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FULL ARTICLE

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Oxygen-dependent delayed fluorescence of protoporphyrin IX measured in the stomach and duodenum during upper gastrointestinal endoscopy

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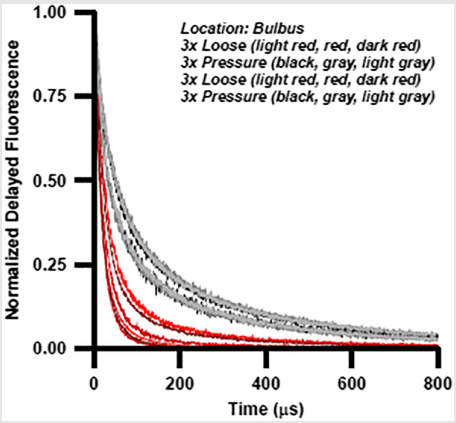
Abstract



Protoporphyrin IX-triplet state lifetime technique (PpIX-TSLT) is a method used to measure oxygen (PO_2) in human cells. The aim of this study was to assess the technical feasibility and safety of measuring oxygen-dependent delayed fluorescence of 5-aminolevulinic acid (ALA)-induced PpIX during upper gastrointestinal (GI) endoscopy. Endoscopic delayed fluorescence measurements were performed 4 hours after oral administration of ALA in healthy volunteers. The ALA dose administered was 0, 1, 5 or 20 mg/kg. Measurements were performed at three mucosal spots in the gastric antrum, duodenal bulb and descending duodenum with the catheter above the mucosa and while applying pressure to induce local ischemia and monitor mitochondrial respiration. During two endoscopies, measurements were performed both before and after intravenous administration of butylscopolamine. Delayed fluorescence measurements were successfully performed during all 10 upper GI endoscopies. ALA dose of 5 mg/kg showed adequate signal-to-noise ratio (SNR) values >20 without side effects. All pressure measurements showed significant prolongation of delayed fluorescence lifetime compared to measurements performed without pressure ($P < .001$). Measurements before and after administration of butylscopolamine did not differ significantly in the duodenal bulb and descending duodenum. Measurements of oxygen-dependent delayed fluorescence of ALA-induced PpIX in the GI tract during upper GI endoscopy are technically feasible and safe.

Louisa J. D. van Dijk and Rinse Ubbink are contributed equally to this manuscript.

Abbreviations: ALA, 5-aminolevulinic acid; CMI, chronic mesenteric ischemia; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyltransferase; GI, gastrointestinal; IMA, inferior mesenteric artery; IQR, interquartile range; NA, numerical aperture; PO_2, oxygen tension; PpIX, protoporphyrin IX; PpIX-TSLT, protoporphyrin IX-triplet state lifetime technique; VLS, visible light spectroscopy.

KEYWORDS
5-aminolevulinic acid (ALA), chronic mesenteric ischemia, COMET, diagnostics, oxygen tension (PO_2), PpIX-TSLT, upper gastrointestinal endoscopy

1 | INTRODUCTION

Protoporphyrin IX (PpIX) is an endogenous compound synthesized in active mitochondria which can be induced by administration of its precursor 5-aminolevulinic acid (ALA). PpIX exhibits oxygen-dependent delayed fluorescence after photoexcitation [1]. The fluorescence lifetime depends directly on oxygen concentration, since oxygen acts as a quencher of excited PpIX molecules. In other words, upon collision of oxygen with an excited PpIX molecule energy transfer to oxygen will relax PpIX to the ground state without emission of a photon. More oxygen leads to more collisions and quenching, thereby shortening the delayed fluorescence lifetime. This phenomenon is described quantitatively by the Stern-Volmer relationship [2], relating the lifetime to the amount of oxygen (eg, oxygen concentration or oxygen tension [PO_2]). A detailed description of the principles of the PpIX-triplet state lifetime technique (PpIX-TSLT) can be found in the article written by Harms et al [3]. Introduction of the Cellular Oxygen METabolism (COMET) monitor (Photonics Healthcare BV, Utrecht, The Netherlands) made PpIX-TSLT clinically available for measurements on the skin [4]. The main goal of this study was to investigate the technical feasibility of delayed fluorescence measurements in the gastrointestinal (GI) tract during endoscopy.

PO_2 measurements in cells of the GI tract during endoscopy could be of great value, for example, for the diagnostic work-up of patients suspected to be suffering from chronic mesenteric ischemia (CMI). CMI is the result of insufficient blood supply to the GI tract caused by obstruction of mesenteric arteries and/or veins [5]. CMI is in >90% caused by atherosclerosis of minimally one mesenteric artery. Typical symptoms of CMI are postprandial abdominal pain and weight loss, however, more atypical symptoms as nausea, constant abdominal discomfort, vomiting, diarrhea or constipation are also reported [5]. Symptoms alone are of limited value for the diagnosis of CMI [6]. Mesenteric artery stenosis can be detected by CT-angiography, however, the presence of mesenteric artery stenosis will not necessarily result in symptomatic disease (CMI) due to an abundant collateral mesenteric network. The prevalence of mesenteric artery stenosis in the asymptomatic general population is high (3%-29%), increasing with age [7, 8]. CMI occurs when extensive mesenteric artery stenosis and/or an insufficient collateral network is present. Therefore, a functional test detecting GI ischemia is highly desired. Currently, visible light spectroscopy (VLS) and tonometry are used as functional tests for the diagnostic work-up of patients suspected to be suffering from CMI.

