Lymphatic Research and Biology

Lymphatic Research and Biology: http://mc.manuscriptcentral.com/lymphatic

Tissue Dielectric Constant and Skin Stiffness Relationships in Lower Extremity Lymphedema

Journal:	*Lymphatic Research and Biology*
Manuscript ID	LRB-2022-0101.R1
Manuscript Type:	Original Contribution
Date Submitted by the Author:	n/a
Complete List of Authors:	Mayrovitz, Harvey; Nova Southeastern University, Dr. Kiran C. Patel College of Allopathic Medicine Shams, Elham; Nova Southeastern University, Dr. Kiran C. Patel College of Osteopathic Medicine Astudillo, Andrea ; Nova Southeastern University, Dr. Kiran C. Patel College of Osteopathic Medicine Jain, Aakangsha; Nova Southeastern University, Dr. Kiran C. Patel College of Allopathic Medicine
Keyword:	Lymphedema, Venous disease, Edema
Manuscript Keywords (Search Terms):	lymphedema, dielectric constant, lower extremity edema, tissue fibrosis, indentation, hardness

Tissue Dielectric Constant and Skin Stiffness Relationships in Lower Extremity Lymphedema

Harvey N. Mayrovitz, PhD, Dr. Kiran C. Patel College of Allopathic Medicine,
Nova Southeastern University

Elham Shams, MS, Dr. Kiran C. Patel College of Osteopathic Medicine
Nova Southeastern University

Andrea Astudillo, MS, Dr. Kiran C. Patel College of Osteopathic Medicine
Nova Southeastern University

Aakangsha Jain, MS, Dr. Kiran C. Patel College of Osteopathic Medicine
Nova Southeastern University

Corresponding Author
Harvey N Mayrovitz, PhD
Nova Southeastern University
Dr. Kiran C. Patel College of Allopathic Medicine
Department of Medical Education
Professor, Division of Physiology
3200 S. University Drive
Davie, Florida 33328
Terry Building Rm 1313
mayrovit@nova.edu

Running Title: Skin stiffness and water relationships

ABSTRACT

Background: Lower extremity lymphedema or edema (LELE) may progressively transition from a state of excess tissue fluid to increased fat accumulation and collagen deposition, with tissue fibrosis and hardening. Such changes may lead to altered tissue water holding and thereby impact tissue dielectric constant (TDC). This study seeks to evaluate the relationship between TDC and indentation force (TIF) in patients with LELE and assess the utility of the leg/arm TDC ratio (LAR) as an indicator of LELE.

Methods and Results: Thirty females (49-91 years) with previously diagnosed LELE were evaluated during a scheduled session. TDC and **TIF** were measured 8-cm proximal to the medial malleolus on the medial and lateral aspects of both legs and on one forearm 8-cm distal to the antecubital fossa. The TDC-TIC relationship and the LAR were subsequently determined. Main results showed an absence of a significant correlation between TDC and **TIF** on medial or lateral leg sites but a positive correlation on the normal forearm site. Further, LAR values exceeded the published proposed threshold of 1.35 for 29/30 patients when using medial-side TDC values and 28/30 patients when using lateral-side TDC values.

Conclusions: Findings suggest that for patients with LELE, TDC values are significantly elevated on medial and lateral standardized sites. The LAR determined using either medial or lateral sites are similar to each other and have values consistent with a lymphedema threshold of 1.35. In edematous legs of the type evaluated herein, there is no apparent relationship between TDC values and indentation force.

CONDENSED ABSTRACT

Lower extremity lymphedema or edema (LELE) may progressively transition from a state of excess tissue fluid to fat accumulation to collagen deposition and tissue fibrosis. Consequently, tissue water is impacted effecting tissue dielectric constant (TDC), indentation force (TIF) and leg-arm TDC ratio (LAR). Findings in 30 females with LELE suggest that TDC values are significantly elevated and LAR, determined using either medial or lateral sites, are similar to each other and have values consistent with a lymphedema threshold of 1.35. In edematous legs of the type evaluated herein, there is no apparent relationship between TDC values and indentation force.

Five KEY WORDS: lymphedema, collagen, tissue fibrosis, dielectric constant, lower extremity edema, indentation, hardness

INTRODUCTION

The potential effects of chronic lymphedema on collagen accumulation were brought to the research community's attention a number of years ago.[1] Studies on mice have shown that lymph stasis is associated with increased fat deposition, collagen deposition, and fibrosis.[2] In this animal model, the role of inflammatory cells has been well described, [3, 4] and more general aspects of tissue changes have been reviewed.[5] Thus, the manifestations of chronic edema or lymphedema may progressively transition from a state of excess tissue fluid to increased fat accumulation and collagen deposition, with associated tissue fibrosis causing hardening of the involved tissue. Such changes may lead to reductions in relative water holding content and tissue dielectric constant (TDC).[6-8] An unanswered question is the extent to which these tissue changes impact measurement methods designed to detect or track lymphedematous changes over time or with treatment. Methods such as bioimpedance spectroscopy (BIS),[9-11] or TDC that use measures of tissue water as indicators of arm,[12-18] or leg,[19-23] lymphedema may be particularly susceptible. In fact, some work has reported a positive correlation between lower extremity skin stiffness and lymphedema stage and tissue water percentage.[24, 25] Thus**,** one of the main aims of the present study was to evaluate possible relationships between lower extremity TDC values and tissue stiffness assessed via tissue indentation force (TIF) in patients diagnosed with lower extremity lymphedema or edema. Because such relationships and their comparisons among groups depend on variability features of both skin stiffness and TDC measurements, variabilities of sequential individual measurements in terms of their coefficient of variation were also of interest.[26] A second aim was to evaluate the utility of a threshold value for the detection of lower extremity lymphedema, calculated from the ratio of leg to forearm

Mary Ann Liebert, Inc., 140 Huguenot Street, New Rochelle, NY 10801

TDC values, based on measurements in non-edematous legs.[21] Because site-dependent variations in TDC values are known to be present in patients with upper extremity lymphedema,[15] the question of whether TDC measurements made on medial vs. lateral leg sites significantly differ in patients with lower extremity edema was also of interest.

