



## Functional testing in the diagnosis of chronic mesenteric ischemia

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## A B S T R A C T

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Chronic mesenteric ischemia (CMI) results from insufficient oxygen delivery or utilization to meet metabolic demand. Two main mechanisms may lead to mesenteric ischemia: occlusion in the arteries or veins of the gastrointestinal tract, or reduced blood flow from shock states or increased intra-abdominal pressure, so-called non-occlusive mesenteric ischemia. Severe stenoses in the three main mesenteric vessels as demonstrated with CT-angiography or MR-angiography are sufficient to prove mesenteric ischemia, for example in patients who present with weight loss, postprandial pain and diarrhea. Still in many clinical situations mesenteric ischemia is only one of many possible explanations. Especially in patients with a single vessel stenosis in the celiac artery or superior mesenteric artery with postprandial pain, mesenteric ischemia remains a diagnosis of probability or assumption without functional proof of actual ischemia. This review is aimed to provide an overview of all past, present and future ways to functionally proof CMI.

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## Introduction

Chronic mesenteric ischemia (CMI) results from insufficient oxygen delivery or utilization to meet metabolic demand. Two main mechanisms may lead to mesenteric ischemia: occlusion in the arteries or veins of the gastrointestinal tract, or reduced blood flow from shock states or increased intra-abdominal pressure, so-called non-occlusive mesenteric ischemia (NOMI). Demonstration of arterial or venous stenoses is now easily achieved with diagnostics like computed tomographic angiography (CTA) or magnetic resonance angiography (MRA). The vessel abnormalities are sufficient to prove mesenteric ischemia, for example in patients with severe stenoses or occlusions in the three main mesenteric vessels who present with weight loss, postprandial pain and diarrhea. Still in many clinical situations mesenteric ischemia is only one of many possible explanations, and one of the more improbable at that. The most challenging patient may be the patient with a single vessel stenosis in the celiac artery (CA) or superior mesenteric artery (SMA) with postprandial pain. Without functional proof

of actual ischemia mesenteric ischemia remains a diagnosis of probability or assumption. The same holds true for NOMI where vessel anatomy is essentially normal and without actual proof of ischemia the diagnosis cannot be made with certainty until end-organ damage is proven by endoscopy showing colonic ischemia or during surgery showing necrotic bowel. This review is aimed to provide an overview of all past, present and future ways to functionally proof ischemic bowel.

## The mesenteric circulation in relation to ischemic function tests

The macro- and micro-vascular properties of GI perfusion, and the response to meals largely dictate the areas and timing to use functional tests.

The macrovascular distribution of gastrointestinal perfusion is characterized by three main arteries and numerous collaterals. The CA is the artery of the foregut and supplies the stomach and duodenal bulb and descending duodenum. The SMA supplies the distal duodenum, small bowel and right colon. The inferior mesenteric artery (IMA) supplies the second part of colon. The normal diameters and grade of stenosis of these vessels are highly relevant when judging the risk of ischemia. The CA and SMA both have diameter of 6 and 7 mm, whereas the IMA normally measures

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1 mm. Consequently, an IMA occlusion would reduce the total mesenteric vessel surface by only 4%, whereas a 70% stenosis of CA and SMA would reduce this by 87% [1]. The venous system involves the inferior mesenteric vein (IMV) and superior mesenteric vein (SMV). Both combine with the splenic vein (SV) to form the portal vein (PV). The liver thus has a dual vascularization from PV and common hepatic artery. This renders the liver resistant to ischemia, as recently demonstrated in large cohort of CMI patients [2]. Because the portal vein receives blood from both the CA and SMA, measurement of blood flow properties of the PV could be used to study insufficient blood flow in either the CA or SMA. For application of functional testing the anatomical distribution dictates that with a normal upper endoscopy only the area perfused by the CA can be inspected. For assessment of the area perfused by the SMA inspection of the more distal duodenum, small bowel or right colon region would be mandatory. This is challenging, because it is widely assumed the SMA is the most important of the mesenteric arteries.

The microvascular perfusion is characterized by larger arteries on the serosal side with large networks of vessels in the outer layers (submucosal, muscular and serosal layers) and a central arteriole with surrounding venules. The mucosal layer has a high metabolic demand and receives app. 80% of the bowel wall blood flow [3,4]. Consequently, the mucosal layer is the first part of the bowel wall affected by reduced perfusion and ischemia leading to early damage in severe ischemia [5,6]. Due to the typical countercurrent organization of the villus the most superficial layers are especially sensitive to ischemic damage. In contrast, ischemic damage to the muscular and serosal layers is a late event in severe ischemia. Consequently, functional test for early ischemia should be focused on the mucosal layer, be it by measuring  $PO_2$  and  $PCO_2$  directly, or by measuring early ischemia-specific proteins and waste products.

The gastrointestinal blood flow response to meals or generalized hypoperfusion is relatively well studied. Following meals, the basal, fasting gastrointestinal blood flow increases from app. 1000 ml/min. to up to 2000 ml/min. The CA blood flow increases and peaks already during the meal and returns to baseline within 20 minutes after the meal, whereas the SMA blood flow increases only after the meal, peaks 30–45 minutes postprandially and returns to baseline within 2–3 h [7,8]. To complicate simple measurement of blood flow it was demonstrated that the increase in blood flow was due to both increased blood flow velocity and increased vessel diameter as well [8]. The duration of the increased SMA blood flow, and the time-to-peak depends on meal composition. Meals with high fat content lead to later and higher peak blood flow and longer duration of increased blood flow [8,9]. The consequences of this pattern of these meal-induced flow patterns are that 1) the time-window for functional tests using test meals would ideally include the meal and first 1–2 h postprandially to cover both the CA and SMA perfusion problems, and 2) that the composition of test meals should be taken into account.

