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GENETIC VARIANTS DISTRIBUTION IN PRIMARY LYMPHEDEMA AND EVALUATION OF HYDROXYTYROSOL AS A CANDIDATE THERAPEUTIC MOLECULE

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ABSTRACT

A chronic inflammatory disease that results from ineffective fluid uptake in peripheral tissues and affects mainly the lower limbs, lymphedema is either primary when caused by genetic mutations, or secondary when it develops following injury, infection or surgery. The genetic tests for the diagnosis of lymphedema have poor efficacy, and specific treatments targeting the molecular pathways involved in its pathogenesis are lacking. Thus, we investigated the genetic variants distribution in a cohort of patients with lymphedema, and designed a molecular pathway diagram that comprises the genes involved in the onset of lymphedema. Finally, we considered the effects of hydroxytyrosol, a polyphenol extracted from olive trees with anti-inflammatory, antimicrobial and antioxidant properties, on the identified molecular pathways. Although further investigations are needed to confirm the efficacy of hydroxytyrosol in treating lymphedema, this preliminary study addresses this natural compound as a promising therapeutic molecule.

Keywords: lymphedema, genetic variants, PI3K/AKT, RAS/MAPK, Rho/ROCK, hydroxytyrosol

INTRODUCTION

Lymphedema is a chronic inflammatory disease characterized by abnormal accumulation of lymph in interstitial fluids caused by ineffective fluid uptake by peripheral lymphatic vessels. Overall, lymphedema affects 140-250 million people worldwide. It can be primary, when caused by genetic mutations, or secondary, when it follows injury, infection or surgery[1, 2]. The prevalence of primary lymphedema is 1/100,000, while the prevalence of secondary lymphedema is 1/1,000[3, 4]. Lymphedema results in debilitating pain, infections, and lymphatic damage. Lymphedema can be both unilateral and bilateral, and it usually affects lower and upper limbs, genitalia, and face[5]. Although several genes are now correlated to syndromic and non-syndromic forms of lymphedema, genetic tests have still a high percentage, up to 43%, of negative results in patients clinically diagnosed for primary lymphedema[6]. This disappointing result clearly indicates that further investigations are needed to identify adequate diagnostic target.[1, 7, 8]

An effective therapy for lymphedema is still missing. Although symptoms are normally alleviated by massages and elastic bands to favor lymphatic drainage, a scientifically-based therapeutic approach targeting the molecular pathways involved in lymphedema's pathogenesis is not yet available, which compromises a resolutive cure for patients[9]. A few pharmacological treatments used to reduce swelling and inflammation include anti-inflammatory drugs, diuretics, antibiotic and analgesics, but with poor results. In some cases, surgical interventions as lymphatic by-pass are required.[10–13]

Hydroxytyrosol (HT), recently proposed for lymphedema treatment[2] is, along with tyrosol, oleuropein and oleocanthal, one of the main phenolic compounds in olives and olive oil.[14] HT was proposed as one of the most important molecules supporting the beneficial effects of the Mediterranean diet[15–17] and is being studied as a nutraceutical in many instances.[18, 19] HT has anti-inflammatory, antioxidant and antimicrobic properties.[2–4] HT inhibits the synthesis of leukotriene B4 (LTB4), a key molecule in lymphedema

pathogenesis.[2] LTB4 reduces the expression of pro-angiogenic factors and reduces the Notch signaling, both important paths for development and functioning of the lymphatic system. Moreover, LTB4 promotes the differentiation of T cells in the Th17 phenotype, which is related to filariasis lymphedema.[5, 13, 20]

The aim of this study is to assess the genetic variants distribution in a cohort of Italian lymphedema patients. Moreover, we aim to study the effects of HT on specific molecular pathways involved in lymphedema pathogenesis. Although few studies have proposed HT as a treatment for lymphedema, its specific molecular effects have yet to be clearly understood. This electronic search could be useful to provide a molecular explanation of HT's beneficial effects, supporting its use in lymphedema treatment.

MATERIALS AND METHODS

4.1. Genetic and Data Analysis

Genetic analysis has been conducted as previously reported.[6, 21] In brief, either peripheral blood or saliva (5 mL) from probands was used for DNA extraction using a commercial kit (Blood DNA kit E.N.Z.A., Omega Biotek, Inc. Doraville, GA, USA or Exgene Clinic SV mini, GeneAll Biotechnology, Seoul, South Korea). PCR and direct sequencing of amplified fragments were used to analyze exons coding for the studied genes, and the respective portions of the intron regions adjacent to the exons.[22] Primer sequences, PCR reaction conditions, and sequencing conditions are available on request. All the samples were then analyzed using NGS Sequencing, and poorly covered target regions were confirmed using Sanger sequencing.[6]

4.2. Creation of Molecular Pathway Diagrams

Information used in this study is based on literature data. A proposal for the most important molecular pathways involved in primary lymphedema has been published recently.[6] We conducted a search on PubMed [23] and Scopus [24] using the following strings: (hydroxytyrosol [Text Word]) AND (PI3K/AKT [Text Word] OR RAS/MAPK[Text Word] OR MAPK[Text Word] OR Rho/ROCK[Text Word] OR ROCK[Text Word]). Articles with detailed description of the effects of HT on the activation of one of these pathways have been included. Moreover, their reference lists were scanned to possibly include other scientific articles. Finally, molecular pathway diagrams were created using PathVisio software.[25]

RESULTS

Genes correlated to lymphedema onset and genetic variants distribution in a cohort of patients with lymphedema

Table 1 reports the genes correlated with the onset of primary lymphedema that participate in the PI3K/AKT, RAS/MAPK and Rho/ROCK pathways. The gene–phenotype relationship, the molecular pathways in which they are involved and their effect on the involved pathways are also reported therein. Figure 1, that reports the genetic variants distribution of the genes reported in Table 1 referred to the patients presented in(6) shows that CELSR1, PTPN14 and PIEZO1 are the most represented genes. Of the 147 lymphedema patients analyzed in(6), 71 presented genetic variants in the genes reported in Table 1.

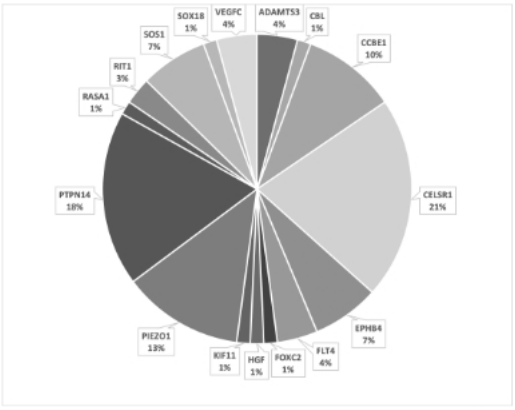

Figure 1: *Genetic variants distribution of the genes reported in Table 1 referred to the patients presented in[6] (n = 71).*

Effect of HT on the molecular pathways involved in primary lymphedema

Figure 2 shows the molecular pathways in which all the proteins encoded by the genes reported in Table 1 participate. Proteins encoded by genes involved in the onset of primary lymphedema participate in three main molecular pathways: the PI3K/AKT, the RAS/MAPK and the Rho/ROCK pathway. Several membrane receptors participate in these signaling, with VEGFR-3 and HGFR (MET) being the most influent in lymphedema molecular pathways and thus probably in lymphedema pathogenesis.[6, 33] HT influences the activation of all the considered molecular pathways.

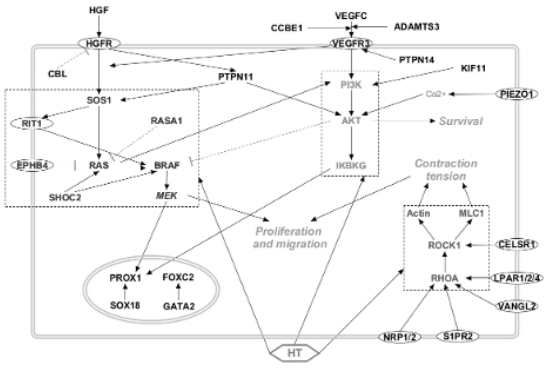

Figure 2: *Molecular pathways involved in primary lymphedema and hydroxytyrosol effects. The proteins coded by the selected genes related to primary lymphedema are represented in a somatic cell and participates in three main pathways: PI3K/AKT (red), RAS/MAPK (blue), Rho/ROCK (violet). Proteins that are not part of the three pathways, but that influence their signaling, are represented in black. Black arrows represent a positive interaction, while red T-bars represent an inhibition.*

DISCUSSION

This study shows the distribution of genetic variants in a cohort of Italian lymphedema patients(6). CELSR1, PTPN14 and PIEZO1 are the most represented genes, being mutated in 21%, 18%, and 13%, respectively, of the patients with genetic mutation in the genes reported in Table 1. CELSR1, PTPN14 and PIEZO1, as well as the other genes reported in Table 1, are involved in the activation of several molecular pathways that may be involved in lymphedema pathogenesis, among which the PI3K/AKT, the RAS/MAPK and the Rho/ROCK pathways(6).

HT and PI3K/AKT pathway

The PI3K/AKT pathway is an essential molecular pathway in many physiological processes, among which inflammation, cell proliferation and apoptosis as well in the lymphangiogenesis process, as mutations in PI3KA and AKT are indeed correlated with the etiopathogenesis of lymphedema(33,48,49). Several reports have studied the effect of HT on the PI3K/AKT pathway. A study by Parra-Perez et al(50) reports that HT inhibits the PI3K/AKT pathway in leukemia T cells stimulating apoptosis through blockade of PI3K, which reduces AKT phosphorylation and subsequently proliferation and survival signals(50). On the other hand, another study suggests that HT activates the PI3K/AKT pathway as HT activates the PI3K/AKT pathway in ischemic rat hearts, inhibiting apoptosis and promoting cells survival(51). Moreover, HT seems to protect cells from oxidative stress and hypoxia-mediated damages activating the PI3K/AKT in several cell types, among which vascular endothelial cells(52–54). Indeed, the PI3K/AKT pathway have been correlated to heart and brain protection under hypoxic condition, suggesting being a viable therapeutic target(55–58). Although new research on lymphatic cells may shed new light on the effects of HT, these studies support the activation of the PI3K/AKT pathway by HT.

HT and the RAS/MAPK pathway

The RAS/MAPK pathway controls several physiological processes, among which cell differentiation, angiogenesis and wound healing(59). Moreover, it promotes lymphangiogenesis, and mutations to several genes involved in this pathway correlates with primary lymphedema onset(60–62). Thus, studying the effect of HT on the RAS/MAPK pathway activation could be important in evaluating its potential therapeutic effect in lymphedema. As for the PI3K/AKT pathway, the evidence on the effect of HT on the RAS/MAPK pathway is contradictory. Indeed, a study by Parra-Perez et al(50) demonstrated that HT induces apoptosis in leukemia T cells and activates the RAS/MAPK pathway, stimulating differentiation of tumoral cells(50). Other research articles report an activating effect of HT on the RAS/MAPK pathway, also in vascular endothelial cells(53,54). On the contrary, a study by Zhang et al. reports that HT reduces the activation of the RAS/MAPK pathway in osteoclasts, inhibiting their differentiation(63). As for the PI3K/AKT pathway, the available research studies sustain that HT influences the RAS/MAPK pathway, but its precise mechanism of action is still unknown. Thus, further studies on lymphatic endothelial cells could be useful to elucidate the effect of HT on the RAS/MAPK pathway.

HT and Rho/ROCK pathway

The Rho/ROCK pathway was recently proposed to be correlated to lymphedema pathogenesis(6). Indeed, the Rho/ROCK pathway controls cell migration and motility, controlling cytoskeleton remodeling of endothelial cells, and is involved in angiogenesis and lymphangiogenesis(64–67). Moreover, the Rho/ROCK pathway controls lymph pumps activation, an important process in lymphedema pathogenesis(68,69). RhoA activates ROCK, which in turn promotes myosin light chain phosphorylation, its interaction with actin, and finally fiber contraction. Rho/ROCK pathway was also demonstrated to activate the PI3K/AKT and the RAS/MAPK pathway(70,71). Retrieving scientific literature, only one research article was found to evaluate the effects of HT on the Rho/ROCK pathway(72). Indeed, the study by Abate et al. proves that HT can stimulate endothelial cell migration in vitro increasing the expression of several proteins, among which ROCK. Moreover, this study suggests that HT stimulates vascular formation increasing the expression of VEGFR2 and of proteins related to the PI3K/AKT pathway(72). Although new studies are needed to confirm that action of HT on the Rho/ROCK pathway, the beneficial effects of HT could also be mediated by this molecular pathway.

CONCLUSION

The low efficacy of genetic tests and the absence of targeted therapies for lymphedema calls for a deeper insight into the molecular mechanisms underlying this pathology. The analysis of the most frequent genetic variants in a cohort of 147 lymphedema patients revealed that CELSR1, PTPN14 and PIEZO1 are the most frequently altered genes. Such variants appear to affect the PI3K/AKT, the RAS/MAPK and the Rho/ROCK pathways, that are therefore proposed of importance in the development of lymphedema. As HT, a polyphenol from olive trees, is deeply involved in the regulation of the aforementioned pathways, it is likely that it may be a valid therapeutical target in the treatment of this debilitating disease. Further investigations are under way to verify this hypothesis.

GENE	OMIM	GENE-PHENOTYPE RELATIONSHIP	REFERENCE	PATHWAYS	REFERENCE	EFFECT
ADAMTS3	605011	Hennekam lymphangiectasia-lymphedema syndrome 3	618154	VEGFR3 pathway	(26)	Activation
AKT1	164730	Proteus syndrome, somatic	176920	Ras pathway	map04014	Inactivation
				PI3K/AKT pathway	map04151	Activation
BRAF	164757	Noonan syndrome 7	613706	MAPK pathway	map04010	Activation
CBL	165360	Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia	613563	Ras pathway	(27)	Inactivation
CCBE1	612753	Hennekam lymphangiectasia-lymphedema syndrome 1	235510	VEGFR3 pathway	(28)	Activation
CELSR1	604523	Lymphatic malformation 9	619319	Planar cell polarity	(29)	Activation
				Rho and cytoskeletal remodelling pathway	(30)	Activation
EPHB4	600011	Capillary malformation-arteriovenous malformation 2	618196	Ras pathway	(31)	Inactivation
		Lymphatic malformation 7	617300			
FLT4	136352	Lymphatic malformation 1	153100	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
FOXC2	602402	Lymphedema-distichiasis syndrome	153400	Transcription factors	(32)	Activation
GATA2	137295	Emberger syndrome	614038	Transcription factors	(33)	Activation
HGF	142409	Primary lymphedema	(8)	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
HRAS	190020	Costello syndrome	218040	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
KIF11	148760	Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation	152950	PI3K/AKT pathway	(34)	Activation
KRAS	190070	Noonan syndrome 3	609942	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
LPAR1	602282	Lymphedema	(35)	PI3K/AKT pathway	map04151	Activation
LPAR2	605110	Lymphedema	(35)	PI3K/AKT pathway	map04151	Activation
LPAR4	300086	Lymphatic vascular development	(36)	PI3K/AKT pathway	map04151	Activation
NRP1	602069	Lymphedema	(37)	Rho and cytoskeletal remodelling pathway	(38)	Activation
NRP2	602070	Lymphedema	(37)	Rho and cytoskeletal remodelling pathway	(38)	Activation
NRAS	164790	Noonan syndrome 6	613224	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
PIEZO1	611184	Lymphatic malformation 6	616843	PI3K/AKT pathway	(39)	Activation
				Lymphatic valve development	(40)	Activation
PIK3CA	171834	CLAPO syndrome, somatic	613089	Ras pathway	map04014	Activation
		CLOVE syndrome, somatic	612918	PI3K/AKT pathway	map04151	Activation
PTPN11	176876	Noonan syndrome 1	163950	Ras pathway	map04014	Activation
				PI3K/AKT pathway	(41)	Activation
PTPN14	603155	Choanal atresia and lymphedema	613611	VEGFR3 pathway	(42)	Activation
RASA1	139150	Capillary malformation-arteriovenous malformation 1	608354	Ras pathway	map04014	Inactivation
RIT1	609591	Noonan syndrome 8	615355	Ras pathway	(43)	Activation
S1PR2	605111	Lymphatic vessels contraction	(44)	Rho and cytoskeletal remodelling pathway	(45)	Activation
SHOC2	602775	Noonan syndrome-like with loose anagen hair 1	607721	Ras pathway	map04014	Activation
SOS1	182530	Noonan syndrome 4	(1)	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation
SOX18	601618	Hypotrichosis-lymphedema-telangiectasia syndrome	607823	Transcription factors	(33)	Activation
VANGL2	600533	Lymphedema	(46)	Rho and cytoskeletal remodelling pathway	(47)	Activation
VEGFC	601528	Lymphatic malformation 4	615907	Ras pathway	map04014	Activation
				PI3K/AKT pathway	map04151	Activation

Table 1: *List of genes involved in lymphedema pathogenesis. Name of the gene, OMIM number, gene-phenotype relationship, relative reference and inheritance, involved pathway, relative reference and effect on the pathway are reported. References are from OMIM, Kegg, or literature.*

REFERENCES

1. Paolacci S., Rakhmanov Y., Maltese P.E., Zulian A., Michelini S., Bertelli M. (2018) Genetic testing for lymphatic malformations with or without primary lymphedema. Eurobiotech J. 2:5–9.

2. Bertelli M., Kiani A.K., Paolacci S., Manara E., Dautaj A., Beccari T., et al. (2020) Molecular pathways involved in lymphedema: Hydroxytyrosol as a candidate natural compound for treating the effects of lymph accumulation. J Biotechnol. 308:82–6.

3. Dhuli K., Ceccarini M.R., Precone V., Maltese P.E., Bonetti G., Paolacci S., et al. (2021) Improvement of quality of life by intake of hydroxytyrosol in patients with lymphedema and association of lymphedema genes with obesity. Eur Rev Med Pharmacol Sci. 25.33–42.

4. Bhusan Tripathi Y., Pandey N., Mishra P., Tripathi P., Coatto M., Anpilogov K., et al. (2021) Effect of a dietary supplement on the reduction of lymphedema-progression in mouse tail-cut model. Eur Rev Med Pharmacol Sci. 25:56–66.

5. Michelini S., Cestari M., Michelini S., Camilleri G., de Antoni L., Sonna W.N., et al. (2020) Study of a supplement and a genetic test for lymphedema management. Acta Biomed. 91:e2020013.

6. Bonetti G., Paolacci S., Samaja M., Maltese P.E., Michelini S., Michelini S., et al. (2022) Low Efficacy of Genetic Tests for the Diagnosis of Primary Lymphedema Prompts Novel Insights into the Underlying Molecular Pathways. Int J Mol Sci. 23:7414.

7. Michelini S., Degiorgio D., Cestari M., Corda D., Ricci M., Cardone M., et al. (2012) Clinical and genetic study of 46 Italian patients with primary lymphedema. Lymphology. 45:3–12.

8. Michelini S., Vettori A., Maltese P.E., Cardone M., Bruson A., Fiorentino A., et al. (2016) Genetic Screening in a Large Cohort of Italian Patients Affected by Primary Lymphedema Using a Next Generation Sequencing (NGS) Approach. Lymphology. 49:57–72.

9. Hespe G.E., Nores G.G., Huang J.J., Mehrara B.J. (2017) Pathophysiology of lymphedema-Is there a chance for medication treatment? J Surg Oncol. 115:96–8.

10. Cavezzi A., Urso S.U., Ambrosini L., Croci S., Campana F., Mosti G. (2019) Lymphedema and nutrition: A review. Veins and Lymphatics. 8.

11. Keeley V. (2008) Pharmacological treatment for chronic oedema. Br J Community Nurs. 13:S4–10.

12. Ramelet A.A. (2000) Pharmacologic aspects of a phlebotropic drug in CVI-associated edema. Angiology. 51:19–23.

13. Executive Committee of the International Society of Lymphology. (2020) The diagnosis and treatment of peripheral lymphedema: 2020 Consensus Document of the International Society of Lymphology. Lymphology. 53:3–19.

14. Karković Marković A., Torić J., Barbarić M., Jakobušić Brala C. (2019) Hydroxytyrosol, Tyrosol and Derivatives and Their Potential Effects on Human Health. Molecules. 24:2001.

15. Kiani A.K., Medori M.C., Bonetti G., Aquilanti B., Velluti V., Matera G., et al. (2022) Modern vision of the Mediterranean diet. J Prev Med Hyg. 63:E36–43.

16. Naureen Z., Bonetti G., Medori M.C., Aquilanti B., Velluti V., Matera G., et al. (2022) Foods of the Mediterranean diet: lacto-fermented food, the food pyramid and food combinations. J Prev Med Hyg. 63:E28–35.

17. Naureen Z., Dhuli K., Donato K., Aquilanti B., Velluti V., Matera G., et al. (2022) Foods of the Mediterranean diet: tomato, olives, chili pepper, wheat flour and wheat germ. J Prev Med Hyg. 63:E4–11.

18. Robles-Almazan M., Pulido-Moran M., Moreno-Fernandez J., Ramirez-Tortosa C., Rodriguez-Garcia C., Quiles J.L., et al. (2018) Hydroxytyrosol: Bioavailability, toxicity, and clinical applications. Food Research International. 105:654–67.

19. Bonetti G., Dhuli K., Michelini S., Michelini S., Michelini S., Ricci M., et al. (2022) Dietary supplements in lymphedema. J Prev Med Hyg. 63:E200–5.

20. Ghanta S., Cuzzone D.A., Torrisi J.S., Albano N.J., Joseph W.J., Savetsky I.L., et al. (2015) Regulation of inflammation and fibrosis by macrophages in lymphedema. American Journal of Physiology-Heart and Circulatory Physiology. 308:H1065–77.

21. Bonetti G., Dhuli K., Ceccarini M.R., Kaftalli J., Samaja M., Precone V., et al. (2022) Next-Generation Sequencing of a Large Gene Panel for Outcome Prediction of Bariatric Surgery in Patients with Severe Obesity. J Clin Med. 11:7531.

22. Dhuli K., Bonetti G., Anpilogov K., Herbst K.L., Connelly S.T. , Bellinato F., et al. (2022) Validating methods for testing natural molecules on molecular pathways of interest in silico and in vitro. J Prev Med Hyg. 63:E279–88.

23. PubMed. Available online: https://pubmed.ncbi.nlm.nih.gov/.

24. Scopus. Available online: https://www.scopus.com/home.uri.

25. PathVisio biological pathway editor. Available online: https://pathvisio.org/.

26. Brouillard P., Dupont L., Helaers R., Coulie R., Tiller G.E., Peeden J., et al. (2017) Loss of ADAMTS3 activity causes Hennekam lymphangiectasia–lymphedema syndrome 3. Hum Mol Genet. 264095–104.

27. Petrelli A., Gilestro G.F., Lanzardo S., Comoglio P.M., Migone N., Giordano S. (2002) The endophilin–CIN85–Cbl complex mediates ligand-dependent downregulation of c-Met. Nature. 416:187–90.

28. le Guen L., Karpanen T., Schulte D., Harris N.C., Koltowska K., Roukens G., et al. (2014) Ccbe1 regulates Vegfc-mediated induction of Vegfr3 signaling during embryonic lymphangiogenesis. Development. 141:1239–49.

29. Robinson A., Escuin S., Doudney K., Vekemans M., Stevenson R.E., Greene N.D.E., et al. (2012) Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis. Hum Mutat. 33:440–7.

30. Nishimura T., Honda H., Takeichi M. (2012) Planar Cell Polarity Links Axes of Spatial Dynamics in Neural-Tube Closure. Cell. 149:1084–97.

31. Amyere M., Revencu N., Helaers R., Pairet E., Baselga E., Cordisco M., et al. (2017) Germline Loss-of-Function Mutations in EPHB4 Cause a Second Form of Capillary Malformation-Arteriovenous Malformation (CM-AVM2) Deregulating RAS-MAPK Signaling. Circulation. 136:1037–48.

32. Missaglia S., Tavian D., Michelini S., Maltese P.E., Bonanomi A., Bertelli M. (2021) Imbalance between Expression of FOXC2 and Its lncRNA in Lymphedema-Distichiasis Caused by Frameshift Mutations. Genes (Basel). 12:650.

