Management of Chronic Gastrointestinal Ischemia

Aria Sana

Management of Chronic Gastrointestinal Ischemia

Aria Sana

ISBN: 978-94-6169-294-8

Layout and printing: Optima Grafische Communicatie, Rotterdam, The Netherlands
Cover: Achid Farooq
Photo cover: *Arghandab* rivier te Kandahar, Afghanistan

The work presented in this thesis was conducted at the Department of Gastroenterology and Hepatology, Erasmus MC University medical Center Rotterdam, the Netherlands.

Financial support for printing this thesis was kindly given by the Department of Gastroenterology and Hepatology of the Erasmus MC University Medical Center Rotterdam, Erasmus University Rotterdam, de Nederlandse Vereniging voor Gastroenterology, Olympus Nederlands B.V., Clinical Devices B.V., Pentax Nederland B.V., J.E. Jurriaanse Stichting.

Management of Chronic Gastrointestinal Ischemia

De diagnostiek en behandeling van chronische gastrointestinale ischemie

Proefschrift

ter verkrijgen van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus Prof. Dr. H.G. Schmidt
en volgens besluit van het college voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 26 september 2012 om 13:30 uur

door

Aria Sana
geboren te Herat, Afghanistan

PROMOTIECOMMISSIE

Promotor	Prof. Dr. E.J. Kuipers
Overige leden	Prof. dr. A.A.M. Masclee
	Prof. dr. E.J.G. Sijbrands
	Prof. dr. H.J.M. Verhagen
Co-promotoren	Dr. P.B.F. Mensink
	Dr. L.M.G. Moons

تقدیم به فامیل دوست داشتنیم

و اهدا به دوانسانیکه موجودیت مرا سبب شدند و در هر مرحلهٔ زندگی یار و مدد گارم هستند

پدر عزیز و مادر مهربان !

من توانائی بیان آن همه محبتها فداکاریها وصبر و شکیبائی تان را ندارم ، اما میخواهم یاد آور شوم که شما عامل اصلی رشد وپیشرفت من هستید.مهربانیها ، تشویقها ، اعتماد ودادن آزادیهای سالم درهر مرحله از زندگی برایم قدرت داد تا خودم را و توانایی هایم را درراه مثبت بکار اندازم و آنگونه که شایستهٔ یک انسان سالم و با دانش است عمل نموده مراحل حساس زندگی را بامشکلاتش با موفقیت سپری کنم وبه آرزوهایم که آرزوی شما نیز هست برسم .

به امید اینکه قطرهٔ ازدریایی محبت تان را روزی بتوانم ادا سازم. همیشه دوست تان خواهم داشت ودختر خوبی برایتان خواهم بود.

Mijn allerliefste familie, ik wil jullie van harte bedanken voor jullie liefde gedurende mijn hele leven. Jullie hebben altijd geduld getoond en klaar gestaan voor mij. Lieve papa en mama, jullie hebben mij altijd gemotiveerd en de kans gegeven om het beste uit me te halen en dat heeft mij gebracht waar ik nu ben. Daarom is dit proefschrift opgedragen aan jullie. Ik hou ontzettend veel van jullie.

Aan mijn lieve ouders en Mojtaba

CONTENTS

Chapter 1

Aims and outline

INTRODUCTION

Gastrointestinal ischemia results from a mismatch of blood supply to the main gastrointestinal arteries and the oxygen demand to maintain adequate metabolism. Three aortic branches supply blood to the gastrointestinal tract: the celiac artery, the superior mesenteric artery, and the inferior mesenteric artery. One of the main causes of chronic gastrointestinal ischemia (CGI) is stenotic or occlusive disease of the supplying gastrointestinal arteries. For a long time it was thought that only occlusive disease of two or more gastrointestinal arteries could lead to CGI. The introduction of functional testing has played a pivotal role in the diagnosis of CGI. Functional testing has shown that CGI is more common than previously thought because it can also be caused by single vessel disease. Further studies showed that a majority of patients with single vessel disease had sustained response after adequate treatment. Moreover, functional testing seems to be pivotal to select patients who will benefit from treatment, whether the cause is single- or multi-vessel disease.

