Title: A Systematic Review of Indocyanine Green Lymphography (ICGL) Imaging for the Diagnosis of Primary Lymphoedema

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Abstract

Objectives. This systematic review aims to evaluate the use of Indocyanine Green Lymphography (ICGL) for the investigation of the lymphatics in the lower limbs of primary lymphoedema patients.

Methods. MEDLINE and EMBASE articles from 01/01/2000 to 01/09/2023 were searched for. A total of 11 studies were included in the review after a two-stage screening process.

Results. Data on patient demographics, ICG contrast injection technique, imaging protocols and imaging outcomes were summarised and reviewed in detail. The review highlights the lack of commonality in protocols used. Factors important for good imaging are highly variable, particularly the number of injections, their location and whether they are delivered intradermally or subcutaneously.

Conclusions. ICGL has strong potential to become a diagnostic tool to diagnose lymphoedema, due to its non-ionising nature and cost-effectiveness. However due to the lack of thorough phenotyping and genotyping of patients included in the studies, uncertainty still exists as to the value of the described imaging features such as splash, starburst and diffuse dermal rerouting patterns. Future studies, therefore, should aim to explore the diagnostic utility of ICGL for lymphoedema further through the imaging of primary lymphoedema patients with a confirmed genetic diagnosis and using standardised imaging protocols.

Advances in knowledge. ICGL is a strong candidate for advancing the diagnosis and understanding of primary lymphoedema, and monitoring response to treatment, but protocol heterogeneity and a lack of consistency in reporting imaging details and patient phenotyping currently hold it back.

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Keywords

Indocyanine green lymphography; Primary lymphoedema; Lower limb; Near-infrared fluorescence; Lymphatic system; Superficial imaging

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Introduction

Lymphoedema is a condition of chronic swelling due to a compromised lymphatic system. Affecting over 66 million people worldwide, there are currently no cures, and treatments aim only to reduce swelling.[1,2] This lack of therapeutic options is partly due to the paucity of knowledge regarding the function and anatomy of human lymphatics, despite its importance for regulating fluid balance, preventing infection, and involvement in conditions ranging from cancer to obesity.[3,4]

Lymphoscintigraphy is the most used imaging technique for diagnosing lymphoedema, offering reliable assessments of lymphatic function.[5,6] Lymphoscintigraphy is however limited by poor image quality.[7] Single Photon Emission Computed Tomography (SPECT), in combination with X-ray Computed Tomography (CT), has also been employed to image lymph nodes due the enhanced anatomical detail and the ability to generate 3D images[7,8]. With the injection of a suitable contrast material, CT alone is capable of providing high-resolution images of lymphatic vessels[8]. However, each of these techniques is limited by the associated exposure to ionizing radiation. In contrast, Magnetic Resonance (MR) lymphangiography is a non-ionizing alternative, either with or without the use of an exogenous contrast agent, providing reasonable spatial resolution. However, it does not enable real-time visualisation of lymphatic flow[9,10].

Indocyanine green (ICG) fluorescence lymphography (ICGL) meanwhile facilitates non-ionising, real-time lymphatic imaging *in vivo*,[11] and has been used to aid sentinel lymph node biopsy for cancer management,[12] identify lymphatics suitable for lymphovenous anastomosis surgery,[13] and to investigate the effectiveness of manual lymphatic drainage.[14] ICGL does not seem to cause lymphatic inflammation or vessel damage,[15] and is hence gaining traction as both a research and clinical tool. ICG has infrared fluorescent properties which are therefore rapidly attenuated within only a few centimeters below the skin surface,[16] an obstacle in patients whose subcutaneous tissue has thickened[17]. Like other 2D imaging techniques, including lymphoscintigraphy, lymphatic vessel depth can also not be obtained[18], but high spatiotemporal resolution visualisation of superficial lymphatic vessels is possible.

Lymphoedema is either primary (PL), due to an intrinsic fault (presumed genetic),[19] or caused by extrinsic damage (secondary), e.g. lymph node removal.[20] The discovery of gene mutations causing lymphatic anomalies has revealed different mechanisms that disturb lymph drainage in PL.[21] Improved management of PL will require definitive imaging of the lymphatic system to identify the pathological mechanisms at play and categorise the lymphatic fault before intervention. ICGL is a potential low-cost, non-ionising candidate for this.

In this study, we comprehensively review the literature describing ICGL in the lower limbs in the context of PL, and highlight its diagnostic potential.

Methods

Paper identification

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[22] A comprehensive search of the ICGL literature was conducted, retrieving Medline and Embase records published between 01/01/2000 and 01/09/2023. Search terms: Diagnos* imag* OR Diagnos* tool* OR Diagnos* method* OR Diagnos* technique* OR Lymphography OR Diagnostic Imaging AND Primary Lymph?edema OR Congen* Lymph?edema OR Lymphan* OR Lymph* malf* AND Indocyanine green OR ICG OR Indocyanine OR Indocyanine Green OR Fluoresc* OR NIRF OR Near-infrared, were used and duplicated articles removed.