33. Brouillard P., Boon L., Vikkula M. (2014) Genetics of lymphatic anomalies. Journal of Clinical Investigation. 898–904.

34. Wei D., Rui B., Qingquan F., Chen C., ping H.Y., Xiaoling S., et al. (2021) KIF11 promotes cell proliferation via ERBB2/PI3K/AKT signaling pathway in gallbladder cancer. Int J Biol Sci. 17:514–26.

35. Meng G., Wuest M., Tang X., Dufour J., McMullen T.P.W., Wuest F., et al. (2020) Dexamethasone Attenuates X-Ray-Induced Activation of the Autotaxin-Lysophosphatidate-Inflammatory Cycle in Breast Tissue and Subsequent Breast Fibrosis. Cancers (Basel). 12:999.

36. Xiang H., Lu Y., Shao M., Wu T. (2020) Lysophosphatidic Acid Receptors: Biochemical and Clinical Implications in Different Diseases. J Cancer. 11:3519–35.

37. Michelini S., Amato B., Ricci M., Kenanoglu S., Veselenyiova D., Kurti D., et al. (2020) Segregation Analysis of Rare NRP1 and NRP2 Variants in Families with Lymphedema. Genes (Basel). 11:1361.

38. Sodhi A., Ma T., Menon D., Deshpande M., Jee K., Dinabandhu A., et al. (2019) Angiopoietin-like 4 binds neuropilins and cooperates with VEGF to induce diabetic macular edema. Journal of Clinical Investigation. 129:4593–608.

39. Chen P., Zhang G., Jiang S., Ning Y., Deng B., Pan X., et al. (2021) Mechanosensitive Piezo1 in endothelial cells promotes angiogenesis to support bone fracture repair. Cell Calcium. 97:102431.

40. Nonomura K., Lukacs V., Sweet D.T., Goddard L.M., Kanie A. , Whitwam T., et al. (2018) Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. Proceedings of the National Academy of Sciences. 115:12817–22.

41. Idrees M., Xu L., Song S.H., Joo M.D., Lee K.L., Muhammad T., et al. (2019) PTPN11 (SHP2) Is Indispensable for Growth Factors and Cytokine Signal Transduction During Bovine Oocyte Maturation and Blastocyst Development. Cells. 8:1272.

42. Au A.C., Hernandez P.A., Lieber E., Nadroo A.M., Shen Y.M., Kelley K.A., et al. (2010) Protein Tyrosine Phosphatase PTPN14 Is a Regulator of Lymphatic Function and Choanal Development in Humans. The American Journal of Human Genetics. 87:436–44.

43. Aoki Y., Niihori T., Banjo T., Okamoto N., Mizuno S., Kurosawa K., et al. (2013) Gain-of-Function Mutations in RIT1 Cause Noonan Syndrome, a RAS/MAPK Pathway Syndrome. The American Journal of Human Genetics. 93:173–80.

44. Kimizuka K., Kawai Y., Maejima D., Ajima K., Kaidoh M., Ohhashi T. (2013) Sphingosine 1-Phosphate (S1P) Induces S1P2 Receptor-Dependent Tonic Contraction in Murine Iliac Lymph Vessels. Microcirculation. 20:1–16.

45. Wang P., Yuan Y., Lin W., Zhong H., Xu K., Qi X. (2019) Roles of sphingosine-1-phosphate signaling in cancer. Cancer Cell Int. 19:295.

46. Gonzalez-Garay M.L., Aldrich M.B., Rasmussen J.C., Guilliod R., Lapinski P.E., King P.D., et al. (2016) A novel mutation in CELSR1 is associated with hereditary lymphedema. Vasc Cell. 8:1.

47. Cheong S.S., Akram K.M., Matellan C., Kim S.Y., Gaboriau D.C.A., Hind M., et al. (2020) The Planar Polarity Component VANGL2 Is a Key Regulator of Mechanosignaling. Front Cell Dev Biol. 8.

48. Cohen MM. (2014) Proteus syndrome review: molecular, clinical, and pathologic features. Clin Genet. 85:111–9.

49. Rodriguez-Laguna L., Agra N., Ibañez K., Oliva-Molina G., Gordo G., Khurana N., et al. (2019) Somatic activating mutations in PIK3CA cause generalized lymphatic anomaly. Journal of Experimental Medicine. 216:407–18.

50. Parra-Perez A.M., Pérez-Jiménez A., Gris-Cárdenas I., Bonel-Pérez G.C., Carrasco-Díaz L.M., Mokhtari K., et al. (2022) Involvement of the PI3K/AKT Intracellular Signaling Pathway in the AntiCancer Activity of Hydroxytyrosol, a Polyphenol from Olea europaea, in Hematological Cells and Implication of HSP60 Levels in Its Anti-Inflammatory Activity. Int J Mol Sci. 23:7053.

51. Pei Y. hao, Chen J., Xie L., Cai X. min, Yang R.H., Wang X., et al. (2016) Hydroxytyrosol Protects against Myocardial Ischemia/Reperfusion Injury through a PI3K/Akt-Dependent Mechanism. Mediators Inflamm. 2016:1–9.

52. Li X., Tian X., Liu T., Li M., Wang W., Wang P., et al. (2022) Hydroxytyrosol Alleviated Hypoxia-Mediated PC12 Cell Damage through Activating PI3K/AKT/mTOR-HIF-1α Signaling. Oxid Med Cell Longev. 2022:1–12.

53. Incani A., Deiana M., Corona G., Vafeiadou K., Vauzour D., Dessì M.A., et al. (2009) Involvement of ERK, Akt and JNK signalling in H2O2-induced cell injury and protection by hydroxytyrosol and its metabolite homovanillic alcohol. Mol Nutr Food Res. 54:788–96.

54. Zrelli H., Matsuoka M., Kitazaki S., Araki M., Kusunoki M., Zarrouk M., et al. (2011) Hydroxytyrosol Induces Proliferation and Cytoprotection against Oxidative Injury in Vascular Endothelial Cells: Role of Nrf2 Activation and HO-1 Induction. J Agric Food Chem. 59:4473–82.

55. Sussman M.A., Völkers M., Fischer K., Bailey B., Cottage C.T. , Din S., et al. (2011) Myocardial AKT: The Omnipresent Nexus. Physiol Rev. 91:1023–70.

56. Caretti A., Bianciardi P., Ronchi R., Fantacci M., Guazzi M., Samaja M. (2008) Phosphodiesterase-5 inhibition abolishes neuron apoptosis induced by chronic hypoxia independently of hypoxia-inducible factor-1alpha signaling. Exp Biol Med (Maywood). 233:1222–30.

57. Milano G., Abruzzo P.M., Bolotta A., Marini M., Terraneo L., Ravara B., et al. (2013) Impact of the Phosphatidylinositide 3-Kinase Signaling Pathway on the Cardioprotection Induced by Intermittent Hypoxia. PLoS One. 8:e76659.

58. Terraneo L., Paroni R., Bianciardi P., Giallongo T., Carelli S., Gorio A., et al. (2017) Brain adaptation to hypoxia and hyperoxia in mice. Redox Biol. 11:12–20.

59. Guo Y., Pan W., Liu S., Shen Z., Xu Y., Hu L. (2020) ERK/MAPK signalling pathway and tumorigenesis (Review). Exp Ther Med.

60. Bui K., Hong Y.K. (2020) Ras Pathways on Prox1 and Lymphangiogenesis: Insights for Therapeutics. Front Cardiovasc Med. 7.

61. Aoki Y., Niihori T., Kawame H., Kurosawa K., Ohashi H., Tanaka Y., et al. (2005) Germline mutations in HRAS proto-oncogene cause Costello syndrome. Nat Genet. 37:1038–40.

62. Cirstea I.C., Kutsche K., Dvorsky R., Gremer L., Carta C., Horn D., et al. (2010) A restricted spectrum of NRAS mutations causes Noonan syndrome. Nat Genet. 42:27–9.

63. Zhang X., Jiang Y., Mao J., Ren X., Ji Y., Mao Y., et al. (2021) Hydroxytyrosol prevents periodontitis-induced bone loss by regulating mitochondrial function and mitogen-activated protein kinase signaling of bone cells. Free Radic Biol Med. 176:298–311.

64. Basile J.R., Gavard J., Gutkind J.S. (2007) Plexin-B1 Utilizes RhoA and Rho Kinase to Promote the Integrin-dependent Activation of Akt and ERK and Endothelial Cell Motility. Journal of Biological Chemistry. 282:34888–95.

65. Basile J.R., Barac A., Zhu T., Guan K.L., Gutkind J.S. (2004) Class IV Semaphorins Promote Angiogenesis by Stimulating Rho-Initiated Pathways through Plexin-B. Cancer Res. 64:5212–24.

66. Valtcheva N., Primorac A., Jurisic G., Hollmén M., Detmar M. (2013) The Orphan Adhesion G Protein-coupled Receptor GPR97 Regulates Migration of Lymphatic Endothelial Cells via the Small GTPases RhoA and Cdc42. Journal of Biological Chemistry. 288:35736–48.

67. Geng X., Yanagida K., Akwii R.G., Choi D., Chen L., Ho Y., et al. (2020) S1PR1 regulates the quiescence of lymphatic vessels by inhibiting laminar shear stress–dependent VEGF-C signaling. JCI Insight. 5.

68. Hosaka K., Mizuno R., Ohhashi T. (2003) Rho-Rho kinase pathway is involved in the regulation of myogenic tone and pump activity in isolated lymph vessels. American Journal of Physiology-Heart and Circulatory Physiology. 284:H2015–25.

69. Maltese P.E., Michelini S., Ricci M., Maitz S., Fiorentino A., Serrani R., et al. (2019) Increasing evidence of hereditary lymphedema caused by CELSR1 loss-of-function variants. Am J Med Genet A. 179:1718–24.

70. Sakurai A., Doci C., Gutkind J.S. (2012) Semaphorin signaling in angiogenesis, lymphangiogenesis and cancer. Cell Res. 22:23–32.

71. Ma Y., Xia Z., Ye C., Lu C., Zhou S., Pan J., et al. (2019) AGTR1 promotes lymph node metastasis in breast cancer by upregulating CXCR4/SDF-1α and inducing cell migration and invasion. Aging. 11:3969–92.

72. Abate M., Pisanti S., Caputo M., Citro M., Vecchione C., Martinelli R. (2020) 3-Hydroxytyrosol Promotes Angiogenesis In Vitro by Stimulating Endothelial Cell Migration. Int J Mol Sci. 21:3657.

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HOW TO QUANTIFY LIMB VOLUME: A NEW OVERVIEW OF MEASUREMENT METHODS

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ABSTRACT

Precise definition of the volume and/or perimeter of a limb is essential to understand the evolution of certain pathologies and to ensure good medical follow-up of the patient. There are many different methods of measuring volume and perimetry, each with its own specificities, but none of them being at the same time accurate, repeatable, reproducible, transportable, fast and easy to use, etc. We then described most used of the methods and then compare them to a new one, the PeriKit® (PK), for taking limb measurements, the main ones being the Water Displacement (WD), Conventional Tape Measure (CTL), Optoelectronic Scanner (OS), Scanner and Magnetic Resonance Imaging (MRI). In order to be able to define which method is the best to use, we compared and ranked them according to 13 criteria of use. The WD (gold standard) appears to be outdated; the PK seems to be the most suitable device for daily measurement.

Keywords: Limb volume, Measurement methods, Volumetry, Perimetry, PeriKit, Water Displacement, Optoelectronic Scanner.

INTRODUCTION

We have always wanted to quantify the measurements we take as accurately as possible. For each type of measurement there is a device (gold standard) that reflects the highest precision we can achieve; and other devices based on this one, which may be less precise, but which value other criteria such as ease and time of use, price, weight and size (and the possibility of moving it), repeatability/reproducibility, fragility, etc. However, some standards have never been re-evaluated over time and are outdated in some respects, no longer optimal for use in everyday medical practice.

Limb measurements can be valuable in a variety of situations: monitoring lymphoedema (LO) or chronic venous insufficiency (CVI),[1-3] quantifying amyotrophy or muscle mass gain,[4-5] observing fat loss,[6] quantifying blood effusion,[7] etc. Lack of clarity about the correct method of limb measurement is an important issue due to the increasing prevalence of LO.[8] Patient self-perception of oedema can be unreliable,[9] requiring accurate objective methods of perimetry or volumetry.[10]

The current gold standard, the WD, remains an extremely reliable method. However, its daily use is almost impossible (too bulky, time consuming, etc.). Accurate quantification of the measurement can provide informations on the nature, stage and severity of swelling,[11] objectivise the effectiveness of treatments (rates and amount of progress), conceive made-to-measure compression garments,[10] and meet the requirements of health and disability insurance.[9-10, 12] Taking perimetry and/or volumetry of a limb is an integral part of the clinical work-up, but seems to be neglected (or even absent) in practice. It is therefore difficult to compare pre- and post-treatment volumes and to judge improvements or worsening compared to previous consultations and the previous assessments, or even between the beginning and the end of the same session if we do not measure. “If you do not measure, you can not prove and if you can not prove, you can not improve” (Joseph Harfouche). It would be essential to establish a new gold standard of limb volume measurement.[8]

It is an advantage for the patient in terms of treatment that a volume and/or a perimetry change is quantified accurately. Multiple studies have shown that patient compliance and cooperation increases when patients are aware of their results and improvements.[10, 13-14]

The diagnosis of LO is usually made by using lymphoscintigraphy, lymphofluoroscopy,[15-16] or by using other devices that measure volume (direct method) or circumferences (indirect method).[2, 13] There are many methods, the most commonly used being the WD and CTL.[3] However, none of them are ideal;[17] none are accurate, fast and affordable simultaneously.[18] Early detection of LO allows early treatment.[19] Sometimes the healthy limb is used in comparison with the ill limb to observe the volume change. A 10%

or 200 millilitres difference of volume, or a perimeter difference of 2 centimeters can be considered as pathological.[2, 20] It is important to highlight that Vaughan observed variations of 2.2% ± 2% between the dominant and non-dominant limb,[21-23] so it's important to be as accurate as possible.

Not all methods are interchangeable over time. The method used varies according to the objectives,[12] diagnosis, prognosis, monitoring or treatment. To be accurate, nowadays, volume measurements should be performed on the same patient by using the same method and with the same operator.

PERIMETRY AND VOLUMETRY INSTRUMENTS

Water volumetry

Gold standard of volumetric measurement of the lower and upper limb, the WD (Figure 1), based on Archimedes' principle, is the only method that gives the exact volume of the limb, including the extremity (hand or foot).[13, 24] Different sizes of reservoirs exist depending on the limb and its size. Once the volumeter has been selected and filled with water (at a controlled and constant temperature), the patient waits until the water surface is stable before slowly immersing the body segment to be measured. When the tank is filled, a weir prevents it from overflowing, while the volume of water displaced by the limb is discharged into an additional container. The volume of water is then collected and read either by the scale on the container (in millilitres) or by weighing it (in grams).[13]

This method is very sensitive but slow,[25] cumbersome and difficult (especially in the lower limb), if not impossible, to perform in a paralysed, spastic or postoperative patient,[15, 26] and does not allow sectorial changes in oedema to be observed.[27] It does not quantify oedema, but describes its short-term, individual variation.[28] It also requires staff trained to perform these measurements under good conditions and strict hygiene measures between 2 consecutive patients.[12] The presence of a wound is a contraindication to the measurement. Also, the water used may be lost and spill when the limb is moved or removed too quickly from the tank.[29]

The University of Paris VI defined the accuracy (0.7%), intra-observer variability (1.3%) and intraclass correlation index (ICC) (0.99) of this device for the measurement of the leg.[30] These excellent results (repeatability of 1.3%) are due, among other things, to the standardisation (position of the leg, time of measurement and water temperature) of the measurement.[28] However, this standardisation remains deficient (variations leading to small differences in results in different studies) because "there are many modalities of water volumetry".[12]

A variant called Reverse Volumetry (so-called subtraction method) was described in 2006.[31] This time the limb is immersed in a partially filled volumeter and the volume of water is topped up to a predefined level, before being removed to calculate the volume. It has high repeatability and reproducibility. Some of the disadvantages of conventional volumetry would be absent with the inverted variant, but the disadvantages related to hygiene, contraindications, device preparation time, and calibration concerns are retained.

A method of "no overflow" volumetry (ValGrado method) is also mentioned, where the limb to be measured is immersed perpendicular to the water surface. Care must be taken to ensure that the patient does not touch the edge of the tank (which would distort the measurement) and that the limb is as stable as possible. The ICC of this method is lower and its use would be questionable.[32] However, a previous study shows better results only for hand volume.[33]

Figure 1: *Measurement of the upper limb volume with water displacement method.*[56]

Classical tape measure method

Proposed by many authors to overcome the problems of Water Volumetry,[12] the CTL (Figure 2) identifies the limb to be measured as a succession of truncated cones (or cylinders) whose volumes are established from other measurements (circumferences) previously determined at regular intervals.[13] To define these circumferences, a simple, flexible tape measure is most commonly used.

This device can therefore be called a Perimeter rather than a Volumeter, as it requires the use of a software to automate the calculation of volumetric values from the perimetry.[13] The

calculation using the cylinder formula would seem to be more efficient but would overestimate the values.[34-35] For the arm, the CTL can only diagnose variations beyond 10% of volume (compared to the healthy arm).[29]

Simple, inexpensive and relatively quick to use,[12] it measures an approximate volume for the extremities (hand or foot) usually excluded from the measurement,[13] hence the composition of specific and very complex mathematical formulae.[36-37] There are more or less accurate "figure of eight" measurements for the hand, ankle and foot.[38-39] Nevertheless, perimetry allows the limb to be segmented and volume variations to be assessed over small areas. This method is not suitable for observing diffuse oedema or for assessing the effectiveness of a treatment alone.[12]

Some authors consider it unreliable, especially in cases of swelling of the limb, as the CTL is difficult to place correctly,[25] but others disagree.[12, 40] There are also problems with standardisation of the methodology: anatomical landmarks, measurement interval, reading the measurement above or below the interval, number of measurements taken, width and stiffness of the CTL,[12] obtaining the same CTL tension (which can alter the circumference by up to 3%).[17, 22] According to the CCIs described in the literature,[41] tape measurements are still a relatively reliable and reproducible method of assessing lower limb circumference, but the placement of markers on the skin, in order to achieve a more reliable measurement, may damage the skin and even stigmatise the patient.[10]

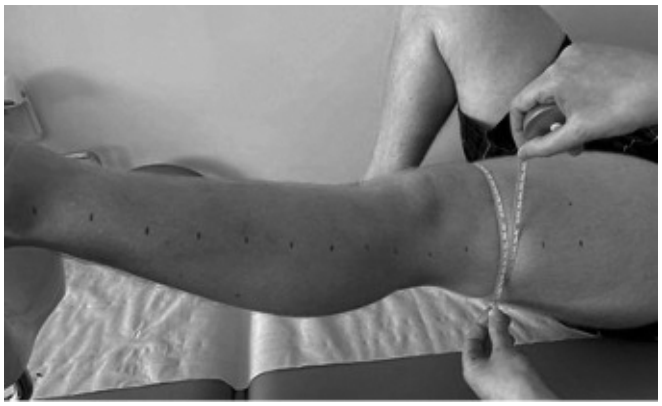

Figure 2: *Measurement of a thigh with the classic tape measure method. Photo taken by Maxime Louys and Maxime Mathieu with the patient's consent.*

Optoelectronic Scanner

The OS (Figure 3) is an infrared system that measures the limb in three dimensions (3D), except for the hand and foot, to define its volume, which is calculated electronically. The frame is mobile, filled with sensors and light emitters, and located above a horizontal base. Once the limb is placed in the frame, it is moved longitudinally to record vertical and horizontal diameters based on circular or elliptical cross-sections; the limb then forms a shadow (blocking light transmission) on the associated receivers.[9, 29] It is essential that the limb is placed perpendicular to the sensors so as not to cause measurement error.[12] It appears difficult to account for the entire lower limb (especially the upper thigh) because of the leg abduction required (mobility restrictions) or because of the thickness of the frame of the device,[42] but the machine is simple and quick to use, ergonomic, efficient and does not require calibration. Data collection and analysis is simplified but they are not widely distributed and not available in most hospitals because of their high price.[29, 43]

Studies on healthy limbs show a very strong correlation (r=0.97) between the OS and the WD for the healthy limb, with the results on the diseased limb also suggesting that this technique can be applied in a clinical setting.[17] The ICC of the WD is between 0.997 and 0.999;[29] therefore this device is reproducible, but not interchangeable with others.

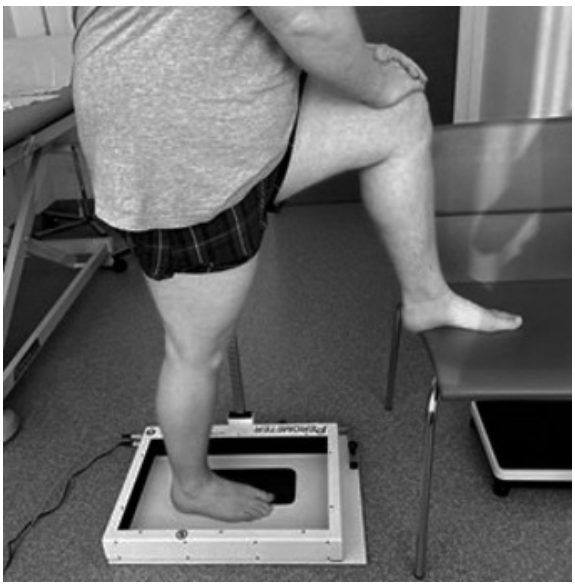

Figure 3: *Patient in the good position for measurement with the optoelectronic scanner method (Perometer® 1000M: Pero-System GmbH, Wupertal, Germany), here the vertical Perometer®. Photo taken by Maxime Louys and Maxime Mathieu with the patient's consent.*

PeriKit®

The PK (Figure 4), invented by Harfouche, is a new patented circumferential measurement device. It consists of 2 main components: the PeriBase (PB) and the PeriTape (PT). The PB eliminates positioning errors when taking circumferential measurements, while the PT is the precision circumference measuring instrument.

The PB is placed at the root of the limb. It is unrolled along the length of the limb or the length of the target segment. It provides a graduated, unstretchable guide line to the wrist or ankle without

marking the skin. In order to avoid reference errors, the PB requires the use of a strap, which is locked into a bony marker at the distal part (e.g. : radial styloid for upper limb and malleolus for the lower limb) creating a stable and repeatable/reproducible "0" reference point.[44] Unlike all other studies that claim to use a bony landmarks but are in fact using a skin landmark overlooking the bone which is, of course, unsteady and unstable which can give erroneous reference points when retaking measurements, the PK is the only device that actually is locked to the bone landmark.[45]

When retaking measurements, the PK has the ability to take exactly the same reference point thanks to the methodology Harfouche, unlike other devices. Because of the conical shape of the limb, any change in the reference point positioning of the measurements can result in an erroneous circumferential measurement that can reach several centimetres.

The guide line of the PB is perforated at every centimetre. Allowing the PT to stick exactly at the place of measurement taking. As the PB guideline is inextensible, one confirmation point is sufficient to ensure the accuracy of all reference points taken. It also offers the possibility of using a skin marker (e.g. mole, scar) as a verification and confirmation point of its correct positioning. It can be adapted to all limb, upper and lower, shapes and lengths. With its fixed reference point, the PB ensures accuracy and reproducibility of measurement locations.

