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The feasibility of assessing perfusion of the bone using quantitative ICG fluorescence imaging

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Abstract:
Background For successful reconstructive surgery, adequate tissue perfusion is necessary. Near infrared (NIRF) imaging, using indocyanine green (ICG) allows perfusion to be visualized of which objective perfusion parameters can be provided after additional measurements. Therefore, it has great potential in predicting adequate tissue perfusion. However, regarding bone tissue the evidence of the feasibility and usefulness of NIRF imaging using ICG is very limited.

Methods A prospective monocentric pilot study was carried out at a tertiary hospital in the Netherlands. Patients undergoing autologous breast reconstruction from August 2021 to August 2022 were included. During surgery ICG (0.1 mg/kg) was injected intravenously and a fluorescent angiogram of four minutes was made directly after injection. Post hoc time-intensity curves were generated of the region of interest (ROI) of 5mm, which was positioned on the cross-sectional lateral surface of the rib.

Results Nine patients and eleven ribs were included for further analyzation. Time intensity curves of the endosteal measurement was performed in ten ribs. Three of the curves show a steep and well-defined ingress and egress. In all other patients, the curves show a much more flattened ingress and egress. Periosteal measurement was performed in nine ribs. No adverse events related to ICG were observed intraoperatively.

Conclusions This feasibility study suggests that quantitative NIRF imaging using ICG can provide objective parameters of endosteal rib perfusion. Larger prospective series are needed to investigate the value of NIRF imaging using ICG to assess bone perfusion intraoperatively and to establish cutoff values for adequate bone perfusion.

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The feasibility of assessing perfusion of the bone using quantitative ICG fluorescence imaging

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Abstract

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Methods A prospective monocentric pilot study was carried out at a tertiary hospital in the Netherlands. Patients undergoing autologous breast reconstruction from August 2021 to August 2022 were included. During surgery ICG (0.1 mg/kg) was injected intravenously and a fluorescent angiogram of four minutes was made directly after injection. Post hoc time-intensity curves were generated of the region of interest (ROI) of 5mm which was positioned on the cross sectional, lateral surface of the rib. The first moment of increase of intensity is defined as t0. Fluorescent parameters included ingress and egress of ICG.

Results Nine patients and eleven ribs were included for further analyzation. Time intensity curves of the endosteal measurement was performed in ten ribs. Three of the curves show a steep and well-defined ingress and egress. In all other patients, the curves show a much more flattened ingress and egress. Periosteal measurement was performed in nine ribs. No adverse events related to the ICG injection were observed intraoperatively.

Conclusions This feasibility study suggests that quantitative NIRF imaging using ICG can provide objective parameters of endosteal rib perfusion. Larger prospective series are needed to investigate the value of NIRF imaging using ICG to assess bone perfusion intraoperatively and to establish cutoff values for adequate bone perfusion.

Keywords: Fluorescence imaging, ICG, bone perfusion

Introduction

Adequate bone perfusion is key for successful surgery. It is important for healing and consolidation, particularly in cases of crush by trauma or when subsequent radiotherapy is necessary.

Over the years, several techniques have been developed to support surgeons in assessing adequate tissue perfusion. One of them is near infrared fluorescence (NIRF) imaging, using indocyanine green (ICG). ICG is a water-soluble fluorescence dye and when administered intravenously, it binds for 98% to plasma proteins and is therefore an ideal tracer to measure perfusion. ICG has a half-life time of 3 to 4 minutes, and it is cleared exponentially by the liver with a clearance rate of approximately 20% per minute [1, 2]. ICG is considered to be very safe for patients, since anaphylactic reactions are rare with an incidence of 0.05% [3]. The light required for the excitation of ICG is generated by a light source which is directly attached to a digital video camera with a specific filter. This light source emits light between 750 and 800nm which is near the infrared range and excites ICG which can be viewed around the maximum peak of 832nm [1, 4]. With NIRF imaging using ICG the absorption of ICG can be recorded real time during surgery and allows perfusion to be assessed. Due to its encouraging results, NIRF imaging has grown in popularity across a variety of surgical specialties, including reconstructive surgery [5-8].

Visualizing bone perfusion is challenging. It would be of great interest, to evaluate the application of NIRF imaging using ICG for osseous tissue. For example, it could be highly valuable when assessing vascularized bone flaps, especially when they need to be osteomized for head and neck reconstructions or extremity reconstructions where adequate perfusion is key for consolidation. Moreover, it could improve the early debridement of necrotic bone in extended trauma cases, it may improve the identification of sequesters and it could be useful in the removal of tissue affected by osteoradionecrosis [9-12].

