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DOES ICG HAVE TIME-DEPENDENT EFFECTS ON THE LYMPHATIC SYSTEM IN WOMEN AFTER BREAST CANCER SURGERY?

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ABSTRACT

Near infrared fluorescence imaging (NIRFI) with Indocyanine Green (ICG) has been shown to detect lymph leakages and patterns in patients with complete axillary lymph node dissection (CALND) for breast cancer. However, ICG has also been demonstrated to have toxic effects in the ophthalmo-logical field and limited data (in animal and human studies) suggest that it can alter functioning of the lymphatic system. The current study investigates pre-operative (Pre) and per-operative (Per) ICG administration and the volumes (V) of liquids collected in drains (Vd) and/or punctures (needle aspiration) (Vp) after breast cancer surgery. Fifty-five patients had one subcutaneous ICG injection in the ipsilateral hand either the day before (group Pre; n = 26) or the day of the surgery (group Per: n = 29). Vd, Vp and Vt (=Vd+Vp) were compared. The two groups did not differ statistically. We observed a statistical tendency (p=0.07) to find lower fluid volumes, overall (Vt) and in aspirations (Vp), when ICG was injected the day before the operation (Pre) compared with the same day (Per). When no fluorescence (no lymph leakage from the arm) was detected in the fluid collections, Vd and Vt were statistically significantly lower in the pre-op group and in the whole group but not in the per-op one. Our correlation results add additional evidence to suggest that ICG may have a causative effect on the lymphatic system depending on the duration of its exposure as observed in the ophthalmological field. Although further study is needed to confirm, lymphologists, angiologists, lymphatic surgeons, vascular surgeons, and others who are using ICG for their NIRFLI evaluations should be aware of possible risks and complications associated with this procedure for use in patients with lymphedema.

Keywords: Near Infrared Fluorescence Lymphatic Imaging; NIRFI; Breast cancer; Lymphatic; Indocyanine Green; ICG imaging

INTRODUCTION

Indocyanine Green (ICG) is an anionic, amphiphilic, water-soluble fluorophore with a molecular weight of 776. It is authorized by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) and is used for determining cardiac output, liver

blood flow, hepatic function from the late 1940s (1). ICG has also been and still is widely used in ophthalmology but with tissues that are then exposed to it for a longer period of time. However, and because worse functional outcomes (reduced visual acuity) and persistence of ICG at the macula and optic nerve (with a higher incidence of retinal pigment epithelium changes and visual field defects) were reported (2-7), it is also in this specialty that its toxicity has been most widely studied (mainly in animal models) and especially "in vitro" (on human and animal cell cultures) with the conclusion of a dose- and time-dependent toxicity on these tissues and/or cell cultures (8-21). This is supported by clinical data in humans, because better functional outcomes were obtained when low dye concentrations and short incubation times were reported (15).

ICG is now also used for Near Infrared Fluorescence imaging lymphatic structures (NIRFLI) after its intra-tissue (e.g., in the mammary gland for the axillary SLN demonstration) (22), intravenous (for metastatic lymph nodes visualisation in breast cancers and other cancers) (23-29) and intracutaneous injection (e.g., for the visualisation and study of the lymphatic vessels and nodes in edematous patients and/or in patients at risk of developing edema) (30-33). In these cases, the risk of allergic response is taken into account and these procedures are considered as safe (34). However, and compared to what can be found for ophthalmic applications, the literature studying ICG toxicity on the lymphatic structures appears to be “poor” with only three debatable papers (in animals) (35-37).

In previous articles, we demonstrated our ability using ICG NIRFLI (after pre-operative and per-operative injection of ICG) to identify the axillary LN draining the upper limb, to detect axillary lymphatic leakage per-operatively and to demonstrate fluorescence in the axillary post-op drains of women who underwent axillary dissection for breast cancer (38-39). We also showed that when no fluorescence (no lymph from the arm) was observed in their drains, associated volumes of liquids collected after surgery in their drains (Vd) and/or ambulatory aspiration punctures (Vp) were lower than when liquids in the drains were fluorescent.

The recent publication of one study reporting data suggestive of an effect of ICG on the lymphatic system after its subcutaneous injection in women with breast cancer related lymphedema (40) has caused us to reconsider the problem in the context of our population and its indications for use. We hypothesized that pre-operative injection of ICG in our patients (the day before and at least 12 hours before their operation) might have effects on the lymphatic system different from those observed with per-operative injection of ICG (at most one hour before the operation) and that the quantities of liquids collected in the post-operative drain (Vd), subsequent aspiration punctures (Vp), and the total volumes of liquids (Vt=Vd+Vp) would be lower in our cases with pre-operative rather than per-operative injections. Because we had access to patients with either pre- or per-operative ICG imaging at our institution, we undertook the current study to investigate the potential impact of such pre-operative and per-operative ICG injections on these volumes of liquids to look for a correlation to postulate on the potential of a causative effect.