The PT is the element responsible for taking circumferential measurements. It rolls around the limb and is attached to the perforated guide line of the PB by a small pin. The pin ensures that circumferential measurements are taken at exactly the right distance, eliminating any errors in positioning the PT when taking measurements again. The PT uses an isotonic spring to provide the same tension each time measurements are taken and retaken. The isotonic spring tension eliminates all errors due to intra- and inter-observer tension differences, giving it high repeatability. Reproducibility was proven in 2017 (ICC of 0.99), showing that 97% of measurements have an error of less than 3 millimetres for circumference.[10]

The PK solves all common and known errors in circumference measurements. It also allows the measurement of extremities (fingers and toes). The measurement is quick and corresponds to the standards described by the social security system. It is affordable (price) and small (transportable). A mobile application also allows the calculation of the volumetry and allows to compare the measurements and adjust the treatment.[10]

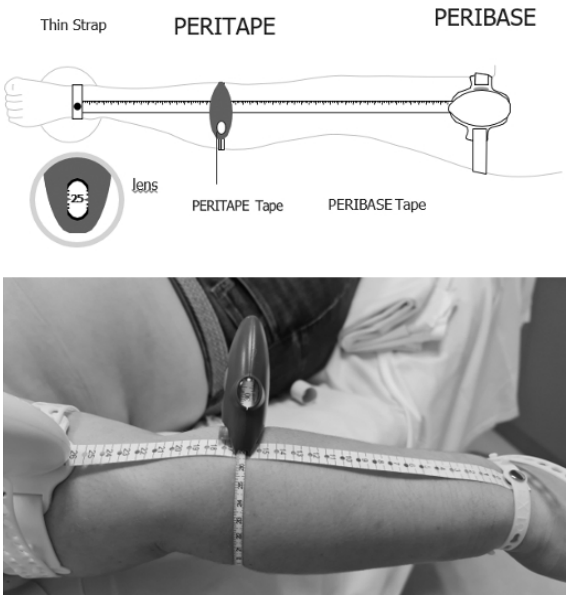

Figure 4: *Diagrams of the use of Perikit® on the lower limb,[58] and Measurement of an arm with the PeriKit® (Prototype No. PKPT7050001: Just A New Health, Hamme-Mille, Belgium). Photo taken by Maxime Louys and Maxime Mathieu with the patient's consent.*

Scanner and Magnetic Resonance Imaging

Initially used to quantify oedema after arterial revascularisation, scanner can also calculate the volume (bone, fat and muscle) of a limb even in the presence of trophic disorders and with less radiation than other techniques. The variations in volume are very small (between 0.1 and 7 millilitres on a leg), making this examination extremely sensitive. In practice, it is only used in the search for or when there is doubt about the diagnosis of LO.[12, 53] It has a key role in the differential diagnosis between LO and lipoedema.[52]

MRI, on the other hand, is a safe and non-invasive method of analysing the composition of human tissue and, like scanner, can differentiate the different pathologies associated with oedema. The analysis depends on the weighting of the signal (T1 or T2) which appears "honeycombed" especially in the subcutaneous fascia.[53] This technique is said to be very reliable and allows early detection of volume variations.[21]

Other processes

There are a number of other, less described and less used procedures. For example, the Leg-O-Meter, designed for calf circumference measurements. It consists of a tape measure attached, always at the same height, to a stand on which the patient stands. Its ICC is 98.28% inter-observer. There is a new, automatic version of this device, but there are no studies on the use of this device at several points.[3, 46-47]

Devoogdt (2010) describes a new circumferential measurement method, which is very similar to the retractable Jobst non stretch tape measure already described in the 1950s by Jobst for taking limb circumferences. It consists of a 50cm long metal bar with tape measures installed perpendicularly every 4cm, to which 20g weights are attached. The metal bar does not follow the curve of the arm, resulting in very large measurement errors. In addition, the fixation point at the olecranon adds inaccuracy between measurements as the skin is particularly mobile at this point.[48]

Also, the Computerized limb evaluation measurement system. This is a mechanical arm instrumented with optical encoders, which allows the volume and shape of a limb to be calculated independently of its position. This method performs cross-sections and then defines the precise shape and volume of each section slice.[25] Automated procedures include CAD (computer-aided design) and CAM (computer-aided manufacturing), which are digitising laser scanners with uncertain reliability.[34] Other examples include laser plethysmography (strain gauge or air),[49] tonometry (electronic device) measuring tissue strength and deformation (indentation) after the application of a mass to define changes in its composition.[19, 21, 50-51]

CONCLUSION

Volumetric measurements calculated from perimetry are considered reliable, repeatable and reproducible. Other techniques, whether experimental, expensive, confidential, not rigorously evaluated or even the current gold standard, are not or no longer suitable for daily use. The choice of one technique over another depends on access to equipment, the operator and the limitations of each method.[12]

Several points appear to be essential for measuring a limb: using the right equipment in the right situation, using the right mathematical formula to go from circumferences to volumes, not only focusing on the volume but also on the circumferences which give essential information on the progression/regression of volume. Volume measurement is not the most suitable for this kind of variability quantification because even if volume is very similar between techniques, there is no guarantee that its distribution is also similar. A volume calculated from circumferences is the best method of measurement in terms of reliability, cost, time and limitation of use.[32]

On the basis of these questions, we have formulated a table (Table 1) comparing some equipment for measuring volumetry and/or limb circumference. We established 13 important criteria found in the literature and ranked the devices according to a rating system (1 being the best and 5 the worst) for each (non-exhaustive) criterion that can influence the quality of the measurement. The criteria are not weighted and some may have more influence than others.

	WD	CTL	SCANNER & IRM	PK	OS (VERTICAL VERSION)
PRICE	3	1	5	2	4
REPEATABILITY	2	5	1	3	4
REPRODUCIBILITY	3	5	1	2	4
FRAGILITY	4	1	5	2	3
TRANSPORTABILITY	4	1	5	2	3
STORAGE SPACE	4	1	5	2	3
TIME USING	5	3	4	2	1
STIGMA/CONTACT WITH THE SKIN	5	4	2	3	1
EASY TO USE	5	2	4	1	3
INVASIVE	4	3	4	2	1
HYGIENIC	5	4	1	2	3
ALLOWS YOU TO TAKE THE CIRCUMFERENCES	5	2	4	1	3
MEASURES THE ENTIRE LIMB	4	2	1	1	5
TOTAL (POINTS)	**53**	**33**	**42**	**25**	**38**
TOTAL (RANKING)	**5**	**2**	**4**	**1**	**3**

Table 1: *Comparison of several devices for measuring the volumetry and/or circumference of the limb. Ranking system (from 1: best, to 5: worst) for each criterion (not exhaustive) that can influence the quality of the measurement. The criteria are not weighted and some may have more influence than others.*

This ranking raises questions: Is the gold standard still relevant in daily measurement? Although it is undoubtedly still an extremely reliable method, it is clear that it no longer meets the expectations of the field. Although it is accurate, all the other criteria to be taken into account classify it as the least interesting equipment to use among those compared.

Is the OS the best placed to replace the current gold standard? No, at least as far as the vertical version of the gold standard is

concerned, which is very inaccurate for lower limb volume and more inaccurate than the scanner, MRI and PK in terms of repeatability. Moreover, the OS values are not interchangeable with those of the gold standard.[29, 55]

Other criteria include ease of use, accessibility of the measurement method, speed of use, cost, reliability, whether the method is invasive or not, whether it is hygienic or not, whether it is suitable for any limb, etc.[23] Despite the good repeatability/reproducibility, it is observed that they are poorly ranked because it is important to take into account a device as a whole.

It appears that the best methods remain those based on perimetry measurements. The use of PK seems to be more interesting than CTL because it is a sensitive, accurate and reproducible device that solves most of the issues in relation with taking measurements.[16] If we couple this with its other advantages, can it become, with its standardised methodology, the new gold standard? Is the PK valid for daily use in LO monitoring? Yes, it meets the main demands and requirements today : Repeatable, reproducible, transportable, requiring little storage space, not time consuming, easy to use not fragile, without contact with the skin, measure the entire limb, allows you to take the circumferences, hygienic, not invasive, not expensive…

REFERENCES

1. Petlund, C. F. (2019). Volumetry of Limbs. In Lymph Stasis: Pathophysiology, Diagnosis and Treatment (pp. 443–452). CRC Press. http://dx.doi.org/10.1201/9780429276200-30

2. Warren, A. G., Brorson, H., Borud, L. J., & Slavin, S. A. (2007). Lymphedema. Ann Plas Surg, 59(4), 464–472.

3. Zuccarelli, F., & Bérard, A. (2000). Test-Retest Reliability Study of a New Improved Leg-O-Meter, the Leg-O-Meter II, in Patients Suffering from Venous Insufficiency of the Lower Limbs. Angiology, 51(9), 711–717. https://doi.org/10.1177/000331970005100902

4. Léger, B., & Gobelet, C. (2011). Schweizerische Zeitschrift. Sportmedizin Und Sporttraumatologie, 59(1), 14–17.

5. Franchi, M. V., Fitze, D. P., Hanimann, J., Sarto, F., & Spörri, J. (2020). Panoramic ultrasound vs. MRI for the assessment of hamstrings cross-sectional area and volume in a large athletic cohort. Sci Rep-UK, 10(1). https://doi.org/10.1038/s41598-020-71123-6

6. González-Ruíz, K., Medrano, M., Correa-Bautista, J., García-Hermoso, A., Prieto-Benavides, D., Tordecilla-Sanders, A., Agostinis-Sobrinho, C., Correa-Rodríguez, M., Schmidt Rio-Valle, J., González-Jiménez, E., & Ramírez-Vélez, R. (2018). Comparison of Bioelectrical Impedance Analysis, Slaughter Skinfold-Thickness Equations, and Dual-Energy X-ray Absorptiometry for Estimating Body Fat Percentage in Colombian Children and Adolescents with Excess of Adiposity. Nutrients, 10(8), 1086. https://doi.org/10.3390/nu10081086

7. Loyd, B. J., Kittelson, A. J., Forster, J., Stackhouse, S., & Stevens-Lapsley, J. (2019). Development of a reference chart to monitor postoperative swelling following total knee arthroplasty. Disabil Rehabil, 42(12), 1767–1774. https://doi.org/10.1080/09638288.2018.1534005

8. Sharkey, A. R., King, S. W., Kuo, R. Y., Bickerton, S. B., Ramsden, A. J., & Furniss, D. (2018). Measuring Limb Volume: Accuracy and Reliability of Tape Measurement Versus Perometer Measurement. Lymphat Res Biol, 16(2), 182–186. https://doi.org/10.1089/lrb.2017.0039

9. Czerniec, S. A., Ward, L. C., Refshauge, K. M., Beith, J., Lee, M. J., York, S., & Kilbreath, S. L. (2009). Assessment of Breast Cancer-Related Arm Lymphedema—Comparison of Physical Measurement Methods and Self-Report. Cancer Invest, 28(1), 54–62. https://doi.org/10.3109/07357900902918494

10. Harfouche, J. (2017). The PeriKit: an innovative connected portable device with high level of accuracy and reliability in taking circumferential limb measurements. Veins and Lymphatics, 6(1). https://doi.org/10.4081/vl.2017.6629

11. Petersen, E. J., Irish, S. M., Lyons, C. L., Miklaski, S. F., Bryan, J. M., Henderson, N. E., & Masullo, L. N. (1999). Reliability of Water Volumetry and the Figure of Eight Method on Subjects With Ankle Joint Swelling. Journal of Orthopaedic & Sports Physical Therapy, 29(10), 609–615. https://doi.org/10.2519/jospt.1999.29.10.609

12. Boulon, C., Becker, F., & Vignes, S. (2010). Comment quantifier un œdème des membres ? J Mal Vascul, 35(3), 163–168. https://doi.org/10.1016/j.jmv.2010.03.003

13. Auvert, J. F., & Vayssairat, M. (2002). La volumétrie :Un examen complémentaire indispensable en lymphologie. Rev Med Interne, 23, 388s–390s. https://doi.org/10.1016/s0248-8663(02)80380-0

14. Abellaneda, S., Baillon, B., Descamps, P.-Y., Florentz, D., Gailly, O., & Vancabeke, M. (2019). Ligament croisé antérieur du genou : comment améliorer la compliance des sportifs non professionnels à suivre le processus de réhabilitation jusqu'à validation des critères de « retour au sport » et contribuer à diminuer le risque de re-rupture de la plastie ? Journal de Traumatologie Du Sport, 36(1), 3–11. https://doi.org/10.1016/j.jts.2019.01.003

15. Giacolone , G., Belgrado, J., Bourgeois, P., Bracale, P., Röh, N., & Moraine, J. (2011). A New Dynamic Imaging Tool to Study Lymphoedema and Associated Treatments. The European Journal Of Lymphology and Related Problems, 22(62), 10–13.

16. Bourgeois, P. (2018). About the EFforT-BCRL-trial... Eur J Obstet Gyn R B, 229, 200. https://doi.org/10.1016/j.ejogrb.2018.07.012

17. Tierney, S., Aslam, M., Rennie, K., & Grace, P. (1996). Infrared optoelectronic volumetry, the ideal way to measure limb volume. Eur J Vasc Endovasc, 12(4), 412–417. https://doi.org/10.1016/s1078-5884(96)80005-0

18. Watteyne, M. (2017). Comparaison de 3 outils de mesure volumétrique que sont le mètre ruban, le Perikit et le scanner. Haute École Bruxelles-Brabant, Unité structurelle paramédicale, Kinésithérapie ISEK.

19. Cornish, B. H., Chapman, M., Mirolo, B., Bunce, I. H., Ward, L. C., & Thomas, B. J. (2001). Early diagnosis of lymphedema using multiple frequency bioimpedance. . Lymphology, 34(1), 2–11.

20. Taylor, R., Jayasinghe, U. W., Koelmeyer, L., Ung, O., & Boyages, J. (2006). Reliability and Validity of Arm Volume Measurements for Assessment of Lymphedema. Phys Ther, 86(2), 205–214. https://doi.org/10.1093/ptj/86.2.205

21. Vaughan, B. F. (1990). CT of swollen legs. Clin Radiol, 41(1), 24–30. https://doi.org/10.1016/s0009-9260(05)80927-4

22. Stanton, A., Badger, C., & Sitzia, J. (2000). Non-invasive assessment of the lymphedematous limb. Lymphology, 33(3), 122–135.

23. Armer, J. M., & Stewart, B. R. (2005). A Comparison of Four Diagnostic Criteria for Lymphedema in a Post-Breast Cancer Population. Lymphat Res and Biol, 3(4), 208–217. https://doi.org/10.1089/lrb.2005.3.208

24. Karges, J. R., Mark, B. E., Stikeleather, S. J., & Worrell, T. W. (2003). Concurrent Validity of Upper-Extremity Volume Estimates: Comparison of Calculated Volume Derived From Girth Measurements and Water Displacement Volume. Phys Ther, 83(2), 134–145. https://doi.org/10.1093/ptj/83.2.134

25. Bednarczyk, J. H., Hershler, C., & Cooper, D. G. (1992). Development and Clinical Evaluation of a Computerized Limb Volume Measurement System (CLEMS). Arch Phys Med Rehab, 73(4), 60–63.

26. Belgrado, J. P., Bracale, P., Bates, J., Röh, N., Rosiello, R., Cangiano, A., & Moraine, J. J. (2010). Lymphoedema: What can be measured and how... overview. The European Journal of Lymphology and Related Problems, 21(0), 3–9.

27. La nomenclature de kinésithérapie—INAMI (Annexe2)., (2020). https://www.inami.fgov.be/fr/professionnels/sante/kinesitherapeutes/Pages/nomenclature- kinesitherapie.aspx#Tests_pour_les_pathologies_de_la_liste_F

28. Nicolaides, A. N. (2000). Investigation of Chronic Venous Insufficiency. Circulation, 102(20). https://doi.org/10.1161/01.cir.102.20.e126

29. Adriaenssens, N., Buyl, R., Lievens, P., Fontaine, C., & Lamote, J. (2013). Comparative Study Between Mobile Infrared Optoelectronic Volumetry With A Perometer® And Two Commonly Used Methods For The Evaluation Of Arm Volume In Patients With Breast Cancer Related Lymphedema Of The Arm. Lymphology, 46(3), 132–143.

30. Vayssairat, M., Maurel, A., Gouny, P., Baudot, N., Gaitz, J. P., & Nussaume, O. (1994). Leg volumetry: A precise method for quantification in phlebology. J Mal Vascul, 19(2), 108–110.

31. Damstra, R. J., Glazenburg, E. J., & Hop, W. C. J. (2006). Validation of the inverse water volumetry method: a new gold standard for arm volume measurements. Breast Cancer Res Tr, 99(3), 267–273. https://doi.org/10.1007/s10549-006-9213-0

32. De Vrieze, T., Gebruers, N., Tjalma, W. A., Nevelsteen, I., Thomis, S., De Groef, A., Dams, L., Van der Gucht, E., Belgrado, J.-P., Vandermeeren, L., & Devoogdt, N. (2019). What is the best method to determine excessive arm volume in patients with breast cancer–related lymphoedema in clinical practice? Reliability, time efficiency and clinical feasibility of five different methods. Clin Rehabil, 33(7), 1221–1232. https://doi.org/10.1177/0269215519835907

33. Martignon, M. V., Fung, L., Vandermeer, L., & Belgrado, J. P. (2018). Evaluation of the reliability of four measuring methods of hand's perimeter and volume: Buoyancy forces valgrado system, circumference measurement, figure-of-eight method and manu3metrix scanner. 8th International Lymphoedema Framework Conference, 8, 58.

34. Lilja, M., Öberg, T., & Johansson, T. (1995). Volumetric determinations with CAD/CAM in prosthetics and orthotics: errors of measurement. J Rehabil Res Dev, 32(2), 141–148.

35. Tewari, N., Gill, P. G., Bochner, M. A., & Kollias, J. (2008). Comparison Of Volume Displacement Versus Circumferential Arm Measurements For Lymphoedema: Implications For The Snac Trial. ANZ J Surg, 78(10), 889–893. https://doi.org/10.1111/j.1445-2197.2008.04686.x

36. Mayrovitz, H., Sims, N., Litwin, B., & Pfister, S. (2005). Foot volume estimates based on a geometric algorithm in comparison to water displacement. Lymphology, 38(1), 20–27.

37. Mayrovitz, H., Sims, N., Hill, C. J., Hernandez, T., Greenshner, A., & Diep, H. (2006). Hand volume estimates based on a geometric algorithm in comparison to water displacement. Lymphology, 39(2), 95–103.

38. Tatro-Adams, D., McGann, S. F., & Carbone, W. (1995). Reliability of the Figure-of-Eight Method of Ankle Measurement. J Orthop Sport Phys, 22(4), 161–163. https://doi.org/10.2519/jospt.1995.22.4.161

39. Devoogdt, N., Cavaggion, C., Van der Gucht, E., Dams, L., De Groef, A., Meeus, M., Van Hemelrijck, R., Heynen, A., Thomis, S., & Orhan, C. (2019). Reliability, Validity, and Feasibility of Water Displacement Method, Figure-of-Eight Method, and Circumference Measurements in Determination of Ankle and Foot Edema. Lymphat Res and Biol, 17(5), 531–536. https://doi.org/10.1089/lrb.2018.0045

40. Mawdsley, R. H., Hoy, D. K., & Erwin, P. M. (2000). Criterion-Related Validity of the Figure-of-Eight Method of Measuring Ankle Edema. J Orthop Sport Phys, 30(3), 149–153. https://doi.org/10.2519/jospt.2000.30.3.149

41. te Slaa, A., Mulder, P., Dolmans, D., Castenmiller, P., Ho, G., & van der Laan, L. (2010). Reliability and reproducibility of a clinical application of a simple technique for repeated circumferential leg measurements. Phlebology: The Journal of Venous Disease, 26(1), 14–19. https://doi.org/10.1258/phleb.2009.009073

42. Stanton, A., Northfield, J., Holroyd, B., Mortimer, P., & Levick, J. (1997). Validation Of An Optoelectronic Limb Volumeter (Perometer®). Lymphology, 30(2), 77–97. https://doi.org/17420

43. Jain, M., Danoff, J., & Paul, S. (2010). Correlation between bioelectrical spectroscopy and perometry in assessment of upper extremity swelling. Lymphology, 43(2), 85–94.

44. Harfouche, J. (2014). An innovative portable device to measure the limb perimeter: reproductibility and accuracy through a blinded study. The 11th NLN International Conference: The Campaign for Lymphedema Care Perspectives, Evidence & Practices.

45. Harfouche, J., Daoud, N., & Velu, T. (2016). The PeriKit: reproductibility and accuracy of an innovative portable device to measure the limb perimeter through a blinded study. The European Journal of Lymphology, 28(74).

46. Berard, A., Kurz, X., Zuccarelli, F., Ducros, J.-J., & Abenhaim, L. (1998). Reliability Study of the Leg-O-Meter, an Improved Tape Measure Device, in Patients with Chronic Venous Insufficiency of the Leg. Angiology, 49(3), 169–173. https://doi.org/10.1177/000331979804900301

47. Guex, J. J., & Perrin, M. (2000). Edema and Leg Volume: Methods of Assessment. Angiology, 51(1), 9–12. https://doi.org/10.1177/000331970005100103

48. Devoogdt, N., Lemkens, H., Geraerts, L., Van Nuland, I., Flour, M. , Coremans, T., Christiaens, M., & Van Kampen, M. (2010). A new device to measure upper limb circumferences: validity and reliability. Int Angiol : A Journal of the International Union of Angiology, 29(5), 401–407.

49. Stephan, D., Tavera, C., Raponsky, J., Moreau, S., Weltin, D., Duver, S., Jeanne, J. F., Ziani, E., & Chauveau, M. (2000). A New Method for Lower Limb Volume Measurement — Laser Plethysmography: Comparison with Two Commonly Used Methods. Phlebology: The Journal of Venous Disease, 15(3–4), 115–121. https://doi.org/10.1177/026835550001500306

50. Marotel, M., Cluzan, R. V., Pascot, M., Alliot, F., Lasry, J. L., & Ghabboun, S. (1998). Transaxial computer tomography of lower extremity lymphedema. Lymphology, 31(4), 180–185.

51. Bates, D. O., Levick, J. R., & Mortimer, P. S. (1994). Quantification of rate and depth of pitting in human edema using an electronic tonometer. Lymphology, 27(4), 159–172.

52. Gniadecka, M. (1996). Localization of dermal edema in lipodermatosclerosis, lymphedema, and cardiac insufficiency. J Am Acad Dermatol, 35(1), 37–41. https://doi.org/10.1016/s0190-9622(96)90493-4

53. Angelhed, J.-E., Strid, L., Bergelin, E., & Fagerberg, B. (2008). Measurement of lower-leg volume change by quantitative computed tomography. Acta Radiol, 49(9), 1024–1030. https://doi.org/10.1080/02841850802427879

54. Gardner, G. C., Nickerson, J. P., Watts, R., Nelson, L., Dittus, K. L., & O'Brien, P. J. (2014). Quantitative and Morphologic Change Associated with Breast Cancer-Related Lymphedema. Comparison of 3.0T MRI to External Measures. Lymphat Res Biol, 12(2), 95–102. https://doi.org/10.1089/lrb.2013.0026

55. Deltombe, T., Jamart, J., Recloux, S., Legrand, C., Vandenbroeck, N., Theys, S., & Hanson, P. (2007). Reliability and limits of agreement of circumferential, water displacement, and optoelectronic volumetry in the measurement of upper limb lymphedema. Lymphology, 40(1), 26–34.