VLS measures the mucosal capillary hemoglobin oxygen saturation during upper GI endoscopy [9, 10] and is less invasive than tonometry measuring luminal pCO_2 with a nasogastric and nasojejunal catheter connected to a capnography [10, 11]. However, the sensitivity of VLS for CMI is 90% and the specificity is 60% [12], indicating the need to improve the accuracy of the diagnostic workup. Since in the original reports of PpIX-TSLT the lifetimes were shown to be related to mitochondrial oxygen levels [1, 13] this technique shows promise for diagnosing CMI. The COMET monitoring device was designed for cutaneous measurements and has been adapted for endoscopic delayed fluorescence lifetime measurements. Therefore, the primary aim of this study was to assess the technical feasibility of delayed fluorescence measurements of the GI tract during upper GI endoscopy. Secondary aims were: (a) to determine the dose of 5-aminolevulinic acid (ALA) needed for endoscopic measurements; (b) to determine the specific measurements locations; (c) to demonstrate oxygen-dependent signal and, (d) to determine the influence of butylscopolamine on the measurements.

2 | METHODS

2.1 | Study design and setting

This work describes a single center study in healthy volunteers. The institutional review board of the Erasmus MC University Medical Center approved this study (NL59177.078.16, NL63050.078.17). The study complies with the Helsinki declaration on research ethics.

2.2 | Participants

Healthy volunteers were recruited by distributing information leaflets. Before inclusion the medical history and existence of GI complaints were evaluated. Blood samples were taken to detect impaired renal function, and liver test abnormalities, defined as estimated glomerular filtration rate (eGFR) <90 mL/min/m^3 or liver test abnormalities >1.5 times upper limit of normal. Healthy volunteers were eligible if they were able to give written informed consent, had an unremarkable medical history, and had no GI complaints.

Volunteers were excluded in case of pregnancy, acute or chronic porphyria, and a known ALA or porphyrin hypersensitivity.

2.3 | Study procedures and setup

The healthy volunteers were fasted for at least 6 hours and ALA (Gliolan, Medac GmbH, Wedel, Germany) was administered orally 4 hours before the upper GI endoscopy. The volunteers were discharged after the endoscopy. They were instructed to report any side effects. Furthermore, they were instructed to avoid exposure to sunlight as much as possible, and take precautions (eg, sunglasses, long sleeves) during the first 24 hours after administration of ALA, since phototoxicity after sunlight exposure is a known side effect of ALA. Adverse events were collected during 7 days.

A probe that could be passed safely through the accessory channel of the endoscope was used for endoscopic detection of fluorescence light (designed by TNO, produced and CE-marked by LightGuideOptics, Mechenheim, Germany). The diameter of the accessory channel of the endoscope is 3.2 to 4.2 mm and therefore the tip of the probe is slightly smaller than 3.2 mm. The probe is a fiber optic assembly with one excitation fiber in the middle directly surrounded by seven detection fibers. The used detection fibers all have a diameter of 365 μm, and the excitation fiber is 470 μm in diameter. The fibers have a numerical aperture (NA) of 0.22 ± 0.2. The tips of the fibers are fixed together in a ferrule. The position of the fiber tips within the ferrule and distance compared to each other is fixed and does not change when pressure is applied on the mucosa with the probe tip or while passing the probe tip through the accessory channel of the endoscope. The excitation fiber of the probe was connected to the light source in the COMET, a pulsed 515 nm laser. The detection fiber was connected to the photomultiplier detection system of the COMET. To protect the highly sensitive detection system from indirect laser light a long pass filter (575 nm with OD 4, Edmund Optics, Barrington, New Jersey) was introduced in the optical path of the detection branch. During endoscopy, peripheral saturation was continuously monitored and oxygen was administered intranasally to maintain a saturation level ≥ 95% (see Figure 1 for the setup).

Delayed fluorescence lifetime measurements were performed similar to VLS measurements at three anatomical locations in the descending duodenum, duodenal bulb and antrum of the stomach [12]. The fiber was positioned approximately 1 to 5 mm above the mucosa of the target area. A perpendicular angle of the fiber tip and the target area is optimal for the signal quality, however, this is not an important parameter for the outcome of the lifetime measurements. To minimize interference by background noise the light of the endoscope was switched off during the measurements. Raw delayed fluorescent traces were stored with an external computer connected through the serial (rs-232) port. A python script written to store the raw data in a comma-separated file was used to read the serial buffer. The raw data were analyzed with software written in LabVIEW (version 13.0, National Instruments, Austin, Texas). Lifetimes were calculated using a rectangular distribution fit, taking into account the heterogeneity in lifetimes underlying the measured delayed fluorescence signal [13] unless stated otherwise. The mitochondrial oxygen tension (mitoPO_2) reading by COMET was not used since COMET is developed and tested for measurement on skin and links the measured lifetimes to the calibration constants for skin cells. Because this is the first report on delayed fluorescence measurements through an endoscope, we judged to exercise some restraint in reporting absolute values of PO_2. Therefore, all delayed fluorescence measurements are represented as reciprocal lifetimes $1/\tau$ (with τ in microseconds) to obtain a positive association between oxygen and lifetime. According to the Stern-Volmer relationship the reciprocal lifetime is linearly related to the actual PO_2. Based on our current understanding of the technology, PO_2 measurements with a reciprocal lifetime $>0.1\ \mu s^{-1}$ should be considered as non-physiological in humans with a normal arterial oxygen tension [1].

2.4 | Determination necessary ALA dose

The appropriate dose of ALA was determined by performing delayed fluorescence lifetime measurements with different doses of ALA. Since PpIX is endogenously present and accumulates in metabolic active cells, such as mucosal cells, measurements were performed during one upper GI endoscopy without administration of ALA. In neurosurgery, 20 mg/kg ALA is used to induce enough PpIX to visually determine the tumor fluorescence under blue light [14]. This was expected to be an abundant dose for detection of delayed fluorescence in the duodenal region because of the high cellular turnover and metabolic rate compared to neurological tissue. Therefore, five upper GI endoscopies were performed using a dose of 20 mg/kg, two upper GI endoscopies were performed after administration of 1 mg/kg ALA and two using a dose of 5 mg/kg. Optimal dosage was based on signal quality and adverse events. Quality is expressed as signal-to-noise ratio (SNR). A SNR >20 is considered adequate [4].