The mesenteric blood flow on the other hand decreases whenever the systemic perfusion pressure lowers, even before actual shock develops [10]. The mesenteric circulation is the first vascular bed to develop vasoconstriction in shock states [11]. This so-called non-occlusive mesenteric ischemia (NOMI) may develop with various low flow and shock states including sepsis, hypovolemia, anaphylaxis, and even strenuous exercise. In the first reported mesenteric ischemia function test, the gastric exercise tonometry, we used exercise to trigger redistribution of blood flow from the GI tract to the exercising muscles to induce ischemia in patients with mesenteric stenoses [12].

Effects of tissue ischemia. The first effect of reduced gastrointestinal perfusion is an increased arteriolar-venous oxygen extraction rate. If this does not suffice to maintain aerobic metabolism then the tissue switches to anaerobic metabolism with

decreased oxygen content in the tissues and at the same time the tissue  $CO_2$  and acidity increases. Next the mitochondrial failure leads to damage to tight junctions, and failing cell membrane pumps. Later, cell swelling and leakage of cell contents in the surrounding tissues and blood stream follow. In later stages re-entry of oxygen following restoration of blood flow can cause reperfusion effects characterized by development of free oxygen radicals and outflow of ischemic products into the systemic circulation. This sequence of events serves as background when assessing the various function tests and their clinical potential, for example as markers for early ischemia or late ischemia.

Functional tests for mesenteric ischemia can be divided into techniques aimed at direct evidence for mucosal ischemia, blood-flow related parameters, and those that measure ischemia-related products. Evidence for mucosal ischemia can be detected by measurement of: 1) reduced mucosal oxygen content, 2) increased mucosal lactate, 3) increased mucosal  $PCO_2$ , 4) evaluation of mucosal biopsies. The main blood-flow parameters that have been used, or are currently under investigation, were aimed at showing both fasting and postprandial blood flow. They include: 1) measurement of mesenteric blood flow, 2) measurement of portal vein blood flow. Measurement of ischemia-derived proteins or waste-products will be briefly discussed, as it is covered extensively in this issue by Dierickx et al.

### The clinical challenge

Proving CMI is a diagnostic challenge in clinical practice. Currently there is no single test with high sensitivity and specificity to diagnose or exclude this condition. Chronic abdominal pain is a common symptom in the general population as are vascular stenoses of the gastrointestinal arteries [13,14]. Occlusive gastrointestinal arterial disease often remains asymptomatic in most cases probably due to the presence of abundant collateral circulation. Only patients with significant arterial stenosis in combination with insufficient collateral circulation may develop clinical signs of gastrointestinal ischemia. This was recently demonstrated in patients with single-vessel stenosis where the presence of large collaterals, visible on non-selective angiography, ischemia was less common than in those without these large collaterals [15]. The same group showed that patients with single-vessel stenosis showed increased flow in the non-affected vessel, indicating a compensatory effect via collateral pathways [16].

The diagnostic approach in patients referred for evaluation of possible CMI focuses on identification of gastrointestinal arterial stenosis and demonstration of mucosal ischemia. Currently, diagnostic approaches include assessment of medical history, clinical symptoms and physical examination, imaging of the gastrointestinal arteries by duplex ultrasound, digital subtraction angiography (DSA), CTA or MRA and assessment of gastrointestinal mucosal perfusion by means of tonometry or Visible Light Spectroscopy (VLS).

Functional testing is not widely available and wider use is often hampered by its cumbersome and invasive nature. Until now, TM and VLS are only used in a limited number of dedicated centers with a CMI program, which means that the majority of CGI patients are still assessed without functional testing. However, development of mucosal perfusion assessment techniques would have additional diagnostic value in identifying and grading the severity of gastrointestinal ischemia.

### Gastrointestinal tonometry

#### Physiological background

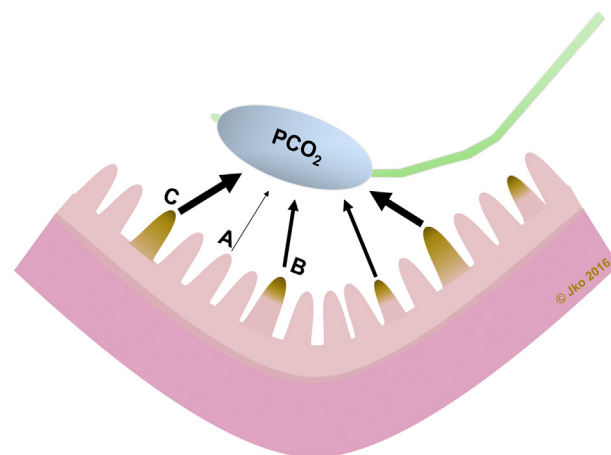
That tissue  $PCO_2$  is an accurate indicator for ischemia has been shown repeatedly in both animal tests and human studies [17]. The