56. Caggiati A, Caggiati L. (2022). Investigations of Lower Limb Edema. In: Approach to Lower Limb Oedema. Singapore: Springer Singapore; p. 65–76. http://dx.doi.org/10.1007/978-981-16-6206-5_6

57. Harfouche J. (2022). PeriKit User's Manual. Hamme-Mille, Belgium.

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QUANTITATIVE ANALYSIS OF CADAVERIC PELVIC LYMPH NODES

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ABSTRACT

INTRODUCTION: *Lymphedema commonly develops as a result of cancer treatments, including surgical removal of lymph nodes. Research suggests there are as many as 450 to 700 lymph nodes throughout the body and there is much variation in the reported ranges per anatomical region. Determining the number nodes to remove and predicting the possible severity of damage can become problematic when the range in one area can vary by up to 30. The purpose of this descriptive study was to investigate more precise ranges of pelvic lymph nodes within cadaver samples.*

MATERIALS AND METHODS: *Quantification of lymph nodes was performed via cadaver dissection and occurred simultaneous with PCOM students' educational dissections. Anatomical landmarks were used to identify and label lymph nodes in the inguinal, iliac, sacral, and lumbar regions of 43 cadavers. Statistical analysis was performed on unpaired (N=43) and paired (N=86) data using Matrix Laboratories and JMP15 data analysis programs.*

RESULTS: *Quantitative analysis revealed a power value of 0.733 for the unpaired lumbar region and a value of 0.954 for the remaining paired regions. Quantification of the number of lymph nodes per region is presented with average ranges, 95% confidence interval ranges, mean, and minimum and maximum values. Furthermore, statistical analysis revealed no correlation between size and number of nodes, minimal differences in male versus female cadavers, and no significant ($p < .001$) difference in left versus right sides of the body.*

CONCLUSIONS: *Compared to previous sources for lymph node quantification, this study demonstrates larger overall ranges, but presents narrower average ranges. Of the regions investigated, only the unpaired lumbar region was shown to utilize an insufficient sample size. Overall, the interpretation of this study's data may provide information for various medical professionals regarding lymph node excision and lymphedema.*

Keywords: Lymphatic system, Lymphedema, Lymphadenectomy, Cadaveric Study, Pelvic Region Anatomy, Matrix Laboratories, JMP15 data analysis

INTRODUCTION

The lymphatic system is a network of vessels and tissues responsible for the removal of fluid, waste, and proteins in the interstitium.[1] This system also provides immunological function by acting as a transportation medium for immune cells.[1, 2] The lymphatic vessels deliver antigen presenting cells and engulfed microorganisms to the lymph nodes in order to initiate an adaptive immunity response within the body.[3] Unfortunately, the lymphatic system also provides an avenue for transportation of malignant disease, or metastasis, to the lymph nodes or other tissues, as well as mechanisms to further the lymphatic system's growth around the malignant cells.[4] Cancer can spread through the lymphatic system when a tumor metastasizes and the cancer cells travel through lymphatic vessels, affecting nearby lymph nodes. The first lymph nodes identified via tracer dye from the primary tumor that drain the cancer site are called sentinel nodes, and they are often removed during biopsies and tumor removal surgeries to diagnose a patient's stage of cancer.[5, 6] For this very reason, previous studies sought to find optimal ranges for the number of lymph nodes required to be removed for an accurate diagnosis of cancer metastasis.[7, 8]

Although there is potential for negative consequences, a lymphadenectomy, or the excision of lymph nodes for the evaluation of micrometastasis, is crucial in diagnosing stages of metastatic disease and selecting the correct treatment course for the patient.[5] Other common scanning procedures, computed tomography (CT) and magnetic resonance imaging (MRI), have poor diagnostic accuracy in regard to metastatic disease.[9] CT or MRI scans possess low sensitivity and can miss metastases in high-risk cancers; thus lymphadenectomies are reported to be the better option.[10] In specific types of cancers, expanded lymphadenectomies are being implemented on patients who are at a higher risk for lymph node involvement in order to better assess the metastasis rather than relying on other diagnostic procedures.[11] Although useful for diagnosis, perioperative complications can be seen in patients receiving lymphadenectomies for certain types of cancer such as lymphocele formation and thromboembolic sequelae.[12, 13] The occurrence of these complications is associated with the number of nodes removed in the lymphadenectomies, with larger node quantities removed yielding greater complications for the patient.[14]

Lymphadenectomies are crucial for proper cancer diagnosis and treatment; yet, can lead to insufficiency of the lymphatic system and create complications in fluid and tissue homeostasis. This alteration to the lymphatic system can lead to lymphedema, a lifelong condition.[15] Lymphedema presents as a progressive swelling, usually of extremities, and may present as painful to the patient. In addition to the swelling, other symptoms may or may not include fatigue, numbness/tingling, heaviness, loss of motion, loss of strength, and discomfort. The inability of the lymphatic system to clear lymph leads to an accumulation in the interstitial spaces which in turn leads to chronic inflammation. Over time, the chronic inflammation can lead to fibrosis and thickening of the tissues and skin, as well as deposition of adipose. [16, 17] Lymphedema can negatively impact a person's quality of life through emotional disturbance and negative self-image as well as negative social impacts such as social isolation and marginalization.[18]

The most common cause of lymphedema in developed countries is secondary to cancer treatments, with incidence as high as 15% following all cancer treatments.[19] While most would agree the pathophysiology of lymphedema is somewhat understood, there is still a significant gap in knowledge about when and if lymphedema occurs in patients undergoing select lymphadenectomy.[1] In one study, ovarian and uterine cancer patients who underwent pelvic lymph node dissection were examined to see if there was a causal relationship between lymphadenectomy and an increase in occurrence of lymphedema. The study concluded no relationship between surgical removal of lymph nodes and the occurrence of lymphedema but rather discovered a statistically significant relationship between post-operative radiotherapy and lymphedema. [19] Other research suggests the number of lymph nodes excised correlates to the incidence of lymphedema. One review [20] noted axillary lymph node dissections resulted in 4 times higher incidence of arm lymphedema than simple sentinel lymph node dissection. Yen et al. [21] showed the removal of 15 nodes in the treatment of breast cancer, compared to removal of up to 5, increased the chances of lymphedema tenfold. McLaughlin et al. [22] reported rates of upper extremity lymphedema with sentinel node biopsy alone as 3% of patients (18 out of 600) as opposed to 27% of patients (91 of 336) who underwent sentinel node biopsy in addition to axillary node lymphadenectomy. The main question is what the least number of lymph nodes required is with a lymphadenectomy to successfully diagnosis, while minimizing the risk of lymphedema. A study by Peyre on esophageal cancer patients,[23] concluded a minimum number of 23 regional nodes are needed to be removed for the highest possible survival rate. Koppie's study on transitional cell carcinoma patients [24] finds there is no minimum number of nodes needed for the maximum survivor rates in cancer patients, which suggests the more lymph nodes dissected, the higher the survivorship rate is for a person.

Sources indicate there may be as many as 450-700 lymph nodes in the body.[25, 26, 27] The reported number of lymph nodes in each anatomical region varies with each source, and often wide possible ranges are denoted. For example, Földi et al.[26] reports 5-27 external iliac lymph nodes whereas Hsu & Itkin[28] reports 9-10 nodes in the same anatomical region. Many cadaveric studies quantify nodes in various regions, however, they utilize small sample sizes and often do not take into account lymph node size or other variables such as selection of cadavers and different methods of tissue preparation and tissue sample selection.[29] There can be wide variation in nodal size, between 0.2 and 3 cm, as well as a failure of nodes to divide during development leading to smaller numbers of larger-sized nodes in anatomical regions.[26] In addition to the aforementioned study limitations of cadaveric lymph node quantification, many studies have inadequately standardized methods and procedures that produce variable lymph node counts.[30]

A more accurate insight into ranges of lymph nodes located in a given region could be paramount in assisting healthcare practitioners' in discussing prognosis related to lymphedema with survivors at future visits. Surgeons and other follow-up care physicians, including oncologist, radiologist, and primary care physicians, could more accurately provide statistics on the likelihood of developing lymphedema. Physicians and therapists (physical, occupational, and speech) might be able to provide more realistic expectations on outcomes from conservative and/or surgical lymphedema treatment.

Through cadaver studies, this study sought to identify more precise ranges for lymph node quantities of the pelvic region specifically. With a targeted range, decisions on how many nodes to remove, more accurate predictions of damage severity, treatment outcomes, and future quality of life may be concluded.

MATERIALS AND METHODS

Embalming and dissection of cadavers

Cadavers used in this study were donated to the Philadelphia College of Osteopathic Medicine's (PCOM) Georgia Campus. Dissections were completed by Doctor of Osteopathic Medicine (DO) students, Physician Assistant students, and Physical Therapy students at PCOM over several quarters during the 2019-2020 academic school year. All students received education on the lymphatic system, the lymph nodes, and on the procedures of the current study by the lead investigator, Dr. Shelley DiCecco, a faculty member from the Physical Therapy Department. All cadavers at PCOM were embalmed onsite utilizing the following procedures and techniques. The cadavers were prepared with a special anatomical solution referred to as the Maryland State Blend (MSB). The solution was prepared by the Hydrol Chemical Company located in Pennsylvania and was carefully blended to meet the requirements and climatic conditions in Georgia. The primary solution was diluted by adding one gallon of water to every ten ounces of MSB prior to injecting the cadavers. Initially, a minimum of 4 to 5 gallons of injection was prepared for most cadavers and injected over 2-3 days. Then, an additional 3-4 gallons of solution was prepared and injected until the

desired saturation of the vascular system was achieved as determined by the Director of Anatomical Donor Services for PCOM. As part of the final prepping, the embalmed cadavers were wrapped with a cotton wrap soaked in a diluted MSB, then in plastic, and stored in a zipper pouch. The cadavers were stored in a refrigeration system for roughly 4 to 8 months until needed for anatomical dissections.

The research team consisted of the lead investigator and 10 students from different programs at PCOM, 4 DO and 6 Master of Science in Biomedical Sciences students (3 left the team for school-related issues). PCOM students performed dissections in the afternoon typically 4-5 days of the work week. The researchers would follow behind these dissections and inspect the bodies in the morning for lymph nodes in the desired regions. Upon locating a lymph node, the researchers would tag the node with a tag gun and document the location and surface area of the node. The nodes were left in place until PCOM students completed the quarter. All areas were revisited on a regular basis to check for new nodes to document. At the end of the quarter, the researchers removed all organs, tissues, and nodes to re-check for any additional nodes in the area not uncovered during regular student dissection. The surface area was calculated using a standard ruler in millimeters for length and width. The size and location of nodes were documented on a grid during the dissection phase and later transferred to a data spreadsheet for analysis.

Cadavers

The demographics of the 43 cadavers were 27 female and 16 male, ages 42 to 102, (mean age 69.65 +/- 12.94) with 39 Caucasian, 3 African American, and 1 of Asian ethnicity. Cause of death for each cadaver was unknown during the experiment as were other external factors in order to prevent bias. The 43 cadavers were examined for paired regions of right and left sacral, inguinal, and iliac region lymph nodes as well as for an unpaired lumbar region. Anatomical landmarks were used to identify each region of nodes.

Anatomical landmarks for identifying lymph nodes per region

Standard anatomical landmarks were used to identify and distinguish the different lymph node regions. The landmarks used were based on anatomical descriptions from multiple references.[26, 27, 28, 31, 32]
A lecture about the research and location of the nodes including illustrations detailing anatomical landmarks were provided to all the students participating in dissections at PCOM, as well as the researchers performing the experiment on the cadavers. **Image 1** depicts the anatomical regions in the demonstration cadaver the students could use along with anatomy images as a reference during the data collection phase.

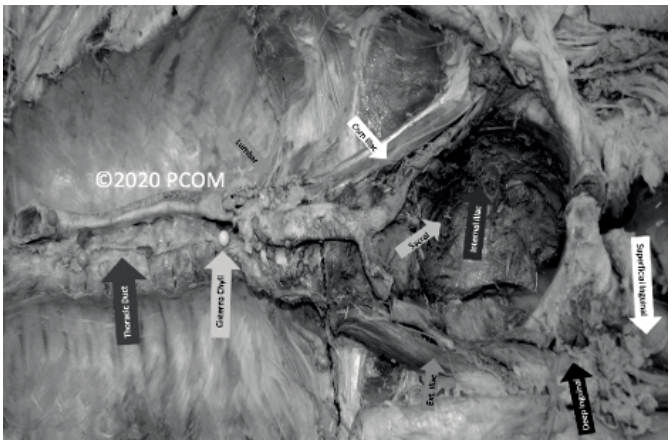

Image 1: *Lymph node regions in demonstration cadaver.*

Inguinal Lymph Nodes
The lymph nodes of the inguinal region were divided into superficial and deep based on placement around the fascia lata. Superficial inguinal nodes resided superficially to the fascia lata and were bound by the Femoral Triangle (inguinal ligament, sartorius muscle, and long adductor muscle). These were not subdivided into superolateral, superomedial, inferolateral, or inferomedial in this study.[26] The deep inguinal nodes were located beneath the fascia lata and cribriform fascia in the iliopectineal fossa along the femoral artery and vein.[28, 31]

Iliac Lymph Nodes
Iliac node regions were determined based on arrangement around iliac blood vessels. The regions are common, internal, and external.[32] Common iliac nodes were found anterior to the psoas muscle, from the iliosacral line to the iliolumbar line along the common iliac blood vessels.[31] Internal iliac nodes were grouped along the anterior and posterior divisions of internal iliac arteries as well as the beginning of the gluteal arteries.[31, 32] In this study, gluteal nodes were also included in the internal iliac region. Lastly, external iliac nodes were found between the iliosacral and inguinal lines along the external iliac vessels, as well as between the obturator branch of the internal iliac artery.[31, 32] Medial, lateral, and intermediate chains of the common, internal, and external iliac nodes were not defined in this study.[26]

Sacral Lymph Nodes
The anatomical landmarks for the sacral lymph nodes were the medial and lateral sacral vessels as well as along the concavity of the sacrum.[31]

Lumbar Lymph Nodes
Lumbar lymph nodes were defined by the region extending from the inferior border of L5 to the superior border of the L1 vertebral body, specifically between the vertebral bodies and both sides of the inferior vena cava and the aorta.[31] In this study, lumbar lymph nodes were not divided into right and left as a large portion of these nodes were located more centrally as opposed to the right or left of the aorta or spine, and due to the inability to visualize the location from where the nodes were draining.[26]

Statistical Analysis

Statistical analysis of the recorded data was performed using MATrix LABoratory (MATLAB) and JMP15 data analysis programs and SPS Analytics. Cadavers were excluded from analysis if they failed to meet predetermined lower boundaries per region which were taken from a variety of sources.[26, 27, 28, 31] These lower boundaries were as follows: common iliac 3, internal iliac 4, external iliac 5, deep inguinal 1, superficial inguinal 4, lumbar 15, and sacral 1. A Wilcoxon signed rank test was used to compare the quantity of nodes per right and left side of the cadavers. A Mann Whitney U test was conducted to determine if there were differences in quantity between female and male cadavers. Both the Wilcoxon signed rank test and Mann Whitney U test were performed with an α value of 0.05. Single factor line regression using the JMP15 program was performed to assess the relationship between the size of nodes and the number of nodes per anatomical grouping. Lastly, a retrospective power analysis was performed to determine if the sample size was great enough to avoid statistical errors.

RESULTS

All quantitative analyses were done using JMP15 and MATLAB. **Table 1** showcases the distribution analysis for each pelvic lymph node section obtained in this study with the mean lymph node number, the average range, the minimum and maximum node values, and a 95% confidence interval range. **Figure 1** shows a graphical representation of overall numerical distribution of lymph nodes in the designated pelvic region. Power analysis revealed a value of 0.733 given a sample size of 43 (unpaired lumbar lymph node region) and a value of 0.954 given a sample size of 86 (paired left and right lymph node regions). Lymph nodes in the lumbar region showed the greatest range of distribution, while the sacral region lymph nodes had the lowest range of distribution. A Wilcoxon signed rank test was performed to determine differences in lymph node quantity between the left and right side of the cadavers. Across all pelvic regions, there were no statistically significant differences ($p < .001$) as shown in **Figure 2. Figure 3** displays pelvic region lymph node quantity differences between male and female cadavers. The Mann Whitney U test was performed to determine differences in lymph node quantity between male and female cadavers. Across all pelvic regions, there were no statistically significant differences ($p < .001$). Values for male cadavers tended to be more evenly distributed within the determined range, whereas in female cadavers, there was less spread. However, the difference is minimal and overall the figure shows a similar distribution of lymph nodes in their respective pelvic area between male and female. Preliminary analysis of the relationship between lymph node quantity and total area was performed in JMP15 as shown in **Figure 4**. Single factor linear regression was used to determine any relationship between the two variables. R2 values ranged from 0.20 - 0.58, indicating minimal linear relationship between cross sectional area and number of nodes for any pelvic lumbar region.

	MEAN ± STD DEV	AVG RANGE	MIN-MAX	95% CI
LUMBAR	24.5±13.4	16-34	0-60	20.3-28.6
COMMON ILIAC	8.2±4.6	5-10	2-26	6.8-9.7
INTERNAL ILIAC	6.0±4.0	3-8	0-22	4.8-7.2
EXTERNAL ILIAC	11.4±4.9	6-15	2-23	9.9-13.0
SUPERFICIAL INGUINAL	10.4±4.4	7-14	3-20	9.1-11.8
DEEP INGUINAL	3.2±1.9	2-4	0-8	2.6-3.7
SACRAL	2.7±1.8	2-4	0-8	2.2-3.3

Table 1: *Lymph node distributions for each pelvic lumbar region.*

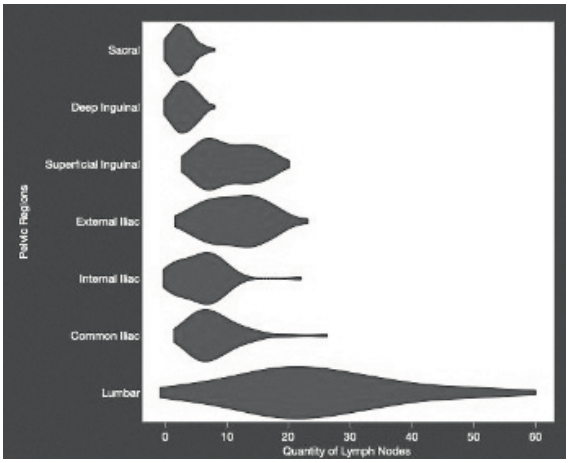

Figure 1: *Distribution of number of pelvic lymph nodes identified per region.*

Figure 2: *Total pelvic lymph node quantity per left and right side.*

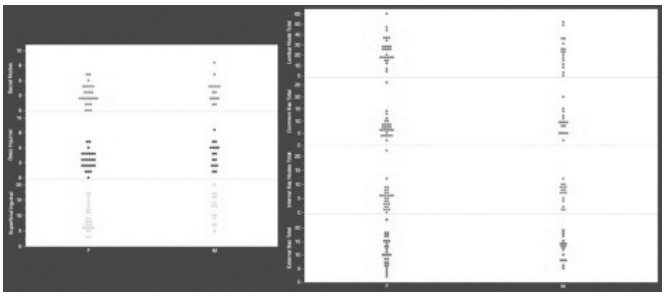

Figure 3: *Pelvic region lymph node quantity per male and female cadavers per pelvic region.*

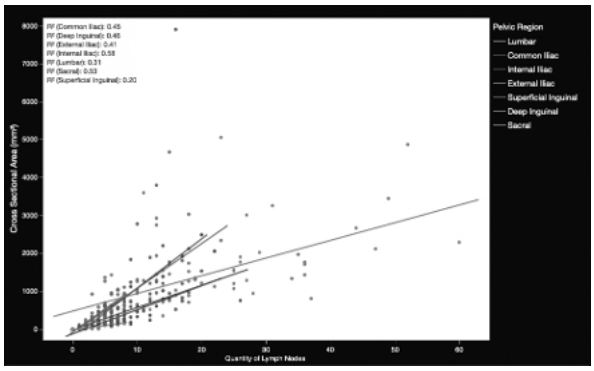

Figure 4: *Pelvic region lymph node quantity with respect to total cross-sectional area in mm.*[3]

DISCUSSION

Currently, various literature sources provide different values for lymph nodes per anatomical region and add to the overall confusion of the quantification of nodes, as seen in **Table 2**. This experiment sought to provide more research toward the understanding of these regions and to produce more concise node ranges for the pelvic region. By contributing to better accuracy, the consequences of lymph node dissections might be further calculated or predicted and negative outcomes such as lymphedema may also be predicted, lessened, or even prevented. This was accomplished by utilizing larger samples sizes, as well as incorporating additional statistical comparisons.

The distribution analysis of the pelvic lymph nodes revealed basic parameters on the data set. The mean number of lymph nodes per region was found to be inside the 95% confidence interval determined by distribution analysis. The confidence interval allows for estimation of the approximate number of nodes in a region, and the average range provides a reference range to compare.

Retrospective power analysis revealed a value of 0.954 for the paired lymph node regions. The sample size of 86 cadavers for this experiment was adequate to avoid Type II statistical errors whereas the value of 0.733 for the unpaired lymph node regions demonstrated a need for a sample size greater than 46 cadavers in order to avoid statistical errors and yield a more concise range of node distribution. The lumbar lymph node region was not of a significant power due to the smaller sample size of 43 cadavers and produced the widest confidence interval of 20.3-28.6 in this study. The limitation of the number of cadavers utilized can explain the lower value for the paired lymph node regions. Most lymph node quantification cadaveric studies utilize small sample sizes which results in such widened confidence intervals; however, the rest of the reported intervals are narrow, demonstrating the increased precision of the point estimate value listed in **Table 1**. Various cadaveric quantification studies reported their statistics in the style of median and a general range of nodes without providing a confidence interval such as Ofo et al. (**EE**) when quantifying central compartment neck lymph nodes. By providing a point of estimate and confidence interval for each pelvic lymph node region, there is only a 5% chance that the true value of nodes per region is not within the reported ranges, albeit the lumbar region needs a greater sample size to produce a better range.

LYMPH NODE ANATOMICAL GROUPING	AVERAGE RANGE FROM CURRENT STUDY	FO'LDI ET AL. (28)	STANDRING (29)	WOLFRAM - GABEL (34)	HSU & ITKIN (30)
SUPERFICIAL INGUINAL	7 - 14	4-25	-	-	4-25
SACRAL	2 - 4	-	1-3	-	-
LUMBAR	16 - 34	-	15-20	-	-
INTERNAL ILIAC	3 - 8	4-6	-	4-8	-
EXTERNAL ILIAC	6 - 15	5-27	-	8-10	9-10
DEEP INGUINAL	2 - 4	1-3	-	-	1-3
COMMON ILIAC	5 - 10	3-16	-	6-10	4-7

Table 2: *Table of lymph node ranges taken from various sources compared to average lymph node ranges from the experiment conducted.*

Table 2 compares the study's lymph node ranges to various literature sources for each pelvic lymph node anatomical grouping. Again, the differences in ranges can be seen when looking at the reported numbers for Földi et al.[26] versus Wolfram-Gabel [32] specifically for external iliac. As shown, the average range from the current experiment tends to fall between the larger reported literature ranges. On the low end of the ranges, the study's count only differs by 1-3 lymph nodes when compared to other studies. There are greater discrepancies when comparing the upper end

values of the lymph node ranges. For example, when comparing the upper end value of the superficial inguinal range from the study's to Földi's, there is an 11 lymph node difference. In **Figure 5**, the aforementioned data is presented in a bar graph to aid in understanding that the majority of the current study's average ranges of lymph nodes per anatomical region fell within the reported literature ranges. The black bar is representative of the total range of lymph nodes found in this study, while the colored boxes are the average lymph node ranges. The colored boxes are superimposed over the total range to visually represent the overlap between each study.

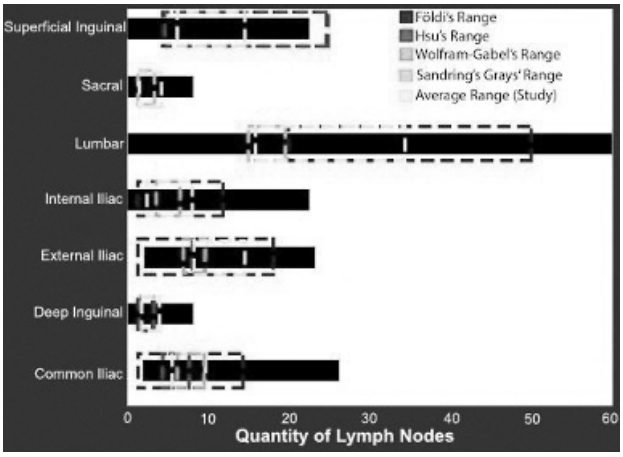

Figure 5: *Lymph node ranges in various sources compared to average ranges from current study.*

This study also demonstrated there was minimal difference in the number of nodes between male and female cadavers and no significant differences between right and left sides of the paired node regions as seen in **Figure 3** and **Figure 2** respectively. This differs from other sources stating there is prominent asymmetry in lymph node distribution with more present on the right side of the body and more particularly noticeable in males.[33] Other studies yielded results demonstrating the size of lymph nodes differs between men and women as well as in distribution of the nodes.[34] Weingartner et al.[35] found interindividual differences between the number of external inguinal nodes per right and left side of cadavers albeit a small difference. However, more current articles examining differences in lymph node distribution, size, and quantity between males and females and left and right sides of the body were not found after an exhaustive database search. Thus, further research is needed to determine how these differences affect the ranges of lymph nodes in the body and how this may impact surgeries and other medical treatments.