2.5 | Determining measurement locations and demonstrating oxygen-dependent signal

Measurements were obtained at each anatomical location with the probe hovering 1 to 5 mm above the mucosal

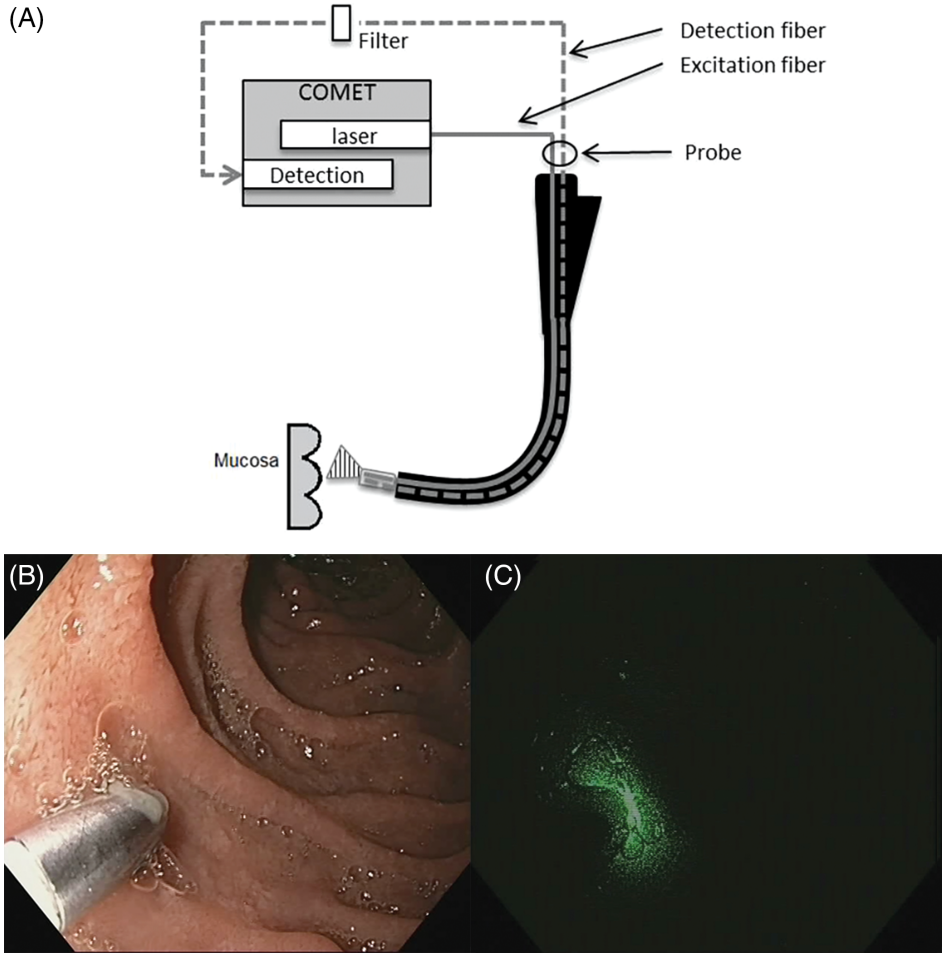


FIGURE 1 The setup for upper gastrointestinal (GI) endoscopic delayed fluorescence measurements: A, Schematic setup, B, Probe in the descending duodenum during upper GI endoscopy, and C, Probe in the descending duodenum during endoscopic measurement (endoscopic light is switched off)

surface. In order to confirm that the measured delayed fluorescence lifetimes depend on the oxygen concentration, measurements were performed while inducing local mucosal ischemia. Temporary ischemia was achieved by applying pressure to the mucosa with the probe. The oxygen supply to the tissue under the probe is compromised by compression of the capillaries. Because the oxygen is used within the cells, and the supply compromised, a prolongation of lifetime was expected due to the decrease in PO_2 during application of pressure. After minimally 2 seconds of pressure, three consecutive measurements were taken while pressure was applied. This process was repeated at two more sites within each anatomical location. In the situation without pressure, the tip of the probe was positioned under view in close proximity of the tissue or just on the tissue without visible deformation of the mucosa. While providing pressure the probe is pushed on the tissue and caused tissue deformation similar to the procedure performed when obtaining tissue biopsies for diagnostic purposes. The exact amount of pressure applied was not controlled or measured. Measurements started in the descending duodenum and subsequently, measurements with and without applying pressure were performed in the duodenal bulb and gastric antrum.

An oxygen consumption curve was made in two volunteers who received 5 mg/kg ALA by taking repeated measurements with an interval of 1 second. Measurements were performed without applying pressure for minimally 5 seconds; the next 20 seconds measurements were performed while applying pressure to visualize mitochondrial respiration [15]. SNR and reciprocal lifetime were used to determine appropriate locations for measurements.

It is known that local oxygen tension will be heterogeneously distributed within the tissue [16]. Further analysis was done on the raw fluorescent traces to determine the effect of local applied pressure on the reciprocal lifetime and its distribution, caused by, for example, oxygen heterogeneity. By assuming the PpIX to be homogenous distributed among the GI tract the underlying lifetime distribution can be obtained with a method described by Golub et al [17]. The trace can be described by a sum of rectangular distributions and the fractional contribution per reciprocal lifetime can be recovered. This method was successfully used by our

group to recover oxygen distribution histograms [16, 18, 19] and is used on specific traces in this study to determine the distribution of (reciprocal) lifetimes in the signal as a measure of heterogeneity in the tissue. The fractional distribution within a trace describes the heterogeneity on a microvascular and cellular scale. To investigate the heterogeneity on a larger tissue scale all data recorded above the tissue is combined in a histogram and the individual contributions are visualized by color.