first mention of this technique was published in 1926 which established that in dogs, the  $\text{PCO}_2$  in the stomach cavity was equal to that in arterial blood. With hypoventilation  $\text{PCO}_2$  in both arterial blood and the stomach went up, while hyperventilation reduced both values. That was lost as soon as the afferent blood flow was interrupted. Now, luminal  $\text{PCO}_2$  rose while the arterial  $\text{PCO}_2$  remained stable [18]. In 1959 Boda published the first study in patients and confirmed both the narrow relation between gastric and arterial  $\text{PCO}_2$ , as well as increased gastric  $\text{PCO}_2$  levels in patients who developed shock or signs of ischemia [19]. In the late 1980's a catheter based measurement device was developed, the tonometer, to allow for simple and clean ex vivo measurement of gastric, small and large bowel  $\text{PCO}_2$  [20]. It was filled with saline, left in the balloon for 20–40 min, then the saline was aspirated and  $\text{PCO}_2$  was measured with a blood gas analyzer. Later, the catheter was attached to a specialized capnograph, the Tonocap, that allowed for automated measurement with 10-minute cycles [21]. Most literature on tonometry focused on NOMI patients in the intensive care or perioperative theater. It was found that tonometry was a reliable indicator of – ischemic – stress ulcerations [22], colonic ischemia following aortic surgery [23,24], complications and mortality in ICU patients [25–27], and complications in acute pancreatitis patients [28]. However, its popularity waned mainly because the technique is relatively complicated – need for acid suppression and complex influence of meals on  $\text{PCO}_2$  – and because  $\text{PCO}_2$ -guided treatment studies were unconvincing. The latter has been challenged recently in meta-analysis suggesting a significant effect of tonometry-based treatment [29]. Still, the technique has virtually disappeared from the intensive care and perioperative care theaters.

Our groups (Enschede and Rotterdam) have mainly focused on occlusive mesenteric ischemia. In this paragraph we will discuss our 20-year experience in over 1500 patients suspected of occlusive mesenteric ischemia. The principle of tonometry is based on measurement of increased  $\text{PCO}_2$  levels during ischemia. This excess  $\text{CO}_2$  stems from buffering of acids, like lactate, in the ischemic tissues by interstitial bicarbonate. With normal oxygenation the cells metabolize sugars as main energy source in the citric acid cycle under formation of  $\text{H}_2\text{O}$  and  $\text{CO}_2$ . This  $\text{CO}_2$  is then removed by the blood flow and exhaled in the lungs. With ischemia this  $\text{CO}_2$  production increases in relation to the severity of ischemia [30]. In numerous studies the strict relation between ischemia and increased  $\text{PCO}_2$  has been demonstrated. Simply stated: ischemia = increased tissue  $\text{PCO}_2$ . In the gastrointestinal tract this excess  $\text{PCO}_2$  will accumulate first in the mucosa but diffuse quickly into the lumen where it can be easily measured using a tonometer. Because mesenteric ischemia is often patchy, tonometry is able to detect the overall excess  $\text{CO}_2$  production, with little influence of exact location of the tonometer catheter (Fig. 1). Our initial work focused on measurement technique and test prerequisites, and validation in healthy volunteers [31].

#### Exercise tonometry

Initial studies with tonometry used an exercise test to elicit mesenteric ischemia. The underlying mechanism is a steal phenomenon from the mesenteric circulation to the exercising muscles [12] with reductions in mesenteric and hepatic blood flow up to 25–50% [32]. Normal values for this gastric exercise tonometry were obtained from a healthy volunteers study. No increase in gastric-arterial difference over the normal upper threshold of 0.8 kPa was seen during exercise provided that lactate levels were below 8 mmol/l. With lactate levels above 8 mmol/l, mesenteric ischemia was seen in 5/11 subjects [33]. The exercise tonometry consists of a 10-minute exercise on a bicycle, aimed at submaximal



**Fig. 1.** Principle of  $\text{PCO}_2$  tonometry. In mucosal ischemia a patchy distribution is characteristic with normal flow (A) reduced flow (B) and no flow (C) areas in close approximation. Because the  $\text{PCO}_2$  in normal perfused mucosa equals blood values, and low flow areas have moderate increased  $\text{PCO}_2$  (B), and no flow areas have very high  $\text{PCO}_2$  values, the overall measured  $\text{PCO}_2$  is an integral of these value. In other words: even early ischemia can be measured, but with worsening ischemia, the  $\text{PCO}_2$  increases.

exercise (70% of maximal). The exercise level is determined by rapid lactate measurements from arterialized blood and is aimed at a lactate level between 4 and 8 mmol/l at 10-minutes exercise. Using this validated gastric exercise test we could establish a 78% sensitivity and 92% specificity in 102 patients with chronic abdominal pain suspected for mesenteric ischemia [34]. Because some patients had SMA stenosis only, we then started to use small bowel tonometry as well. The tonometer catheter is placed beyond either in the distal duodenum or jejunum to reach the ‘SMA perfusion area’. Using the same exercise protocol, we established a normal upper limit of jejunal-arterial  $\text{PCO}_2$  difference of 1.4 kPa [35]. We used this same test in patients with celiac compression and found the gastric exercise test to be accurate in selecting patients who later benefitted from treatment by crural release [36]. Of 43 referred patients with celiac compression, 30 had abnormal tonometry of whom 29 were operated. After a median follow-up of three years 83% were free of abdominal pain. The main draw-backs of exercise tonometry is first the need to exercise up to submaximal levels and some patients cannot exercise, or not enough to reach that level. Secondly, although many patients with mesenteric ischemia complain of post-exercise abdominal pain, this complaint did not differentiate between ischemic and non-ischemic subjects with mesenteric stenoses [37]. Post-prandial pain on the other hand, is a key complaint in the vast majority of mesenteric ischemia patients [37,38]. We therefore focused our attention on development of a reliable and reproducible tonometry test using meals as ischemic trigger (see Table 1).

