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Vascular diseases of the gastrointestinal tract

Jihan Harki

Abbreviations
A-M

ADL	activities of daily life
ALT	aminotransferases
Akt	protein kinase B
APA	antiphospholipid antibody
BMI	body mass index
CA	celiac artery
CACS	celiac artery compression syndrome
CGI	chronic gastrointestinal ischemia
CI	confidence interval
CRP	C-reactive protein
C-statistic	concordance statistic
CTA	computed tomography angiography
CT-scan	computed tomography scan
CVD	cardiovascular disease
DEXA	dual energy X-ray absorptiometry
DM	diabetes mellitus
DDD	defined daily dose
DVT	deep vein thrombosis
ER	emergency room
ES	essential thrombocytosis
EVL	endoscopic variceal ligation
FOX04	forkhead transcription factor 04
FVL	factor V Leiden
FVIII	factor VIII
GET	gastric exercise tonometry
GEVB	gastro-oesophageal variceal bleeding
GI	gastrointestinal
GSK3B	glycogen synthase kinase 3B
HA	hepatic artery
HE	hepatic encephalopathy
HIF-1α	hypoxia inducible factor-1α
IBD	inflammatory bowel disease
IC	ischemic colitis
ICU	intensive care unit
IMA	inferior mesenteric artery
IQR	interquartile range
IV	intravenous
LDH	lactate dehydrogenase
MFIa	microvascular flow index of all vessels
MFIs	microvascular flow index of small vessels
MPN	myeloproliferative neoplasm
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging

Vascular diseases of the

Vasculaire ziekten van het

gastrointestinal tract

gastrointestinale stelsel

Jihan Harki

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Vascular Diseases of the Gastrointestinal Tract
Vasculaire ziekten van het gastrointestinale stelsel

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copromotoren
Dr. E.T.T.L. Tjwa
Dr. D. Leemreis-van Noord

Contents

General introduction

Adapted from
Jihan Harki, Eric T.T.L. Tjwa and Désirée van Noord, Functional testing in the diagnosis of chronic mesenteric ischemia, *Mesenteric Vascular Disease: Current Therapy*. New York, Springer, 2015:63-77

Chronic gastrointestinal ischemia

Chronic gastrointestinal ischemia (CGI) results from insufficient blood supply to the stomach, small intestine and colon. In most cases this is caused by stenosis of the supplying arteries with lack of sufficient collateral circulation. Most often the stenosis is due to atherosclerosis[1], but other non-occlusive causes are also known[2,3]. Three direct branches of the abdominal aorta are responsible for the arterial blood supply of the gastrointestinal tract: the celiac artery (CA), the superior mesenteric artery (SMA) and the inferior mesenteric artery (IMA).

CGI is a diagnostic challenge. 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 population, as are stenoses of the mesenteric arteries. Development of mucosal perfusion assessment techniques may be of additional diagnostic value in identifying and grading the severity of mesenteric ischemia. These functional tests may help to select patients who will benefit from treatment by revascularization. Until recently it was thought that CGI could only occur in the presence of minimal two occluded mesenteric arteries, but studies on diagnostic strategies including functional tests have shown that a significant stenosis of a single artery with insufficient collateral circulation can also lead to clinically relevant mesenteric ischemia[4,5].

Therefore, a diagnostic approach combining assessment of clinical symptoms, radiological imaging of the mesenteric arteries and functional testing is recommended in the work-up of CGI suspected patients[6].

Diagnosis of CGI

The current established approach for diagnosing CGI is based on three main components. The first includes assessment of medical history, clinical symptoms and physical examination. The second component concerns radiological imaging of the mesenteric arteries and the third aims at detection of mucosal ischemia by means of a functional test[6,7]. All patients and procedures will be discussed in a dedicated multidisciplinary team consisting of a vascular surgeon, intervention radiologist and gastroenterologist, all specialized in CGI, leading to a final expert-based consensus diagnosis[6,8,9].

The diagnosis CGI is made if patient fulfils 2 of the 3 following criteria[9]:

1 Distinctive clinical presentation including presence of post-prandial pain and otherwise unexplained weight loss of >5% of the normal body weight.

2 Significant stenosis of >70% of at least one of the mesenteric arteries demonstrated by radiological evaluation.

3 Mucosal ischemia detected by tonometry or visible light spectroscopy (VLS).

A definitive diagnosis of CGI is made after persistent relief of symptoms on follow-up after treatment. This is not the ideal gold standard, however it is currently the most reliable way to establish CGI.

Visible light spectroscopy

Background

Functional tests such as gastric exercise tonometry (GET) and 24-hour tonometry are both accurate for the detection of CGI[1,4,10,11]. Unfortunately, the wider use of tonometry is hampered by its cumbersome and invasive nature, therefore the need for a better, more patient-friendly test remained. This led to the development of VLS. VLS, also known as reflectance spectrophotometry, enables direct measurement of the adequacy of mucosal perfusion[12]. It is a relatively new technique that non-invasively measures capillary

hemoglobin oxygen saturation using white light delivered by a fiberoptic probe during endoscopy. The marked difference in the absorption spectra of oxygenated and deoxygenated hemoglobin makes direct measurement of the percent saturation of the mucosal hemoglobin possible. Using real-time signaling, artifacts as those caused by scattering can also be eliminated [12]. Connected to a device, continuous display of the mucosal oxygen saturation on a screen is possible [9,12].

VLS has been used for evaluation of intensive care patients, assessment of anastomotic strength in esophageal and colorectal anastomoses and to determine microvascular perfusion during reconstructive surgery [13-15]. It can also increase endoscopic detection of mesenteric tumors [16].

Furthermore, VLS appears to be of great value as a new and less invasive diagnostic tool in patients suspected of CGI [9]. Since the measured oxygen saturation measurements reflect the adequacy of mucosal perfusion, events that decrease the delivery of oxygen to the mesenteric mucosa (i.e. mesenteric artery stenosis) will result in lower mucosal hemoglobin oxygen saturations [9]. VLS can easily be incorporated in diagnostic strategies as endoscopy is often performed early in the diagnostic work-up of these patients with abdominal pain.

Procedure

VLS is performed during upper endoscopy under conscious sedation [9,12]. Patients are not allowed to drink or eat before the procedure. Butylscopalamin is admitted intravenously before the start of VLS measurements in order to prevent luminal spasms and optimize the readings. Peripheral oxygen saturation and heart rate are continuously monitored and to minimize the effect of confounding factors of concomitant cardiopulmonary diseases peripheral saturation should be above 94%. If necessary, oxygen (FiO2 21%) can be administered.

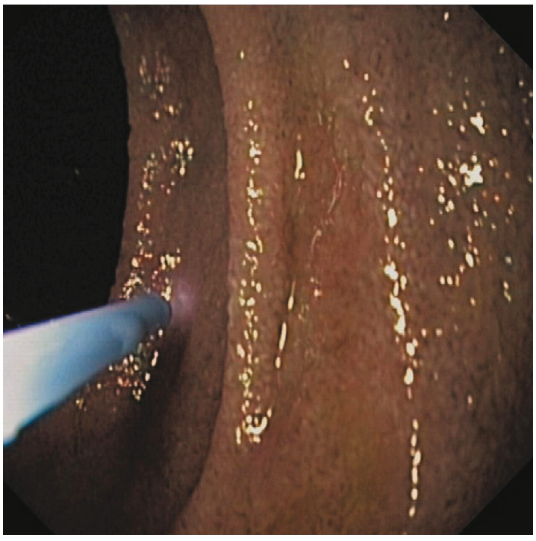
The VLS measurements are performed using a fiberoptic catheter-based oximeter that can be passed through the endoscope.

After irrigation of the target area to remove bile remnants, point-measurements of the oxygen saturation are performed at three locations: antrum of the stomach, duodenal bulb and descending duodenum. The probe is positioned approximately 1-5 mm above the mucosa ⊡ **1**. Once a stable reading is obtained with less than 5% variation in read-out as seen on the display, the actual measurement can be performed. Three repeated readings will be taken of each location. The average of the three readings per location will be regarded as the most accurate reflection of mucosal oxygen saturation of that specific location.

Based on the cut-off values determined in a large cohort study in CGI suspected patients, measurements are positive for ischemia when the measured saturation is:

- < 63% in the antrum and/or,
- < 62% in the duodenal bulb and/or,
- < 58% in the descending duodenum[9].

⊡ **1** Mucosal oxygen saturation using VLS. The probe is placed 1-5mm directly above the mucosa of the stomach and duodenum



Diagnostic value of VLS

VLS is a validated diagnostic method to correctly detect CGI with a sensitivity and specificity of 90% and 60% respectively[9]. The high sensitivity and low specificity are the consequence of the established cut-off values for each of the specific sites of the mesenteric tract as calculated by van Noord et al[9]. These cut-off values were based on a trainee data set of patients diagnosed with CGI using mesenteric tonometry and were additionally validated in a confirmation cohort.

With these cut-off values, the ability to distinguish patients with CGI from those without is the highest and no patients with CGI were missed, as earlier studies have shown that undiagnosed and untreated patients with ischemia have higher morbidity and mortality rates[1,7]. However, the higher sensitivity is at the expense of the specificity resulting in higher rate of false-negatives.

After successful intervention, improved VLS measurements can be observed in patients with CGI[9]. Among the patients with relief of symptoms one year after intervention, 80% showed improved or even normalization of the oxygen saturation measurements by VLS measurements. At the same time, in all patients with persistent symptoms after intervention no improvement in the oxygen saturation measurements was observed[9]. Furthermore, selection for treatment based on standardized diagnostic work-up including radiological imaging and oxygen saturation measurements by VLS accommodates for a sustained response in 70% of the patients with the clinical suspicion of CGI[17].

Chronic gastrointestinal ischemia and liver injury

Hypoxic liver injury in an acute setting of cardiac, circulatory or respiratory failure, also known as hypoxic or ischemic hepatitis, is caused by insufficient oxygen uptake by hepatocytes and is usually defined as a sharp increase in serum aminotransferases (ALT) up to 20 times the upper limit of normal due to centrilobular hepatocyte necrosis in the absence of other potential causes of hepatitis[18]. It occurs in up to 20% of critically ill patients with low cardiac output and has a high 30-day mortality rate of 50%[19]. It is unknown whether hypoxic liver injury only pertains to the acute or also to the chronic setting. Chronically compromised mesenteric arterial flow is hallmark of CGI[6]. The main cause of CGI is significant stenosis (≥70%) at the level of the origin of the mesenteric vasculature (CA and/or SMA) due to atherosclerosis, but also non-occlusive mesenteric ischemia (NOMI) due to cardiac forward failure may be observed. It is not known to what degree liver injury occurs in patients with CGI.

Chronic gastrointestinal ischemia and portal vein thrombosis

Portal vein thrombosis (PVT) is a infrequent vascular disorder caused by deposition of blood clot in the extra-hepatic portal vein. Etiological factors include systemic and local prothrombotic factors such as portal vein stasis in the presence of liver cirrhosis. PVT can be classified as either acute or chronic, depending on the presence of a portal cavernoma[20,21]. A feared complication of PVT is gastrointestinal ischemia. Gastrointestinal ischemia can result in intestinal infarction, a life-threatening complication that often requires immediate surgical intervention[22]. Treatment of acute PVT therefore aims at vascular recanalization, preventing the occurrence of gastrointestinal ischemia and portal hypertension[20]. The actual prevalence of gastrointestinal ischemia in patients with PVT is unknown. Studies, mainly conducted in patients with acute PVT, report prevalences of intestinal infarction of 2%-32%, with mortality rates of 0%-20%[21,23-27]. Regarding patients with chronic PVT, studies report conflicting results. As patients with chronic PVT often have an extensive venous collateral circulation, it is assumed that gastrointestinal ischemia is less likely to occur[22]. However, in a large prospective study in splanchnic vein thrombosis, 26% of patients presented with intestinal infarction. In nearly half of these patients a portal cavernoma was detected, suggesting gastrointestinal ischemia is also a frequent complication in chronic PVT[24].

Vascular diseases and the liver

Portal hypertension is defined as an increase in porto-systemic pressure gradient in any portion of the portal venous system. A major cause of portal hypertension is liver cirrhosis. A severe complication of portal hypertension is gastroesophageal variceal bleeding (GEVB). There is a high chance of rebleeding in these patients, which is associated with high morbidity and mortality[28,29]. Therefore, management should be directed at the prevention of rebleeding. Currently, the secondary prevention of GEVB is endoscopic treatment with variceal ligation (EVL) for esophageal varices and N-butyl cyanoacrylate injection for gastric varices, in combination with β-blocker therapy. When this fails, transjugular intrahepatic portosystemic shunt (TIPS) placement is the recommended as the second-line therapy[30]. Recent studies have shown that TIPS is more effective in reducing the risk recurrent variceal bleeding, but is associated with a higher risk of hepatic encephalopathy and does not improve survival[31,32].

However, next to clinical effectiveness, it is important to evaluate costs in order to fully determine which treatment is more superior as a secondary prevention therapy for variceal rebleeding. In these times when healthcare is getting increasingly expensive and more focus is on economic viability of new treatments, cost studies provide insight into the distribution of costs and thus cost reducing measures.

Aims and outline

The aim of this thesis is to explore various aspects and diagnostic methods for the detection of CGI, by means of functional testing using VLS, a prediction model for CGI, and hypoxia-inducible factor-1α (HIF-1α) as a marker for CGI. This is followed by studies of CGI due to portal vein thrombosis and CGI induced liver injury. Furthermore, treatment of other vascular diseases of the gastrointestinal tract such as variceal bleeding, are discussed.

In the standard work-up for patients suspected of CGI, mucosal oxygen saturation measurements using VLS are performed in fasting conditions. However, detection of CGI using VLS could be optimized using food as provocation. It is known that patients with CGI with a 70% to 99% stenosis of the superior mesentery artery have significantly lower increase in postprandial peak systolic velocity[33]. Due to this lack of postprandial increase in the splanchnic blood flow, not enough oxygen can be delivered to provide for the metabolic demand of the gastrointestinal tract and consequently resulting in gastrointestinal mucosal ischemia. Therefore, stimulation with food with a high caloric content will lead to an increased metabolic demand of the gastrointestinal tract and subsequently require a higher blood flow which cannot be met in patients with gastrointestinal ischemia and could result in different, possibly lower oxygenation saturation measurements of the gastrointestinal tract using VLS. In **chapter 2** the predictive value and the diagnostic ability of postprandial mucosal saturation measurements using VLS is described. Furthermore, several studies have shown that microcirculatory alterations are related to hemodynamic changes which have been reported to impair intestinal perfusion[34-36]. In these patients, the sublingual capillary perfusion seems well correlated to the hypoxic state of the gastric mucosa[37]. In **chapter 3** we study the association between sublingual microcirculatory alterations, as triggered by luminal feeding stimulation, and chronic hemodynamic changes such as in patients with CGI.

The role of clinical symptoms alone in the diagnosis of CGI is limited[10,38]. The combination of postprandial pain, weight loss and presence of an abdominal bruit is known as the 'classical' triad of CGI. This is however only present in 16-21% of patients and has limited predictive value for diagnosing CGI[10,39,40]. In **chapter 4** we present a prediction model for CGI, based on the combination of medical history, symptoms and radiological evaluation of the mesenteric arteries.

Currently, histological examination of biopsy material plays no definitive role in the diagnosis of CGI. A histological pathognomonic marker for CGI would be of great value as a non-invasive diagnostic method. HIF-1α is a significant intracellular regulator of oxygen hemostasis and is expressed in cells under low oxygen tension[41]. Expression of HIF-1α in gastric or intestinal tissue may thus identify patients with CGI. In **chapter 5** we performed a pilot study to investigate the expression of HIF-1α in chronic ischemic tissue by immunohistochemistry.

Hypoxic liver injury occurs in up to 20% of critically ill patients in an acute setting of cardiac, circulatory or respiratory failure[19]. It is unknown whether hypoxic liver injury only pertains to the acute or also to the chronic setting. In **chapter 6** we aimed to determine the incidence of hypoxic hepatitis in a well-defined cohort of CGI patients.

Gastrointestinal ischemia is a feared complication of PVT. In **chapter 7** we describe the clinical presentation and characteristics of gastrointestinal ischemia in patients with PVT.

Gastroesophageal variceal bleeding (GEVB) is a severe complication of portal hypertension. There is a high chance of rebleeding in these patients, which is associated with severe morbidity and mortality. In **chapter 8** we focus on the quality of life and cost-effectiveness of treatment with EVL and TIPS in patients with GEVB.

Finally, in **chapter 9** and **chapter 10** the conclusions of this thesis and suggestions for further research are discussed.

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Postprandial mucosal saturation measurements using endoscopic visible light spectroscopy in patients with gastrointestinal ischemia and healthy controls: a proof of concept study

Jihan Harki, Louisa J.D. van Dijk, Désirée van Noord, Eric T.T.L. Tjwa, Ernst J. Kuipers , Marco J. Bruno

Submitted

Abstract

Background

Chronic gastrointestinal ischemia (CGI) is the result of decreased mucosal perfusion, in most cases due to atherosclerotic stenosis of the supplying gastrointestinal arteries. Visible light spectroscopy (VLS) enables direct measurement of mucosal oxygenation saturation during upper endoscopy. No data is available on the hemodynamic changes using VLS in patients with CGI after intake of food. In this proof of concept study, we aimed to investigate the effect of food administration on VLS measurements in patients with CGI and healthy controls.

Methods

Consecutive patients with the diagnosis of CGI and healthy controls were prospectively included between September 2014 and August 2015. All patients received the standard work-up for CGI, consisting of assessment of medical history and symptoms, radiological imaging of the gastrointestinal arteries, and VLS measurements. VLS measurements in postprandial state were compared to VLS measurements in fasting state.

Results

We performed 168 VLS measurements in 12 patients with CGI (median age 63.2 (IQR 48.8-70.4) years, 67% male) and 16 controls (32.9 (IQR 27.7-39.8.2) years, 44% male). There was no significant increase between postprandial and baseline VLS measurements in CGI patients (baseline antrum median 58.5 (IQR 53.5-59.0) vs postprandial antrum median 57.5 (IQR 54.0-60.8), p=0.25 ; baseline duodenal bulb median 53.0 (IQR 48.8-54.0) vs postprandial duodenal bulb median 53.5 (IQR 50.3-56.8), p=0.89; baseline descending duodenum median 49.5 (IQR 45.3-52.8) vs postprandial descending duodenum median 54.0 (IQR 47.3-57.8), p=0.33 , respectively). In the healthy controls, there was a trend towards higher VLS measurements in the postprandial state.

Conclusion

This is the first comparative prospective study showing the hemodynamic changes in patients with CGI and healthy controls after stimulation with food using VLS. We found no difference in VLS measurements before and after caloric challenge in patients with CGI, however there was a trend in increase in VLS measurements in the healthy controls. The current results show insufficient discrimination between patients with CGI and healthy controls.

Introduction

Chronic gastrointestinal ischemia (CGI) results from insufficient blood supply to the gastrointestinal tract, in most cases caused by atherosclerotic stenosis of the supplying arteries[1].

The diagnosis of CGI remains a challenging quest. This is firstly because the role of clinical symptoms alone in the diagnosis of CGI is limited[2,3]. Furthermore, there is no single test with high sensitivity and specificity to diagnose or exclude this condition. Currently, a diagnostic approach combining assessment of clinical symptoms, radiological imaging of the gastrointestinal arteries and functional testing is recommended in the work-up of patients with the clinical suspicion of CGI[1]. Functional tests, such as tonometry and visible light spectroscopy (VLS), have been reported to be reliable tests in establishing gastrointestinal mucosal ischemia[3,9-11]. VLS enables direct measurement of mucosal haemoglobin oxygen saturations during upper endoscopy[4]. It is a validated diagnostic method and has a sensitivity of 90% for the detection of CGI[5].

Currently, VLS measurements are performed in a fasting state during endoscopy. However, studies have shown that patients with CGI may have less increase in splanchnic blood flow after oral caloric stimulation compared to healthy subjects leading to mucosal ischemia of the gastrointestinal tract. Consequently, this leads to the presence of postprandial pain, a typical presenting symptom for CGI[3,6-9]. Therefore, the hypothesis rises that mucosal ischemia only occurs postprandially, in response to an increased metabolic demand, or exercise related, indicating a time-dependent relation. Consequently, patients with CGI with less impaired mesenteric blood flow could thus show low-normal or even normal mucosal saturation measurements in this fasting situation and can therefore be missed using VLS.

In this proof of concept study, we aimed to investigate the effect of food administration on mucosal oxygenation measured by VLS in patients with CGI and healthy controls. Assuming mucosal ischemia occurs when metabolic demand exceeds the oxygen

supply during postprandial state, we hypothesize that VLS measurements after stimulation with food results in different, possibly lower VLS measurements in patients with CGI compared to healthy subjects.

Methods

Study population

Patients with the diagnosis of CGI were prospectively and consecutively included between September 2014 to August 2015. All patients received the recommended work-up of CGI consisting of thorough assessment of medical history and clinical symptoms, radiological imaging using computed tomography (CT)- angiography, or in case of contra-indications magnetic resonance (MR)- angiography, and functional testing by VLS. All patients and assessment results were discussed in a multidisciplinary team consisting of vascular surgeons, intervention radiologists and gastroenterologists, all experienced in the management of CGI. This yielded a final expert-based consensus diagnosis in each case. The diagnosis CGI was established if a patient fulfilled two of the three following criteria: (a) distinctive clinical presentation including presence of postprandial pain and otherwise unexplained weight loss of >5% of the normal body weight, (b) significant stenosis of >50% of at least one of the mesenteric arteries, and (c) functional signs of mucosal ischemia demonstrated by VLS. In case of one-vessel disease, three of the above criteria has to be fulfilled for the diagnosis of CGI Mucosal ischemia by VLS was defined as mucosal oxygen saturation <58%, <62%, or <63% for measurements in the duodenum, duodenal bulb and the antrum of the stomach respectively. The definitive diagnosis of CGI was made after sustained relief of symptoms during follow-up in treated patients.

Non-smoking healthy subjects with patent gastrointestinal arteries as determined by abdominal duplex ultrasound, no gastrointestinal complains, and unremarkable medical history were asked to participate in our study on voluntary basis.

Patients with history of gastric (bypass) surgery, cardiac arrhythmias or cardiac conduction disease, or who were unable to give informed consent were excluded from the study. Approval for the study was obtained from the Institutional Review Board of the Erasmus MC University Medical Centre Rotterdam, the Netherlands.

VLS measurements
We performed mucosal oxygen saturation measurement with VLS in CGI suspected patients and healthy volunteers at baseline (standard care) and after stimulation with luminal feeding.
In standard fashion patients receive midazolam 2.5-5 mg intravenously and combined with fentanyl 0.05 mg for sedation. To prevent luminal spasms butylscopalamine 20 mg is admitted intravenously before the start of VLS measurements. As to minimize the effect of confounding factors of cardiopulmonary diseases we aim to keep peripheral oxygen saturation >95% by administering oxygen intra-nasally to every patient and systemic oxygen saturation is monitored by pulse oximetry.

The baseline VLS measurements were performed during upper endoscopy using a fiberoptic catheter-based oximeter (T-Stat 303 Microvascular Oximeter, Spectros, Portola Valley, California, USA), which can be passed through the accessory channel of the endoscope. Measurements of the oxygen saturation were performed at three sites in the stomach and duodenum: antrum of the stomach, descending duodenum and duodenal bulb. After irrigation of the target area and positioning the probe approximately 1-5 mm above the mucosa, three repeated readings were taken of different areas of each location once a stable reading is obtained with less than 5% variation in panel read-out. The average of the three readings per location is regarded as the actual measurement of that specific location.

Subsequently, a feeding tube was endoscopically placed in the antrum. Over the feeding tube, patients were given 300 ml (= 450 kcal) compound liquid food (Nutrison Energy ®, Nutricia Koninklijke Numico N.V., WTC Schiphol, the Netherlands), a solution of carbohydrates, protein and fat with a low volume and a high caloric content. Patients were seated in upright position while the receiving the liquid compound food and they were monitored extensively.

Simultaneous to the luminal feeding stimulation, patients received erythromycin 250 mg in order to improve motility and clear gastric contents more rapidly.

The postprandial VLS measurements were performed 60 minutes after the baseline VLS measurements and were done the same fashion as described previously. In case of liquid food remnants in the stomach, these were thoroughly removed by irrigation and suction first before performing the VLS measurements.

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Data were expressed as mean and standard deviation (SD), median and interquartile range (IQR), or count and percentage (%), when appropriate. Mann-Whitney test was applied to calculate the differences in baseline and postprandial VLS measurements between patients with CGI and healthy volunteers. Wilcoxon signed rank test was performed to study the differences between baseline and postprandial VLS measurements in CGI patients and in healthy volunteers.

Statistical analysis was performed using SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). A p-value of <0.01 was considered significant in order to correct for multiple endpoints (antrum, duodenal bulb and descending duodenum).

Results

Patient characteristics

During the study period, 49 consecutive patients with the clinical suspicion of CGI were eligible for the study. Informed consent was obtained from 31 (63%) patients. Reasons for non-participation were mostly poor condition of the patient or anxiety to undergo multiple upper endoscopies. Three (6%) patients failed to complete this work-up, leaving a total of 28 (57%) patients. The results of the diagnostic work-up of 28 patients in the study were discussed in a multidisciplinary expert panel. A consensus diagnosis of CGI was made in 16/28 (57%) patients and in 12/28 (43%) patients CGI was ruled out (see ⊙ **1**, flowchart of study).

During follow-up (median 3.0 (IQR 1-6) months), two patients were lost to follow-up and in two patients the diagnosis of Non-Occlusive Mesenteric Ischemia (NOMI) had to be rejected due to an alternative diagnosis, leaving a total of 12 patients with CGI (11 patients with occlusive CGI and one patient with NOMI).

Intervention was offered to all 11 patients with occlusive ischemia, which consisted of endovascular treatment for seven patients with atherosclerotic stenosis of the gastrointestinal arteries and surgical treatment for four patients diagnosed with Celiac Artery Compression Syndrome (CACS). The patient diagnosed with NOMI received vasodilatory medication. All patients had sustained relief of symptoms after symptoms. Thus, in total 12 patients were diagnosed with a definitive diagnosis of CGI. For characteristics of patients and volunteers see ☰ **1**.

Baseline and postprandial VLS measurements

The baseline VLS measurements in patients with CGI were below the established cut-off values for that specific site in the gastrointestinal tract, indicating mucosal ischemia: gastric antrum median 58.5 (IQR 53.5-59.0), duodenal bulb median 53.0 (IQR 48.8-54.0), and descending duodenum median 49.5 (IQR 45.3-52.8). In the controls, these values were significantly higher in the gastric antrum (median

61.0 (IQR 59.3-63.0); p=0.007) and in the duodenal bulb 54.0 (IQR 49.0-56.0); p=0.003), but not in the descending duodenum (median 54.0 (IQR 49.0-56.0); p=0.04) ⊡ **2**.

Postprandial VLS measurements showed a significant difference in only the measurements in the duodenal bulb (median 53.5 (IQR 50.3-56.8) for CGI patients vs median 59.5 (IQR 54.8-61.5) for the controls, p=0.004) ⊡ **3**.

Despite a trend to an increase in VLS measurements after stimulation with food, there was no significant increase between postprandial and baseline VLS measurements in patients with CGI (baseline antrum median 58.5 (IQR 53.5-59.0) vs postprandial antrum median 57.5 (IQR 54.0-60.8), p=0.25; baseline duodenal bulb median 53.0 (IQR 48.8-54.0) vs postprandial duodenal bulb median 53.5 (IQR 50.3-56.8), p=0.89; baseline descending duodenum median 49.5 (IQR 45.3-52.8) vs postprandial descending duodenum median 54.0 (IQR 47.3-57.8), p=0.33, respectively). In the healthy controls, a trend was observed towards higher postprandial VLS measurements in the descending duodenum (baseline descending duodenum median 58.0 (IQR 54.0-62.8) vs postprandial descending duodenum median 59.5 (IQR 54.8-61.5), p=0.03), but not in the antrum and duodenal bulb (baseline antrum median 61.0 (IQR 59.3-63.0) vs postprandial antrum median 63.0 (IQR 58.5-65.0), p=0.57, and baseline duodenal bulb median 54.0 (IQR 49.0-56.0) vs postprandial duodenal bulb median 56.0 (IQR 52.8-59.5), p=0.93, respectively).

There was no difference in Δ VLS after food stimulation between patients with CGI and controls (p=0.66 for the antrum, p=0.94 for the duodenal bulb, and p=0.98 for the descending duodenum, respectively) ⊡ **4**.

1 Flowchart of study

1 Baseline characteristics of CGI patients and controls

	CGI (n=12)	controls (n=16)	*p*-value[e]
age (mean, SD)	63.2 (48.8-70.4)	32.9 (27.7-39.8)	0.001
gender			0.2
male	8 (67)	7 (44)	
female	4 (33)	9 (56)	
BMI (kg/m²)	24.2 (21.5-28.3)	23.0 (20.7-24.9)	0.2
symptomatology			
abdominal pain	10 (83)	0	<0.001
postprandial pain	9 (75)	0	<0.001
exercise-induced pain	4 (33)	0	0.013
weight loss	7 (58)	0	<0.001
weight loss (kg/months)[a]	2.0 (1.1-3.0)	0	<0.001
diarrhea	2 (17)	0	0.09
nausea	5 (42)	0	0.004
duration of symptoms (months)[a]	10.0 (6.0-21.0)	0	0.004
cardiovascular comorbidity			
history of CVD	8 (67)	0	<0.001
hypertension	7 (58)	1 (6)	0.003
DM type II	3 (25)	0	0.03
hypercholesterolemia	6 (50)	0	0.001
family history of CVD	5 (42)	3 (19)	0.1
smoking[d]	10 (83)	0	0.04
pack years[a]	162 (0-356)	0	<0.001
alcohol use	6 (50)	11 (69)	0.3
alcohol (U/day)[b]	0 (0-1.8)	0.5 (0-1.0)	<0.001
mesenteric artery stenosis[c]			
no stenosis	1 (8)	16	<0.001
single artery stenosis	4 (33)	0	<0.001
multi artery stenosis	7 (58)	0	<0.001

values represent number and percentage (%) of patients unless otherwise specified

a median, interquartile range (IQR)

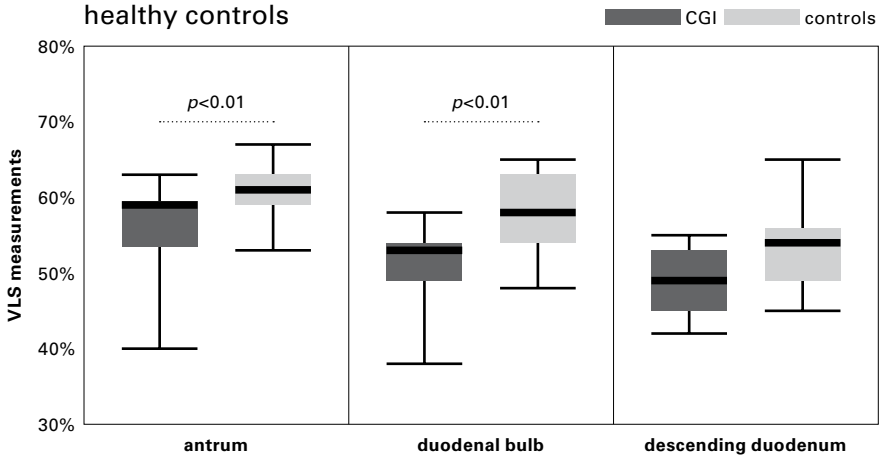
b median, range

c stenosis of the mesenteric arteries is defined as >70% lumen reduction on CT- or MR-angiography

d includes present and former smokers

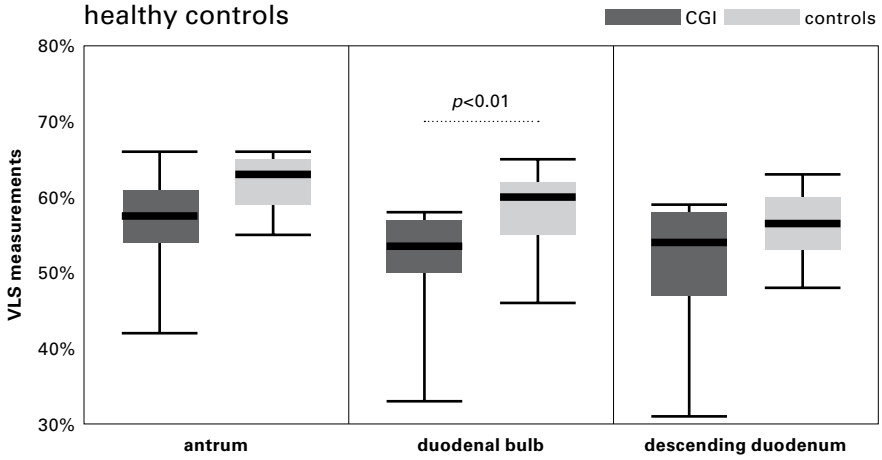
e *p*-value <0.01 was considered significant

⊙ 2 Baseline VLS measurements in patients with CGI and healthy controls



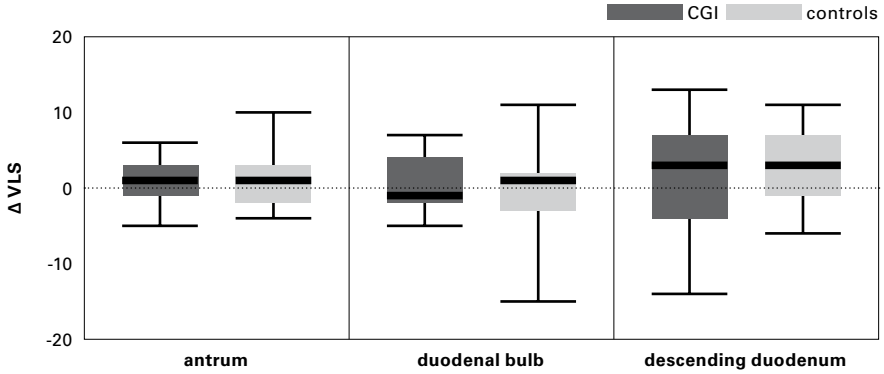
VLS measurements performed at three locations in the gastrointestinal tract for both patients and controls in the fasting state. VLS measurements were significantly decreased for patients with CGI compared to the controls in the gastric antrum and duodenal bulb, but not in the descending duodenum. Boxes represent medians with interquartile range, whiskers extend to the most and least extreme scores respectively.