2.6 | Determining influence of butylscopolamine

Intravenous administration of butylscopolamine is commonly used during upper GI endoscopy to reduce intestinal contractions. Since butylscopolamine is known to induce tachycardia causing increased cardiac output, GI tract tissue oxygen levels could be influenced. This possible influence was evaluated in two volunteers receiving 5 mg/kg ALA. All measurements mentioned before were performed in the descending duodenum, duodenal bulb and gastric antrum. After intravenous administration of 20 mg butylscopolamine, the measurements were repeated in exactly the same manner.

2.7 | Statistical analyses

The SNR and reciprocal lifetime were not normally distributed and therefore described as medians and interquartile ranges (IQR). Differences in reciprocal lifetimes with and without pressure and before and after administration of butylscopolamine were determined using the Wilcoxon-Mann-Whitney test. A two-tailed P-value of $<.05$ was considered significant. Statistical analysis was performed using R (version 3.4.2) [20].

3 | RESULTS

3.1 | Participants

Ten upper GI endoscopies were performed in six healthy volunteers. Nine out of 10 upper GI endoscopies were performed in females, with a median age of 30 years (IQR 28.5-34.0 years). All endoscopies were performed in non-smokers and all healthy volunteers gave informed consent. No oxygen or sedatives were administered during endoscopy. Delayed fluorescence lifetime measurements were successfully performed during all 10 endoscopies.

3.2 | Determining necessary ALA dose

The SNR for each specific location and dose of ALA are presented in Table 1 and Figure 2. The endogenous amount of PpIX showed to be insufficient to measure delayed fluorescence, since SNR was below 20 at all locations. The dose of 1 mg/kg ALA also showed to be insufficient with SNR values of the first quartile reaching maximal 18 in the descending duodenum and a median SNR of 29 just above the lower limit of 20.

SNR in duodenal bulb and descending duodenum were adequate in the 5 and 20 mg/kg group, however, phototoxic side effects occurred in three out of five volunteers in the 20 mg/kg group (Table 1). The phototoxicity was observed on the skin of the face exposed to sunlight after the experiment in all three volunteers with phototoxicity and looked similar to a sunburned like reaction. Side effects resolved within 24 hours in all volunteers. Phototoxicity was not observed in the GI tract and volunteers did not experience abdominal complaints.

It was concluded that 5 mg/kg was the best dose with sufficient SNR values and without reported side effects. This dose leaves a margin for suboptimal circumstances, for example, less conversion of PpIX in patients and losses in the detection system due to the use of adaptors needed to couple the measuring probe to the COMET system.

3.3 | Determining measurement locations

The SNR at the gastric antrum location was low compared to the SNR of the duodenal bulb and descending duodenum. Only in the 20 mg/kg group, the upper quartile of the values reached an SNR >20 in the gastric antrum, therefore, the signal quality in the gastric antrum showed insufficient for reliable measurements. The lifetime calculation became more inaccurate if the SNR dropped below 20, resulting in a scatter of lifetimes with a tendency to non-physiological lifetimes, see Figure 3. Since the SNR values in the gastric

TABLE 1 Signal-to-noise ratio (SNR) for measurements with probe position 1 to 5 mm above tissue, data presented as median [interquartile range], total of 10 upper gastrointestinal endoscopies

Dose	0 mg/kg	1 mg/kg	5 mg/kg	20 mg/kg
SNR antrum	2 [1-3]	4 [3-6]	6 [4-13]	19 [8-37]
SNR duodenal bulb	3 [2-7]	233 [165-533]	702 [179-900]	617 [269-921]
SNR descending duodenum	6 [5-6]	29 [18-56]	215 [156-684]	110 [77-248]
Adverse events	0/1	0/2	0/2	3/5 phototoxicity

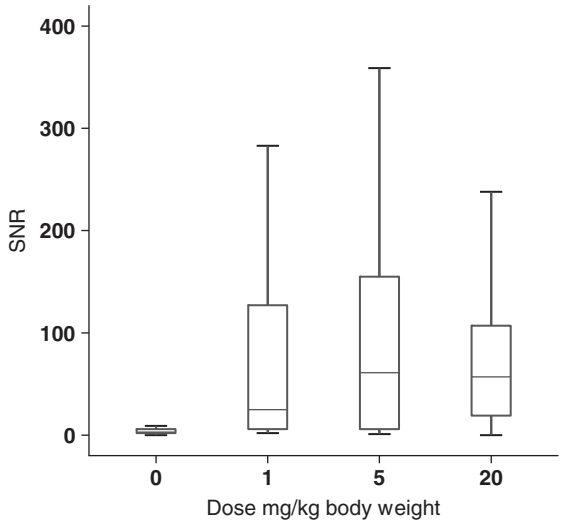


FIGURE 2 Signal-to-noise ratio (SNR) sorted by oral dose 5-aminolevulinic acid (ALA) combined for all measurement locations (gastric antrum, duodenal bulb, descending duodenum). Measurements are performed without pressure applied. Outliers are not presented since SNRs in 5 and 20 mg/kg go up to 6000

antrum were too low and caused non-physiological lifetimes, measurements in gastric antrum were not included in the analysis of the influence of butylscopolamine.

3.4 | Demonstrating oxygen-dependent signal

Raw delayed fluorescence lifetime traces were recorded. When the probe was "loose" hovering above the GI tissue short lifetimes were measured. While the pressure was applied the lifetime increased, shown in the Bulbus in Figure 4.

