Identifying diagnostic criteria for upper- and lower-limb lymphoedema

(ABRIDGED VERSION)

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BACKGROUND

Bioimpedance spectroscopy (BIS) assesses resistance (impedance) to the flow of an electrical current. Through the measurement of impedance to currents at low (R0) and high (Rinf) frequencies, extracellular fluid (ECF) and total body fluid (TBF) can be measured, respectively, and intracellular fluid (Ri = ICF) subsequently extrapolated (TBW = ECF + ICF).

Measuring bilateral upper-limb or lower-limb secondary lymphoedema following cancer is complicated by the unavailability of a comparable, unaffected limb. Availability of normative BIS data for all 4 limb segments would enable an extension of BIS in the diagnosis of bilateral upper-, as well as lower-limb lymphoedema.

The purpose of this study was to describe normative arm to leg, arm to arm and leg to leg impedance ratios and to determine optimal cut-off thresholds for diagnosing lymphoedema by testing the accuracy of cut-offs based on normative means plus or minus 0.5 to 3 SDs to diagnose known cases of lymphoedema.

STUDY 1: Investigation of normative bioimpedance data for all four limb segments

AIMS

1) To calculate ratios of impedance of ECF between arm and leg on the dominant side and between arm and leg on the non-dominant side, as well as between arms and between legs, in a convenience sample of healthy men and women.
2) To describe the normal change in ratios of impedance of ECF between arms and legs, between arms and between legs over a 3-month follow-up period.
3) To present the above outcomes for all subjects and stratified by sex, age and body mass index (BMI).

METHODS

This work brings together data collected in three cross-sectional studies and two repeated measures studies, involving Australian and New Zealand samples. Chief investigators collaborated to generate two datasets for the purposes of this work; the first representing cross-sectional BIS values from individuals without a known cancer diagnosis at any point in time (normative dataset) and the other including range of change in impedance over time (normative change dataset). The 5 initial datasets are described below:

Dataset A: A convenience sample (n=33) of men and women participated in this repeated measures cohort study conducted over a three-month period between September and December 2008. Participants were 18+ years and were staff or students of the Queensland University of Technology. Females were measured up to 22 times and males up to 8 times. Females were sampled more regularly so that any potential change associated with menstruation was captured. Data were collected with Impedimed SFB7 devices.

Dataset B: A convenience sample (n=491) of men and women contributed to this cross-sectional dataset. Data were collected between 2003 and 2013. Participants were recruited through the University of Queensland, and were University staff and students or members of the general public. Data were collected with Impedimed SFB7 or SFB3 devices.

Dataset C: A convenience sample (n=63) of women contributed to this cross-sectional dataset. Data were collected between July and August 2010. Participants were recruited from the James Cook University and Anne Street Gospel Chapel communities if they were aged over 40 years and able to provide consent. Data were collected with Impedimed SFB7 devices.

Dataset D: Data from 90 women were measured up to 5 times over a 12-month period between May 2001 and April 2002. Repeated measures from 3 occasions within this time period were included in this analysis. Participants were volunteers from the population, aged between 25 and 85 years and were age-matched to five-year age-group proportions for women diagnosed with breast and gynaecological cancers. Data were collected with Impedimed SFB3 devices.

Dataset E: A convenience sample (n=252) of men and women participated in this collaborative study between Fonterra and the New Zealand Government with Auckland University carrying out the cross-sectional study to assess body composition. Data were collected on 1 occasion between November 2013 and June 2014. Participants were aged 45-70 years of either Caucasian or Chinese ethnicity, and healthy by self-report. Exclusion criteria included morbid obesity (>BMI 40kg/m2) or significant current disease including muscle wasting syndromes. The cohort was recruited via newspaper advertising, online advertising, email circulation and word of mouth. Data were collected with Impedimed U400 devices.

