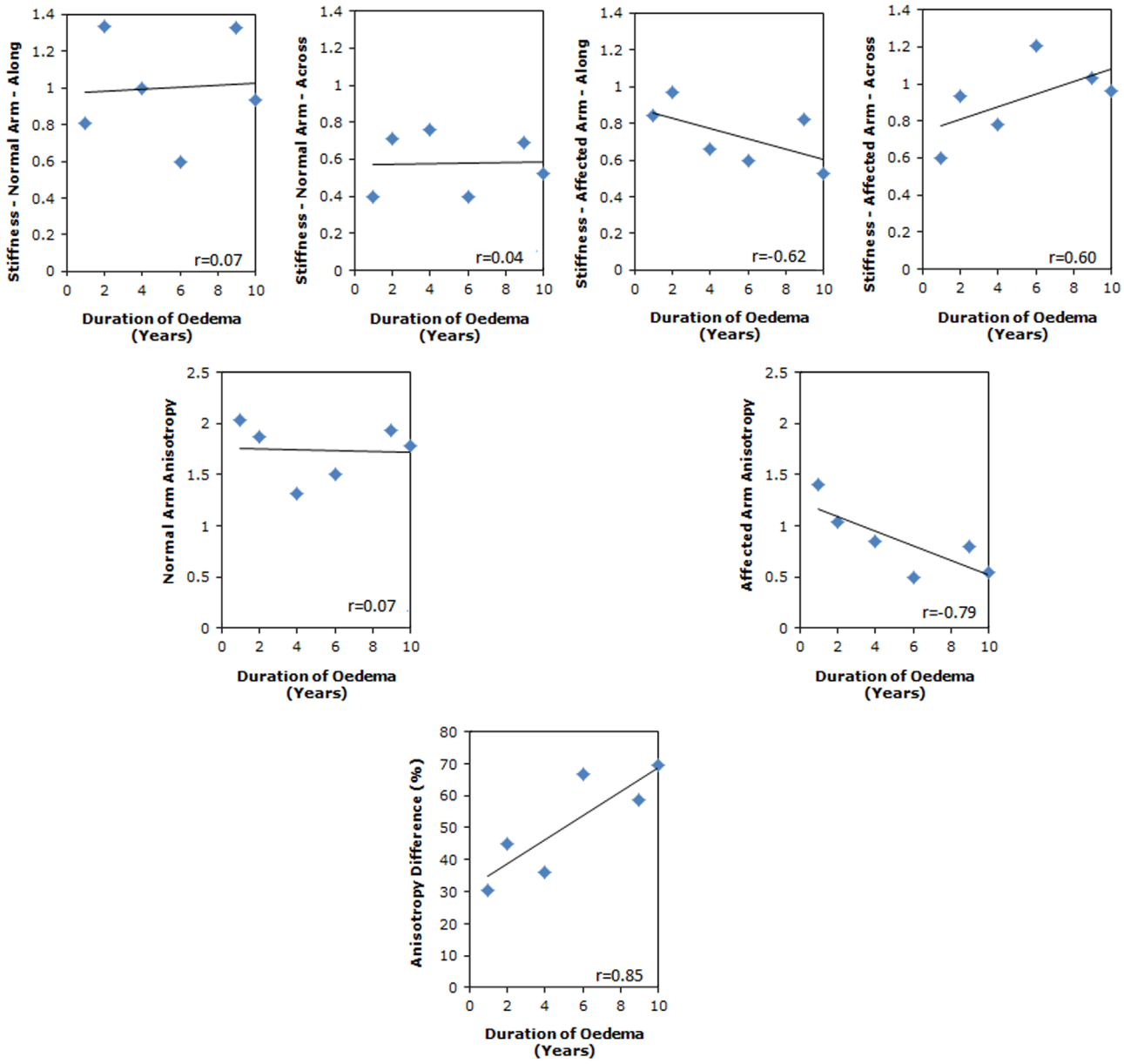

Fig. 4. Stiffness measured in different directions on normal (contralateral) and affected arms (first row), allowing anisotropy calculation in normal and affected arms (second row) and percentage difference between arms (third row). All data are plotted against duration of oedema in years.

row shows the percentage difference in anisotropy between arms, calculated from the data in the second row, showing that the difference in anisotropy increases with duration ($r=0.85$, $p=0.03$).