⊙ 3 Postprandial VLS measurements in patients with CGI and healthy controls



VLS measurements performed at three locations in the gastrointestinal tract for both patients and controls in the postprandial state. VLS measurements were significantly decreased for patients with CGI compared to the controls in the duodenal bulb, but not in the gastric antrum and descending duodenum. Boxes represent medians with interquartile range, whiskers extend to the most and least extreme scores respectively.

4 Difference (Δ) between postprandial VLS measurements and baseline VLS measurements per location in the gastrointestinal tract in patients with CGI and in healthy controls



VLS measurements performed at three locations in the gastrointestinal tract for both patients and controls. Δ VLS was calculated as postprandial VLS minus baseline VLS. In both the patients as well as in the controls, there was no significant difference between postprandial and baseline VLS measurements in all locations of the gastrointestinal tract. Boxes represent medians with interquartile range, whiskers extend to the most and least extreme scores respectively. P-value <0.01 was considered significant.

Discussion

In this proof of concept study, we aimed to investigate the effect of caloric challenge on VLS measurements in patients with CGI and healthy controls. We found that there is no significant increase or decrease in VLS measurements after stimulation with food in patients with CGI whereas in healthy controls VLS measurements in the duodenal bulb rose significantly after food stimulation.

Currently VLS measurements are validated only in a fasting state. Measurements for the postprandial state have not been investigated and its diagnostic discriminatory potential to correctly identify those with CGI from healthy controls, and ultimately patients with abdominal complaints that are not caused by gastrointestinal ischemia, has not been assessed. Our hypothesis was that, contrary to healthy volunteers, metabolic demand in patients with CGI exceeds mucosal oxygen supply when calorically challenged. The ability to maintain or increase postprandial mucosal oxygenation might therefore offer a more sensitive means to diagnose patients with gastrointestinal ischemia. We found that there is no significant decrease in VLS measurements after stimulation with food in patients with CGI. However, in line with our hypothesis, we did observe higher VLS measurements in healthy controls suggestive of a physiological hyperemic response induced by food stimulation [1,10,11].

To our knowledge, this is the first study which quantitatively assesses gastrointestinal ischemia in the postprandial state using VLS. VLS measures capillary hemoglobin oxygen saturation and enables to objectively and quantitatively determine the presence and extent of gastrointestinal mucosal ischemia. It is a validated diagnostic method and has a sensitivity of 90% and specificity of 60% for the detection of CGI and has established cut-off values for each of the specific sites of the gastrointestinal tract, which are previously calculated by van Noord et al [5].

Although our original hypothesis was confirmed from a (patho)physiological point of view, showing a statistically signifi-

cant difference in postprandial duodenal bulb VSL measurements between patients with CGI and healthy volunteers, the numerical overlap is considerable making postprandial measurements not yet suitable for clinical use. For this, several issues need to be taken into consideration.

In this proof-of-concept study we tested a limited number of individuals. Inclusion of more patients and controls may increase the power to statistically differentiate between both groups, for example also by measurements of the antrum and duodenum.

VLS measurements in patients with CGI sometimes show substantial variation. Therefore, the average of three repeated measurements of each location is regarded as the most accurate reflection of the mucosal oxygen saturation of that location[5]. In addition, VLS is limited to point measurements. Mucosal ischemia might be patchy and could therefore be missed or underestimated. In addition, VLS measurements may be further hampered by food remnants. In case of liquid food remnants in the stomach, these were thoroughly removed by irrigation and suction before performing the VLS measurements. Simultaneous to luminal feeding, patients received erythromycin 250 mg in order to improve motility and clear gastric contents more rapidly. Other events that may hamper the VLS measurements are luminal spasms and bile acid remnants. Therefore, butylscopolamine was administered intravenously prior to the start of VLS measurements and the VLS measurements were performed after irrigation of the target area.

Studies have shown that the increase in abdominal discomfort in patients with CGI occurs after 15-30 minutes after digestion of food and can persist for one to three hours[2,10,12,13]. In our study, repeated VLS measurements were performed during this time range, that is after 60 minutes after caloric stimulation. This time period was chosen in order to minimize the effect of food remnants in the stomach and consequently to optimize the patient safety e.g. to reduce the risk of aspiration during the second upper endoscopy. However, it might be that the time point of the repeated VLS measurements were not optimally aligned with the time point at which

metabolic demands are maximal and hence mucosal oxygen supply most severely challenged. Therefore choosing a different time point for postprandial VLS measurements might enhance its discriminatory capacity.

This is the first study describing the hemodynamic changes in patients with CGI after stimulation with food using VLS. We found that there is no significant increase or decrease in VLS measurements after stimulation with food in patients with CGI whereas in healthy controls VLS measurements in the duodenal bulb rose significantly after food stimulation. Although these observations seem to confirm our initial hypothesis that indeed the physiological hyperemic response induced by food stimulation is impaired in patients with CGI, it currently provides to little discriminatory power for clinical use. Modifications of the VLS assessment protocol, in particular the timing of postprandial measurements, may improve its applicability.

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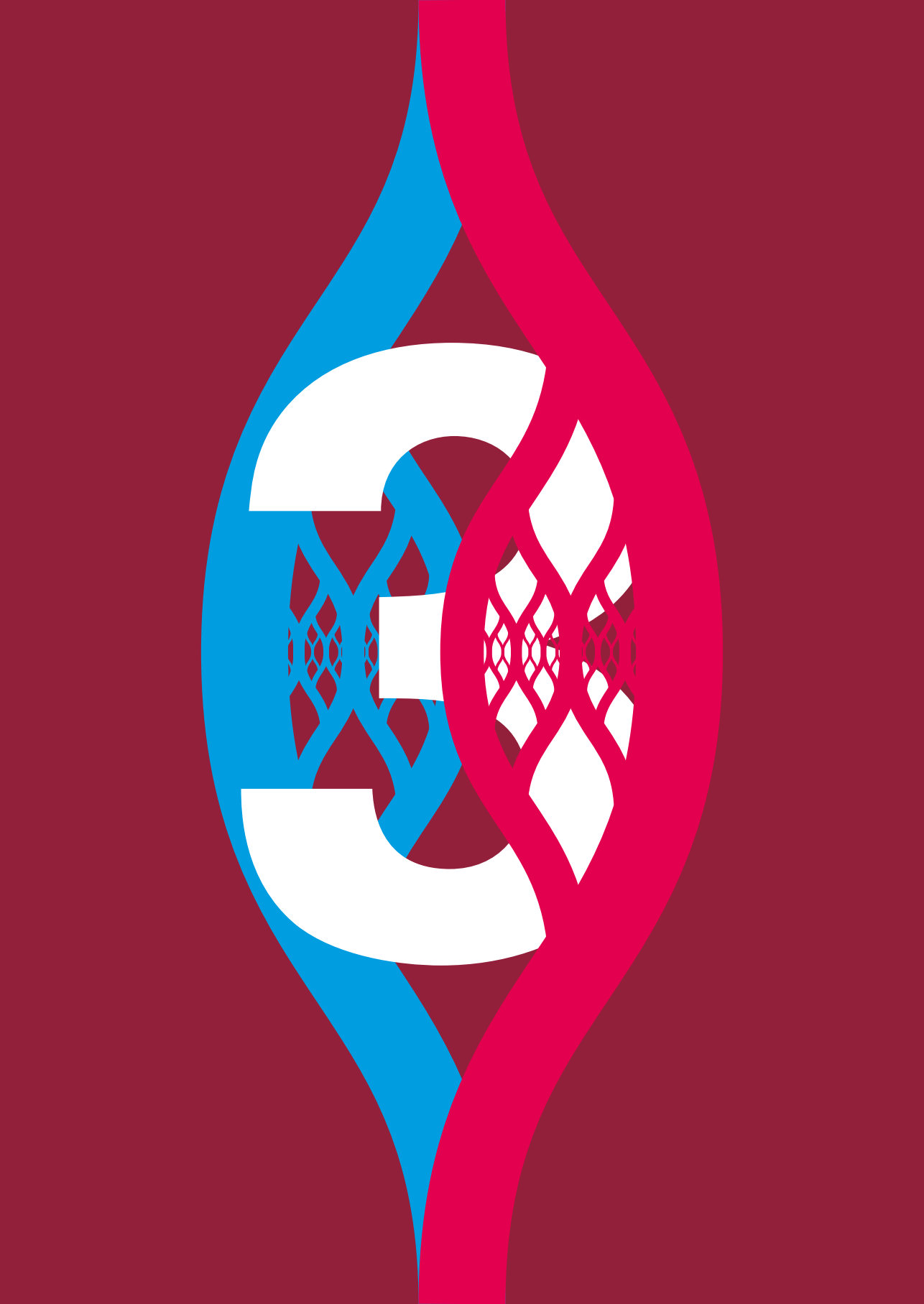


Patients with chronic gastrointestinal ischemia have an altered sublingual microcirculation

Jihan Harki, Mustafa Suker, M. Sherezade Tovar Doncel, Louisa J.D. van Dijk, Casper H.J. van Eijck, Marco J. Bruno, Ernst J. Kuipers, Can Ince

Submitted

Abstract

Background

Chronic gastrointestinal ischemia (CGI) is the result of insufficient blood supply to the gastrointestinal tract. The majority of CGI patients has systemic disorders of the circulatory system including hypertension, diabetes and other cardiovascular risk factors. Studies in patients with acute gastrointestinal ischemia, found a correlation between intestinal ischemia and sublingual microcirculatory alterations. However, little is known about the microcirculatory alterations in patients with CGI. We hypothesize that patients with CGI may suffer of systemic microcirculation disturbances. We further hypothesized that sublingual microcirculation is responsive to enteral caloric challenges and as such provides a means to indirectly assess the intestinal microcirculation.

Methods

Consecutive patients with CGI were prospectively included between September 2014 and August 2015. All patients received the standard work-up for CGI, consisting of assessment of medical history and symptoms, radiological imaging of the gastrointestinal arteries, and endoscopic visible light spectroscopy (VLS) for assessment of gastroduodenal mucosal oxygen saturation. The sublingual microcirculation was evaluated before (T0) and 20 minutes after enteral feeding[1]. Total vessel density (TVD (mm/mm^2)), perfused vessel density (PVD (mm/mm^2), proportion of perfused vessels (PPV (%)), microvascular flow index (MFI (AU)) were assessed.

Results

We included 12 consecutive patients with CGI. After caloric challenge, a significant increase in the PVD of the small (PVDs) vessels and all vessels (PVDa), and in the PPV of the small vessels (PPVs) was observed (PVDs median 16.3 (IQR 13.3-22.1) mm/mm^2 vs 19.9 (IQR 14.2-26.2) mm/mm^2, p=0.008; PVDa median 19.1 (IQR 16.2-23.6) mm/mm^2 vs 22.2 (16.5-28.9) mm/mm^2, p=0.02; PPVs median 84.8% (IQR 75.3-90.4) vs 91.0% (80.1-93.8), p=0.01).

Conclusion

Patients with CGI show significant alterations in the sublingual microcirculation after stimulation with food. This non-invasive tool may offer differential diagnostic opportunities to diagnose patients with CGI.

Introduction

Chronic gastrointestinal ischemia (CGI) is the result of insufficient blood supply to the stomach, small intestine and colon leading to decreased mucosal perfusion and oxygenation. In most cases this is caused by stenosis of the supplying arteries due to atherosclerosis, chronic heart failure and pulmonary conditions can further contribute or predominate (so-called non-occlusive chronic ischemia (NOMI))[2].

It is believed that patients with CGI have a lack of postprandial increase in the splanchnic blood flow after ingestion of food causing a misbalance between sufficient oxygen delivery and metabolic demand[3]. Hence the postprandial discomfort many CGI patients experience after ingestion of food[3]. Until recently it was thought that CGI could only occur in the presence of minimal two occluded mesenteric arteries, but studies have shown that a significant stenosis of a single artery can also lead to clinically relevant gastrointestinal ischemia[4,5]. In these patients with single-vessel CGI, the occurrence of symptoms is thought to be related to insufficient collateral circulation[2]. Another underlying cause might be that patients with CGI have microcirculatory alterations associated with systemic disorders of the circulatory system. The majority of patients with CGI is known with hypertension, diabetes and other cardiovascular risk factors[6]. Therefore, microvascular pathology in this patient group is very likely and might explain why single-vessel stenosis can lead to clinical symptoms even in presence of sufficient collateral circulation.

However, there is little insight regarding the microcirculatory alterations in patients with CGI. Several studies, mainly investigating patients in hemodynamic shock, found a correlation between intestinal ischemia and sublingual microcirculatory alterations[7-9]. These alterations consisted of decreased microvessel density and erythrocyte velocity which were similar in both the sublingual and gut regions. Furthermore, the sublingual capillary perfusion seemed well correlated to the hypoxic state of the gastric mucosa

in these patients[9-11]. This may be expected since the gastrointestinal tract and the sublingual microcirculation share a common embryogenic origin[7].

No studies have been performed to investigate the relationship between sublingual microcirculation and hemodynamic changes such as in patients with CGI. Based on these findings we formulated the hypothesis patients with CGI may also exhibit microcirculatory alterations. We hypothesize that sublingual microcirculation is responsive to enteral caloric challenges and as such provides a means to indirectly assess the intestinal microcirculation. Assessment of the sublingual microcirculation may provide a non-invasive diagnostic opportunity since it may ultimately provide a non-invasive tool to diagnose or refute the diagnosis of CGI in a more patient friendly way.

Methods

Study population

Patients with confirmed CGI were prospectively included between September 2014 and August 2015. All patients received the recommended work-up of CGI consisting of thorough assessment of medical history and clinical symptoms, radiological imaging of the mesenteric arteries using computed tomography (CT)- angiography, or in case of contra-indications magnetic resonance (MR)- angiography. Gastroduodenal mucosal oxygen saturation was further functionally tested by VLS. Patients work-up were discussed in a multidisciplinary team consisting of vascular surgeons, intervention radiologists and gastroenterologists, all experienced in the management of CGI. This yielded a final expert-based consensus diagnosis. A diagnosis CGI was made if a patient fulfilled two of the three following criteria:

1 distinctive clinical presentation including presence of postprandial pain and otherwise unexplained weight loss of >5% of the normal body weight;

2 significant stenosis of >50% of at least one of the mesenteric arteries;

3 functional signs of mucosal ischemia demonstrated by VLS.

Mucosal ischemia by VLS was defined as mucosal oxygen saturation < 58%, <62%, or <63% for measurements in the duodenum, duodenal bulb and the stomach, respectively. The diagnosis CGI was established if a patient fulfilled two of the three following criteria:

1 distinctive clinical presentation including presence of postprandial pain and otherwise unexplained weight loss of >5% of the normal body weight;

2 significant stenosis of >50% of at least one of the mesenteric arteries;

3 functional signs of mucosal ischemia demonstrated by VLS.

Mucosal ischemia by VLS was defined as mucosal oxygen saturation < 58%, <62%, or <63% for measurements in the duode-

num, duodenal bulb and the antrum of the stomach respectively based on previous assessments in patients and healthy controls. A definitive diagnosis of CGI was made after sustained relief of symptoms during follow-up in patients after appropriate treatment, which includes revascularization in patients with stenotic CGI and vasodilatory medication in patients with NOMI .

Informed consent was obtained from all patients. Approval for the study was obtained from the Institutional Review Board of the Erasmus MC University Medical Centre Rotterdam, The Netherlands.

Assessment of microcirculation

We measured the sublingual microcirculation using a Cytocam-IDF device ⊡ **1** [12]. The measurement was performed both before (T0) and 20 minutes after enteral feeding (T1). Patients received enteral feeding through an endoscopically placed feeding tube in the antrum. Over the feeding tube, patients were given 300 ml compound liquid food (Nutrison Energy ®, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands), a solution of carbohydrates, protein and fat with a low volume and a high caloric content (= 450 kcal). At each time point, microcirculation was filmed in three different locations on the sublingual mucosa for a minimum of 2 seconds per sequence as previous described [13]. Great care was taken to clear saliva before image acquisition. Film sequences were repeatedly taken until three sequences of good quality. The quality of the images were assessed using the Massey score for micro-circulation image quality [14]. Video-image analysis was performed blindly by one independent researcher according to protocol using Automated Vascular Analysis (AVA) 3.0 [15]. The following parameters were obtained: total vessel density (TVD (mm/mm^2), perfused vessel density (PVD (mm/mm^2), proportion of perfused vessels (PPV (%)), and microvascular flow index (MFI (AU)), respectively, of small vessels and all vessels.

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Data were expressed as median and interquartile range (IQR), or count and percentage (%), when appropriate. Wilcoxon signed rank test was performed to study the differences between baseline and postprandial parameters of the microcirculation in CGI patients. Statistical analysis was performed using SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). A two-sided *p*-value of <0.05 was considered significant.

1 The Cytocam-IDF captures the sublingual microcirculation

1 Baseline characteristics of CGI patients and controls

	CGI n=12
age (mean, SD)	63.2 (48.8-70.4)
gender	
male	8 (67)
female	4 (33)
BMI (kg/m²)	24.2 (21.5-28.3)
symptomatology	
abdominal pain	10 (83)
postprandial pain	9 (75)
exercise-induced pain	4 (33)
weight loss	7 (58)
weight loss (kg/months)[a]	2.0 (1.1-3.0)
diarrhea	2 (17)
nausea	5 (42)
duration of symptoms (months)[a]	10.0 (6.0-21.0)
cardiovascular comorbidity	
history of CVD	8 (67)
hypertension	7 (58)
DM type II	3 (25)
hypercholesterolemia	6 (50)
family history of CVD	5 (42)
smoking[d]	10 (83)
pack years[a]	162 (0-356)
alcohol use	6 (50)
alcohol (U/day)[b]	0 (0-1.8)
mesenteric artery stenosis[c]	
no stenosis	1 (8)
single artery stenosis	4 (33)
multi artery stenosis	7 (58)
VLS measurement	
antrum	58.5 (53.5-59.0)
duodenal bulb	53.0 (48.8-54.0)
descending duodenum	49.5 (45.3-52.8)

values represent number and percentage (%) of patients unless otherwise specified

a median, interquartile range (IQR)

b median, range

c stenosis of the mesenteric arteries is defined as >70% lumen reduction on CT- or MR-angiography

d includes present and former smokers

Results

Patient characteristics

During the study period, 49 consecutive patients with the clinical suspicion of CGI were eligible for the study. Informed consent was received from 31 (63%) patients. Reasons for non-participation were mostly poor condition of the patient or anxiety to undergo multiple investigations. Three (6%) patients failed to complete this work-up, leaving a total of 28 (57%) participants in the study. The results of the diagnostic work-up of the 28 patients in the study were discussed in a multidisciplinary expert panel. A consensus diagnosis of CGI was made in 16/28 (57%) patients whereas in 12/28 (43%) patients CGI was ruled out.

During follow-up (median 3.0 (IQR 1-6) months), two patients were lost to follow-up and in two patients the diagnosis of NOMI had to be adjusted leaving a total of 12 patients with CGI (11 patients with occlusive CGI and one patients with NOMI ☰ **1**).

Sublingual microcirculation

Absolute data and ranges for the microvascular variables of all patients are provided in ☰ **2**.

The PVD of the small vessels and all vessels significantly increased after caloric challenge (T1) compared to baseline (T0) (PVDs median 16.3 (IQR 13.3-22.1) mm/mm^2 vs 19.9 (IQR 14.2-26.2) mm/mm^2, p=0.008; PVDa median 19.1 (IQR 16.2-23.6) mm/mm^2 vs 22.2 (16.5-28.9) mm/mm^2, p=0.02) ☰ **3** ⊙ **2**. Furthermore, there is a significant increase in the PPV of the small vessels after caloric challenge (median 84.8% (IQR 75.3-90.4) vs 91.0% (80.1-93.8), p=0.01). This did not pertain to the PPV of all vessels (median 85.4% (IQR 79.8-91.5) vs 90.4% (IQR 81.4-94.4), p=0.05) ⊙ **3**. In addition, there was a trend towards significance in the increase in TVD of the small vessels after caloric challenge (median 20.8 (IQR 17.6-23.8) mm/mm^2 vs 22.4 (IQR 17.8-32.2) mm/mm^2, p=0.09).

≣ 2 Individual patient data of sublingual microcirculation

		microcirculation							
patient	**time point[a]**	**TVDs**	**TVDa**	**PVDs**	**PVDa**	**PPVs**	**PPVa**	**MFIs**	**MFIa**
1	0	19,13	24,22	17,19	22,25	90,50	92,27	2,83	2,94
	1	18,76	21,81	16,29	19,24	86,48	87,66	3,00	2,84
2	0	15,36	18,00	13,58	16,17	87,58	89,20	3,00	2,61
	1	13,87	15,01	13,32	14,46	95,97	96,17	3,00	2,90
3	0	17,64	21,08	13,27	16,71	75,34	79,35	2,67	2,56
	1	17,51	21,93	14,33	17,95	82,80	83,28	2,50	2,80
4	0	23,95	25,18	21,78	23,01	91,44	91,78	3,00	2,97
	1	34,90	38,03	32,31	35,44	92,92	93,46	3,00	3,00
5	0	23,45	25,04	22,25	23,84	94,80	95,20	3,00	3,00
	1	25,42	28,44	24,96	27,98	98,05	98,36	3,00	3,00
6	0	20,54	24,13	10,82	13,43	52,52	55,79	1,58	1,94
	1	19,90	23,94	12,58	16,21	62,63	66,41	2,33	2,58
7	0	21,11	23,87	15,35	17,00	73,64	72,98	2,75	2,50
	1	16,50	18,31	15,47	17,26	92,81	93,74	3,00	2,94
8	0	31,68	34,26	28,80	31,37	89,98	90,70	3,00	3,00
	1	34,45	35,76	31,09	32,20	90,40	90,08	3,00	2,93
9	0	11,88	15,65	8,98	12,75	75,25	81,11	2,83	2,70
	1	18,52	20,77	14,21	16,10	77,20	78,16	2,89	2,49
10	0	23,66	25,68	19,24	21,25	81,95	83,09	2,67	2,89
	1	27,78	27,78	25,43	25,24	91,57	90,79	3,00	2,89
11	0	28,70	31,06	22,72	25,08	79,47	81,06	2,75	2,75
	1	33,67	36,33	26,51	29,17	79,22	80,79	2,58	2,82
12	0	17,56	19,01	15,12	16,26	88,39	87,66	3,00	2,85
	1	24,85	27,32	23,42	25,88	94,11	94,62	3,00	3,00

a time point 0 is at baseline before caloric challenge, time point 1 is after caloric challenge

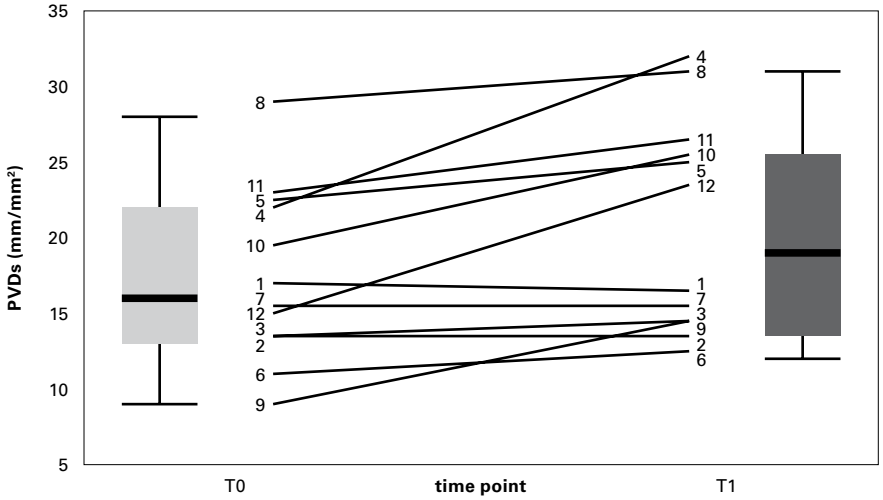
3 Difference in sublingual microcirculatory parameters before and after caloric challenge

		CGI (n=12)	
microcirculation	**time point[a]**	**median (IQR)**	***p*-value[b]**
TVDs	0	20.8 (17.6-23.8)	
	1	22.4 (17.8-32.2)	0.09
TVDa	0	24.2 (19.5-25.6)	
	1	25.6 (21.0-33.9)	0.18
PVDs	0	16.3 (13.3-22.1)	
	1	19.9 (14.2-26.2)	0.008
PVDa	0	19.1 (16.2-23.6)	
	1	22.2 (16.5-28.9)	0.02
PPVs	0	84.8 (75.3-90.4)	
	1	91.0 (80.1-93.8)	0.01
PPVa	0	85.4 (79.8-91.5)	
	1	90.4 (81.4-94.4)	0.05
MFIs	0	2.8 (2.7-3.0)	
	1	3.0 (2.7-3.0)	0.17
MFIa	0	2.8 (2.6-3.0)	
	1	2.9 (2.8-3.0)	0.14

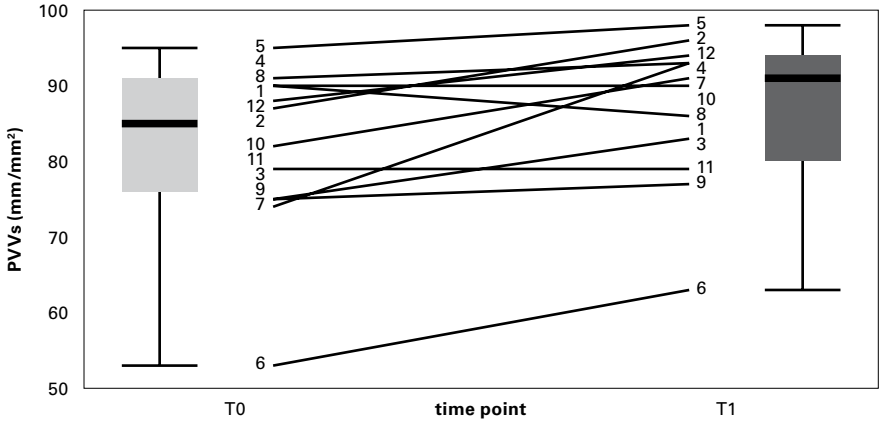
a time point 0 is at baseline before caloric challenge, time point 1 is after caloric challenge

b *p*-value <0.05 was considered significant

2 Perfused vessel density of the small vessels (PVDs) in mm/mm² of all patients at baseline (T0) and after caloric challenge (T1)



3 Proportion of perfused vessels of the small vessels (PPVs) in mm/mm² of all patients at baseline (T0) and after caloric challenge (T1)



boxes represent medians with interquartile range, whiskers extent to the most and least extreme scores respectively.

Discussion

In this proof of concept study, we aimed to investigate the sublingual microcirculation of CGI patients during fasting and caloric challenge. We found that patients with CGI have a significant increase in the perfused vessel density and proportion of perfused vessels after stimulation with food.

Studies in patients with CGI have focused on macrovascular alterations[16-18]. It is believed that patients with CGI have a lack of postprandial increase in the splanchnic blood flow after ingestion of food leading to insufficient oxygen delivery to provide for the metabolic demand of the gastrointestinal tract in the presence of insufficient collateral circulation[3]. Another underlying cause might be that patients with CGI have microcirculatory alterations associated with systemic disorders of the circulatory system and that this also attributes to the presence of clinical symptoms[6]. Therefore, microvascular pathology in this patient group is very likely and might explain why single-vessel stenosis can lead to clinical symptoms even in presence of sufficient collateral circulation. We therefore hypothesized that patients with CGI may also exhibit microcirculatory alterations. We hypothesize that sublingual microcirculation is responsive to enteral caloric challenges and as such provides a means to indirectly assess the intestinal microcirculation.

Our study shows that patients with CGI have a significant increase in the microvascular parameters after a caloric challenge and thus have a hyperaemic response to ingestion of food with a high caloric composition. In addition, we have reason to assume that patients with CGI may have impaired sublingual microcirculation compared to healthy volunteers. A previous study investigated the sublingual microvessel density and flow in healthy volunteers[19]. The investigators observed a mean PPV for the small vessels of 94.0%, whereas the PPV of small vessels in healthy volunteers is 100%[20]. In our study, the median PPV for the small vessels was considerably lower (84.8%). Therefore, it might be that patients with CGI have a poor microcirculation to begin with, despite the increase

in microcirculatory parameters after a caloric challenge. Further studies with the inclusion of more patients and healthy controls using the current protocol are needed to further illustrate this matter.

Our study has limitations. Patients with CGI were under conscious sedation with intravenous midazolam and fentanyl at time of the second sublingual microcirculatory measurements after caloric challenge but not during the baseline sublingual measurements. A recent study showed that the sublingual microcirculation in patients with shock was greater after the infusion of midazolam[21]. In this study, the proportion of perfused vessels was significantly greater after infusion of midazolam (PPV median 96.4% (IQR 93.7-97.6) vs 92.7% (88.3-94.7), $p<.005$). Therefore, the hyperaemic response in our patients after a caloric challenge might partly be caused by the effect of midazolam.

To our knowledge, this is the first study to investigate the sublingual microcirculation in patients with CGI. Patients with CGI might have a worse microcirculation to begin with compared to healthy controls. Furthermore, we found that patients with CGI have a significant increase in the perfused vessel density and proportion of perfused vessels after stimulation with food and thus have a hyperaemic response to ingestion of food. This study provides us more insight in the relationship between the microcirculation and hemodynamic changes in the gastrointestinal tract. These findings may lead to development of non-invasive, rapid, and less-burdensome diagnostic methods for the detection of CGI. We believe it would be interesting to assess the diagnostic ability of sublingual measurements of the microcirculation in patients with the diagnosis of CGI and to compare it to the current diagnostic modalities such as VLS. Future studies with more patients and healthy are needed to increase the generalizability of our findings and to provide more discriminatory power for clinical use.

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4

A prediction model to identify patients with chronic gastro-intestinal ischemia

Jihan Harki, Yvonne Vergouwe, Johannes A. Spoor,
Peter B. Mensink, Marco J. Bruno, Désirée van Noord,
Ernst J. Kuipers, Eric T.T.L. Tjwa

Submitted

Abstract

Background

No golden diagnostic standard is available to diagnose Chronic Gastrointestinal Ischemia (CGI). We aimed to establish an accurate prediction model for CGI, based on clinical symptoms and radiological evaluation of the mesenteric arteries.

Methods

We prospectively included consecutive patients with clinical suspicion of CGI in a tertiary referral center. Predictors for CGI were obtained by comparing clinical parameters to the diagnosis of CGI. Multivariable logistic regression was used to combine the strongest predictors in a model. A score chart based on the prediction model was provided to calculate the risk of CGI.