Aims

In this thesis we aimed to study different aspects of diagnosis and treatment of CGI. We studied the predictive value of functional testing in diagnosis of CGI, and determined the diagnostic accuracy of a new minimally invasive technique to detect ischemia in order to optimize the diagnosis of CGI. Furthermore, we assessed the risk factors for atherosclerotic disease of the abdominal arteries, being one of the main causes of CGI. We also evaluated the clinical success of revascularization in single vessel disease and response to vasodilation therapy in patients with non-occlusive CGI.

Patients clinically suspected of CGI may present with typical symptoms such as postprandial pain resulting in fear of eating and subsequently weight loss, however they can also present with less typical symptoms such as exercise related pain, diarrhea, and nausea. The classic triad of CGI, consisting of postprandial pain, weight loss, and an abdominal bruit, is present in only around 20% of patients diagnosed with CGI. Thus, based on presenting symptoms and signs, CGI remains a clinical challenge, and currently there is no single test, which is sensitive and specific enough to diagnose CGI. Therefore, the proposed diagnostic approach in patients clinically suspected of CGI includes radiologic imaging of the celiac artery and the mesenteric arteries, and a functional test for assessment of mucosal perfusion. Radiologic imaging may identify gastrointestinal arterial stenosis, and a functional test may detect gastrointestinal mucosal ischemia. In **chapter 2** we assess the diagnostic value of items from history and physical examination, radiological imaging and tonometry as a functional test for the diagnosis of CGI.

Tonometry is not widely used as a functional test in the diagnosis of CGI because of its time consuming and invasive nature. Recently, visible light spectroscopy (VLS) was introduced as a new and minimally invasive technique to detect mucosal ischemia. VLS enables measurements of mucosal capillary hemoglobin oxygen saturation during endoscopy. First studies using VLS measurements in diagnosis of patients with CGI showed that lowered mucosal oxygen saturation could be used to detect ischemia in these patients. In **chapter 3** we present the results of a study, which was designed to prospectively evaluate the diagnostic accuracy of VLS for detection of ischemia in a large cohort of patients clinically suspected of CGI.
In a second large cohort we evaluated the treatment response of patients clinically suspected of CGI who were selected for treatment with a standard diagnostic work-up including radiological imaging, and VLS, and additionally we determined predictors of positive response to treatment in these patients. The results of this study are shown in **chapter 4**.

A single artery stenosis is generally not considered to cause CGI due to the abundant collateral circulation of the splanchnic vascular bed. Furthermore, stenosis of a single gastrointestinal artery is highly prevalent in the general -asymptomatic- population. Nevertheless, the concept that single artery stenosis never induces CGI is currently being challenged. Firstly, functional studies using gastrointestinal tonometry or endoscopic VLS, have consistently observed signs of mucosal ischemia in a proportion of patients with single artery disease, with a correlation with symptoms. Secondly, several studies have reported clinical success of treatment in patients with single vessel disease. The introduction of functional testing can help to indentify those with single artery stenosis who would benefit most from treatment. In **chapter 5** we evaluate the clinical success rates of revascularization of a stenosis of either the celiac artery or superior mesenteric artery in patients with unexplained refractory gastrointestinal symptoms.

CGI is in the majority of cases caused by narrowing of the gastrointestinal arteries, with atherosclerosis being the most common underlying cause. Atherosclerosis is a generalized disease, and any ischemic event in a certain vascular bed is a strong independent risk factor for a new cardiovascular event. Studies focused on a cardiovascular work-up of classical risk factors for atherosclerosis in patients with CGI are missing. Therefore, in **chapter 6** we present the results of a case-control study, which was designed to determine the contribution of classical atherosclerotic risk factors to atherosclerotic CGI, and the mortality risk in treated patients.