METHODS

Subjects

Inclusion criteria for participation in this study was that the subject be female, with previously diagnosed lower extremity lymphedema or edema, **who was scheduled for a** treatment session at a participating local lymphedema treatment clinic. **Thirty females participated and each was** advised of the nature of the study prior to starting measurements, and each signed an approved university institutional review board informed consent (#2019-515). Patients were included sequentially as available, subject to the following exclusionary criteria: (1) any open wound near an intended measurement site or (2) any implanted wires or electronic medical devices. **The 30 patients evaluated ranged in age** from 49 to 91 years, with body mass index (BMI) ranging from 14.9 to 65.9 Kg/m^2. The duration of their lower extremity condition ranged from 1 to 40 years. (**Table 1)** summarizes the main presenting features and the target leg and arm sides measured. **Of the 18 patients with lower extremity edema, all had diagnoses of chronic venous insufficiency and of the 12 patients with lower extremity lymphedema, all had diagnoses of secondary lymphedema.**

Measurements

TDC measurements were done with a compact hand-held device (MoistureMeterD Compact, Delfin Technologies, Kuopio, Finland), the operating principles and use of which have been previously described.[27] In brief, measured TDC values are largely dependent on water within the measured local volume and is a dimensionless number equal to the ratio of tissue permittivity to vacuum permittivity. The effective measurement depth of the device used is between 2.0 and 2.5 mm, so that epidermis and dermis are in their measurement volume. The TDC measuring device operates as an open-ended transmission line.[6, 28] In use, a low-level 300 MHz signal is activated and transmitted into the skin when the sensor part of the device is in contact with the skin. Certain features of the reflected signal allow for the calculation of the complex reflection coefficient from which TDC values are determined.[7, 29, 30] After about five seconds of skin contact, the percentage of tissue water that is calculated from the measured TDC value, is displayed on a digital screen on the device. In the present study, TDC measurements were made in triplicate at each of three standardized sites (two leg sites and one arm site), with measured values reported as the average of the triplicate values. **The standard** leg sites **used** were located eight cm proximal to the **middle of the** medial malleolus with one measurement site on the medial aspect (shown in **figure 1**) and the other on the lateral aspect approximately opposite (180^{o}) to the medial site (not shown). **The middle of the medial malleolus as a reference point was chosen to be consistent with prior TDC measurement data. The standard** leg sites **used** were located eight cm proximal to the **middle of the** medial malleolus with one measurement site on the medial aspect (shown in **figure 1**) and the other on the lateral aspect approximately opposite (180^{o}) to the medial site (not shown). **The middle of the medial malleolus as a reference point was chosen to be consistent with prior TDC measurement data.**[21] These measurements were made on the leg deemed to be visually the most edematous. TDC measurements were also made on the contralateral leg at the medial and lateral locations corresponding to the target leg standard sites. The arm measured was on the same side as the most edematous leg, and the location on the arm was the volar forearm five cm distal to the antecubital fossa. Measurements were done with the patient supine but not started until they had been supine for at least 10 minutes. The temporal measurement order was forearm, medial leg, and lateral leg. Following completion of all TDC measurements, TIF was determined at the same anatomical sites and in the same measurement order using the SkinFibrometer (Delfin Technologies, Kuopio, Finland) that is based on refinements to a skin indentation approach.[31-34] The SkinFibrometer measures skin stiffness or firmness based on the indentation force in milliNewtons (mN) required to indent skin 1.3 mm. With this hand-held device, the skin is lightly touched, whereupon a two mm diameter indentor is caused to deform skin inwardly with the resultant force recorded and displayed.[24, 25, 35, 36] The device is equipped with internal sensors that accept measurements only within prescribed limits of force and force application velocity. Thus, if an applied pressure is too large or applied too slowly or rapidly, internal software prompts to repeat the measurement. The standard device internally averages five consecutive measurement values and displays the average. In this study each of the five consecutive repeated values was individually recorded so that the measurement coefficient of variation could be determined. The average of the five was used to represent the TIF value at each site. All measurements were done while participants were supine for at least 10 minutes on a padded examination table with arms resting at their sides and shoes and socks removed. **Leg circumferences were** measured with a **Gulick II tape measure** at **the location where the TDC measurements were made on both legs**.

Analyses

Coefficient of variation of individual measurements (CVI) was calculated for each site based on the three repeated TDC measurements and the five repeated TIF measurements. Overall TDC and TIF variability was then determined as the overall mean and SD of the CVI for the entire group of 30 patients. Tests for relationships between TDC and TIF were based on regression analysis done separately for each measured site. Tests for differences in TDC between medial and lateral leg sites were based on paired t-tests with statistical significance based on a p-value < 0.05. LAR were calculated as the leg value/forearm value for each patient using TDC values measured on the target leg lateral and medial aspects. These ratios were compared to the lymphedema threshold LAR of 1.35 previously reported.[21] **To determine the number of subjects to be included in the present study, TDC values and their standard deviations previously measured at the medial and lateral gaiter area in 22 non-edematous legs were used to calculate an overall mean and standard deviation that was 36.7 ± 5.05.** [21] **The effect size (ES) was based on being able to detect a 10% difference (3.67) at an α-level of 0.05 with a power (1-β) of 95%. The number of subjects needed for the calculated ES (0.7267) was determined to be N=27 using the software GPower, version 3.1.97.** [37] **It was decided to increase this by 10% yielding an N of 30.**

RESULTS

TDC and TIF Values and Variability

Table 2 summarizes leg TDC values measured at medial and lateral leg sites and TIF values measured at the leg lateral site along with the CVI. At the standard leg site (eight cm proximal to the **middle of the** medial malleolus) TDC values did not differ between medial and lateral measurements. Average values ranged narrowly from 48.3 ± 8.3 at the standard medial site to 47.5 ± 7.7 at the lateral site. TDC values on the **contralateral** leg differed slightly between medial and lateral sites presumably due to the slightly lower value measured at the medial location. However, corresponding values between the target and **contralateral** leg did not significantly differ, suggesting that most of **the** patients may have had bilateral conditions. **The CVI tended to be greater at lateral vs. medial sites but was statistically greater ($p<0.05$) only on the target leg. Forearm** TDC values (**28.0 ± 4.0)** were significantly less than **all measured** leg values ($p < 0.001$). TIF values, measured at the standard lateral site, were quite similar on both legs and on average differed by less than 15 mN with CVI values both less than 20% on average. Forearm TIF values were significantly less than at any leg site ($p<0.001$) and had a slightly lower CVI.