Results

CGI was present in 171/436 (39%) patients (67 (54-74) years; 27% male). Strongest predictors for CGI were increasing age (OR1.24, 95%CI 0.88-1.73), female gender (OR1.86, 95%CI 1.18-2.95), weight loss (OR1.87, 95% CI1.18-2.97), nausea (OR1.27, 95%CI 0.82-1.98), concomitant cardiovascular disease (OR2.15, 95%CI 1.35-3.44), and positive family history for cardiovascular disease (OR1.36, 95%CI 0.87-2.12). Duration of symptoms >12 months (OR0.90, 95%CI 0.82-0.99) and use of analgetics (OR0.80, 95%CI 0.45-1.44 for opioid use and OR0.41, 95%CI 0.19-0.87 for non-opioid use) lowered the risk of having CGI. A model based on clinical symptoms alone showed limited discriminative ability for diagnosing CGI (c-statistic 0.62). Adding radiological imaging of the mesenteric arteries improved the discriminative ability (c-statistic 0.76).

Conclusion

Clinical symptoms alone are insufficient to predict the risk of CGI. Radiological evaluation of the mesenteric arteries is essential. This tool may be useful for clinicians to assess the risk of CGI and to decide whether further diagnostic work-up for CGI is needed.

Introduction

Chronic gastrointestinal ischemia (CGI) is the result of decreased mucosal perfusion, in most cases caused by atherosclerotic stenosis of the supplying arteries[1]. In some cases, hypoperfusion and/or hypo-oxygenation is due to underlying cardiac or pulmonary disease. The diagnosis of CGI is a clinical challenge. This is firstly because patients with CGI may present with a variety of symptoms. Furthermore, there is no single, accurate test to diagnose or exclude CGI. The role of clinical symptoms alone in the diagnosis of CGI is limited[2,3]. The combination of postprandial pain, weight loss and presence of an abdominal bruit is known as the 'classical' triad of CGI. This is however only present in 16-21% of patients and has limited predictive value for diagnosing CGI[3-5]. Studies to identify predictive symptoms and risk stratification methods have been based on relatively small cohorts of selected patients. The current models thus only rendered modest discriminative capacity[2,3]. Even though larger cohorts would allow further specification, it is unlikely that this will raise the predictive probability of a model based on symptoms alone.

This supports the current diagnostic approach that combines assessment of clinical symptoms with radiological imaging of the gastrointestinal arteries and functional testing by tonometry or visible light spectroscopy (VLS)[6]. This seemingly simple approach is however limited by a number of challenges. Functional testing is not widely available, and is furthermore burdensome and costly. The use of the more patient-friendly and less cumbersome VLS technique also has its limitations. VLS has a sensitivity of 90% for the detection of CGI[7]. However, this is at the loss of specificity, which ranges around 60%. Random abdominal imaging, especially in the elderly, frequently reveals mesenteric artery stenosis in variable degrees. This is often an incidental finding with uncertain clinical relevance when typical symptoms of CGI are lacking.

A more accurate CGI prediction model is needed. This should take the finite availability of functional tests in general hospitals into

account[2,3]. We therefore aimed to establish a prediction model for CGI, based on the combination of medical history, symptoms and radiological evaluation of the mesenteric arteries. For that purpose, we used a large cohort of patients in order to identify low, intermediate and high-risk patients for CGI.

Methods

Study population

Consecutive patients with a clinical suspicion of CGI referred to a tertiary care center were prospectively included in the study cohort between November 2006 to March 2013. Suspicion for CGI was defined upon discretion of the referring physician based on presence of one or more of the following criteria:

1 presence of postprandial abdominal pain

2 otherwise unexplained weight loss (>5% of normal body weight)

3 significant stenosis of >50% of at least one of the gastrointestinal arteries on radiological evaluation[3].

In all patients, upper endoscopy, colonoscopy and radiological imaging had not revealed an alternative diagnosis. The standard CGI work-up included assessment of medical history and symptoms by a structured questionnaire. This was followed by radiological imaging by CT- or MR-angiography. The latter was done in case of contra-indications for CT-angiography, in particular contrast allergy or renal failure. Finally, patients were functionally assessed by gastrointestinal tonometry or VLS. Approval for the study was obtained from the Institutional Review Board of the Erasmus MC University Medical Centre Rotterdam, the Netherlands.

Consensus diagnosis and definitive diagnosis of CGI

All patients and assessment results were discussed in a multidisciplinary team consisting of vascular surgeons, intervention radiologists and gastroenterologists, all experienced in the management of CGI. This yielded a final expert-based consensus diagnosis in each case.

A diagnosis CGI was established if a patient fulfilled two of the three following criteria:

1 distinctive clinical presentation including presence of postprandial pain and otherwise unexplained weight loss of >5% of the normal body weight

2 significant stenosis of >50% of at least one of the mesenteric arteries

3 functional signs of mucosal ischemia demonstrated by tonometry or VLS.

Pathological tonometry findings were defined as gastric or jejunal PCO2 above respectively 12.0, 13.6, or 10.6 kPa after breakfast, dinner, or compound solution meal, respectively[8]. Mucosal ischemia by VLS was defined as mucosal oxygen saturation < 58%, <62%, or <63% for measurements in the duodenum, duodenal bulb and the antrum of the stomach respectively. After reaching a consensus diagnosis, CGI patients were sub-classified as having occlusive disease CGI (≥ 1 stenotic mesenteric artery) or non-occlusive mesenteric ischemia (NOMI). Patients with occlusive CGI were eligible for endovascular or surgical revascularization. In patients with NOMI, vasodilatory therapy was considered[9,10]. Upon either treatment, all patients received standard follow-up consisting of scheduled visits at the outpatient clinic. The definitive diagnosis of CGI was made after sustained relief of symptoms during follow-up in treated patients.

Questionnaire

All patients were asked to complete a standardized questionnaire prior to their first visit to our outpatient clinic. The questionnaire was specifically designed for collection of relevant medical and family history, abdominal symptoms, and medication use[1,3]. Medical history findings that were considered relevant for CGI assessment included a history of cardiovascular (both coronary and peripheral) arterial disease. Family history findings that were considered relevant included a known history of first-degree relatives with cardiovascular arterial disease. Participants were asked for the presence of gastrointestinal symptoms in general and of symptoms specific for CGI such as postprandial and exercise-related pain. Severity of these symptoms was assessed on a 5-point Likert-type scale (0 = no pain; 1 = pain, but no interference with Activities of Daily Living (ADL); 2 = pain, with small impairment of ADL; 3 = pain,

with major impairment of ADL, 4 = pain, with complete impairment of ADL).

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Data were expressed as mean and standard deviation (SD), median and interquartile range (IQR) or count and percentage (%), when appropriate. Missing observations were imputed multiple times using the R-software. The imputation model used all the variables that we considered as potential predictors and the diagnosis CGI. Univariable and multivariable associations of the candidate predictors for CGI were assessed using logistic regression analysis. The associations were estimated as Odds Ratios (OR). For the univariable analysis the OR for the following variables were studied: age, gender, presence of postprandial pain and exercise-induced pain (both yes/no, Likert-scale) weight loss per month defined as total amount of weight loss divided by the period (in months) in which the weight loss occurred, diarrhea (yes/no), nausea (yes/no) and duration of symptoms in months, smoking (yes/no, pack years (py)), use of alcohol in units, use of analgetics (yes/no), use of antithrombotics (yes/no), concomitant cardiovascular disease (yes/no), hypercholesterolemia (yes/no), diabetes mellitus (DM) type II (yes/no) and hypertension (yes/no). For continuous variables, the OR was calculated for the interquartile range (IQR). In all these categorical variables, absence of variable was used as reference category.

For the multivariate analysis, we developed two models: model A (clinical model), solely based on clinical parameters obtained from medical history and questionnaire, and model B, based on clinical parameters plus mesenteric artery imaging.

In agreement with the rule of thumb to use no more than one variable per ten outcomes in the less frequent outcome category, we considered no more than 17 variables for the clinical model A. Based on literature and clinical knowledge, we chose clinical symptoms of CGI and risk factors for atherosclerosis but also use of analgetics and antithrombotic therapy.

Variable selection was performed using backward stepwise selection using a liberal p-value of 0.20 in order to increase the power of selecting true predictors and to exclude variables with small effects[11]. The discriminative ability of the model was estimated with a measure of concordance, the c-statistic. The c-statistic is identical to the area under the receiver operating characteristic (ROC) curve for dichotomous outcomes, and indicates to what extent patients with CGI can be distinguished from patients without CGI[12]. We used bootstrapping to assess and correct for optimism[11,13].

Based on the multivariable analysis for model A and model B, we developed a score chart based on the original regression coefficients[14].

Results

Patient characteristics

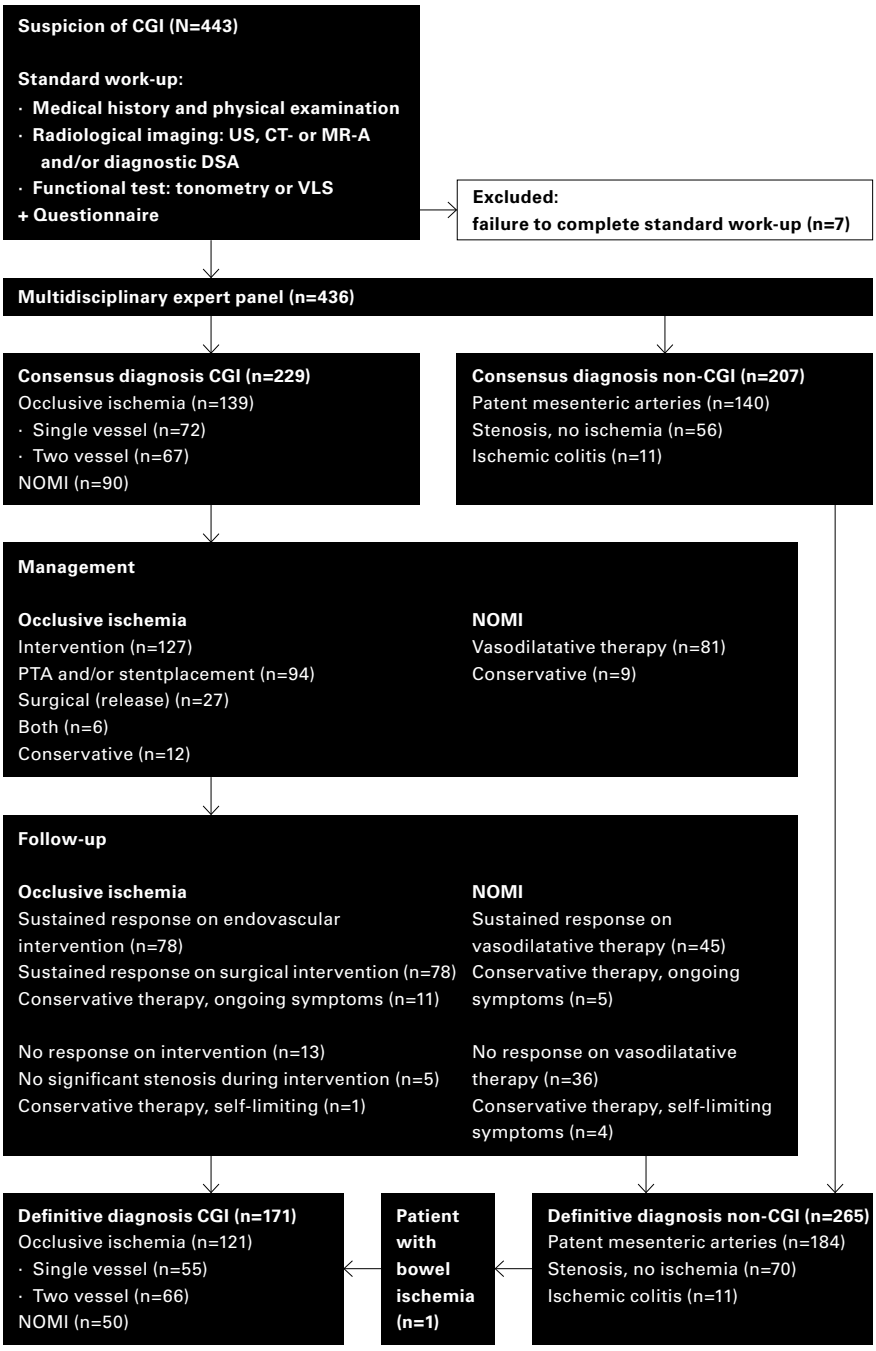
During the study period 443 consecutive patients with the clinical suspicion of CGI received the protocolized work-up. Seven patients failed to complete this work-up and were excluded from analysis, leaving a total of 436 patients in the study ◘ **1**. The median age of the referred population was 61 (IQR 50-72) years, and 151 (35%) patients were male ≣ **1**.

Forty-two percent of the total population had a history of cardiovascular disease. Analgetics, including opioids, and non-opioids and over-the-counter drugs, were used in 25% of patients. Antithrombotics were used by 50% of patients, with platelet aggregation inhibitors being mostly used (39%) followed by oral anticoagulants (10%) ≣ **1**.

Clinical symptoms

The most commonly reported symptoms were presence of abdominal pain (83%), postprandial pain (79%), and exercise-induced pain (76%). Presence of these symptoms was similar between patients with CGI and without CGI. Weight loss (median 1.6 (IQR 0.8-3.5) kg/month) was present in 286 (66%) patients and was more prevalent in patients with CGI compared to patients with non-CGI (75% vs 61%; median weight loss 1.8 (IQR 1.0-3.7) vs 1.6 (IQR 0.8-3.3) kg/month). A significant stenosis of at least 1 mesenteric artery was present in 195 (45%) patients and was present in 123 (72%) patients with CGI compared to 72 (27%) patients with non-CGI. Fifty-five patients were diagnosed with CGI based on single-vessel arterial stenotic disease. Stenosis of the CA was present in 43 (78%) patients. Mucosal ischemia was detected in 37 (86%) of these patients with CA stenosis. Stenosis of the SMA was present in 12 (22%) patients. In only one patient of this group mucosal ischemia was not detected by means of functional testing. Multi-vessel disease was present in 66 patients (46 patients with concomitant stenosis of the

1 Flowchart of study



CA and SMA, and 20 patients with concomitant stenosis of the CA, SMA and inferior mesenteric artery (IMA), respectively).

Management of patients with CGI

The results of the diagnostic work-up of the 436 included patients in the study were discussed in a multidisciplinary expert panel. A consensus diagnosis of CGI was made in 229 (53%) patients and in 207 (47%) patients CGI was excluded. As listed in ≣ **1**, 208/229 patients with the consensus diagnosis of CGI were eligible for treatment. Intervention was offered to 127 patients with occlusive ischemia, of whom 94 patients received angioplasty and/or stenting of the mesenteric arteries, and 27 patients were treated surgically. A further six patients were treated surgically after endovascular treatment had failed. The remaining twelve patients were unfit for intervention and received conservative therapy. During follow-up of these patients, 109 patients with occlusive CGI had sustained relief of symptoms after treatment. Of these patients, 19/109 had restenosis during follow-up. Stent revision was successful in 10/17 and surgical bypass in five patients. Despite multiple attempts for bypasses and relaparatomies for bowel resection, three patients died of acute-on-chronic ischemia. In one patient with instentstenosis, surgical treatment was not possible. He was treated conservatively and died of ischemia-related causes. Fourteen patients who were diagnosed with occlusive CGI, died of non-ischemia related causes during follow-up. Eleven patients with occlusive CGI who were not eligible for treatment, had ongoing symptoms during follow-up. Furthermore, one of these 11 patients died of acute-on-chronic ischemia. This patient was admitted for sudden, severe abdominal pain. Bowel resection was performed, however this was not successful and this patient died during admission.

NOMI patients received medical treatment with vasodilative therapy (n=81) or a expectative approach (n=9) ⊡ **1**. The first line therapy for patients with NOMI consists of isosorbide dinitrate 20 or 40mg od. The first line therapy is ceased in case of side effects or no clinical improvement after four weeks, and is replaced by

1 Patient characteristics for the total group and subgroups according to definitive diagnosis of CGI/non-CGI

	observed (n)	total population (n=436)	CGI (n=171 (39))	non-CGI (n=265 (61))
age (years)[a]	436	61 (50-72)	67 (54-74)	58 (45-71)
sex	436			
male		151 (35)	47 (27)	104 (39)
female		285 (65)	124 (73)	161 (61)
postprandial pain	427			
no pain		92 (22)	33 (20)	59 (23)
pain, no hindrance in ADL		79 (19)	29 (17)	50 (19)
pain, small impairment ADL		141 (33)	55 (33)	86 (33)
pain, major impairment ADL		77 (18)	32 (19)	45 (17)
pain, complete impairment ADL		38 (9)	19 (11)	19 (7)
exercise-induced pain	415			
no pain		99 (24)	39 (24)	60 (24)
pain, no hindrance in ADL		70 (17)	20 (13)	50 (20)
pain, small impairment ADL		134 (32)	58 (36)	76 (30)
pain, major impairment ADL		84 (20)	28 (18)	56 (22)
pain, complete impairment ADL		28 (7)	15 (9)	13 (5)
BMI (kg/m^2)[a]	424	22.4 (19.5-25.9)	21.5 (18.6-25.0)	20.8 (18.8-23.6)
weight loss	431	286 (66)	126 (75)	160 (61)
weight loss (kg/month)[a]	271	1.6 (0.8-3.5)	1.8 (1.0-3.7)	1.6 (0.8-3.3)
diarrhea	416	111 (37)	39 (24)	72 (28)
nausea	429	141 (33)	66 (47)	75 (29)
duration of symptoms (months)[a]	420	12.0 (6.0-36.0)	12 (6.0-26.0)	12 (6.0-24.0)
cardiovascular disease	436	185 (42)	93 (54)	92 (35)
hypertension	436	232 (53)	108 (63)	124 (47)
DM type II	436	63 (14)	29 (17)	34 (13)
hypercholesterolemia	434	171 (39)	80 (16)	91 (35)
family history CVD	425	280 (66)	119 (71)	161 (63)
smoking	433	317 (73)	133 (78)	184 (70)
smoking (pack years)[a]		11 (0-26)	13 (2-26)	10 (0-25)
alcohol	393	161 (41)	65 (42)	96 (40)
alcohol (U/day)[b]		0 (0-30)	0 (0-10)	0 (0-24)
analgetics	435			
none		326 (75)	134 (78)	192(73)
opioids		64 (15)	26 (15)	38 (14)
non-opioids		45 (10)	11 (6)	34 (13)
antithrombotics	436			
none		216 (50)	66 (39)	150 (57)
platelet aggregration inhibitors		169 (39)	82 (48)	87 (33)
oral anticoagulants		45 (10)	20 (12)	25 (9)
both		6 (1)	3 (2)	3 (1)
mesenteric artery stenosis	436			
no stenosis		241 (55)	48 (28)	193 (73)
single artery stenosis		122 (28)	57 (33)	65 (25)
multi artery stenosis		73 (17)	66 (39)	7 (2)

	observed (n)	total population (n=436)	CGI (n=171 (39))	non-CGI (n=265 (61))
functional mucosal ischemia[c]	409			
present		317 (73)	152 (89)	165 (62)
absent		92 (21)	14 (8)	78 (29)
endoscopic mucosal appearance[d]	428			
normal		282 (66)	108 (64)	174 (67)
edema		3 (1)	1 (1)	2 (1)
erythema		64 (15)	19 (11)	45 (17)
erosion		58 (14)	29 (17)	29 (11)
ulceration		21 (5)	12 (7)	9 (4)

a median, interquartile range (IQR)

b median, range

c functional mucosal ischemia as detected by functional testing using tonometry or VLS

d endoscopic (morphological) appearance of the gastric mucosal during upper GI endoscopy

second-line treatment consisting of ketanserin 20mg or 40 mg od. Clinical response was defined as complete or partial relief of the major persisting symptoms. The etiologies of the NOMI were mostly of cardiac origin, however there were also patients with pulmonary pathology (3 patients with chronic obstructive disease), two patients with vasospasms of the mesenteric arteries and one patient with a low-flow state based on chronic kidney insufficiency. Five patients with NOMI were treated conservatively. Four of the five patients had ongoing symptoms and one patient died of cardiac failure during follow-up. Of the 45 patients with NOMI and a sustained response on vasodilatative therapy, four patients eventually received percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass procedure during follow-up as the final treatment of their complains. One patient was eligible for PTCA, however after two unsuccessful attempts it was decided to omit this. Two patients died of terminal heart failure during follow-up.

At follow-up (median 5.6 (IQR 1.4-18.6) months), sustained relief of symptoms was achieved in 78/94 patients endovascularly treated and in 31/33 patients surgically treated. A sustained response was observed in 45/81 (56%) patients with NOMI. During follow up, five (2%) patients with the consensus diagnosis CGI became free of symptoms without any treatment and were classified as definitive diagnosis non-CGI. One patient with the consensus diagnosis of non-CGI was hospitalized with ischemia-related morbidity during follow-up and was therefore reclassified as CGI patient. Thus, from the 436 included patients, 171 (39%) were definitely diagnosed with CGI of whom 121 (71%) patients with occlusive CGI and 50 (29%) patients with NOMI (see supplementary ≣ **1** for patient characteristics of the groups).

Management of patients with non-CGI

Among the patients in whom CGI was ruled out, 140 (68%) had patent mesenteric arteries, 51 (25%) patients had a single-vessel stenosis, and 5 (2%) patients had multi-vessel stenosis. In the patients with patent mesenteric arteries, 59 patients had normal

oxygen saturation levels. Sixty-six patients had signs of local hyposaturation but a diagnosis of CGI was rejected based on atypical symptomatology in combination with patent arteries. In the remaining patients, functional testing was not performed due to a known history of (partial) gastrectomy or due to atypical symptomatology in combination with patent arteries. In the 51 patients with a single-vessel stenosis, functional testing showed normal mucosal oxygen saturation in ten patients. Functional testing was not performed in five patients due to a history of gastrectomy in combination with an atypical symptomatology. In 36 patients with single-vessel stenosis, functional testing showed hypo-oxygenation. However, these patients had a very atypical symptomatology. In the patients with multi-vessel mesenteric stenosis, functional testing showed normal mucosal oxygen saturation in one patient in combination with an atypical symptomatology. Four patients had atypical symptomatology and hypo-oxygenation on functional testing. In these patients, CGI was ruled out due to an alternative diagnosis. The alternative diagnosis for non-CGI patients was in most cases presence of malignancy with or without metastases, inflammatory bowel disease, or (chronic) pancreatitis. Five patients were classified as non-CGI after an initial consensus diagnosis of CGI, but were however not eligible for treatment and were thus treated conservatively. During follow-up the symptoms were self-limiting, which in return made the diagnosis of CGI very unlikely in these patients.

Prediction model for CGI

In the univariate regression analysis, clinical parameters showing strong association with CGI were increasing age (OR 1.61, 95% CI 1.21-2.16), female gender (OR 1.70, 95% CI 1.12-2.58), weight loss (OR 1.91, 95% CI 1.24-2.92), nausea (OR 1.65, 95% CI 1.10-2.49), duration of symptoms (OR 0.88, 95% CI 0.80-0.99), presence of concomitant cardiovascular disease (OR 2.24, 95% CI 1.51-3.32), hypertension (OR 1.95, 95% CI 1.32-2.89), hypercholesterolemia (OR 1.68, 95% CI 1.13-2.48), smoking (OR 1.36, 95% CI 1.01-1.84) and use of non-opioid analgetics (OR 0.47, 95% CI 0.23-0.95) ☰ **2**.

2 Predictors for CGI

	univariable analysis OR [95% CI][a]	model A OR [95% CI][a]	model B OR [95% CI][a]
age (years)[a]	1.61 [1.21-2.16]	1.24 [0.88-1.73]	0.99 [0.68-1.43]
female gender	1.70 [1.12-2.58]	1.86 [1.18-2.95]	1.30 [0.76-2.21]
postprandial pain			
no pain	reference		
pain, no hindrance in ADL	0.99 [0.53-1.84]		
pain, small impairment ADL	1.09 [0.63-1.87]		
pain, major impairment ADL	1.25 [0.67-2.32]		
pain, complete impairment ADL	1.86 [0.87-4.00]		
exercise-induced pain			
no pain	reference		
pain, no hindrance in ADL	0.65 [0.34-1.25]		
pain, small impairment ADL	1.17 [0.69-1.99]		
pain, major impairment ADL	0.81 [0.44-1.49]		
pain, complete impairment ADL	1.78 [0.72-4.39]		
BMI (kg/m²)[a]	0.98 [0.77-1.26]		
weight loss	1.91 [1.24-2.92]	1.87 [1.18-2.97]	1.72 [0.02-2.90]
weight loss (kg/month)[a]	0.99 [0.85-1.14]		
diarrhea	0.79 [0.49-1.26]		
nausea	1.65 [1.10-2.49]	1.27 [0.82-1.98]	1.78 [1.07-2.95]
duration of symptoms (months)[a]	0.88 [0.80-0.98]	0.90 [0.82-0.99]	0.90 [0.81-1.00]
cardiovascular disease	2.24 [1.51-3.32]	2.15 [1.35-3.44]	1.55 [0.90-2.69]
hypertension	1.95 [1.32-2.89]		
DM type II	1.38 [0.81-2.38]		
hypercholesterolemia	1.68 [1.13-2.48]		
family history CVD	1.44 [0.95-2.19]	1.36 [0.87-2.12]	1.39 [0.84-2.31]
smoking	1.49 [0.95-2.32]		
smoking (pack years)[a]	1.36 [1.01-1.84]		
alcohol	1.06 [0.71-1.59]		
alcohol (U/day)[b]	1.03 [0.90-1.19]		
analgetics			
none	reference		
opioids	0.99 [0.57-1.70]	0.80 [0.45-1.44]	0.71 [0.35-1.41]
non-opioids	0.47 [0.23-0.95]	0.41 [0.19-0.87]	0.60 [0.27-1.36]
antithrombotics			
none	reference		
platelet aggregration inhibitors	2.14 [1.41-3.25]		
oral anticoagulants	1.82 [0.94-3.50]		
both	2.27 [0.45-11.56]		
mesenteric artery stenosis			
no stenosis	reference		reference
single artery stenosis	3.53 [2.19-5.68]		3.56 [2.15-5.92]
multi artery stenosis	37.91 [16.35-87.89]		34.60 [14.15-84.63]
c-statistic of model		0.62	0.76

a values represent Odds Ratio [95% Confidence Interval]

b for continuous variables, values represent OR [95% CI] for interquartile range (IQR)

c concordance statistic, equivalent to area under the receiver operating characteristic (ROC) curve, after internal validation

In the multivariable logistic regression analysis using backward stepwise selection, age, female gender, weight loss, nausea, concomitant cardiovascular disease and positive family history for cardiovascular disease were the strongest predictors for CGI. Duration of symptoms >12 months and use of analgetics were adversely associated with CGI. Note that some 95% confidence intervals cross one, which is the result of the liberal p-value of 0.20 used to select the predictors. Combining the predictors in model A showed a c-statistic of 0.62. The model adding radiological imaging to assess the patency of the mesenteric arteries (no stenosis, single- or two-vessel arterial stenotic disease) to the self-reported clinical parameters (Model B) improved the discriminative ability of the model, with a c-statistic of 0.76 after optimism correction. For the false positive and false negative rates based on Model B see supplementary ≣ **2**.

Based on Model A and B, a score chart was developed for predicting CGI ≣ **3**. Based on the total score, risks of having CGI are categorized ≣ **4a**. A total score of -4 to 1 points indicates a predicted risk of 18% of having CGI, 2-7 points to 32% and 8 points or more to an absolute risk 50% of having CGI. As an example, a 65-year old (1 point) female (3 points) with no history of cardiovascular disease (0 points) and weight loss of 4 kg since 3 months (3 point), but who uses analgetics including over-the-counter medication (-4 points), has a total score of 3 points, indicating a low risk of having CGI.

The prediction model including radiological evaluation of the mesentery arteries allows further distinction of patients in having low (0%-17% absolute risk of CGI), intermediate (18%-38%) or high risk (>70%) of CGI ≣ **4b**.

3 Score chart for the prediction of CGI based on questionnaire

predictors	scoring points
age	
< 60 years	0
≥ 60 years	1
sex	
male	0
female	3
cardiovascular disease	
no	0
yes	1
family history of cardiovascular disease	
no	0
yes	1
weight loss	
no	0
yes	3
nausea	
no	0
yes	1
duration of symptoms	
≤ 12 months	1
> 12 months	0
use of analgetics	
none	0
opioids	-1
non-opioids	-4
mesenteric artery stenosis	
no stenosis	0
single artery stenosis	6
multi artery stenosis	10

4 Prediction model for CGI: model A with self-reported clinical variables and model B with self-reported clinical variables and radiological evaluation of the mesenteric arteries and the absolute risk (%) for CGI

model A	
total score	**absolute risk**
–4-1	18%
2-7	32%
8+	50%

model B	
total score	**absolute risk**
–4-1	18%
2-7	32%
8+	50%

Discussion

We present a prediction model to identify patients with CGI based on a large prospective study. Our study shows that advanced age, female gender, presence of nausea and weight loss, recent onset of symptoms, concomitant cardiovascular disease, positive family history for cardiovascular disease, and no use of analgetics are predictors for CGI. However, medical history and clinical symptoms alone are only moderately predictive of the risk of CGI and including radiological evaluation of the mesenteric arteries in the model considerably improves the accuracy of risk assessment of CGI. Therefore, we established a model based on clinical symptoms and radiological evaluation of the mesenteric arteries to identify low, intermediate and high-risk patients for CGI.

There is an unmet need for widely accepted criteria for the diagnosis of CGI[15-17]. Until now, evaluation for CGI is in particular reserved for patients who report specific clinical symptoms. This may however result in underdiagnosis, continuation of testing for other conditions, and delay of appropriate treatment[18-20]. This large prospective study shows that medical history and clinical symptoms alone are only moderately predictive of the risk of CGI. Including radiological evaluation of the mesenteric arteries in the model considerably improved the accuracy of risk assessment of CGI. We established a model based on these variables to identify low, intermediate and high-risk patients for CGI. The strengths of this model are its high performance for predicting the risk of CGI constructed on the largest patient population diagnosed with CGI. Furthermore, it also applies to patients with non-occlusive mesenteric ischemia NOMI, a disease entity that has not earlier been included[21-23]. As patients with NOMI still have the same symptomatology, the model is designed to identify them as risk-patients, urging the clinician not to refrain from further investigation. A next step could then be the performance of functional testing such as gastric tonometry of VLS.

In this study, patients with a clinical suspicion of CGI referred for further evaluation to a tertiary center were included. All patients, also non-CGI patients, received the same work-up which makes this an unique cohort suitable for developing a prediction model. In line with earlier studies, our study shows that 'classical' symptoms such as postprandial pain and presence of weight loss are the most prevalent symptoms in patients with CGI but also in non-CGI patients[1-3,24]. As a consequence, postprandial abdominal pain is a weak clinical predictor for CGI. However, this finding may be confounded by selection. Our data corroborate with earlier reports that a cardiovascular risk profile predisposes for CGI and earlier onset of symptoms[1,2,19,25].

Our study confirms that the diagnostic value of clinical variables alone in the diagnosis of CGI is limited (c-statistic 0.62)[26]. This is similar to previous models (c-statistics of 0.60-0.62) assessing only these clinical variables[2,3]. Combining clinical variables with presence of mesenteric arterial stenosis in a second model allows discriminating more accurately between patients with and without CGI (c-statistic of 0.76). Based on this, we have developed a simple score chart that takes into account the limited availability of functional tests in most hospitals[8,27,28]. This score may be used in clinical decision making. This could allow a wait-and-see policy in patients with a low score[2]. In patients with intermediate and high score a more aggressive approach (including vascular intervention) may be advocated[29,30].

A possible limitation of this study regards incorporation bias[31]. All test results such as reported symptoms, imaging of mesenteric vasculature, and mucosal saturation levels were considered to establish the diagnosis of CGI. This may have led to overestimation of the accuracy of the studied variables for the prediction model for CGI[32,33]. This illustrates the necessity for prospective, external validation of our model in a new cohort of patients. However, this is expected to be difficult as large well-defined cohorts are lacking and will also be limited by the absence of a golden standard for CGI. Another limitation concerns the duration of follow-up after

reaching the consensus diagnosis. The consensus diagnosis of CGI was changed into non-CGI in 46 subjects, as treatment of CGI did not result in a sustained response[3,24,34,35]. Also, most patients with a consensus diagnosis of non-CGI were discharged from follow-up but were instructed to report when ischemic complications had occurred[1,34]. This may have resulted in underdiagnosis of CGI in follow-up. Nevertheless, we believe this will not affect the prediction model as we expect this to occur at a low rate[3,34].