To provide objective perfusion parameters and fluorescence intensity curves of bone tissue, this study investigated the perfusion of the human rib with NIRF imaging using ICG. The rib is easily accessible when anastomosing a free flap in autologous breast reconstruction. Bones have a bipartite blood supply consisting of an endosteal and periosteal network which are connected through small capillaries [13-15]. The endosteal blood supply is provided by the nutrient artery which is a branch of the posterior intercostal artery. The nutrient artery enters the medullary canal of the rib just beyond the tubercle which is posteriorly [16, 17]. The internal mammary vessels, which arise from the subclavian vessels, branch into the inferior intercostal vessels **Figure 1.** The periosteal circulation of ribs is based on dual blood supply provided by the inferior intercostal vessels and the superior supracostal vessels. These vessels have interconnecting arterioles covering the whole surface of the rib [18]. It is thought that the nutrient artery is necessary for the survival of the rib [19]. Conversely, studies have shown that the viability of the rib is sustained on periosteal blood supply alone [20-24].

Objective fluorescence parameters of bone perfusion are sparse [25]. There are solely five studies in which outcomes are reported, including relative perfusion and absolute perfusion [26-30]. One study reported the relative perfusion parameter defined as the fluorescence intensity at a region of interest divided by the background fluorescence [26]. Others defined absolute perfusion parameters as maximum fluorescence intensity over time [27-29]. Some of these outcomes are investigated in human and others in animal studies. Quantitative interpretation of fluorescence imaging has as main limitation that it is subject to inter-user interpretation. Qualitative parameters may overcome this limitation. There is no consensus on a standardized manner or which parameters should be used to assess bone perfusion. However, dynamic perfusion parameters such a fluorescence intensity curves seemed effectively for evaluating perfusion[31]. At this moment, these dynamic parameters have not yet been studied for bone perfusion in humans.

The aim of this pilot study is to assess the feasibility of fluorescence imaging of the rib, in order to provide objective perfusion parameters of bone.

Materials and methods

Participants

This feasibility study was carried out at a tertiary hospital in the Netherlands. A total of thirteen patients undergoing primary or secondary autologous breast reconstruction from August 2021 to August 2022 were included before surgery. Exclusion criteria were: < 18 years, hyperthyroidism, autonomic thyroid adenoma, epilepsy, renal failure with eGFR < 60, severe liver failure or patients who are allergic to ICG, iodine or shellfish. All data was collected in Castor (CDMS version 2022.3). The study was approved by the Ethics Committee of Amsterdam University Medical Center (2021.0142). All patients provided written informed consent. Preoperative data recorded included patient’s age, height, weight, body mass index (BMI), medical history, history of smoking, and family history. Intraoperative recorded data included vital parameters such as blood pressure, heart rate, saturation and use of vasopressors during administration of ICG.

Surgery and fluorescence imaging

In all patients undergoing autologous breast reconstruction, the medial part of approximately 2 cm of the 2nd or 3rd rib was removed to perform the arterial and venous anastomoses of the flap to the internal mammary vessels **Figure 2.** After revascularization, the rib was exposed, and the camera was fixed at approximately 30cm above the rib and pointed transverse to the rib with periosteum visible. 0.1mg/kg ICG (Verdye 25mg) was injected intravenously. Subsequently, fluorescent intensity was captured by Tivato 700 microscope (Carl Zeiss Meditec AG, 2019, Jena Germany) for four minutes **Figure 3**. During the fluorescent assessment, ambient light was dimmed.

Quantification of the fluorescent signal

Postoperatively, the video images were quantified using a tailor-made software written in the Python v3.8 programming language (Python Software Foundation, https://www.python.org/). For endosteal measurement, the region of interest (ROI) of 5mm was positioned on the cross sectional, lateral surface of the rib by (DFB). The ROI was also positioned by a second observer (MM) to analyze inter-observer reliability. Also, a ROI was positioned on the anastomosed blood vessels where blood perfusion is considered to be optimal and if feasible, also on a region of bone with intact periost for periosteal measurement. For all ROIs the software generated time-intensity curves of the measured intensity in arbitrary units (a.u). From these curves, perfusion parameters were extracted of which an explanation is given in **Figure 4.** The ingress was defined as the increase of fluorescence intensity per second, from baseline to maximum fluorescence intensity (Imax). Relative perfusion was defined as the maximum fluorescence intensity of ROI at anastomosed blood vessel divided by maximum fluorescence intensity at ROI. Mean slope is calculated as Δintensity / Δtime. Normalized maximum slope is calculated by dividing the mean slope at the steepest point of the ingress curve by the total slope of the ingress curve ((Δintensity / Δtime) / Imax – I0)). The egress was defined as the decrease of ICG fluorescence intensity per second, from Imax until last measurement. The starting time of the curves (t0) was defined as the first moment of increase of intensity compared to baseline.

Patients with videos of blurry image were excluded.

The median values of the measured intensities in the ROI for each video were calculated. Statistical analysis was performed using IBM SPSS 26.0 (IBM, Armonk, NY, U.S.A). Normality was assessed using Shapiro-Wilk-test and data is presented as median with minimum and maximum. For continuous variables the Wilcoxon signed rank test was used. The inter-observer reliability has been calculated with intraclass correlation coefficient using an absolute agreement definition.

Source of funding

No external funding was received for this study.

Results

Patient characteristics and outcomes

Thirteen patients were included of which four patients were excluded. Two were excluded due to videos of blurry image and two were excluded due to a different surgical technique used to remove the rib fragment.