TIF-TDC Relationship

Paired leg TIF-TDC values are plotted in (**figure 2)** along with the associated regression lines for TDC values measured at lateral and medial standard leg sites. In figure 2 the absence of a correlation between TDC and TIF is visually observed and quantified by the regression equation for the lateral leg site shown in 2A as TDC = 46.5 + 0.005 TIF ($r^2 = 0.0078$, $p = 0.664$) and the medial leg site shown in 2B as TDC = 49.1 – 0.004 TIF ($r^2 = 0.0049$, $p = 0.714$). Contrastingly, as shown in (**figure 3)** there was a significant positive correlation between TIF and TDC as measured on the forearm and characterized by the linear regression equation shown in figure 3 as TDC = 23.7 + 0.089 TIF, $r^2 = 0.196$, $p =0.014$.

Leg-to-Arm TDC Values and Distributions

Average leg-to-arm TDC ratios (mean ± SD) were similar, whether the leg medial site was used (1.787 ± 0.363) or the lateral site was used (1.751 ± 0.287). (**Figure 4)** shows the distribution of LAR in comparison to the previously determined threshold level of 1.35[21] that is indicated in the figure as the horizontal dashed line. At the medial leg site 29/30 ratios (96.7%) exceeded the 1.35 threshold whereas at the lateral site 28/30 ratios (93.3%) exceeded the threshold.

DISCUSSION

Comparison of TDC values

Absolute TDC values to the depth measured on the forearm in the present study (28.0 ± 4.0) were similar to those reported (28.0 ± 2.4) in non-edematous forearms of 40 young healthy women (23.8 ± 2.7 years),[38]and reported (28.9 ± 4.1) in non-edematous forearms of 30 mature women (58.0 ± 12.0 years).[39] This suggests that the presence of lower extremity edematous or lymphedematous conditions has not altered the upper extremity skin water state and forearm values are representative of a normal hydration state in the presently studied group. Lower extremity TDC values on healthy non-edematous legs have been reported for 30 mature women as 29.4 ± 4.7 on medial calf at a site similar to that herein measured.[21] Thus, the lower extremity values for the presently evaluated edematous group are notably greater than normally measured, with a medial leg TDC value of 48.3 ± 8.3, that is significantly greater than TDC values of the previously measured healthy group ($p < 0.05$).

Comparison of TIF values

Contrastingly TIF values measured on anterior forearms in a group of 30 mature women (56.4 ± 7.6 years) yielded TIF values (43.8 ± 14.9 mN),[40] which were closer to those presently measured. Overall, the present forearm TIF values are consistent with most previously reported values. Fewer studies have provided TIF data on legs. One study estimated the average TIF in patients with cancer-related lower extremity lymphedema by determining TIF as the average of measurements at 20 systematically arranged points on the leg.[24] These values ranged from 71.1 ± 8.7 mN for stage I lymphedema to 160.5 ± 30.4 mN for stage III. A similar study in which 56 women with lower extremity lymphedema were evaluated indicated a leg average TIF value of 160 ± 70 mN in lymphedematous legs as compared to 90 ± 30 mN in contralateral apparently non-affected legs.[25] These prior values are somewhat less than herein measured at a single standardized lateral leg sites, but are not directly comparable since the prior values include both upper and lower leg measurements.

Coefficient of Variation Among Measurements

The mean CVI of TDC measurements expressed as percentage was less at the standard medial site than at the lateral site (3.38 ± 2.3 vs. 5.15 ± 4.9) and least at the forearm (2.7 ± 2.6). Contrastingly, the overall mean CVI of the five TIF measurements at the leg lateral site was 17.6 ± 10.4 and somewhat less at the forearm site (14.0 ± 8.6). The CVI of the present TDC measurements are consistent with previously reported measurements on forearm and legs[41]. Although there is data in the literature regarding variability of sequentially obtained five value TIF averages,[24, 25, 36, 42-44] this is the first report of the coefficient of variation of sequentially obtained individual values. These values provide an estimate of device measurement precision on lymphedematous lower extremities potentially useful in future assessment comparisons.

TDC-TIF Dependence

A new finding based on the present paired TDC-TIF measurements reveals the absence of an identifiable relationship between tissue dielectric constant and tissue indentation force in the lymphedematous legs evaluated. Contrastingly, a significant positive correlation between these two parameters was found on non-edematous forearms. Aside from the differences in anatomical locations, one possible functional explanation for these findings may relate to the role of water and tissue properties of the two sites since both water content and tissue properties affect tissue indentation force. Evaluation of non-edematous arms will likely require proportionately greater force to indent to a given depth for small differences in water content due to water's low compressibility. However, at already much higher water content levels, as was present to varying degrees with the edematous legs evaluated, the impact of incremental water content changes on required indention force is likely diminished. A test of this concept would be if the positive correlation between TIF and TDC herein observed in non-lymphedematous arms would be diminished or lost in breast cancer related arm lymphedema. Such a comparison study could be conducted with women who have various degrees of upper extremity unilateral lymphedema. Such a study would seem warranted to clarify the issue.

Leg-to-Arm TDC Ratios

As already noted, prior measurements of leg-to-arm TDC ratios of a group of 60 healthy subjects indicated that all ratios were less than a threshold value of 1.35[21]. In the same study six patients with congestive heart failure (CHF) were also measured and it was found for this small group, all but one had ratios greater than 1.35. The present results extend confidence in this threshold as an indicator parameter of the presence of lower extremity edema or lymphedema.