Occlusive disease of the gastrointestinal arteries is one of the most common causes of CGI. However, the introduction of dedicated functional tests for detection of mucosal ischemia by means of gastrointestinal tonometry and VLS has shown that the spectrum of CGI may also include patients without macrovascular pathology, defined as having non-occlusive CGI.

Non-occlusive CGI is thought to be caused by a chronic state of hypoperfusion, either related to vasospasm of the small branches of gastrointestinal arteries, atherosclerotic disease of the gastrointestinal microvascular arteries, or hyposaturation either as a result of low cardiac output or hypo-oxygenation due to severe anemia or pulmonary failure. In theory, patients with non-occlusive CGI as a result of hypoperfusion could benefit from vasodilating medication. **Chapter 7** discusses clinical characteristics of this particular group as well as the effectiveness of vasodilating medication.

Ischemic colitis accounts for half of all forms of gastrointestinal ischemia. It results from an inadequate blood flow to the colon to meet the metabolic demands of the colon. This could be due to occlusion of the mesenteric arteries, mechanical obstruction or systemic low flow states. Endoscopy and histopathology often show nonspecific abnormalities in diagnosis of ischemic colitis. A potential marker may be hypoxia inducible factor 1 alpha (HIF-1 alpha), which is induced by hypoxia. HIF-1 alpha could be a marker, which can help us with an early diagnosis of ischemic colitis, and start of adequate treatment. In **chapter 8** we compare the expression of HIF-1 alpha in normal colon, ischemic colitis, as well as in Crohn's disease (CD), ulcerative colitis (UC), and infectious colitis. Furthermore, we investigated if there is a correlation between the degree of inflammation and expression level of HIF-1 alpha.

Finally in **chapter 9** you will find a summary and discussion of the main findings of the studies presented in this thesis. Moreover, suggestions for future studies are also discussed in this chapter.

Chapter 2

Diagnosing chronic gastrointestinal ischemia: value of clinical features, radiological imaging, and gastrointestinal tonometry

A. Sana MD[1], Y. Vergouwe PhD[2], D. van Noord MD[1], L.M.G. Moons MD PhD[1], P.M.T. Pattynama MD PhD[3], H.J.M. Verhagen MD PhD[4], E.J. Kuipers, MD PhD[1,5],,P.B.F. Mensink MD PhD[1]

Departments of Gastroenterology and Hepatology[1], Center for Medical Decision Sciences, Department of Public Health[2], Interventional Radiology[3], Vascular Surgery[4], Internal Medicine[5] Erasmus MC - University Medical Center, Rotterdam, The Netherlands

Clinical Gastroenterology and Hepatology 2011;9:234-241

ABSTRACT

Background & aims: The diagnosis of chronic gastrointestinal ischemia (CGI) remains a clinical challenge. We aimed to assess the diagnostic value of clinical features, visualization of the gastrointestinal arteries and evaluation of mucosal perfusion in patients clinically suspected of CGI.

Methods: 186 patients referred for suspicion of CGI were prospectively included and followed. All patients had an extensive diagnostic work up, including visualization of the gastrointestinal arteries with CT-, MR- or conventional angiography, and mucosal perfusion with tonometry. The reference standard for CGI was persistent clinical response after adequate therapy. The diagnostic value of individual and combined tests was assessed with multivariable logistic regression analysis.

Results: 116 (62%) patients were diagnosed with CGI. In a multivariable model solely based on clinical features, the strongest predictors for CGI were presence of postprandial pain, weight loss per month in kg, concomitant cardiovascular disease, and presence of an abdominal bruit. However, this model showed limited discriminative ability for presence or absence of CGI (c-statistic 0.62). Adding radiological imaging to the prediction model improved the discriminative ability substantially (c-statistic 0.81). Adding tonometry to the prediction model further improved the discriminative ability of the model (c-statistic 0.90). The combination of clinical features and tonometry with a c-statistic of 0.88 approximated the discriminative ability of the latter model.