In conclusion, our study confirms that clinical symptoms alone are insufficient to predict the risk of CGI. Radiological evaluation of the mesenteric arteries improves predictions substantially. We present a prediction model based on clinical variables and radiological imaging of the mesenteric arteries with acceptable accuracy. This may be a useful tool for clinicians to assess the risk of CGI and to decide whether further diagnostic work-up and treatment is indicated.

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≣ s1 Patient characteristics of patients with CGI according to occlusive CGI and NOMI

	observed n	total population n=171 (100%)	CGI n=121 (71%)	non-CGI n=50 (29%)
age (years)[a]	121	67 (54-74)	63 (54-74)	60 (52-72)
sex	121			
male		47 (27)	25 (21)	22 (44)
female		124 (73)	96 (79)	28 (56)
postprandial pain	119			
no pain		33 (20)	22 (19)	11 (22)
pain, no hindrance in ADL		29 (17)	18 (15)	11 (22)
pain, small impairment ADL		55 (33)	47 (40)	8 (16)
pain, major impairment ADL		32 (19)	24 (20)	8 (16)
pain, complete impairment ADL		19 (11)	8 (7)	11 (22)
exercise-induced pain	112			
no pain		39 (24)	27 (24)	12 (25)
pain, no hindrance in ADL		20 (13)	14 (13)	6 (13)
pain, small impairment ADL		58 (36)	40 (36)	18 (38)
pain, major impairment ADL		28 (18)	23 (21)	5 (10)
pain, complete impairment ADL		15 (9)	8 (7)	7 (15)
BMI (kg/m²)[a]	116	21.5 (18.6-25.0)	22.2 (19.5-25.1)	22.5 (18.5-27.8)
weight loss	119	126 (75)	88 (74)	38 (76)
weight loss (kg/month)[a]	83	1.8 (1.0-3.7)	1.6 (0.8-3.6)	1.9 (1.0-5.6)
diarrhea	112	39 (24)	26 (23)	13 (26)
nausea	119	66 (47)	45 (38)	21 (42)
duration of symptoms (months)[a]	118	12 (6.0-26.0)	12 (5.6-24.0)	12 (5.5-39.0)
cardiovascular disease	121	93 (54)	56 (46)	28 (56)
hypertension	121	108 (63)	79 (65)	29 (58)
DM type II	121	29 (17)	20 (17)	9 (18)
hypercholesterolemia	121	80 (16)	58 (48)	22 (44)
family history CVD	118	119 (71)	83 (70)	36 (72)
smoking	121	133 (78)	96 (79)	37 (74)
smoking (pack years)[a]	118	13 (2-26)	15 (3-28)	15 (0-31)
alcohol	110	65 (42)	48 (44)	17 (38)
alcohol (U/day)[b]	118	0 (0-10)	0 (0-2)	0 (0-2)
analgetics	112			
none		134 (78)	95 (79)	39 (78)
opioids		26 (15)	19 (16)	7 (14)
non-opioids		11 (6)	7 (6)	4 (8)
antithrombotics	112			
none		66 (39)	46 (38)	20 (40)
platelet aggregration inhibitors		82 (48)	58 (48)	24 (48)
oral anticoagulants		20 (12)	16 (13)	4 (8)
both		3 (2)	1 (1)	2 (4)

values represent number and percentage (%) of patients.

a median, interquartile range (IQR)

b median, range

≡ s2 False positive and false negative rates for the diagnosis of CGI according to Model B

	CGI +	CGI –	total	PPV
–4-7 points CGI +	25	133	158	0.16
8-13 points CGI +	50	102	152	0.33
>14 points	96	30	126	0.76
total	171	265	436	


5

Hypoxia-inducible factor 1-α in chronic gastrointestinal ischemia

Jihan Harki*, Aria Sana*, Désirée van Noord, Paul J. van Diest, Petra van der Groep, Ernst J. Kuipers, Leon M.G. Moons, Katharina Biermann*, Eric T.T.L. Tjwa*

* Authors contributed equally to this work

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Abstract

Background

Chronic gastrointestinal ischemia (CGI) is the result of decreased mucosal perfusion. Typical histological characteristics are lacking which hampers its early diagnosis. Hypoxia-inducible factor-1α (HIF-1α) is expressed under acute hypoxia. We investigated HIF-1α expression in chronic ischemic and inflammatory conditions of the human gastrointestinal (GI)-tract.

Materials and methods

Immunohistochemical expression of HIF-1α was analyzed in 61 patients, including patients with CGI, *Helicobacter pylori*-gastritis, ischemic colitis (IC), infectious colitis, inflammatory bowel disease (IBD) and 22 controls. HIF-1α expression in >10% of the cells was regarded as positive staining, and expression <10% of the cells was considered as negative staining.

Results

In the upper GI-tract, HIF-1α expression was found in 5/20 CGI-patients, but not in controls (p=0.08). The sensitivity and specificity of HIF-1α expression for diagnosing CGI was 25% and 84%. In the lower GI-tract, in HIF-1α was expressed in all patients with IC, infectious colitis and in majority of IBD-patients. This was also in 7/12 controls. The sensitivity and specificity of HIF-1α for diagnosing IC was 100% and 51%. HIF-1α expression was more observed in patients with inflammation on histology (p=0.02) in the lower GI-tract.

Conclusions

HIF-1α is expressed in acute and chronic ischemic tissue, but also in normal colon tissue and inflammatory disorders.

Introduction

Chronic gastrointestinal ischemia (CGI) is characterized by a broad range of clinical symptoms including chronic abdominal pain and weight loss and is the result of decreased mucosal perfusion by the gastrointestinal vasculature. The majority of patients with CGI is only diagnosed after extensive evaluation, often over prolonged periods, but it may be fatal when not timely recognized [1,2].

Currently, there is no single test with high sensitivity and specificity to diagnose or exclude CGI. A diagnostic approach combining assessment of clinical symptoms, radiological imaging of the gastrointestinal arteries and functional testing by tonometry or spectroscopy, is currently performed in the work-up of CGI suspected patients [3]. At present, a definitive diagnosis of CGI can only be made after persistent relief of symptoms on follow-up after treatment. Histology does not seem to play a major role in the diagnosis of CGI [4]. Markers for acute ischemia such as lactate dehydrogenase (LDH), leukocyte counts, C-reactive protein (CRP), and D-dimer have limited value for diagnosing CGI [5-8]. Against the lack of specificity of symptoms and diagnostic tests, further research is needed to assess typical histology for CGI.

Hypoxia-inducible factor-1α (HIF-1α) is expressed in acute ischemia [9]. HIF-1α is a significant intracellular regulator of oxygen hemostasis and is expressed in cells under low oxygen tension [10]. It may exert a tissue-protective effect under these circumstances by upregulation of cytoprotective genes that help to control metabolism, enhance oxygen delivery, and angiogenesis [11-13]. Immunohistochemical staining revealed presence of HIF-1α in several organs, including the gastrointestinal (GI)-tract, under hypoxic conditions [14,15]. Expression of HIF-1α in gastric or intestinal tissue may thus identify patients with CGI. To address this issue, we performed a pilot study to investigate the expression of HIF-1α in chronic ischemic tissue by immunohistochemistry.

Materials and methods

Study design

A histological study in which the immunohistochemical expression of HIF-1α was analyzed in 61 patients and 22 controls. This study was approved by the Institutional Review Board of the Erasmus MC University Medical Centre and adherent to Good Clinical Practice.

Participants and controls

Immunohistochemical expression of HIF-1α in the gastrointestinal tract was analyzed in the upper or lower GI tract of 61 patients and 22 controls. These included 20 patients with CGI, nine patients with *H. pylori*-gastritis, nine patients with ischemic colitis (IC), five patients with infectious colitis and 18 patients with inflammatory bowel disease (IBD). All patients underwent either upper endoscopy with paired gastric and duodenal biopsies or colonoscopy with biopsy sampling.

Consecutive patients with CGI were selected. Diagnosis of CGI was based on clinical history, presence of mucosal ischemia assessed by functional testing, and/or significant stenosis of ≥1 gastrointestinal artery. Patients with *H. pylori*-gastritis and infectious colitis were randomly selected from the hospital database. Presence of *H. pylori*-gastritis was confirmed by histology. Infectious colitis was confirmed by means of stool culture and histology confirmation: *Clostridium difficile* (n=3), *Campylobacter jejuni* (n=1), and *Escherichia coli (0157 strain)* (n=1). Patients with IC were selected when clinical presentation, course of the disease, and the endoscopic appearance indicated ischemia and this was confirmed by histology. Patients with IBD consisted of 9 patients with M. Crohn, and 9 with ulcerative colitis. Tissue samples for patients with ulcerative colitis were collected during endoscopy due to complains (n=5), and surveillance (n=3), or were resection specimen (n=1). For patients with M. Crohn, biopsy-sampling occurred during endoscopy due to complains (n=4), surveillance (n=4) and in one patient during surgery (n=1).

Twenty-two subjects served as the control group in the study, and were unmatched. For the upper GI-tract, consecutive patients with unexplained abdominal symptoms (n=10) requiring upper endoscopy were selected. In these patients CGI, H. pylori- gastritis and other pathology was excluded. For the lower GI-tract, tissue samples were collected during double balloon enteroscopy due to gastrointestinal bleeding (n=6) and unexplained abdominal symptoms (n=6).

Endoscopy

Upper GI endoscopy was performed in all patients with CGI, *H. pylori*-gastritis and 10 controls. Colonoscopy with biopsy sampling was performed in all other patients. Prior to biopsy sampling, the endoscopic appearance was evaluated by grading into: no inflammation, presence of oedema and erythema, ulceration, or necrosis. Biopsy sampling was performed according to a standardized protocol during endoscopy. Biopsies from the upper GI-tract included a biopsy from the descending duodenum, and one from the greater curvature of the antrum within 2-3 cm from the pylorus or corpus.

Histology

Presence of inflammation was systemically scored for the upper and lower GI-tract. Gastric biopsies of patients with CGI were scored by the Houston-updated Sydney System [16]. In biopsies of the lower GI-tract, the presence of inflammation was scored by the 6 grade classification system by Geboes et al. [17] All scoring was performed by an expert GI pathologist (KB), blinded for the patient characteristics.

Immunohistochemistry

Formaldehyde-fixed, paraffin-embedded tissue samples were cut and prepared according to protocol. Antigen retrieval was achieved as was previously described [18]. After an overnight incubation with mouse monoclonal antibody against human HIF-1α (1:10 for upper

GI tract and 1:200 respectively for colon samples; BD Transductions Laboratories, Sparks, Maryland, USA), binding of the primary antibody was visualized with the Novocastra Polymer Detection System (Novocastra Laboratories Ltd., New castle, United Kingdom). Peroxidase activity was developed with DAB (BD Pharmingen, Transductions Laboratories, Sparks MD, U.S.A.). Between each step the slides were washed in phosphate buffered saline. Finally, after rinsing with deionized water, the slides were counterstained with hematoxylin, and dehydrated before being mounted. Ductal-type breast cancer cell lines served as positive controls, and negative controls were achieved by omitting the primary antibody [19]. To test the specificity of the primary antibody, hepatocytes were stained for HIF-1α expression, as these cells are known for not expressing HIF-1α [20]. All slides were scored by the same pathologist (KB), blinded for the results of the clinical work-up. The slide order was randomized, minimizing the risk of bias as much as possible. Immunohistochemical staining of HIF-1α expression was evaluated as previously described [21]. Staining in the nuclei and cytoplasma of the cells was evaluated. The number of positive cells was assessed in all optical fields at magnification x200, and the mean value of all fields was used to obtain the final score. Stain intensity and stain pattern was assessed. Expression > 10% of the cells was regarded as positive staining, and expression < 10% of the cells was regarded as negative staining. The overall staining intensity was graded on a 4-point system by Allred et al. [22]

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Differences in presence of HIF-1α expression and inflammation were assessed using Kruskal-Wallis or Mann-Whitney test. Differences in presence of inflammation according presence of HIF-1α expression was assessed using Mann-Whitney test. Statistical analysis was performed using the SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

Results

Patients characteristics

Immunohistochemical expression of HIF-1α in the gastrointestinal tract was analyzed in 61 patients and 22 controls. These included 20 patients with CGI, nine patients with *H. pylori*-gastritis, nine patients with ischemic colitis (IC), 18 patients with inflammatory bowel disease (IBD) and five patients with infectious colitis. Ten controls served for the upper GI-tract and 12 controls for the lower GI-tract respectively. Characteristics of patients and controls are summarized in ≣ **1a** and ≣ **1b**.

≣ **1a** Patients characteristics according to localization in the GI-tract

	upper GI-tract n=39	**lower GI-tract n=44**
age (mean, SD)	61.6 (16.1)	51 (18)
gender		
male	17 (44%)	22 (50%)
female	22 (56%)	22 (50%)
diagnosis		
ischemia[a]	20 (51%)	9 (21%)
infection[b]	9 (23%)	5 (11%)
inflammation[c]	n.a.	18 (41%)
controls	10 (26%)	12 (27%)
endoscopic appearance		
normal	15 (38%)	15 (34%)
edema/ erythema	12 (31%)	8 (18%)
ulceration	12(31%)	11 (25%)
necrosis	0 (0%)	10 (23%)

values represent number and percentage (%) of patients

a chronic gastrointestinal ischemia and/or ischemic colitis

b *H. pylori*-gastritis and/or infectious colitis

c inflammatory bowel disease

Endoscopy

Overall, endoscopic pathological findings were found in 24 (61%) and 29 (66%) patients of the upper and lower GI-tract group. The endoscopic appearance of patients with CGI did not differ significantly from patients with *H. pylori*-gastritis and the controls. Interestingly, the majority of patients with CGI had no or only mild inflammation on endoscopy and in 50% of patients the endoscopic appearance was completely normal. In patients with *H. pylori*-gastritis, ulceration (44%) was mostly seen on endoscopy. The endoscopic appearance of the controls was normal in 3 (30%) subjects. However, mild erythema and edema was observed in 50% of the healthy subjects.

1b Clinical characteristics of patients: upper and lower GI-tract

	CGI n=20	H. pylori-gastritis n=9	controls upper GI n=10
inflammatory parameters			
CRP, mg/l[a]	2.0 (1.0-4.0)	1.0 (1.0-3.0)	1.0 (1.0-3.0)
leukocytes, $\times 10^9$/l[a]	6.8 (5.8-10.1)	7.9 (6.5-8.4)	5.7 (4.9-7.1)
mesenteric arterial stenosis[b]			
absent	1 (5%)	–	9 (90%)
single-vessel	6 (30%)	–	–
multi-vessel	13 (65%)	–	1 (10%)
unknown	–	9 (100%)	–
medication			
immunesuppressives	3 (15%)	3 (33%)	–
anti-inflammatory drugs	11 (55%)	4 (44%)	2 (20%)
antacids	16 (80%)	3 (33%)	7 (70%)
smoking habit			
current	4 (20%)	1 (11%)	2 (22%)
former	9 (45%)	–	4 (44%)
never	7 (35%)	4 (44%)	3 (33%)
unknown	–	4 (44%)	–

values represent number and percentage (%) of patients

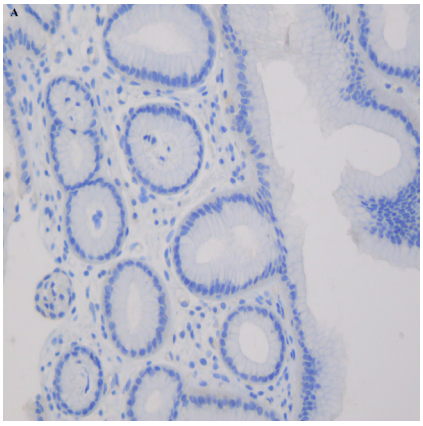
a median, interquartile range (IQR)

b mesenteric arterial stenosis defined as significant stenosis ≥50%

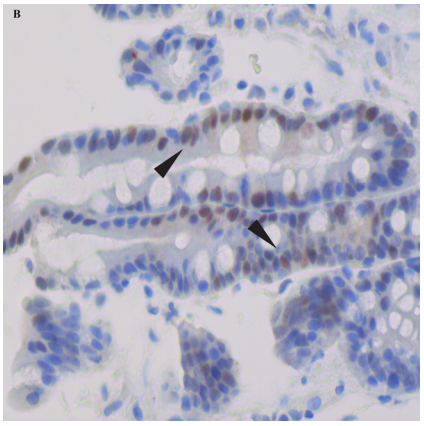
In all IC patients abnormal findings were present on endoscopy. Ulceration was present in most of the patients with IC (n=67%). All patients with infectious colitis had pathologic endoscopic appearance, either presence of erythema and edema (n=2/5) or even necrosis (n=3/5). The majority of patients with IBD had abnormal findings on endoscopy, varying from presence of erythema to necrosis. However, in 5 patients no signs of inflammation were observed in the colon on endoscopy. In the majority of the controls (83%), no abnormalities were seen in the colon on endoscopy.

	IC	UC	IBD	CD	infectious colitis	controls lower GI
	n=9	n=9		n=9	n=5	n=12
	72 (40-175)	2 (1-47)		17 (4-37)	66 (38-480)	1 (1-6)
	11.7 (8.4-13.4)	8.1 (5.2-15.0)		9.0 (7.4-9.6)	10.9 (10.2-27)	5.3 (4.9-7.3)
	2 (22%)			–	1 (20%)	2 (17%)
	–			–	–	1 (8%)
	1 (11%)			–	–	–
	6 (66%)		18 (100%)		4 (80%)	9 (75%)
	–	7 (78%)		6 (67%)	2 (40%)	4 (40%)
	4 (44%)	6 (67%)		6 (67%)	2 (40%)	5 (46%)
	7 (78%)	1 (11%)		1 (11%)	2 (40%)	9 (90%)
	2 (33%)	1 (11%)		1 (11%)	–	5 (42%)
	1 (17%)	–		1 (11%)	–	1 (8%)
	2 (33%)	6 (66%)		1 (11%)	2 (40%)	1 (8%)
	1 (17%)	2 (22%)		6 (66%)	3 (60%)	5 (42%)

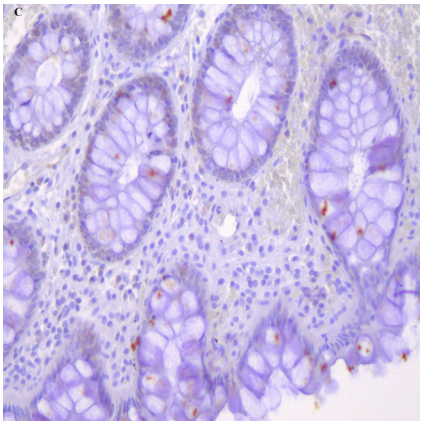
⊡ 1 Serial sections of gastrointestinal tract tissue analyzed for expression of HIF-1α by immunohistochemistry



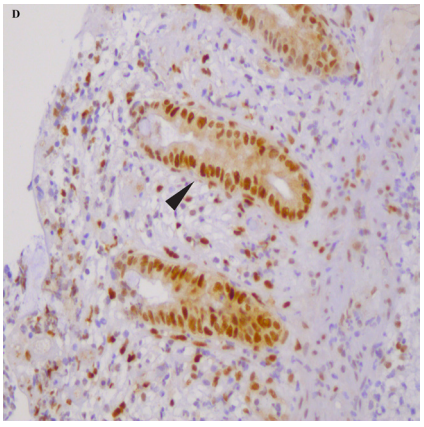
a normal stomach mucosa
magnification 400 ×



b chronic gastrointestinal ischemia
magnification 400 ×



c normal colon mucosa
magnification 400 ×



d ischemic colitis
magnification 200 ×

Expression of HIF-1α

Expression of HIF-1α was found in 5 out of 20 (25%) patients with CGI. HIF-1α expression was not present in the controls ⊙ **1a,b**. Expression of HIF-1α did not significantly differ between these groups ≣ **2**.

Expression of HIF-1α was more present in the lower GI-tract. In all patients with IC, and infectious colitis HIF-1α was upregulated ⊙ **1c**. Presence of HIF-1α was also seen in majority of IBD patients (67%). This was also in 7 out of 12 controls ⊙ **1d**.

Assessing the diagnostic performance of HIF-1α expression for the diagnosis of gastrointestinal ischemia, the sensitivity and specificity of HIF-1α were 25% and 84%. The sensitivity of HIF-1α for indicating presence of IC is 100%, with a specificity of 51%.

≣ **2** Expression of HIF-1α in the GI-tract

	HIF-1α +	P-value
upper GI-tract		
CGI	5 (25%)	
H. pylori-gastritis	3 (33%)	0.65
controls	0	0.08
lower GI-tract		
IC	9 (100%)	
infectious colitis	5 (100%)	0.48
IBD	12 (67%)	0.05
controls	7 (58%)	0.03[a]

values represent number and percentage (%) of patients

a *p*-value <0.05 was considered significant

3a Inflammation in the upper GI-tract according to diagnosis and expression of HIF-1α

	no inflammation	mild-moderate inflammation	moderate-severe inflammation
CGI	13 (65%)	7 (35%)	0
H. pylori-gastritis	0	4 (44%)	5 (56%)
controls	5 (50%)	5 (50%)	0
HIF-1α present	2 (11%)	4 (25%)	2 (40%)
HIF-1α absent	16 (89%)	12 (75%)	3 (60%)

values represent number and percentage (%) of patients

a *p*-value <0.05 was considered significant. Inflammation was scored as no inflammation (Sydney grade 0), mild to moderate (Sydney grade I-II) and moderate to severe inflammation (Sydney grade III-IV). Sydney grading was performed as previously published[16].

3b Inflammation in the upper GI-tract according to diagnosis and expression of HIF-1α

	no inflammation	chronic inflammation	acute inflammation	crypt destruction
IC	1 (11%)	1 (11%)	1 (11%)	6 (67%)
infectious colitis	0	2 (40%)	0	3 (60%)
IBD	3 (17%)	3 (17%)	1 (5%)	11 (61%)
controls	10 (83%)	2 (17%)	0	0
HIF-1α present	9 (64%)[a]	3 (38%)[a]	2 (100%)[a]	19 (95%)[a]
HIF-1α absent	5 (36%)	5 (62%)	0	1 (5%)

values represent number and percentage (%) of patients

a *p-value* <0.05 was considered significant. Inflammation was scored as no inflammation (Geboes grade 0), chronic (Geboes grade 1), acute (Geboes grade 2-3) and crypt destruction (Geboes grade 4-5). Scoring of inflammation in IBD as previously published[17].

Presence of inflammation

In 65% of patients with CGI histologic examination showed no sign of inflammation (grade 0 of the Sydney Classification Score). In the remaining patients (n=7), mild to moderate inflammation (Sydney grade I-II) was observed. Mild to severe inflammation (Sydney grade III-IV) was present in all patients with *H. pylori*-gastritis. Remarkably, in 50% of the controls mild to moderate inflammation (Sydney grade I-II) could be observed on histology ☰ **3a**.

In the lower GI-tract, majority (68%) of patients showed some degree of inflammation on histology ☰ **3b**. In all but one patient of the IC-group inflammation was present on histology. In 67% (n=6) of these patients ulceration and crypt destruction was present. This was comparable to the IBD and infectious colitis group, in which ulceration and crypt destruction was present in 61% (n=11) and 60% (n=3), respectively. In 83% (n=10) of the healthy subjects no abnormalities were observed on histology.

Inflammation and HIF-1α expression

HIF-1α expression was more frequently observed in 53% of patients with any degree of inflammation on histology in the upper GI-tract, and in 73% of patients in the lower GI-tract ☰ **3a**. The sensitivity and specificity of HIF-1α for indicating presence of inflammation is 29% and 89% respectively, in the upper GI-tract, compared to 80% and 36% for the lower GI-tract respectively. In the lower GI-tract, HIF-1α expression was significantly more observed in patients with inflammation on histology (p=0.02) ☰ **3b**.

Discussion

Only limited studies are performed to investigate the role of HIF-1α in the gastrointestinal tract, with most of them focusing solely on ischemia-reperfusion injury or neoplasia, but none in CGI[23,24]. The present study demonstrates the presence of HIF-1α in chronic ischemic and inflammatory diseases of the gastrointestinal tract.

We performed a pilot study to investigate the expression of HIF-1α in chronic ischemic tissue in order to identify patients with CGI. Our results show that HIF-1α is present in ischemic tissue, but cannot differentiate between ischemic, inflammatory diseases and controls. Furthermore, there is a weak correlation between HIF-1α expression and presence of inflammation seen on histology.

HIF1-α is markedly expressed in acute gastric ischemia, but expression of HIF-1α in chronic ischemic tissue of the GI-tract has not been described before[25]. In this study, patients with acute colonic ischemia and chronic gastric ischemia were included. It would have been ideal to include chronic colitis, being a long-term complication after an episode of acute ischemic colitis, but its incidence is very low and therefore rare[26]. Hence, to evaluate the expression of HIF-1α in ischemic tissue, we opted to explore its expression under both acute and chronic hypoxic conditions in the two most encountered forms of gastrointestinal ischemia.

This study demonstrates that HIF-1α is a marker for ischemia, showing high sensitivity for correctly identifying patients with IC, but poor sensitivity for diagnosing CGI. The difference in the expression of HIF-1α between these disease entities could be attributed to several variables.

First, the cellular responses involving HIF-1α appear to be different in acute and chronic conditions of hypoxia. Prolonged periods of hypoxia, such as in CGI, activate feedback mechanisms that decrease HIF-1α, such as enhanced protein kinase B (Akt) phosphorylation and consequent glycogen synthase kinase 3B (GSK3B), or forkhead transcription factor (FOX)04 activation. Second, upregulation of prolyl hydroxylase domains[27], encoding for

enzymes involved in HIF1-α hydroxylation, seem to be responsible for decrease of HIF-1α under chronic hypoxia. This is in contrast to their downregulation during short term hypoxic periods[28,29].

Furthermore, exposure to hypoxia elicits tissue inflammation by activation of genes encoding pro-inflammatory molecules. In case of acute hypoxia, such as in IC, it can be hypothesized that the pro-inflammatory reaction is more severe compared to chronic exposure to hypoxia.

Indeed, presence of inflammation might attribute to the enhanced expression of HIF-1α[30]. Expression of HIF-1α was present in all patients with ischemic colitis and infectious colitis, and was also significantly more present in patients with *H. pylori*-gastritis compared to CGI and healthy subjects. We found that patients with enhanced expression of HIF-1α also showed more inflammation on histology. In line with this, patients with expression of HIF-1α showed significantly more inflammation on histology in the lower GI-tract. Multiple factors associated with inflammation can cause limited oxygen availability, such as vasoconstriction, edema, and vasculitis with HIF-1α expression as result[31,32]. Also, inflammatory cells at the site of inflammation enhance the local oxygen consumption leading to hypoxic conditions[33]. Studies have detected immunoreactive HIF-1α in human macrophages. Bacterial exposure induces the production of NO and TNF-α production by macrophages, which not only generate inflammation, but also further stabilize HIF-1α in myeloid cells in the infectious focus[34]. This could also explain why in patients with infectious colitis, as well as gastritis, and in patients with IBD, expression of HIF-1α is present, even under normoxia. This is in line with earlier studies concerning expression of HIF-1α in colonic and infected upper GI-tract tissue[35-37].

A possible limitation of the study is the use of HIF-1α-modifying drugs during time of endoscopy and biopsy sampling. Use of non-steroidal anti-inflammatory drugs (NSAID) and proton pump-inhibitors (PPI) results in under expression of HIF-1α through upregulation of the von Hippel-Lindau factor (pVHL) which enhances degradation of HIF-1α, and by inhibition of angiogenesis[38-40]. As

not all patients could be stratified for (on-demand) PPI and NSAID use, this may have introduced a bias. Furthermore, the study is limited by the small sample population limiting the ability to detect significant differences between patients with chronic ischemia, controls and patients with inflammation. Further research using a larger sample size is needed.

In addition, it would be preferred to conduct a second independent technique such as western blot analysis or polymerase chain reaction (PCR) to corroborate our immunohistochemical findings. Demonstrating stable HIF-1α by such techniques is challenging because the ubiquitination of HIF-1α is a dynamic process and HIF-1α is a short-lived protein. As a solution, deubiquitination through pVHL-interacting protein deubiquitinating enzyme-2 (VDU2) could be considered. However, this required careful attention as it may result in artefacts[41]. Given the extent of these and possible other confounders, we believe performing these additional techniques is beyond the scope of this pilot study.

The results in our study confirm previous studies showing no expression of HIF-1α in normal tissue of the upper GI-tract[40,42]. Concerning the expression of HIF-1α in normal colon tissue, different results are demonstrated ranging from no expression to 90% expression in samples of normal colon[15,35]. In our study, HIF-1α expression in the colon was present in 57% of the healthy subjects. It is suggested that HIF-1α may play a role in the physiology of normal colon tissue. Due to the anaerobic environment in the lumen of the colon, capillaries in the surface regions of the colon epithelium are unable to provide for the oxygen demand of the tissue. As a result, these hypoxic areas may induce HIF-1α in order to promote angiogenesis and glycolysis[15].

Our study shows that HIF-1α identifies ischemia. However, no significant difference between patients with CGI and controls could be seen. HIF-1α has a high sensitivity for correctly identifying patients with IC; however, it cannot differentiate between ischemia and inflammation.

In conclusion, this study shows that HIF-1α is expressed in ischemic tissue, but also in normal colon tissue, and various inflammatory disorders. Therefore, the value of HIF-1α as a marker for ischemia remains to be further investigated.

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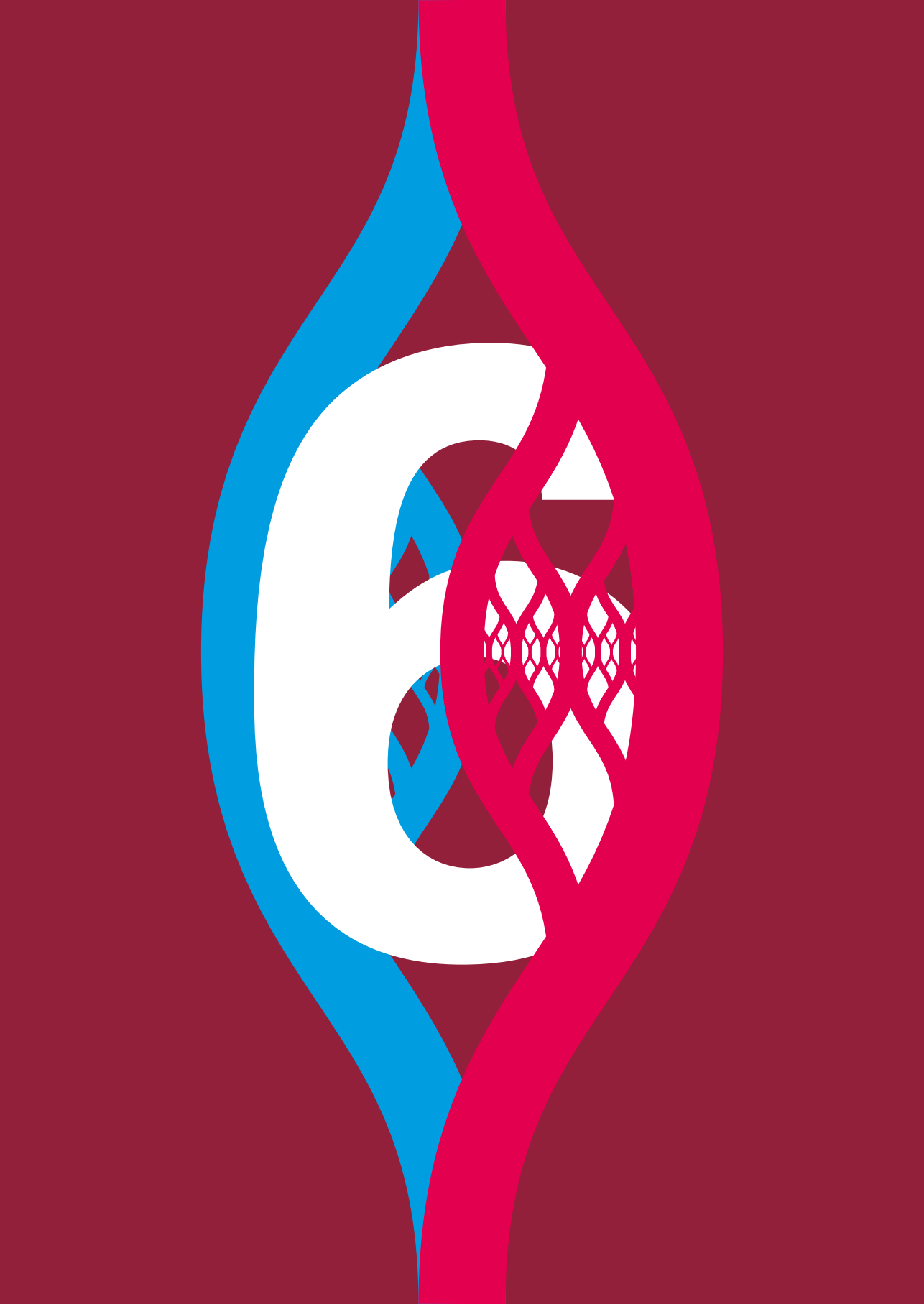

Liver injury is uncommon in chronic gastrointestinal ischemia

Adapted from:
Jihan Harki, Ernst J. Kuipers, Désirée van Noord, Hence J.M. Verhagen, Eric T.T.L. Tjwa

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Introduction

Hypoxic liver injury in an acute setting of cardiac, circulatory or respiratory failure, also known as hypoxic or ischemic hepatitis, is caused by insufficient oxygen uptake by hepatocytes and is usually defined as a sharp increase in serum aminotransferases (ALT) up to 20 times the upper limit of normal due to centrilobular hepatocyte necrosis in the absence of other potential causes of hepatitis[1]. It occurs in up to 20% of critically ill patients with low cardiac output and has a high 30-day mortality rate of 50%[2]. It is unknown whether hypoxic liver injury only pertains to the acute or also to the chronic setting. Chronically compromised mesenteric arterial flow is hallmark of a disease entity known as chronic gastrointestinal ischemia (CGI)[3]. The main cause of CGI is significant stenosis (≥70%) at the level of the origin of the mesenteric vasculature (celiac artery (CA) and/or superior mesenteric artery (SMA)) due to atherosclerosis, but also non-occlusive mesenteric ischemia (NOMI) due to cardiac forward failure may be observed. The incidence of CGI is estimated at 13/100.000 person-years and may progress in the vast majority from asymptomatic to symptoms of abdominal pain and malnutrition, with some progressing to visceral infarction and death within 6 years[4]. It is not known to what degree liver injury occurs in CGI. We aimed to observe the extend of liver injury in a well-defined cohort of CGI patients.