Study Limitations

There are several limitations to the present study that should be considered. The study included patients with diagnoses of lymphedema or edema. Thus, definitive statements regarding specific conditions cannot be fairly made. Further studies in which each of these conditions is dealt with separately would seem warranted to provide additional specificity. However, it can be stated that the proposed threshold is indicative of lower extremity excess fluid accumulation.

Another possible limitation is that only females were included. This was a practical limitation dictated by the nature of patient availability. However, one implication of this is that lower extremity lymphedematous conditions related to prostate surgery or radiation were excluded from evaluation. Another potential limitation was that TDC and TIF data sets were obtained on a given day with no attempt to repeat the measurements on subsequent days. Thus, the data represent a snap-shot view and further test-retest studies may be warranted to confirm the stability of the present findings.

CONCLUSION

In conclusion, the present findings suggest the following.

(1) In patients with lower extremity lymphedema or edema, TDC values are elevated compared to normal and average TDC values are similar as measured on medial or lateral standardized sites.

(2) The leg to forearm TDC ratios determined using either medial or lateral sites are similar to each other and have values consistent with a lymphedema threshold of 1.35 as previously reported.

(3) In edematous legs of the type evaluated herein, there is no apparent relationship between TDC values and indentation force.

AUTHOR CONTRIBUTIONS:

All authors contributed to and reviewed this manuscript and approved the final article.

AUTHOR DISCLOSURE STATEMENTS

Each author declares to have not conflict of interest and no competing financial interests.

FUNDING

This study was not funded.

REFERENCES

1. Rockson SG: The unique biology of lymphatic edema. Lymphat Res Biol. 2009;7:97-100.
2. Zampell JC, Aschen S, Weitman ES, et al.: Regulation of adipogenesis by lymphatic fluid stasis: part I. Adipogenesis, fibrosis, and inflammation. Plast Reconstr Surg. 2012;129:825-834.
3. Avraham T, Zampell JC, Yan A, et al.: Th2 differentiation is necessary for soft tissue fibrosis and lymphatic dysfunction resulting from lymphedema. FASEB J. 2013;27:1114-1126.
4. Ghanta S, Cuzzone DA, Torrisi JS, et al.: Regulation of inflammation and fibrosis by macrophages in lymphedema. Am J Physiol Heart Circ Physiol. 2015;308:H1065-1077.
5. Azhar SH, Lim HY, Tan BK, Angeli V: The Unresolved Pathophysiology of Lymphedema. Front Physiol. 2020;11:137.
6. Alanen E, Lahtinen T, Nuutinen J: Measurement of dielectric properties of subcutaneous fat with open-ended coaxial sensors. Phys Med Biol. 1998;43:475-485.
7. Gabriel S, Lau RW, Gabriel C: The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. Phys Med Biol. 1996;41:2251-2269.
8. Stuchly MA, Kraszewski A, Stuchly SS, Smith AM: Dielectric properties of animal tissues in vivo at radio and microwave frequencies: comparison between species. Phys Med Biol. 1982;27:927-936.
9. Cornish BH, Chapman M, Hirst C, et al.: Early diagnosis of lymphedema using multiple frequency bioimpedance. Lymphology. 2001;34:2-11.
10. Rockson SG: Tissue changes, bioimpedance, and acquired lymphedema. Lymphat Res Biol. 2013;11:195.
11. Ward LC, Koelmeyer LA, Moloney E: Staging Breast Cancer-Related Lymphedema with Bioimpedance Spectroscopy. Lymphat Res Biol. 2022;20:398-408.

12. De Vrieze T, Gebruers N, Nevelsteen I, et al.: Reliability of the MoistureMeterD Compact Device and the Pitting Test to Evaluate Local Tissue Water in Subjects with Breast Cancer-Related Lymphedema. Lymphat Res Biol. 2019.

13. Johansson K, Darkeh MH, Lahtinen T, Bjork-Eriksson T: Two-Year Follow-up of Temporal Changes of Breast Edema After Breast Cancer Treatment with Surgery and Radiation Evaluated by Tissue Dielectric Constant (TDC). The European Journal of Lymphology. 2015;27:15-21.

14. Karlsson K, Johansson K, Nilsson-Wikmar L, Brogardh C: Tissue Dielectric Constant and Water Displacement Method Can Detect Changes of Mild Breast Cancer-Related Arm Lymphedema. Lymphat Res Biol. 2021.

15. Koehler LA, Mayrovitz HN: Spatial and Temporal Variability of Upper Extremity Edema Measures After Breast Cancer Surgery. Lymphat Res Biol. 2019;17:308-315.

16. Liu Y, Long X, Guan J: Tissue Dielectric Constant Combined With Arm Volume Measurement as Complementary Methods in Detection and Assessment of Breast Cancer-Related Lymphedema. Lymphat Res Biol. 2021.

17. Mayrovitz HN, Arzanova E, Somarriba S, Eisa S: Factors affecting interpretation of tissue dielectric constant (TDC) in assessing breast cancer treatment related lymphedema (BCRL). Lymphology. 2019;52:92-102.

18. Mayrovitz HN, Weingrad DN, Lopez L: Patterns of temporal changes in tissue dielectric constant as indices of localized skin water changes in women treated for breast cancer: a pilot study. Lymphat Res Biol. 2015;13:20-32.

19. Birkballe S, Jensen MR, Noerregaard S, Gottrup F, Karlsmark T: Can tissue dielectric constant measurement aid in differentiating lymphoedema from lipoedema in women with swollen legs? Br J Dermatol. 2014;170:96-102.

20. Jensen MR, Birkballe S, Norregaard S, Karlsmark T: Validity and interobserver agreement of lower extremity local tissue water measurements in healthy women using tissue dielectric constant. Clin Physiol Funct Imaging. 2012;32:317-322.