Conclusion: Clinical features alone have a limited value to correctly assess CGI. Visualization of the gastrointestinal arteries and evaluation of mucosal perfusion substantially improve the diagnosis of CGI. The strongest diagnostic contribution comes from mucosal perfusion assessment.

INTRODUCTION

The diagnosis of chronic gastrointestinal ischemia (CGI) remains challenging to establish. Postprandial pain is thought to be the typical presenting symptom for chronic gastrointestinal ischemia (CGI). This pain causes fear of eating, which results in reduced intake and consequent weight loss. However, patients with CGI can also present with less typical symptoms as exercise-related abdominal pain, diarrhea and nausea. Physical examination may reveal an abdominal bruit and low body mass index (BMI).[1-4] The combination of postprandial pain, weight loss and an abdominal bruit is known as the 'classical' triad of CGI, but recent studies in larger cohorts of CGI patients have shown that this triad is only present in around 20% of CGI patients.[3,5-7] Consequently, based on presenting symptoms and signs, CGI remains a difficult diagnosis to establish.

Currently, there is no single test which is sensitive and specific enough to diagnose CGI. The proposed diagnostic approach for patients clinically suspected of CGI includes radiological imaging of the celiac trunc and mesenteric arteries, and a functional test for mucosal perfusion.[4, 8, 9] Radiologic imaging may identify gastrointestinal arterial stenosis, which can be visualized by means of computed tomography angiography (CTA), magnetic resonance angiography (MRA), duplex ultrasound, or conventional digital subtraction angiography (DSA). [1-2, 8, 10] A functional test may detect low oxygen saturation within areas of the gastrointestinal mucosa. Twenty-four hour gastrointestinal tonometry has been shown to be an accurate functional test for mucosal perfusion, enabling identification of a considerable proportion of patients with CGI.[3, 11]

The relative contribution of these diagnostic modalities for the assessment of patients with possible CGI is unknown. In the present study we therefore aimed to assess the diagnostic value of items from history and physical examination, radiological imaging, and functional testing for the diagnosis of CGI.

METHODS

Consecutive patients with a clinical suspicion of CGI were included after informed consent, and prospectively followed. All patients were seen in a single tertiary referral center with a catchment area of 4.4 million subjects. In all patients more common causes of upper gastrointestinal complaints had been excluded by upper endoscopy, colonoscopy, and abdominal ultrasound or abdominal computed tomography. Suspicion of CGI was defined as having at least two of the following characteristics: 1) postprandial upper abdominal pain, 2) unexplained weight loss (> 5% of standard weight), and 3) significant stenosis of at least one of

the gastrointestinal arteries on radiological evaluation. The Institutional Review Board of the Erasmus MC- University Medical Center approved the study.

Standard diagnostic work-up

All consecutive patients referred to our center with suspicion of CGI were prospectively evaluated and all data were collected in a prospective manner. All patients underwent a standard work up by means of a thorough medical and physical examination, and an extensive questionnaire concerning clinical complaints and medical and family history. All patients underwent radiological evaluation by means of CTA, MRA and/or conventional angiography to visualize the gastrointestinal arteries (celiac artery, superior mesenteric artery and inferior mesenteric artery) in combination with a 24-h gastric and jejunal tonometry. A significant stenosis of the abdominal arteries was defined as a luminal reduction of >70 %. Gastrointestinal tonometry was performed to assess mucosal PCO_2 measurements, both in fasting and postprandial state with gastric and jejunal catheters. All patients had meals at standard times during gastrointestinal tonometry: liquid compound meal (400 ml) (12.00 p.m.), bread meal (18.00 p.m.), breakfast (8.00 a.m.), liquid compound meal (10.00 a.m.) and dinner (12.00 p.m.) [12]. The patients were instructed to eat their meals within 15 minutes. The criteria for a pathologic response was a gastric or jejunal PCO_2 > 12.0, 13.6 and 10.6 kPa after breakfast (or bread meal), dinner or compound solution, respectively as described previously[11]. A positive (abnormal) tonometry test was defined as: 1) a pathologic response after 3 or more meals, or 2) a combination of one or two pathologic responses after meals combined with a median PCO_2 > 8.0 kPa in between meals. This was all done according to previously published standards[11].