For the lifetime distribution analysis a base of 15 reciprocal lifetimes equally distributed over a corresponding physiological PO_2 range (0-150 mm Hg) assuming a quenching constant of 830 mm Hg^{-1} and lifetime of spontaneous relaxation of 800 µs was taken [1]. The distribution analysis was done on the 2 times 3 loose and 2 times 3 pressure traces. The averaged distributions of both the loose and pressure measurements are shown in Figure 4. While pressure was applied a fraction of 0.55 of the signal fell in the bin with central reciprocal lifetime 0.005 μs^{-1}. Such lifetimes around 200 µs and even longer, as in this bin, indicate that a large part in the measurement volume is ischemic. The decrease in oxygen resulted in a distribution that has shifted to the left (lower PO_2) compared to the loose situation [3]. To analyze the heterogeneity between different spots of mucosal tissue, all "loose" data was combined into a histogram. To visualize the contribution of individual volunteers the data is color categorized per subject, shown in Figure 5.

All measurements performed while applying pressure on the mucosa were significantly lower compared to measurements performed without pressure at all measurements locations (Table 2). Figure 6 displays a decrease of reciprocal lifetime when pressure is applied with values decreasing further when pressure is maintained for a longer period of time. The oxygen disappearance curve (Figure 7) supports this finding and shows a decrease of reciprocal lifetime over time, demonstrating the disappearance of oxygen due to ongoing oxygen consumption, while temporary ischemia is induced.

3.5 | Determining influence of butylscopolamine

Delayed fluorescence before and after administration of butylscopolamine did not differ significantly in the duodenal

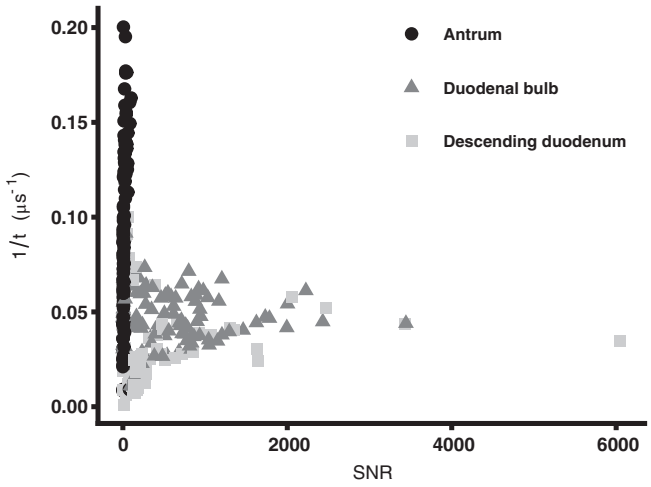


FIGURE 3 Reciprocal lifetimes $1/\tau$ (with lifetime τ in microseconds) versus signal-to-noise ratio (SNR) of all measurements without pressure applied specified for each measurement location