As a result, nine patients were included in whom eleven ribs were analyzed. From these patients, there were seven DIEP (deep inferior epigastric artery perforator) reconstructions including two bilateral DIEP, one SGAP (superior gluteal artery perforator) reconstruction and one omental flap reconstruction. The video of patient 5 was only suitable for periosteal measurement and of patient 2 and 5 only for endosteal measurement.

Patient characteristics are displayed in **Table 1.** No adverse events related to the ICG injection were observed intraoperatively. Intraoperative vital parameters and patient specifications are shown in **Table 2.**

Time-intensity curves

Time intensity curves of the endosteal measurement was possible in ten ribs and is shown in **Figure 5**. There are two distinct patterns in the ICG ingress phase. First, a steep slope reached within 10s after t0 is observed in patient 2, patient 6 and patient 7. In other patients, the ingress is less steep and turns into a flattened slope.

Regarding the ICG egress phase, there are three distinct patterns. Three curves show a steep slope which turns quickly into a flattened slope, (patient 2, patient 6 and patient 7). In four curves, the egress is clearly prolonged (patient 1b, patient 3, patient 4 and patient 9b). In the three remaining curves (patient 1a, patient 8 and patient 9a), it seems that the egress has not started within the 240s of measurement.

To summarize: patients 2, 6 and 7 have a steep and well-defined ingress and egress. In all other patients, the curves show a much more flattened ingress and egress.

Quantitative analysis

The outcomes of quantitative analysis on the time intensity curves of the endosteal and periosteal region of interest are shown in **Table 3.** The median maximum intensity was 94.6 a.u in the endosteal ROI as compared to 89.1 a.u in the periosteal ROI ($p = 0.889$). The median mean slope and the median normalized maximum slope were slightly higher in the periosteal ROI (0.8 vs. 1.2, $p > 0.726$; 0.1 vs. 1.5, $p > 0.161$. Among the observers (DFB and

MM), there was an excellent agreement about the positioning of the ROI with an intraclass correlation coefficient of 94.3%.

Discussion

In this pilot study we have been able to quantify perfusion of the human rib with NIRF imaging using ICG. This was shown with measurements in two different regions of interests (ROI): endosteal and periosteal. According to several anatomical studies, the osseous blood supply is bipartite and depends on the endosteal and periosteal blood supply [14, 15, 32]. In the eleven measurements, the endosteal and periosteal parameters show much agreement with no statistically significant differences. Numerous clinical applications in soft tissue surgery have been studied for Near Infrared Fluorescence (NIRF) imaging using Indocyanine green (ICG) [33-36]. There are only five previous studies that report on objective NIRF outcomes for bone perfusion [26-30]. It's notoriously difficult to assess the viability of bone based on clinical signs, but accurate debridement of non-viable bone is crucial. The relative perfusion of 2.5% (endosteal) and 3.1% (periosteal) confirm that perfusion of the rib is quite low as compared to the perfusion at the level of the anastomosed blood vessels.

Thorough debridement without wasting additional bone is extremely important to accomplish optimal bone healing, bone reconstruction and cure. Fluorescence imaging may play a role in debridement after trauma injury of the extremities, long-lasting infectious disease of the tibia, femur and humerus or osteoradionecrosis after breast (rib), rectum (sacrum), oropharyngeal tumors ((neo-)mandible) [12]. Moreover, it may have an interesting role in osseous reconstructions such as free vascularized fibula grafts, especially when multiple osteotomies are necessary [28]. The scientific evidence for using NIRF to visualize bone perfusion is sparse [25]. Yoshumatsu et al. evaluated bone perfusion in cadaveric femoral medial condyle [37]. They compared the penetration depth of methylene blue with NIRF imaging using ICG. Following injection of methylene blue and ICG into the descending genicular artery, the cancellous area was visible with NIRF imaging due to tiny perforators that penetrated the periosteum in contrary to the blue dye which was solely visible in the periosteum. Nguyen et al. was able to demonstrate that endosteal perfusion and viability of vascularized bone flaps can be assessed with NIRF imaging using ICG [26]. This was shown in the osteomyocutaneous forelimb flaps and in fibula flaps of female Yorkshire pigs. **They compared a devascularized bone flap, in which the is pedicle had been ligated, to a vascularized bone flap using NIRF imaging. The vascularized flap showed NIRF perfusion at the osteotomy site, whereas the devascularized flap showed a lack of fluorescence.** Gitajn et. al demonstrated in porcine models that bone perfusion can be measured quantitatively from endosteal and periosteal sources, with NIRF imaging using ICG [29]. Fichter et al. and Gitajn et al. defined absolute perfusion values as maximum fluorescence in number of units at a specific moment in time and extracted time-intensity curves, whereas Valerio et al. focused on maximum fluorescence at a single unspecified time point without extracting time-intensity curves [27, 28, 29] . Gitajn et al. and Elliot et al. studied ICG fluorescence curves of regions of interest in bone under various damage situations, and a new kinetic model was created and used [29,30]. The underlying idea behind this model is that the bone ICG fluorescence curve reflects both ‘early’ and ‘late’ bone perfusion and once again represents the bone's bipartite blood supply network. Gitajn et al. demonstrated that when the periosteal blood supply was disrupted by stripping soft tissue from the bone, maximum fluorescence intensity decreased with 50% and time to reach maximum fluorescence intensity increased [29]. This suggests that ‘late’ bone perfusion is connected to endosteal blood supply, while ‘early’ bone perfusion and periosteal blood supply are connected to one another.