Screening stage 1

Abstracts were screened using the inclusion criteria summarised in Table.1. Conference abstracts/reports, reviews, letters/replies, book chapters, and single case studies were excluded, as were abstracts not mentioning ICG imaging and lymphoedema, or related terms. Abstracts referencing the use of animal or cadaveric subjects were also removed.

Screening stage 2

Full texts were then obtained. Further single case reports, papers not reporting original ICGL data or detailed imaging methods, were removed as were studies of healthy controls and/or secondary lymphoedema cases only. The remaining papers were analysed independently by

two assessors (GB and PO) and only papers describing detailed imaging methods for the purpose of diagnosing lower limb primary lymphoedema were retained. Papers using ICGL for other purposes (*e.g.* interoperative ICG imaging used during lymphatic surgery) were excluded.

Results

Study inclusion

The initial Medline and Embase searches yielded 410 records (Figure 1). After duplicate removal, 258 abstracts were reviewed, of which 82 were retained after having passed screening stage I. After full text review, 11 studies focusing on ICGL of the lower limbs in PL were included in this systematic review (Table 2). For these studies, data on patient recruitment, diagnosis, ICG contrast injection, imaging protocol, and imaging outcomes are presented.

Patient cohorts

Among the 11 studies, four enrolled patients with PL only.[23-26] The remainder included patients with primary and secondary lymphoedema.[27-33] Number of cases, their age and sex, and limbs imaged are summarised in Table.2.

ICG Injection Protocol

The most commonly used fluorescent agent (7/11) was Diagnogreen (Table 3). Verdye was used in two studies, one study used ICC-Pulsion and another did not specify. Some diluted the ICG in saline[31,33] or water [23], but most did not specify. ICG agents were administered at 0.5% (6/11) or 0.25% (4/11) concentrations, or not reported.

All studies reported the volume of ICG fluorescent agent injected per site, ranging from 0.05mL to 1mL. In five, the administered volume varied between participants. Over half (7/11) injected the agent subcutaneously, the others intradermally. Some studies mentioned the use of local numbing with lidocaine,[29] xylocaine[23] or a topical cryogenic numbing device.[31,33]

Injection sites

Between 1-4 injection sites per foot were employed, including at least one of the web spaces of the toes (Table 4). Of the seven sites used (Figure 2), the most common was the 1st web space of the toes (8/11). Five studies also injected into the second and/or fourth web spaces, and one injected in the third. The second most common site was laterally, towards the rearfoot near the lateral malleolus and Achilles tendon. One study reported an injection into the lateral side of the superior edge of the knee in addition to two foot injections.[30]

Imaging protocols

Most commonly (8/11), imaging commenced immediately after ICG contrast injection (Table 5). The duration of imaging was not consistently reported. One study reported the exam lasts 10-15 minutes,[29] whilst others reported ~1hr.[31,33]

Two studies repeated imaging after 2 hours, while three studies reimaged patients after 6-24 hours. It was noted if the lymphoedema was unilateral or bilateral, but they did not comment on how the lymphatic imaging patterns differed between these periods.[23,25] Others reported that repeat imaging 24h post-injection was comparable to the early imaging.[33]

Only a few studies disclosed the position of the patient (standing, supine, lateral or prone) when imaged,[25,27,29,33] and, when imaging both limbs, if they were injected and imaged simultaneously.[23,27-29,34]

Lymph flow is often delayed at the ankle joint,[25] and exercise or massage is considered to purposefully encourage ICG contrast uptake. Some have described improved visualisation of lymphatic pathways after 30 min of manual lymphatic stimulation in secondary lymphoedema upper limb imaging.[35] Of the papers reviewed, some employed toe and ankle flexions,[25] while others experimented with exercise on a treadmill to improve uptake of ICG contrast[27] and thus reduce imaging time. Two studies reported checking for spontaneous movement of ICG contrast immediately after injection, then after 10 min the imaging would continue while manual lymphatic drainage (MLD) was performed.[31,33] It was suggested that the application of ICG contrast guided manual lymphatic drainage reduces imaging time.[33]

Imaging features and outcome measures

Functional lymphatic vessels

All studies agree that in healthy limbs, lymphatic vessels appear on ICGL as a linear pattern spreading from the injection site (Figure 3A) toward the groin. Interestingly, a third of 'unaffected' clinically healthy limbs in patients with unilateral lymphoedema showed abnormality on ICGL,[23] and age-related declines in lymphatic function were demonstrated.[24]