MATERIALS AND METHODS

This prospective monocentric study was approved by the Investigational Review Board (IRB) of the Jules Bordet Institute (CE2876) and was registered at the European Clinical Trials Database (EudraCT number 2018-002862-38). Between June 2019 and June 2021, fifty-five women (mean age = 58.5, range = 23-89 years) were successively enrolled and provided written informed consent. All patients were scheduled to undergo either mastectomy (n = 21) or lumpectomy (n = 34) with complete axillary node dissection for a histologically proven mammary tumor (CALND: at least levels I-II were removed). Exclusion criteria were: (1) History of iodine allergy or anaphylactic reactions to insect bites or medication; (2) Hyperthyroidism; (3) Severe cardiac or pulmonary disease; (4) Significant renal failure (creatinine > 400 µmol/l); (4) Pregnancy.

Patients were not limited in their normal behavior, diet, or medication intake before the study.

ICG (0.2 ml from 25mg of ICG diluted by 5.0 ml of sterile water for injection) was injected sub-cutaneous in the first interdigital space of the hand of operated side, either the day before the operation, or just before the operation (at the induction of anesthesia or within the half hour before), depending on the patient's hospitalization time. A specialized near-infrared camera system (PDE, Hamamatsu) is used for fluorescence imaging. A light-emitting diode light source set to a wavelength of 760 nm is used and the detector is a charge-coupled device (CCD) camera with a filter set to detect light with a wavelength of 820 nm. The fluorescent signal is sent to a digital video processor to be displayed on a monitor in real time. The camera was held directly by the surgeon at a standard distance from the operative specimen.

After axillary lymph node dissection and before closing a compress was put inside the axilla to verify the lymph loss. If fluorescence was detected on the compress using our near-infrared camera system, it was considered positive for a lymph loss. After surgery, we recorded and analysed drainage volumes and liquid fluorescence (or lack thereof) using our near-infrared camera system. The drain was then removed when fluid production was less than 50 ml per day. Each patient was seen one week after surgery and then weekly or more frequently. Aspirations (needle punctures) of axillary collections were performed as long as necessary. The follow-up period was at least 12 months.

The outcomes were measurements of the quantities of volumes captured in the drains (Vd) and/or in the aspirates (Vp) and correlated with a) the timings of injections of ICG (pre- and per-operative), and b) the presence or absence of fluorescence in the axillary drains.

Statistical analysis

Our analysis is based on a set of statistical tests to assess the impact of the temporal variation in ICG administration on the main parameters. The parameters of interest were divided into categorical variables (fluorescence or not of the SLN biopsy, fluorescence or not of the axillary LN in the CALND, pN+ status) and quantitative variables (number/percentage of fluorescent lymph nodes in the CALND). The standard Chi-Square test was used to assess whether the categorical parameters depend on the timing of ICG injection. However, when the number of patients for a given category is too small, the Fisher exact test was used. The Kruskal-Wallis non-parametric test was used to determine if the distributions of the quantitative variables are different depending on the moment of the ICG injection. A nonparametric method was applied when the Shapiro-Wilk test led to the rejection of the normality hypothesis. All statistical analyzes were carried out using the R software. We consider 0.05 as the level of significance for all statistical tests (41).

RESULTS

A total of 55 patients were enrolled in the study with 26 patients receiving ICG imaging prior to operation and 29 patients with ICG imaging at the time of operation. The clinical characteristics of patients in the pre-operative and per-operative sub-groups do not differ significantly for any measurement (*Table 1*).

Fluid volumes were collected post-operatively on all patients and examined. Comparison of pre-operative ICG injected sub-group volumes to the per-operative sub-group, we observe a statistical tendency (p=0.07) to find lower overall fluid volumes (Vt) and in punctures (aspirations) (Vp) in patients when the ICG was injected the day before the operation (Pre) rather than on the same day (Per) (*Table 2*). Fluid volume in the drains (Vd) did not differ significantly.