Absolute and relative perfusion values do not offer insight in how intensity changes over time. Additionally, absolute perfusion is dependent of the measured fluorescence intensity and hence prone to several influencing factors including camera distance and camera angle [31, 38]. Among the endosteal and periosteal region of interests that we obtained, extracted time-intensity curves demonstrated several patterns of ICG ingress and ICG egress. In the endosteal view, a steep ingress was recognized with a steep egress in three ribs and the majority of the observed curves demonstrated a prolonged egress. In others, the ingress is less steep and the egress is flat or does not start at all. For several subjects in this study, four minutes which was insufficient to observe the initiation of venous outflow. In other studies, these flat curves with a slow ingress and slow or absent egress, represent bad perfusion with suboptimal inflow and outflow [39-42].

Quantitative perfusion analysis in studies focusing on esophagus, ileum and colon tissue often show a good inflow and a well-defined egress in case of good tissue perfusion. We find these curves in three of the ribs that we studied. The majority of ribs however show a slow ingress and a flat or absent egress. This may be explained by a more medial ostectomy, at the level where the rib is most cartilaginous. Cartilage is notoriously bad perfused. As a result, the intensity curves in cartilaginous bone tissue may be different from the curves that are often found in well vascularized tissue during gastrointestinal surgery [39-42]. The well-defined curves with a steep ingress and well-defined egress may match the patients in whom the rib was more spongious, c.q. the patients in whom the ostectomy of the rib was extended laterally. However, this cannot be confirmed in hindsight. Other factors may influence the shape of the curve are iatrogenic damage of the periosteum during surgical dissection, environmental influences such as light, heat and manipulation leading to vasoconstriction, or blood dripping onto or ROI.

Some limitations were observed in this study. The measurement of maximum fluorescence intensity is influenced by multiple environmental factors, such as camera distance, camera angle, ambient light, blood pressure and use of peroperative medication. This may also explain why the fluorescence intensity curves do not start from zero. Due to the small sample size of this study, it was not possible to establish the effect of all these parameters on the fluorescence intensity and its dynamics. Moreover, it was hard to position the camera adequately to capture an endosteal and periosteal ROI in one view. This resulted in two ribs in which only one ROI was visible. Despite these limitations, this study provides insight into the possibility of quantification of bone perfusion showing promising results. This study demonstrated that it is challenging, but feasible to use NIRF imaging to study the rib. This is a step towards NIRF imaging using ICG in providing surgeons quantitative parameters for assessing bone perfusion.

Based on our first experience of quantifying rib perfusion we recommend the following: keep all external parameters stable, prolong the video for more than five minutes and make sure there is a ROI with sufficient perfusion available in the field of view as well. A larger cohort is needed to investigate the value of the inflow parameters in the assessment of bone perfusion, to correlate divergent parameters to patient-specific factors and for the prediction of clinical outcomes. It would be of great interest to measure bone perfusion in for example a healthy fibula graft, so the movement artifacts due to breathing are minimized. Taken this into account, it should be possible to establish reliable cutoff values for normal bone perfusion. Therefore, more research is needed to investigate the possibilities of NIRF imaging using ICG to assess bone perfusion intraoperatively. Cutoff values are needed to be guide a surgeon in the debridement of affected bone or reconstructive surgery with vascularized bone.

Conclusion

This study demonstrated the feasibility of quantification of perfusion in human ribs, using NIRF imaging with ICG. The result of our study suggests that NIRF imaging using ICG can provide surgeons objective parameters of bone perfusion. It is a challenge to implement our NIRF imaging results of bone perfusion into surgery.

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Figure captions

Figure 1: Diagram showing the anatomy of the rib, including the Internal mammary artery also known as Internal thoracic artery.

Figure 2. Intraoperative photo throughout autologous breast reconstruction of the right breast, showing the visual before (a) and after (b) removal of rib fragment in which the lateral cross-sectional surface becomes visible.

Figure 3. NIRF imaging using ICG peroperative in a patient showing the visual (a) and NIRF fluorescence (b) in the rib. (X: Endosteal ROI, Y: Periosteal ROI)

Figure 4. Time-intensity curve with extracted perfusion parameters. Imax is maximum intensity.

Figure 5. Time intensity curves of endosteal measurement of the rib.