Retrograde flow in the collector vessels

Transport of lymph should be a one directional flow from absorbing initial lymphatics through ever enlarging lymphatic vessels to lymph nodes. Larger main limb lymphatics or collecting vessels ensure flow against gravity due to lymphatic contractility and lymphatic valves. If they fail, retrograde or reverse lymph flow can result. One study, recording the presence of valves and the direction of flow, was able to assess collector vessel function. Faulty contractility and retrograde or reverse flow across incompetent valves could be imaged and recorded by ICGL.[31]

Dermal backflow

Any hold-up of downstream flow can result in reverse or retrograde flow of lymph back toward initial lymphatics in the dermis. This is dermal backflow (DB) and is a diagnostic sign of lymphoedema. Visible on ICGL as a vessel network within the skin extending well beyond the injection sites, DB can be seen as soon as 4-5 minutes after contrast injection[29] and can spread and mask underlying vessels.[30] Generally the more extensive the dermal backflow, the more severe the disruption to limb lymph flow and so the severity of lymphoedema. In addition to the retrograde filling of dermal lymphatic vessels, appearances on ICGL may also be due to the diffusion of ICG out of the lymphatic vessels into the interstitial tissues.[36]

Dermal backflow in lymphoedema has been grouped into three different patterns: 'splash', 'stardust', and 'diffuse' (Figure 3B-D)[34] and these definitions were adopted by some of the studies (Table 6). Others use definitions like 'distal' or 'proximal' dermal backflow to define its location, and 'less enhancement' or 'no enhancement' to convey a degree of vessel hypoplasia or aplasia.[23-26] There were no significant sex-related differences reported between the different lymphography patterns.[24]

Transport capacity

Some studies offered measures of transport capacity such as time to groin (Table 6): the time taken to visualise the inguinal nodes after ICG injection.[24,25,27] In healthy limbs the superficial inguinal lymph nodes may be observed within 10-15 minutes, becoming more delayed as lymphoedema worsens.[24,25,28] However, with exercise, these could also be observed after 15 minutes in lymphoedema patients.[27]

ICG contrast distribution

In non-lymphoedematous limbs, drainage of ICG contrast appears to follow predictable routes based on the location of injection.[37] Alternative drainage routes may appear as a result of lymphoedema[32,33], however, a particular pattern (labelled the "print sign") was observed in some PL cases where signal distal to the injection site on the foot plantar surface and plantar and dorsal surface of the toes was recorded. The authors suggest this feature could be of diagnostic utility.[23]

Discussion

Lymphoscintigraphy has shown use in phenotyping, and improving understanding of the causal mechanisms of primary lymphoedema.[38] The objective of this review was to explore whether ICGL has been used for this purpose, or what features may be useful in this regard. We limited our investigation to imaging in the lower limbs of PL patients (the most commonly swollen region) and this review provides evidence that ICGL of lymphatic vessels is capable of demonstrating altered flow dynamics and drainage in these cases.

Protocol standardisation

This systematic review shows that ICGL protocols are variable; including the ICG agent used, the concentrations or volumes administered, and injection sites and depth.

ICG Agent

The most commonly used manufacturer of ICG was Diagnogreen. Reasons for this were not clearly described but are usually related to local availability and cost. ICG is usually provided in a sterile powder and administered diluted with water or saline and local anaesthetic to reduce discomfort of injection. Though saline has been used in some studies reported here, anecdotal concerns regarding ICG solubility and spectral characteristics have been raised

and dilution with water may be preferable. The concentration and volume per injection were found to vary substantially. In a study conducted by Visconti et al. (2017), it was reported that diluting ICG powder (ICC-Pulsion, Pulsion Medical System, Germany) with 5 mL of sterile water containing 0.5% xylocaine significantly reduces the pain associated with intradermal injections in patients with primary and secondary lymphoedema[39].

To what extent these impact performance could not be determined, however that administered concentration influences the fluorescent properties of ICG is known.[40] Future studies investigating the optimal conditions of ICG contrast solution and dilution would be beneficial in humans, as has been done in animals.[41,42]

Anatomical injection sites

Shinaoka and colleagues studied lymphatic drainage routes in non-lymphoedematous cadavers with 19 injections in the foot, allowing them to classify four distinct lymphatic drainage routes: anteromedial, anterolateral, posteromedial, and posterolateral.[37] It is suggested that in addition to injecting into the web space between the toes, injection sites in the medial, lateral, and posterior aspects of the foot are also needed for full evaluation of all the lymphatic pathways to improve our understanding of leg lymphoedema. The number of injections varied across the 11 studies, with only one study[33] covering all four main drainage routes. This suggests that studies only using injection in the web spaces between the toes could fail to visualise some of the lymph drainage pathways. Thus, interpretation and comparison of results from ICGL studies need to consider this.