Further analysis examined potential differences between patients who had fluorescence identified in the drains (Vd) compared to those who did not as a potential measure of the lymphatic system function in the limb. This analysis identified significantly lower volumes in the total overall population for those

TABLE 1
Characteristics of Patients with Pre-Operative (Pre) and Per-Operative (Per) Injection of ICG

		Injection of ICG			Pre/Per p-value
		Pre	Per	Total	
Patients (number)		26	29	55	
Age	median	59.5	64	60	0.527
	mean	58.9	60.9	58.5	
	range	33-89	23-85	23-89	
BMI	median	27.3	26.8	25.1	0.483
	mean	28.5	27.6	25.7	
	range	17-41	17-41	17-41	
Lateralization	Right	17	17	34	0.812
	Left	9	12	21	
Tumor Size (mm)	median	27.5	28.5	30	0.684
	mean	34.2	32.9	33.3	
	range	14-70	8-87	8-87	
Histology	Ductal	21	27	48	0.23
	Lobular	5	2	7	
Molecular sub-type	Luminal A	2	0	2	0.77
	Luminal B	16	13	29	
	HER2 +	2	9	11	
	HER2 -	6	7	13	
Grade	3	10	18	28	0.155
	2	12	10	22	
	1	4	1	5	
Surgery	Lump.	15	19	34	0.75
	Mast.	11	10	21	
Number of Lymph Nodes Removed	median	7.5	10	12	0.259
	mean	9.8	11.3	12.8	
	range	4-29	5-27	4-29	
Axillary Status	pN+	11	8	19	0.388
	pN-	15	21	36	

Abbreviations: ICG- indocyanine green; pre-preoperative; per-peroperative

TABLE 2
Fluid Volumes in Drains (Vd), Punctures (Aspirations) (Vp), and Total (Vt) for Patients with Pre, Per, or Total ICG Injections

		Injection of ICG			Per/Pre np-value
		Pre	Per	Total	
Vd	mean	201	214	208	NS
	median	125	210	200	
	range	0-615	20-510	0--615	
Vp	mean	216	545	390	0.063
	median	140	250	185	
	range	0-1000	0-3500	0-3500	
Vt	mean	418	760	598	0.066
	median	320	5540	490	
	range	0-1470	20-3620	0-3620	

Abbreviations: ICG- indocyanine green; pre-preoperative; per-peroperative; NS-no significant

patients who did not have any fluorescence in their drains (drain, aspirate, and total) compared to those who did. In addition, only in the group where the ICG was injected pre-operatively was there a significant difference for the drains, aspirates, and total with those patients having no fluorescence again being lower than those who did have fluorescence in their drains (*Table 3*). Finally, analysis examining the patients who had fluorescence identified in the drains (Vd) compared to those who did not for both the use of aspirations and the number of aspirations revealed a significant increase in the number of patients with aspirations and the number of aspirations for both the pre-operatively ICG injected group as well as for the total group, but not for the per-operatively ICG injected group (*Table 4*).

DISCUSSION

Although this study is not able to directly measure causation of ICG injection for lymphatic system alterations, it does allow indirect measurement of the impact with comparison of pre- and per-operative patients using direct measurement of the post-operative fluid from these patients. There are several lines of other evidence to consider for postulating that ICG may impact functioning of the lymphatic system.

ICG Toxicity in Ophthalmology

Analysis of ophthalmological literature about the effects of ICG is made difficult by the multiplicity of parameters that vary from one study to another: in vitro cultured cells of different characteristics (retinal pigment epithelial cell, retinal ganglion/Muller cell, of human or animal origin), ICG concentrations ranging from 5 µg/ml to 20 mg/ml, exposure time to the ICG solution ranging from a minimum of 1 minute to a maximum of 2-3 weeks, injection of ICG into the vitreous cavity of rat eyes, differing tissular or cellular characteristics studied (light-induced oxidative stress and senescence, cellular apoptosis, degeneration of all retinal layers in the central retinal area, blood-retinal barrier function measured through transepithelial electrical resistance), (8-21). However, it is clear from that analysis that ICG shows a dose-dependent toxicity and time exposure-dependent toxicity (and light

TABLE 3
Patients with Fluorescence or Not in the Post-Operative Drains (Pre, Per, or Total for ICG Injection) and Volumes of liquids collected in drains (Vd) and punctures (aspirations) (Vp) and Total (Vt)

		Injection of ICG							
		Pre		Per		Total			
Fluorescence in Post-op Fluid		**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	If Yes, pre/per p-value	If No pre/per p-value
Number of patients		21	5	23	6	44	11		
Vd	mean	235	60	227	166	231	118	NS	0.12
	median	170	15	220	172	2027	115		
	range	20-615	0-260	10-510	20-370	10-615	0-370		
Yes versus No p-value		**0.01**		NS		**0.02**			
Vp	mean	257	44	614	173	444	173	0.13	NS
	median	150	0	300	50	240	50		
	range	0-1000	0-200	0-3500	0-1010	0-3500	0-1010		
Yes versus No p-value		**0.02**		NS		**0.02**			
Vt	mean	403	104	841	445	675	290	0.15	0.07
	median	362	15	565	362	525	70		
	range	20-1470	0-460	20-3620	0-1220	20-3620	0-1220		
Yes versus No p-value		**<0.01**		NS		**0.01**			

Abbreviations: ICG- indocyanine green; pre-preoperative; per-peroperative; NS-no significant

toxicity) (8,16,18).

