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A NEW DYNAMIC IMAGING TOOL TO STUDY LYMPHOEDEMA AND ASSOCIATED TREATMENTS

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ABSTRACT

The quest of imaging the superficial lymphatic network with the help of contrasting products started already more than 200 years ago. Because of the physiological specificity, the limpidness and the smallness of lymphatic vessels, researchers have always had a difficult task in the search of a specific tracer, that could be sensitive enough for lymphatics, affordable and suitable for in vivo examinations with a minimum of risk. Indocyanine green has already been used in angiology, but is new in lymphology. Its application in lymphology completes in an original manner the arsenal of imaging tools in this field. A specific camera, equipped with an infra-red diode and an uv-captor allows a mapping of the functional superficial lymphatic network after injection of Indocyanine green. A physiotherapist collaborating with the examinator by exerting manual lymphatic drainage during the examination, increases the amount of information produced by the images. Therefore, the surgeon, the clinician and the physiotherapist get precious indications for the follow-up and the therapeutic choices in the treatment of lymphoedema.

INTRODUCTION

Because of their small size and their invisibility to the naked eye, lymphatic vessels were the last to be identified among all macro-anatomical vascular structures. From the beginning, anatomists needed lot ingenuity in order to analyze this tiny and fragile, but dense network. The examination of its topography requires sophisticated technology. Historically it seems that the mesenteric lymphatics were the first to be discovered due to their less transparent contents: the chyle (a milky substance). The researcher realized soon that he needed a specific colored fluid to visualize this transparent vascular network of incredible density.
Different contrast fluids such as glue (Mascagni [1]), mercury (Cruveilhier [2]), the mass of Gerota (Gerota) and potassium iodide [3] were used over the years but were interesting means as long as they were applied on cadavers or animals, but could unthinkably be used in the in-vivo study of human physiology and anatomy.

Fig. 1 -
Parietal and neck Lymphatic's visualized by mercury injections. Musée d'anatomie, Université Libre de Bruxelles - Dir. Pr. M. Rooze.

Later on, lymphatics were visualised in-vivo by injection and canulation of a selected lymphatic by iodine oil (Lipiodol) by Kindmont in 1940.
The «Lymphography» was born. Thereupon this technique provided the basis for the contemporary lymphology because it became the main method to illustrate and analyze the lymphatic anatomy.
Another advantage was the imaging of anatomical dysfunction related to the pathology of lymphatics. However, nowadays the lymphography has been largely eliminated as diagnostic technique in the lymphedema because of its invasive and harmful trait [4].
Therefore, «blue dye» is currently used by surgeons in-vivo to visualize lymphatics [5], but it has unfortunately also some side effects: the coloration could mask underlying deeper anatomical structures; it colors the skin for 3 to 6 months and might trigger allergic reactions and some authors report skin and parenchymal necrosis when dye is injected in a subdermal fascia [6].
For the diagnosis of lymphatics physiology and physiopathology, the lymphoscintigraphy substitutes since the late seventies the