Exclusion criteria for all studies included being pregnant or lactating, or having any of the following: history of cancer, history of surgery or radiotherapy to the regional lymph nodes, history of lymphoedema, a pacemaker or defibrillator, pins, plates or knee/hip replacements, allergies to Elastoplasts, Band-Aids or adhesive materials. Appropriate ethical approval was obtained for the conduct of all initial studies, as well as the conduct of the work outlined in this report.

Data collection

All contributing studies implemented the same, established, validated protocol, outlined by Impedimed, to measure impedance to TBW (Rinf), ICF (Ri) and ECF (R0) in arms and legs using a portable, Impedimed SFB7, SFB3 or U400 BIS machine. SFB3 data were converted to equivalent SFB7 data using the conversion factors provided by Ward [1-3]. BIS measurements were taken with the participant lying supine with legs apart. For any given measure, 2 measurement electrodes were placed at either end of the arm or leg (i.e., wrist for arm measures and ankle for leg measures) with a current drive electrode placed approximately 10 cm distally to the base of the middle finger or toe, respectively, and another placed on the ipsilateral or contralateral foot or hand, respectively [4]. Each limb segment was measured in this manner and the resistance corresponding to ECF volume (R0) and to TBW (Rinf) were determined; ICF impedance (Ri) was subsequently calculated from R0 and Rinf using the manufacturer's software (Bioimp 4.15.0.0). Participants were routinely asked to empty their bladder within the previous hour to data collection, avoid a higher than normal intake of alcohol or caffeine, vigorous exercise or more exercise than usual, in the previous 24 hours. Age, sex, height (measured to the nearest mm using a stadiometer), weight (measured to the nearest 100 g using a calibrated scale) and side of dominance were also recorded for each participant.

Data analysis

Normative data were assessed to determine the normal range of BIS ratios for dominant arm/dominant leg (R0 and Ri:R0), non-dominant arm/non-dominant leg (R0 and Ri:R0), dominant leg/non-dominant leg (R0) and dominant arm/non-dominant arm (R0). Normative change data (generated by subtracting subjects' lowest ratios from their highest ratios when repeated measures were recorded) were assessed to determine the normal range of change in the above measures.

For each set of outcomes, bootstrapping analysis was performed (5000 iterations) with R Project Version 3.1.1 to calculate the mean and SD of bootstrap distributions. Bootstrap mean, bootstrap SD and bootstrap mean plus and minus 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 bootstrap SDs (indicative of approximately 38.3%, 54.7%, 68.3%, 86.6%, 95.4%, 98.8% and 99.7% of the normative population, respectively) are presented for all outcomes.

Unpaired two-sample t-tests were used to determine whether differences between males and females were statistically significant. Paired two-sample t-tests were used to determine whether differences between dominant and non-dominant limbs were statistically significant. Multiple linear regressions, adjusted for sex, were used to investigate the effects of age and BMI as continuous variables on BIS ratios. Assumptions of normality, independence, constant variance and noncollinearity between predictor variables were tested and met for all outcomes. The significance level was set at 0.05 for all analyses.

RESULTS

Subject selection and characteristics

The normative dataset, which combined cross-sectional data from 5 datasets, consisted of a total of 934 subjects. Of these, 5 subjects were excluded due to missing characteristics and 20 were excluded due to having no BIS data. Table 1 shows characteristics of included participants (n=909) from database A through E and the combined sample. In total, data from 313 men (33%) and 596 women (66%) were included. The age range of participants was wide (18-87), 94% were right side dominant and there were approximately 50% of participants in the healthy or underweight BMI category.