ICG "Toxicity" on Lymphatic Structures in Animal Models

Although there is limited data in the literature on the subject and this needs to be carefully analysed, Aldrich et al. (36) were unable to detect changes in lymphatic vessel function (in lymphatic propulsive velocity or frequency) from the inguinal lymph node to the axillary lymph node in mice after (free) ICG injections in various concentrations (0.32, 0.50, 0.625, and 1.3 mM: 10 µL per injection site), but within the (one) minute after ID injection.

In studies examining *ex-vivo* isolated rat mesenteric lymphatic vessels, the effects of ICG (diluted in a physiological NaCl solution at a concentration of 32 µM, 320 µM and 1.3 mM, whether containing or not albumin), Gashev et al. (35) showed that ICG inhibits the contraction of these vessels in a dose-dependent manner, but after a delay of several minutes.

Weiler and Dixon's study (37) appears to be interesting in this context. These authors analysed *in-vivo* the impact on the function (contraction, velocity) of rat tail lymphatic vessels after ICG injection (pre-mixed with BSA) as well as the effects on lymph nodes accumulating this tracer (but at 1, 2 and 4 weeks after injection). ICG remained visible in the tails of the animals for up to 2 weeks after injection and was accompanied (compared to controls) by significant decreases in lymphatic function at week 1 and enlargement of the lymph nodes draining this ICG (with an in-

TABLE 4
Patients with Fluorescence or Not in the Post-Operative Drains (Pre, Per, or Total for ICG Injection) and Both Yes/No for Aspirations and Number of Aspirations

	Injection of ICG							
	Pre		Per		Total			
Fluorescence in Post-op Fluid	Yes	No	Yes	No	Yes	No	If Yes, pre/per p-value	If No, pre/per p-value
number	21	5	23	6	44	11		
Patients with Aspiration								
No	1	3	2	1	3	4	NS	NS
Yes	20	2	21	5	41	7		
Yes/No p-value	**0.014**		NS		**0.024**			
Number of Aspirations								
Mean	3.38	1	5.30	3.33	4.39	2.27	NS	NS
Median	3	0	4	2.5	4	1		
Range	0-6	0-4	0-22	0-10	0-22	0-10		
Yes/No p-value	**0.017**		NS		**0.015**			

Abbreviations: ICG- indocyanine green; pre-preoperative; per-peroperative; NS-no significant

crease in size of more than 350% at week 1 and nearly 200% at week 2).

These studies highlight that the influence of ICG injection on the lymphatic system therefore seems to be delayed rather than immediate and seems to require a long pre-exposure to the molecule to show an effect.

ICG Near Infrared Fluorescence Lymphatic Imaging "Toxicity" in Humans?

Devoogdt et al. (40) used (free) ICG (two injections of 0.2 ml in the hand at a concentration of 80 µg/mL per injection site) to highlight superficial lymphatic structures of edematous upper limbs secondary to breast cancer surgery and to study changes in the areas of dermal reflux, the number of vessels (draining these areas), and lymph nodes (accumulating the tracer) in 3 groups of patients subjected to different physiotherapeutic protocols (a "placebo" group, a group with traditional MLD, and a group with fluoro-guided MLD). They compared the basal situation to those observed after 3 weeks of intensive treatment and 6 months of maintenance treatment. Their initial hypothesis was that patients receiving fluoro-guided drainage would have an increase in the number of lymphatic vessels draining the areas of dermal reflux, a decrease in these areas of reflux, and an increase in the number of lymph nodes receiving lymph from these areas of lymphatic stasis. However, they observed a significantly decreased number of lymphatic vessels in their whole group after their intensive phase (and borderline after their maintenance phase) and a significant decrease in dermal backflow and number of lymph nodes visualized (both after their intensive phase and after their maintenance phase). These authors do not discuss their observations, all of which may nevertheless suggest a negative effect on the lymphatic structures of the limb and more specifically on the ability of the initial lymphatics and pre-collectors at the injected site to eliminate, uptake, and transport it into the vessels of the limbs, in the areas of reflux, and axillary lymph nodes.