The relationship between lymph node quantity and size was analyzed and showed a minimal linear correlation with a coefficient of determination of 0.20-0.58 as seen in **Figure 4**. Thus, the number of nodes per anatomical region showed no influence on the area measured in this experiment. This agreed with Grey et al.[36] which specifically examined inguinal lymph nodes and concluded there was no correlation between nodal size and number. However, in this study, possible malignant lymph nodes were counted in the data and possibly modified the linear correlation due to the potential size decrease or increase. The preservation process itself can also alter tissues leading to variation to lymph node size.[37]

The range of distribution of pelvic lymph nodes was examined and found to be greatest in the lumbar region and the least in the sacral region. There is known variability between individuals' quantity of lymph nodes per anatomical region such as an increase in nodes in patients with higher body mass indexes seen in specifically retroperitoneal lymph node dissection.[38, 39] This variability aids in explaining the increased or decreased distribution in this experiment. In addition, this experiment did not account for the causes of death and comorbidities which may have possibly influenced individual lymph node counts, further increasing the range of distribution. Possible lymph node removal during the anatomical dissections performed by students could further obscure the data recorded regarding the distribution of nodes. Lastly, microscopy or imaging, such as intranodal lymphangiogram and dynamic contrast enhanced magnetic resonance lymphangiography, was not used in this experiment to visualize nodes.[40] Thus, lymph nodes were quantified based on visual identification only, potentially failing to identify all nodes.

Limitations such as reduced sample size in analysis of paired lumbar lymph nodes and unknown comorbidities of the cadavers did impact the recorded data; however, the results complimented known sources as the data presented more precise ranges with confidence intervals of 95% and narrow average ranges within the various sources' reported lymph node ranges.[26, 27, 28]
Further research is needed in defining the ranges of lymph nodes in the pelvic region in non-cadaveric subjects. This data could assist in determining the min and max number of nodes needed for lymphadenectomies in the pelvic region to appropriately diagnosis with the least amount of lymphatic dysfunction. Other medical professionals should also benefit from more accurate average ranges of lymph nodes in the pelvic region. Primary care physicians self-reported less knowledge concerning lymphedema associated with breast cancer than oncologists and surgeons primarily handling the patient.[41] With the increasing number of breast cancer survivors and the need for long term follow up care, primary care physicians need to be heavily educated on lymphedema associated with cancer to better serve this patient population. Physicians, including primary care, and lymphedema therapists can use these ranges to aid in providing survivors with a more accurate prognosis of outcomes with conservative and surgical interventions for lymphedema. Overall, this improved information regarding the lymphatic system and nodal quantity in general could lead to a reduced number of individuals being diagnosed with lymphedema and improved post-care treatment or expectations for those diagnosed with cancer related lymphedema.

CONCLUSION

The lymphatic system is responsible for the drainage of interstitial fluid and is necessary for the prevention of edema. The system is frequently disrupted for the assessment of metastasis and/or the treatment of cancer. A significant complication for both is lymphedema. Here we quantified the lymph nodes of the pelvic region, reporting larger ranges of lymph nodes in their respective regions compared to previous illustrations, but presenting more succinct average ranges. Additionally, we found no significant differences when investigating left to right side comparison or male to female comparison, nor significant correlation between size and number of lymph nodes. Interpretation of these findings may provide insight for physicians when considering surgical or medical procedures involving the lymph nodes of the pelvic region as well as improved post-care treatment for patients after said procedures by medical professionals.

LIST OF ABBREVIATIONS

CT	Computed tomography
DO	Doctor of Osteopathic Medicine
MATLAB	Matrix Laboratory
MRI	Magnetic resonance imaging
MSB	Maryland State Blend
PCOM	Philadelphia College of Osteopathic Medicine

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REFERENCES

1. Suami H. (2020) Anatomical theories of the pathophysiology of cancer-related lymphoedema. Cancers (Basel).12(5),1338. https://doi.org/10.3390/cancers12051338

2. Swartz MA. (2001) The physiology of the lymphatic system. Adv Drug Deliv Rev. 50(1-2):3-20. https://doi.org/10.1016/s0169-409x(01)00150-8

3. Liao S, von der Weid PY. (2015) Lymphatic system: an active pathway for immune protection. Semin Cell Dev Biol. 38:83-9. https://doi.org/10.1016/j.semcdb.2014.11.012

4. McAllaster JD, Cohen MS. (2011) Role of the lymphatics in cancer metastasis and chemotherapy applications. Adv Drug Deliv Rev. 63(10-11):867-75. https://doi.org/10.1016/j.addr.2011.05.014

5. Rossi EC, Kowalski LD, Scalici J, Cantrell L, Schuler K, Hanna RK, et al. (2017) A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. Lancet Oncol. 18(3):384-92. https://doi.org/10.1016/s1470-2045(17)30068-2

6. Nezhat F, Mahdavi A, Pejovic T. (2006) Laparoscopic procedures. In: Bieber EJ, Sanfilippo JS, Horowitz IR, editors. Clinical Gynecology. Elsevier Inc. p. 549-567.

7. Terrone C, Guercio S, De Luca S, Poggio M, Castelli E, Scoffone C, et al. (2003) The number of lymph nodes examined and staging accuracy in renal cell carcinoma. BJU Int. 91(1):37-40. https://doi.org/10.1046/j.1464-410x.2003.04017.x

8. Giuliano AE, Dale PS, Turner RR, Morton DL, Evans SW, Krasne DL. (1995) Improved axillary staging of breast cancer with sentinel lymphadenectomy. Ann Surg. 222(3):394–401. https://doi.org/10.1097/00000658-199509000-00016

9. Hövels AM, Heesakkers RA, Adang EM, Jager GJ, Strum S, Hoogeveen YL, et al. (2008) The diagnostic accuracy of CT and MRI in the staging of pelvic lymph nodes in patients with prostate cancer: a meta-analysis. Clin Radiol. 63(4):387-95. https://doi.org/10.1016/j.crad.2007.05.022

10. Borley NC, Fabrin K, Sriprasad S, Mondaini N, Thompson PM, Muir GH, et al. (2003) Laparoscopic pelvic lymph node dissection allows significantly more accurate staging in 'high-risk' prostate cancer compared to MRI or CT. Scand J Urol Nephrol. 37(5):382-6. https://doi.org/10.1080/00365590310006309

11. Heidenreich A, Varga Z, Von Knobloch R. (2002) Extended pelvic lymphadenectomy in patients undergoing radical prostatectomy: high incidence of lymph node metastasis. J Urol. 167(4):1681-6. https://doi.org/10.1016/s0022-5347(05)65177-4

12. Panici PB, Basile S, Maneschi F, Lissoni AA, Signorelli M, Scambia G, et al. (2008) Systematic pelvic lymphadenectomy vs no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. J Natl Cancer Inst. 100(23):1707-16. https://doi.org/10.1093/jnci/djn397

13. Musch M, Klevecka V, Roggenbuck U, Kroepfl D. (2008) Complications of pelvic lymphadenectomy in 1,380 patients undergoing radical retropubic prostatectomy between 1993 and 2006. J Urol. 179(3):923-9. https://doi.org/10.1016/j.juro.2007.10.072

14. Franchi M, Ghezzi F, Riva C, Miglierina M, Buttarelli M, Bolis P. (2001) Postoperative complications after pelvic lymphadenectomy for the surgical staging of endometrial cancer. J Surg Oncol. 78(4):232-40. https://doi.org/10.1002/jso.1158

15. Achouri A, Huchon C, Bats AS, Bensaid C, Nos C, Lécuru F. (2013) Complications of lymphadenectomy for gynecologic cancer. Eur J Surg Oncol. 39(1):81-6. https://doi.org/10.1016/j.ejso.2012.10.011

16. Cheng M, Chang DW, Patel KM. (2015) Principles and practices of lymphedema surgery. Elsevier Inc. Chapter 1, An introduction to principles and practices of lymphedema surgery; p. 1-2.

17. Greene AK, Slavin SA, Brorson H, editors. (2015) Lymphedema: presentation, diagnosis, and treatment. Switzerland: Springer International Publishing.

18. Fu MR, Ridner SH, Hu SH, Stewart BR, Cormier JN, Armer JM. (2013) Psychosocial impact of lymphedema: a systematic review of literature from 2004 to 2011. Psychooncology. 22(7):1466-84. https://doi.org/10.1002/pon.3201

19. Tada H, Teramukai S, Fukushima M, Sasaki H. (2009) Risk factors for lower limb lymphedema after lymph node dissection in patients with ovarian and uterine carcinoma. BMC Cancer. 9:47. https://doi.org/10.1186/1471-2407-9-47

20. DiSipio T, Rye S, Newman B, Hayes S. (2013) Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. Lancet Oncol. 14(6):500-15. https://doi.org/10.1016/S1470-2045(13)70076-7

21. Yen TW, Fan X, Sparapani R, Laud PW, Walker AP, Nattinger AB. (2009) A contemporary, population-based study of lymphedema risk factors in older women with breast cancer. Ann Surg Oncol. 16(4):979–88. https://doi.org/10.1245/s10434-009-0347-2

22. McLaughlin SA, Wright MJ, Morris KT, Sampson MR, Brockway JP, Hurley KE, et al. (2008) Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: patient perceptions and precautionary behaviors. J Clin Oncol. 26(32):5220-6. https://doi.org/10.1200/JCO.2008.16.3766

23. Peyre CG, Hagen JA, DeMeester SR, Altorki NK, Ancona E, Griffin SM, et al. (2008) The number of lymph nodes removed predicts survival in esophageal cancer: an international study on the impact of extent of surgical resection. Ann Surg. 248(4):549-56. https://doi.org/10.1097/SLA.0b013e318188c474

24. Koppie TM, Vickers AJ, Vora K, Dalbagni G, Bochner BH. (2006) Standardization of pelvic lymphadenectomy performed at radical cystectomy: can we establish a minimum number of lymph nodes that should be removed? Cancer. 107(10):2368-74. https://doi.org/10.1002/cncr.22250

25. Zuther JE. (2004) Lymphedema management: the comprehensive guide for practitioners. New York: Thieme. p. 5-12.

26. Fo¨ldi M, Fo¨ldi E, Stro¨ssenreuther RH, Kubik S, editors. (2012) Fo¨ldi's textbook of lymphology: for physicians and lymphedema therapists. 3rd ed. Mu¨nchen: Elsevier Urban & Fischer.

27. Standring S, editor. (2016) Grays anatomy: the anatomical basis of clinical practice. 41st ed. Elsevier.

28. Hsu MC, Itkin M. (2016) Lymphatic anatomy. Tech Vasc Interv Radiol. 19(4):247-54. https://doi.org/10.1053/j.tvir.2016.10.003

29. Ofo E, Thavaraj S, Cope D, Barr J, Kapoor K, Jeannon J, et al. (2016) Quantification of lymph nodes in the central compartment of the neck: a cadaveric study. Eur Arch Otorhinolaryngol.273(9):2773-8. https://doi.org/10.1007/s00405-015-3827-y

30. Ahmadi O, McCall JL, Stringer MD. (2013) Does senescence affect lymph node number and morphology? A systematic review. ANZ Journal of Surgery. 83(9):612-8. https://doi.org/10.1111/ans.12067

31. Moore KL, Dalley AF, Agur AM. (2018) Clinically Oriented Anatomy. 8th ed. Philadelphia: Wolters Kluwer.

32. Wolfram-Gabel R. (2013) Anatomie du système lymphatique pelvien [Anatomy of the pelvic lymphatic system]. Cancer Radiother.17(5-6):549-52. https://doi.org/10.1016/j.canrad.2013.05.010

33. Sapin MR. (1980) Asimmetriia limfaticheskikh uzlov u cheloveka i ee prikladnoe znachenie [Human lymph node asymmetry and its applied importance]. Arkh Anat Gistol Embriol. 79(11):58-63.

34. Shvetsov EV. (1991) Anatomiia i topografiia naruzhnykh podvzdoshnykh limfaticheskikh uzlov u vzroslogo cheloveka [Anatomy and topography of external iliac lymph nodes in adults]. Arkh Anat Gistol Embriol. 100(7-8):50-7.

35. Weingartner K, Ramaswamy A, Bittinger A, Gerharz EW, Voge D, Riedmiller H. (1996) Anatomical basis for pelvic lymphadenectomy in prostate cancer: results of an autopsy study and implications for the clinic. J Urol. 156(6):1969-71. https://doi.org/10.1016/s0022-5347(01)65406-5

36. Grey AC, Carrington BM, Hulse PA, Swindell R, Yates W. (2000) Magnetic resonance appearance of normal inguinal nodes. Clin Radiol. 55(2):124-30. https://doi.org/10.1053/crad.1999.0330

37. Gosomji IJ, Omirinde JO, Hena SA, Wanmi N, Azeez IA. (2018) Saturated salt solution an alternative reagent in reducing formaldehyde concentration in embalming. MOJ Anat Physiol. 5(3):205-7. http://doi.org/10.15406/mojap.2018.05.00192

38. Capitanio U, Suardi N, Shariat SF, Lotan Y, Palapattu GS, Bastian PJ, et al. (2009) Assessing the minimum number of lymph nodes needed at radical cystectomy in patients with bladder cancer. BJU International. 103(10):1359-62. https://doi.org/10.1111/j.1464-410x.2008.08212.x

39. Thompson RH, Carver BS, Bosl GJ, Bajorin D, Motzer R, Feldman D, et al. (2012) Body mass index is associated with higher lymph node counts during retroperitoneal lymph node dissection. Urology. 79(2):361-4. https://doi.org/10.1016/j.urology.2011.04.050

40. Dori Y. (2016) Novel lymphatic imaging techniques. Tech Vasc Interv Radiol. 19(4):255-61. https://doi.org/10.1053/j.tvir.2016.10.002

41. Tam EK, Shen L, Munneke JR, Ackerson LM, Partee PN, Somkin CP, et al. (2012) Clinician awareness and knowledge of breast cancer-related lymphedema in a large, integrated health care delivery setting. Breast Cancer Res Treat. 131(3):1029-38. https://doi.org/10.1007/s10549-011-1829-z

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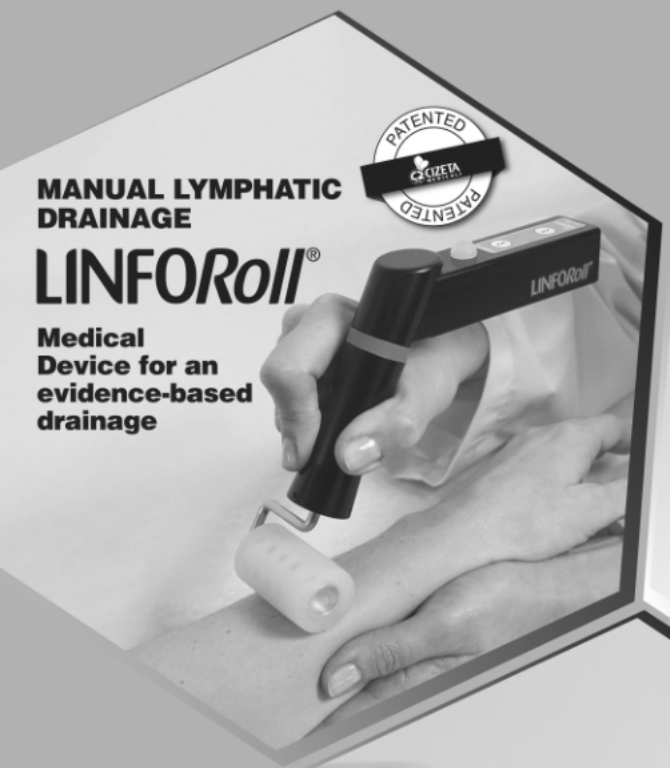

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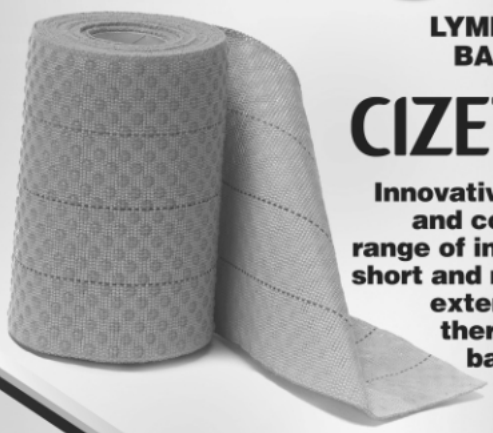

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THE LYMPHOSCINTIGRAPHIC STUDY OF THE DEEP LYMPHATIC CIRCULATION IN THE DIFFERENTIAL DIAGNOSIS OF OEDEMA OF THE LOWER LIMBS

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ABSTRACT

BACKGROUND: *Lymphoscintigraphy is the main investigation for visualization of lymphatics, assessment of their function, diagnosis of lymphoedema. Till now there is no international agreed standard. Even if there are two peripheral lymphatic circulations (superficial and deep), with connections and the potentiality of compensations in case of lymphatic damage, commonly only the superficial lymphoscintigraphy is performed. This could lead to a wrong diagnosis of the origin of a leg oedema.*

AIM OF THE STUDY: *Demonstration of the importance of performing lymphoscintigraphic study of both superficial and deep peripheral circulation (two compartment lymphoscintigraphy).*

MATERIAL & METHODS: *A retrospective study was carried out on lymphoscintigraphic exams pooled from Nuclear Medicine Units of three Italian hospitals (Udine, Venice, Trento), performed between 2013 and 2018, for diagnosis in case of established or suspected lymphoedema of lower limbs.*
Lymphoscintigraphy of superficial and deep lymphatic systems has been performed on 142 patients.
Of these, 106 are females (75%) and 36 are males (25%), with average age of 52.7 ± 17.7
Eighty one patients have been diagnosed with lymphoedema, in congenital form for 37 and secondary for 33; 11 patients have been diagnosed with lipoedema and 61 are affected by leg oedemas of unknown origin.
The lymphoscintigraphic assessment of the superficial circulation was followed, after few days, by the examination of the deep system. In both studies, the images were taken immediately and after 60 and 120 minutes. They were evaluated semiquantitatively using a modified Transport Index (TI) score, with analysis of tracer kinetics, distribution pattern, and visualization of lymph nodes and lymphatic vessels. Normal TI score was <9. The resulting data were subjected to descriptive statistical analysis.

RESULTS: *In 31 cases the study of superficial and deep lymphatic networks was normal (TI <9) for both limbs.*
In pathological cases (TI ≥ 9), the involvement of the deep lymphatic system (mono- or bilateral, associated or not with similar disturbance of the superficial system) was found in 100 cases; Involvement of the deep system alone was found in 29 cases (13 unilateral and 16 bilateral); 7 of these belonged to the group with oedema of unknown origin.
The exclusive involvement of the superficial system was found in 11 cases.
In the group of patients with leg oedema of unknown origin, lymphoedema was excluded in 18 cases, while in the remaining 43 cases there were all the combinations of superficial and deep lymphatic damage.

CONCLUSIONS: *Our study demonstrates a high prevalence of deep lymphatics damage in case of leg lymphoedema. Furthermore, a two-compartment lymphoscintigraphy allows the detection of isolated deep lymphatic impairment in case of leg oedemas of unknown origin, with early addressing to adequate care path.*

Keywords: Two compartment lymphoscintigraphy, deep lymphatic system, leg oedema, leg lymphoedema.

INTRODUCTION

Oedemas of the lower limbs represent a disorder found at any age, even if they are more frequent in the elderly. They can be divided into two types, depending on the kind of lymphatics insufficiency: oedema depending on functional disturbance (venous oedema, with low level of proteins) and that due to mechanical impairment (oedema that is rich of proteins).[1] In the first case it is a sign of an overload of a normal-functioning lymphatic system, with a lot of different possible causes; in the second case we face a well-defined clinical entity: lymphoedema.
The differential diagnosis of these oedemas can become an important challenge, especially in mild and unilateral forms, since lymphedema can have at first stage a clinical expression similar to that of venous oedema. Lymphoscintigraphy, a minimally invasive diagnostic tool used for quantitative and qualitative study of lymphatic circulation, can be of great help.
It allows the assessment of pathways of lymphatic drainage, quantification of lymphatic flow and differentiation of lymphedema from other oedematous conditions.[2, 3]
Its protocol is not standardized: main differences include the choice of radiotracer, use of dynamic and static acquisitions and acquisition times.[4]
Another difference is if lymphoscintigraphy is performed for the study of the superficial circulation only, or if it includes also an examination of the deep lymphatic flow.
The lymphatic circulation is organized in a superficial and a deep network, that at extremities run respectively above and under the muscular fascia.
The two lymphatic networks have some connections and one can compensate a malfunctioning of the other.[5]
The study of both superficial and deep lymphatic networks is called two compartment lymphoscintigraphy.[6] Inclusion of the deep lymphatic vessels in the lymphoscintigraphic study has shown to increase diagnostic accuracy.[7]
In order to confirm the importance of the assessment of deep lymphatic circulation for lymphoedema detection, we present our casuistry, relating to established lymphoedema conditions, lipoedemas and leg oedemas of unknown origin.

MATERIAL AND METHODS

A retrospective analysis was performed on 142 two compartment lymphoscintigraphies of the lower limbs, carried out in the period 2013-2018 and pooled from Nuclear Medicine Units of three Italian hospitals (Udine, Venice, Trento).
We included all the exams performed during the target period to obtain the largest available sample. Most of patients were addressed to the institutes by expert lymphologists. Other subjects were sent by specialists in Vascular Surgery or Infectious Diseases.
Lymphoscintigraphy was performed after a clinical evaluation with the purpose of confirmation and prognostic assessment in diagnosed conditions (primary and secondary lymphoedema, lipoedema) or for understanding the aetiology of oedemas of unknown origin.

Casuistry

We studied 142 patients (36 males and 106 females) with suspected or clinically diagnosed lymphoedema. The average age of the sample was 52.7 years (± 17.7).
The anamnestic and clinical evaluation prior to the examination made it possible to specify the diagnosis in 81 cases: 37 primary lymphoedema, 33 secondary forms and 11 lipoedema with doubtful mechanical insufficiency of the lymphatics.
In 61 cases, two compartment lymphoscintigraphy was performed for diagnostic clarification in mono- or bilateral oedemas of the lower limbs of unknown origin. (Table n.1)

Technique of the lymphoscintigraphic examination

The same examination protocol was used at the three hospitals, with lymphoscintigraphy of the lower limbs performed in two phases: a study of the superficial network followed, after a few days, by the study of the deep one.
For the assessment of the superficial circulation, an intradermal injection was performed at the 2nd intermetatarsal space of the distal portion of the dorsum of the foot; for the second study, the injection was performed subfascially, at the posterior portion of the sole of the feet.
At any site was injected a solution of 0,5 ml, containing colloidal nanoparticles of human albumin (NANOTOP, product of ROTOP PHARMAKA GmbH), labelled with 99mTc (37 MBq) and diluted with 0.9% NaCl solution.
For image acquisition a Gamma Camera SYMBIA SIEMENS was used, with a collimator LEAR.
Static images were acquired in 3 phases:
- Time 0: immediately after the injection (at injection point, injection point+leg+knee, knee, thigh, pelvis);
- Time 1: at 60' exactly, after 45' of walking (at injection point, injection point+leg+knee, knee, thigh, pelvis, total body);
- Time 2: at 120' exactly, after rest (at injection point, injection point+leg+knee, knee, thigh, pelvis, total body).

Data evaluation

A modified Transport Index was used to differentiate pathological and normal pictures.
Since the used protocol does not allow calculation of time to visualize the lymph nodes (T), only transport kinetics (K), distribution of the tracer (D), visualization of lymph nodes (N) and visualization of lymph vessels (V) were assessed, as described by Kleinhans.[8] TI scores were considered pathological if ≥ 9.
The scores were assigned by a Nuclear Medicine medical doctor belonging to the Institute, who is expert in the execution and reporting of lymphoscintigraphies.

Statistical evaluation

ITo describe the sample, mean and standard deviation were used for age, while frequency distribution were reported to describe the sample at the categorical level: gender, type of exam performed (deep or superficial) diagnosis, type and localization of the oedema. The descriptive statistical analysis was performed using Excel 2013 (Microsoft Corporation, Redmond, WA).