#### 24-hour tonometry

There are several methodological issues when performing postprandial tonometry. First, acid suppression must be maximal, because persistent acid production leads to increased  $\text{PCO}_2$  from buffering by bicarbonate [39,40]. There are three potential bicarbonate sources: the gastric mucosal layer, small bowel content or the ingested food itself. We therefore use high-dose PPI treatment of Omeprazole 80 mg bolus followed by 8 mg per hour. Because it was shown that  $\text{PCO}_2$  increase after meals could be related to

**Table 1**  
Exercise tonometry.

<b>Preparation</b>
PPI intravenous (80 mg bolus, 8 mg/h, >2 h before test)
<b>Exercise</b>
Duration 10 min, aimed at 70–80% submaximal at least three minutes
<b>Measurements</b>
Heart rate
ECG continuous with ST segment detection (avoid coronary events)
Gastric and jejunal PCO <sub>2</sub> every 10 min
Arterialized blood gas baseline and peak exercise
Arterialized lactate baseline, at 5–7–9 minutes (monitor exercise level, aim at 3–8 mmol/l at peak exercise)
<b>Interpretation</b>
Lactate 3–8 mmol, and
Gastric-arterial PCO <sub>2</sub> difference >0.8 kPa → ischemia
Jejununal-arterial PCO <sub>2</sub> difference >1.4 kPa → ischemia
Lactate levels <3 mmol/l: insufficient exercise level with normal PCO <sub>2</sub> gaps (risk: false-negative)
Lactate levels >8 mmol/l: maximal exercise, chance of physiological ischemia (risk: false-positive)

acids in food as well [40], we tested different test meals in vitro to minimize these effects. The ideal candidate was Nutridrink<sup>®</sup>, a standard liquid nutrition [39]. We then used the Nutridrink as a standard meal and established normal values in a 24-hour tonometry test in healthy subjects. The normal baseline of gastric PCO<sub>2</sub> and small bowel PCO<sub>2</sub> levels [41] as well as the response to meals were assessed. The cut-off value for baseline PCO<sub>2</sub> in stomach and small bowel was <8.0 kPa. The cut-off values for the maximum PCO<sub>2</sub> from 0 to 120 min after meals were for the stomach: 11.3 after Nutridrink, 11.4 after dinner and 12.1 kPa after breakfast. For the small bowel these values were 10.6, 13.6 and 12.0 kPa [41]. An abnormal test was defined as [1] pathologic responses after three or more (standard) meals, or [2] a combination of one or two pathologic responses after (standard) meals combined with a median PCO<sub>2</sub> > 8.0 kPa, measured in between meals. Using these cut-off values a sensitivity of 76% and specificity of 94% was calculated. In a larger study Sana et al. confirmed the value of 24-h tonometry and showed an overall accuracy of 87% (sensitivity 92%, specificity 77%). This function test was incorporated in the decision with a greater impact than the medical history or radiological findings [42]. This study re-emphasized the importance of a consensus diagnosis using a multidisciplinary approach with a diagnostic accuracy >90% on follow-up, when all elements (anamnesis, radiology and tonometry) were included in decision making.

In patients with end-stage CMI two patterns can be observed. Typically, the baseline PCO<sub>2</sub> is grossly elevated, with long and high PCO<sub>2</sub> peaks after meals, indicating imminent or ongoing mesenteric infarction. In patients with acute ischemia for longer duration a very low PCO<sub>2</sub> pattern with hardly any variation was observed. The latter can be seen in non-ischemic subjects, but was observed in several patients with ongoing, and yet undetected, bowel infarction. In the latter years, we have not seen this pattern as patients with persistent pain, or pain unassociated with meals are now treated immediately and do not undergo tonometry anymore (Fig. 2, Table 2).

#### Tonometry after intervention

Only one study investigated the effects of treatment on tonometry. In the study on CACS patients, Mensink et al. showed improved tonometry in all patients who responded to treatment. In contrast, in those without improvement of pain after treatment 50% was unchanged, 25% improved and 25% worsened [36].

#### Procedure

The tonometer, a balloon-tipped catheter can be placed via the nose in stomach and small bowel, using a guide wire placed endoscopically, and checked by fluoroscopy or with fluoroscopy alone. A second tonometer is placed in the stomach and the placement is checked by fluoroscopy. The catheter is then attached to a specialized capnograph, the Tonocap, that measures the PCO<sub>2</sub> every 10 min. The patient should be fasting for 6 h, and use of intravenous high-dose PPI is advised to prevent false-positive PCO<sub>2</sub> peaks from buffering of gastric acid [39,43]. The Tonocap is now connected to a PC with software that captures the data directly. After measurement the data are exported from this PC to MMS software (Medical Measurement Systems, Enschede, the Netherlands) and analyzed using this software program that was developed for manometry and pH-impedance studies.