In our population of patients undergoing complete axillary lymph node dissection for breast cancer, ICG (250 µg of ICG in 0.2 ml) was injected subcutaneously in the first interdigital space of the hand (final concentration = 50µg/ml) to identify the axillary LN draining the upper limb with the associated risk of lymphatic leakage, to detect axillary lymphatic leakage per-operatively, and to demonstrate fluorescence in the axillary post-op drains (38-

39). It was hypothesized that, if no fluorescence (therefore no lymph transported from the arm) was observed in the post-op drain, that associated volumes of liquids collected after surgery in the axillary drains (Vd) and/or ambulatory punctures (aspirations) (Vp) would be lower than if the drains were fluorescent. Our findings support this hypothesis across all groups (regardless of whether ICG was injected pre or per-operatively), with significantly lower volumes found when no ICG fluorescence was observed in the post-operative drains and, for the volumes in the drains (Vd) and total volumes (Vd+Vp), when no ICG leaks were observed on the compress placed per-operatively in the axilla. However, we also observed that a decrease in the volumes was found when ICG was injected pre-operatively compared to per-operatively and when no ICG was observed leaking in the drain (for the total group) (*Tables 2 and 3*). These observations suggest that ICG has an effect on the lymphatic vessels of the arm and/or on the axillary LN taking up the fluorescent ICG lymph, but that this effect also appeared only after a delay of at least 15 hours.

Limitations of our study

The limitations of our study are found in the small numbers of subjects in our pre- and per-op groups, but also in the numbers of patients without fluorescence in the post-op drains. We believe fluorescence in the drains reflects a leakage and a lymphatic supply to the volumes collected in these drains and the possible subsequent aspirations which result in larger volumes and we then observe at best only trends. However, these remain suggestive of an effect of ICG on the fluid volumes found in drains and aspirates when it is injected the day before the operation.

NIFRLI with ICG and allergies?

Intra-Venous injection of ICG exposes tissues and cells only for short periods of time, usually several minutes and in series published now 30 years ago (42-43), adverse reactions to ICG are said rare and mild occurring in 0.15% of the exposed patients with moderate reactions in 0.2% and severe reactions in 0.05% to 0.07%. However, anaphylactic shocks were still recently reported in the literature (44-45). Although the amounts of ICG (in mg) injected in lymphatic imaging are much lower than in IV injections, a group of specialists brought together in a DELPHI study and more specifically surgeons using ICG for the visualization of lymphatic vessels during lymphatic venous anastomoses have acknowledged having witnessed cases of allergic reactions (34). The risk therefore seems to exist, even if it is rare, and it should not be forgotten even in a purely diagnostic approach to lymphedemas because lymphatico-venous shunts may be present (46).

CONCLUSION

Although ICG is widely utilized in the field of lymphology, there is good published data on the effects of ICG in the ophthalmological field (both on cells and tissues) and on lymphatic structures (both in an animal model and in women) that supports the idea that the injection of ICG into the skin does indeed have a "negative" effect on cells that are durably exposed. This fact should be of high interest in particular to the field of lymphology where lymphatic structures (the initial lymphatics and/or the pre-collectors and/or the lymphatic vessels and/or the lymph nodes?) may be impacted.

The implications of these observations are manifold. On the positive side, and in the case of patients requiring axillary dissection, it can be considered that the reduction of lymphatic drainage of the limb and associated fluid leakage (and axillary fluid volumes in drains or aspirates) might represent an advantage, regardless of the fact that the intraoperative detection of such leaks paves the way for their cauterization, ligation, or (lymphatic to vein) anastomoses with the additional benefit of decreasing the risk of lymphedema development (47-49). On the negative side, these effects of ICG on lymphatic vessels and their lymph nodes raise several questions. The use of ICG could thus have the consequence of masking the effect of different therapeutic

approaches for lymphedema as for instance in the works by Devoogdt's team (who concluded that MLD had no effect on BRCL edemas) (50). At worst, this decrease in lymphatic drainage could aggravate these edemas (especially in case of repeated injections?) and/or modify the images we have of them (on dermal backflows, on lymphatic collaterals).

These questions deserve to be answered (and our results to be confirmed) through dedicated studies based on larger numbers of cases than in our series. In a more fundamental approach, it should also be tried to reproduce the studies carried out with the ICG by ophthalmologists on the cellular elements of the initial lymphatic components, lymphatic pre-collectors, (and maybe more specifically and rather than on) lymphatic vessels and lymph nodes.

Finally, lymphologists, angiologists, vascular surgeons, etc. who are using ICG for their NIRFLI should be aware of possible risks and complications associated with this procedure, at least when using it in patients with lymphedema.

CONFLICT OF INTEREST

All authors declare no financial conflicts of interest regarding the publication of this paper.

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