RESULTS

In 31 (22%) cases we found totally normal pattern for both limbs (TI <9). (Table n. 2)
A pathological pattern of only one leg was found in the 32% of altered exams: in 16 cases of both networks (superficial and deep), in 13 only at the deep and in 6 only at the superficial circulation.
In the 68% of cases, we found TI pathological values at both lower limbs: the involvement regarded

- In 20 cases (18% of pathological exams) only deep circulation at one side and both superficial and deep circulation at the other;
- In 19 (17%) both circulations (deep and superficial) at both sides
- In 16 (14%) solely deep system at both sides
- In 10 (9%) only superficial at one side and both circulation on the other side
- In 6 (5%) only superficial at one side e only deep at the other
- In 5 (4%) cases only superficial on both sides.

In pathological cases, the involvement of the deep lymphatic system (uni- or bilateral, associated or not with similar disturbance of the superficial system) was found in 100 cases (90%). Damage of the deep system alone was found in 29 cases (26%): 13 unilateral and 16 bilateral. The exclusive involvement of the superficial network was found in 11 cases (10%).
In the group of 61 patients with oedema of unknown origin (and with suspicion of lymphoedema) according to the TI score, lymphoedema was excluded in 18 cases (29,5% of this group), while in the remaining 43 cases there were all the combinations of damage (superficial / deep, mono- / bilateral), with greater frequency of exclusive bilateral involvement of the deep circulation (see table n. 2).

DISCUSSION

Lymphoscintigraphy is the main investigation for visualization of lymphatics, assessment of their function, diagnosis of lymphoedema. Till now there is no international agreed standard in relation to tracer, sites of injection, times of image acquisition.
Even if there are two peripheral lymphatic circulations (superficial and deep), with connections and with the possibility of compensations in case of lymphatic damage, commonly only the superficial lymphoscintigraphy is performed. This can lead to misdiagnosis of the origin of an oedema. In the absence of a standard reference for diagnosis a diagnostic and accurate study is not feasible, but the present research is a cue to open a discussion on which should be the best modality to examine patients.
The possibility of studying superficial and deep lymphatic circulation of the leg was already presented by Bräutigam in the nineties (5, 6); what he called two compartment lymphoscintigraphy was developed in order to assess type and stage of leg oedemas. With this approach he could differentiate primary lymphoedema and early and advanced stages of postthrombotic syndromes.
Diagnosis of a leg oedema can be challenging at any age. Actually, there are many factors to take in account: duration, mono- or bilaterality, location (proximal or distal), fovea and Stemmer signs, other known patient's pathologies (heart, renal or liver failure; chronic venous insufficiency, hypothyroidism and others). Important is the possibility of a detection and confirmation of a lymphoedema, but in early stages this pathology may have clinical characteristics like those of a venous oedema.
In our experience, lymphoscintigraphy of the lower limbs is performed for diagnostic confirmation and prognostic evaluation of a clinically diagnosed lymphoedema, for an early diagnosis of mechanical insufficiency in lipoedema at the 2nd and 3rd stages, and for differential diagnosis in oedemas of unknown origin. For this last indication, we learned to perform the study early, particularly in case of mild, unilateral, distal oedema, which often proves to be a primary lymphedema at the 1st or at the second initial stage.
Since 2013 at the Nuclear Medicine Institute of University Hospital of Udine lymphoscintigraphy of the lower limbs involves the study of both the superficial and the deep circulation, in the belief that the data obtained allow better understanding of the lymphatic function and clinical correlations.

The high prevalence of deep lymphatic damage confirms the importance of the two-compartment examination: of the 111 people with pathological lymphoscintigraphic exam, 100 cases showed a uni- or bilateral deep circulation impairment, associated or not with alteration of the superficial network. This data agrees with that recently published by Campisi, in which in a series of 248 two compartment lymphoscintigraphies the deep lymphatic vessels were damaged in the vast majority of patients (88% - 98%), with a pathological TI for either the deep subfascial vessels alone, or both the superficial and deep vessels.[9]
In 29 cases we found a damage concerning only the deep lymphatics. This means that in more than a quarter of patients submitted to a study of the superficial circulation alone, diagnosis of lymphatic impairment would be missed. This is in accordance with Villa's report: in his study deep vessel abnormalities were found in approximately 30% of the patients with normal superficial system.[10]
A correlation has been demonstrated in the past between isolated damage of the deep lymphatics of the lower limbs and initial stages of postthrombotic syndrome; in advanced stages also the impairment of superficial lymphatics was found, as consequence of chronic

functional overload of this network. A less frequent isolated alteration of the deep lymphatics was found also in case of primary lymphedema.[5] Our patient had neither history nor clinical features of a postthrombotic syndrome.

In our group of patients with oedema of unknown origin, the examination was diagnostic in 43 of 61 cases, confirming the importance of two compartment lymphoscintigraphy in the differential diagnosis of oedema, especially in the initial, clinically mild forms. Bräutigam was able to distinguish different lymphoscintigraphic patterns, finding: in case of idiopathic cyclic oedema, a marked increase in lymphatic flow in the two networks; increased flow in the superficial circle in case of phlebedema; in postthrombotic syndrome reduced flow in the deep network, while in the superficial it was normal or reduced depending on the duration of the condition; normal activity in obesity.[6]

The possibility of distinguishing the origin of the swelling can allow early adoption of adequate therapeutic strategies (especially in the case of lymphoedema) or address to further investigations, in case of venous oedema of still unrecognized origin.

This possibility is also important in all those cases in which an objective demonstration of a lymphoedema ensures specific benefits, provided by healthy systems.

The completion of lymphoscintigraphy with the study of the deep network can have implications for the surgical approach to lymphoedema. Derivative interventions between lymphatics and veins can be performed in two ways: connection of one superficial collector to one small venule in multiple sites (supermicrosurgery), or multiple lymphatic-venous anastomoses involving superficial and deep lymphatic vessels into a vein branch.[11-13] Knowledge of the functional state of the superficial and deep lymphatic vessels could guide the choice of one or the other surgical approach.

Now there are not protocols of conservative treatment of deep lymphatics disfunctions, even if some maneuvers of manual lymphatic drainage are specifically addressed to this network.

The awareness of the frequent involvement of deep lymphatics in lymphedema and the possibility of their morphological and functional study will lead to the search for specific conservative treatments.

Our study presents some limitations. One of these is the modification of criteria for calculation of Transport Index, eliminating the T (time to visualize the lymph nodes). It was due to our modality of study performance, that not allows the computation of this parameter. Other authors have already used a modified TI score, without specification about the change, and with a cut off of 5.[14]

The fact that lymphoscintigraphy is a non-standardized examination exposes to evident difficulties in applying score systems. In his study on scintigraphic exam of deep lymphatics, in case of leg oedema with normal superficial scintigraphic pattern, Barbieux used a different method to discriminate normal from pathological conditions. The absence of popliteal lymphnodes visualization after the second phase of his lymphoscintigraphic protocol was used as criterion for this differentiation (specificity and sensitivity of 89% in patients with unilateral lower limb oedema and without associated venous symptoms). He could also demonstrate that, in the casuistry of 32 patients, 20 had a pathological deep lymphatics scintigraphy after a normal result of the study of superficial network. This study gives a new approach for diagnosis of deep lymphatic circulation impairment and confirms the importance of the two compartment lymphoscintigraphy for an adequate diagnosis of peripheral oedemas of unknown origin.[15]

We could not make a correlation with the clinical parameters (extent of swelling, features of the skin) since, being a retrospective study, it was not possible to obtain all the necessary data. A study on this correlation will be carried out in further prospective studies.

CONCLUSION

Lymphoscintigraphy is an important tool for diagnosis of lymphoedema. Nevertheless, it is still lacking of a standardization. Moreover, due to many reasons, in most cases only an investigation of the superficial lymphatic circulation is performed. This can lead to misdiagnosis, especially in case of mild leg oedema of unknown origin. Our study demonstrates that in 90% of pathological studies there is a damage of the deep lymphatic circulation. This could be of interest not only for a diagnostic reason, but also for the choice of the best surgical approach to lymphedema and for development of specific therapeutic strategies addressed to deep lymphatics impairment.

	PRIMARY	SECONDARY	LIPOEDEMA	OEDEMA OF UNKNOWN ORIGIN	TOTAL
AGE	39.09 ± 14.02	67.8 ± 8.43	57.5 ± 17.4	51.82 ± 18.04	52.7 17.7
MALE/FEMALES	8/29	15/18	0/11	14/47	37/105
BILATERAL	13(35%)	13(39%)	11(100%)	37(61%)	74(52%)
UNILATERAL	24(65%)	20(61%)	0(0%)	24(39%)	68(48%)
RIGHT	10(42%)	10(50%)	0(0%)	9(38%)	29(43%)
LEFT	14(58%)	10(50%)	0(0%)	15(62%)	39(57%)
OVERALL	37 (26%)	33 (23%)	11(8%)	61(43%)	142 (100%)

Table n.1: *Sample description*

	S+S	S+SD	N+N	SD+SD	D+D	D+S	N+SD	N+D	SD+D	N+S	TOTAL
PRIMARY LYMPHEDEMA	0	4	4	7	4	0	4	5	8	1	**37**
SECONDARY LYMPHEDEMA	2	3	5	7	2	0	6	2	5	1	**33**
LIPEDEMA	1	0	4	0	3	1	0	0	2	0	**11**
OEDEMA OF UNKNOWN ORIGIN	2	3	18	5	7	5	6	6	5	4	**61**
TOTAL	**5**	**10**	**31**	**19**	**16**	**6**	**16**	**13**	**20**	**6**	**142**

Table n.2: *Different patterns relating to damage to one and/or both legs (damage: S = superficial, D = deep, SD= superficial and deep; N = none). For example, S+SD means damage of superficial lymphatic circulation on one side and damage of both superficial and deep circulation on the other side.*

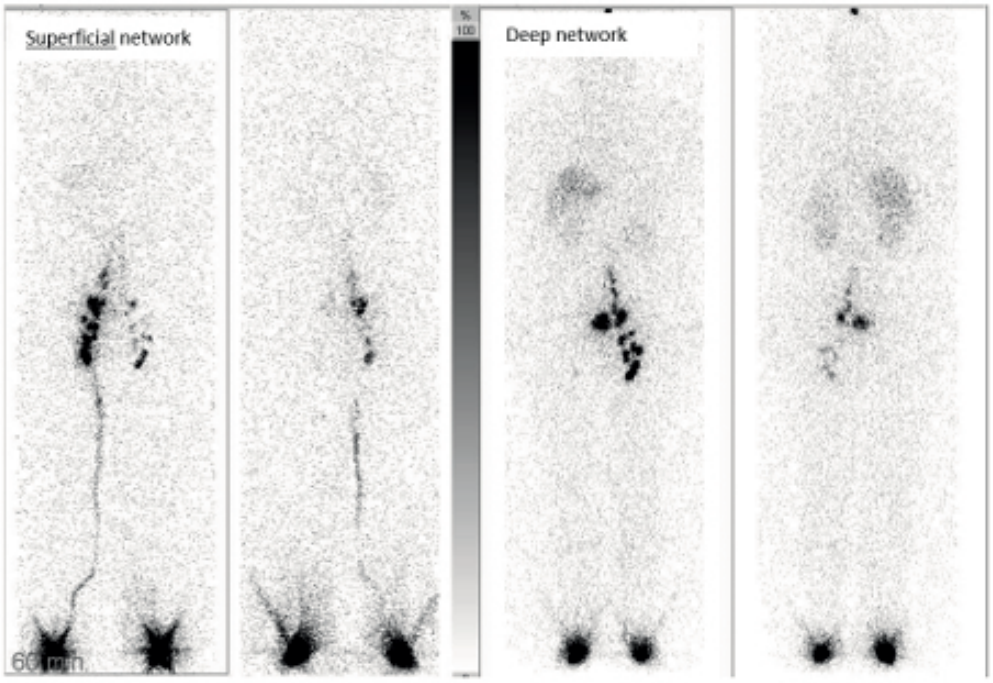

Figure n.1: *Lymphoscintigrams of superficial and deep network of lower limb in an oedema of unknown origin, at 60 minutes from injection. Due to our examination technique, Transport Index scores are assessed for K (= transport kinetics), D (= distribution of the tracer), N (= visualization of lymph nodes), V (= visualization of lymph vessels), not for T (= time to visualize the lymph nodes). The scores for these exams are: right superficial: K=3, D=0, N=0, V=0 (total = 3); left superficial: K=5, D=3, N=5, V=9 (total = 22); right deep K=9, D=0, N=9, V=9 (total = 27); left deep K=3, D=0, N=3, V=9 (total = 15)*

REFERENCES

1. Ely JW, Osheroff JA, Chambliss ML, Ebell MH.: Approach to Leg Edema of Unclear Etiology. J Am Board Fam Med. 2008 Jan-Feb; 21(1):86.

2. Yoshida RY, Kariya S, Ha-Kawa S, Tanigaw N: Lymposcintigraphy for imaging of the lymphatic flow disorders. Tech Vasc Interv Radiol 2016; 19 (4): 273-6

3. Williams WH, Witte CL, Witte MH, McNeil GC: Radionuclide lymphangioscintigraphy in the evaluation of peripheral Lymphoedema. Clin Nuc Med 2000, 25: 451-64.

4. Keeley V: The use of lymphoscintigraphy in the management of chronic oedema. Journal of Lymphoedema, 2006, Vol 1 (1): 42-57.

5. Bräutigam P, Vanscheidt W, Földi E, Krause T, Moser E.: The importance of the subfasciai lymphatics in the diagnosis of lower limb edema: Investigations with semiquantitative lymphoscintigraphy. Angiology. 1993 Jun;44(6): 464-70.

6. Bräutigam P, Földi E, Schaiper I, Krause T, Vanscheidt W, Moser E.: Analysis of lymphatic drainage in various forms of leg edema using two compartment lymphoscintigraphy. Lymphology. 1998 Jun;31(2): 43-55.

7. Erba PA, Sollini M, D'Errico G e al: In Mariani G, Manca G, Orsini F et al.: Methodological aspects of lymphoscintigraphy: bicompartimental versus monocompartimental radiocolloid administration. Milan, Italy: Atlas of lymphoscintigraphy and sentinel node mapping, Springer, 2013.

8. Kleinhans E, Baumeister RG, Hahn D, Siuda S, Büll U, Moser E: Evaluation of Transport Kinetics in Lymphoscintigraphy: Follow-Up Study in Patients with Transplanted Lymphatic Vessels. Eur J Nucl Med, 1985; 10(7-8): 349-52.

9. Campisi CC, Ryan M, Villa G1, Di Summa P2, Cherubino M, Boccardo F, Campisi C.: Rationale for Study of the Deep Subfascial Lymphatic Vessels During Lymphoscintigraphy for the Diagnosis of Peripheral Lymphedema. Clin Nucl Med. 2019 Feb;44(2): 91-98.

10. Villa G, Campisi CC, Ryan M, Boccardo F, Di Summa P, Frascio M, Sambuceti G, Campisi C: Procedural Recommendations for Lymphoscintigraphy in the Diagnosis of Peripheral Lymphedema: the Genoa Protocol. Nucl Med Mol Imaging. 2019 Feb;53(1):47-56.

11. Campisi C, Boccardo F. : Lymphedema and microsurgery. Microsurgery. 2002; 22: 74-80.

12. Mehrara BJ, Zampell JC, Suami H, et al.: Surgical management of lymphedema: past, present, and future. Lymphat Res Biol. 2011; 9: 159-167.

13. Cormier JN, Rourke L, Crosby M, et al.: The surgical treatment of lymphedema: a systematic review of the contemporary literature (2004-2010). Ann Surg Oncol. 2012; 19: 642-651.

14. Cambria RA, Gloviczki P, Naessens JM, Wahner HW.: Noninvasive evaluation of the lymphatic system with lymphoscintigraphy: A prospective, semiquantitative analysis in 386 extremities. J Vasc Surg. 1993 Nov;18(5): 773-82.

15. Barbieux R, Roman MM, Rivière F, et al.: Scintigraphic Investigations of the Deep and Superficial Lymphatic Systems in the Evaluation of Lower Limb Oedema. Sci Rep 2019 Sep 23; 9 (1):13691.

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ULTRASOUND ASSESSMENT OF UPPER LIMB LYMPHEDEMA AFTER BREAST CANCER TREATMENT

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ABSTRACT

Objective: *Cross-sectional observational study comparing volumetry and ultrasound for lymphedema assessment in female with unilateral breast surgery.*

Method: *Khunke method (Volumetry) and thickness ultrasonography were performed on both upper limbs.*

Results: *Ninety females, mean age 59.23a. 70 patients (77.8%) didn't show clinical signs of lymphedema. Mean volume was 2828.36cc (1667.0 - 4895.9) for healthy limb and 2958.85cc (1767.6 - 5296.0) for affected limb. Volumetric differences between both upper limbs were: -15.70% and 69.7% (Mean = 4.54; SD 10.56). Mean dermo-epidermal thickness: 0.10cm in the healthy arm; 0.26cm on the affected side; mean thickness in the hypodermis: 0.88cm in the healthy arm and 1cm in the affected arm. Pearson showed positive correlation between volume and ultrasound thickness in both EESS ($p < 0.05$) except in dermal thickness in arm. Also, there were positive correlation between volumetric difference between unaffected and affected limb and ultrasound thickness for affected limb ($p < 0.05$).*

Conclusions: *The thickness tissue by ultrasonography is related with volumetric measurement in both upper limbs of female with unilateral breast surgery. The ultrasound tissue thickness of the affected limb is higher if there is clinical lymphedema.*

Keywords: lymphedema, lymphatic vessels, ultrasonography, upper limb, breast cancer, diagnostic, assessment.

INTRODUCTION

Breast cancer is the most frequent cause of lymphedema. It is estimated that 20% of these patients will develop lymphedema in the upper extremities. Incidence of lymphedema increases from 13,5% in two years to 41,1% in ten years. Moreover, between 15% and 22% develop fluctuating or intermittent lymphedema with asymptomatic periods.[1,2]

The benefit of the lymphoedema units of public hospitals Physical Medicine and Rehabilitation Services is recognized for the early care of patients who have undergone surgery for breast cancer. However, there is significant variability in the assessment system. The measurement of displaced water volume is considered the reference[3] technique but is not routinely used due to technical and economic limitations. Lymphoscintigraphy has been considered the gold standard for diagnosing lymphedema[4], but it is not a technique commonly used in rehabilitation consultations. Impedance Imaging is a simple technique but is also not implemented in clinical practice, and the cost of CT and MRI for the assessment of lymphedema limits its use.

In clinical practice, lymphedema is most often diagnosed by clinical history, physical examination of the tissue by palpation and measurement of the increased volume of the limb. There may not be sufficient volume increase in the early subclinical stage to be considered diagnostic. Therefore, it is essential to investigate other methods of early detection of lymphedema to minimize impairment effects on health and quality of life resulting from the increased extracellular fluid characteristic of lymphatic system dysfunction.

Diagnostic ultrasound (US) is based on obtaining images of the human body from high-frequency sound waves that are inaudible to the human ear (> 20,000Hz) from the echoes generated by the reflection of these waves as they hit the tissues.

The beginnings of the use of the US in medicine had a therapeutic purpose in the middle of the last century. However, in the '70s, it began to be developed to assess the locomotor system, with an exponential increase in its application in numerous medical specialities, as a non-invasive, fast, economic, repeatable, dynamic diagnostic imaging tool without exposure to radiation.

Musculoskeletal ultrasound has become a valuable tool in the daily clinical practice of specialists in Physical Medicine and Rehabilitation because it can contribute significantly to a patient's diagnostic and therapeutic algorithm. Thanks to technological advances in improving image quality, musculoskeletal ultrasound is a real option for the appropriate diagnosis of soft tissues.

Ultrasound can be used with the usual 13 Mhz linear probes (7-14) [5] to assess the changes in the skin and subcutaneous cellular tissues that occur in chronic lymphoedema due to alterations affecting the extracellular matrix with hypertrophy and accumulation of protein-rich fluid. The intra- and inter-observer reliability of the technique in assessing the forearm and upper arm in healthy subjects has been confirmed.[6]

Suehiro et al. established a classification based on the echogenicity of the dermo-epidermal and hypodermal layers in patients with lymphoedema in the legs, with a high degree of correlation with the staging of the International Society of Lymphology classification.[7]

For subclinical cases in which circometry does not show differences between the upper extremities, ultrasound could be a standardized and reproducible tool to be implemented in rehabilitation clinics. Hypothetically, establishing baseline ultrasound measurements would speed up the assessment of lymphoedema in the rehabilitation clinic. Therefore, the following study compares the circometric/volumetric measurement (commonly used method) and ultrasound assessment between the healthy and the affected limb in women treated for breast cancer and referred to the lymphoedema unit.

MATERIALS AND METHOD

Subjects: Women who underwent surgery for unilateral breast cancer were referred to the "lymphoedema consultation" of the Rehabilitation Service of a university hospital.

Design: Cross-sectional observational study over one calendar year (June 2020 to June 2021). Patients with bilateral involvement, cases of tumour recurrence in the contralateral breast and patients with lymphoedema of non-breast cancer etiology are not included in the study.

Procedure: data collection in medical consultation, regardless of the time since surgery, in which the following methodology is applied.

Method:

- Protocolized clinical assessment including Body Mass Index (BMI), inspection and comparative palpation of both upper extremities (UE), shoulder girdle and pectoral-breast area, joint balance and muscle balance of UE, neurological examination, recording of pain, recording of infectious, radio- or chemotherapy-related complications and axillary web syndrome.

- Volumetric assessment using Kuhnke's simplified formula based on the circumferential measurement according to the modified Mortimer method on the healthy and affected limbs.[8,9]

- Ultrasound study (General Electrics Logic C5 Premium™) with a linear probe (7-14 Hz) of the forearm at the inner surface of the upper 1/3 and the upper arm at the level of the posterior surface of the middle 1/3. Recording of dermo-epidermal thickness and subcutaneous cellular tissue (SCT) and morphology according to the Suehiro technique.

Statistics:

- Descriptive study of the sample using mean and standard deviation statistics for quantitative variables and absolute and relative frequency for qualitative variables:
    - Sociological data of the patients; tumour characterization (histological type, tumour staging; surgical procedure; adjuvant therapies); clinical data from the physical examination of lymphoedema based on the ad hoc protocol described.
    - Ultrasound features of both upper limbs: thickness and morphology of the skin and hypodermis of the healthy and affected limbs.

- Pearson's correlation for:
    - Determine the correlation between ultrasound thicknesses and volume for each limb,
    - Determine whether there is a correlation between the thicknesses found on ultrasound and the volumetric difference between the two UE,
    - Assess the bias that may exist between dermo-epidermal and SCT thicknesses concerning BMI.

PSPP 1.4.0[10] was used for statistical processing.
The degree of significance was considered to be p= 0.05.

The Ethics Committee has approved the work that it complies with the ethical regulations on biomedical research involving human subjects.

RESULTS

Descriptive study of the sample:

Clinical variables

The sample consisted of 90 women aged between 36 and 87 years (mean: 59.23; SD: 12.52) who underwent surgery for unilateral breast cancer; three of them had homolateral recurrence at the time of assessment. In 54.4% of cases (49 patients), the affected side was the right side, which corresponds to the side of manipulative dominance in 82.2% of cases. Regarding previous physical activity, 60% of the sample performed some activity, either mild aerobic exercise (23 patients) or moderate-intense exercise in a gym (27 patients). In two-thirds of the sample (70.1%), axillary dissection was performed (63 patients), and 87.8% received radiotherapy (79 patients) with total doses between 40 and 50Gy. In addition, thirteen patients received brachytherapy. Sixty-five patients (72.2%) received hormone therapy, and fifty patients received adjuvant or neoadjuvant chemotherapy (55.6%), with the combination of anthracyclines and taxanes being the most commonly used option. **Table 1** shows descriptive data on the histological type of breast tumour, staging and the therapeutic procedures.

The mean time for patients to be assessed since surgery was 42.35 days (SD: 69; 15-372).