Injection depth

A mix of subcutaneous and intradermal injections were employed in the reviewed studies. Sub-epidermal injections (high dermis) for lymphoscintigraphy result in faster lymphatic uptake and flow[43] but for quantitative results, *e.g.* lymph node uptake and limb lymph drainage function, subcutaneous injections appeared better.[44] To what extent this is the same for ICGL is not known but in theory access and uptake to superficial lymphatics ought to be better with intradermal injections. Only four of the studies used intradermal injections.

Time of imaging

In addition to the injection sites and depth, timing of imaging is essential. Some carried out repeat images 6h to 24h post injection and reported that repeat imaging was comparable to the early imaging,[33] however, on late imaging DB may mask underlying vessels and thus not give the full picture of the status of the lymphatic network.

Interpretation of imaging

Dermal backflow (reflux) patterns

Despite the heterogeneity of ICGL protocols used, all studies were able to visualise lymphatic vessel and DB patterns. The linear lymphatic pattern was the most reported structural finding and is thought to represent the normal superficial lymphatic network, as evidenced by all healthy controls displaying this pattern.[45] Different backflow patterns from 'splash', 'stardust' to 'diffuse' are suggested to grade the severity of disease,[34] and some studies also tried to classify the DB by location (distal vs proximal). However, no clear classification linking these to specific PL phenotypes has been attempted. Regardless of the definitions utilised, it will be interesting in future studies to see how these can be used to categorise different phenotypic or genotypic forms of PL.

Retrograde flow in collector vessels

Dysfunction in the lymph-collecting vessels resulting in a reversal of lymphatic flow has been described commonly in the literature as a pathological feature of primary lymphoedema especially in patients with lymphoedema distichiasis syndrome.[46] Contrary to the accepted knowledge of the pathological alterations in PL, retrograde lymph flow with valve incompetence in the lymphatic vessels was rarely reported in the selected studies. However, only one study included a method for the observation of retrograde flow, which they observed in two patients with confirmed lymphoedema distichiasis syndrome, and they discussed valve incompetence as a potential feature to diagnose lymphoedema.[31] Thus, exploring ICGL utility according to lymphoedema pathogenesis, and analysing the signature imaging features for each genotype, could establish retrograde lymph flow analysis as a useful diagnostic measure in ICGL in combination with the clinical presentation.

Flow of lymph

Other measures for lymphatic function could relate to the speed with which the ICG gets transported or lymphatic contractility. In 2010, Unno and colleagues estimated lymphatic pumping pressure in human subjects with ICGL via the application of pressure cuffs to occlude lymphatic vessels.[47] Shortly thereafter, ICGL was used to measure lymphatic contractile frequency and the speed of ICG contrast boluses in the vessels.[48,49] Pumping frequency was also reported in an ICGL study of rats following lymph node removal and X-ray irradiation, showing increased and more irratic lymphatic pumping following lymphatic injury.[50] None of the 11 studies attempted measurements as detailed as these, but a few investigated the time taken for the ICG contrast to reach the groin. For this to be a useful tool and to allow comparison between individuals or between studies, the protocol needs standardising, particular regarding exercise which can greatly influence the speed.[24,25,27] It should also be noted that these measures of speed relate only to transport of ICG via the superficial lymphatics which are detectable with ICGL.

Diagnostic utility

The ability of ICGL to demonstrate abnormal lymphatic vasculature was clearly demonstrated within this review, and ICGL has also been shown sensitive enough to detect subtle lymphatic anomalies prior to clinical signs of lymphoedema.[14] In all 11 articles, patients were reported as presenting with swelling prior to the described imaging. The ability to diagnose lymphoedma in the absence of evident disease was not explored, though some reported ICGL was used to confirm the lymphoedma diagnosis.[24,25]

Phenotyping through imaging

With the established imaging patterns and methods for assessment of lymph transport, ICGL could possibly aid phenotyping of primary lymphoedema. However, there is little published on this. The 11 studies in this review included over 460 reported cases of PL but only two papers specified the type. One reported the inclusion of two lymphoedema distichiasis cases,[31] and the other listed eleven cases with genetic variants identified in known PL genes, however causality was not confirmed.[23]

Some case studies, excluded from the systematic review, used ICGL to confirm the presence or absence of lymphoedema in genotyped family members,[51,52] however, the reports

included too few cases to enable any meaningful genotype-phenotype correlations. Thus, there are no studies in the literature that systematically look at genotyped PL cases with ICGL to determine the pathology.

Based on a previous lymphoscintigraphy study, clear phenotypic differences between Milroy disease and lymphoedema distichiasis syndrome were demonstrated on imaging.[38] We believe ICGL can be used in similar ways to define genetic groups. However, if the studies do not genotype, or as a minimum thoroughly describe the phenotypic details of their patients, then the ICGL can only distinguish whether a patient has lymphoedema or not.