21. Mayrovitz HN: Assessing Lower Extremity Lymphedema Using Upper and Lower Extremity Tissue Dielectric Constant Ratios: Method and Normal Reference Values. Lymphat Res Biol. 2019;17:457-464.

22. Mayrovitz HN, Davey S: Changes in tissue water and indentation resistance of lymphedematous limbs accompanying low level laser therapy (LLLT) of fibrotic skin. Lymphology. 2011;44:168-177.

23. Tugral A, Viren T, Bakar Y: Tissue dielectric constant and circumference measurement in the follow-up of treatment-related changes in lower-limb lymphedema. Int Angiol. 2018;37:26-31.

24. Sun D, Yu Z, Chen J, Wang L, Han L, Liu N: The Value of Using a SkinFibroMeter for Diagnosis and Assessment of Secondary Lymphedema and Associated Fibrosis of Lower Limb Skin. Lymphat Res Biol. 2017;15:70-76.

25. Yu Z, Liu N, Wang L, Chen J, Han L, Sun D: Assessment of Skin Properties in Chronic Lymphedema: Measurement of Skin Stiffness, Percentage Water Content, and Transepidermal Water Loss. Lymphat Res Biol. 2020;18:212-218.

26. van Belle G, Martin DC: Sample Size as a Function of Coefficient of Variation and Ratio of Means. The American Statistician. 1993;47:165-167.

27. Mayrovitz HN, Mikulka A, Woody D: Minimum Detectable Changes Associated with Tissue Dielectric Constant Measurements as Applicable to Assessing Lymphedema Status. Lymphat Res Biol. 2019;17:322-328.

28. Stuchly MA, Athey TW, Stuchly SS, Samaras GM, Taylor G: Dielectric properties of animal tissues in vivo at frequencies 10 MHz--1 GHz. Bioelectromagnetics. 1981;2:93-103.

29. La Gioia A, Porter E, Merunka I, et al.: Open-Ended Coaxial Probe Technique for Dielectric Measurement of Biological Tissues: Challenges and Common Practices. Diagnostics (Basel). 2018;8.

30. Nuutinen J, Ikaheimo R, Lahtinen T: Validation of a new dielectric device to assess changes of tissue water in skin and subcutaneous fat. Physiol Meas. 2004;25:447-454.

31. Arokoski JP, Surakka J, Ojala T, Kolari P, Jurvelin JS: Feasibility of the use of a novel soft tissue stiffness meter. Physiol Meas. 2005;26:215-228.

32. Iivarinen JT, Korhonen RK, Julkunen P, Jurvelin JS: Experimental and computational analysis of soft tissue stiffness in forearm using a manual indentation device. Med Eng Phys. 2011;33:1245-1253.

33. Iivarinen JT, Korhonen RK, Jurvelin JS: Experimental and numerical analysis of soft tissue stiffness measurement using manual indentation device--significance of indentation geometry and soft tissue thickness. Skin Res Technol. 2014;20:347-354.

34. Viren T, Iivarinen JT, Sarin JK, Harvima I, Mayrovitz HN: Accuracy and reliability of a hand-held in vivo skin indentation device to assess skin elasticity. Int J Cosmet Sci. 2018;40:134-140.

35. Mayrovitz HN, Yzer JA: Local Skin Cooling as an Aid to the Management of Patients with Breast Cancer Related Lymphedema and Fibrosis of the Arm or Breast. Lymphology. 2017;50:56-66.

36. Kim MA, Kim EJ, Lee HK: Use of SkinFibrometer((R)) to measure skin elasticity and its correlation with Cutometer((R)) and DUB((R)) Skinscanner. Skin Res Technol. 2018;24:466-471.

37. Faul F, Erdfelder E, Lang AG, Buchner A: G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-191.

38. Mayrovitz HN, Lorenzo-Valido C, Pieper E, Thomas A: Forearm and biceps circumferential variations in skin tissue dielectric constant and firmness. Lymphology. 2020;53:204-211.

39. Mayrovitz HN: Assessing Lower Extremity Lymphedema Using Upper and Lower Extremity Tissue Dielectric Constant Ratios: Method and Normal Reference Values. Lymphat Res Biol. 2019.

40. Mayrovitz HN, Wong J, Fasen M: Age and Hydration dependence of jowl and forearm skin firmness in young and mature women. J Cosmet Dermatol. 2017.

41. Mayrovitz HN: Assessing Upper and Lower Extremities Via Tissue Dielectric Constant: Suitability of Single Versus Multiple Measurements Averaged. Lymphat Res Biol. 2019;17:316-321.

42. Douglass J, Graves P, Gordon S: Intrarater Reliability of Tonometry and Bioimpedance Spectroscopy to Measure Tissue Compressibility and Extracellular Fluid in the Legs of Healthy Young People in Australia and Myanmar. Lymphat Res Biol. 2017;15:57-63.

43. Mayrovitz HN, Corbitt K, Grammenos A, Abello A, Mammino J: Skin indentation firmness and tissue dielectric constant assessed in face, neck, and arm skin of young healthy women. Skin Res Technol. 2017;23:112-120.

44. Seo SR, Kang NO, Yoon MS, Lee HJ, Kim DH: Measurements of scar properties by SkinFibroMeter((R)) , SkinGlossMeter((R)) , and Mexameter((R)) and comparison with Vancouver Scar Scale. Skin Res Technol. 2017;23:295-302.

Figure Legends

Figure 1. Measurement Sites

The forearm measuring site is shown in A) and the medial leg measurement site is shown in B). The lateral leg measuring site (not shown) is circumferentially located 180^o from the medial site. The **tissue dielectric constant (**TDC) measuring device is shown in B) as it would be applied.

Figure 2. TDC – Indentation Force Relationships on Leg

In A) Individual paired **tissue dielectric constant – tissue indentation force (**TDC-TIF) values measured on the target leg at the lateral site along with its calculated regression line (dashed). In B) is the corresponding data as measured on the medial leg site. As may be visualized by the near horizontal regression lines there is no significant correlation between the two parameters at either site. The corresponding regression equations are given in the text.