Tables

Table 1. Patient characteristics	
Characteristics	*Number of patients (n=9)*
Mean age (years, SD)	53.1 (4.6)
Mean BMI (kg/m^2, SD)	28.2 (3.1)
Diabetes mellitus	0
Hypertension	2
Hypercholesterolemia	0
Active smoking	0

Type of autologous breast reconstruction	
DIEP flap	7
SGAP flap	1
Omental flap	1

Abbreviations: SD, standard deviation; *BMI, body mass index;* DIEP, deep inferior epigastric perforator; SGAP, super gluteal artery perforator.

Table 2. Patient specifications

Characteristics				Vital parameters		
	Radiation therapy	Smoking	Cardiovascular comorbidity	Blood pressure (mmHg)	Heart rate (beats/min)	Saturation (%)
Patient 1*	No	Former	No	95/55	57	100
Patient 2	No	No	No	113/59	65	100
Patient 3	No	No	No	100/60	80	98
Patient 4	No	No	HT	118/63	60	99
Patient 5	No	No	No	93/50	81	100
Patient 6	Yes	Former	No	115/60	65	98
Patient 7	No	No	No	96/53	46	100
Patient 8	No	Former	No	110/63	53	97
Patient 9*	No	No	HT	93/50	54	98

Abbreviations: HT, hypertension

Vital parameters were collected at the time of injecting of ICG.

Patient is former smoker when quit smoking more than 1 month ago.

*Patient undergoing a bilateral DIEP and whom will continue as patient 'a' and patient 'b'.