TABLE 1. Characteristics of subjects in normative dataset

	Total	Dataset A	Dataset B	Dataset C	Dataset D	Dataset E
N	909	33	489	63	89	235
			Mean (SD) Range			
Age (y)	48 (15)	38 (12)	42 (15)	55 (11)	54 (13)	58 (7)
	18, 87	21, 60	18, 87	40, 82	24, 83	44, 69
BMI (kg/m^2)	25.7 (4.4)	25.1 (5.1)	25.8 (4.4)	27.5 (5.6)	26.7 (4.3)	24.5 (3.6)
	16.1, 46	17.9, 41.1	16.1, 46	16.4, 42.7	18.6, 38.5	17.2, 37.4
Number of times BIS measured	2 (2)	12 (6)	1 (0)	1 (0)	2 (1)	1 (0)
	1, 23	5, 23	1, 1	1, 1	1, 3	1, 1
			N (%)			
Female	596 (66)	22 (67)	273 (56)	63 (100)	89 (100)	149 (63)
Right side dominant	853 (94)	31 (94)	465 (95)	55 (87)	81 (91)	221 (94)
BMI categories (kg/m^2)						
Underweight (<18.5)	68 (7)	6 (18)	37 (8)	6 (10)	4 (4)	15 (6)
Healthy weight (18.5-24.9)	377 (41)	11 (33)	191 (39)	15 (24)	33 (37)	127 (54)
Overweight (25.0-29.9)	322 (35)	13 (39)	179 (37)	25 (40)	32 (36)	73 (31)
Obese (30.0+)	142 (16)	3 (9)	82 (17)	17 (27)	20 (22)	20 (9)

Bioimpedance measurements

While 909 subjects were assessed, not all subjects had BIS data for all outcomes of interest. Furthermore, 13 BIS ratios from 10 subjects were excluded from the final analysis due to data indicating measurement error. Ratios of ipsilateral limbs were considered as erroneous or abnormal if a limb was less than a quarter or more than 4 times the size of the ipsilateral limb (i.e., ratios of <0.25 or >4.0). These criteria led to the exclusion of:

- 1 out of 900 (0.1%) dominant arm/leg R0 ratios
- 2 out of 724 (0.3%) non-dominant arm/leg R0 ratios
- 1 out of 897 (0.1%) dominant arm/leg Ri:R0 ratios
- 1 out of 721 (0.1%) non-dominant arm/leg Ri:R0 ratios

Ratios of comparable limbs (leg/leg or arm/arm) were considered as erroneous or abnormal if a limb was less than half or more than twice the size of the comparable limb (i.e. ratios of <0.5 or >2.0). These criteria led to the exclusion of:

- 2 out of 719 (0.3%) dominant arm/non-dominant arm R0 ratios
- 6 out of 722 (0.8%) dominant leg/non-dominant leg R0 ratios

The final numbers of subjects (N) analysed for each outcome are shown in the results tables. BIS ratios are shown for the following groups:

a) all subjects
b) stratified by sex
c) stratified by sex and age (<50, 50-59, 60+)
d) stratified by sex and BMI[1] (<25, 25-29, 30+)
e) stratified by sex, age and BMI

[1] Due to low subject numbers in the underweight category for BMI, the underweight and healthy weight groups were combined (i.e. BMI <25 kg/m^2) for presentation of BIS data.

TABLE 2. Normative arm to leg ratios of R0 – dominant side

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
899	1.132	0.176	1.044	1.220	1.000	1.264	0.955	1.308	0.868	1.396	0.780	1.485	0.692	1.573	0.603	1.659

TABLE 3. Normative arm to leg ratios of R0 – non-dominant side

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
722	1.167	0.173	1.080	1.253	1.037	1.296	0.994	1.340	0.908	1.426	0.822	1.512	0.736	1.598	0.649	1.685

TABLE 4. Normative arm to leg ratios of Ri:R0 – dominant side

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
896	1.197	0.452	0.971	1.422	0.858	1.535	0.745	1.649	0.519	1.875	0.293	2.100	0.067	2.327	-0.158	2.551

TABLE 5. Normative arm to leg ratios of Ri:R0 – non-dominant side

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
720	1.127	0.371	0.942	1.312	0.849	1.405	0.755	1.498	0.570	1.683	0.385	1.868	0.199	2.054	0.013	2.241

TABLE 6. Normative arm to arm ratios of R0 – dominant over non-dominant

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
717	0.987	0.068	0.953	1.021	0.936	1.038	0.919	1.055	0.885	1.089	0.851	1.123	0.817	1.157	0.783	1.191