Figure 3. TDC – Indentation Force Relationships on Forearm

Individual paired **tissue dielectric constant – tissue indentation force** (TDC-TIF) values measured on the forearm and the calculated regression line (dotted) with the associated linear regression equation shown in the figure demonstrating a significant positive correlation between TDC and TIF.

Figure 4. Leg-to-Arm TDC Ratios (LAR)

Individual **leg-to-arm ratios (**LAR), are calculated using the lateral leg measurements (circles) and using the medial leg measurements (triangles). The short solid horizontal lines represent the means and the longer dashed line represents an LAR proposed threshold value of 1.35. Based on the medial leg data 29/30 ratios (96.7%) exceeded the 1.35 threshold whereas using the lateral site data 28/30 ratios (93.3%) exceeded the threshold.

Tables

	Lower Extremity Edema	Lower Extremity Lymphedema	p-value	Combined
Diagnosis, N (%)	18 (60%)	12 (40%)		30
Age (years)	77.8 ± 11.4	76.6 ± 11.2	0.755	77.3 ± 11.2
BMI (Kg/m²)	32.1 ± 10.0	32.6 ± 11.3	0.884	32.3 ± 10.4
Duration (years)	6.6 ± 6.2	14.7 ± 12.4	0.079	9.8 ±9.9
Target Leg Measured = Right	7 (38.9%)	8 (66.7%)		
Arm Measured = Dominant	7 (38.9%)	7 (58.3%)		

Table 1. Patient Features

Table values are mean ± SD. Duration is the number of years the lower extremity condition has been present. The p-values are based on Mann-Whitney tests. The target leg was the leg that had the greater visually observed swelling and the forearm measurement was on the same side as the target leg. Right indicates the right leg was the target otherwise it was the left. Dominant indicates if the arm measurement was on the dominant side otherwise it was on the non-dominant side.

	Standard Site of Target Leg			Standard Site of **Contralateral** Leg			Forearm		
Circumference (cm)	27.9 ± 6.6**			24.9 ± 5.2			24.8 ± 4.0	Leg/Forearm TDC Ratios	
	Medial	Lateral	Average	Medial	Lateral	Average	Anterior	Medial	Lateral
TDC values	48.3 ± 8.3	47.5 ± 7.7	47.9 ± 7.1	41.8 ± 11.1	46.8 ± 8.9*	44.3 ± 9.2	28.0 ± 4.0	1.787 ± 0.363	1.751 ± 0.287
TIF values (mN)		216.9 ± 149.8			229.6 ± 167.9		48.4 ± 19.9		
TDC_CVI (%)	3.38 ± 2.3	5.15 ± 4.94*		4.11 ± 7.4	4.78 ± 4.08		2.7 ± 2.6		
TIF_CVI (%)		17.6 ± 10.4			16.4 ± 11.9		14.0 ± 8.6		

Table 2. TDC and TIF values and individual measurement coefficients of variation

Table entries are mean ± SD. TDC is the tissue dielectric constant (dimensionless) and TIF is the tissue indentation force in mN. TDC_CVI and TIF_CVI are their corresponding coefficients of variation (CV) expressed as a percentage. CVI is based on the average of multiple individual measurements for each of the 30 patients. Asterix (*) denotes significantly different between medial and lateral sites at a p-value < 0.05 and ** denotes leg difference at a p-value < 0.01.

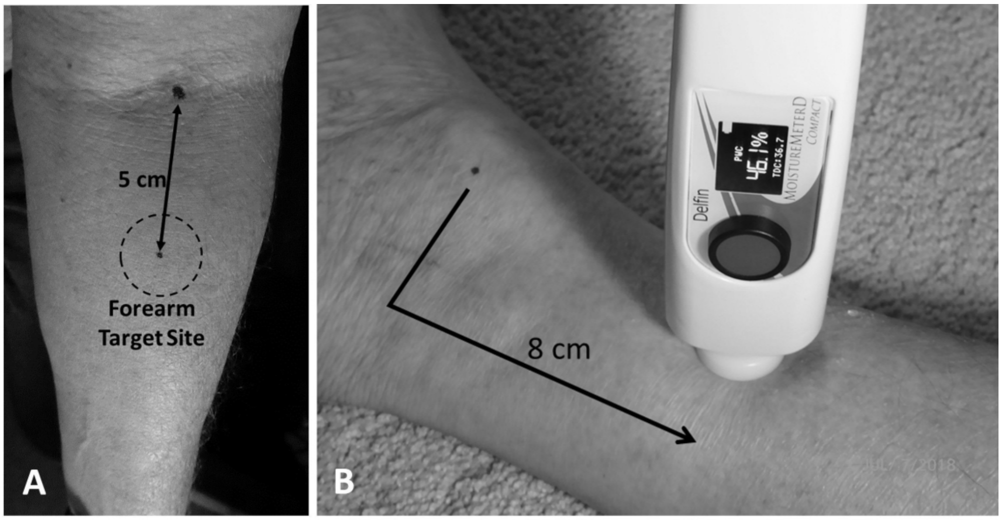

Figure 1. Measurement SitesThe forearm measuring site is shown in A) and the medial leg measurement site is shown in B). The lateral measuring site on the leg (not shown) is circumferentially located 180^{o} from the medial site. The tissue dielectric constant (TDC) measuring device is shown in B) as it would be applied.