The first row of Fig. 5 shows the measured arm circumference, for each arm, against duration of oedema in years. Circumference appears to reduce with duration in the normal arm ($r=0.31$, $p=0.55$), but increase with duration in the affected arm ($r=0.38$, $p=0.46$), however, these observations are not statistically significant, possibly due to variation in levels of body fat between individuals. However, when these data were used to compare the difference in circumference between arms, the difference in circumference increases significantly with duration ($r=0.93$, $p=0.007$), shown in the second row of Fig. 5.

4. Discussion

Fig. 3(a) shows that, in healthy volunteers, anisotropy is very similar in both arms which leads to the assumption that in lymphoedema patients, had the affected arm not been affected with lymphoedema, the anisotropy would have been the same as that observed in the contralateral arm. The Extensometer results give preliminary evidence of a significant difference in anisotropy between lymphoedema and healthy tissue. This would indicate that a more comprehensive study is worthwhile, with a much larger number of cases.

In this study, initial loading cycles had to be applied to each sample, to determine the amount of low stiffness slack present in the skin, typically varying due to posture. This initial pre-strain had to be applied to remove the corresponding slack, prior to the initiation of strain in the higher stiffness region, where stiffness was measured. Such a method would not be ideal for routine clinical use, and future work should evaluate opportunities to improve the method for the best possible patient experience. For example, the need for initial loading cycles to determine the pre-strain could be eliminated by use of a force (rather than solely displacement) controlled device, analysing the force–displacement data in real time, as it is accumulated. In addition, fitting the full

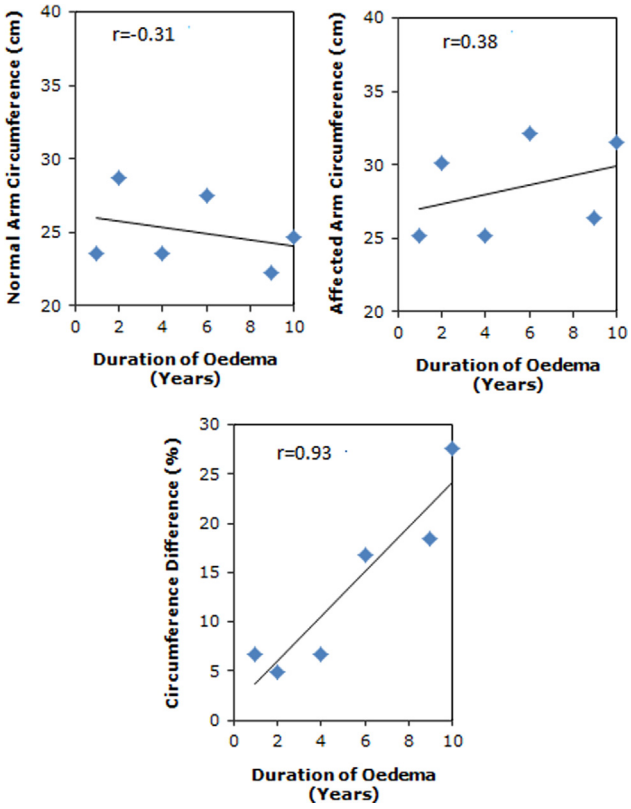

Fig. 5. Arm circumference measured on normal (contralateral) and affected arms (first row), allowing percentage difference between arms to be calculated (second row). All data are plotted against duration of oedema in years.

range of force-displacement data to a non-linear model, such as the Ogden model (Ogden, 1997), may result in more accurate estimation of skin stiffness than the higher stiffness gradient method described in this paper.

With the changes in stiffness observed in this study, there results a positive correlation between the percentage difference in anisotropy, between contralateral and affected arms, and the duration for which the patient has suffered with oedema ($r=0.85$, $p=0.03$, third row of Fig. 4). The difference in circumference between patients' affected and contralateral arms was also found to correlate positively with duration ($r=0.93$, $p=0.007$, second row of Fig. 5). The study measured smaller mean circumference change with BCRL than previous studies, for example Mellor et al. (2004), who measured a mean circumference change of 21.3+/−20.9%, compared to 13.5+/−8.9% for this study. However this may be a consequence of the preference of the patients recruited to the current study being only very recently diagnosed with BCRL.