Methods

We performed a retrospective observational study of 156 consecutive patients diagnosed with CGI between November 2006 and March 2013. All patients received a standard CGI work-up including assessment of clinical symptoms and lifestyle parameters, laboratory tests, radiological imaging (abdominal ultrasound and/or computed tomography angiography (CT-A) and validated gastroduodenal mucosal saturation measurements[5]. We excluded patients with known liver disease. Liver injury was defined as ALT >1x upper limit of normal (ULN), corrected for gender.

Baseline characteristics were calculated using descriptive statistics. Data were expressed as mean and standard deviation (SD), median and interquartile range (IQR) or count and percentage (%), when appropriate. Logistic regression analysis was used to assess associations between potential risk factors and presence of liver injury. The associations were estimated as Odds Ratios (OR) with 95% confidence interval (CI). Statistical analysis was performed using the SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). A two-sided p-value of <0.05 was considered statistically significant.

The study was approved by the institutional reviewing board of the Erasmus MC University Medical Centre, Rotterdam, the Netherlands.

Results

In total, 156 patients were eligible for inclusion (median age 64.1 (IQR 53.1-73.6) years; 74% female, median ALT 0.49x ULN (IQR 0.36-0.67) and 90% had gastro-intestinal mucosal ischemia ≣ **1**). Radiological imaging did not reveal any considerable liver abnormalities. Stenotic CGI was present in 107 (69%) patients and NOMI in 49 (31%) patients.

Elevated ALT >1x ULN was present at baseline in 15 (11%) patients (10 stenotic CGI; five NOMI). Mucosal ischemia was detected in 12 of these patients. None had an ALT level more than 3x ULN. Elevated ALT >1x ULN was not significantly associated with presence of mucosal ischemia, significant stenosis of the CA, SMA, or both CA and SMA. Neither was there an association between ALT >1x ULN and presence of known predisposed conditions of steatotic liver injury such as body mass index (BMI), alcohol consumption and diabetes mellitus (DM) type II, nor between ALT >1x ULN and increasing age ≣ **2**. Twelve of these 15 patients received treatment (endovascular, surgical or medical treatment according to cause of CGI). At follow-up (median 17.6 months), only 2 of these patients kept ALT >1x ULN (mean 75 IU/ml). Both patients were diagnosed with NOMI and were irresponsive to treatment.

1 Baseline characteristics

	total population (n=156)	ALT <1x ULN[a] (n=141)	ALT >1x ULN[a] (n=15)
age (years)[b]	64.1 (53.1-73.6)	64.2 (52.9-73.7)	61.8 (57.6-72.4)
sex			
male	40 (26)	37 (26)	3 (20)
female	116 (74)	104 (74)	12 (80)
BMI (kg/m²)[b]	22.4 (19.4-25.9)	22.5 (19.5-26.0)	21.6 (18.7-25.8)
duration of symptoms (months)[b]	12 (6.0-25.5)	12 (6-24)	24 (6.0-78)
alcohol (U/day)[c]	0 (0-20)	0 (0-20)	0 (0-5)
increased alcohol intake[d]	37 (24)	35 (25)	2 (13)
DM type II	26 (16)	24 (17)	2 (13)
diagnosis			
NOMI	49 (31)	44 (31)	5 (33)
stenotic CGI	107 (69)	97 (69)	10 (67)
mesenteric artery stenosis			
no stenosis	47 (30)	42 (30)	5 (33)
single artery stenosis	52 (33)	47 (33)	5 (33)
multi artery stenosis	67 (37)	52 (37)	5 (33)
mucosal ischemia			
present	141 (90)	129 (92)	12 (80)
absent	13 (8)	10 (7)	3 (20)
not performed	2 (1)	2 (1)	0

values represent number and percentage (%) of patients

a ALT >1x ULN, corrected for gender (33 U/l for females, 44 U/l for males)

b median (interquartile range (IQR))

c median (range)

d increased alcohol intake is defined as >1 units/dag for females and >2 units/day for males

☰ 2 Univariable analysis for ALT >1x ULN

	OR (95% CI)	*p*-value[a]
age (years)	0.98 (0.96-1.04)	0.91
age >60 years	0.96 (0.32-2.85)	0.94
female gender	1.42 (0.38-5.33)	0.60
BMI (kg/m²)	0.96 (0.86-1.07)	0.96
BMI >25 (kg/m²)	1.09 (0.35-3.39)	0.88
duration of symptoms (months)	1.01 (0.99-1.02)	0.23
alcohol (U/day)	0.79 (0.52-1.22)	0.29
increased alcohol intake[b]	0.45 (0.10-2.09)	0.31
DM type II	0.75 (0.16-3.54)	0.72
mesenteric artery stenosis		
≥70% stenosis CA	0.59 (0.20-1.73)	0.34
≥70% stenosis SMA	0.66 (0.20-2.19)	0.49
≥70% stenosis both CA and SMA	0.84 (0.34-3.83)	0.84
presence of mucosal ischemia	2.69 (0.67-10.86)	0.17

a *p*-value <0.05 was considered significant

b increased alcohol intake is defined as >1 units/day for females and >2 units/day for males

Conclusion

In this study, we show that liver injury is uncommon in CGI. The results of the current study shows that serum aminotransferases were very mildly raised in only 10% of the total cohort of 156 patients with well-defined CGI, which may have normalized after successful treatment of CGI.

The liver has a unique dial blood supply by both the hepatic artery (HA), which stems from the CA and provides up to 30% of the total blood supply to the liver, and the portal vein which provides the remaining 70-85% of blood to the liver[6]. In contrast to most other organs, hepatic perfusion does not follow principles of supply and demand. Blood flow in the HA is inversely related to blood flow in the portal vein and is regulated by the presence adenosine, which is capable of controlling arterial tonus. In case of decreased portal flow, arterial blood flow increases due to dilation of the HA as a result of decreased washout of adenosine from the periportal space of Mall[7]. This is not reciprocal in case of decreased arterial flow (such as in CGI) as the PV cannot control its blood flow (being the outflow tract of the extrahepatic splanchnic organs)[8]. It is tempting to speculate that in CGI liver injury may be prevented by increased oxygen extraction and less by enhancement of portal blood flow[9].

Interestingly, although patients with CGI are known to have a high cardiovascular risk profile, no association could be found between elevated serum aminotransferases and risk factors for steatohepatitis. The observed BMI in this patient population might be beneficial and could be explained by involuntary weight loss due to CGI. However, no association could either be observed for BMI>25kg/m^2.

In conclusion, in contrast to acute ischemic conditions, liver injury is not a common feature of CGI. Further studies need to the confirm the generalizability of this observation.

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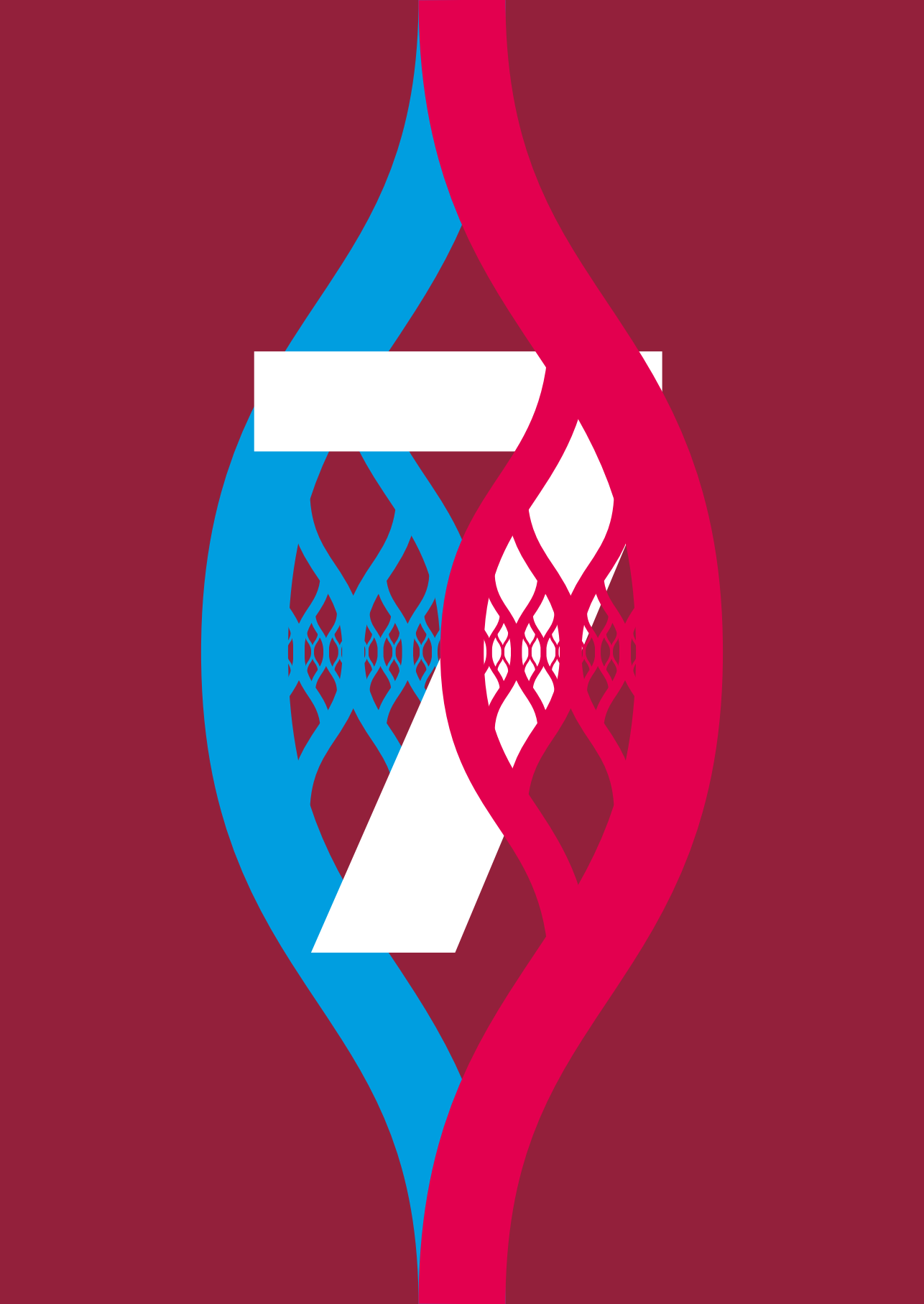

Gastrointestinal ischemia in patients with portal vein thrombosis: a prospective cohort study

Jihan Harki*, Elisabeth P.C. Plompen*, Désirée van Noord, Jildou Hoekstra, Ernst J. Kuipers, Harry L.A. Janssen, Eric T.T.L. Tjwa

* Authors contributed equally to this work

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Abstract

Background

Gastrointestinal ischemia is a concerning complication of portal vein thrombosis (PVT). Minimally invasive techniques, such as visible light spectroscopy (VLS), have greatly improved the ability to diagnose gastrointestinal ischemia. The aim of this study was to assess the clinical presentation and characteristics of gastrointestinal ischemia in patients with PVT.

Methods

Patients with non-cirrhotic, non-malignant PVT were included in this prospective cohort study. Clinical symptoms of gastrointestinal ischemia were assessed by a structured questionnaire, VLS, and radiological evaluation of the mesenteric vasculature. VLS measurements were compared to those in patients with cirrhosis and a reference population.

Results

We included 15 patients with chronic PVT and one patient with acute PVT (age 46.1 [IQR 30.9-53.7] years; 44% male). Decreased mucosal oxygenation in at least one location of the gastrointestinal tract was found in 12/16 (75%) patients. Compared to the reference population (median 60.0 [56.2-61.7]), VLS measurements were mostly decreased in the descending duodenum for patients with PVT (median 55.5 [52.3-58.8], p=0.02) and patients with cirrhosis (median 52.0 [46.5-54.0], p=0.003). Symptoms typical for gastrointestinal ischemia, such as postprandial pain and exercise-induced pain, were reported in 10/16 (63%) patients with PVT. In patients with extension of thrombosis into the superior mesenteric vein and splenic vein and/or presence of hypercoagulability, decreased VLS measurements were observed compared to the historical controls.

Conclusions

In patients with chronic PVT, gastrointestinal ischemia is frequent. VLS enables objective and quantitative determination of gastrointestinal mucosal ischemia. Onset of abdominal symptoms such as postprandial pain should prompt the physician to re-evaluate extent, cause and treatment of PVT.

Introduction

Portal vein thrombosis (PVT) is an infrequent vascular disorder that often leads to portal hypertension. Etiological factors include systemic and local prothrombotic factors[1]. PVT can be classified as acute or chronic. The latter is characterized by the presence of portal cavernoma, which depends on the duration of existence of the thrombus in the portal vein (PV)[1-3].

Gastrointestinal ischemia is a concerning complication of PVT. It can result in intestinal infarction, a life-threatening complication that often requires immediate surgical intervention[2]. Treatment of acute PVT therefore aims at vascular recanalization, preventing occurrence of gastrointestinal ischemia and portal hypertension[1]. The actual prevalence of gastrointestinal ischemia in patients with PVT is unknown. Studies, mainly conducted in patients with acute PVT, report prevalences of intestinal infarction of 2%-32%, with mortality rates of 0%-20%[3-9]. Regarding patients with chronic PVT, studies report conflicting results. As patients with chronic PVT often have an extensive venous collateral circulation, it is assumed that gastrointestinal ischemia is less likely to occur[2]. However, in a large prospective study in splanchnic vein thrombosis, 26% of patients presented with intestinal infarction. In nearly half of these patients a portal cavernoma was detected, suggesting gastrointestinal ischemia is also a frequent complication in chronic PVT[4].

Typical complaints suggesting gastrointestinal ischemia are postprandial and/or exercise related abdominal pain, weight loss, and diarrhea. However, some patients present with more atypical symptoms, which limits the diagnosis of gastrointestinal ischemia based on clinical symptoms alone[10]. In addition, clinical signs of gastrointestinal ischemia are often difficult to differentiate from symptoms due to PVT. Also, it has been suggested that signs of ischemia in patients with PVT depend largely on the extent of the occlusion, the size of the vein, and the involvement of the superior mesenteric vein (SMV) and/or splenic vein (SV)[2,11].

With the development of minimally invasive techniques, such as visible light spectroscopy (VLS), the ability to diagnose gastrointestinal ischemia has greatly improved [12]. VLS enables direct measurement of the adequacy of mucosal perfusion in the gastrointestinal tract [13]. It is a new technique that non-invasively measures capillary hemoglobin oxygen saturation using white light delivered by a fiberoptic probe during gastroscopy [12,13]. The oxygen saturation reflects the adequacy of mucosal perfusion. Therefore, venous occlusion, hampering oxygenation of the gastrointestinal mucosa, may result in lower mucosal hemoglobin oxygen saturations [12]. VLS is a validated diagnostic method to detect gastrointestinal ischemia with a sensitivity and specificity of 90% and 60%, respectively [12,13].

Due to the rarity of the disease and lack of adequate diagnostic tools in the past, most data on gastrointestinal ischemia in patients with PVT stem from retrospective studies in selected patient populations [3-7]. VLS enables us to directly quantify gastrointestinal ischemia in patients with PVT. Therefore, we performed a prospective cohort study in patients with PVT using VLS, radiological examination, and questionnaires. The objective of this study was to assess the presence, clinical presentation and characteristics of gastrointestinal ischemia in patients with PVT.

Methods

Study population

Patients with non-cirrhotic, non-malignant PVT were studied in this single-center prospective cohort study in a tertiary care center. Patients aged 18 years and over with non-cirrhotic, non-malignant PVT were included in the study from 2009 until 2010. Acute and chronic PVT were defined according to Baveno V criteria as the presence of PVT with or without portal cavernoma and portal hypertension[14]. Patients with isolated SV or SMV thrombosis were excluded. All patients underwent radiological evaluation within two months of the VLS measurements. All patients with PVT received scheduled visits at the out-patient clinic. For this cohort study, we adhered to the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) initiative[15].

Patients with liver cirrhosis and portal hypertension with patent mesenteric vasculature were included as cirrhotic patient controls. Portal hypertension was defined as: presence of varices and/or splenomegaly and/or ascites and/or hepatic hydrothorax and/or increased hepatic venous pressure gradient (>12mm Hg) AND in the absence of an intrahepatic shunting stent (i.e. TIPS).

For further comparison, VLS measurements of a historical reference population were used. These were participants in the original cohort of van Noord et al., of whom mucosal saturation measurements using VLS were obtained to establish the present cut-off values for gastrointestinal ischemia in the different locations in the gastrointestinal tract. These were consecutive patients referred for evaluation of possible chronic gastrointestinal ischemia who underwent the standard diagnostic work-up for gastrointestinal ischemia consisting of evaluation of symptoms, radiological imaging, and gastrointestinal tonometry in addition to VLS. In all these patients, more common causes of upper gastrointestinal symptoms had been previously excluded. A diagnosis of chronic gastrointestinal ischemia was established if a patient fulfilled two of the three following criteria:

1 distinct clinical presentation including presence of postprandial pain and otherwise unexplained weight loss of >5% of the normal body weight.
2 significant stenosis of >50% of at least one of the mesenteric arteries.
3 functional signs of mucosal ischemia demonstrated by VLS.

None of the historical controls fulfilled the required criteria. The VLS measurements were performed in identical fashion as in the previous study of the same group[12].

Informed consent was obtained from all patients. Approval for the study was obtained from the Institutional Review Board of the Erasmus MC University Medical Centre Rotterdam, the Netherlands. The study was registered at the ISRCTN registry (study ID ISRCTN14235960).

Diagnostic work-up and data collection

All patients with PVT received the standard work-up. This consisted of screening for etiologic factors, laboratory testing, abdominal ultrasound, and gastroscopy.

Standardized abdominal ultrasound was performed to assess the liver parenchyma, PV, hepatic artery, hepatic veins, SV, SMV, and to exclude the presence of cirrhosis and assess features of portal hypertension. Gastroscopy was performed to determine the possible presence of varices.

Use of antithrombotic medication was evaluated for all patients. Antithrombotic medication was initiated at the discretion of the treating physician and/or according to standard care. Time of antithrombotic medication use was assessed from diagnosis of PVT to time of VLS measurements. For each patient, a covered time (in %) of antithrombotic use was calculated by dividing the time (in months) of antithrombotic medication use by time (in months) since diagnosis of PVT.

Assessment of gastrointestinal ischemia

Clinical symptoms of gastrointestinal ischemia were assessed by a structured questionnaire, VLS measurements and radiological evaluation of the mesenteric arteries.

The questionnaire was specifically designed to collect relevant medical and family history, medication use, and gastrointestinal symptoms [10,16]. To avoid missing data, medical history notes on gastrointestinal symptoms were also included.

Radiological evaluation of the mesenteric vasculature was performed by CT- A, or MR-angiography (MR-A), with a maximum slice thickness of 3mm. Site and extension of the PVT, as well as involvement of the SMV and SV, were also evaluated. Stenosis of the mesenteric arteries of ≥70% of vessel lumen was considered significant.

Mucosal saturation measurements

Gastroscopy was performed in all patients. VLS measurements were performed during gastroscopy as described before [12,17]. In short, cleansing of remnant bile, fluids or food remnants was done prior to measuring along with administration of butylscopolamine 20 mg intravenously to halt upper gastrointestinal motility. Measurements were performed at three specific locations: descending duodenum, duodenal bulb and gastric antrum. We averaged three repeated readings per location, with every measurement within 5% variation of panel read-out once a stable reading was obtained. This was considered the most accurate reflection of the mucosal saturation at that location. The sites of the VLS measurements per location were standardized in the way that in every patient the same anatomical regions were used. Peripheral oxygenation was kept above 94%. Mucosal ischemia was defined as: mucosal saturation <63%, <62% and <58%, for measurements in the gastric antrum, duodenal bulb, and descending duodenum, respectively [12]. The physician performing the VLS measurements was not blinded to the patient characteristics. However, he was not the treating physician of the patient and was therefore not familiar

with the specifics of the radiological findings of these patients. At time of inclusion, only two physicians in our center were specialized in performing the VLS measurements. The second physician was trained by the other physician, limiting a possible inter-observer variability.

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Data were expressed as mean and standard deviation (SD), median and interquartile range (IQR) or count and percentage (%), when appropriate. Differences in VLS measurements per location in the GI-tract between the different groups were assessed with linear regression analysis. Statistical analysis was performed using SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results

Study population

In this prospective cohort study, we included 16 patients with PVT (age 46.1 [30.9-53.7] years; seven patients were male). Baseline characteristics of these patients, at the moment of VLS measurement, are displayed in ≣ **1**. At diagnosis, five patients had an acute PVT and in 11 patients a portal cavernoma was observed. At the moment of VLS measurement, 15 patients (94%) had chronic PVT.

In 14 patients (87%), at least one prothrombotic risk factor was present, including an inherited hypercoagulable risk factor in six patients of whom three with a factor V Leiden mutation. A local risk factor, such as inflammatory bowel disease or pancreatitis, was found in four patients.

Treatment with heparin followed by oral anticoagulants (OAC) was administered in 12 patients (75%). In nine of these patients (75%), anticoagulants were started at diagnosis and was continued at least until the moment of VLS measurement (covered time 99-100%). In two of these 12 patients, administration of OAC was delayed by 6 and 9 months respectively, which resulted in treatment with anticoagulants with a covered time <99-100%. In the remaining patient, OAC was directly administered, but discontinued early because of an upper gastrointestinal bleeding, resulting in a covered time of 16.7%. The absolute length and percentage covered time of anticoagulation use is further described in ≣ **1**. In two patients, acetylsalicylic acid was started because of presence of a myeloproliferative neoplasm (MPN). Before administering oral anticoagulants, thrombolysis was unsuccessfully attempted in one patient. All patients who initially presented with an acute PVT were treated with OAC from diagnosis until at least the moment of VLS measurement.

To determine whether the VLS measurements in patients with PVT are affected only by presence and extent of the portal and mesenteric venous thrombosis or also by portal hypertension, we included a cirrhotic patient control group consisting of five patients

1 Baseline characteristics

			PVT				
ID	**age**	**sex**	**site**[a]	**presentation**[b]	**underlying liver disease**	**degree of occlusion**	**extrahepatic involvement**
1	30	F	**3**	chronic	none	complete	SMV+SV
2	38	M	**2a**	chronic	none	complete	SMV+SV
3	48	F	**1**	chronic	none	complete	SMV
4	34	F	**3**	chronic	none	partial	SMV
5	53	F	**2b**	acute	none	partial	SMV
6	62	M	**3**	chronic	none	complete	None
7	26	F	**1**	chronic	none	complete	None
8	53	F	**3**	chronic	none	complete	SMV+SV
9	54	M	**3**	chronic	none	complete	SMV+SV
10	30	F	**2a**	chronic	none	partial	SMV
11	48	M	**3**	chronic	none	complete	SMV+SV
12	43	F	**3**	chronic	none	complete	SMV+SV
13	53	F	**3**	chronic	none	complete	SMV+SV
14	20	M	**3**	chronic	none	complete	None
15	19	M	**3**	chronic	none	complete	None
16	54	M	**3**	chronic	none	complete	SMV+SV
17	61	M	none	none	cirrhosis	none	none
18	55	M	none	none	cirrhosis	none	none
19	59	F	none	none	cirrhosis	none	none
20	64	M	none	none	cirrhosis	none	none
21	67	F	none	none	cirrhosis	none	none

a site of thrombotic involvement of the portal vein. Type 1=thrombosis of the trunk; type 2a=thrombosis of one branch; type 2b=both branches; type 3=thrombosis of both portal trunk and branches

b presentation at moment of VLS measurement, as diagnosed by radiological imaging (CT or abdominal ultrasound)

c one patient (ID 4) had elevated antiphospholipid (anti-cardiolipin) antibodies and ID 8 had elevated levels of factor VIII. Three patients had an FVL mutation

d MPN NOS: an MPN is present, but the type could not be classified into more detail by histological examination of bone marrow

e amount of time on anticoagulants (oral anticoagulants and/or heparin) from diagnosis until VLS measurement divided by total amount of time from diagnosis until VLS measurement

f absolute length of anticoagulation use from moment of inclusion until end of follow-up, in months

g all patients with varices had oesophageal varices, except for ID 2, who was diagnosed with duodenal varices. ID 1 had both gastric (GOV-1) and oesophageal varices

hyper-coagulability[c]	Jak2 mutation	MPN[d]	anticoagulant time (%)[e] / length (mon)[f]	anti-platelets	varices[g]
–	+	NOS	0/0	–	+
–	–	–	38.5/6	–	+
FVL	–	–	100/66	–	+
APA	na	na	99/55	–	–
na	na	na	100/62	–	–
–	+	PV	100/26	+	+
FVL	na	na	0/0	–	–
FVIII	+	ET	100/59	–	+
PC	–	na	16.7/1	–	+
–	–	–	100/8	–	–
–	–	–	100/23	–	–
–	+	PV	0/0	+	+
–	+	ET	100/57	–	–
–	–	–	0/0	–	+
–	–	–	100/31	–	–
FVL	+	ET and PV	20.8/22	–	+
na	na	na	–	–	+
na	na	na	–	–	+
na	na	–	–	–	+
na	na	na	–	–	+
na	na	na	–	–	+

(age 61.0 [57.5-66.1] years (p=0.02 compared to patients with PVT), three patients were male (p=0.2)) with liver cirrhosis without PVT. All of these patients had Child-Pugh C cirrhosis and were screened for liver transplantation. In these five patients, liver cirrhosis was caused by hepatitis B virus infection (n=2), hepatitis C virus infection (n=2) or primary sclerosing cholangitis (n=1). All patients in the cirrhotic patient control group had oesophageal varices. There were no liver function abnormalities in all patients with PVT.

2 Clinical symptoms and risk factors for gastrointestinal ischemia at time of VLS measurements

	cases (n=16)	cirrhotic patient controls (n=5)	historical controls (n=29)
abdominal pain	10 (63)	0	28 (97)
postprandial pain	9 (56)	0	15 (52)
exercise-induced pain	8 (50)	0	13 (45)
weight loss	7 (44)	1(20)	19 (66)
weight loss (kg/month) [a]	5 [2.0-6.7]	–	0.6 [0-1.1]
loose stools	12 (75)	3 (60)	8 (28)
BMI (kg/m^2) [a]	25.4 [21.9-26.8]	28.4 [24.0-33.5]	20.9 [19.6-23.9]
coronary and/or peripheral arterial disease	0	1 (20)	7 (24)
family history of CVD [b]	4(27)	5 (100)	12 (44)
DM type II	1 (6)	2 (40)	3 (10)
hypertension [c]	9 (56)	1 (20)	10 (35)
hypercholesterolemia [d]	4 (27)	1 (25)	6 (21)
smoking	3 (19)	1 (20)	13 (46)
mesenteric arterial stenosis [e]	0	0	4 (14)

values represent number of patients (percentage %), unless otherwise specified

a median, interquartile range

b positive family history is defined as first-degree relatives with history of coronary and/or peripheral arterial disease. Family history of cardiovascular disease was missing in one patient with PVT

c hypertension is defined as systolic blood pressure of >140mm Hg and/or diastolic blood pressure of ≥90mm Hg or use of anti-hypertensive medication

d hypercholesterolemia is defined as serum LDL cholesterol ≥190 mg/dl or use of statins. Serum LDL-cholesterol was missing in one cirrhotic patient control

e mesenteric arterial stenosis is defined as a significant stenosis of ≥70% of the vessel lumen of the celiac trunk (CT), superior mesentery artery (SMA) or inferior mesentery artery (IMA) on CT-angiography with maximum slice thickness of 3 mm

Clinical features of gastrointestinal ischemia

Abdominal pain was reported by ten out of 16 (63%) patients with PVT ☰ **2**. Postprandial pain, typical for gastrointestinal ischemia, was reported by nine out of 16 (56%). Furthermore, exercise-induced pain and weight loss (median 5.0 [2.0-6.7] kg/month) were reported in eight (50%) and seven (44%) patients with PVT. None of the cirrhotic patient controls reported any presence of postprandial abdominal or exercise-induced pain. Of the 11 PVT patients with extension into the SMV and SV, eight reported postprandial or exercise-induced abdominal pain. None of the PVT patients without involvement of the SMV and SV reported abdominal pain.

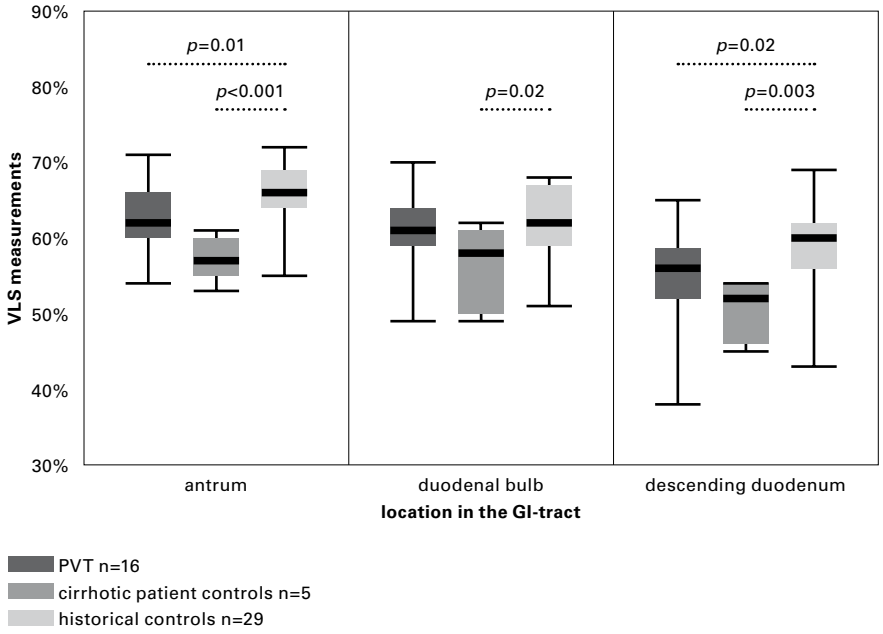
Abdominal pain was the presenting symptom and reason for referral in the vast majority of the 29 historical controls (38% male (p=0.09 and p=0.09 compared to patients with PVT and cirrhotic patient controls, respectively), age 56.5 [43.9-66.4] years (p=0.7 and p=0.4, respectively)). However, the occurrence of significant weight loss (median 0.6 [0-1.1] kg/month) and loose stools (28%) was significantly lower in this group compared to patients with PVT (p=0.001 and p=0.002, respectively). Mesenteric arterial stenosis was ruled out in the patients with PVT and the cirrhotic patient controls. Four historical controls were referred because of a stenosis of the mesenteric arteries, however the diagnosis of gastrointestinal ischemia was excluded during the diagnostic work-up.