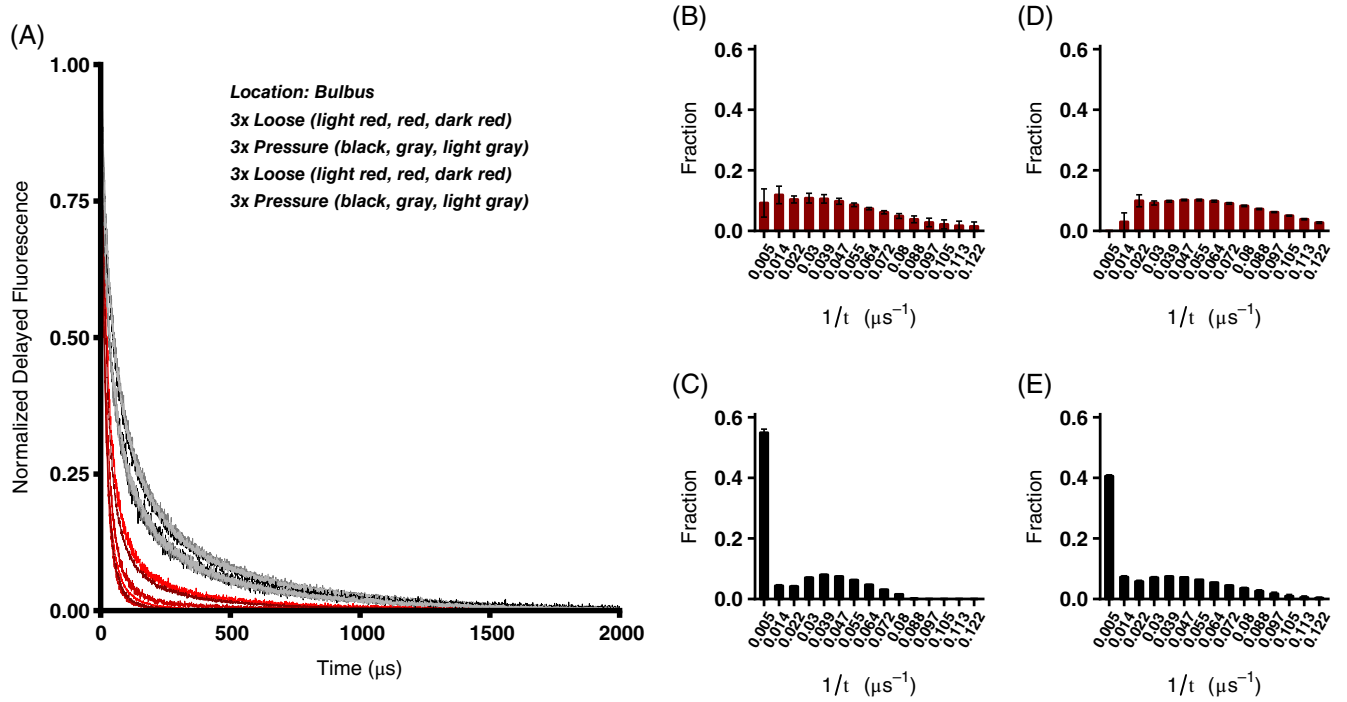


FIGURE 4 A, The normalized delayed fluorescence traces recorded in the Bulbus with 5 mg/kg 5-aminolevulinic acid. The traces represent three loose followed by three measurements while pressure was given with the probe on the tissue to induce local ischemia. This sequence was repeated for a second time. For the distribution analysis (histograms) a base of 15 reciprocal lifetimes equally distributed over a corresponding physiological PO_2 range (0-150 mm Hg) assuming a quenching constant of 830 mm Hg^{-1} and lifetime of spontaneous relaxation of 800 µs was taken [1]. Histograms show reciprocal lifetime distributions of traces of the first panel. B, and D, Average of three loose measurements. C and E, Average of three measurements while pressure was applied on the tissue with the optical probe. Histogram data presented as mean ± SEM

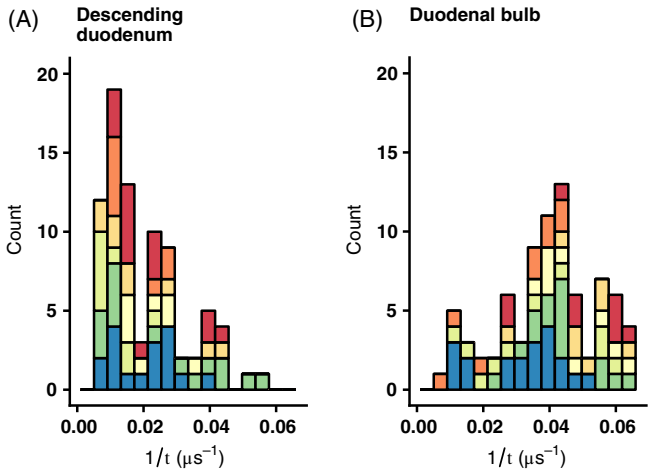


FIGURE 5 Histogram, 16 bins distributed over physiological reciprocal lifetimes, of the two measurement locations, included data is 5 and 20 mg/kg 5-aminolevulinic acid (ALA) administration. The presented data is from two measurement location, A, Descending duodenum, B, duodenal bulb, with the measurement probe hovering 5 mm above the tissue. The colors indicate the individual measurement subject

bulb (Figure 8). In the descending duodenum, reciprocal lifetimes before administration of butylscopolamine were significantly lower than after administration of butylscopolamine (Figure 8). Measurements before and after administration of butylscopolamine did no longer differ significantly ($P = .281$) when the lowest duodenal values before administration of butylscopolamine are excluded. These measurements were performed while unintended pressure was applied to the mucosa due to intestinal contractions.

4 | DISCUSSION

This study showed that delayed fluorescence measurements of ALA-induced PpIX in the GI tract during upper GI endoscopy are technically feasible and that the measured signal is oxygen-dependent. We determined an optimal dose of 5 mg/kg ALA and duodenal bulb and descending duodenum as suitable measurement locations. Administration of butylscopolamine during upper GI endoscopy did not influence the measured GI delayed fluorescence signal (reciprocal lifetimes).

ALA-induced enhancement of mitochondrial PpIX provided the first method to measure mitochondrial PO_2 in living cells [1]. In a time window of some hours after administration of ALA the PpIX is confined in the mitochondria as shown in animal studies in rat liver [18] and rat heart [19]. Since PpIX diffuses slowly out of the

TABLE 2 Reciprocal lifetime (in units, $1/\tau\ \mu s^{-1}$) of gastrointestinal (GI) tissue PO_2 measurements with and without pressure applied for each measurement location

Location	1/τ loose median [interquartile range]	1/τ pressure median [interquartile range]	*P*-value
Antrum	0.092 [0.061-0.132]	0.052 [0.032-0.069]	<.001
Duodenal bulb	0.041 [0.032-0.049]	0.013 [0.010-.0.022]	<.001
Descending duodenum	0.016 [0.011-0.026]	0.009 [0.008-0.011]	<.001

Note: 5-Aminolevulinic acid dose is 5 and 20 mg/kg, total of seven upper GI endoscopies.

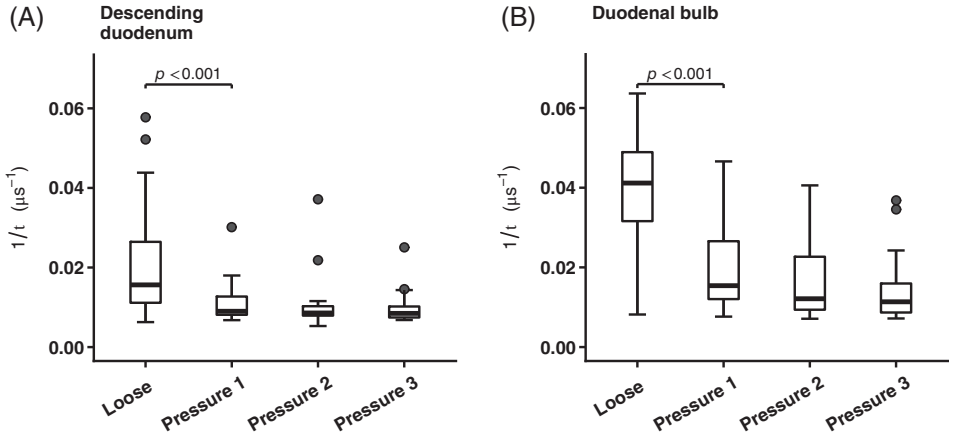


FIGURE 6 A, Measurements in the descending duodenum. The x-axis shows measurements just above tissue (loose) and three consecutive measurements during pressure application (pressure 1, pressure 2, pressure 3). B, Measurements in the duodenal bulb. The x-axis shows measurements just above tissue (loose) and three consecutive measurements during pressure application (pressure 1, pressure 2, pressure 3)