lymphography. Lymphoscintigraphy became thenceforth the ultimate technique permitting the representation not only of the topography of the lymphvessels and lymphnodes, but also their physiology and physiopathology.
It is specific and sensitive, employing a radio-tracer such as rhenium sulfide or more commonly Tc99 agglutinated to human albumin. Because of their important molecular weight, these tracers progress - after injection in the dermal or subcutaneous space -mainly in the lymphatic network.
The modalities of the injection have to be respected strictly on account of their influence on the progression of the tracer inside of the lymphatics [7].
Lymphoscintigraphy helps oncologists not only to identify sentinel lymphnodes due to its high specifity and sensitivity, but first as the gold standard tools for the diagnosis of lymphatic's pathologies and to develop a more selective surgery, i.e. less destructive, respecting much more the lymphatic system. Since it has been more largely used, the incidence of limb's lymphedema linked to oncological surgery has decreased to 2% [8]. Lymphoscintigraphy has also supported scientists to study the efficiency of specific treatment of the lymphatic system such as manual lymphatic drainage, multilayer bandaging or intermittent compression therapy.
However, even though lymphoscintigraphy persists as the reference imaging for lymphatics, we can't ignore its ionizing effects on the patients and physician even if they are minimal. Moreover, the radiocolloids are expensive and sometimes difficult to obtain. The examination requires imperatively the competence of a nuclear physician accustomed to the lecture of lymphatic vessels that sometimes can show quite original topographies.
Actually, the field of lymphatic imaging is constantly growing; new contrast agents and techniques in ultrasound, PET, and MR imaging are available, able to represent both: lymphatic vessels and lymphnodes [9]. But by reason of the very small diameter of the main branches of the lymphatic tree, the sensitivity of all those techniques and their precise rule in the field of lymphology remain still to be determined more exactly.
During the 80's a new tracer, called «fluorescein-dextran» has been applied. By virtue of the fluorescence, it was possible to examine under microscope the initial lymphatic vessels in the derm and other anatomical structure as synovia [10]. But the complicated optical systems necessary to its application limited its use. Anyhow fluorescence remains a very interesting method of investigation of the superficial lymphatics because of its innocuousness compared to radio-isotopes and the instantaneous mapping on the patient.
Recently, another fluorescent substance named "Indocyanine green" who is a dye used primarily in cardiac output determinations, and in ophthalmic angiography has found an appliance in the field of oncology and lymphology: It is first used for the screening of sentinel lymphnodes in the intestine cancer [10], from 3 years, oncologist are using these technique for the screening of the SNL in breast cancer [11] that reaches acceptable sensitivity and specificity comparable to conventional methods. [12]
Interpeeled by this application, an idea crossed our mind: could a subcutaneous injection of indocyanine (such as the radio colloid injection effected in lymphoscintigraphy) help in the mapping of the superficial lymphatic network? If that was possible we would have found a new non-invasive method how to examine the anatomy of lymphatic vessels, their physiology and physiopathology. We started a pilot study in order to verify the interest of this new approach in line with our practice: imaging, surgery and physical treatment.
Just lately, we realized that our colleagues from the Stanford University Texas discovered in 2009 that the near-infrared fluorophore Indocyanine green can image the lymphatic system. Compared to us, these colleagues concentrated only on the visualization of superficial lymphatics of the arm, but did not deepen the larger appliance of this method.
Pursuiting this train of thoughts, we realized movements of manual lymphatic drainage in order to verify, on one hand if the lympho-fluoroscopy (this expression seems to be the most appropriate name) could really conduce to a mean of objectivation of this method of physical treatment and on the other hand, if manual lymphatic drainage was able to bring out more lymphatic ways than a passive situation.

MATERIAL AND METHOD

1. Principles of the «Lympho-fluoroscopy»

The method consists of the injection of Indocyanine green, a highly fluorescent substance, when excited by an infra-red (IR) radiation emitting at a wavelength of 760 nm.
As soon as Indocyanine green is injected into the subcutaneous space, it is conjoining with the local free albumin. Those so called nanoparticles are resorbed and transported exclusively by the lymphatic system.
With the aid of a special camera emitting IR, the operator is filming the patient's skin from a distance of at least 15 to 30 cm. Then it is possible, from the point of injection on, to observe the progression of the substance inside of the superficial lymphatic vessels located under the skin up to a profundity of +/- 1 cm. The signal is filtered and amplified and shows a dynamic image of the superficial lymphatic network.

2. Methods

We accomplished 12 lympho-fluoroscopies on 6 volountary patients presenting an unilateral secondary lymphedema of the upper limb after adenectomy for breast cancer.
Written informed consent was obtained from all patients.
Firstly, we effected a local anesthesia with lidocain at 1% into the second interdigital space. Secondly, we injected in the same place Indocyanine green monosodium salt (ICG-Pulsion® 25mg) added at 5ml of acqua purificata [15].
Subsequently, we observed the progress of the tracer in the subcutaneous area with the help of the photodynamic eye (PDE) system (Hamamatsu Photonics, Hamamatsu, Japan). (Fig. 2)


Fig. 2 - PDE Camera observing the injection area.