Conclusion

Depending on the outcome measures of interest, ICGL seems overall to be a suitable tool for visualising lymphatic vessels and could prove useful for the phenotyping of primary lymphoedema phenotypes. There is a clear lack of consensus in injection protocols, particularly regarding anatomical injection sites, that will greatly affect which superficial lymphatic pathways can be visualised with ICGL. Robust outcome measures, i.e. consensus on criteria for determining lymphatic abnormalities, are also lacking. This limits the current utility of ICGL for the diagnosis of lymphoedema. Suami and colleagues' proposals for injection that will allow ICG contrast to reach each of the 4 main lymphatic drainage pathways are recommended.[33] The depth of injection influences lymphatic access and also needs careful consideration. Future research should look at optimising and implementing the best ICGL imaging protocols, and developing a range of objective measures for quantifying and subjective measures for describing imaging features. Studies applying this technique for phenotyping primary lymphoedema patients could also then be explored.

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Figure 1

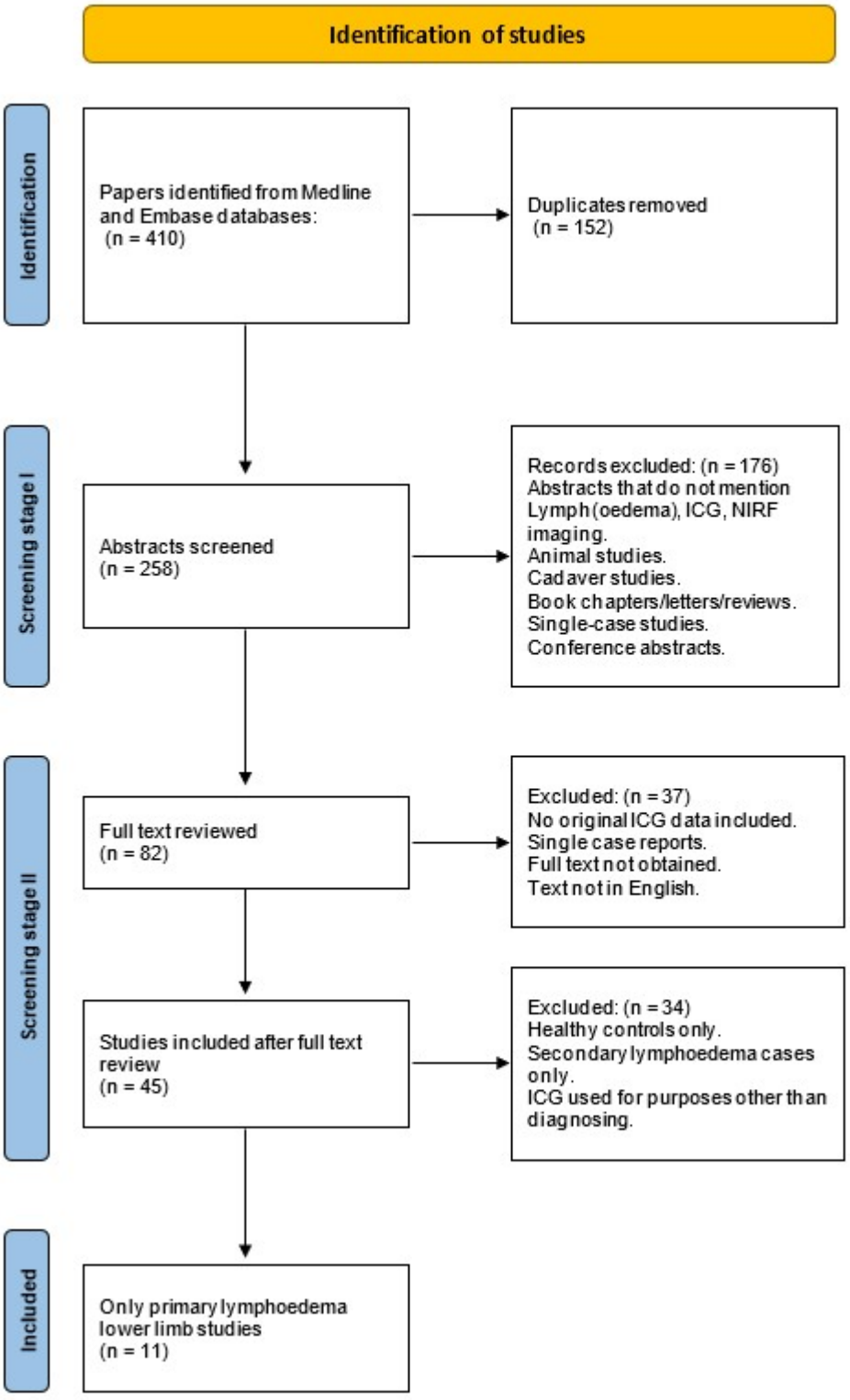

Figure 1 Study selection flow chart. Medline and Embase databases revealed a total of 410 sources. After the application of inclusion and exclusion criteria, a total of 11 articles were shortlisted for this review. Note that some single-case reports were removed in screening stage 2, as it was only after full-text retrieval that it became clear the article reported only one case.