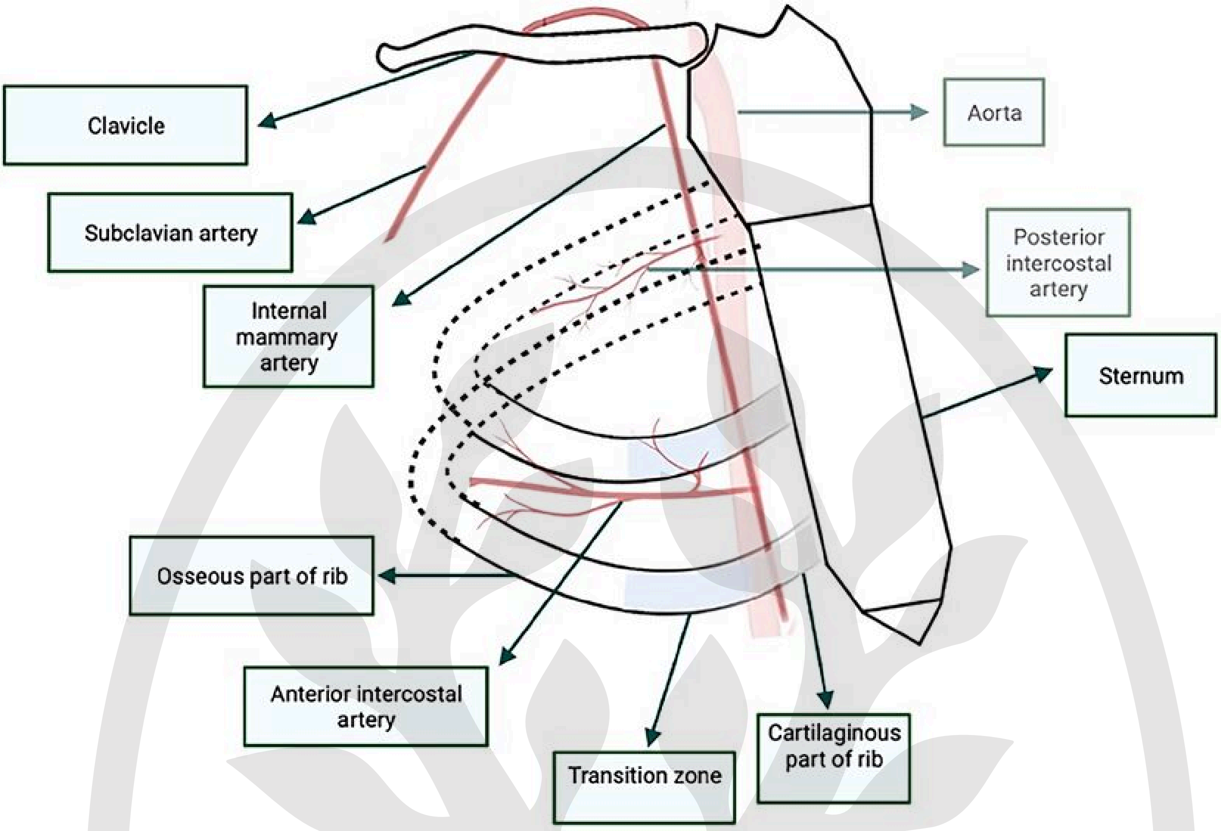
Table 3. **Quantitative assessment**

NIRF imaging

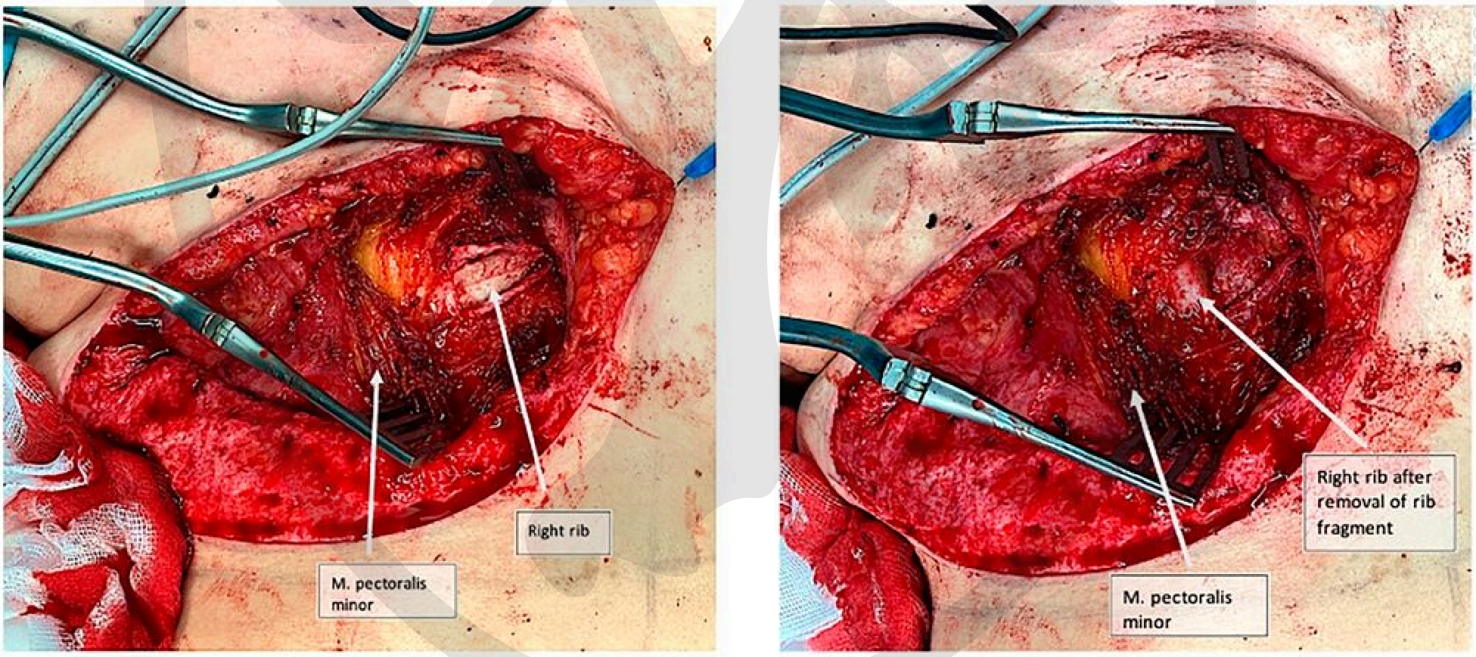
Parameter Ingress	*Endosteal*	*Periosteal*
	Median (min - max)	*Median (min - max)*
Imax (a.u)*	94.6 (41.5 - 182.4)	89.1 (44.4 – 110.71)
Time from t0 to Imax (s)	54.4 (1.8 - 230.8)	59.4 (1.0 – 224.3)
Relative perfusion (%)	2.5 (1.2 - 4.7)	3.1 (1.7 - 4.6)
Mean slope	0.8 (0.2 - 17.4)	1.2 (0.2 - 63.9)
Normalized maximum slope	0.1 (0.0 - 3.9)	1.5 (0.1 - 3.1)
Parameter Egress		
	Number of ribs (n = 10)	*Number of ribs (n =9 ribs)*
90% Imax	7	6
80% Imax	6	6
70% Imax	5	5
60% Imax	2	3
50% Imax	2	1

Abbreviations: Imax, maximum intensity.

*Median maximum intensity at blood vessel was 224.0 a.u (163.6 – 274.7)