TABLE 7. Normative leg to leg ratios of R0 – dominant over non-dominant

N	Mean	SD	-0.5SD	+0.5SD	-0.75SD	+0.75SD	-1SD	+1SD	-1.5SD	+1.5SD	-2SD	+2SD	-2.5SD	+2.5SD	-3SD	+3SD
716	1.004	0.071	0.968	1.039	0.951	1.057	0.933	1.075	0.897	1.111	0.862	1.146	0.826	1.182	0.791	1.218

STUDY 2: Selection of optimal binary cut-off thresholds for the diagnosis of lymphoedema

AIMS

1) To optimise cut-off thresholds for diagnosing lymphoedema by testing the accuracy of cut-offs based on normative means plus or minus 0.5 to 3 SDs to diagnose known cases of lymphoedema.

METHODS

Binary cut-offs of mean normative ratios (arm R0/leg R0, arm Ri:R0/leg Ri:R0, dominant arm R0/non-dominant arm R0, dominant leg R0/non-dominant leg R0) plus and minus 0.5 to 3.0 standard SDs, calculated in Study 1 of this report, were evaluated for their ability to diagnose lymphoedema. Cut-offs were tested against a sample of 28 subjects with clinically diagnosed, secondary upper- or lower-limb lymphoedema.

Data collection

R0 and Rinf were measured, and Ri was subsequently calculated, for all limbs as described in Study 1. The following data were collected from each participant: date of lymphoedema diagnosis, duration and stage (0-4) of lymphoedema, the affected limb(s) (upper-limb, lower-limb, left/right/ bilateral), whether lymphoedema was pitting/non-pitting and/or whether there was skin thickening (yes/no). Additional information collected included patient weight and height, and details that can affect BIS readings (e.g., emptied bladder, high fat meal, alcohol or caffeine intake, vigorous exercises, pregnant or lactating).

Data analysis

Characteristics of subjects within the lymphoedema dataset were summarised and compared to subjects of the normative dataset using t-tests and chi-squared tests for continuous and categorical data, respectively.

Arm R0/leg R0, arm RiR0/leg RiR0, arm R0/arm R0 and leg R0/leg R0 ratios were calculated for all lymphoedema affected limbs and compared to ratios of normative limbs (extracted from Study 1) using two-sample t-tests. The significance level was set at 0.05.

The bootstrap means plus and minus 0.5, 0.75, 1, 1.5, 2, 2.5 and 3 bootstrap SDs of normative limb ratios (arm R0/leg R0, arm Ri:R0/leg Ri:R0, arm R0/arm R0, leg R0/leg R0) were used as binary cut-offs to diagnose lymphoedema in 28 subjects with clinically diagnosed lymphoedema. The sensitivity, specificity and area under the curve (AUC) were determined for all cut-offs using the R packages epiR and pROC [5]. The reference standard used for calculation of sensitivity and specificity was previous diagnosis of upper- or lower-limb secondary lymphoedema by a health care provider (an experienced lymphoedema therapist). From these results, optimal cut-off thresholds were selected.

RESULTS

Subject selection and characteristics

Twenty-eight subjects (26 females, 2 males) with clinically diagnosed, secondary lymphoedema were assessed. The prevalence of upper- and lower-limb lymphoedema were 71% and 29%, respectively. Table 15 shows characteristics of the included subjects.

The mean age and BMI of subjects in the lymphoedema group were significantly higher than for the normative dataset (61 versus 48 years, $t=6.0$, $p<0.001$; 29.0 versus 25.7 kg/m^2, $t=3.0$, $p=0.006$). Differences in BMI were also reflected by the obese category having the highest proportion of subjects in the lymphoedema group, compared to the healthy weight category having the highest proportion in the normative dataset. There were more females in the lymphoedema group compared to the normative dataset (93% versus 66%, $\chi^2=7.9$, $p=0.005$). There was no difference in the proportion of subjects with right side dominance (96% versus 94%, $\chi^2=0.03$, $p=0.9$).