Consensus and definitive diagnosis of CGI

Medical history, complaints, and the results of all diagnostic procedures were discussed in a dedicated multidisciplinary team consisting of a vascular surgeon, intervention radiologist and gastroenterologist, all specialized in CGI. The discussion resulted in a final expert-based consensus diagnosis of CGI or non-CGI. Patients with the diagnosis CGI had either occlusive or non-occlusive CGI. Non-occlusive CGI was defined as mesenteric ischemia in the absence of significant vascular stenoses, such as can be observed in low-flow states in patients with insufficient cardiac output. Patients diagnosed with occlusive CGI were offered revascularization of the vascular obstruction by either surgery or by endovascular stent placement. Patients diagnosed with non-occlusive CGI were offered medical treatment with vasodilation therapy, consisting of isosorbide dinitrate 20 or 40 mg od. In case of side effects or no clinical improvement after 4 weeks use of isosorbide dinitrate 40 mg od, the isosorbide dinitrate was replaced by ketanserin 20 mg or 40 mg od for the same period of time. Conservative treatment was considered in patients diagnosed with CGI who declined intervention or were not-eligible for interventional therapy. In these patients proton pomp inhibitors were prescribed

to decrease metabolic demand of the stomach and proximal gastrointestinal tract. All patients diagnosed with CGI were prospectively followed up at the out-patient clinic with scheduled visits at six weeks, three months, six months and one year after treatment for assessment of clinical status and repeated duplex ultrasound scanning of the gastrointestinal arteries. After this period, patients were assessed once yearly at our out-patient clinic or referred for yearly clinical assessment by their referring physician. The referring physicians were instructed to report recurrent symptoms, and were asked to present and / or confirm follow-up data at the end of the follow-up period. The definitive CGI diagnosis was made after persistent relief of symptoms on long-term follow-up of at least 12 months after initiation of therapy. Persistent relief of symptoms was defined as: complete disappearance of postprandial pain and / or weight gain or weight stabilization after therapeutic intervention. Patients diagnosed by the expert panel as not having CGI had no intervention and were discharged from follow-up. Follow-up data of these patients was obtained by means of a survey, which was conducted by contacting the primary care or referring physician. The survey focused on current health status, presence of any persisting symptoms, abdominal weight, further diagnostic procedures, and events such as hospital admission and death. The definite diagnosis non-CGI was only made if patients did not encounter gastrointestinal ischemia-related morbidity and / or mortality at follow-up of at least 12 months. During follow-up, patients could switch from an initial diagnosis of CGI to a final diagnosis of non-CGI in case of persistent complaints after technical successful revascularization and treatment with vasodilating agents, or from an initial diagnosis of non-CGI to a final diagnosis of CGI in case of occurrence of gastrointestinal ischemia-related complications or death during follow-up. The latter was seen in none of the patients at the definite diagnosis. The definitive diagnosis assessed at follow-up was used in the current analyses.

Statistical analysis

Missing observations were imputed five times with multiple imputation.[13-15] We used the areg.impute algorithm, which works with R software.[16] The imputation models used all the variables that we considered as potential predictors and the diagnosis CGI (yes/no). Patient's characteristics were compared using Mann-Whitney *U* test or X^2 test. Univariable and multivariable associations of the candidate predictors with CGI were quantified with logistic regression analysis. The associations were estimated as regression coefficients and odds ratios. For the univariable analysis the following patients characteristics were studied: age, gender, reported weight loss, weight loss per month, which is defined as the total amount of weight loss (in kg) a patient had from symptom onset divided by the period (in months) in which the weight loss occurred, postprandial pain, exercise related pain, diarrhea, nausea, smoking, family history of cardiovascular disease, known cardiovascular disease, presence of abdominal bruit, BMI after complaints, the classical triad, results of radiologic evaluation (significant stenosis yes/no) and gastrointestinal tonometry (ischemia present yes/no). For