#### Limitations of tonometry

Although the data for tonometry as measure for mesenteric ischemia are rather convincing, the technique never was really accepted in the medical community. There are several limitations that hampered its wider use. First, in the first years the technique involved measurement of manually infused and aspirated saline by blood gas analyzer, and was notoriously cumbersome, error-prone, and laborious [31]. The switch to automated air tonometry was a clear improvement [21]. Second, tonometry was mainly used for critical care patients, but failed to impact the outcome significantly. Third, the technique is still challenging, with need for strict testing conditions (acid suppression, meals, timing, analysis). Fourth, even with these strict conditions the current device is far from fail safe, and slight kinking of the catheter, or occasional leakage in the balloon results in failed measurement and need for repetition. Fifth, the need for two, small and soft, catheters trans-nasally is burdensome for patients, as is the setup with a large PC and two Tonocaps next to the bed, limiting the freedom of movement for 24 h. Finally, the current producer has stopped making and maintaining the Tonocaps, limiting its future to the very near future. A new 'balloon-less' technique has been described by the late Professor Boda [44,45]. We have done some ex vivo studies using this technique and found that it was able to measure multiple compartments with short response times (unpublished data) and are currently developing further validation and pilot studies. Still, further studies are needed to evaluate its actual diagnostic accuracy and clinical applicability.

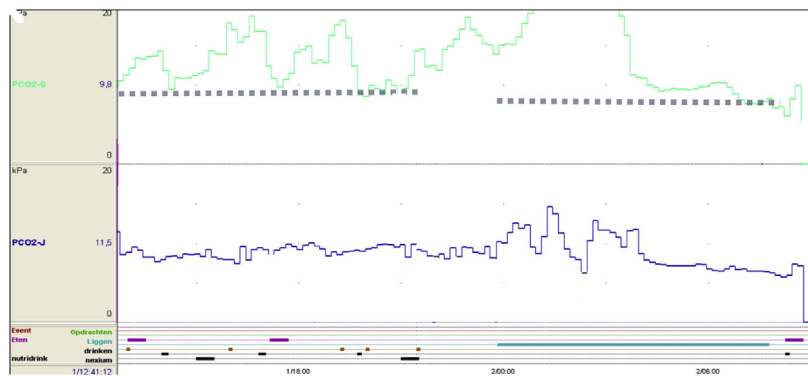
#### Visible light spectroscopy

##### Physiological background

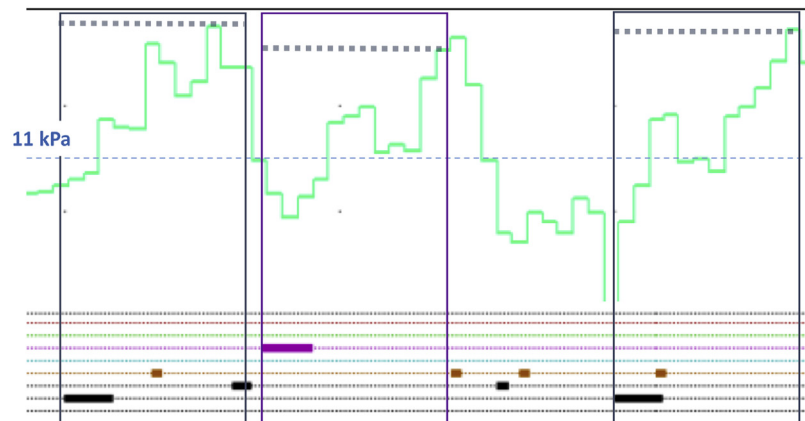
Measurement of mucosal oxygen saturation dates back to the early 1950's, and was used for skin measurement mainly. Measurement of gastrointestinal mucosal oxygen saturation became possible with microfiber technique combining a light source and external spectrometer [46]. This technique is used in the reflectance spectrophotometer (RS) that can be used endoscopically, and is known as visible light spectroscopy (VLS). VLS is a relatively new technique that enables non-invasively measurements of mucosal capillary hemoglobin oxygen saturations during upper endoscopy. Unlike near-infrared spectroscopy (NIRS) or pulse oximetry (SpO<sub>2</sub>%), that are mostly used for tissue measurement, VLS is based on locally absorbed, shallow-penetrating visible light for measurement of microvascular hemoglobin oxygen saturation, and was



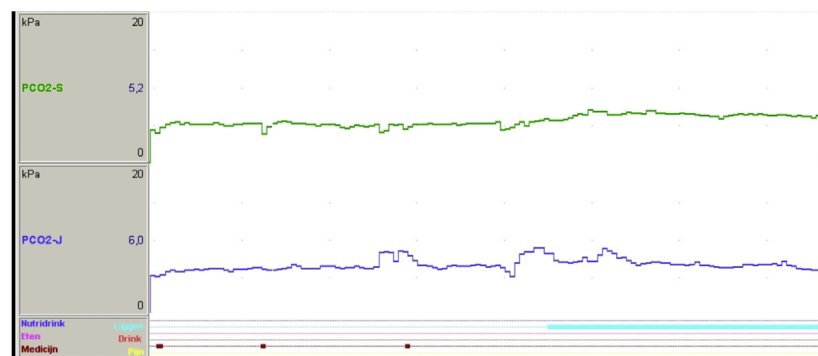
## A. 24-hr tonometry: baseline



## B. Analysis of meal-related peaks



## C. Flat curve; severe ischemia



**Fig. 2.** Analysis of 24-hr tonometry. A. 24 hour read-out with the stomach PCO<sub>2</sub> in the top and the jejunal PCO<sub>2</sub> in the bottom panel. The baseline values during day- and night time are indicated by the dotted lines. B. Detail of the stomach and jejunal PCO<sub>2</sub> response with meals (twice Nutridrink and one dinner). The PCO<sub>2</sub> within a 2-hour period of meals is analyzed. The response is abnormal if the maximum PCO<sub>2</sub> exceeds the upper limit of normal (11 kPa with Nutridrink). C. The typical PCO<sub>2</sub> pattern in end-stage ischemia. A flat, hardly changing PCO<sub>2</sub> curve can be seen, indicative of ischemic cell death.