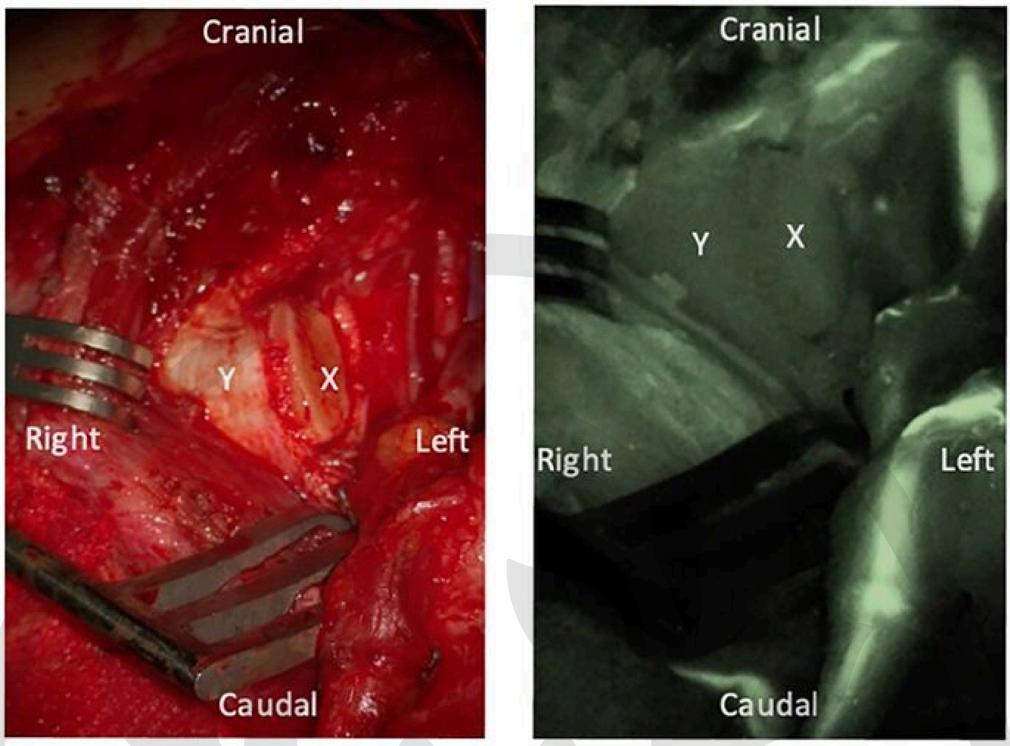


Clavicle
Subclavian artery
Internal mammary artery
Aorta
Posterior intercostal artery
Sternum
Osseous part of rib
Anterior intercostal artery
Transition zone
Cartilaginous part of rib