the multivariable analysis we developed three models: model A (clinical model) consisted of clinical features only, i.e. clinical model. Clinical features were defined as items obtained from medical history including complaints of abdominal angina, questionnaire and physical examination; subsequently, we assessed the additional value of radiological evaluation in correctly diagnosing of CGI. This additional value was assessed compared to a model with only clinical features, so model B included predictors of model A together with radiological evaluation added; model C included the predictors of model B and tonometry results added. A third model C included predictors of model B together tonometry. Further, we developed alternative models to assess the added diagnostic value of tonometry on top of the clinical features and to assess the value of single- and multivessel stenosis separately in models B and C. For the clinical model (model A), we considered seven variables, in agreement with the rule of thumb to use no more than one variable per ten outcomes in the less frequent outcome category.[17] In our case the less frequent outcome category consisted of patients without CGI (n = 70). Based on the literature and clinical knowledge, we chose typical symptoms and signs of CGI and risk factors for atherosclerosis, namely postprandial pain, weight loss per month, BMI, family history of cardiovascular disease, smoking, concomitant cardiovascular disease, and presence of an abdominal bruit. Starting with a model using these seven variables, variable selection was performed using backward elimination with p-value <0.20. We used a relatively high p-value, because stepwise elimination with the standard p-value (0.05) may lead to a loss of information by excluding important predictors. Selection with higher p-values is considered a sensible alternative, which increases the power of selecting true predictors and excluding only those variables with small effects.[18]. If a predictive model is developed in a relatively small dataset as in our case, the model performance estimated in that dataset is too optimistic. This "optimism" is a well-known phenomenon in prediction research.[17-19] We used bootstrapping to assess the optimism in c-statistic and to assess a shrinkage factor.[18,20].

The discriminative ability of the model was estimated with a measure of concordance, the c-index. The c-index indicates to which extent patients with CGI can be distinguished from patients without CGI. This means: in case of two random patients one of them having CGI and the other one not having CGI; the c-statistic gives a higher probability for having CGI to the patient who actually has CGI than to the patient who does not have CGI. The c-statistic shows how well a model can distinguish between a patient with CGI and a patient without CGI. The c-statistic is identical to the area under the receiver operating characteristic (ROC) curve for a dichotomous outcome.[21]

RESULTS

Patients

From June 2006 to January 2009, 186 patients were referred for evaluation of suspected CGI and included in the present study (Figure 1; flow chart of the study). The median age was 63 (range 17 – 87) years and 69(37%) patients were male (Table 1). After initial diagnostic work-up, a *consensus diagnosis CGI* was made in 128 (69%) patients. Majority of patients with the consensus diagnosis of occlusive CGI (n = 99) were offered intervention by vascular surgery or endovascular revascularization. One patient was not found eligible for intervention and received conservative treatment. Four patients underwent first endovascular treatment, but surgical intervention was followed at a later stage, leading to persistent relief of symptoms in all four. Five patients suspected of occlusive CGI had no, or non-significant, arterial stenosis at angiography. The latter patients were then defined as non-occlusive CGI. Thus, eventually from 128 patients who initially had the consensus diagnosis CGI, 99 occlusive, and 29 patients non-occlusive, the diagnosis of occlusive and non-occlusive CGI was established in 94 and 34 patients, respectively. Of occlusive CGI patients, 93 (99%) had endovascular (n = 66) or surgical intervention (n = 27), and 1 patient had conservative treatment. All 34 patients with non-occlusive CGI received conservative treatment, usually consisting of vasodilatory medication (Figure 1).

Table 1: Patient characteristics for the total group, CGI patients and non-CGI patients.