Figure 2

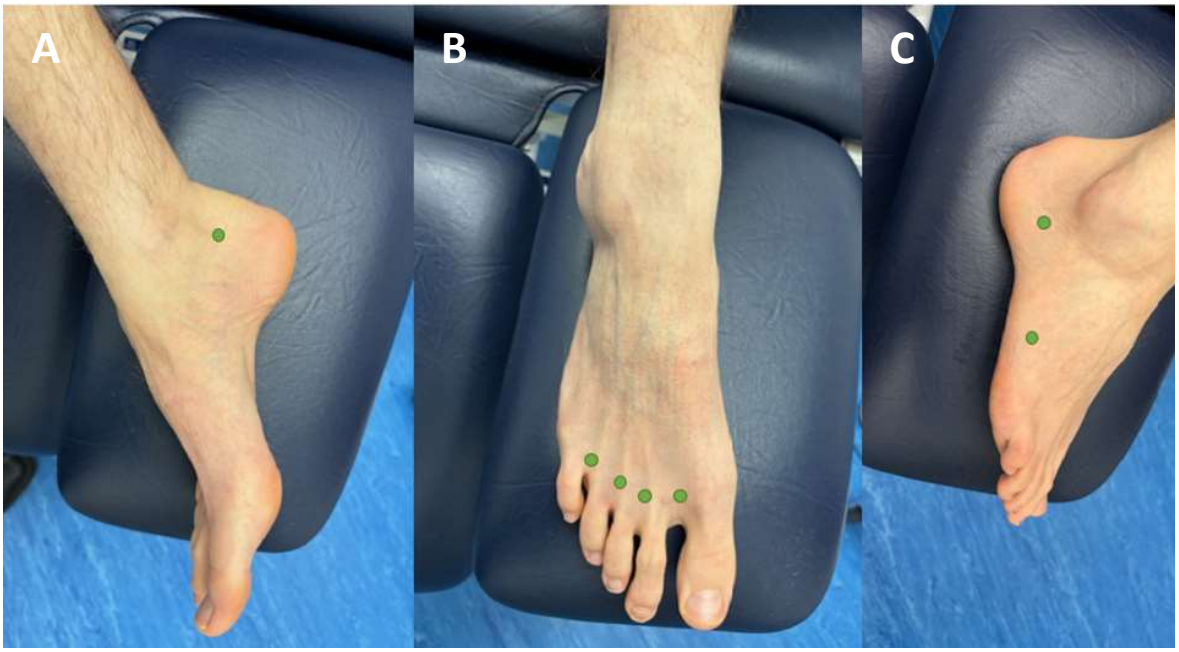

Figure 2 The eleven studies use a combination of seven sites for ICG contrast injections in the foot, marked here with a green dot. (A) shows the medial side of the foot, (B) shows injections in the forefoot and (C) shows the lateral aspect of the foot indicating midfoot and rearfoot injections.