The median duration of lymphoedema among subjects was 4 years. Upper-limb lymphoedema was secondary to breast cancer (18), melanoma (1) and an unknown diagnosis (1). Lower-limb lymphoedema was secondary to cervical (2), endometrial (1), melanoma (2) and uterine (3) cancers. Bilateral lymphoedema was present in only 1 subject with upper-limb lymphoedema (5%), but nearly all subjects with lower-limb lymphoedema (88%). Stage of lymphoedema ranged from I to III, with 57% of subjects having stage II lymphoedema. Over half of the subjects had pitting lymphoedema (64%) and thickening of the skin (68%).

TABLE 8. Characteristics of subjects with clinically diagnosed lymphoedema

	Total	Upper-limb	Lower-limb
N	28	20	8
		Mean (SD) Range	
Age (y)	61 (11)	59 (12)	63 (7)
	37-77	37-77	51-76
BMI (kg/m^2)	29.0 (5.9)	30.0 (5.9)	26.3 (5.1)
	19.9-41.9	19.9-41.9	19.9-33.5
		N (%)	
Female	26 (93)	19 (95)	7 (88)
Right side Dominant	27 (96)	19 (95)	8 (100)
BMI categories (kg/m^2)			
Underweight (<18.5)	-	-	-
Healthy weight (18.5-24.9)	8 (29)	5 (25)	3 (38)
Overweight (25.0-29.9)	8 (29)	5 (25)	3 (38)
Obese (30.0+)	11 (39)	10 (50)	1 (13)
Unknown	1 (4)	-	1 (13)
		Median (IQR) Range	
Duration of lymphoedema (years)	4 (1-8)	4 (1-7)	7 (4-9)
	0.1-34	0.1-34	1-21
		N (%)	
Lymphoedema secondary to			
Breast cancer	18 (64)	18 (90)	-
Cervical cancer	2 (7)	-	2 (25)
Endometrial cancer	1 (4)	-	1 (13)
Melanoma	3 (11)	1 (5)	2 (25)
Uterine cancer	3 (11)	-	3 (38)
Unknown	1 (4)	1 (5)	-
Side of lymphoedema			
Dominant only	13 (46)	12 (60)	1 (12)
Non-dominant only	7 (25)	7 (35)	-
Bilateral	8 (29)	1 (5)	7 (88)
Stage			
I	8 (29)	6 (30)	2 (25)
II	16 (57)	12 (60)	4 (50)
III	4 (14)	2 (10)	2 (25)
IV	-	-	-
Pitting	18 (64)	13 (65)	5 (63)
Non-pitting	3 (11)	3 (15)	-
Pitting and non-pitting	7 (25)	4 (20)	3 (38)
Thickening	19 (68)	14 (70)	5 (63)

Bioimpedance data of subjects with clinically diagnosed lymphoedema

BIS data from 28 subjects with clinically diagnosed lymphoedema are summarised and compared to normative ratios in Table 16. The mean arm R0/leg R0 ratio differed significantly between lymphoedema affected arms and normative arms on the dominant side only (p=0.002), while arm R0/arm R0 comparisons were significantly different on dominant (p<0.001) and non-dominant (p=0.003) sides of the body.

For lower-limb lymphoedema, both the arm R0/leg R0 and arm RiR0/leg RiR0 methods showed significant differences between lymphoedema affected and normative limbs on dominant (R0: p=0.031, Ri:R0: p<.001) and non-dominant (R0: p=0.049, Ri:R0: p=0.008) sides of the body. Use of the leg R0/leg R0 method to compare lymphoedema affected legs (7 out of 8 subjects had bilateral lymphoedema) to normative legs failed to indicate a significant difference.