Mucosal saturation measurements

VLS measurements were performed in all patients with PVT and in the five patients with severe cirrhosis and portal hypertension. VLS measurements of the historical controls were used as reference population. Decreased VLS measurements in at least one location of the gastrointestinal tract were found in 12 out of 16 (75%) patients with PVT. Compared to the reference population (median 60.0 [56.2-61.7]), mucosal saturation measurements were mostly decreased in the descending duodenum for both patients with PVT (median 55.5 [52.3-58.8], p=0.02) and patients with cirrhosis (median 52.0 [46.5-54.0], p=0.003) ⊡ **1**. The median VLS measurement of patients

with PVT (median 62.0 [60.0-65.8]) and cirrhosis (median 57.0 [55.0-60.0]) were also significantly lower in the antrum compared to the historic reference population (median 65.9 [64.2-69.3], p=0.01 and p<0.001, respectively). There was a significant difference in median VLS measurements in the duodenal bulb between patients with cirrhosis and portal hypertension (median 58.0 [50.5-61.0]) compared to the historic reference population (median 62.9 [59.2-66.9], p=0.02), but not between patients with PVT (median 60.5 [59.3-64.0]) and the reference population (p=0.5)). There

1 Visible light spectroscopy (VLS) measurements in patients with portal vein thrombosis (PVT), cirrhotic patient controls and in the reference population



VLS measurements performed at three locations in the gastrointestinal tract for all three groups of patients. VLS measurements were significantly decreased for patients with PVT compared to the reference population in the antrum and descending duodenum, but not in the duodenal bulb. Compared to the reference population, patients with portal hypertension had decreased VLS measurements in all locations, indicating mucosal ischemia. Boxes represent medians with interquartile range, whiskers extend to the most and least extreme scores respectively.

seemed to be an association between presence of postprandial abdominal pain and/or exercise-induced abdominal pain and decreased VLS measurements in the descending duodenum. Patients with abdominal pain tended to have lower VLS measurements than patients without abdominal pain (median 54.0 [48.8-57.3] vs median 58.5 [53.8-60.5], p=0.09). Of the ten patients with PVT with postprandial abdominal pain and/or exercise-induced abdominal pain, nine (90%) had decreased VLS measurements, of which six at more than one location in the gastrointestinal tract.

Characteristics of ischemic colitis

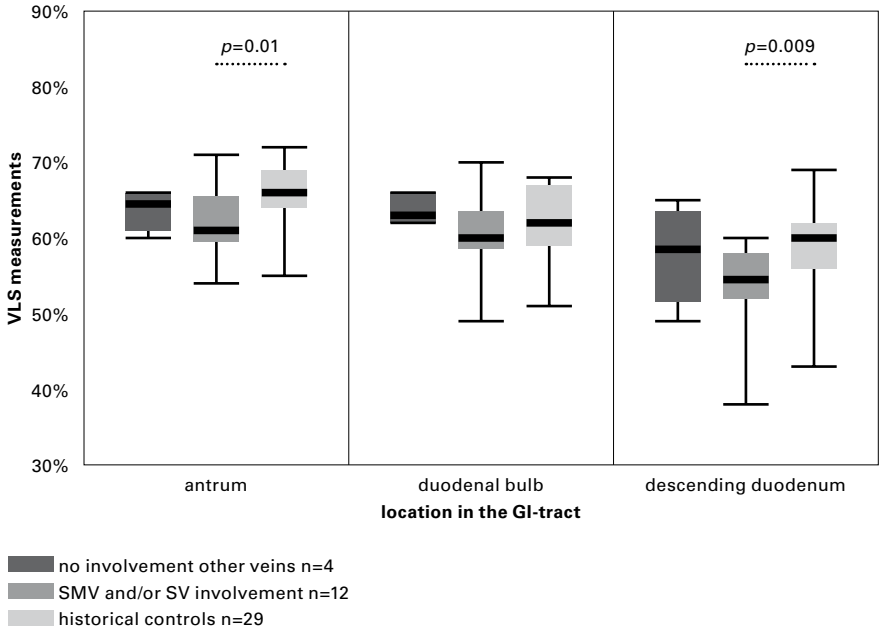
Colonoscopy was performed in three out of 16 patients with PVT at the moment of VLS measurements. Two patients had abnormal findings. In one patient, signs of ulceration and colitis were seen in the descending colon attributed to ischemia, which was confirmed by histological tissue evaluation. This patient (ID 2) was diagnosed with a complete occlusion of a side branch of the PV, SMV, and SV. This patient also had decreased VLS measurements in the descending duodenum. In the second patient (ID 3), colonoscopy revealed mucosal edema and erythema in the distal colon and sigmoid, suggestive for colitis due to congestion. This patient had a complete occlusion of the PV and SMV, and decreased VLS measurements of the duodenum. None of the cirrhotic patients had signs of ischemic colitis during colonoscopy.

Factors associated with VLS measurements

Patients with an extension of the thrombus into the SMV and/or SV had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population, indicating mucosal ischemia (p=0.01 and p=0.009, respectively) ⊡ **2**. There was no difference in VLS measurements between patients with PVT without extension compared to the historical controls. Furthermore, no difference in VLS measurements was observed in patients with extension of the PVT into the SMV and/or SV compared to patients without extension of thrombus outside the PV.

Patients who were treated with OAC had higher VLS measurements compared to those who were not or partly (covered time <99%) treated, (median 58.0 [53.5-59.5] vs 53.0 [49.0-57.0], p=0.096 in the descending duodenum) ⊡ **3**. Patients who were not or partly treated with OAC had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population, indicating mucosal ischemia (p=0.007 and p=0.003, respectively). The use of OAC in patients with PVT results in a relative risk difference of 19% in decrease of VLS measurements. Hence, the number needed to treat with anticoagulants to prevent decreased VLS measurements in one patient is 5.3.

⊡ **2** VLS measurements and extent of venous thrombosis

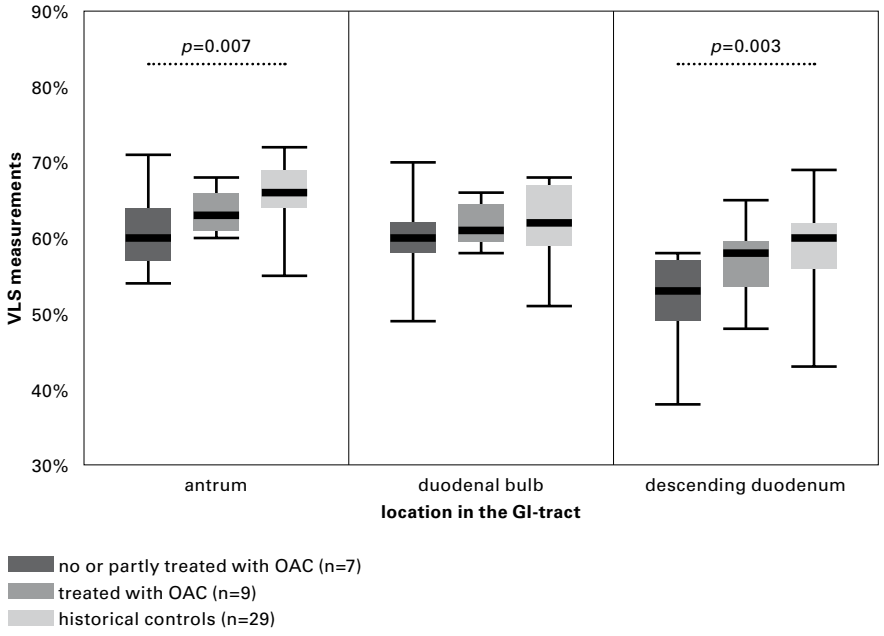


Patients with PVT with an extension of the thrombus into the superior mesenteric vein (SMV) and/or splenic vein (SV) had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population, indicating mucosal ischemia. Boxes represent medians with interquartile range, whiskers extend to the most and least extreme scores respectively.

Abdominal pain was reported in a lower proportion of patients using OAC compared to patients without anticoagulation, but this difference was not statistically significant (56% vs 71%, p=0.6).

Presence of an inherited hypercoagulable risk factor resulted in decreased VLS measurements at all locations in three out of six (50%) patients. In two of the three remaining patients with an inherited risk factor, decreased measurements in the descending duodenum – and antrum in one patient – were observed. One patient with an inherited risk factor did not have decreased VLS measurements, however this patient had an incomplete occlusion of the PV. Patients with a known inherited hypercoagulable risk factor more

3 VLS measurements in relation to use of antithrombotic therapy



Patients who were not or partly treated with oral anticoagulation (OAC) had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population, indicating mucosal ischemia. Boxes represent medians with interquartile range, whiskers extent to the most and least extreme scores respectively.

often reported abdominal pain typical for gastrointestinal ischemia compared to patients without an inherited hypercoagulable risk factor (83% vs 44%, p=0.3).

Follow-up after VLS measurements

After a median follow-up time of 51 (11-59) months, 15 of the 16 patients with PVT were still alive. One patient developed multi-organ failure due to renal insufficiency and died of pneumonia and sepsis.

Ischemic events occurred in two patients. One patient (ID 13) presented with an acute small bowel ileus at time of diagnosis of PVT, which was treated conservatively. Four months after diagnosis, due to ongoing symptoms, an explorative laparotomy was performed which showed a complete stenosis of the jejunum as well as thrombosis of the draining mesenteric veins. A side-to-side jejuno-jejunostomy was performed proximally of the stenosis. The second patient (ID 2) presented with melena one month after the VLS measurements. Distal enteroscopy showed signs of ischemic colitis for which the patient was treated conservatively. In both patients decreased VLS measurements were observed in at least one location of the gastrointestinal tract.

Gastrointestinal bleeding events occurred in three patients and were all related to variceal bleeding (ID 1, 2, and 9). One patient did not use OAC at time of bleeding. However, in the fourth year of follow-up, the patient developed deep vein thrombosis (DVT), for which OAC treatment was initiated. Two patients were using OAC at time of bleeding. In one of these patients, OAC had to be discontinued, which resulted in a DVT five months later.

Discussion

Gastrointestinal ischemia is a concerning complication of PVT. The occurrence and risks of gastrointestinal ischemia have in particular been acknowledged in patients with acute PVT[1-3,5,6]. The clinical spectrum of gastrointestinal ischemia in patients with chronic PVT is hitherto unknown. Therefore, we performed a unique prospective study in a well-characterized cohort of PVT patients in which clinical symptoms for gastrointestinal ischemia were assessed using a structured questionnaire, imaging of the mesenteric vasculature and VLS measurements. The latter enabled us to objectively and quantitatively determine the presence and extent of gastrointestinal mucosal ischemia. Patients presenting with non-malignant, non-cirrhotic PVT are quite rare, but we were able to include 15 patients meeting our inclusion criteria during a period of 24 months. The rarity of the disease made it difficult to compose a homogenous group. Therefore, we included patients with both acute and chronic PVT, patients on anticoagulation versus no anticoagulation, and complete versus partial occlusion of PV.

Nevertheless, this is one of the few studies with prospective data on the occurrence of gastrointestinal ischemia in a population of patients with PVT. In our study, 15 out of 16 patients were known with chronic PVT at time of the VLS measurements. We found that postprandial abdominal pain and exercise-induced pain are common in patients with chronic PVT and that mucosal ischemia is often present in these patients, especially in the antrum and descending duodenum. We also found that patients with a thrombus extending into the SMV and/or SV more often report abdominal pain than patients without extension of the thrombus. Also, patients with extension of the thrombus into the SMV and/or SV had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population.

The findings presented in this study warrant caution in the patient with PVT: onset of abdominal symptoms such as postprandial pain should prompt the physician to re-evaluate extent, cause

and treatment of PVT. For instance, one should be vigilant of a possible thrombus extension into the SMV, as this is associated with worse outcome[18]. The results of our study show that involvement of the SMV and/or SV in patients with PVT can be detected by VLS measurements, however this was not the case in patients with PVT without thrombus extension. This suggests that mucosal ischemia could be more prevalent in patients with PVT with thrombus extension. Also, one should be aware of presence of a systemic hypercoagulable state, as this is associated with thrombosis of the smaller venules. Hypercoagulability results in thrombosis beginning in the intramural venules, venous arcades, and vasa recta, often without involvement of the larger venous vessels. Occlusion of these small veins, easily missed on conventional CT-A or MR-A[19], results in compromised venous drainage and is associated with an increased risk of bowel infarction[2,11,20]. Indeed, we found decreased VLS measurements in five out of six patients with an inherited hypercoagulable risk factor. Finally, we observed that VLS measurements were higher in patients treated with OAC compared to patients who were not or only partly treated, although this difference was not statistically significant, suggesting that there might be a positive effect of OAC on gastrointestinal ischemia in patients with chronic PVT. The reported NNT needs to be interpreted with caution given it is based on a very limited number of patients. Combined with the demonstrated beneficial effects of anticoagulation in chronic PVT in previous studies, showing a reduction in thrombotic events and splanchnic venous infarction without increasing the risk of gastrointestinal bleeding, it is tempting to advocate for a more prominent role of OAC in the treatment of patients with chronic PVT[21,22].

In order to extrapolate our findings into daily practice, it is important to address a few issues. First, patients with chronic PVT by definition have portal hypertension leading to venous congestion, which may result in decreased VLS measurements. This might be attributed to the presence of portal hypertension, since decreased VLS measurements were also observed in patients with cirrhosis. Furthermore, patients with cirrhosis had even lower VLS

measurements than patients with PVT. Gastrointestinal ischemia in patients with portal hypertension might be attributed to the substantial circulatory imbalance between vasoactive mediators, such as endothelin and nitric oxide synthetase, due to the affected liver parenchyma in cirrhosis that may lead to vascular dysfunction in the gastric antrum[23,24]. Nonetheless, intestinal infarction is not a common complication of cirrhotic portal hypertension. This suggests compensatory mechanisms in these patients, which are lacking in patients with non-cirrhotic portal hypertension such as PVT. However, an in-depth studiy of the differences between cirrhotic and non-cirrhotic portal hypertension was beyond the scope of this study.

Second, we used historical controls as a reference group for VLS measurements as concurrent controls were not available. These controls were derived from the study by van Noord et al.[12] The VLS measurements in this group were performed in the same fashion as in the patients with PVT and cirrhotic patient controls, using the same protocol of measuring, devices, and equipment. Nevertheless, we cannot exclude the possibility that the inclusion of historical controls, instead of concurrent controls, might have led to a selection bias. Therefore, caution is needed in the interpretation of these results.

Finally, cardiovascular risk factors for atherosclerosis, especially DM type II and obesity, are associated with occurrence of PVT and intestinal infarction[20,25,26]. In our study, only one patient had diabetes. However, given the limited number of patients, we cannot exclude the possibility that cardiovascular risk profile is associated with PVT-related gastrointestinal ischemia.

To our knowledge, this is the first study which quantitatively assessed the presence of gastrointestinal ischemia in patients with PVT. VLS measures capillary hemoglobin oxygen saturation and is a validated diagnostic method with a high sensitivity to assess the presence of gastrointestinal ischemia. The established cut-off values for each of the specific sites of the mesenteric tract were previously calculated by van Noord et al. These cut-off values were

based on a trainee data set obtained from patients diagnosed with gastrointestinal ischemia using gastric tonometry and the values were additionally validated in a confirmation cohort[12]. Events that decrease the delivery of oxygen to the mesenteric mucosa (i.e. concomitant cardiopulmonary diseases) will result in lower mucosal hemoglobin oxygen saturations. Therefore, peripheral oxygen saturation and heart rate are continuously monitored during VLS measurements. In order to minimize the effect of modifying factors such as concomitant cardiopulmonary diseases, peripheral saturation is kept above 94% by administering oxygen (FiO2 21%), if necessary. Factors that may affect VLS measurements are luminal spasms and bile acid remnants. Therefore, butylscopolamine is administered intravenously prior to the start of VLS measurements and these measurements are performed after irrigation of the target area. There is no difference in VLS measurements between younger and older patients, as VLS measurements are known to be affected only by the above mentioned factors and by presence of a significant stenosis of the mesenteric vasculature. As VLS measurements were performed at one time point only, it would be interesting to study the time course of gastrointestinal ischemia in patients with PVT, for instance before and after initiation of anticoagulation. Nonetheless, we were able to collect follow-up data in all patients, enabling us to assess occurrence of bleeding events, recurrent thrombosis, ischemic complications, and mortality.

In future studies, it would be interesting to perform colonoscopy in all patients with PVT to assess ischemic changes in the colonic mucosa. Because bloody diarrhoea is only rarely observed in gastrointestinal ischemic patients, colonoscopy is now only included in our standard work up of the patient in case of lower GI symptoms. Furthermore, we believe it would be interesting to quantify the potential presence of ischemia in the colon by colonoscopy and also by VLS in future studies. However, currently VLS measurements are only validated in the upper GI tract and have not yet been validated in the colon.

In addition, future studies should look into interactions between the factors associated with VLS measurements. Unfortunately, we were not able to look into this further due to the limited number of patients included in our cohort.

In conclusion, gastrointestinal ischemia in patients with chronic PVT is more frequently encountered than previously assumed. Albeit in a small cohort, mucosal ischemia was detected in 75% of patients with PVT. Furthermore, two patients experienced an ischemic event. To increase generalizability, future studies assessing patients with PVT should put more focus on the diagnosis and treatment of gastrointestinal ischemia.

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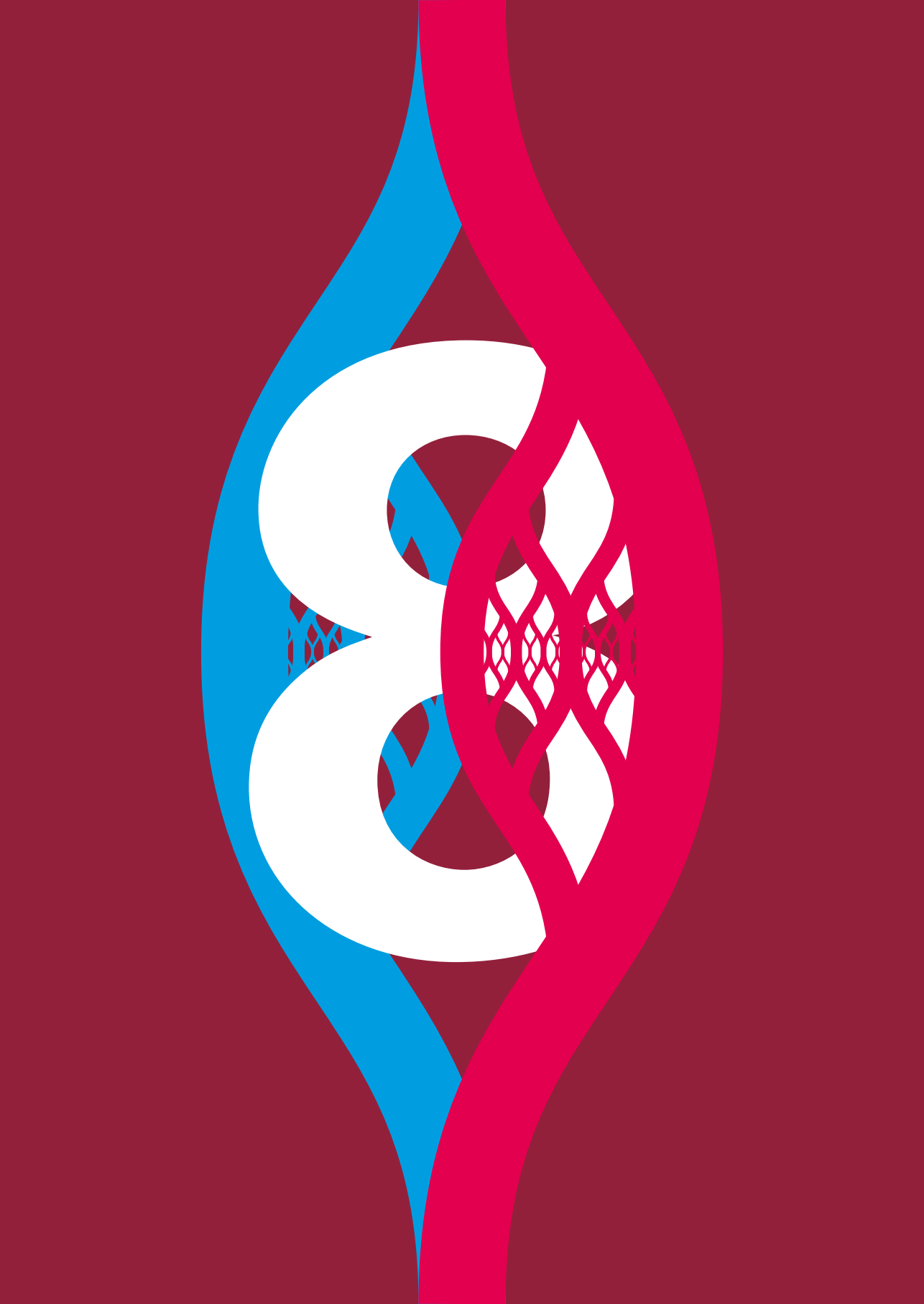

Cost study of covered transjugular intrahepatic portosystemic shunt (TIPS) versus endoscopic treatment + β-blocker for secondary prevention of gastro-oesophageal variceal bleeding

Jihan Harki*, I. Lisanne Holster*, Suzanne Polinder, Adriaan Moelker, Henk R. van Buuren, Ernst J. Kuipers*, Eric T.T.L. Tjwa*

* Authors contributed equally tot his work

Submitted

Abstract

Background

Endoscopic variceal ligation (EVL) is the accepted first line therapy for the secondary prevention of gastroesophageal variceal bleeding (GEVB). Recent data suggest that transjugular intrahepatic portosystemic shunt (TIPS) is more effective and may become the preferred treatment. However, the comparative costs of these treatment strategies have not been well defined. We aimed to compare the total health care costs of TIPS placement versus EVL + β-blocker for the secondary prevention of GEVB in the first year following the index bleeding.

Methods

Health care consumption data were based on observed data in 52 patients (25 TIPS/27 endoscopy) surviving an acute first or second variceal bleeding due to liver cirrhosis-related portal hypertension in a multicenter, randomized controlled trial. The primary endpoint of this study was defined as mean total health care costs per patient for the two treatment strategies.

Results

Twenty-five patients (age 56 (IQR 49-60) years; 13 males) were randomized to TIPS placement versus 27 patients (age 54 (IQR 49-63) years; 16 males) to treatment with EVL. The mean total costs per patient were significantly higher in the TIPS group compared to the EVL group (€ 27.746 vs € 16.818, p=0.006). The highest cost category for the TIPS group were costs of the intervention (mean cost per patient € 8.673 for TIPS placement vs € 328 for EVL, p<0.001) and re-intervention (mean cost per patient per TIPS revision were € 1.388). Intramural care, consisting of hospital admissions, ICU care, and treatment in daycare setting were the highest cost categories for the EVL treatment arm (mean total cost per patient € 5.612 and € 1.440 for patients treated with EVL for admission on ward in an academic hospital and community hospital, respectively ≣ **1**).

Conclusion

Treatment with TIPS after an acute episode of GEVB has significantly higher treatment costs in the first year after treatment compared to patients treated with EVL. This cost consideration should play a role in selection of the most optimal treatment. From an economic point of view, treatment with endoscopic variceal band ligation is recommended for the secondary prevention of gastroesophageal variceal rebleeding in patients with portal hypertension.

Introduction

A severe complication of portal hypertension is gastroesophageal variceal bleeding (GEVB). There is a high chance of rebleeding in these patients, which is associated with high morbidity and mortality[1,2]. Therefore, management should be directed at the prevention of rebleeding. Currently, the secondary prevention of GEVB is endoscopic treatment with variceal ligation [EVL] for esophageal varices and N-butyl cyanoacrylate injection for gastric varices, in combination with β-blocker therapy. When this fails, transjugular intrahepatic portosystemic shunt (TIPS) placement is the recommended as the second-line therapy[3].

Previous research showed that TIPS placement is an effective and safe treatment for variceal rebleeding in patients with portal hypertension due to cirrhosis[4]. This was recently confirmed in a randomized controlled study compared TIPS placement versus EVL for the secondary prevention of gastroesophageal variceal rebleeding[5]. The latter study also showed that TIPS is more effective in reducing the risk of variceal rebleeding in cirrhotic patients with GEVB, but is associated with a higher risk of hepatic encephalopathy and does not improve survival after a follow-up of two years[5].

Thus, TIPS placement has shown to be more effective in the prevention of rebleeding in patients with a previous episode of GEVB than treatment with EVL. Yet, there was no difference in survival between both treatment groups. However, next to clinical effectiveness, it is important to evaluate costs in order to fully determine which treatment is more superior as a secondary prevention therapy for variceal rebleeding. Health care costs associated with the treatment of variceal bleeding are significant as these patients need intensive and time-consuming treatments. EVL requires multiple sessions to achieve successful eradication of varices and is associated with frequent rebleeding from varices or banding ulcers, and probably has thus higher hospitalization costs. TIPS on the other hand is an expensive procedure that carries a risk, albeit low, of severe complications including bleeding, liver injury, and

heart failure but is associated with a relatively high risk of hepatic encephalopathy (HE)[4,6]. Hence it might be that TIPS has higher costs regarding complications due to hepatic encephalopathy.

Up till now, only a few studies have focused on the costs for different treatment options in this patient group. However, these studies have primarily focused on the cost-effectiveness of TIPS vs surgical portacaval or splenorenal shunts. Therefore, we aimed to compare the total costs of TIPS placement versus EVL + β-blocker for the secondary prevention of gastroesophageal variceal bleeding in the first year following the index bleed by determining direct medical costs from a multicenter randomized trial.

Methods

Study design and population

We performed an analysis of direct medical costs based on observed data from a multicenter, randomized controlled trial comparing long-term EVL or glue injection + β-blocker treatment with TIPS placement in patients with a first or second episode of gastric and/or esophageal variceal bleeding[5].

Health care consumption data were based on observed data in 52 patients (25 TIPS/27 endoscopy + β-blocker) surviving an acute first or second variceal bleeding due to liver cirrhosis-related portal hypertension. These 52 patients were included in one academic centre and its surrounding community hospital, and were therefore more uniform regarding their treatment plan. Details of patients selection are described previously[5]. In summary, patients were eligible for the study if they were between 18-75 years and presented with a first or second episode of endoscopically documented esophageal or gastric variceal bleeding[5,7].

Exclusion criteria have been extensively described before[5]. They mainly included a history of serious or refractory hepatic encephalopathy unrelated to gastrointestinal bleeding, a history of significant heart failure, portal hypertension due to other causes than liver disease (e.g. portal or splenic vein thrombosis), and previous TIPS placement.

Costs of all patients were calculated from inclusion until twelve months after randomization, death, or moment of liver transplantation. In the first year after inclusion, patients were followed with 3-monthly intervals. The trial was performed in accordance with the provisions of the Declaration of Helsinki and local regulations. The institutional review board at each center approved the protocol, and written informed consent was obtained from all patients.

Treatment

Endoscopic treatment of esophageal varices consisted of EVL (6-Shooter Saeed Multi-band Ligator®, Cook Medical, Winston-Salem, North Carolina, USA) in combination with a β-blocker. Gastric varices were injected with cyanoacrylate glue (HistoAcrylTM, B. Braun, Germany) with lipiodol (Lipiodol®, Guerbet, France). A non-selective β-blocker (preferably slow release propranolol, titrated to the maximum tolerated dose aiming to decrease the heart rate in rest by 25%, with a lower limit of 50 bpm) was started at day 5 after the index bleeding, unless a contraindication was present (severe arrhythmia, severe obstructive COPD or known intolerance). Elective EVL sessions started 2 weeks after the index bleeding and were performed every 2-4 weeks thereafter until eradication of varices. In patients allocated by randomization to endoscopic treatment, further endoscopic management occurred on site by gastroenterologists experienced in variceal ligation.

TIPS placement was performed under general anesthesia after transhepatic portography. Polytetrafluoroethylene (PTFE)-covered stents (Viatorr, W.L. Gore & Associates, Flagstaff, Arizona, USA) were used. Post-TIPS monitoring of shunt patency and function was done according to local guidelines.

Outcome measurements

Health care consumption data were compared between the two treatment arms; the endoscopy + β-blocker arm and the TIPS arm. The primary endpoint of this study was defined as mean total health care costs per patient for the two treatment strategies. Total costs included the costs for intervention as well as healthcare costs from the day of randomization until twelve months after randomization, or until the occurrence of death, or moment of liver transplantation. Costs of treatment failure of one arm (switch to other therapy arm) were calculated for the randomization arm. The costs for initial stabilization of the index bleeding were excluded, because they were assumed to be comparable in both arms.

The study was performed in accordance with the Dutch guidelines for economic evaluation studies[8]. Direct healthcare costs (inpatient and outpatient costs) were calculated from a health care perspective. Data on health care consumption were obtained from the follow-up survey conducted until 24 months after randomization and was again inventoried in all participating centers.

Direct healthcare costs were calculated by multiplying the volumes of health care (e.g. length of stay) with the corresponding unit costs (e.g. costs per day in hospital). All unit costs were estimated in agreement with national guidelines for health care costing[8] or a detailed inventory of all cost items, reflecting real resource use ☰ **1**. Cost prices of the different treatment options (e.g. TIPS and EVL) were inventoried in one center (Erasmus MC) and used for all centers. All costs estimated were adjusted for inflation with the use of the Dutch Consumer Price Index Statline CBS (Consumentenprijsindex Statline CBS, The Hague, the Netherlands). The costs applied to the financial year of 2014 and are in euros.

Data on hospital costs consisted of intervention costs, hospital admissions, diagnostics and medication:

· Interventional costs consisted of all the costs associated with TIPS placement or EVL. The cost price per unit for the procedures consisted of detailed measurement of investments in manpower, equipment, material costs, maintenance, number of years of use and discounting the number of procedures per year, housing, personnel costs, and overhead.

· Hospital stays included intensive care unit (ICU) ad,missions, non-ICU admissions, visits to the emergency department and visits to the outpatient clinic. The length of hospital stay was multiplied with the cost price with differentiation for admission to an academic medical center or to a community hospital.

· Costs of laboratory testing and other diagnostics were also included, such as radiological imaging as well as ascites taps and aspirations.

· Medication costs were calculated for all relevant medication associated with portal hypertension (e.g. terlipressin, albumin),

1 Unit costs

	unit costs (€)
admissions	
admission ward[a]	634
admission ward[b]	480
admission ICU	1.572
treatment in daycare setting	276
consultations	
ER	167
outpatients clinic[c]	142
outpatients clinic[d]	71
telephone consulting	30
radiological imaging	
chest X-ray	53
abdominal ultrasonography	78
abdominal ultrasonography ward	77
CT-scan	223
MRI	261
angiography	14.725

	unit costs (€)
diagnostics	
laboratory testing	14
gastroscopy[e]	303
colonoscopy	469
ascites puncture	172
liver biopsy	133
liver transplantation screening[g]	9.858
percutaneous manometry[h]	2.160
pleural puncture/aspiration	180
pulmonary function test	41
electroencephalography	222
electrocardiography	352
cardiac ultrasonography	77
treatment	
EVL	328
TIPS placement	8.395
thrombocyte transfusion	502
red blood cells transfusion	216
plasma transfusion	186

values represent full cost prizes in euro in financial year 2014

a admission to the ward of an academic medical center

b admission to the ward of a community hospital

c visit to the outpatients clinic in an academic medical center

d visit to the outpatients clinic in a community hospital

e includes diagnostic gastroscopies without EVL

f includes ascites aspirations as well as taps.

g liver transplantation screening includes extensive laboratory testing, blood cultures, urine cultures, gastroscopy, colonoscopy, abdominal ultrasound, Dual Energy X-ray Absorption (DEXA) scan, CT-scan, dental and sinus X-ray, if needed a mammogram, as well as visits to the infectiologist, surgeon, orthognathic surgeon, otolaryngologist, social worker and hepatologist. It also includes an admission of 5 work days in an academic medical center.

h percutaneous transhepatic balloon angioplasty with manometry in the portal vein

hepatic encephalopathy (e.g. lactulose syrup or enema, rifaximin) and complications related to portal hypertension such as infection or rebleeding (e.g. antibiotics, octreotide, but not β-blockers). It was calculated in Defined Daily Dose (DDD) and was multiplied by the cost per DDD. The cost per DDD was different for inpatient or outpatient use of medication ≣ **s1**. The price for outpatient use of medicine was determined by calculating the mean price of the medicine[9].