Regarding clinical characteristics, the mean body mass index was 27.94 kg/m2 (SD: 5.08). No skin alterations were detected on inspection in 94.4% of cases, compared to 5.6% (5 patients) with radiotherapy dermatitis. In 66.7% of the cases (60 patients), palpation of the surgical scar showed no significant alterations, while one-third of the sample (29 patients) had some scar alteration (dysesthesia, hyperalgesia, adhesions). In 74.4% of the cases, palpation of the upper extremities was symmetrical between the two UE. Sensory examination showed some alteration in the affected limb in 34.5% of the cases (31 patients), with a predominance of hypoalgesia in the posterior area of the upper arm. In addition, 8.9% of the patients had sensory alterations due to chemotherapy applied (chemotherapy-related neuropathy). Among the causes of pain in patients who underwent surgery, postmastectomy syndrome stood out in 27 patients (30%); 7 patients had orthopaedic pain, and 6 had symptoms compatible with axillary web syndrome. The shoulder joint balance was not limited in 52 patients (57.8%); a third of the cases (30%: 27 patients) presented mild limitation (< 30% of the joint arc for the healthy side) without functional repercussion, while ten patients (11.1%) had severe limitation of shoulder mobility due to different causes (pain, previous osteoarticular pathology, stroke). For the most part, strength in UE was not reduced in the non-painful joint mobility range.

At the time of assessment, 77.8% of patients (70 cases) had no clinical signs of lymphoedema according to the International Society of Lymphology[11] classification (subclinical stage), 14.4% (13 patients) were classified as stage 1, four patients (4.4%) were considered upper limb stage 2A and three patients were classified as stage 2B (3.3%) according to the international classification.

Ultrasound variables

The mean volume of the UE in the 90 patients studied, obtained from circumferential measurement, was 2828.36cc (1667.0-4895.9; SD: 662.18) for the healthy side and 2958.85cc (1767.6-5296.0; SD: 740.87) in the affected limb. The volumetric differences between the two UE ranged from -15.70% to 69.7% (mean 4.54; SD 10.56). **Figure 1** shows the frequency histogram of the volumetric differences between the two UE.

Table 2 shows the mean values of dermo-epidermal and hypodermal thicknesses in the assessed areas of the forearm and upper arm in both UE.

Regarding ultrasound morphology, according to Suehiro's description, 77.8% of the sample (70 patients) showed no alterations (stage 0), 13 patients (14.4%) had an ultrasound pattern compatible with stage 1, and 5 patients had an ultrasound morphology labelled as stage 2.

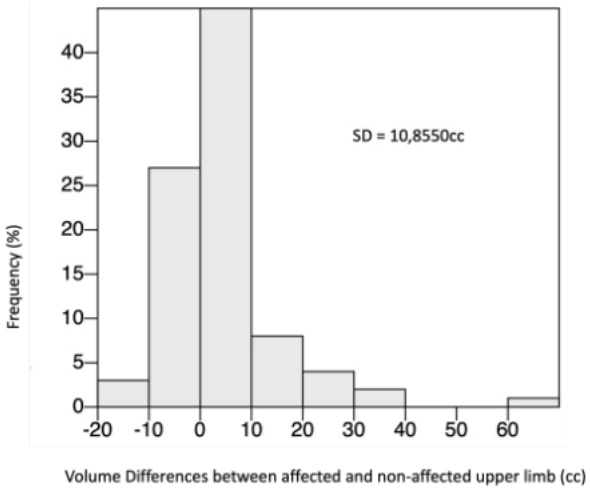

Figure 1: *Hystogram of frequencies of volume difference in percentage between healthy and affected upper limb.*

Statistical variables

Pearson correlation on the affected and healthy side between ultrasound thicknesses and volumetric values

Table 3 shows the values of Pearson's bivariate analysis of the thicknesses of the dermo-epidermal and hypodermal layers for each limb to the volumes and the volumetric difference between the two UE. From this analysis, it can be seen that:

- On the affected side, the greater the volume of the limb, the greater the thickness of the dermo-epidermal layers and the SCT on the forearm and the thickness of the SCT on the upper arm; the correlation is not significant between the volume of the affected limb and the dermal thickness on the upper arm.

- On the healthy side, the same trend is observed: the greater the volume of the limb, the greater the thickness of the dermo-epidermal layers and the SCT in the forearm and the thickness

of the SCT in the upper arm; there is no significant correlation between the volume of the healthy side and the dermo-epidermal thickness in the upper arm.

- The thickness of the dermis in the forearm of the affected extremity correlates with the more significant the volumetric difference between the two arms, but not in the healthy limb.

- The hypodermis thickness at the upper arm and forearm level correlates significantly more remarkably with the volumetric difference between the two UE, but not on the healthy side.

- The more significant difference in volume between the healthy and affected limb shows no significant correlation with the thickness of the dermis at the level of the arm, both on the healthy and affected side.

Pearson's correlation between BMI and dermo-epidermal and hypodermal thicknesses for both UE

Table 4 shows the bivariate analysis with Pearson correlation between the BMI of the patients studied and the thicknesses of the dermo-epidermal and hypodermal layers assessed in the forearm and upper arm of both healthy and diseased limbs for each patient. This analysis showed a strong correlation between the thickness of the hypodermis and BMI in the forearm and upper arm in both the affected and healthy limbs. In contrast, the dermis thickness showed a low correlation with BMI, especially at the upper arm level, in both limbs.

DISCUSSION

In rehabilitation consultations, the diagnosis of lymphoedema continues to be clinical, based on a comparative study between the affected limb and the contralateral limb, healthy in the case of unilateral edema. However, this method has some limitations, such as in cases where both sides are affected. In addition, there may not be a sufficient increase in volume to meet the diagnostic criteria in the early, subclinical stage, nor is it helpful in assessing structural changes in the subcutaneous tissues. Women with mild lymphedema are three times more likely to have moderate or severe lymphoedema than women without lymphoedema[2]. Therefore, it is essential to investigate other cost-effective methods for the study and early detection of lymphoedema that minimize the impairment effects of increased extracellular fluid on health and quality of life.

Ultrasound can be used to assess, with the usual 13 MHz linear probes (7-14), the essential morphological characteristics of the layers of the skin and subcutaneous cellular tissues, and the reliability of the technique has been confirmed intra- and inter-observer in the evaluation of the forearm and arm in healthy subjects. In recent years, several studies have been published defending the usefulness of ultrasound for the diagnosis of lymphoedema of the upper extremity in patients who have undergone breast cancer surgery.[13, 14, 15, 16]

Thus, this study aimed to compare the circometric/volumetric measurement (commonly used method) and ultrasound assessment between the healthy limb and the affected limb in women treated for breast cancer referred to the rehabilitation unit. Hypothetically, establishing reference ultrasound measurement parameters would speed up the assessment of lymphoedema and allow more accurate treatment and follow-up guidelines about the stage of lymphoedema. In this study, the measurements of the healthy side have been assumed as the standard values.

In the sample studied, 77.8% did not show clinical signs compatible with lymphedema according to the SIL classification, a percentage identical to that of cases in which the morphology of the dermal and hypodermal layers of the UE are described as usual according to the criteria proposed by Suehiro. For this reason, given that only 22.2% of the sample showed morphological alterations characteristic of lymphedema, Pearson's bivariate analysis was not performed. Instead, we opted to evaluate the ultrasound thicknesses and their possible correlation in anticipation of the development of lymphedema before it develops clinically.

Pearson's bivariate analysis shows a significant positive correlation between thickness and volume, i.e. the greater the thickness, the greater the volume for each limb, healthy and affected, at the dermo-epidermal level of the forearm (not the upper arm) and in the SCT of the forearm and upper arm. These data are consistent with other authors such as Mander et al.[14] A retrospective study of 287 patients who underwent surgery for unilateral breast cancer found a significant increase in dermo-epidermal thickness of the forearm of the affected limb with that of the healthy limb.

Similarly, Polat et al.[15] assessed the usefulness of elastography for diagnosing lymphoedema in correlation with the clinical stage of lymphedema based on the thickness of the cutaneous and subcutaneous layers of the affected limb about the healthy limb.

On the other hand, bearing in mind that obesity leads to an increase in adipose tissue, it was decided to assess the possible bias between dermo-epidermal and SCT thicknesses to BMI using Pearson's correlation analysis. In this way, it was found that there is a strong correlation between the thickness of the hypodermis and BMI in the forearm and upper arm in both the affected and healthy limbs. In contrast, the dermis thickness showed a low correlation with BMI, especially in the upper arm in both limbs **(Table 3)**. From this data, it could be inferred that dermo-epidermal thickness may be the ultrasound variable that can provide the most information with the diagnosis of lymphedema, as its thickness does not depend on the degree of obesity of the patients studied. Several ultrasound studies of lipedema determine the thickness of the SCT as a diagnostic criterion to distinguish between lipedema and lymphedema.[17, 18]

Pearson's analysis was performed to assess whether there is a correlation between the difference in the volume of the affected limb concerning the healthy limb and the ultrasound thicknesses.

This analysis showed a significant positive correlation in the affected limb, specifically between dermal and hypodermal thicknesses in the forearm and the thickness of the SCT of the upper arm. The same does not occur in the healthy limb, where the Pearson analysis shows no correlation between the difference in volume between the healthy and affected arms and the thickness of the skin layers. From all this, it could be inferred that the more significant the difference in volume between the healthy and affected limbs (clinical criterion of lymphoedema), the more significant the difference in thickness of the dermis and SCT in the forearm and the more significant the difference in thickness of the SCT of the affected arm compared to the other side.

From the statistical analysis carried out, we could consider that, given that the dermis does not correlate with BMI, it could be the most relevant ultrasound parameter for discriminating the existence of subclinical lymphedema, specifically at the level of the forearm, in cases where the thickness of the hypodermis is related to BMI and not to incipient lymph accumulation, where the ultrasound morphology is not altered. These results are consistent with Soo-Yeon et al.[16], who found a positive correlation between circometry and soft tissue ultrasound area at 10 cm below the elbow, with moderate to excellent intra- and interclass coefficients.

Limitations of the study:
It is a cross-sectional study. The data analyzed are from a single clinical episode, which should be contrasted with future longitudinal studies to contrast the ultrasound/volumetric correlation over time. Hypothetically, including patients who underwent reoperation could generate a particular bias in the analysis; however, the number of cases is minimal (3 cases), and, on the other hand, the study presented does not aim to assess the risk factors of lymphedema but to compare two methods of characterizing it.
In this regard, the literature details that the determining factor in the appearance of lymphedema is axillary lymph node dissection; in this study, 71.1% (64 patients) had undergone such surgery, so the sample is considered representative of the study of the development and evaluation of lymphedema.
The fact that the study was carried out early after surgery in the majority of cases (mean: 6 weeks) could be considered a limitation in that there is not enough time to develop lymphedema; however, the chronological variability in the presentation of lymphedema is well known, and the statistical study presented here points to the usefulness of ultrasound as a technique to anticipate the clinical confirmation of lymphedema, an attitude advocated by various authors who have demonstrated the usefulness of ultrasound for the diagnosis of lymphedema. In our case, this hypothesis should be corroborated with follow-up studies of the sample presented.

A possible weakness of the ultrasound technique is the variation in measured thickness depending on the pressure applied by the ultrasound probe. In this regard, Soo-Yeon found, with the application of maximum and minimum pressures on the soft tissues, that the ratio of the differences in thickness between the affected side and the healthy side maintained the correlation with the circometry at 10 cm below the elbow, but not at 10 cm above the elbow.
Another aspect to consider is the definition of the areas to be studied by ultrasound, which, although there are similarities between the authors consulted, the procedure is not standardized.[14, 16, 19]
In this study, two areas of interest have been chosen based on clinical experience with the highest frequency of lymphedema localization, excluding the hand, to facilitate the homogenization of the sample.

CONCLUSIONS

Ultrasonography of UE in women who underwent surgery for breast cancer shows a significant positive correlation between ultrasound thickness and clinical volume, i.e.:

- The greater the clinical volume, the greater the ultrasound thickness both on the affected side and on the healthy limb in the dermo-epidermal layers of the forearm and the SCT of the forearm and upper arm.

- The more significant the difference in volume between the affected limb and the healthy limb, the greater the ultrasound, dermal and hypodermal thickness in the affected limb compared to the healthy limb.

- Dermal thickness in the forearm of the affected limb may be the most useful diagnostic parameter for subclinical lymphedema.

Ultrasound could be helpful in the early diagnosis of lymphedema. Studies are needed to confirm the usefulness of implementing ultrasound in the rehabilitation clinic as an early diagnostic technique for lymphedema in the upper extremity after surgery for breast cancer.

CONFLICT OF INTEREST

Non-existent.

Histological type		Tumour staging		Lymphatic invasion		Metastasis	
DCIS	4 (4,4)	**G I**	30 (33)	**N0**	31 (34,4)	**M0**	87 (96,7)
IDC	67 (74,4)	**G II**	38 (42,2)	**N1**	38 (42,2)	**M1**	2 (2,2)
DCIS + IDC	5 (5,5)	**G III**	20 (22,2)	**N2**	17 (18,9)	**NV**	1 (1,1)
ILC	12 (15,4)			**N3**	4 (4,4)		
ISLC + ILC	1 (1,1)	**No registered**	2				

CIRUGÍA			
CS + SLNB	**CS + LNS**	**MASTECTOMY + SLNB**	**MASTECTOMY + LNS**
24 (26,6)	39 (43,4)	3 (3,3)	24 (26,7)

RADIOTHERAPY	
RT	**RT + BRACHITERAPY**
79 (87,8)	13 (14,4)

HORMONAL THERAPY			
TAMOXIFEN	**AROMATASE INHIBITORS**		**HT ALTERNATION**
	NON STEROIDAL	**EXEMESTANE**	
28 (31,1)	33 (36,6)	3 (3,3)	2 (2,2)

CHIMIOTHERAPY				
AC ± T	**AC + T + MA**	**FEC ± MA**	**T + MA**	**COMBINATIONS (Mtx; Cb; CT)**
30 (33,3)	8 (8,9)	3 (3,3)	4 (4,4%	4 (4,4)

Table 1: Descriptive study: tumor characterization and therapeutical procedures (number in brakets: percentage)
DCIS: Ductal carcinoma in situ; IDC: Invasive ductal carcinoma ISLC: In situ lobular carcinoma Carcinoma lobulillar in situ; ILC: Invasive lobular carcinoma; CS: Conservative surgical; SLNB: Sentinel lymp node biopsy; LNS: Lymph Node Surgery; RT: Radiotehrapy; HT: Hormonal therapy; AC: anthracyclines; T: taxane; MA: Monoclonal antibodies; FEC: Fluorouracil + epirubicin + cyclophosphamide; Mtx Methotrexate; Cb: Carboplatin; CT: Clinical trial.

Forearm				Arm			
DERMIS		HYPODERMIS		DERMIS		HYPODERMIS	
Healthy UL	Affected UL	Healthy UL	Affected UL	Healthy UL	Affected UL	Healthy UL	Affected UL
0,07 (0,02)	0,09 (0,04)	0,51 (0,31)	0,61 (0,41)	0,13 (0,11)	0,43 (2,83)	1,26 (0,45)	1,39 (0,44)

Table 2: Dermo-epidermal and hypodermal thicknesses in the assessed areas of the forearm and upper arm in both upper limbs.
Mean echographic thicknesses values (cm). In brackets: estándar deviation.
UL: Upper limb

Pearson	Ultrasound Thickness							
	AFFECTED LIMB				HEALTHY LIMB			
	Forearm		Arm		Forearm		Arm	
	D-E	HYD	D-E	HYD	D-E	HYD	D-E	HYD
CIRCOMETRY	0,432*	0,551*	0,110	0,611*	0,209*	0,469*	0,085	0,638*
VOLUMEN DIFFERENCE (healthy/affected limb)	0,531*	0,507*	0,156	0,252*	-0,103	0,077*	0,067	-0,104

Table 3: Pearson correlation on the affected and healthy side between ultrasound thicknesses and volumetric values.
*Significance 0,05
D-E: Dermoepidermis; HYD: Hypodermis

BMI	Forearm				Arm			
	DERMO-EPIDERMIS		HYPODERMIS		DERMO-EPIDERMIS		HYPODERMIS	
	Healthy UL	Affected UL	Healthy UL	Affected UL	Healthy UL	Affected UL	Healthy UL	Affected UL
Pearson	0,343 (0,001)	0,401 (0,000)	0,600 (0,000)	0,582 (0,000)	0,242 (0,023)	0,160 (0,136)	0,475 (0,000)	0,582 (0,000)

Table 4: Pearson correlation between body mass index and dermo-epidermal and hypodermal thicknesses for both upper limbs
(in brackets: Pearson significance level)

REFERENCES

1. Ana Carolina Padula Ribeiro Pereira, Rosalina Jorge Koifman and Anke Bergmann. Incidence and risk factors of lymphedema after breast cancer treatment: 10 years of follow up. The Breast 2017(36):67-73

2. Trace DiSipio, Sheree Rye, Beth Newman and Sandi Hayes. Incidence of inilateral arm lymphoedema after breast cancer:a systematic review and methaanalysis. The Lancet Oncol 2013 May 14(6): 500-15. Doi:10.1016/S1470-2045(13)70076-7

3. Levenhagen K, Davies C, Perdomo M, Ryans K, Gilchrist L. Diagnosis of Upper-Quadrant Lymphedema Secondary to Cancer: Clinical Practice Guideline From the Oncology Section of APTA. Rehab Oncol 2017;35:E1–E18.

4. Nicola´s Pereira C. y Isao Koshima. Linfedema: actualizacio´n en el diagno´stico y tratamiento quiru´rgico. Rev Chil Cir 2018;70(6):589-597.

5. Forner-Cordero I, Cuello-Villaverde E y Forner-Cordero A. Linfedema: diagnóstico diferencial y pruebas complementarias. Rehabilitación 2010;44(S1):14–20.

6. Hwang JH, Lee CH, Lee HH, Kim SY. A new soft tissue volume measurement strategy using ultrasonography. Lymphat Res Biol. 2014;12:89-94.

7. Suehiro K, Morikage N, Murakami M, Yamashita O, Samura M,and Hamano K. Significance of Ultrasound Examination of Skin and Subcutaneous Tissue in Secondary Lower Extremity Lymphedema. Ann Vasc Dis. 2013;6(2):180–188.

8. Mortimer PS. Investigation and management of lymphoedema. Vasc Med [revista electrónica]. 1990;1:1–20. [consultado 13/02/2010]. Disponible en: http://vmj.sagepub.com/.

9. Kuhnke E. Determination of volume from circumferential measurements [in German]. Folia Angiol 1976; 24:228–232.

10. https://www.gnu.org/software/pspp/

11. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology. Lymphology. 2013;46:1-11.

12. Guía de Orientación Diagnóstica y Terapéutica del Linfedema. Capi´tulo Espan˜ol de Flebologi´a y Linfologi´a ISBN:978-84-697-4329-4.

13. Jonhson KC, DeSarno M, Ashikaga T, Dee J, Henty SM. Ultrasound and clinical measures for lymphedema. Lymphat Res Biol 2016; 14(1):8-17.

14. Mander A, Venosi S, Menegatti E, Byung-Boong L, Neuhardt D, Maietti E, Gianesini S. Upper limb secondary lymphedema ultrasound mapping and characterization. Int Angiol 2019; 34(4):334-342.

15. Ahmed Veysel Polat, Mesut Ozturk, Ayfer Kamali Polat, Ufuk Karabacak, Tumay Bekci, Naci Murat. Efficacy of ultrasound and shear wave elastography for the diagnosis of breast cancer-related lymphedema. JUltrasound Med 2020; 39(4):795-803.

16. Soo-Yeon Kim, Chang-Hyung Lee, Sung Jin Heo, Myung-Hoon Moon. The clinical usefulness of lymphedema measurement technique using ultrasound. Lymphat Res Biol.2021;19(4):340-346.

17. Naouri M, Samimi M, Atlan M, et al. High-resolution cutaneous ultrasonography to differentiate lipoedema from lymphoedema. Br J Dermatol 2010; 163: 296–301.

18. Alexandre Campos Moraes Amato, Dumitri Zunino Saucedo, Keller da Silva Santos, Daniel Augusto Benitti. Ultrasound criteria for lipedema diagnosis. Phlebology 2021;36(8):651-658.

19. Suehiro K, Morikage N, Yamashita O, Harada T, Samura M, Takeuchi Y, et al. Skin and Subcutaneous Tissue Ultrasonography Features in Breast Cancer-Related Lymphedema. Annals of Vascular Diseases 2016;9(4):321-316.

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VALIDATION OF A "RISK-SCORE" FOR THE IDENTIFICATION OF PATIENTS AT RISK OF LYMPHEDEMA SECONDARY TO BREAST CANCER TREATMENT

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INTRODUCTION

What is upper limb secondary lymphedema? - This kind of lymphedema is a chronic condition characterized by the development of swelling and eventually affected alterations due to accumulation of lymph in the tissues due to acquired abnormalities of lymphatic drainage (not congenital). This leads to inefficient lymphatic transport, fluid accumulation and increased limb volume. The most frequent etiology is an iatrogenic nature which is secondary to the treatments received: surgery, radiotherapy, chemotherapy, etc. This category includes secondary lymphedema due to breast cancer treatment. With the persistence of the condition and in the absence of targeted interventions, there is a progressive worsening. The initial stage is characterized by superficial changes and mild symptoms such as heaviness, discomfort, swelling. As the swelling progresses, it becomes more extensive, not reversible and accompanied by the development of skin changes and lesions, infections, lymphatic loss, reduced motility and function of the limbs.[1, 2]

What is the extent of the problem? - The incidence of the problem is still not entirely clear and it is underestimated due to the many mistakes made in measurement procedures and the absence of common and shared systems of definition and diagnosis. 45,000 cases/year in Italy and 50 million worldwide (OMS) are estimated. All studies agree that incidence increases in patients exposed to known risk factors.

What women are at risk? - Women treated with demolitive surgical treatments, radiation treatments, chemotherapy, insane lifestyle drivers (obesity, lack of hygiene and skin care, sedentary lifestyle) are at greater risk of developing secondary lymphedema.[3-10] Other factors must be subjected to further studies and insights about their correlation with the risk of developing lymphedema: temperature, hypertension, ethnicity, age, genetics...

Is secondary lymphedema curable? - Lymphedema is an uncurable but manageable condition if diagnosed and treated early: intervening early and allowing an increase in the perceived quality of life bring the best possible cohabitation by surviving women. The first and best therapeutic approach is conservative, with "complex or combined decongestive therapy" that includes: manual lymphatic drainage, compressive therapy, hygiene and skin care, therapeutic education for long-term condition management and physical activity.[11] When conservative treatment is not effective and in the advanced stages of lymphedema, reconstructive or ablative surgical treatment is recommended.

What is the role of nursing in this context? - Recent data in the literature show that the knowledge and skills of the nursing figure in this context have proved to be insufficient.[12] It is necessary to be present through a training process aimed at the present knowledge deficits. The nursing role is in fact the pivotal point of the prevention or optimal management of a condition developed through the implementation of educational interventions, follow-up meetings in the long term and psychosocial support. Therapeutic education aims to reduce the risk exposure of healthy women (primary prevention), promote early diagnosis and treatment for women suffering from pathology (secondary prevention), ensure post-acute stability (tertiary prevention). The long-term follow-up must be based on meetings planned for evaluation, educational and decision-making purposes. Psycho-social support promotes acceptance, adaptation and coexistence with pathology.

Is it possible to predict early risk of secondary lymphedema in women treated for breast cancer? - This is the research question where the observational study conducted comes from. In a context where the lengthening of the average life expectancy of patients after a diagnosis of breast cancer, due to the diagnostic-therapeutic improvements introduced, has led to an increase in the possibility of developing complications secondary to the treatments received, the

most feared and common problem is lymphedema of the upper limb, a condition that is widespread but underestimated and neglected by health professionals. Hence the need for more attention in estimating the risk of developing complication, so as to implement preventive strategies and interventions, in order to reduce the risk as much as possible and ensure the highest possible level of quality of life for survivors.

MATERIALS AND METHODS

The cornerstones of the fight against secondary lymphedema are the identification of patients at high risk and prevention of the same. Nowadays, the main precautionary measures suggested to patients after breast cancer surgery consist in the implementation of standard strategies not related to the grade of personal risk of development lymphedema (high, medium, low).

The objective of the study is to propose a Lymphedema Risk-Score (LRS) to estimate the risk of developing breast cancer related lymphedema, better orientation of healthcare staffs in clinical practice and early and personalized preventive action.