TABLE 9. Bioimpedance ratios for lymphoedema affected limbs compared to normative limbs

	Lymphoedema	Normative	p*	Lymphoedema	Normative	p*
Upper-limb	Dominant			Non-dominant		
Arm R0/leg R0, N Mean (SD)	13 0.994 (0.176)	899 1.132 (0.176)	**0.002**	8 1.088 (0.193)	722 1.167 (0.173)	0.291
Arm RiR0/leg RiR0, N Mean (SD)	13 1.138 (0.283)	896 1.197 (0.452)	0.475	8 1.100 (0.340)	720 1.127 (0.371)	0.833
Arm R0/arm R0, N Mean (SD)	13 0.790 (0.090)	717 0.987 (0.068)	**<0.001**	8 1.111 (0.080)	717 0.987 (0.068)	**0.003**
Lower-limb	Dominant			Non-dominant		
Arm R0/leg R0, N Mean (SD)	8 1.309 (0.185)	899 1.132 (0.176)	**0.031**	7 1.515 (0.376)	722 1.167 (0.173)	**0.049**
Arm RiR0/leg RiR0, N Mean (SD)	8 0.832 (0.160)	896 1.197 (0.452)	**<0.001**	7 0.819 (0.210)	720 1.127 (0.371)	**0.008**
Leg R0/leg R0, N Mean (SD)	8 1.140 (0.323)	717 1.004 (0.071)	0.271	7 1.170 (0.337)	717 1.004 (0.071)	0.239

*Two sample t-tests were used to compare mean limb ratios between the lymphoedema and normative datasets.

Comparison of binary cut-offs to diagnose subjects in lymphoedema dataset

Tables 10 and 11 present the sensitivity, specificity and AUC of different limb comparison methods (i.e. arm R0/leg R0, arm Ri:R0/leg Ri:R0, arm R0/arm R0 and leg R0/leg R0) applied using seven different cut-off thresholds ranging from 0.5 to 3 SD ± mean to diagnose upper- or lower-limb lymphoedema, respectively. In the upper-limb setting, sensitivity reached 100% for the arm/arm R0 method with cut-offs based on the normative mean + 0.5 SD and mean + 0.75 SD. Specificity reached 100% for the arm/leg R0 method with cut-offs above mean + 0.75 SD, for the arm/leg RiR0 methods with cut-offs above mean + 2 SD and for the arm/arm R0 method with cut-offs above mean + 1.5 SD. Area under the curve (AUC), which is calculated from a plot of sensitivity versus 1-specificity is considered an effective measure of diagnostic accuracy. The highest AUC was 0.95, suggesting that the optimal method for diagnosing upper-limb lymphoedema was the dominant arm R0/non-dominant arm R0 method with a cut-off of mean ± 1.5. These data suggest that if the ratio of dominant arm R0 to non-dominant arm R0 is outside of 86.6% of the normal population (i.e. <0.885 on the dominant side or >1.089 on the non-dominant side) lymphoedema can be diagnosed with a sensitivity of 90% and a specificity of 100%.

In the lower-limb setting, sensitivity was highest (75%) for the arm/leg R0 method with cut-offs of mean + 1, 2 or 3 SD, and for the leg/leg R0 method with a cut-off of mean ± 1 SD. Specificity of 100% was achieved for the arm/leg R0 method with cut-offs above mean + 2 SD, for the arm/leg RiR0 method with cut-offs above mean +2 SD and for the leg/leg R0 method with a cut-off of mean + 3SD. The highest AUC was 0.80, suggesting that the optimal method for diagnosing lower-limb lymphoedema was the arm R0/leg R0 method with a cut-off of mean + 1. These data suggest that if the ratio of arm R0 to leg R0 is outside of 68.3% of the normal population (i.e. >1.308 on the dominant side or >1.340 on the non-dominant side) lymphoedema can be diagnosed with a sensitivity of 75% and a specificity of 85%.