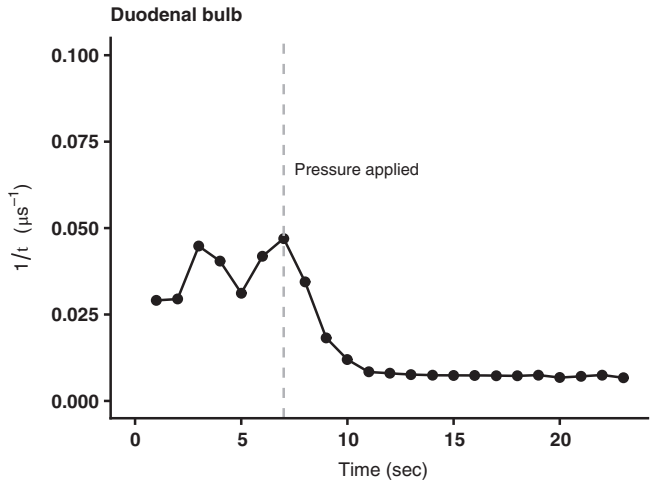


FIGURE 7 Oxygen consumption curve performed in the duodenal bulb

mitochondria, it will not solely remain in the mitochondria, making the localization of the signal less specific over time. The COMET system provides the clinical parameter "mitoPO_2" (mitochondrial oxygen tension), to distinguish the measurement from, for example, hemoglobin-based "tissue oxygen" measurements, since such parameters provide other information from different tissue compartments [4]. However, the reader should keep in mind that the origin of the PpIX delayed fluorescence signal is not only mitochondrial and depends on tissue type, and the amount of time after ALA administration.

Administration of ALA is needed for reliable endoscopic delayed fluorescence lifetime measurements since the amount of endogenously present PpIX is not sufficient for measurements using the current setup. The optimal dose of ALA is a compromise between sufficient signal quality and no or few side effects. The signal quality was high for a dose of 20 mg/kg ALA, however, phototoxicity was reported in 60%. Measured reciprocal lifetime values were disproportionally high at a dose of 1 mg/kg ALA compared to the values measured with 5 or 20 mg/kg ALA. This finding suggests the occurrence of disproportional high reciprocal lifetime values at low SNRs due to insufficient ALA dose. An explanation may be the occurrence of PpIX photobleaching, caused by the white xenon light source of the endoscope plus the potential photobleaching effect of the green laser light. The total amount of light exposure depends on the endoscopic observation time. But despite the endoscopic light source, the measurements could be performed with sufficient signal quality. With technical and medical considerations in mind, we advise an ALA dose of 5 mg/kg for endoscopic oxygen-dependent delayed fluorescence measurements, since we showed reliable delayed fluorescence measurements without any side effects after administration of this dose.

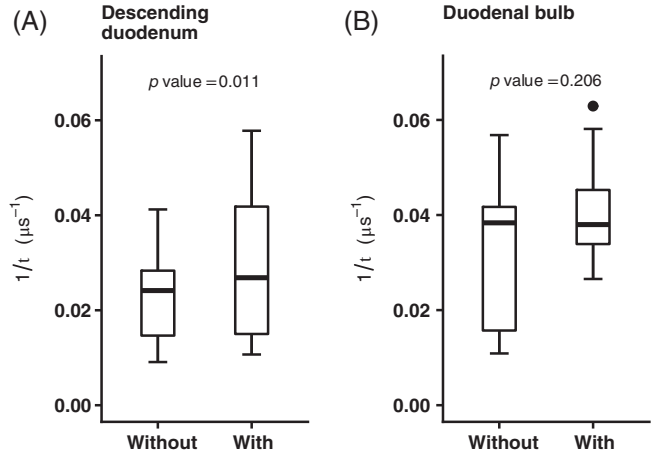


FIGURE 8 A, measurements with 5 mg/kg 5-aminolevulinic acid (ALA) in duodenal bulb without butylscopolamine versus measurements with butylscopolamine and B, measurements with 5 mg/kg ALA in descending duodenum without butylscopolamine versus measurements with butylscopolamine

The COMET device determines mitoPO_2 by the delayed fluorescence lifetime of PpIX after excitation with a laser pulse. The measurement has been calibrated for skin and in that case, reciprocal lifetime is linearly proportional to oxygen tension in mm Hg [1]. The calibration constants in the human gastro-duodenal tract are unknown, as a consequence, all data is represented in reciprocal lifetimes $1/\tau$ (μs^{-1}). The measured lifetimes in the gastric antrum were short, with a median value of 10.8 μs, that would have corresponded with non-physiological high PO_2 values if the currently available calibration constants were used [1]. The reason why the gastric antrum has short delayed fluorescent lifetimes has not been studied. We consider the histological composition and function of the mucosa could interfere with the excitation light of the delayed fluorescent signal itself. It could be that the ability of these cells to accumulate PpIX is lower, e.g. due to a relatively slow cellular turnover, resulting in low SNRs and unreliable short lifetimes.