Statistical analysis

Baseline characteristics were calculated using descriptive statistics. Data were expressed as mean and standard deviation (SD), median and interquartile range (IQR) or count and percentage (%), when appropriate. Non-parametric tests were used to compare costs per patient in the TIPS treatment group versus the EVL treatment group. Data analyses were carried put using the SPSS v21.0 program (SPSS Inc., Chicago, IL, USA). A two-sided p-value of <0.05 was considered statistically significant.

Results

Patient characteristics

We used data of 52 patients (age at randomization 54 (interquartile range (IQR) 49-61) years; 29 males) with an acute first or secondary GEVB randomized to either TIPS (n=25, age 56 (IQR 49-60) years; 13 males) or standard care with EVL + β-blocker (n=27, age 54 (IQR 49-63) years; 16 males). Characteristics of these patients, at the moment of randomization, are displayed in ≣ **2**. At randomization, 14 patients (56%) in the TIPS group vs 10 patients in the EVL (37%) group were known with ascites (p=0.27). Two patients in the EVL group and none of the patients in the TIPS group had experienced a previous episode of hepatic encephalopathy (p=0.38).

≣ **2** Characteristics of 52 patients randomized to either TIPS placement or endoscopic therapy with EVL after an acute first or second episode of GEVB after one year

	TIPS placement n=25	EVL n=27
gender		
male	13 (52)	16 (59)
female	12 (48)	11 (41)
age at randomization (years)[a]	56 (49-60)	54 (49-63)
etiology		
alcohol	8 (32)	12 (44)
hepatitis B/C	2 (8)	1 (4)
auto-immune liver/biliary disease[b]	9 (36)	7 (26)
other[c]	6 (24)	7 (26)
Child-Pugh score at randomization[d]	7,8 ± 2,27	7,2 ± 1,99
pre-existent ascites	14 (56)	10 (37)
previous episode of HE	0	2 (8)

data is derived from Holster et al.[5], with the exclusion of one patient of which follow-up data was insufficient for this paper. Values represent number of patients and percentage (%), unless otherwise specified

a values represent median, IQR

b this includes primary biliary cirrhosis, primary sclerosing cholangitis and auto-immune hepatitis

c this includes NASH, combined alcoholic and viral hepatitis, and cryptogenic liver disease.

d the Child-Pugh score ranges from 5 to 15. Values represent mean ± standard deviation.

Initial costs

The initial costs of the two treatments, including all personnel, equipment, material, housing, and overhead costs were € 8.395 for TIPS placement and € 328 for EVL + β-blocker . The higher costs for TIPS placement were mainly caused by the higher material costs e.g. purchase costs of the stent is € 3.228. Also, the costs for personnel, equipment, and other materials were significantly higher in the TIPS group. In addition, treatment with TIPS also requires a pre-TIPS computed tomography (CT-) angiography (€ 188), and a post-TIPS stay on the post-anesthesia care unit (€ 2.324), costs that are not involved in EVL. For a detailed overview of the costs per treatment see ≣ **3**.

≣ **3** Full costs prices of the two treatments groups

	TIPS placement n=25	**EVL n=27**
personnel [a]	€ 921	€ 87
equipment [b]	€ 393	€ 51
materials [c]	€ 4120	€ 130
housing/overhead [d]	€ 2962	€ 61
total costs	€ 8395	€ 328

values represent full cost prizes in euro in financial year 2014

a for TIPS placement personnel includes costs for interventional-radiologist, registered nurse, registered in anesthesiology, and medical clerk. For EVL treatment this includes a gastroenterologist, registered nurse, and medical clerk

b equipment for TIPS placement includes anesthesia equipment (breathing/ventilation support, monitoringsystem, two pumps, hotline, warm air, monoplane X-ray system, and Ultrasound Esaote Mylab Twice), and costs for pre-TIPS computed tomography (CT) angiography. Equipment for EVL treatment includes use of a gastroscope, processor, monitor, pulsoximeter, computer, and datascope

c materials for TIPS placement include use of PTFE Stent (€ 3228), pigtail catheter, cobra catheter, Amplatz wire, Terumo enter wire, TIPS needle and sheet, portal vein needles, coils, sterile and non-sterile cloths, and sterile and non-sterile gloves. Materials for EVL include iv catheters, six shooter, endoscopic injector needle, scalpel, cryanoactrylate glue, mouth guard, non-sterile cloths, suction reservoir and wire, gloves, and personal protective equipment

d housing for TIPS placement includes post-TIPS admission on the post-anesthesia care unit. The overhead rate for both TIPS placement and EVL was 42%

Total costs in the first year

The mean total costs per patient in the first year after index bleeding were significantly higher in the TIPS group compared to the EVL + β-blocker group (€ 27.415 vs € 16.762, p=0.006). The highest cost categories for the TIPS group were costs of the intervention (mean cost per patient € 8.395 for TIPS placement vs € 803 for EVL, p<0.001) and re-intervention (mean cost per patient per TIPS revision € 1.343 for TIPS group vs € 0 for EVL + β-blocker group, p=0.07). Intramural care, consisting of hospital admissions, ICU care, and treatment in daycare setting were the highest cost categories for the EVL treatment arm (mean total cost per patient € 5.612 and € 1.440 for admission on ward in an academic hospital and community hospital, respectively). However, patients in the TIPS group had significantly higher costs per admission when compared to patients in the EVL treatment group (mean total days of admission on an academic ward for the TIPS group 16 vs 9 days for the EVL + β-blocker group; mean total cost for admission on academic ward € 10.093 for the TIPS group vs € 5.612 for the EVL group, p=0.005). Costs for treatment in daycare setting were significantly higher in the EVL treatment group (mean total days of treatment in daycare setting for the EVL group 2 days vs 0 days for the TIPS group; the mean total cost per patient € 552 for the EVL group vs € 0 for the TIPS group, p<0.001). There were no differences in costs in hospital consultations and radiological imaging between both groups. In the EVL group, more diagnostic gastroscopies were performed compared to the TIPS group (p<0.001).

A detailed overview of the mean costs of treatment per patient per treatment group is shown in ☰ **4**. For the distribution of the total costs per patient per treatment group see ⊡ **s1**.

Sensitivity analysis

The TIPS treatment arm and the EVL treatment arm were re-stratified into patients with and without pre-existent ascites. Fourteen patients (56%) in the TIPS group were known with ascites against 10 patients (37%) in the EVL + β-blocker group. The mean total cost

per patient with ascites in the TIPS group was € 35.581 vs € 26.623 (p=0.5) in the EVL group ☰ **s2**. The mean total cost per patient for both treatment groups was higher in patients with pre-existent ascites.

4 Mean costs of healthcare use per patient

	TIPS placement n=25		EVL n=27		
cost category	volume[a]	costs[b] (€)	volume[a]	costs[b] (€)	*p*-value[c]
admissions					
admission ward[d]	15,9	10.093	8,9	5.612	0.005
admission ward[e]	1,2	826	3,0	1.440	0.022
admission ICU	1,3	2.075	1,3	2.038	0.59
treatment in daycare setting	0,0	11	2,0	552	<0.001
consultations					
ER	0,5	80	0,2	37	0.77
outpatients clinic[f]	2,6	364	1,7	247	0.29
outpatients clinic[g]	1,2	85	1,7	121	0.29
outpatients clinic and daycare[h]	0,0	0	0,3	124	0.09
telephone consulting	0,5	16	0,5	16	0.72
radiological imaging					
chest X-ray	1,8	93	0,7	35	0.26
abdominal ultrasonography	1,5	119	1,3	98	0.61
CT-scan	0,8	187	0,4	83	0.59
MRI	0,2	52	0,1	19	0.21
angiography	0	0	0,1	34	0.17
diagnostics					
laboratory testing	16,7	234	10,5	147	0.08
gastroscopy[i]	0,1	24	1,4	438	<0.001
colonoscopy	0,1	56	0	0	0.30
ascites puncture[j]	0,6	96	0,4	64	0.44
liver biopsy	0	5	0	0	0.30
liver transplantation screening[k]	0,1	789	0,1	1.095	0.71
percutaneous manometry[l]	0,1	173	0	0	0.14
pleural puncture/aspiration	0	7	0	0	0.30
pulmonary function test	0	2	0	0	0.30
electroencephalography	0,2	44	0	8	0.26
electrocardiography	0,3	99	0,2	65	0.12
cardiac ultrasonography	0,2	12	0	3	0.14
initial treatment					
EVL	0	0	2,4	803	<0.001
TIPS placement	1,0	8.395	0	0	<0.001
other treatments					
switch therapy[m]	0,1	349	0,2	1.555	0.11
TIPS revision[n]	0,2	1343	0	0	0.07
thrombocyte transfusion	0,6	301	0,5	260	0.81
red blood cells transfusion	3,3	708	4,7	1.016	0.79
plasma transfusion	2,0	365	2,9	537	0.69

	TIPS placement n=25		EVL n=27		
cost category	volume	costs (€)	volume	costs (€)	*p*-value
medication					
laxatives[o]	936,8	237	42,9	43	0.07
albumin[p]	9,8	40	26,5	27	0.79
terlipressin[q]	122,0	23	0	0	0.07
octreotide[r]	0,1	89	223,5	223	0.80
antibiotics[s]	13,2	20	21,8	29	0.54
total costs	–	27.415	–	16.762	0.006

a values represent volume of admission days, visits to the outpatient clinic, ER visit, or number of times of radiological imaging, diagnostics, and treatment. In case of medication, volumes represent the amount of defined daily dose (DDD) unless otherwise specified

b values represent full cost prizes per patient in euro in financial year 2014

c *p*-value <0.05 was considered significant

d admission to the ward of an academic medical center

e admission to the ward of a community hospital

f visit to the outpatients clinic in an academic medical center

g Visit to the outpatients clinic in a community hospital

h combined visit to the outpatients clinic and treatment in daycare setting in an academic medical center

i includes diagnostic gastroscopies without EVL

j includes ascites aspiration as well as taps

k liver transplantation screening includes extensive laboratory testing, blood cultures, urine cultures, gastroscopy, colonoscopy, abdominal ultrasound, Dual Energy X-ray Absorption (DEXA) scan, CT-scan, dental and sinus X-ray, if needed a mammogram, as well as visits to the infectiologist, surgeon, orthognathic surgeon, otolaryngologist, social worker and hepatologist. It also includes an admission of 5 work days in an academic medical center

l percutaneous transhepatic balloon angioplasty with manometry in the portal vein.

m failure of the initial treatment arm for which switch therapy to the other treatment arm

n revision of the TIPS placement to rule out occlusion or stenosis of the stent

o includes the administration of lactulose as well as rifaximin in DDD used in the clinical setting and in the outpatient setting

p includes albumin supplementation used in the clinical setting in ml used

q includes terlipressin supplementation in DDD used in the clinical setting

r includes octreotide administration in DDD in the clinical setting

s includes the use of the following antibiotics in DDD in case of bleeding related infection: amoxicillin/clavulanic acid, cefotaxim, ceftriaxone, cefuroxim, cefazolin, ciprofloxacin, clindamycin, erythromycin, gentamycin, norfloxacin, meropenem, and piperacilline/tazobactam

Discussion

We aimed to compare the total costs of TIPS placement versus EVL + β-blocker for the secondary prevention of gastroesophageal variceal bleeding. We found that patients treated with TIPS after an acute episode of GEVB have significantly higher treatment costs in the first year after treatment compared to patients treated with the standard care EVL + β-blocker.

In the current era with rapidly expanding healthcare costs and more focus on economic viability of new treatments, cost studies provide insight into the distribution of costs and may allow to identify cost reducing measures. This is the first detailed cost inventory based on a randomized clinical trial on the secondary prevention of variceal rebleeding by means of TIPS or EVL + β-blocker. Mean total costs per treatment arm varied between € 16.762 and € 27.415, and was significantly higher for patients with TIPS placement. This is the result of the significant higher intervention costs in the TIPS group, which also includes higher costs for personnel, material, and post-TIPS care at the post-anesthesia care unit, as well as higher costs for intramural care compared to patients with EVL + β-blocker.

Up till now, no insight in the costs was involved when comparing the two treatment options, TIPS or EVL + β-blocker, for variceal rebleeding due to portal hypertension in patients with liver cirrhosis. Only a few studies aimed to determine the costs for different treatment options in this patient group. These studies primarily focused on the cost-effectiveness of TIPS vs surgical portacaval or splenorenal shunts [10-12]. The average costs per life year was between $17.771 and $35.691 [11,12]. However, these studies were limited by the use of bare stents during TIPS placement. One study provided the actual costs accounted for covered stent TIPS placement, including costs for personnel, medical supply, costs of anesthesia, hospitalization, and amortization expense [10]. In this study, the costs for a mean of 1.1 stent per patient, was $12.211 and included $4.746 for a covered stent, $3.168 for medical supply, $1.119 for staff, $1.143 for a mean length of admission of 2,5 days, and $2.035 for amortiza-

tion expense. When converted to the euro currency and adjusted for the use of 1 stent, the price for TIPS placement is similar to our study except for the price and length of stay of admission. A recent randomized clinical trial from the United States, the direct costs of endoscopic therapy versus portocaval shunts in the elective setting and TIPS placement versus portocaval shunts in the emergency setting were calculated [13]. In this study, the total charges were greater in patients treated with endoscopic therapy (mean total overall charges of $168.100) or TIPS compared to emergency portocaval shunt placement (mean total overall charges of $39.400). However, a factor that may hamper the generalizability of the study is that it focused on treatment of gastric varices only. Another study only focused on the outcomes of TIPS through the left branch vs the right branch of the portal vein [14].

Our study offers insight into the economic burden that is associated with GEVB treated with EVL + β-blocker or TIPS. The costs in this study are based on the direct costs of a randomized controlled trial, which included patients with esophageal varices as well as gastric varices bleeding. Complete charges were obtained for every patient entered in the study. In addition, a detailed cost overview for inpatients as well as outpatient costs is considered. We also performed a sensitivity analysis for patients with pre-existent ascites. There were more patients known with ascites in the TIPS group than in the EVL + β-blocker group. However, this difference was not significant as the data is based on a randomized controlled trial. The mean costs per patient for both treatment groups were higher in patients with pre-existent ascites compared to patients without ascites. The mean costs per patients were higher in patients with pre-existent ascites receiving TIPS-placement, however this difference was not statistically significant compared to the mean costs per patient in the EVL-group.

Our study had some limitations. Firstly, we calculated the direct costs for patients during the first 12 months after intervention. The costs after the first year were not considered.

Secondly, direct non-healthcare costs (travel costs) and indirect costs (productivity loss) were not calculated. This may affect the total costs per treatment group as TIPS placement is only performed in tertiary referral centers and EVL in almost all community hospitals. Consequently, this may result in higher travel expenses in the TIPS group. However, productivity loss may not have a large effect on the mean costs of treatment after a year since most of these patients were already known with severe liver cirrhosis that prevented them from employment early on before treatment with TIPS or EVL + β-blocker .

Furthermore, the cost calculations were performed based on data in Dutch centers. Cost prices differ per country, but are probably comparable.

In addition, the study is limited by the limited number of individuals. Inclusion of more patients might assure an even more precised cost overview by reducing the effect of outliers (four patients in TIPS group, highest total cost per patient € 114.392 and two patients in the EVL group, highest cost prize per patient € 94.505).

TIPS placement is more effective in the prevention of rebleeding in patients with a previous episode of GEVB than treatment with EVL + β-blocker, currently the standard care[4,5]. However, there was no difference in survival between both treatment groups. In addition, TIPS placement led to higher prevalence of hepatic encephalopathy in these patients. This study implies that TIPS placement has significant higher costs than EVL + β-blocker. Considering this finding, one should be vigilant to treat patients with GEVB with TIPS as standard care.

However, there are possibilities to overcome these issues. First, there is the possibility to perform TIPS by administration of conscious sedation with intravenous midazolam and fentanyl[15]. Performing the TIPS procedure under conscious sedation rather than general anesthesia, considerably reduces the initial costs prize for treatment with TIPS, as conscious sedation is relatively cheap and does not require certified staff in anesthesiology and post-TIPS admission on the PACU is no longer needed. Second, studies have

shown that the placement of a TIPS stent in the left portal vein branch may be more reasonable for decreasing the development of hepatic encephalopathy [14]. Patients undergoing TIPS through the left branch of the portal vein had lower incidence of encephalopathy, less rehospitalization and lower costs after TIPS implantation compared with patients undergoing TIPS through the right branch of the portal vein.

In conclusion, treatment with TIPS after an acute episode of GEVB is significantly more costly in the first year after treatment compared to EVL treatment. Given the similar effectiveness of TIPS and EVL in combination with β-blocker in the secondary treatment of variceal rebleeding, cost consideration should play a role in selection of the most optimal treatment. Future studies should put more focus on full cost comparisons as well as data on quality of life in this patient group.

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s1 Costs per DDD of medication according to inpatient or outpatient use and prescription in an academic or community medical center

medication	administration	costs per DDD (€) [a]	costs per DDD (€) [b]
cefuroxim	IV	2,04	12,27
octreotide	IV	4,45	17,23
octreotide	SC	6,44	34,65
augmentin	IV	2,46	9,66
augmentin	O	0,40	0,77
albumin	IV	27,30	75,65
norfloxacin	O	0,30	0,53
ciprofloxacin	IV	3,00	29,68
ciprofloxacin	O	0,34	0,25
erytromycin	IV	13,65	0,62
lactulose	O	0,074	0,147
lactulose (enema)	R	0,0142	0,07
clindamycin	O	2,36	1,94
rifaximin	O	6,92	6,71
terlipressin	IV	327,67	964,24
gentamycin	IV	3,24	5,52
cefotaxim	IV	4,60	22,20
piperacilline/tazobactam	IV	7,84	52,69
ceftriaxon	IV/P	1,15	20,27
meropenem	P	10,26	46,91
cefazolin	P	2,16	8,58

values represent cost prizes per DDD in euro in financial year 2014

a cost per DDD in an clinical setting

b cost per DDD prescribed in the outpatient clinic

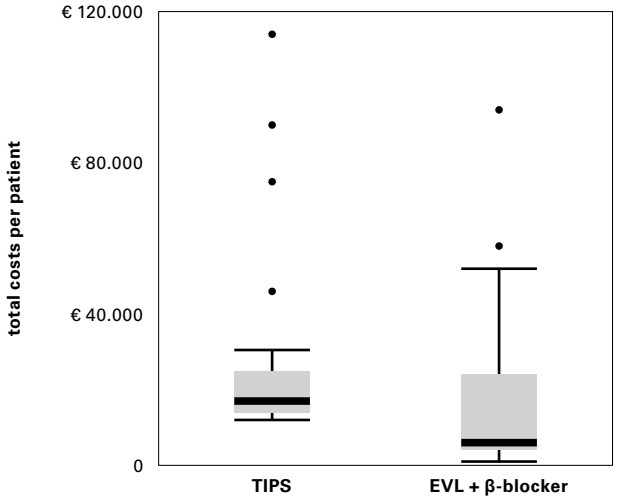
≡ s2 Mean costs of healthcare use per patient with ascites

	TIPS placement n=14	EVL n=10
cost category	costs[a] (€)	costs (€)
admissions		
admission ward[b]	14.174	8.879
admission ward[c]	1.474	1.296
admission ICU	3.481	5.030
treatment in daycare setting	20	552
consultations		
ER	119	67
outpatients clinic[d]	274	227
outpatients clinic[e]	117	85
outpatients clinic and daycare[f]	0	0
telephone consulting	9	21
radiological imaging		
chest X-ray	148	42
abdominal ultrasonography	156	78
CT-scan	271	112
MRI	75	26
angiography	0	3.492
diagnostics		
laboratory testing	329	197
gastroscopy[g]	43	394
colonoscopy	101	0
ascites puncture[h]	160	103
liver biopsy	10	0
liver transplantation screening[i]	704	986
percutaneous manometry[j]	309	0
pleural puncture/aspiration	13	0
pulmonary function test	0	0
electroencephalography	79	0
electrocardiography	126	141
cardiac ultrasonography	22	8
initial treatment		
EVL	0	953
TIPS placement	4.701	0
other treatments		
switch therapy[k]	349	2.519
TIPS revision[l]	1.007	0
thrombocyte transfusion	538	703
red blood cells transfusion	987	2.462
plasma transfusion	545	1.451

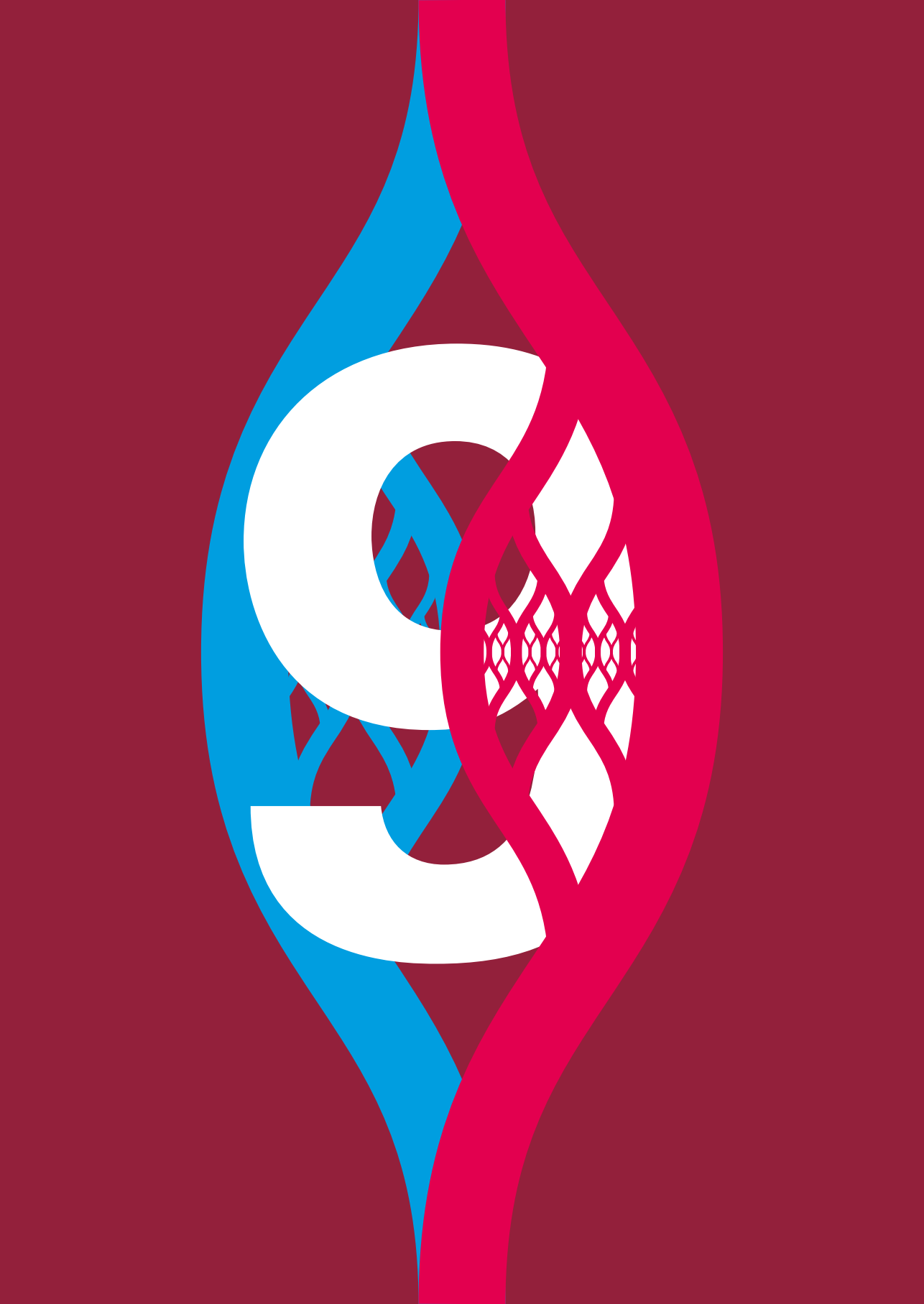
	TIPS placement n=14	EVL n=10
cost category	costs[a] (€)	costs (€)
medication		
laxatives[m]	217	58
albumin[n]	67	59
terlipressin[o]	41	0
octreotide[p]	133	69
antibiotics[q]	25	16
total costs	35.581	26.623

a values represent full cost prizes per patient in euro in financial year 2014
b admission to the ward of an academic medical center
c admission to the ward of a community hospital
d visit to the outpatients clinic in an academic medical center
e visit to the outpatients clinic in a community hospital
f combined visit to the outpatients clinic and treatment in daycare setting in an academic medical center
g includes diagnostic gastroscopies without EVL
h includes ascites aspirations as well as taps
i liver transplantation screening includes extensive laboratory testing, blood cultures, urine cultures, gastroscopy, colonoscopy, abdominal ultrasound, Dual Energy X-ray Absorption (DEXA) scan, CT-scan, dental and sinus X-ray, if needed a mammogram, as well as visits to the infectiologist, surgeon, orthognathic surgeon, otolaryngologist, social worker and hepatologist. It also includes an admission of 5 work days in an academic medical center
j percutaneous transhepatic balloon angioplasty with manometry in the portal vein
k failure of the initial treatment arm for which switch therapy to the other treatment arm
l revision of the TIPS placement to rule out occlusion or stenosis of the stent
m includes the administration of lactulose as well as rifaximin in DDD used in the clinical setting and in the outpatient setting
n includes albumin supplementation used in the clinical setting in ml used
o includes terlipressin supplementation in DDD used in the clinical setting
p includes octreotide administration in DDD in the clinical setting
q includes the use of the following antibiotics in DDD in case of bleeding related infection: amoxicilline, cefotaxim, ceftriaxone, cefuroxim, cefazolin, ciprofloxacin, clindamycin, erythromycin, gentamycin, norfloxacin, meropenem, and piperacilline/tazobactam

s1 Distribution of total costs per patient per treatment group in euros (€)



boxes represent medians with interquartile range, whiskers extent to the most and least extreme scores respectively. Dots represent the outliers



General discussion and conclusions

Introduction

Chronic gastrointestinal ischemia (CGI) results from insufficient blood supply to the gastrointestinal tract, in most cases caused by atherosclerotic stenosis of the supplying arteries[1] but may also be due to hypo-perfusion and/or hypo-oxygenation caused by an underlying cardiac or pulmonary disease[2,3]. Three direct branches of the abdominal aorta are responsible for the blood supply of the gastrointestinal tract: the celiac artery (CA), the superior mesenteric artery (SMA) and the inferior mesenteric artery (IMA).

Until recently it was thought that CGI could only occur in the presence of two or more occluded arteries, but recent studies using functional testing have shown that a significant stenosis of a single artery with insufficient collateral circulation can also lead to clinically relevant gastrointestinal ischemia.[1,4-6]. Functional testing such as gastric tonometry and visible light spectroscopy (VLS) have been incorporated in the standard work-up to optimize the diagnosis of CGI in patients with chronic abdominal symptoms. Both methods seem to be accurate for detection of gastrointestinal ischemia in combination with radiological imaging[1,4,7,8].

Currently, the established approach for diagnosing CGI is based on three main components including assessment of medical history and clinical symptoms, radiological imaging of the mesenteric arteries and detection of mucosal ischemia by means of a functional test such as VLS[9,10].

A definitive diagnosis of CGI is made after persistent relief of symptoms on follow-up after treatment.

In **chapter 1** an introductory summary on CGI and other vascular diseases of the gastrointestinal tract are provided.

Chapter 2 addresses the effect of caloric challenge on VLS measurements in patients with CGI and healthy controls. Consecutive patients with a diagnosis of CGI and healthy controls were prospectively included between September 2014 and August 2015. All patients received the standard work-up for CGI, consisting of assessment of medical history and symptoms, radiological

imaging of the gastrointestinal arteries, and VLS measurements. VLS measurements in postprandial state were compared to VLS measurements in fasting state. We performed 168 VLS measurements in 12 patients with CGI (median age 63.2 (IQR 48.8-70.4) years, 67% male) and 16 controls (32.9 (IQR 27.7-39.8.2) years, 44% male). There was no significant increase between postprandial and baseline VLS measurements in CGI patients (baseline antrum median 58.5 (IQR 53.5-59.0) vs postprandial antrum median 57.5 (IQR 54.0-60.8), p=0.25; baseline duodenal bulb median 53.0 (IQR 48.8-54.0) vs postprandial duodenal bulb median 53.5 (IQR 50.3-56.8), p=0.89; baseline descending duodenum median 49.5 (IQR 45.3-52.8) vs postprandial descending duodenum median 54.0 (IQR 47.3-57.8), p=0.33, respectively). In the healthy controls, there was a trend towards higher VLS measurements in the postprandial state. We found no difference in VLS measurements before and after caloric challenge in patients with CGI, however there was a trend in increase in VLS measurements in the healthy controls. The current results indicate that a caloric challenge does not improve the discrimination between patients with CGI and healthy controls.

In **chapter 3** we studied the sublingual microcirculation in 12 patients with CGI using a hand-held microscope based on Incident Dark Field (Cytocam). The microcirculation was assessed before (T0) and 20 minutes after enteral feeding (T1). Total vessel density (TVD (mm/mm^2)), perfused vessel density (PVD (mm/mm^2), proportion of perfused vessels (PPV (%)), and microvascular flow index (MFI (AU)), respectively, of small vessels and all vessels, and before and after a caloric challenge. There was a significant increase in the PVD of the small vessels and all vessels, and in the PPV of the small vessels after caloric challenge compared to baseline (PVDs median 16.3 (IQR 13.3-22.1) mm/mm^2 vs 19.9 (IQR 14.2-26.2) mm/mm^2, p=0.008; PVDa median 19.1 (IQR 16.2-23.6) mm/mm^2 vs 22.2 (16.5-28.9) mm/mm^2, p=0.02; PPVs median 84.8% (IQR 75.3-90.4) vs 91.0% (80.1-93.8), p=0.01). We found that patients with CGI have a significant increase in the perfused vessel density and proportion of perfused vessels after stimulation with food and thus have a hy-

peraemic response to ingestion of food. These findings may lead to development of more rapid, less-burdensome and more advanced diagnostic methods for the detection of CGI. Future studies with more patients and healthy are needed to increase the generalizability of our findings and to provide more discriminatory power for clinical use.

In **chapter 4** we present a prediction model to identify patients with CGI based on a large prospective study consisting of 436 patients. Our study shows that advanced age, female gender, presence of nausea and weight loss, recent onset of symptoms, concomitant cardiovascular disease, positive family history for cardiovascular disease, and no use of analgetics are predictors for CGI. Our study confirms that the diagnostic value of clinical variables alone in the diagnosis of CGI is limited (c-statistic 0.62)[11]. Combining clinical variables with presence of mesenteric arterial stenosis in a second model allows discriminating more accurately between patients with and without CGI (c-statistic of 0.76). A prediction model for CGI that takes into account clinical variables and radiological assessment of the mesenteric arteries may also be a useful tool for clinicians to assess the risk of CGI and to decide whether further diagnostic work-up and treatment is indicated.

In **chapter 5** we performed a pilot study to investigate the expression of HIF-1α in chronic ischemic tissue by immunohistochemistry. Immunohistochemical expression of HIF-1α in the gastrointestinal tract was analyzed in 20 patients with CGI, nine patients with H. pylori-gastritis, nine patients with ischemic colitis (IC), 18 patients with inflammatory bowel disease (IBD) and five patients with infectious colitis. Ten controls served for the upper GI-tract and 12 controls for the lower GI-tract respectively. In the upper GI-tract, HIF-1α expression was found in 5/20 CGI-patients, but not in controls (p=0.08). The sensitivity and specificity of HIF-1α expression for diagnosing CGI was 25% and 84%. In the lower GI-tract, in HIF-1α was expressed in all patients with IC, infectious colitis and in majority of IBD-patients. This was also in 7/12 controls. The sensitivity and specificity of HIF-1α for diagnosing IC was 100% and 51%.