TABLE 10. Sensitivity, specificity and AUC for binary cut-off thresholds for the diagnosis of upper- limb lymphoedema

Cut-off criterion	Dominant side cut-off	Non-dominant side cut-off	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Arm R0 / leg R0					
Mean - 0.5 SD	<1.044	<1.081	0.75 (0.51-0.91)	0.75 (0.35-0.97)	0.75 (0.56-0.94)
Mean - 0.75 SD	<1.000	<1.037	0.55 (0.32-0.77)	1.00 (0.52-1.00)	0.78 (0.66-0.89)
Mean - 1.0 SD	<0.956	<0.994	0.45 (0.23-0.68)	1.00 (0.52-1.00)	0.72 (0.61-0.84)
Mean - 1.5 SD	<0.868	<0.908	0.35 (0.15-0.59)	1.00 (0.52-1.00)	0.68 (0.57-0.78)
Mean - 2.0 SD	<0.780	<0.822	0.10 (0.01-0.32)	1.00 (0.52-1.00)	0.55 (0.48-0.62)
Mean - 2.5 SD	<0.692	<0.735	0.00 (0.00-0.24)	1.00 (0.52-1.00)	0.50 (0.50-0.50)
Mean - 3.0 SD	<0.603	<0.649	0.00 (0.00-0.24)	1.00 (0.52-1.00)	0.50 (0.50-0.50)
Arm Ri:R0 / leg Ri:R0					
Mean - 0.5 SD	<0.971	<0.942	0.45 (0.23-0.68)	0.12 (0.00-0.53)	0.36 (0.28-0.44)
Mean - 0.75 SD	<0.858	<0.849	0.30 (0.12-0.54)	0.12 (0.00-0.53)	0.40 (0.32-0.48)
Mean - 1.0 SD	<0.745	<0.755	0.10 (0.01-0.32)	0.50 (0.16-0.84)	0.35 (0.25-0.45)
Mean - 1.5 SD	<0.519	<0.570	0.00 (0.00-0.24)	0.88 (0.47-1.00)	0.44 (0.32-0.56)
Mean - 2.0 SD	<0.293	<0.385	0.00 (0.00-0.24)	1.00 (0.52-1.00)	0.50 (0.50-0.50)
Mean - 2.5 SD	<0.067	<0.199	0.00 (0.00-0.24)	1.00 (0.52-1.00)	0.50 (0.50-0.50)
Mean - 3.0 SD	<-0.158	<0.013	0.00 (0.00-0.24)	1.00 (0.52-1.00)	0.50 (0.50-0.50)
Arm R0 / arm R0					
Mean 0.5 SD	<0.953	>1.021	1.00 (0.76-1.00)	0.50 (0.16-0.84)	0.75 (0.56-0.94)
Mean +/- 0.75 SD	<0.936	>1.038	1.00 (0.76-1.00)	0.62 (0.24-0.91)	0.81 (0.63-0.99)
Mean +/- 1.0 SD	<0.919	>1.055	0.95 (0.75-1.00)	0.75 (0.35-0.97)	0.85 (0.68-1.00)
Mean +/- 1.5 SD	**<0.885**	**>1.089**	**0.90 (0.68-0.99)**	**1.00 (0.52-1.00)**	**0.95 (0.88-1.00)**
Mean +/- 2.0 SD	<0.851	>1.123	0.70 (0.46-0.88)	1.00 (0.52-1.00)	0.85 (0.75-0.95)
Mean +/- 2.5 SD	<0.817	>1.157	0.50 (0.27-0.73)	1.00 (0.52-1.00)	0.75 (0.64-0.86)
Mean +/- 3.0 SD	<0.783	>1.191	0.25 (0.09-0.49)	1.00 (0.52-1.00)	0.62 (0.53-0.72)

TABLE 11. Sensitivity, specificity and AUC for binary cut-off thresholds for the diagnosis of lower-limb lymphoedema