Patient characteristics	Total group		CGI		Non-CGI	
	n = 186		n = 116		n = 70	
Age (years)*	63	(49 - 71)	63	(50-74)	60	(47 - 70)
Male gender	69	(37)	42	(36)	27	(39)
Reporting weight loss	133	(72)	87	(75)	46	(66)
Weight loss (kg/month)*	1.0	(0.7-2.5)	1.0	(0.7-3.0)	1.0	(0.6-2.5)
Postprandial pain	132	(71)	90	(78)	42	(60)
Exercise related pain	70	(38)	44	(38)	26	(37)
Nausea	76	(41)	46	(40)	30	(43)
Diarrhea	49	(26)	25	(22)	24	(34)
Smoking	132	(71)	81	(70)	51	(73)
Family history CVD	80	(43)	49	(42)	31	(44)
CVD	78	(42)	53	(46)	25	(36)
Abdominal bruit	41	(22)	32	(28)	9	(13)
BMI (kg/m^2)*	21.9	(19.1-25.7)	22.2	(19.0-25.0)	21.3	(19.4 -26.0)
Classical triad	24	(13)	19	(16)	5	(7)
Stenosis	108	(58)	89	(77)	19	(27)
Abnormal tonometry	123	(66)	107	(92)	16	(23)

n (%) unless indicated otherwise;

* values are median (25-75 percentile); data calculated only in patients with weight loss

BMI = body mass index; CVD = cardiovascular disease; CGI = chronic upper gastrointestinal ischemia; Classical triad = combination of postprandial pain, weight loss and presence of abdominal bruit