developed as a catheter based measurement device [47]. The technique uses white light delivered by a fiber optic probe to directly measure intra-mucosal hemoglobin saturation, relying on the marked difference in absorption spectra of oxygenated and deoxygenated hemoglobin. It was indeed shown that

gastrointestinal mucosal ischemia by hypotension, smoking, or injection of vasoconstricting substances could be detected by reflectance spectroscopy [48]. Using RS Kamada et al. found decreased oxygenated hemoglobin in patients with stress ulcers [48], around the same time that Fiddian Green published a similar

**Table 2**  
24-hr tonometry.

<b>Preparation</b>	
PPI intravenous (80 mg bolus, 8 mg/h, >2 h before test, continued throughout test)	
<b>Meals</b>	
At least two liquid compound meals 400 ml (Nutridrink, Nutricia, the Netherlands), normal dinner, normal breakfast (two slices of bread, tea or coffee). No acidic or CO <sub>2</sub> containing beverages.	
<b>Measurements</b>	
Baseline PCO <sub>2</sub> (lowest stable measurement of PCO <sub>2</sub> for at least 60 minutes)	
PCO <sub>2</sub> peaks after meals (window: start of meal – 2 h after end of meal)	
PCO <sub>2</sub> peaks (>11 kPa) not associated with meals	
Pain episodes	
<b>Interpretation</b>	
Threshold values (upper levels of normal) PCO <sub>2</sub> [41]	
Gastric:	Baseline <8.0 kPa Postprandial: breakfast: 12.1 kPa, dinner: 11.4 kPa, Nutridrink: 11.3 kPa Associated with pain: 11.0 kPa
Jejunum:	Baseline <8.0 kPa Breakfast: 12.0 kPa, dinner: 12.0 kPa, Nutridrink: 10.6 kPa Associated with pain: 11.0 kPa
<b>Abnormal test when</b>	
- Three or more peaks, postprandial peaks or peaks associated with pain, above threshold	
- Baseline PCO <sub>2</sub> above threshold, at least one postprandial peak or peak associated with pain above threshold.	

relationship using tonometry in stress ulcer patients [22]. Both studies thus noticed the importance of gastric hypoperfusion in the development of stress ulcers. The relation between gastric mucosal perfusion and mucosal oxygen saturation was later confirmed in ICU-model during cardiac bypass surgery [49].

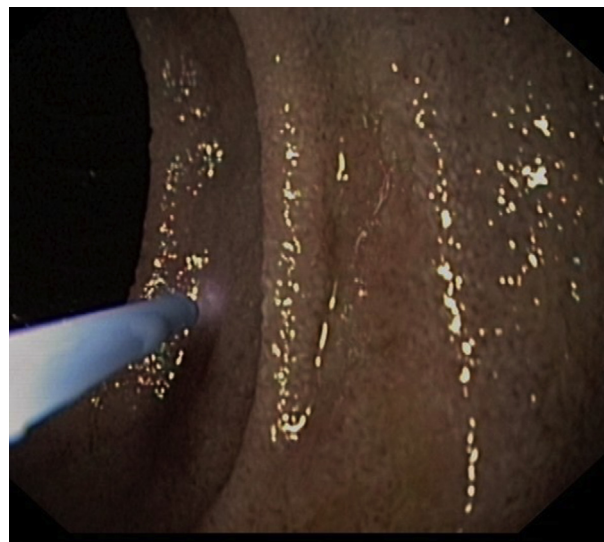
The data on human mesenteric ischemia using VLS is limited. It is expected that gastrointestinal arterial stenosis would decrease the delivery of oxygen to the gastrointestinal mucosa resulting in lower mucosal hemoglobin oxygen saturations. Indeed, a pilot study showed promising results: VLS was able to demonstrate mesenteric ischemia in three patients [50]. The authors developed normal values using healthy volunteers and laid a foundation to further develop upon. The major drawback of their paper is that the three patients with abnormal VLS all had end-stage mesenteric ischemia, all with subtotal stenosed or occluded CA and SMA and end-organ damage (gastric ulcers in two and bloody diarrhea in the third), as pointed out in commentary following the paper [51]. However, following this pilot study, VLS did detect mucosal hypoxemia in patients clinically suspected for CMI in a large cohort, showing excellent correlation with the established ischemia work-up using tonometry [52].

### Procedure

VLS is performed during upper endoscopy under conscious sedation. The peripheral saturation level has to be above 94%. After irrigation of the target area to remove any bile remnants, the fiberoptic catheter-based visible light spectroscopy oximeter (T-Stat 303 Microvascular Oximeter, Spectros, California, USA) can be passed through the accessory channel of the endoscope. After administration of Butylscopolamine to prevent luminal spasms, point measurements of the oxygen saturation are performed at three locations: antrum of the stomach, duodenal bulb and descending duodenum. The probe is positioned 1–5 mm above the mucosa. Once a stable reading is obtained with less than 5% variation in panel read-out, the actual measurement can be started. Then three repeated readings of each location will be taken and averaged. This average per location is regarded as most accurate reflection of mucosal oxygen saturation. The oxygen saturation measurements are regarded positive for ischemia below the following cut-off values: 63% in antrum and/or 62% in duodenal bulb and/or 58% in descending duodenum (Fig. 3).