Cut-off criterion	Dominant side cut-off	Non-dominant side cut-off	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Arm R0 / leg R0					
Mean + 0.5 SD	>1.220	>1.254	0.75 (0.35-0.97)	0.60 (0.36-0.81)	0.68 (0.48-0.87)
Mean + 0.75 SD	>1.264	>1.297	0.75 (0.35-0.97)	0.80 (0.56-0.94)	0.78 (0.59-0.96)
Mean + 1.0 SD	**>1.308**	**>1.340**	**0.75 (0.35-0.97)**	**0.85 (0.62-0.97)**	**0.80 (0.62-0.98)**
Mean + 1.5 SD	>1.396	>1.427	0.62 (0.24-0.91)	0.90 (0.68-0.99)	0.76 (0.57-0.95)
Mean + 2.0 SD	>1.484	>1.512	0.50 (0.16-0.84)	1.00 (0.76-1.00)	0.75 (0.56-0.94)
Mean + 2.5 SD	>1.572	>1.598	0.50 (0.16-0.84)	1.00 (0.76-1.00)	0.75 (0.56-0.94)
Mean + 3.0 SD	>1.661	>1.685	0.25 (0.03-0.65)	1.00 (0.76-1.00)	0.62 (0.46-0.79)
Arm Ri:R0 / leg Ri:R0					
Mean + 0.5 SD	>0.971	>1.312	0.00 (0.00-0.48)	0.80 (0.56-0.94)	0.40 (0.31-0.49)
Mean + 0.75 SD	>1.535	>1.405	0.00 (0.00-0.48)	0.90 (0.68-0.99)	0.45 (0.38-0.52)
Mean + 1.0 SD	>1.649	>1.498	0.00 (0.00-0.48)	0.95 (0.75-1.00)	0.48 (0.43-0.52)
Mean + 1.5 SD	>1.875	>1.683	0.00 (0.00-0.48)	0.95 (0.75-1.00)	0.48 (0.43-0.52)
Mean + 2.0 SD	>2.100	>1.868	0.00 (0.00-0.48)	1.00 (0.76-1.00)	0.50 (0.50-0.50)
Mean + 2.5 SD	>2.327	>2.054	0.00 (0.00-0.48)	1.00 (0.76-1.00)	0.50 (0.50-0.50)
Mean + 3.0 SD	>2.551	>2.241	0.00 (0.00-0.48)	1.00 (0.76-1.00)	0.50 (0.50-0.50)
Leg R0 / leg R0					
Mean ± 0.5 SD	<0.969	>1.040	0.75 (0.35-0.97)	0.50 (0.27-0.73)	0.62 (0.43-0.82)
Mean ± 0.75 SD	<0.951	>1.057	0.62 (0.24-0.91)	0.70 (0.46-0.88)	0.66 (0.46-0.87)
Mean ± 1.0 SD	<0.933	>1.075	0.62 (0.24-0.91)	0.80 (0.56-0.94)	0.71 (0.51-0.91)
Mean ± 1.5 SD	<0.898	>1.111	0.50 (0.16-0.84)	0.95 (0.75-1.00)	0.72 (0.53-0.92)
Mean ± 2.0 SD	<0.862	>1.146	0.50 (0.16-0.84)	0.95 (0.75-1.00)	0.72 (0.53-0.92)
Mean ± 2.5 SD	<0.826	>1.182	0.50 (0.16-0.84)	0.95 (0.75-1.00)	0.72 (0.53-0.92)
Mean ± 3.0 SD	<0.790	>1.217	0.50 (0.16-0.84)	1.00 (0.76-1.00)	0.75 (0.56-0.94)

REFERENCES

1. Hayes, S., D. Battistutta, and B. Newman, *Objective and subjective upper body function six months following diagnosis of breast cancer.* Breast cancer research and treatment, 2005. **94**(1): p. 1-10.

2. Hayes, S., B. Cornish, and B. Newman, *Comparison of methods to diagnose lymphoedema among breast cancer survivors: 6-month follow-up.* Breast cancer research and treatment, 2005. **89**(3): p. 221-6.

3. Ward, L.C., *Inter-instrument comparison of bioimpedance spectroscopic analysers.* The Open Medical Devices Journal, 2009. **1**: p. 3-10.

4. Cornish, B.H., et al., *Optimizing electrode sites for segmental bioimpedance measurements.* Physiological measurement, 1999. **20**(3): p. 241-50.

5. Stevenson, M., et al., *epiR: An R package for the analysis of epidemiological data* 2015.