HIF-1α expression was more observed in patients with inflammation on histology (p=0.02) in the lower GI-tract. Therefore, HIF-1α is not a pathognomonic marker for the presence of ischemia.

In **chapter 6** we aimed to determine the incidence of hypoxic hepatitis in a well-defined cohort of CGI patients. In total, 156 patients were eligible for inclusion (median age 64.1 (IQR 53.1-73.6) years; 74% female, median ALT 0.49x ULN (IQR 0.36-0.67). The results of the current study shows that serum aminotransferases were very mildly raised in only 10% of the total cohort of 156 patients with CGI, which normalized after successful treatment of CGI. Elevated ALT >1x ULN was not significantly associated with presence of mucosal ischemia, significant stenosis of the CA, SMA, or both CA and SMA. Neither was there an association between ALT >1x ULN and presence of known predisposed conditions of steatotic liver injury such as body mass index (BMI), alcohol consumption and diabetes mellitus (DM) type II, nor between ALT >1x ULN and increasing age. Twelve of these 15 patients received treatment (endovascular, surgical or medical treatment according to cause of CGI). At follow-up (median 17.6 months), only 2 of these patients kept ALT >1x ULN (mean 75 IU/ml). Both patients were diagnosed with NOMI and were irresponsive to treatment. Therefore, liver injury is not a common feature of CGI. When present, it is mild and mostly normalizes after appropriate treatment of CGI.

In **chapter 7** we describe the clinical presentation and characteristics of gastrointestinal ischemia in patients with portal vein thrombosis (PVT). We performed a prospective cohort study in which patients with non-cirrhotic, non-malignant PVT were included. Clinical symptoms of gastrointestinal ischemia were assessed by a structured questionnaire, VLS, and radiological evaluation of the mesenteric vasculature. VLS measurements were compared to those in patients with cirrhosis and a reference population. We included 15 patients with chronic PVT and one patient with acute PVT (age 46.1 [IQR 30.9-53.7] years; 44% male). Decreased mucosal oxygenation in at least one location of the gastrointestinal tract was found in 12/16 (75%) patients. Compared to the reference

population (median 60.0 [56.2-61.7]), VLS measurements were mostly decreased in the descending duodenum for patients with PVT (median 55.5 [52.3-58.8], p=0.02) and patients with cirrhosis (median 52.0 [46.5-54.0], p=0.003). Symptoms typical for gastrointestinal ischemia, such as postprandial pain and exercise-induced pain, were reported in 10/16 (63%) patients with PVT. We also found that patients with a thrombus extending into the SMV and/or SV more often report abdominal pain than patients without extension of the thrombus. Also, patients with extension of the thrombus into the SMV and/or SV had significantly lower VLS measurements in the antrum and descending duodenum compared to the reference population. In addition, we found decreased VLS measurements in five out of six patients with an inherited hypercoagulable risk factor. Finally, we observed that VLS measurements were higher in patients treated with OAC compared to patients who were not or only partly treated, although this difference was not statistically significant, suggesting that there might be a positive effect of OAC on gastrointestinal ischemia in patients with chronic PVT. Therefore, in patients with PVT gastrointestinal ischemia is frequent. Onset of abdominal symptoms such as postprandial pain should prompt the physician to re-evaluate the extent, cause and treatment of PVT.

In **chapter 8** we aimed to compare the total costs of TIPS placement versus EVL for the secondary prevention of gastro-oesophageal variceal bleeding in the first year following the index bleed by determining direct medical costs from a multicenter randomized trial in which EVL was compared with TIPS placement. We performed a post-hoc analysis of direct medical costs of 52 consecutive patients (25 TIPS/27 endoscopy) surviving an acute first or second variceal bleeding due to liver cirrhosis-related portal hypertension. The primary endpoint of this study was defined as mean total health care costs per patient for the two treatment strategies. Total costs included the costs for intervention as well as healthcare costs from the day of randomization until twelve months after randomization, or until the occurrence of death, or moment of liver transplantation.

The cost prizes of the two treatment groups, including all personnel, equipment, material, housing, and overhead costs were € 10.589 and € 385 for TIPS placement and EVL, respectively. The mean total costs per patient were significantly higher in the TIPS group compared to the EVL group (€ 27.746 vs € 16.818, p=0.006). Therefore, from an economic point of view, treatment with endoscopic variceal band ligation is recommended for the secondary prevention of gastro-oesophageal variceal rebleeding in patients with portal hypertension.

Conclusions

The diagnosis of chronic gastrointestinal ischemia (CGI) is a challenge. Currently, there is no single test with high sensitivity and specificity to diagnose or exclude this condition. Therefore, a diagnostic approach combining assessment of clinical symptoms, radiological imaging of the mesenteric arteries and functional testing using VLS is recommended in the work-up of CGI suspected patients[9]. However, a definitive diagnosis of CGI is only to be made after persistent relief of symptoms on follow-up after treatment. This is not the ideal reference standard, however given the absence of any other generally accepted reference standard, it is currently the only reliable way to diagnose true CGI. Therefore, further studies should focus on improvement of the pre-treatment diagnosis of CGI. VLS appears to be of value as a minimal invasive diagnostic tool in patients suspected of CGI[5]. It has a high sensitivity and specificity for the diagnosis of CGI. Currently, VLS measurements are performed when patients are in a fasting state. Postprandial VLS measurements after 60 minutes of caloric intake did not improve the discrimination between patients with CGI and healthy controls. Finding a correlation between sublingual microcirculation alterations and presence of CGI could also lead to a change in approach in patients clinically suspected for CGI prompting more institutions and gastroenterologists to consider and test for CGI. A prediction model for CGI that takes into account clinical variables and radiological assessment of the mesenteric arteries may also be a useful tool for clinicians to assess the risk of CGI and to decide whether further diagnostic work-up and treatment is indicated. Studies on the value of HIF-1α as a marker for ischemia remains to be further investigated as HIF-1α is expressed in ischemic tissue, but also in normal colon tissue, and various inflammatory disorders.

We have also shown that the effect of CGI on the liver is mild and that in contrast to acute ischemic intestinal conditions, liver injury is not a common feature of CGI. When present, it is mild and mostly normalizes after appropriate treatment of CGI. In patients

with PVT however, gastrointestinal ischemia is frequent. Albeit in a small cohort, mucosal ischemia using VLS was detected in 75% of patients with PVT. Onset of abdominal symptoms such as postprandial pain should prompt the physician to re-evaluate the extent, cause and treatment of PVT. We also compared the total costs of TIPS placement versus EVL for the secondary prevention of gastro-oesophageal variceal bleeding. The highest costs were found in the TIPS group, due to higher personnel, equipment, material, and housing costs. From an economic point of view, treatment with endoscopic variceal band ligation is recommended for the secondary prevention of gastro-oesophageal variceal rebleeding in patients with portal hypertension.

Discussion and future directions

This thesis has some limitations. Firstly, all studies in this thesis were conducted in a single center setting. The Erasmus MC is one of the two referral centers in the Netherlands with a dedicated CGI program. To increase power of our studies and generalizability of our findings, further collaboration between the two referral centers should be explored.

This also applies to the studies regarding the VLS. With the current cut-off values and the high sensitivity of 90%, no patient with CGI should be missed[5]. However, this high sensitivity is at the expense of a low specificity and thus results in a higher rate of false-positives. In addition, the mean of mucosal saturation measurements in patients with CGI shows substantial variation and the range of measurements within an individual patient is large. Furthermore, there are small small changes in the position of the probe during the measurements which can elicit the possibility of not acquiring the actual mucosal oxygen saturation. Also, the performance of VLS are based on data from only one large cohort study and has not yet been reproduced by other groups[5]. This illustrates the necessity for prospective validation of the established cut-off values in a new cohort of patients with the suspicion of CGI. Improved VLS measurements can be observed in patients with CGI after successful intervention[5]. However, repeated upper endoscopy including VLS measurements, was performed in only a small number of patients. Therefore, future studies should focus on the role of repeated VLS measurements in the follow up of patients with CGI after treatment.

Another limitation of the VLS is that the current technique causes restrictions regarding the place and time of the measurements. Mucosal ischemia might be patchy and because VLS is limited to point measurements, it could be that mucosal ischemia might be missed with VLS. Continuous measurement of the mucosal oxygen saturation using VLS could overcome this problem. Moreover, the time-dependent relationship of VLS measurements

and gastrointestinal blood flow can be further investigated by direct cessation of blood flow in the celiac artery and superior mesenteric artery in animal models. In addition, there might be a time-dependent relation between mucosal ischemia and oral caloric stimulation. In our study, repeated VLS measurements were performed after 60 minutes after caloric stimulation. We found that there is no significant increase or decrease in VLS measurements after stimulation with food in patients with CGI whereas in healthy controls VLS measurements in the duodenal bulb rose significantly after food stimulation. Although these observations seem to confirm our initial hypothesis that indeed the physiological hyperemic response induced by food stimulation is impaired in patients with CGI, it currently provides too little discriminatory power for clinical use. Modifications of the VLS assessment protocol, in particular the timing of postprandial measurements, may improve its applicability.

Furthermore, portal vein flow measurement using phrase contrast, with and without caloric stimulation, might also provide additional information on the time-dependent relationship between caloric intake, gastrointestinal arterial blood flow, and mucosal ischemia.

Several studies have shown that microcirculatory alterations are related to hemodynamic changes which have been reported to impair gastrointestinal perfusion [12-14]. In these patients, the sublingual capillary perfusion seems well correlated to the hypoxic state of the gastric mucosa [15]. Further research is required to illustrate and to validate the hypothesis that there is a correlation between impaired sublingual microcirculation and decreased endoscopic VLS measurements in patients with CGI. Although VLS is minimally invasive and can be performed in almost all patients, sublingual VLS measurements are less invasive, more patient-friendly and less time consuming than endoscopic VLS and therefore have the potential to be of great value in diagnosing patients with CGI [5,9].

We established a prediction model for CGI based on clinical symptoms and radiological evaluation of the mesenteric arteries. However, a possible limitation of this study is incorporation bias

which may have led to overestimation of the accuracy of the studied variables for the prediction model for CGI[17,18]. This illustrates the necessity for prospective, external validation of our model in a new cohort of patients. However, this is expected to be difficult as large well-defined cohorts are lacking and these studies will also be limited by the absence of a golden standard for CGI.

Another point of focus for future studies should be the further elucidation of gastrointestinal ischemia in patients with PVT. It would be interesting to perform VLS measurements to study the time course of gastrointestinal ischemia in patients with PVT, for instance before and after initiation of anticoagulant therapy. In addition, colonoscopy is now only included in our standard work up of the patient in case of lower gastrointestinal symptoms. It would be interesting to perform colonoscopy in patients with PVT to assess ischemic changes in the colonic mucosa.

The use of endovascular techniques for revascularization of chronic stenosis and occlusions of the gastrointestinal arteries has rapidly evolved and endovascular therapy with stenting has become the most common method in the treatment of CGI. Nowadays, use of bare metal stents is the standard care although the patency of these stents is limited. According to retrospective data the patency of covered stents is significantly higher compared to bare metal stents. It would be interesting to compare the outcome of revascularisation of the gastrointestinal arteries using covered stents compared to bare-metal stents in patients with CGI. Of great interest would also be to assess the role of fractional flow reserve (FFR) for the detection of significant stenosis in the gastrointestinal arteries. FFR is a technique used in coronary catheterization to measure pressure differences across a coronary artery stenosis to determine the likelihood that the stenosis impedes oxygen delivery to the organ. This could lead to a new approach in that only in case of high FFR patients with the suspicion of CGI would receive endovascular treatment. Another way of correctly identifying a significant stenosis of the gastrointestinal arteries would be using direct upper gastrointestinal sonography.

A point of discussion has always be the treatment of single vessel CGI. Studies using functional testing in the diagnosis of CGI have shown that a significant stenosis of a single artery with insufficient collateral circulation can lead to clinically relevant gastrointestinal ischemia[4,6]. However, a randomized controlled trial including intervention by means of endovascular treatment, or a release operation in case of the celiac artery compression syndrome (CACS), and a sham procedure will present concrete evidence that patients with single vessel CGI would benefit from treatment. Future studies should also focus on the follow-up of these patients, especially regarding anti-coagulation. Nowadays, the use of anticoagulation consist of lifelong use of platelet aggregration inhibitor, such as aspirine, in combination with an oral, thienopyridine-class antiplatelet agent such as clopidogrel. However, no sound scientific evidence existent on the duration of use of the latter in patients with CGI.

In addition, studies on cost-effectiveness and quality of life in patients with CGI are lacking. Future studies on these topics should be performed to elucidate these issues further.

Cost studies in patients with portal hypertension has shown that early TIPS placement has higher costs than EVL in the prevention of rebleeding in patients with portal hypertension. However, data on quality of life in these patients are lacking. Future research should focus on the cost-effectiveness and quality of life in this patient population.

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10

Summary in Dutch

Samenvatting

Het stellen van de diagnose chronische gastrointestinale ischemie (CGI) is een klinische uitdaging. Er bestaat geen enkel- en eenvoudig onderzoek waarvan de sensitiviteit hoog genoeg is voor het stellen van de diagnose. Daarom wordt evaluatie van de combinatie van klinische symptomen, radiologische beeldvorming van de mesenteriale slagaders en functionele testen aanbevolen in de diagnostiek van CGI verdachte patiënten[1]. Echter een definitieve diagnose van CGI kan pas gesteld worden nadat de patiënt aanhoudend verlichting van symptomen ondervindt na de behandeling. Dit is niet de ideale gouden standaard voor het stellen van de diagnose, maar gezien het ontbreken van een andere aanvaarde standaard, het is momenteel de meest betrouwbare manier om de diagnose van CGI te bevestigen. Daarom moet verder onderzoek zich richten op verbetering van de diagnose van CGI.

Hoofdstuk 1 geeft een korte inleiding en doelen van dit proefschrift weer.

Hoofdstuk 2 richt zich op het effect van calorische intake op VLS metingen bij patiënten met CGI en gezonde controles. Opeenvolgende patiënten met de diagnose van CGI en gezonde controles werden prospectief geïncludeerd tussen september 2014 en augustus 2015. Alle patiënten kregen de standaard diagnostische work-up voor CGI, bestaande uit uitvoerige anamnese en medische voorgeschiedenis, radiologische beeldvorming van de gastrointestinale bloedvaten en VLS metingen. VLS metingen na calorische intake, oftewel postprandiale VLS waarden, werden vergeleken met VLS metingen in nuchtere toestand. We voerden 168 VLS metingen uit bij 12 patiënten met CGI (gemiddelde leeftijd 63,2 (IQR 48,8-70,4) jaar, 67% mannen) en 16 controles (32,9 (IQR 27.7-39.8.2) jaar, 44% mannen). Er was geen significante toename in postprandiale VLS metingen en VLS metingen in nuchtere toestand in patiënten met CGI (nuchter antrum mediaan 58,5 (IQR 53,5-59,0) versus postprandiale antrum mediaan 57,5 (IQR 54,0-60,8), p=0,25; nuchter bulbus duodeni mediaan 53,0 (IQR 48,8 -54,0) versus postprandiale bulbus duodeni mediaan 53,5 (IQR 50,3-56,8), p=0,89; nuchter duodenum descendens mediaan 49,5 (IQR 45,3-52,8) versus postprandiale

duodenum descendens mediaan 54,0 (IQR 47,3-57,8), *p*=0,33, respectievelijk). We vonden geen verschil in VLS metingen vóór en na calorische intake bij patiënten met CGI, maar er was een trend in toename VLS metingen in de gezonde controles. De huidige resultaten tonen onvoldoende onderscheid tussen patiënten met CGI en gezonde controles.

Hoofdstuk 3 beschrijft de sublinguale microcirculatie in 12 patiënten met bewezen CGI. Beoordeling van de sublinguale microcirculatie gebeurde met behulp van een handmicroscoop (Cytocam). Patiënten met CGI hadden een toename in het aantal kleine bloedvaten en een toename in de perfusie van deze kleine bloedvaten na calorische intake.

In **hoofdstuk 4** presenteren we een predictiemodel voor patiënten met een klinische verdenking op CGI. Het predictiemodel is gebaseerd op een grote prospectieve studie bestaande uit 436 patiënten met de verdenking op CGI en die verwezen zijn naar ons centrum voor nadere analyse. Onze studie toont aan dat toenemende leeftijd, vrouwelijk geslacht, misselijkheid, gewichtsverlies, recent begin van de symptomen, belaste cardiovasculaire voorgeschiedenis alsook een positieve familie anamnese voor cardiovasculaire ziekten sterke voorspellers zijn voor het ontwikkelen van CGI. Daarentegen pleit het gebruik van analgetica tegen het krijgen van CGI. Onze studie laat zien dat de diagnostische waarde van klinische variabelen alleen in de diagnose van CGI beperkt is (c-statistic 0,62), maar dat een combinatie van klinische variabelen en radiologische evaluatie van de mesenteriale vaten een nauwkeurigere voorspelling kan doen voor het krijgen van CGI (c-statistic van 0,76). Op basis hiervan ontwikkelden we een eenvoudige vragenlijst die voor clinici een nuttig hulpmiddel kan zijn om het risico van CGI in te schatten en om te beslissen of verdere diagnostiek en behandeling van deze patiënten geïndiceerd is.

Hoofdstuk 5 laat de resultaten zien van een studie waarin de expressie van HIF-1α is onderzocht in het gastrointestinale stelsel. Immunohistochemische expressie van HIF-1α werd geanalyseerd in 20 patiënten met CGI, 9 patiënten met H. pylori-gastritis, 9 patiënten

met ischemische colitis (IC), 18 patiënten met inflammatoire darmziekte (IBD), 5 patiënten met infectieuze colitis en 22 controles. In de bovenste tractus digestivus werd HIF-1α expressie in 5/20 CGI-patiënten gezien, maar niet in de controlegroep (p=0,08). De sensitiviteit en specificiteit van HIF-1α expressie voor de diagnose CGI was 25% en 84%, respectievelijk. In de lage tractus digestivus werd HIF-1α expressie gezien in alle patiënten met IC, infectieuze colitis, in de meerderheid van de IBD-patiënten en in een gedeelte van de controles. De sensitiviteit en specificiteit van HIF-1α voor de diagnose IC was 100% en 51%, respectievelijk. HIF-1α expressie werd sterk waargenomen in patiënten met histologische inflammatie van de lage tractus digestivus (p=0,02). Onze resultaten tonen aan dat HIF-1α expressie aanwezig is in ischemisch weefsel, maar bij inflammatie en in normaal colon weefsel.

In **hoofdstuk 6** geeft de resultaten weer van de frequentie van leverschade in een welomschreven cohort van 156 patiënten met CGI. In slechts 10% van de patiënten was een zeer mild verhoogde serum transaminasestijging te zien. Verhoogde ALT> 1 x ULN was niet significant geassocieerd met de aanwezigheid van mucosale ischemie, significante stenose van de CA, SMA of van zowel CA als de SMA. Evenmin was er geen associatie tussen ALT> 1x ULN en verhoogde BMI, alcoholconsumptie, DM type II en toenemende leeftijd. Na behandeling van CGI, normaliseerde de ALT in 10/12 (83%) patiënten. In tegenstelling tot acute ischemische aandoeningen, komt leverschade in slechts 10% van de patiënten met CGI voor. De leverschade is mild en normaliseert na een goede en passende behandeling van CGI.

In **hoofdstuk 7** worden de resultaten beschreven van een prospectieve cohort studie waarin de klinische presentatie en kenmerken van gastrointestinale ischemie in patiënten met vena porta trombose (PVT) wordt beschreven. In deze studie werden 15 patiënten met chronische PVT en een patiënt met acute PVT geïncludeerd. Klinische symptomen van ischemie werd beoordeeld met behulp van een gestructureerde vragenlijst, radiologische evaluatie van de mesenteriale vaten en VLS metingen. De VLS metingen

werden vergeleken met een historische controlegroep en met een groep patiënten met levercirrose. Symptomen typisch voor ischemie, zoals postprandiale pijn en inspannings-geïnduceerde pijn, werd in 10/16 (63%) patiënten met PVT waargenomen. Verlaagde VLS metingen in ten minste één plaats in het bovenste tractus digestivus werd 12/16 (75%) patiënten gezien. Patiënten met PVT hadden met name verlaagde VLS metingen in het duodenum vergeleken met de controlegroep en patienten met levercirrose(mediaan 55,5 [52,3-58,8] vs mediaan 60,0 [56,2-61,7], p=0,02 en mediaan 52,0 [46,5-54,0], p=0,003, respectievelijk). Gastrointestinale ischemie is frequent aanwezig in patiënten met chronische PVT. Mucosale ischemie werd in 75% van de patiënten met PVT gedetecteerd. VLS maakt objectieve en kwantitatieve bepaling van mucosale ischemie mogelijk.

De huidige maatschappij vraagt om slimme en betaalbare behandelmogelijkheden. In **hoofdstuk 8** hebben we de totale kosten van een transjugulaire intrahepatische portosystemische shunt (TIPS) plaatsing versus endoscopische rubberbandligatie (EVL) vergeleken in de secundaire preventie van gastro-oesofageale bloedingen. Directe medische kosten van 52 opeenvolgende patiënten (25 TIPS / 27 EVL) in het eerste jaar na de eerste bloeding werden berekend. De hoogste kosten werden gevonden in de TIPS groep (gemiddelde kosten per patiënt € 27.746 vs € 16.818 voor de EVL groep), als gevolg van hogere personeelskosten, uitrusting-, materiaal- en huisvestingskosten.

In het laatste **hoofdstuk 9** worden de belangrijkste bevindingen uit de voorgaande hoofdstukken in dit proefschrift samengevat en worden aanbevelingen voor toekomstig onderzoek beschreven.

Appendix

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Rotterdam, 16 december 2015

List of publications

1
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7

J. Harki, I.L. Holster, S. Polinder, A. Moelker, H.R. van Buuren, E.J. Kuipers, E.T.T.L. Tjwa; Cost study of covered transjugular intrahepatic portosystemic shunt (TIPS) versus endoscopic treatment + β-blocker for secondary prevention of gastro-oesophageal variceal bleeding. *Submitted*

8

J. Harki, M. Suker, M.S. Tovar Doncel, L.J.D. van Dijk, C.H.J. van Eijck, M.J. Bruno, E.J. Kuipers, C. Ince; Patients with chronic gastrointestinal ischemia have an altered sublingual microcirculation. *Submitted*

PhD portfolio

Name	Jihan Harki
Promotor	prof. dr. E.J. Kuipers and prof. dr. M.J. Bruno
Co-promotor	dr. E.T.T.L. Tjwa and dr. D. van Noord-Leemreis
Affiliation	department of Gastroenterology and Hepatology
PhD period	2012-2015

Oral presentations

2015 Endoscopic visible light spectroscopy in patients with chronic gastrointestinal ischemia and healthy controls, *United European Gastroenterology Week*, Barcelona, *Oral Free Paper Prize*

2014 Development of a prediction model to assess the risk of chronic gastrointestinal ischemia in referred patients, *United European Gastroenterology Week*, Vienna

2014 Development of a prediction model to assess the risk of chronic gastrointestinal ischemia in referred patients, *Dutch Society of Gastroenterology*, Veldhoven

Poster presentations

2015 Endoscopic visible light spectroscopy in patients with chronic gastrointestinal ischemia and healthy controls, *United European Gastroenterology Week*, Barcelona

2015 Ischemic hepatitis in patients with chronic gastrointestinal ischemia, *Digestive Disease Week*, Washington

2014 Development of a prediction model to assess the risk of chronic gastrointestinal ischemia in referred patients, *Digestive Disease Week*, Chicago

2014 Gastrointestinal ischemia in patients with portal vein thrombosis, *Digestive disease week*, Chicago

2014 Gastrointestinal ischemia in patients with portal vein thrombosis, *International Liver Congress*, London

Courses, workshops and seminars

2015 Certificate of 'Basiskwalificatie onderwijs',
Erasmus MC Desiderius School, Rotterdam
2015 Training 'Teach the Teacher',
Erasmus MC Desiderius School, Rotterdam
2015 Training 'Hoorcollege geven',
Erasmus MC Desiderius School, Rotterdam
2015 Training 'Individuele begeleiding',
Erasmus MC Desiderius School, Rotterdam
2014 Biomedical English writing and communication,
Erasmus MC, Rotterdam
2014 Research Integrity, Erasmus MC, Rotterdam
2013 Training 'Omgaan met Groepen',
Erasmus MC Desiderius School, Rotterdam
2013 Research Management, Erasmus MC, Rotterdam
2013 Good Clinical Practice (BROK), Erasmus MC, Rotterdam
2013 Prognostic research, NIHES Winter program, Rotterdam
2013 Regression for clinicians, NIHES Winter program, Rotterdam
2013 Biostatistics for clinicians, NIHES Winter program, Rotterdam
2013 Gastroenterologic year, Erasmus MC, Rotterdam
2012 Erasmus Liverday, Erasmus MC, Rotterdam

Memberships

2014 American Gastroenterology Association
2014 European Association for the Study of the Liver
2012 Dutch Society of Gastroenterology

Bursaries

2015 'Oral Free Paper prize' Endoscopic visible light spectroscopy in patients with chronic gastrointestinal ischemia and healthy controls, *United European Gastroenterology Week*, Barcelona
2015 'Poster in the Spotlight' Endoscopic visible light spectroscopy in patients with chronic gastrointestinal ischemia and healthy controls, *United European Gastroenterology Week*, Barcelona
2014 EASL Young Investigator Bursary

Teaching

2015 Chronic Gastrointestinal Ischemia, i.o. Zorgacademie, Erasmus MC, Rotterdam

2015 Course on SPSS, i.o. Molecular Medicine, Erasmus MC, Rotterdam

2014-15 Lecturer Medical Psychology for interns, department of Medical Psychology, Erasmus MC, Rotterdam

2014 Chronic gastrointestinal ischemia, Regionaal onderwijsavond MDL, Rotterdam

2012-13 Supervising Joram A. Spoor, medical student Erasmus University Rotterdam, Master's thesis

Review activities

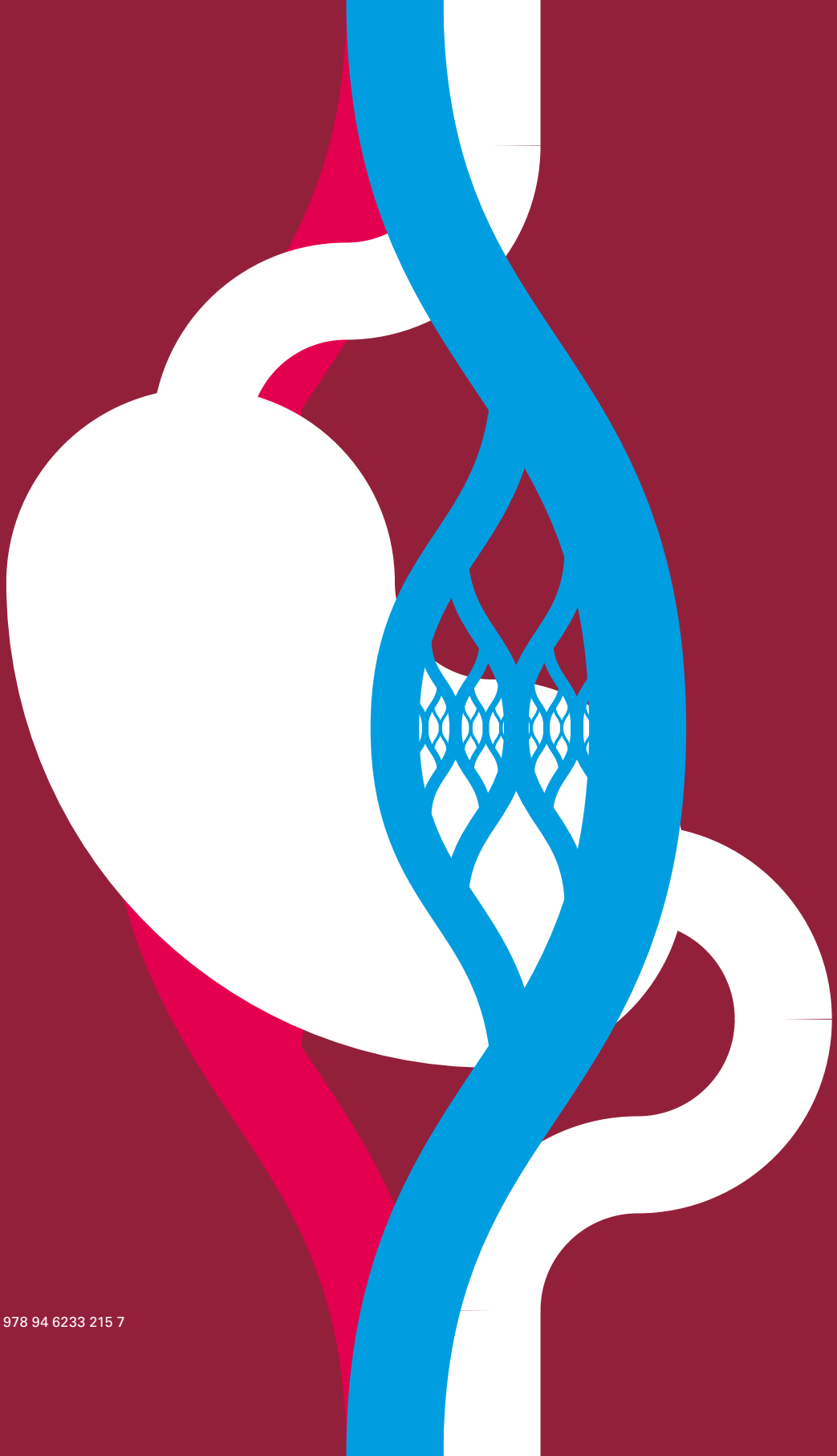
Acta Radiologica

UEG Journal

About the author

Jihan Harki was born on October 8th 1987 in Al-Sulaymaniya and grew up in Erbil, in Iraqi-Kurdistan. She was six years old when her family decided to leave the country and move to the Netherlands. She graduated at the Stedelijk Dalton Lyceum (Gymnasium) in Dordrecht in 2005, after which she attended medical school at the Erasmus University in Rotterdam. During her medical study, she soon became interested in Gastroenterology and Hepatology. She was involved in several studies in this field, which also included her master's thesis study on the assessment of regulatory T-cells in liver diseases at the Queen Elizabeth Hospital in Birmingham, United Kingdom (supervision prof. dr. H.L.A. Janssen and prof. dr. D.H. Adams). She obtained her medical degree in September 2012 and started her PhD trajectory as described in this thesis at the department of Gastroenterology and Hepatology of the Erasmus MC University Medical Center Rotterdam, under the supervision of prof. dr. E.J. Kuipers, dr. E.T.T.L. Tjwa and dr. D. van Noord, and in 2014 also by prof. dr. M.J. Bruno. As of January 2016, she will start with her two-year Internal Medicine residency at the Sint Franciscus Gasthuis in Rotterdam (program director dr. A.P. Rietveld). Hereafter, she will continue her training in Gastroenterology and Hepatology at the Erasmus MC University Medical Center Rotterdam (program director dr. R.A. de Man).

NA	not available
NOMI	non-occlusive mesenteric ischemia
NOS	not otherwise specified
NPV	negative predictive value
NSAID	non-steroidal anti-inflammatory drugs
O	oral
OAC	oral anticoagulation
OR	odds ratio
P	parenteral
PC	protein C deficiency
PCR	polymerase chain reaction
PHD	prolyl hydroxylase domain
PPI	protein pump inhibitors
PPV	positive predictive value
PPVa	proportion of perfused vessels of all vessels
PPVs	proportion of perfused vessels of small vessels
PS-ratio	platelet spleen ratio
PTA	percutaneous transluminal angioplasty
PTFE	polytetrafluoroethylene
PV	portal vein
PVDa	perfused vessel density of all vessels
PVDs	perfused vessel density of small vessels
pVHL	Von Hippel-Lindau factor
PVT	portal vein thrombosis
Py	pack years
R	rectal
ROC-curve	receiver operating characteristic curve
SC	subcutaneous
SD	standard deviation
SMA	superior mesenteric artery
SMV	superior mesenteric vein
STROBE	strengthening the reporting of observational studies in epidemiology
SV	splenic vein
TIPS	transjugular intrahepatic portosystemic shunt
TVDa	total vessel density of all vessels
TVDs	total vessel density of small vessel
ULN	upper limit of normal
VDU2	deubiquitinating enzyme-2
VLS	visible light spectroscopy



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