Whilst in the current analysis, the anisotropy and circumference difference with duration have been modelled linearly, further participant testing may further determine the shape of the responses, especially at recent onset duration. Using a linear trend line for percentage difference in anisotropy versus duration, the intercept at time equals zero indicates percentage difference in anisotropy of approximately 30%, which would indicate a large change in anisotropy at onset of lymphedema. In comparison, using a linear trend line for circumference difference versus duration, the intercept at time equals zero indicates a percentage difference in circumference of approximately 3%, in the region of difference in circumference between arms in healthy participants (see Table 1, mean 1.99+/−0.58% 1SD). The difference in anisotropy between arms in healthy participants is also fairly small (4.65+/−2.54% 1SD). These findings indicate that with BCRL, early on there is a large change in anisotropy but not in circumference, therefore anisotropy measurement appears to have potential to be useful for early detection. However this observation is offered tentatively, given the small dataset.

The first row of Fig. 4 shows separately the stiffness in the along and across arm test directions against duration for contralateral arms and affected arms from the lymphoedema patients. In the majority of cases, the stiffness in the along arm test direction (normally the stiffest direction) reduces with lymphoedema, while the stiffness in the across arm test direction (normally the softest direction) increases with lymphoedema. The net result is that the skin on the lymphoedema patients' affected arms loses the stiffness anisotropy. Following the first couple of years, the magnitude of anisotropy becomes lower than one, meaning that for the two directions tested, the previously stiffest direction has become the least stiff direction. Measurement in only two directions appears sufficient for determining BCRL presence in a clinical setting, however from a scientific perspective, a further study would appear to be worthwhile to measure stiffness in more test directions, to investigate whether intermediate directions are even stiffer or softer and to determine the true dominant stiffness orientation and how it varies with duration to further understanding of the condition and causes of the mechanical changes observed, for example from build-up of fluid and collagen in the dermis (Levick, 2003; Mortimer, 1998) or extra fibre cross-linking, which would act to obscure the predominant collagen fibre orientation.

The work described in Coutts et al. (2013) tested the reproducibility of stiffness measurements made with the Extensometer in healthy volunteers. This indicated that the standard deviation of stiffness measurements on the forearm was equal to 12% of the mean stiffness. For anisotropy measurements, the standard deviation from each of the two stiffness measurements (in the two directions) can be combined to give an expected total variation, equal to the square root of the sum of the squares of the standard deviation of each of the two stiffness measurements. This indicates

that anisotropy should be measurable to within 17% of the true anisotropy, which is smaller than the differences between affected and unaffected arm anisotropy measured in this study (mean 51%, see third row of Fig. 4). The anisotropy for healthy volunteers' dominant arms (shown on the contralateral arm axis) has been plotted against the anisotropy for healthy volunteers non-dominant arms (shown on the affected arm axis) in Fig. 3(a), to establish whether there is a trend. The anisotropy in the non-dominant arm tends to be significantly higher than the anisotropy in the dominant arm (mean increase of 4.1%, paired *t*-test, $p=0.06$). This may mask reduction in anisotropy during the early stages of BCRL, if the non-dominant arm is the arm affected with BCRL, therefore consideration of which arm is dominant should be taken into account in future use of the technique. In addition, where understanding of the following parameters was limited to only healthy subjects for this study, for thorough future investigation of the potential of the technique, these parameters should also be investigated in skin of BCRL patients: (1) variation in repeat measurements and (2) the delay required for BCRL patients' skin to return to the original resting condition following a loading cycle.

Although these differences in stiffness anisotropy were measured without the use of imaging techniques, the implications of these differences for future use in elastography are important. When measuring stress or solving the inverse problem, the direction of tissue strain should be standardised, so that variation in mechanical properties with test direction do not confuse strain measurements. In addition, changes in the level of anisotropy, such as those found with BCRL, may be overlooked if imaging measurements were recorded solely in one test direction.

For this study, the normal arm was measured along with the affected arm, as is typically performed for the circumference measurement technique, but this could be a problem where other conditions are present. It would appear possible however, to develop a technique that can diagnose the condition by just measuring anisotropy in the affected arm, as anisotropy in BCRL is so different to that of normal tissue (see Fig. 3a). This would not be possible with the circumference measurement technique (see Fig. 3b).

Conflict of interest statement

There are no conflicts of interest to disclose from any author included in the manuscript.

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