### Diagnostic value

VLS is a validated diagnostic method to correctly detect CMI with a sensitivity of 90% and a specificity of 60% [52]. Because missing a mesenteric ischemia patient was considered more harmful than having false-positives the cut-off values were chosen with high sensitivity and low specificity. The rationale being that undiagnosed and untreated patients with ischemia have a higher morbidity and mortality rate. Harki et al. [53] confirmed the diagnostic value of VLS in distinguishing patients with CMI from patients without CMI and healthy subjects. Patients with CMI have significantly lower mucosal oxygenation saturation measurements using VLS compared to patients without CMI and healthy controls. Sana et al. investigated the response of patients with occlusive CMI to treatment after evaluation by radiologic imaging of the vasculature and functional testing using VLS [54]. A corpus mucosal saturation level <56% (OR, 4.84) was one of the strongest predictors of a positive response to treatment.



**Fig. 3.** VLS measurements using a fiberoptic catheter-based visible light spectroscopy oximeter. The catheter is passed through the accessory channel of the endoscope and positioned approximately 1–5 mm above the mucosa.

### VLS after intervention

After successful treatment, improved repeated VLS measurements can be observed, 80% of patients with relief of symptoms showed improvement of even normalization of VLS measurements [52]. In patients with persistent symptoms no change in mucosal perfusion was observed.

### Limitations of VLS

VLS has some limitations and unsolved issues. First, VLS is a relatively new test method with few studies to support its value. All published data from the Rotterdam group are based on a trainee data set and additionally confirmed in a validation cohort. Still, there is only one large single center cohort and the data are not yet reproduced by other research groups.

Second, current specificity is rather low, and could lead to treatment of too many non-ischemic patients. Third, the intra- and inter-observer variability of VLS measurements has never been investigated. This may be of importance because small changes in the position of the probe can cause small variations in the oxygen saturation. Moreover, the mean of mucosal saturation measurements in patients with CGI showed great variation and the range of measurements is large [52]. Therefore, it is sometimes quite hard to classify a measurement as positive or negative for mucosal ischemia when the obtained average value is around the cut-off value. Fourth, mucosal ischemia might be patchy and could be missed because VLS is limited to repeated point measurements. A very large number of repeated point measurements might in theory increase the diagnostic yield of VLS, this remains to be established in future research. Furthermore, the hypothesis rises that mucosal ischemia only occurs postprandially, in response to an increased metabolic demand or exercise related, indicating a time-dependent relation. Currently, VLS measurements are performed in a fasting state due to the nature of the test as performed during upper endoscopy. In theory, patients with CGI with less impaired gastrointestinal blood flow could show normal mucosal saturation measurements in this fasting condition and could therefore be missed or underestimated. VLS measurements after luminal feeding stimulation may overcome these false negative results, thereby enhancing the specificity of the test. Currently, the diagnostic accuracy of postprandial VLS measurements is investigated, the results of this study will follow during the next year.

### Total splanchnic blood flow

In Scandinavian countries invasive measurement of mesenteric and hepatic blood flow has been used as functional test for several decades. This so-called total splanchnic blood flow (SBF) can be determined by catheterization of the liver vein and subsequent blood sampling after infusion of indocyanine green or radio-active markers and measured by Fick's principle. Hansen reported in 1977 that an increase in SBF after test meal of less than 250 ml/min in SBF indicated CMI [55]. In that first paper the six patients with <250 ml/min postprandial SBF increase all had severe 2- and 3-vessel CMI. The blood flow increase in single-vessel CMI patients did not differ from healthy controls. They also observed a wide variation in basal SBF (from 700 to 1500 ml/min) and postprandial increase (from 410 to 880 ml/min). In a carefully conducted study Zacho et al. confirmed the wide variation in SBF in healthy volunteers, and could not relate them to age, body weight or sex [56]. In a study in 46 patients the same group found an abnormal increase after meals, but again only in patients with multi-vessel disease [57]. We therefore think that this technique has no place as functional test in suspected single-vessel CMI.

### Future, new function tests

#### Endomicroscopy of the microcirculation

Recently, studies in patients with acute gastrointestinal ischemia demonstrated a correlation between intestinal ischemia and sublingual microcirculatory alterations [58]. There is increasing data and interest in assessing microcirculation changes, especially in critical care patients. The current most applicable technique for critical patients is a hand-held microscope that can easily investigate the sublingual mucosal capillaries [59]. With this technique a tissue endomicroscopy is targeted at the mucosa after removal of all fluids (saliva, bowel content) and avoidance of pressure on the mucosa. It is recommended to measure five different areas for 20 s with steady image. The filmed fragments are then analyzed for patterns of microvascular flow and particularly the presence of stopped or intermittent flow. Various parameters have been described in literature, including vessel diameter, vessel length, capillary perfusion patterns (no flow, intermittent flow, sluggish flow or continuous flow), and vessel density [58,60].

It is unclear whether measuring sublingual perfusion will be of value in occlusive mesenteric ischemia. There is data that suggests that sublingual perfusion is closely related to gastrointestinal perfusion. Animal studies in shock using tonometry found significant relations between sublingual and gastric perfusion [61,62]. Patients with CMI may suffer of systemic microcirculation disturbances, therefore a correlation with a decrease in sublingual oxygen saturation, as captured by the Cytocam-incidental dark field illumination handheld microscope ([63]), can theoretically be possible in patients with CMI with decreased mucosal oxygen measurements indicating impaired intestinal perfusion and is currently investigated. We could identify two studies that looked at gastrointestinal microcirculatory patterns in human patients. Kara et al. studied 22 septic patients with confocal microscopy of both sublingual and intestinal mucosa. The latter could be obtained because all 22 had either an ileostomy or colostomy. It was observed that during shock and resuscitation of postoperative septic patients, the response of sublingual and intestinal microcirculation differed, where the former followed generalized perfusion failure and restoration. Finally, a distorted perfusion pattern in small bowel villi was associated with adverse outcome.