Figure 3

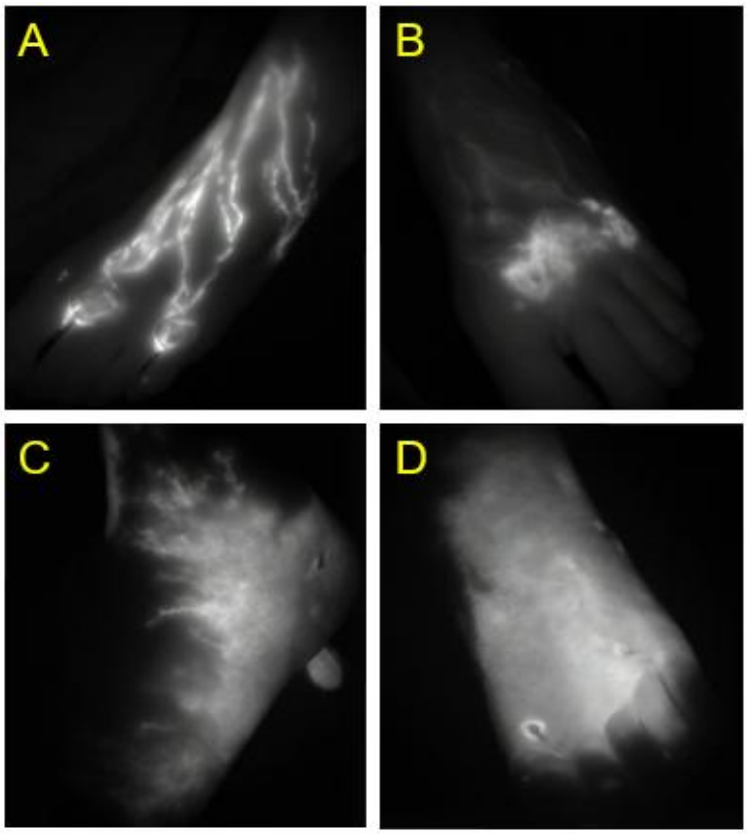

Figure 3. Lymphography patterns observed with the near infrared detector camera following the definitions of Yamamoto and colleagues.[34] (A) Linear, the normal superficial lymphatic pattern; and the abnormal lymphatic patterns: (B) splash; (C) stardust; and (D) diffuse, collectively called dermal backflow patterns and indicate greater disease severity in order of appearance (from B to D). (Images from lower limb ICGL in primary lymphoedema shared by St George's Lymphovascular Research Group).

Table.1. List of inclusion criteria applied in this systematic review.

Inclusion Criteria

1.	Records including ICG imaging in Primary Lymphoedema.
2.	Human studies only.
3.	Lower limb ICGL findings with descriptions.
4.	The imaging method was described by the authors.
5.	Manuscripts from 1st January 2000 to 1st September 2023.

Table 2

Table. 2. Overview of papers shortlisted in the systematic review including a summary of the number of individuals included in the studies ns, not specified; [a] average age for primary lymphoedema cases only; * suspected a subset of Yoshida et al 2020[25]

Reference	Female	Male	Age range (years)	Average Age (years)	Age of onset (average age years)	Primary lymph-oedema (number of individuals)	Secondary lymph-oedema (number of individuals)	Limbs imaged in the primary lymphoedema cases
Akita *et al.,* 2013	115	19	9-82	58.5	ns	39	95	Unilateral and bilateral lower and upper limb
Gentili *et al.,* 2021	26	6	18-73	38	ns	6	26	Lower limb only
Hara and Mihara, 2020	96	7	11-82	57.8	ns	10	93	Lower limb only
Mackie *et al.,* 2022	ns	ns	ns	ns	ns	88	478	Lower limb (n=87), upper limb (n=1)
Mangialardi *et al.,* 2018	19	1	ns	43.4	14-70	20	0	Unilateral and bilateral lower and upper limb
Matsumoto *et al.,* 2019	59	4	20-78	56	ns	6	57	Lower limb only
Pons *et al.,* 2019	77	5	ns	45.5	ns	21	61	Lower and upper limbs
Suami *et al.,* 2022	215	63	ns	47.1[a]	ns (34.4)	112	166	Unilateral and bilateral lower limb
Yamamoto *et al.,* 2015	20	11	12-82	42.5	0-78 (28)	31	0	Unilateral and bilateral lower limb
Yoshida *et al*., 2020[25]	48	26	33-95	73.6	25-93 (68)	74	0	Unilateral and bilateral lower limb
Yoshida *et al*., 2020[24]	35	21	33-95	73.1	25-93 (68)	56*	0	Unilateral and bilateral lower limb

Table. 3. Summary of ICG contrast injection protocols used in selected studies.

Reference	ICG manufacturer	Concentration	Volume per injection	Injection plane
Akita *et al.,* 2013	Not specified	Not specified	0.3mL	Subcutaneous
Gentili *et al.,* 2021	Diagnogreen	0.5%	0.2-0.3mL	Subcutaneous
Hara and Mihara, 2020	Diagnogreen	0.5%	0.05mL	Subcutaneous[#]
Mackie *et al.,* 2022	Verdye	0.5%[*]	0.05-0.1mL	Intradermal
Mangialardi *et al.,* 2018	ICC-Pulsion	0.5%	0.2-1mL	Intradermal
Matsumoto *et al.,* 2019	Diagnogreen	0.5%	0.05mL	Intradermal
Pons *et al.,* 2019	Diagnogreen	0.5%	0.2-0.4ml	Subcutaneous
Suami *et al.,* 2022	Verdye	0.25%[**]	0.05-0.1mL	Intradermal
Yamamoto *et al.,* 2015	Diagnogreen	0.25%	0.2mL	Subcutaneous
Yoshida *et al*., 2020[25]	Diagnogreen	0.25%	0.2mL	Subcutaneous
Yoshida *et al*., 2020[24]	Diagnogreen	0.25%	0.2mL	Subcutaneous

*25mg Verdye mixed with 5ml saline;[34] **25mg Verdye mixed with 10ml saline;[33] [#]Protocol based on previous publication by the authors.[35]

Table 4

Table. 4. Summary of injection sites in the feet for imaging of the lower limbs.