We determined the influence of administration of butylscopolamine on the endoscopic delayed fluorescence signal since butylscopolamine is commonly administered intravenously in clinical practice if intestinal contractions hamper the endoscopy. Delayed fluorescence lifetimes before and after administration of butylscopolamine did not differ significantly in the duodenal bulb, however, in the descending duodenum, the reciprocal lifetimes before administration of butylscopolamine were significantly lower than after administration. These lower values were due to unintended application of pressure during measurements, caused by peristaltic movements of the bowels. During the endoscopic GI delayed fluorescence measurements, the green light emitted by the laser is visible when the endoscopic light is switched off. When peristaltic movements of the bowels were present we frequently observed fading or even disappearance of the green light when the GI mucosa pressed against the fiber. At the same time, lower reciprocal lifetime values were noted. Since the endoscopic light has to be switched off during GI delayed fluorescence measurements, correcting the position of the probe to avoid contact with the mucosa is difficult in contractile bowels. After excluding the low values caused by unintended application of pressure, no significant differences in reciprocal lifetime before and after butylscopolamine remained. Therefore, we conclude that butylscopolamine does not influence the endoscopic GI tissue reciprocal lifetime values, however, when intestinal contractions are present measurements can be affected by unintended application of pressure. We advise to administer butylscopolamine during duodenal delayed fluorescence measurements when intestinal contractions are present, to increase the reliability of the measurements. Furthermore, future design of the probe with development of pressure sensors on the probe tip to avoid measurements during unintended application of pressure with the probe tip on the GI mucosa could be a solution for this problem.

This study has some limitations. First, the measured GI tract delayed fluorescence measurements in this study are presented as reciprocal lifetime since the calibration constants for the mucosa of the GI tract are unknown yet. Second, during the GI tissue measurements, the light of the endoscope has to be switched off to minimize background noise. Furthermore, the probe tip currently consists of sharp edges, which can possibly damage the GI mucosa. Finally, the amount of endogenous PpIX is far from sufficient to measure GI delayed fluorescence using COMET. Necessity to administrate ALA causes a delay between oral intake and the first measurement opportunity, aside from potential phototoxic effects. Fortunately, we did not register any adverse events with 5 mg/kg ALA. The use of a commercial device limited our choice to an excitation wavelength of 515 nm, since this is the excitation wavelength of the COMET device, which is designed for cutaneous delayed fluorescence measurements after topical application of ALA [4]. This wavelength is a tradeoff between melatonin and hemoglobin absorption, topical applied ALA penetration depth, and measurement volume depth. The penetration depth of green light in the gut is very limited [21], and in our application the use of 515 nm excitation light likely limits the measurement depth to the mucosa. However, since ALA is systemically administered in this application, other excitation wavelengths could provide more efficient photo excitation and improve SNR, or alter the penetration depth and increase measurement volume.

For the interpretation of the signal in terms of quantitative oxygen measurements additional research into the

localization of PpIX and the photo-physical properties of the delayed fluorescence would be very helpful. For example, Vinklárek et al described a Singlet Oxygen Feedback-Induced mechanism (SOFDF) that influences the delayed fluorescence of PpIX under certain non-physiological circumstances [22]. It is currently unknown to what extent other mechanisms than T-type delayed fluorescence, like this SOFDF mechanism, contribute to the in vivo delayed fluorescence signal. Although we are determined to improve our measurement and eventually hope to be able to convert the measured lifetimes to PO_2 levels this in itself is not necessary for successful clinical application. The only relevant question here is whether the measured lifetimes in healthy subjects differ from patients with, for example, CMI. Sensitivity and specificity of the technique could equally well be studied and calculated on reciprocal lifetimes instead of oxygen tensions.

In conclusion, measurement of oxygen-dependent delayed fluorescence of ALA-induced PpIX during upper GI endoscopy is feasible and safe. Further research is needed for the clinical applicability of this technique in the diagnostic work-up of CMI, starting with endoscopic GI delayed fluorescence measurements in patients suspected of CMI to determine its discriminative ability and to determine cut-off values for the inverse lifetime measurements for mucosal ischemia. Further research has to determine whether a more accurate diagnosis of CMI can be established when the presence of mucosal ischemia detected by endoscopic delayed fluorescence measurements is combined with the symptoms and imaging of the mesenteric arteries, and ultimately whether its use results in less patients treated for suspected CMI (with the associated risks and costs of revascularization therapy) who will not experience relief of symptoms since they are false positively diagnosed by current standards.

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AUTHOR CONTRIBUTIONS

L. J.D.D. is planning and conducting the study, collecting, interpreting and analyzing data, drafting the manuscript. R.U. is planning and conducting the study, collecting, interpreting and analyzing data, drafting the manuscript. L.G.T. is conducting the study, collecting, interpreting and analyzing data, drafting the manuscript. D.N. is planning and conducting the study, interpreting data and critical revision of the manuscript. E.G.M. is planning the study, interpreting data and critical revision of the manuscript. M.J.B. is planning the study, interpreting data and critical revision of the manuscript. All authors approved the final draft submitted.

CONFLICT OF INTEREST

L.J.D.D., L.G.T. D.N. declare no potential conflict of interest. R.U. is shareholder of Photonics Healthcare, a company aimed at making the delayed fluorescence lifetime technology available to a broad public. Photonics Healthcare B.V. is the company that developed and markets the COMET device. Photonics Healthcare B.V. holds the exclusive licenses to several patents regarding this technology, filed and owned by the Academic Medical Center in Amsterdam and the Erasmus Medical Center in Rotterdam, The Netherlands. E.G.M.is a one of the founders and shareholder of Photonics Healthcare, a company aimed at making the delayed fluorescence lifetime technology available to a broad public. Photonics Healthcare B.V. is the company that developed and markets the COMET device. Photonics Healthcare B.V. holds the exclusive licenses to several patents regarding this technology, filed and owned by the Academic Medical Center in Amsterdam and the Erasmus Medical Center in Rotterdam, The Netherlands. M.J. B. The author reports having received lecturer and consultant fees (Boston Scientific, Cook Medical, Pentax Medical and 3 M) and having received grants for investigator-initiated studies (Boston Scientific, Cook Medical, Pentax Medical and 3 M) and industry initiated studies (Boston Scientific, Cook Medical).

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