3. Protocol

The patient is lying in a supine position during the whole session. Firstly, we concentrate on the healthy side, where the tracer is injected into the second interdigital space, then we apply the whole protocol of manual lymphatic drainage. Secondly, we repeat the same procedure on the edematous side.

During the first 5 minutes the patient remains immobile. The camera is focused on the site of injection waiting for the appearance of the first lymphatic vessels that absorbed the fluorescent nanocolloid.

Afterwards, the patient executes active movements of flexion-extension of his fingers (without moving the rest of the arm). Meanwhile, the camera sways up and down in order to follow the tracer's progression and the appearance of other subcutaneous lymphatic vessels or lymphnodes, from the injection point on along the hand, the wrist, the forearm… (Fig. 3).

Fig. 3

Then, after 10 minutes, we initiate the third sequence of the protocol: 20 minutes of manual lymphatic drainage according to Leduc (drainage of the proximal lymphnodes, inciting technique till the edema, resorption technique on the edema's area, inciting technique back to the proximal lymphnodes).

In the meantime, the camera follows alternately the therapist's hands and observes any change in the progression of the tracer inside the lymphatic vessels from the root of the limb till the point of injection.

We recorded the images of the examination from the first injection till the end after 30 minutes.

RESULTS

The injections are well tolerated by the patients.

Around the site of injection, the derm stays slightly green colored for about 6 days. Then, it disappears completely. Immediately after injection, a small pustule appears due to the pressure of injection. It flattens as soon as the tracer is evacuated into the lymphatic system.

On the healthy side

Observations during the first five minutes

After injection of the Indocyanine green, lymphatic collectors are appearing immediately on the dorsal and the palmar side of the hand (Fig. 4).

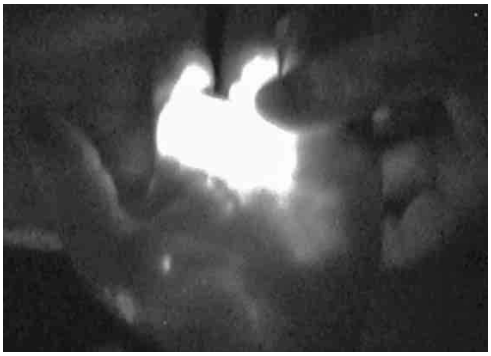

Fig. 4 - The palmar side of the hand after intergidital space injection of ICG.

Due to the pressure of the injection, during the following minutes, a network of lymphatic collectors shows up little by little in the region of the wrist following the diffusion of the tracer from the initial lymphatics to the collectors. At that instance, we have repeatedly observed contractions of lymphangions generated by the expulsion of the lymph from lymphangion to lymphangion.

Observations after five minutes

After 5 minutes of passive progression, when the patient started the muscle contractions, the speed of diffusion of the lymph in increased visibly showing up more and more the density of the lymphatic network.

Observations after 10 minutes

At 10 minutes we started the manual lymphatic drainage. In the beginning, we effected the drainage of the proximal lymphnodes in the axilla, followed by the inciting technique from the root of the arm till the experimental edema on the hand. Until this moment, we did not observe any modification. Only when we started the resorption technique on the point of injection («edema's area»), we noticed a great elevation of the resorption of the product and an increase of the flow inside of all collectors up to the forearm (Fig. 5).

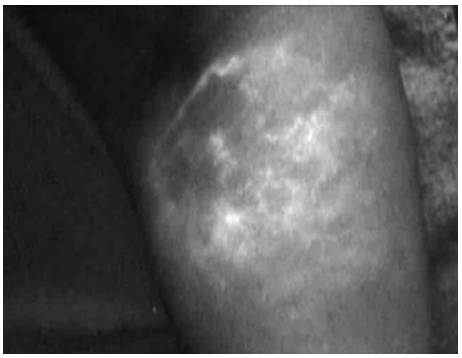

Fig. 5 - The dermal back flow on the anterior part of the forearm.