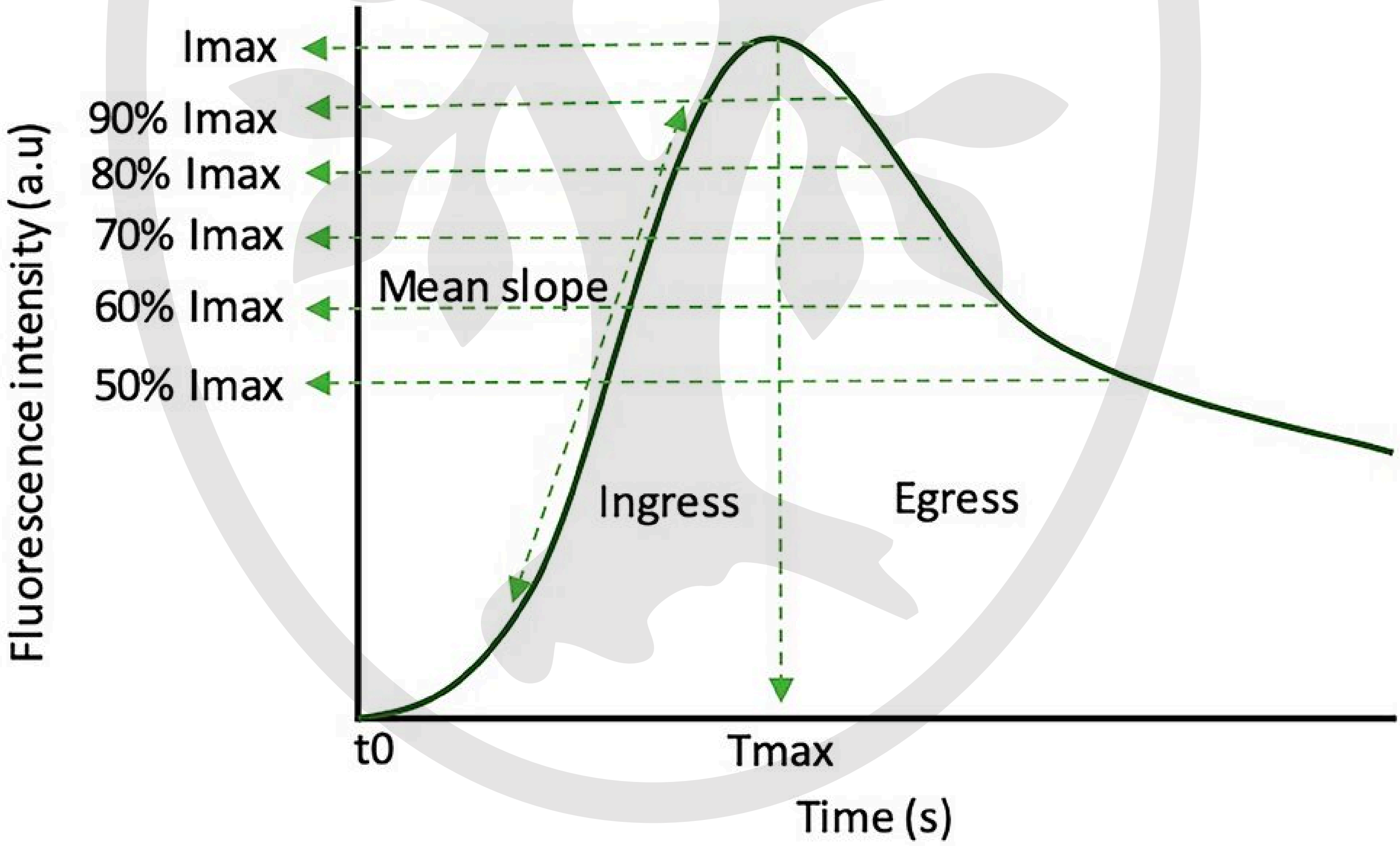


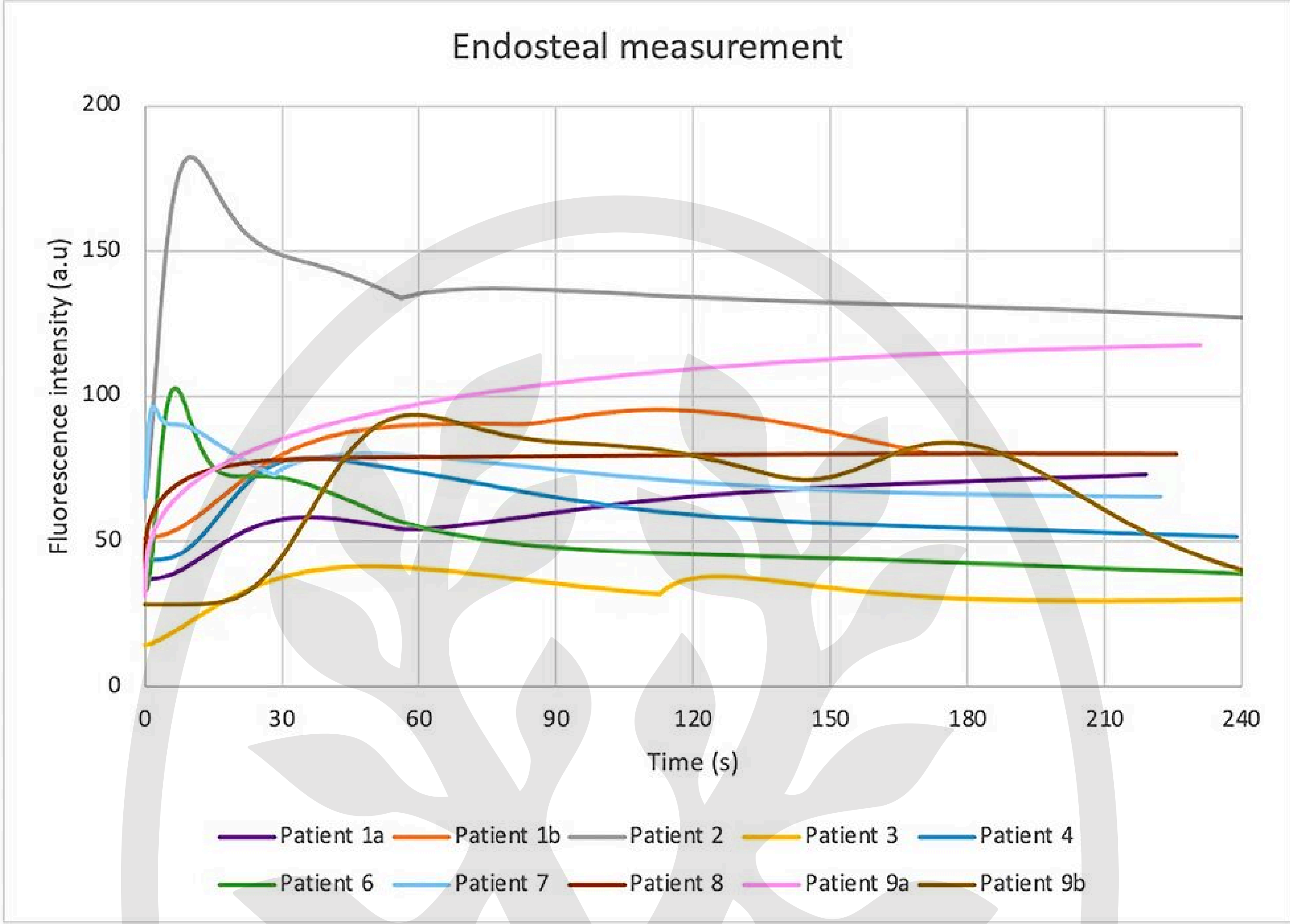
Right rib
M. pectoralis minor
Right rib after removal of rib fragment
M. pectoralis minor

(a) (b)


Cranial
Y
X
Right
Left
Caudal
Cranial
Y
X
Right
Left
Caudal

(a) (b)


Imax
90% Imax
80% Imax
70% Imax
60% Imax
50% Imax
Mean slope
Ingress
Egress
Fluorescence intensity (a.u)
t0
Tmax
Time (s)



Endosteal measurement
Fluorescence intensity (a.u)
200
150
100
50
0
0
30
60
90
120
150
180
210
240
Time (s)
Patient 1a
Patient 1b
Patient 2
Patient 3
Patient 4
Patient 6
Patient 7
Patient 8
Patient 9a
Patient 9b