Reference	Total number of injections	Web space of the toes				The lateral aspect of the foot		Medial aspect	Other injection sites
		1st	2nd	3rd	4th	Midfoot	Towards rearfoot		
Akita *et al.,* 2013	1	x							
Gentili *et al.,* 2021	2		x		x				
Hara and Mihara, 2020	3	x					x		Lateral side of the superior edge of the knee
Mackie *et al.,* 2022	4	x	x	x	x				
Mangialardi *et al.,* 2018	2		x				Border of AT		
Matsumoto *et al.,* 2019	4	x			x	x	Posterior side of the ankle		
Pons *et al.,* 2019	2		x		x				
Suami *et al.,* 2022	4	x				x	x	Below medial malleoli	
Yamamoto *et al.,* 2015	2	x					Border of AT		
Yoshida *et al*., 2020[25]	2	x					Border of AT		
Yoshida *et al*., 2020[24]	2	x					Border of AT		

AT, Achilles tendon.

Table 5

Table. 5. Summary of imaging protocols used in our selected studies.

Reference	Initial imaging Time after injection	Repeat imaging Time after initial contrast injection	Near infrared detector camera
Akita *et al*., 2013	1hr	2hr	PDE
Gentili *et al*., 2021	Immediately	Not specified	PDE
Hara and Mihara, 2020	Immediately	2hr	PDE
Mackie *et al.,* 2022	Immediately	Not specified	PDE Neo II
Mangialardi *et al.,* 2018	12-18hr	Not specified	PDE
Matsumoto *et al.,* 2019	Immediately	6 times after a 5-minute exercise period*	PDE
Pons *et al.,* 2019	Not specified	Not specified	PDE
Suami *et al.,* 2022	Immediately	Not specified	PDE Neo II
Yamamoto *et al*., 2015	Immediately	12-18hr	PDE
Yoshida *et al*., 2020[25]	Immediately	6hr and 24hr	PDE
Yoshida *et al*., 2020[24]	Immediately	12-18hr	PDE

PDE, Photo Dynamic Eye.

* Each additional imaging session was carried out after 5 minutes of treadmill (2km/h) exercise with a total of 30 min exercise per imaging session.

Table 6

Table. 6. ICG imaging features and outcome measures used to evaluate lymphoedema.

Reference	Linear	Dermal backflow			Other dermal backflow definitions	Time to groin	Other types of measures
		Splash	Stardust	Diffuse			
Akita *et al*., 2013		x	x	x			
Gentili *et al*., 2021					x		
Hara and Mihara, 2020	x				x		% of linear pattern
Mackie *et al.,* 2022					x		Retrograde flow, patent vessels, contractility
Mangialardi *et al.,* 2018	x		x	x	NE, LE, DDB, PDB		
Matsumoto *et al.,* 2019	x				x	Yes	Dermal backflow appearance rate
Pons *et al*., 2019	x	x	x				Collateral vessels
Suami *et al.,* 2022					x		Compensatory drainage regions
Yamamoto *et al.*, 2015	x				NE, LE, DDB, PDB		
Yoshida *et al*., 2020[25]	x				LE, dDB, eDB	Yes	
Yoshida *et al*., 2020[24]	x				LE, dDB, eDB	Yes	

DDB, distal dermal backflow; eDB, extended dermal backflow; LE, low enhancement; NE, no enhancement; PDB, proximal dermal backflow (similar to eDB).

PRISMA 2020 Main Checklist

Topic	No.	Item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Page 1, line 3-4
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Page 3, 60-66
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	page 3, 68-69
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Line 89-95
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Line 75
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Line 76-80
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Line 82-95

Topic	No.	Item	Location where item is reported
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Line 82-95
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Line 98-104
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Line 112-115
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Line 92
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Line 83-95
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item 5)).	Line 83-95
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Line 83-95
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Line 83-95
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Line 83-95

Topic	No.	Item	Location where item is reported
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Line 83-95
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Line 83-95
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	N/A
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Line 99-104
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Line 89-91
Study characteristics	17	Cite each included study and present its characteristics.	Line 93-95
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Line 107-109
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Line 107-109
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Line 107-109
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A

Topic	No.	Item	Location where item is reported
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Line 99-104
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Line 99-104
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Line 107-151
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Line 107-151
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Line 288-293
	23b	Discuss any limitations of the evidence included in the review.	Line 309-312
	23c	Discuss any limitations of the review processes used.	Line 309-312
	23d	Discuss implications of the results for practice, policy, and future research.	Line 316-328
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	N/A
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	N/A
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Line 331-332
Competing interests	26	Declare any competing interests of review authors.	Line 333

Topic	No.	Item	Location where item is reported
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

PRIMSA Abstract Checklist

Topic	No.	Item	Reported?
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesize results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes

Topic	No.	Item	Reported?
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	No

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. MetaArXiv. 2020, September 14. DOI: 10.31222/osf.io/v7gm2. For more information, visit: www.prisma-statement.org