We tried then to execute the same resorption technique on the fluorescent collectors of the forearm and observed a significant increase of the lymphatic flow inside of those vessels.

Underneath the therapist's hand, the fluorescent lymphatic vessel faded, while downstream, it swoll instantly up.

The inciting technique applied on these collectors provoked the same effects as the resorption technique, but we couldn't observe any «succion» effect upstream of the draining hand as it is expected to do.

On the edematous side

We observed different situations

Three out of the 6 patients showed the same diffusion and progression of the tracer on the edematous side and on the healthy side. The only peculiarity was a little lesser density of the fluorescent network on the edematous side at the end of the protocol.
One out of the 6 patients presented a pronounced edema of the back of the hand. In this case, the tracer diffused through the whole hand defining a fine, dense and disorganized lymphatic network without appearance of any bigger collector and without any progression into the rest of the upper limb despite of manual lymphatic drainage. These images corresponded exactly to those obtained by lymphography with Lipidiol in case of dermal backflow.
One out of the 6 patients suffering from an edema of the forearm after a large adenectomy, showed a fluorescent axilla and a clearly visible lymphatic network in the whole upper limb.
Two other patients with a slight edema of the back of the hand and a pronounced edema of the forearm, presented collectors of the hand and the wrist and later also of the arm, but not of the forearm that gave an image of dermal backflow (Fig. 6). In this case, we found a lymphatic way of Mascagni (Fig. 7) and one of Caplan.
In 3 of the 6 patients epitrochlear lymphnodes appeared during the period of manual lymphatic drainage (Fig. 8).

Fig. 6 - Increase of the flow inside of all collectors up to the forearm.

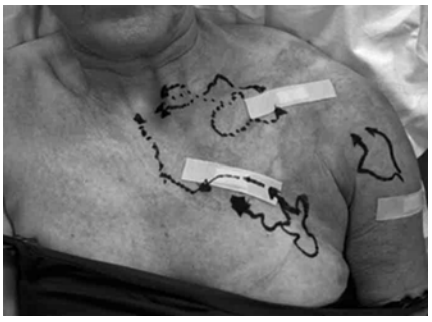

Fig. 7 - Way of mascagni and subclavian ways.

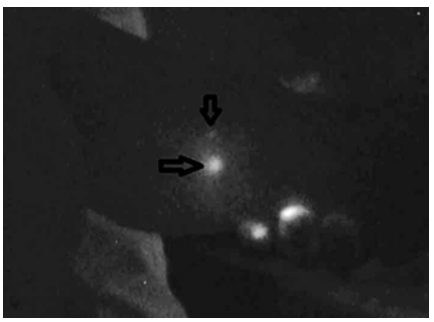

Fig. 8 - Epitrochlear lymphnodes.