The study conducted between May to September 2022 involved a sample of 84 patients treated at the University Hospital Policlinico San Martino in Genoa with regular outpatient examinations for the control or treatment of breast cancer related lymphedema. Data were collected by proposing questions in interviews to patients who meet the established inclusion criteria (female, breast cancer of previous diagnosis, surgical treatment received with association of SLNB or ALND, consent to participation) opting for frontal or telephone mode depending on their availability. All participants were first informed through a brief introduction about objectives and methods of conducting the study, possibilities for voluntary participation and confidentiality of personal data. All the women involved expressed their consent before being included in the study.

The review of the available scientific literature and international guidelines[13-15] identified and selected the main risk factors chosen as items of the proposed Lymphedema risk-score (LRS) tool (Table 1):

1. Age;
2. Body mass index;
3. Previous operations on the homolateral limb;
4. Sentinel lymph node biopsy (SLNB);
5. Axillary lymph node dissection (ALND);
6. Lymphangitis;
7. Peripheral lymphatic insufficiency;
8. Signs of peripheral lymphatic stasis;
9. Radiation therapy;
10. Chemotherapy

For each of the listed factors a score (0= no exposure; 1-2-3= present risk factor) will be assigned indicating the patient's exposure or not. At the end of the evaluation of all the items listed, the total score obtained will stratify the risk of the patient to develop secondary lymphedema in 3 levels: low, moderate or high. The higher the number of risk factors the patient is exposed to, the higher the total score achieved and consequently the risk and probability of developing the complication.

1. Age >65 **1**
2. BMI >30 **3**
3. Previous operations:
   a. Sentinel node biopsy **1**
   b. Homolateral limb interventions (orthopaedic, soft tissue, etc.) **1**
4. Sentinel lymph node **1**
5. Complete lymph node dissection **3**
6. Previous lymphangitis **2**
7. Positive history of peripheral lymphatic insufficiency 1
8. Signs of peripheral lymphatic stasis (edema) **2**
9. Radiotherapy (previous or planned) **3**
10. Chemotherapy (previous or planned) **2**

Associated risk:
- Low risk **1-2**
- Moderate risk **3-4**
- High risk **= or >5**

Table 1: *Lymphedema risk-score (LRS)*

RESULTS

The collected data have been subjected to statistical analysis in order to study predictivity, sensitivity, specificity of the proposed score and reliability of the variables that constitute it.

- Lymphedema Risk-Score prediction for lymphedema development has been evaluated using the unchanged Cox regression model.

- The rank-hazard plot was used to identify the score value beyond which there was an increase in relative risk for lymphedema development.

- The contribution of each parameter for the calculation of lymphedema risk score related to the diagnosis of lymphedema was estimated by calculating the area underlying the ROC curves (AUC: area under curve) 95% confidence intervals. Statistical significance was assumed with P value <0.05.

The statistical analysis was carried out using the open-source environment R *(version 4.0.3. The R Foundation for Statistical Computing. Vienna, Austria. 2020).*

1-LRS prediction (unchanged Cox regression model)

The Cox model has been used to measure and determine the likelihood that the risk of developing lymphedema, calculated

through the use of the score, will occur in the presence of different risk factors in the study. Therefore, once the risk score (Score) was obtained with LRS and subjected to Cox regression model analysis for the development of lymphedema, the result was a significant predictive potential of the same.

2-Sensitivity and specificity LRS (rank-hazard plot model)

The rank-hazard plot model made it possible to identify the progress of the score obtained with LRS (Score) in relation to the actual progressive increase in the relative risk arising for the development of lymphedema. From the analysis of the data, it has been evidenced that in the population in study there has been an increase of the relative risk for the development of lymphedema in the entire case study only with values of LRS >9. This cut-off corresponds to the median of the score for the whole series (average: 8.12 +/- 4.95; median: 9; range: 1-17). It could therefore be assumed that the assumption/expectation of a high risk already for values of score 5 reflects a potential overestimation, configuring the score as a tool tendentially to high sensitivity (ability to identify patients with lymphedema) and reduced specificity (poor ability to identify patients not affected). Further single centre/multicentre studies in which a substantial number of patients are enrolled would be desirable to deepen/refine the calibration of the score.

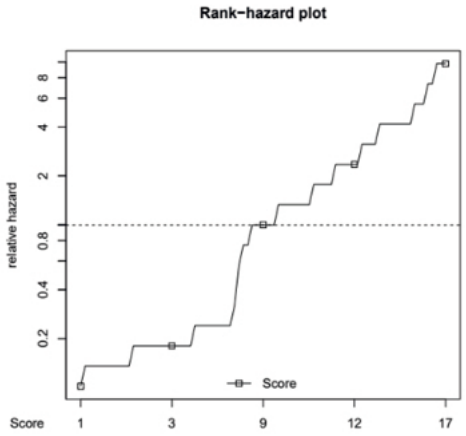


Figure 1: *Cox's regression model rank-hazard plot for the LRS score, in relation to the relative risk of developing lymphedema in the post-surgical treatment period for breast cancer.*

3-Analysis of the contribution of LRS variables in the diagnosis estimation of lymphedema (area underlying ROC curves)

Considering the different variables that contribute in the calculation of the Lymphedema Risk-Score and the different scores attributed to them, the contribution of each in the diagnosis of lymphedema through ROC (Receiver Operating Characteristic) curves has been estimated. The ROC curve is a graphic representation with a system of Cartesian axes in which sensitivity is placed on the axis of the ordinates and specificity on that of the abscissa. For each value of the score variable taken into account, different sensitivity and specificity values will correspond depending on the cut-off value considered by intersecting its axes. It is therefore a graph that relates the sensitivity and specificity to the variation of the cut-off value, in which joining the corresponding points to the different cut-off values considered is constructed the ROC curve. The accuracy of the variables is represented by the area subtended to the ROC curve (AUC), resulting in greater proximity of the same to the upper left corner of the graph representing the cut-off value that maximizes sensitivity and specificity (AUC=1). Below is represented the subtended area of each ROC curve related to the association with the diagnosis of lymphedema. The accuracy of the ROC curve increases as the value of AUC increases. Values of AUC 0.70 attest to the progressive reliability of the risk estimation.

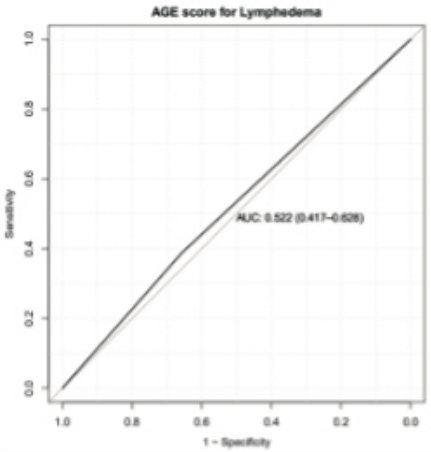


Figure 2: *ROC curve calculated for the age of the patients enrolled in the study (AGEsc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on Lymphedema Risk Score*

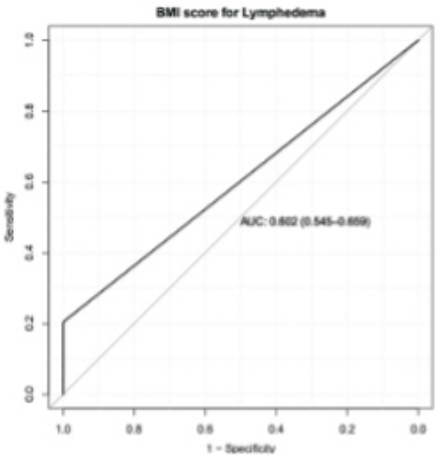


Figure 3: *ROC curve calculated for the body mass index of patients enrolled in the study (BMIsc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on Lymphedema Risk Score*

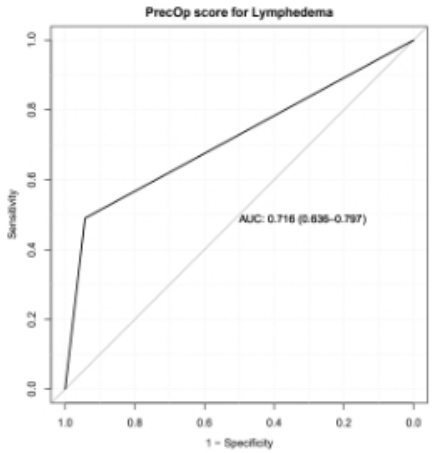


Figure 4: *ROC curve calculated for the presence of previous operations in patients enrolling in the study (PrecOpsc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on Lymphedema Risk Score*

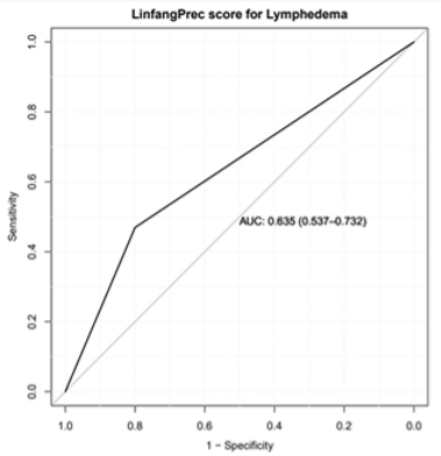


Figure 7: *ROC curve calculated for the presence of previous lymphangitis (LinfangPrecSc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on the Lymphedema Risk Score.*

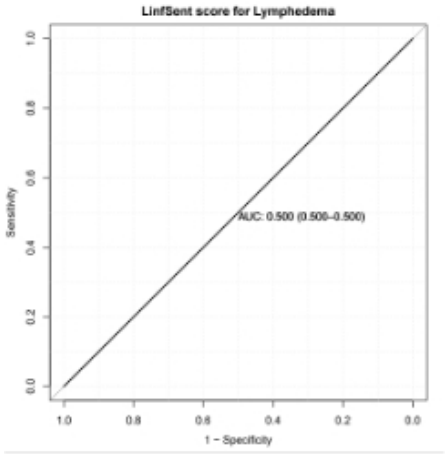


Figure 5: *ROC curve calculated for sentinel lymph node removal (LinfSentSc) in relation to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on Lymphedema Risk Score.*

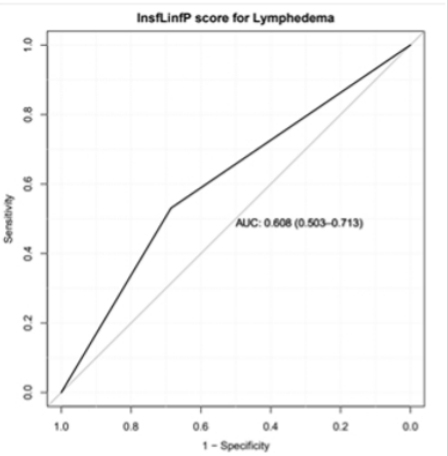


Figure 8: *ROC curve calculated for the presence of peripheral lymphatic insufficiency (InsfLinfPSc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on the Lymphedema Risk Score.*

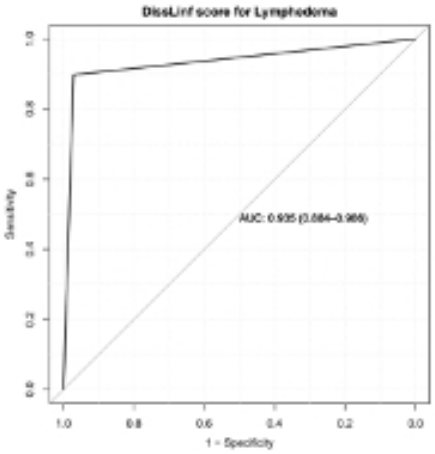


Figure 6: *ROC curve calculated for axillary lymph node dissection (DissLinfSc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on Lymphedema Risk Score.*

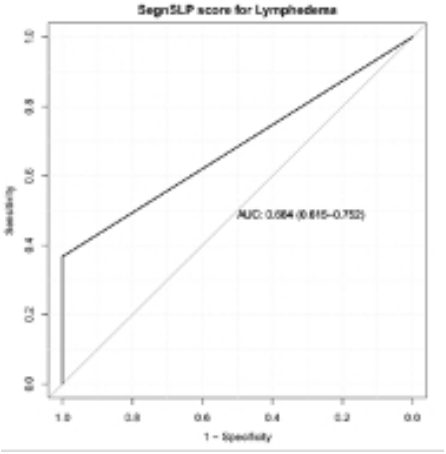


Figure 9: *ROC curve calculated for the presence of signs of peripheral lymphatic stasis (SegnSLPSc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on the Lymphedema Risk Score.*

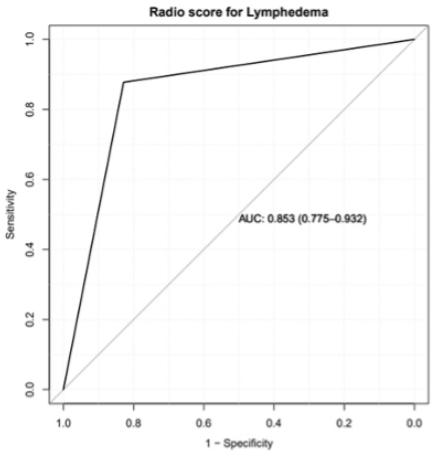


Figure 10: *ROC curve calculated for radiotherapy treatment (RadioSc) related to the diagnosis of lymphedema in the post-surgical treatment period for Lymphedema-based breast cancer Risk Score.*

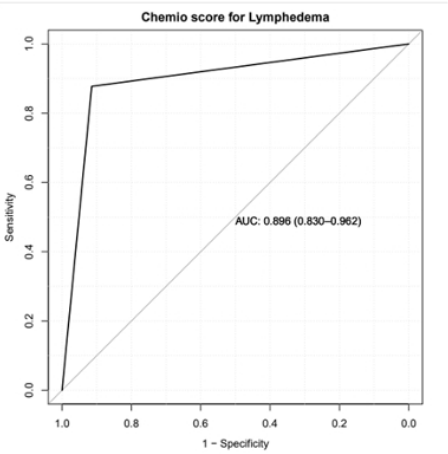
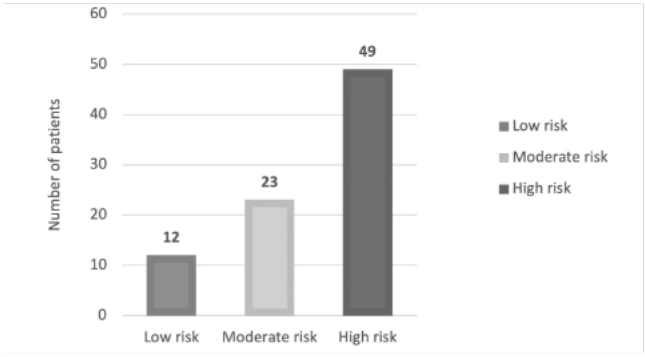
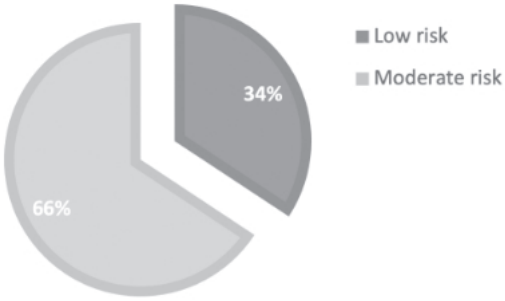


Figure 11: *ROC curve calculated for chemotherapy treatment (ChemioSc) related to the diagnosis of lymphedema in the post-surgical treatment period for breast cancer based on the Lymphedema Risk Score.*

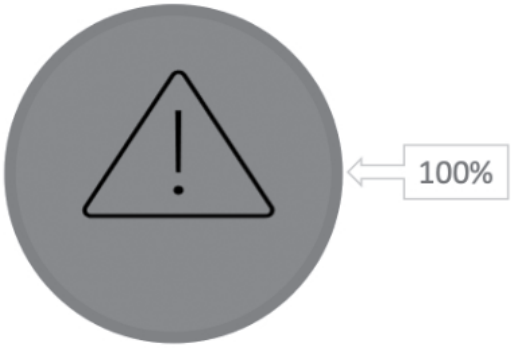
The results obtained, in terms of risk classes, clearly show that patients who do not show signs of post-treatment breast cancer complications on regular follow-up visits carried out, according to the LRS score, do have a low risk. On the contrary, all patients with a high risk have been diagnosed with lymphedema (stage II-III) with the need for targeted treatment.



Graph 1: *Distribution of the total sample of patients according to the risk level presented (according to Lymphedema Risk-score).*



Graph 2: *Distribution of the sample of patients without lymphedema development depending on the risk level presented (according to Lymphedema Risk-score).*



Graph 3: *Distribution of the sample of patients with lymphedema development depending on the level of risk presented (according to Lymphedema Risk-score).*

DISCUSSION AND CONCLUSIONS

The tool proposed by the study and applied in a local hospital context has significant predictive potential, a high level of sensitivity and of low specificity.

As can be seen in the previous curves (Figure 20-27), the variables that from the study results are particularly accurate and predictive in estimating the risk of developing lymphedema (AUC 0.70) are related to the type of treatment received and are represented, consistently with

the literature, by axillary lymph node dissection (AUC: 0.935), chemotherapy treatment (AUC: 0.896) and radiotherapy (AUC: 0.853).

The number of lymph nodes removed and therefore the extent of the surgical damage from the patient has been previously subjected to, have been statistically significant for the risk of developing lymphedema confirming as valid risk predictors, as well as lymphatic damage resulting from radiation and chemotherapy treatments. 89% of patients treated with axillary lymph node dissection have developed lymphedema in the limb, as well as 88% of patients treated with radiotherapy and 93% of patients with chemotherapy.

They follow the other LRS variables that seem to be less predictive in identifying risk. In order to reduce diagnostic accuracy, according to the study, we find the following parameters to be considered: previous operations carried out, signs of peripheral lymphatic stasis, previous lymphangitis, positive history of peripheral lymphatic insufficiency, BMI.

There appears to be a not important correlation with patient age and sentinel node biopsy. Age has been analyzed by several studies but identified by most of them as a non-prognostic factor in the development of lymphedema, despite the fact that with increasing age it seems to increase the risk of exposure to vascular diseases and comorbidities. The removal of sentinel lymph node alone, however, is not a variable that contributes to the increased risk of developing lymphedema, remaining in line with what is stated in the literature, because determining a more limited impact on the lymphatic system than axillary lymph node dissection exposes the patient to lower risk of developing secondary complications.

Depending on the risk score estimated through the LRS, a personalized treatment path will be set up for the patients:

- women with low risk will undergo regular follow-up visits;
- women with mild to moderate risk will be directed towards early interventions to modify the risk factors possessed to minimize their risk and conservative treatment (compressive therapy);
- women with moderate-high risk in addition to other recommendations will be candidates for surgical treatment.

Through this work we tried to propose a scoring system that would estimate the probability of developing post-treatment lymphedema by evaluating the main risk factors emerged from the scientific literature and guidelines.[16-18] The tested instrument has proved to be simple, easy and quick to apply in clinical care practice.

In the conduct of the study, it emerged that patients with low risk, based on the proposed score, do not seem to have developed to date post-treatment complications for breast cancer as opposed to patients with lymphedema development who, according to the score, revealed a high risk.

It is important to underline that the application of the score, object of this study, is at a very preliminary phase and the sample under analysis is limited for a reliable applicability of the score. This tool can therefore currently represent a potential additional aid to the management of clinical risk for the specific type of patients enrolled in the study, pending more in-depth analysis and validation of the study on larger cases, hopefully also using multicentric studies. Risk stratification could help future studies, using the most significant results risk factors, to predict the occurrence of lymphedema, reduce risk, guide decisions about the best therapeutic strategies. Early risk recognition is important for the prevention and reduction of discomfort, resulting from neglect of the problem19. The nursing figure has an extremely important role in this context and it is necessary to possess adequate educational-relational technical skills for the overall and continuous management of the patients considered.

REFERENCES

1. https://www.aimac.it/libretti-tumore/il-linfedema

2. https://www.grupposandonato.it/news/2020/novembre/linfedema-sintomi-cause-trattamenti.

3. McLaughlin SA, DeSnyder SM, Klimberg S et al. Considerations for clinicians in the diagnosis, prevention, and treatment of breast cancer-related lymphoedema, recommendations from an expert panel: part 2: preventative and therapeutic options. Ann Surg Oncol. 2017; 24:2827-2835;

4. Krag DN, Anderson SJ, Julian TB, et al. Sentinel-lymph-node resection compared with conventional axillary-lymph-node dissection in clinically node-negative patients with breast cancer: Overall survival findings from the NSABP B-32 randomized phase 3 trial. Lancet Oncol. 2010; 11:927–933;

5. Gillespie TC, Sayegh HE, Brunelle CL, Daniell KM, Taghian AG. Breast cancer-related lymphedema: risk factors, precautionary measures, and treatments. Gland Surg. 2018; 7(4):379-403;

6. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. Lancet Oncol. 2013; 14 (6); 500-515;

7. Schmitz KH, Troxel AB, Dean LT, et al. Effect of Home-Based Exercise and Weight Loss Programs on Breast Cancer-Related Lymphedema Outcomes Among Overweight Breast Cancer Survivors: The WISER Survivor Randomized Clinical Trial. JAMA Oncol. 2019; 5(11):1605-1613;

8. McLaughlin SA, Wright MJ, Morris KT et al. Prevalence of lymphedema in women with breast cancer 5 years after sentinel lymph node biopsy or axillary dissection: patient perceptions and precautionary behaviors. J Clin Oncol. 2008; 26 (32): 5220-5226;

9. F Al-Niaimi, N Cox. Cellulitis and lymphoedema: a vicious cycle. J Lymphoedema, 4 (2009), pp. 38-42;

10. Park JH, Lee WH, Chung HS. Incidence and risk factors of breast cancer lymphoedema. J Clin Nurs. 2008; 17 (11): 1450-1459.

11. Mobarakeh ZS, Mokhtari-Hesari P, Lotfi-Tokaldany M, Montazeri A, Heidari M, Zekri F. Combined decongestive therapy and reduction of pain and heaviness in patients with breast cancer-related lymphedema. Support Care Cancer. 2019; 27:3805–3811.

12. J.E. Maree, D. Beckmann. Just live with it: having to live with breast cancer related lymphedema. Health Gesondheid 21 (2016) 77-85.

13. Executive Committee of the International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2020 Consensus Document of the International Society of Lymphology. Lymphology 2020; 53 (1): 3-19;

14. Boccardo F. An overview of the treatment of primary and secondary lymphatic diseases: the effort of the ESL to put some order. EJLRP vol.29, n.77, pp.1-9, 2017.

15. Ministero della salute, con nota del 12 luglio 2016, accordo ai sensi dell'articolo 4 del decreto legislativo 28 agosto 1997, n. 281. Linee di indirizzo sul Linfedema e patologie correlate (37 pagine), 2016

16. Villa G, Campisi CC, Ryan M, Boccardo F, Di Summa P, Frascio M, Sambuceti G, Campisi C, Procedural Recommendations for Lymphoscintigraphy in the Diagnosis of Peripheral Lymphedema: the Genoa Protocol, Nucl Med Mol Imaging, 53(1), 47-56, 2019.

17. Boccardo, F., Casabona, F., DeCian, F., Friedman, D., Murelli, F., Puglisi, M., C. Campisi, C., Molinari, L., Spinaci, S., Dessalvi, S. e Campisi, C., Lymphatic Microsurgical Preventing Healing Approach (LYMPHA) for primary surgical prevention of breast cancer-related lymphedema: Over 4 years follow-up, Microchirurgia, 34, 421-424, 2014.

18. McLaughlin SA, DeSnyder SM, Klimberg S, Alatriste M, Boccardo F, Smith ML, Staley AC, Thiruchelvam PTR, Hutchison NA, Mendez J, MacNeill F, Vicini F, Rockson SG, Feldman SM, Considerations for Clinicians in the Diagnosis, Prevention, and Treatment of Breast Cancer-Related Lymphedema, Recommendations from an Expert Panel: Part 2: Preventive and Therapeutic Options, Ann Surg Oncol, 24(10), 2827-2835, 2017.

19. Boccardo FM, Casabona F, Friedman D, et al, Surgical prevention of arm lymphedema after breast cancer treatment, Ann Surg Oncol, 18, 2500–2505, 2011.

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