Schmidt et al. studied 10 septic patients using an endoscope-based technique. The imaging probe was introduced through the working channel of any standard endoscope. The probe is attached to a laser scanning unit, and an acquisition and image analysis device [60]. The patients were injected with 1 ml fluorescein 10% i.v. 10 min before measurement. They observed a reduction in number of perfused vessels as well as their diameter in septic patients compared to healthy controls. Further developments, including image stabilization [64] might make the technique clinical feasible. Still, studies in occlusive mesenteric ischemia are lacking. A pilot study is currently initiated by our groups.

#### Mitochondrial PO<sub>2</sub> measurements

Another approach is to measure oxygen at the site where it is utilized, the mitochondria. Mik et al. introduced the protoporphyrin IX-triplet state lifetime technique for measuring PO<sub>2</sub> in mitochondria (mitoPO<sub>2</sub>) [65]. The technique resulted in the development of the COMET monitor, a clinical monitor for assessment of Cellular Oxygen METabolism, which allows cutaneous mitoPO<sub>2</sub> measurements to be made in humans [66]. This non-invasive technique is not yet tested in the stomach and small intestine. If it is possible to measure mitochondrial oxygen tension in the gastrointestinal tract, for example during endoscopy, mitoPO<sub>2</sub> measurements could be used in the work-up of CMI. Whether it will be an improvement over

VLS remains to be determined. A pilot study is currently initiated by our groups.

#### *Volatile organic compounds (VOC's)*

An interesting development in the diagnostic arsenal is measurement of exhaled VOC's. Pathological conditions often cause metabolic changes in the body resulting in measurable changes in the blood. At the lung surface, there is an intensive exchange of organic compounds between the blood and the air in the lungs. They are referred to as volatile organic compounds (VOC's). Using exhaled air as potential diagnostic indicator is an emerging activity in detection of malignancies, infections and inflammatory bowel disease [67–70]. There are two animal studies to VOC detection of the changes in metabolic activity following ischemia. The first used an occlusive model (SMA occlusion) and detected various VOC's during ischemia [71]. A second study studied the effects of hypovolemic shock in VOC patterns in rats and detected different molecules in early ischemia, reperfusion and during recovery [72]. A pilot study to VOC detection for mesenteric ischemia is currently initiated by our groups.

#### *Mesenteric and hepatic blood flow*

Another approach to functionally test the bowel's perfusion would be to measure blood flow under fasting and postprandial conditions. The normal fasting bowel receives an average blood flow of 1000 ml/min. After meals this blood flow can double. Theoretically, it would be interesting to see whether blunted postprandial increase, or lower basal blood flow could be used as indicator for CMI.

#### *Magnetic resonance imaging*

Less invasive alternatives to measure GI blood flow response to meals would be to measure PV blood flow using MRI. MRI can be used to non-invasively measure both the SMA, SMV and PV blood flow. The PV is the common outflow vein from both CA and SMA. Therefore, insufficient mesenteric perfusion should be detectable by MRI. The variation in basal blood flow varies widely [73]. After meals the PV and SMV flow increases. The 250% increase in SMV flow seems the main determinant of the 70% increase in PV flow after meals [74]. The latter increase was later confirmed in a study in healthy subjects, where the increase in PV after a meal was mean 800 ml (66% of baseline) with 95% CI of 570–1030 ml/min [75]. In patients with CMI the mesenteric blood flow is suspected to increase less postprandially due to the hemodynamically significant stenosis of the mesenteric vessels. Currently the feasibility to assess the hemodynamics of the mesenteric arteries and portal vein using MR flow measurements is investigated by the Rotterdam group and seems a promising technique.

#### **Summary**

Functional ischemia tests are crucial for patients with single-vessel and non-occlusive mesenteric ischemia. The two functional tests tonometry and visible light spectroscopy have important limitations including availability, accuracy and complexity. New tests that are currently under development include balloon-less tonometry, endomicroscopy of microcirculation, mitochondrial PO<sub>2</sub> measurement and VOC detection.

#### **Conflicts of interest**

None.

#### **Practice points**

- The diagnostic approach in patients referred for evaluation of possible chronic mesenteric ischemia focuses on identification of gastrointestinal arterial stenosis by CT-angiography or MR-angiography and demonstration of mucosal ischemia.
- Assessment of gastrointestinal mucosal perfusion adequacy is performed by means of a functional test: Tonometry or Visible Light Spectroscopy.
- Exercise tonometry and 24-hour tonometry are established techniques that measure increased PCO<sub>2</sub> levels during ischemia, as induced by exercise or a meal, by a balloon-tipped catheter placed via the nose in stomach and small bowel.
- Visible Light Spectroscopy is a relatively new technique that enables non-invasively measurements of mucosal capillary hemoglobin oxygen saturations during upper endoscopy by a fiberoptic catheter-based oximeter that can be passed through the accessory channel of the endoscope.

#### **Research agenda**

- Balloon-less tonometry.
- VLS measurement after luminal feeding stimulation.
- Intra- and inter-observer variability of VLS measurement.
- Endomicroscopy of microcirculation.
- Mitochondrial PO<sub>2</sub> measurement.
- Volatile organic compounds detection.
- Hemodynamics of the mesenteric arteries and portal vein using MR flow measurements.

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