DISCUSSION

Lympho-fluoroscopy after subcutaneous injection of Indocyanine seems to be an innovating and promising mean for lymphology. It allows the mapping of superficial lymphatic pathways and lymphnodes. Moreover, it identifies clearly areas of dermal backflow on limbs.
It turns up as a particularly interesting imaging technique compared to blue dye or lymphoscintigraphy: it is economical and not ionizing as isotopes. But the risk of the use of iodine has to be considered.
The actual report quotes the risk of this examination at 1/320 000.
Recently, we came upon a study [9] that pointed out the risk of Indocyanine to reduce or even block the flow of the intestinal lymphatics of mice. Our experience on the lymphatic network of the human limb cannot support that conclusion. After a subcutaneous injection of the tracer into the second interdigital space, it takes more or less 10 minutes to reach the root of a human limb. The values are the comparable to those obtained in lymphoscintigraphy.One can also observe the contraction of the lymphangion and the originated flowing rather than a stagnation or block. But the remarks of these authors need to be taken into account and evaluated accurately.
Without surgery, lympho-fluoroscopy is limited to the superficial lymphatic system.
Lymphofluoroscopy is a qualitative and not quantitative method such as is lymphoscintigraphy.
All collectors immerging into the adipose tissue are invisible to that examination, unless one interposes an amplifying lens that permits a view into 2 cm deepness. Unfortunately, it compresses the vessels and interrupts the lymphatic flow during a dynamic exam.
The deep lymphatic network that in certain circumstances might disencumber the superficial system, is unvisible.
But coupled to lymphoscintigraphy this weakness can become strength.
In fact, if both tracers (Indocyanine and radiocolloids) were injected simultaneously, it might be possible to distinguish the collectors from the lymphnodes.
Successive studies will certainly explore more widely these possibilities.
Lympho-fluoroscopy does not exclude lymphoscintigraphy, but fortifies previous imaging techniques in lymphology.
In the same way, the intervention of a physiotherapist during the examination countenances the clinical interest of this imaging technique: applying manual lymphatic drainage on sectors of the lymphatic network activates the tracer's progression inside of those lymphatic vessels that are still functional. Subsequently, the results can help to modulate therapeutic protocols and focus the action of the manual lymphatic drainage on functional lymphatics.
It might also be interesting to use this examination in order to follow-up the therapeutic care. For example, it could verify if new lymphatic vessels appear after the treatment or contrary, disappear.
Our preliminary study has confirmed that inciting and resorption movements described in the technique of manual lymphatic drainage according to A. Leduc [16] are efficient concerning the resorption of the edema and the acceleration of the lymphatic flow inside of the collectors. For the moment this research work

remains qualitative, but we started already other studies targeting the quantification of these phenomenon.
Finally, with the help of lympho-fluoroscopy, other techniques of manual lymphatic drainage or other treatment might find a tool that could help them to emerge out of their empiricism.
This manner of approaching the diagnosis and the treatment of lymphedema requires even more studies in order to valid the different steps and the interpretation of the images, but from our point of view, lympho-fluoroscopy has already now demonstrated its major interest in the development of lymphology.
Clinicians and researchers, physicians and physiotherapists have been waiting for a long time for a simple, economic and ethically acceptable mean to study in detail manual lymphatic drainage, other instrumental techniques, surgery… aiming at the success of decongestive treatment.
At last, we would like to indicate the interest of this technique for the anatomical mapping of lymphatic vessels before and during surgical interventions in order to respect this system, avoid lymphedema or help their treatment.

CONCLUSIONS

Lympho-fluoroscopy is a new efficient imaging tool for the study of the superficial lymphatics. Its use still appertains to the medical staff and as any medications – even with a diagnostic purpose – there is a certain risk of intolerance, such as allergic reactions to iodine.
Oncologists study the properties of this new approach in order to complete or substitute partially lymphoscintigraphy concerning the localization of the sentinel lymphnode.
The advantage of lympho-fluoroscopy consists in its few invasive and non-ionizing character. It is more accessible than other imaging techniques in lymphology.
It replenishes the lymphatic vessels and lymphnodes at which it designs its network while its progress through the lymphpaths.
The intervention of a competent physiotherapist during the examination increases considerably the information acquired during the lympho-fluoroscopy.
Functional lymphatic vessels can be detected in lymphedema and dermal backflows become easy to localize.
The whole topography of the superficial lymphatic network linked to the injected anatomical region, appears like a route map that – given to a surgeon, a clinician or a physiotherapist – guides them in their therapeutical choice.
Our preliminary study supported us in the confirmation that techniques such as manual lymphatic drainage and others will benefit from lympho-fluoroscopy, because it will assist them in the reinforcement of the evidence based science by the justification of their specific applications.
In conclusion, lympho-fluoroscopy opens the floodgates to a wider research in the field of diagnosis and treatment of lymphatic diseases.

Conflict of interests note

This study has not benefited from any financial help. All injection products were financed by scientific budgets from the laboratory of physiotherapy of the Faculté des Sciences de la Motricité de l'Université libre de Bruxelles - Belgium and the Dpt. of Vascular Surgery of the ST. Dimpna Hospital, Geel - Belgium.
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