98x51mm (600 x 600 DPI)

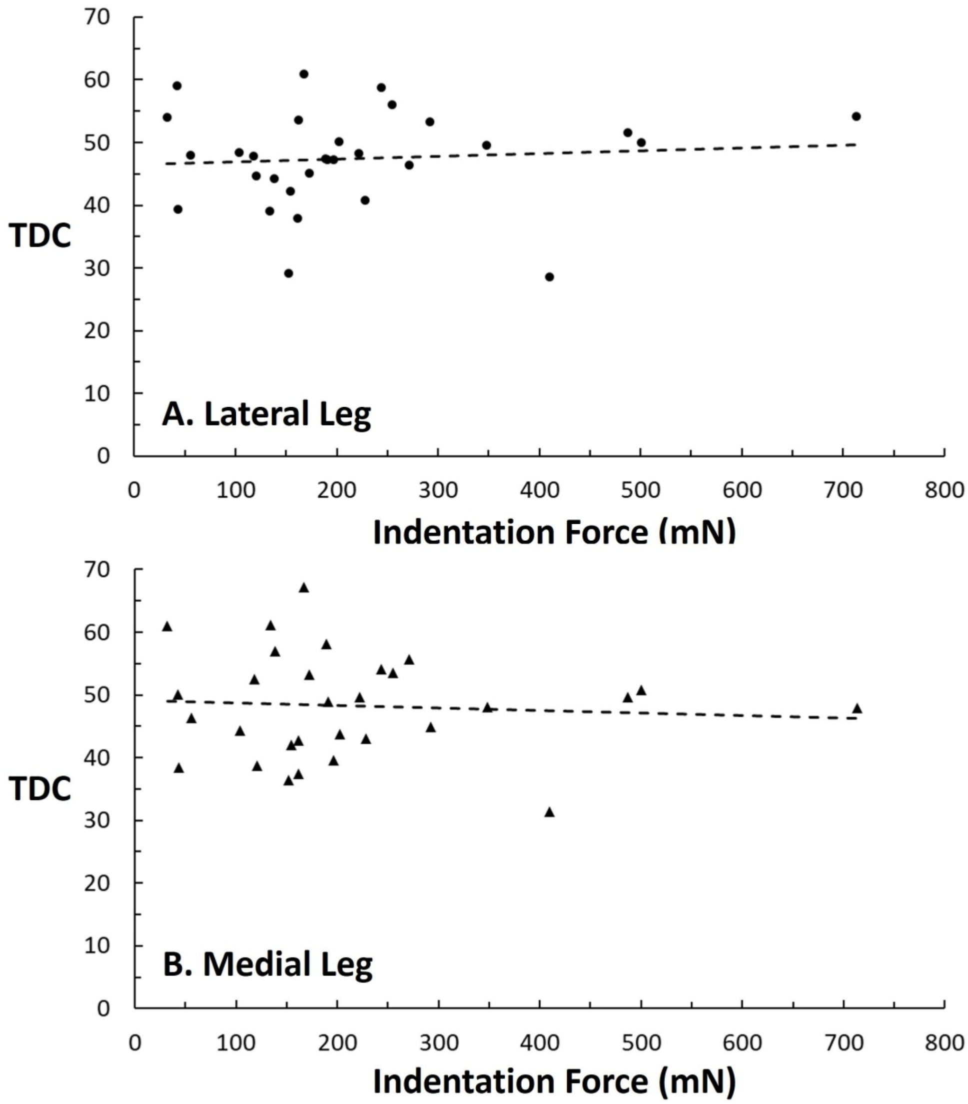

Figure 2. TDC – Indentation Force Relationships on LegIn A) Individual paired tissue dielectric constant-tissue indentation force (TDC-TIF) values measured on the target leg at the lateral site along with its calculated regression line (dashed). In B) is the corresponding data as measured on the medial leg site. As may be visualized by the near horizontal regression lines there is no significant correlation between the two parameters at either site. The corresponding regression equations are given in the text.

92x104mm (600 x 600 DPI)

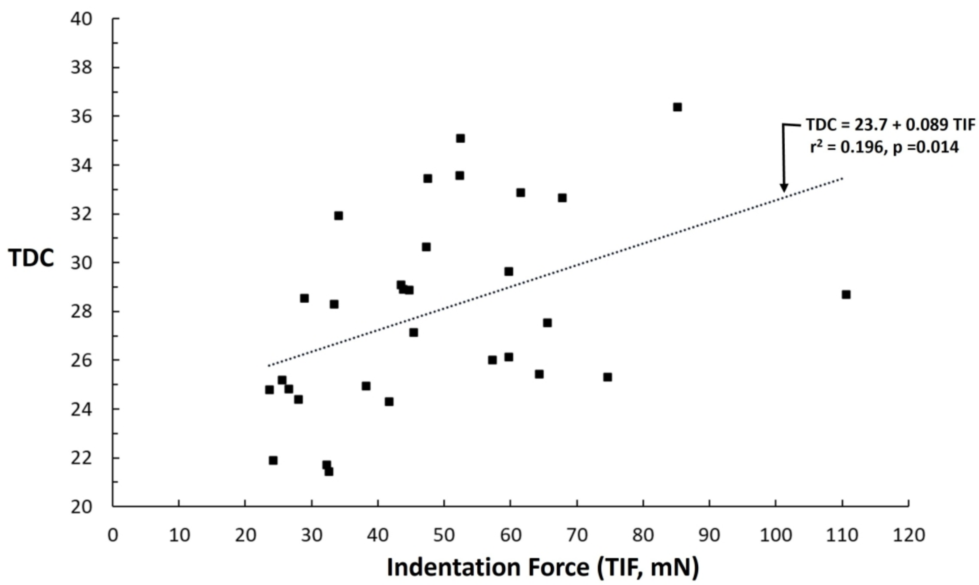

Figure 3. TDC – Indentation Force Relationships on ForearmIndividual paired tissue dielectric constant-tissue indentation force (TDC-TIF) values measured on the forearm and the calculated regression line (dotted) with the associated linear regression equation shown in the figure demonstrating a significant positive correlation between TDC and TIF.

227x146mm (330 x 330 DPI)

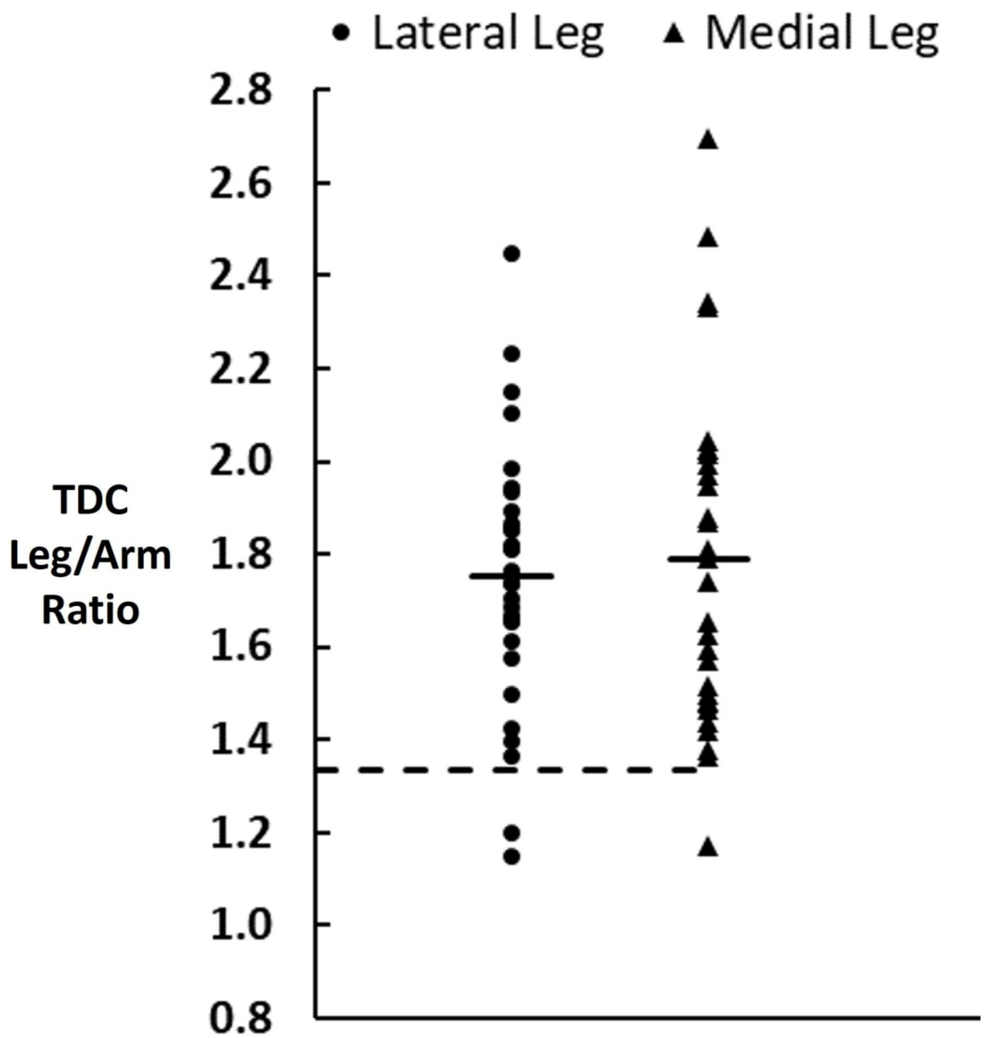

Figure 4. Leg-to-Arm TDC Ratios (LAR)Individual leg-to-arm ratio (LAR) values calculated using the lateral leg measurements (circles) and using the medial leg measurements (triangles). The short solid horizontal lines represent the means and the longer dashed line represents an LAR proposed threshold value of 1.35. Based on the medial leg data 29/30 ratios (96.7%) exceeded the 1.35 threshold whereas using the lateral site data 28/30 ratios (93.3%) exceeded the threshold.

76x82mm (600 x 600 DPI)

	Lower Extremity Edema	Lower Extremity Lymphedema	p-value	Combined
Diagnosis, N (%)	18 (60%)	12 (40%)		30
Age (years)	77.8 ± 11.4	76.6 ± 11.2	0.755	77.3 ± 11.2
BMI (Kg/m^2)	32.1 ± 10.0	32.6 ± 11.3	0.884	32.3 ± 10.4
Duration (years)	6.6 ± 6.2	14.7 ± 12.4	0.079	9.8 ±9.9
Target Leg Measured = Right	7 (38.9%)	8 (66.7%)		
Arm Measured = Dominant	7 (38.9%)	7 (58.3%)		

Table 1. Patient Features

Table values are mean ± SD. Duration is the number of years the lower extremity condition has been present. The p-values are based on Mann-Whitney tests. The target leg was the leg that had the greater visually observed swelling and the forearm measurement was on the same side as the target leg. Right indicates the right leg was the targert otherwise it was the left. Dominant indicates if the arm measurement was on the dominant side otherwise it was on the non-dominant side.

	Standard Site of Target Leg			Standard Site of Contralateral Leg			Forearm		
Circumference (cm)	27.9 ± 6.6**			24.9 ± 5.2			24.8 ± 4.0	Leg/Forearm TDC Ratios	
	Medial	Lateral	Average	Medial	Lateral	Average	Anterior	Medial	Lateral
TDC values	48.3 ± 8.3	47.5 ± 7.7	47.9 ± 7.1	41.8 ± 11.1	46.8 ± 8.9*	44.3 ± 9.2	28.0 ± 4.0	1.787 ± 0.363	1.751 ± 0.287
TIF values (mN)		216.9 ± 149.8			229.6 ± 167.9		48.4 ± 19.9		
TDC_CVI (%)	3.38 ± 2.3	5.15 ± 4.94*		4.11 ± 7.4	4.78 ± 4.08		2.7 ± 2.6		
TIF_CVI (%)		17.6 ± 10.4			16.4 ± 11.9		14.0 ± 8.6		

Table 2. <u>TDC and TIF values and individual measurement coefficients of variation</u>

Table entries are mean ± SD. TDC is the tissue dielectric constant (dimensionless) and TIF is the tissue indentation force in mN. TDC_CVI and TIF_CVI are their corresponding coefficients of variation (CV) expressed as a percentage. CVI is based on the average of multiple individual measurements for each of the 30 patients. Asterix (*) denotes significantly different between medial and lateral sites at a p-value < 0.05 and ** denotes leg difference at a p-value < 0.01.