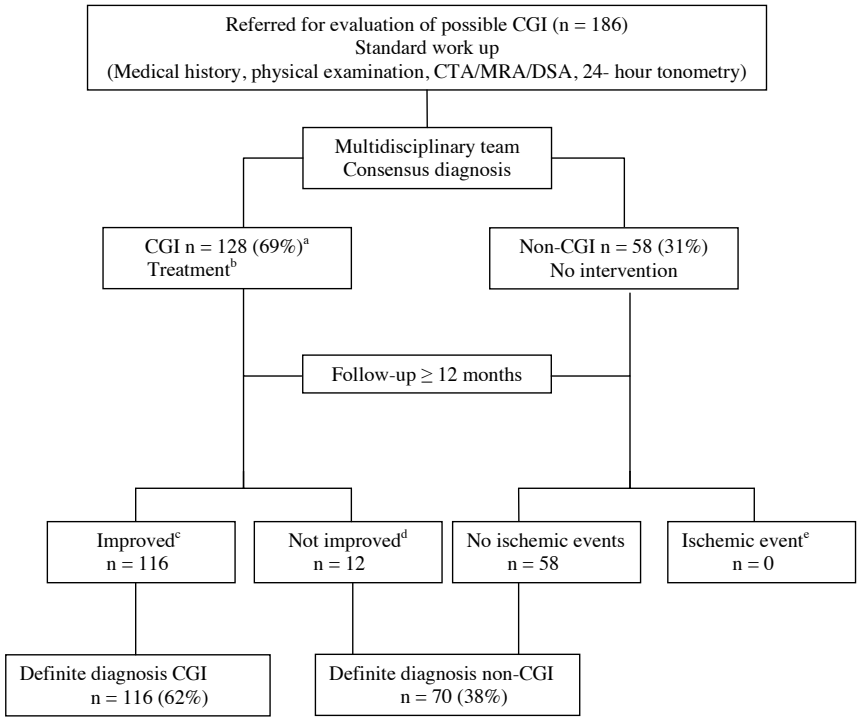


Figure 1: Flow-chart of the study

CGI = Chronic gastrointestinal ischemie;
CTA = computed tomography angiography;
MRA = magnetic resonance angiography;
DSA = digital subtraction angiography;
TM = 24-hours gastric and jejunal tonometry;
a 99 (77%) with occlusive CGI; 29 (23%) with non-occlusive CGI
b Occlusive CGI: intervention: n = 98; stent placement (n = 75), in 4 patients this was followed by surgery and in 5 patients no stenosis was seen during angiography and these patients were treated as non-occlusive ischemia); surgery (n = 23); conservative treatment in 1 patient
Non-occlusive CGI; vasodilating agents (n = 24); conservative treatment (n = 10)
c Symptoms improvement; stent placement 62/66, surgery 26/27; vasodilating agents 21/24; other 6/10
d Symptoms unchanged despite adequate treatment, 12
e Non ischemia related morbidity and mortality occurred during the follow-up
A definite diagnosis of CGI was made if a patient was free of symptoms after adequate therapy for at least 12 months of follow-up; A definite diagnosis of non-CGI was made if a patient was not diagnosed or hospitalized with ischemia related morbidity or mortality after follow-up

After a median follow-up of 21 (range 12 – 44) months, the *definitive diagnosis CGI* was confirmed in 116 patients (62% of total, 91% of initial CGI group). Relief from postprandial pain was achieved in 93% of patients with occlusive CGI and 88% of patients with non-occlusive CGI. After treatment, weight gain or weight stabilization occurred in 93% and 74% of patients with occlusive and non-occlusive CGI, respectively. Five patients, who received endovascular or surgical revascularization, did not experience symptom improvement during follow-up despite technical successful revascularization: three of these patients were treated for single vessel stenosis, and two for multivessel stenosis. In seven patients who were treated for non-occlusive CGI symptoms did also not improve. Therefore, in total 12 (9%) patients switched from the diagnosis CGI tot non-CGI after follow-up. (Figure 1).

Follow-up in these patients showed spontaneous improvement in three patients, a diagnosis of Giardia infection in one, celiac disease in one, and *Helicobacter pylori* gastritis in one patient, which were all successfully treated. In five patients symptoms persisted without an alternative diagnosis on follow-up. One patient was lost in follow-up. Moreover, during follow-up in none of the patients with the consensus diagnosis non-CGI, ischemia related morbidity was noted. The *definitive diagnosis* occlusive CGI was diagnosed in 89 (77%) and non-occlusive

Table 2: Patient characteristics of CGI patients with occlusive and non-occlusive disease.

Patient characteristics	**CGI**			
	Occlusive		**Non-occlusive**	
	n = 89		n = 27	
Age (years)*	63	(51-74)	63	(45-71)
Male gender	33	(37)	9	(33)
Reporting weight loss	65	(73)	22	(82)
Weight loss (kg/month)*	1.2	(0.7-3.0)	1.0	(0.4-2.4)
Postprandial pain	68	(76)	22	(82)
Exercise related pain	30	(34)	14	(52)
Nausea	34	(38)	12	(44)
Diarrhea	20	(23)	5	(19)
Smoking	64	(72)	17	(63)
Family history CVD	36	(40)	13	(48)
CVD	40	(45)	13	(48)
Abdominal bruit	28	(32)	4	(15)
BMI (kg/m^2)*	22.0	(19.0-25.0)	22.5	(18.9-25.6)
Classical triad	16	(18)	3	(11)
Stenosis	89	(100)	0	
Abnormal tonometry	80	(90)	27	(100)

n (%) unless indicated otherwise;
* values are median (25-75 percentile); data calculated only in patients with weight loss
BMI = body mass index; CVD = cardiovascular disease; CGI = chronic upper gastrointestinal ischemia;
Classical triad = combination of postprandial pain, weight loss and presence of abdominal bruit;
** two-tailed significance

Table 3: Univariable association of patient's characteristics and test results with presence of CGI.

Patient characteristics	Coding	CGI		Non-CGI		OR*	95 % CI*
		n = 116		n = 70			
Age**	49-71 years	-		-		1.34	0.90 - 1.98
Gender	Female	74	(63%)	43	(37%)	1.11	0.60 – 2.04
	Male	42	(61%)	27	(39%)		
Weight loss	Yes	87	(65%)	46	(35%)	1.57	0.85 - 2.89
	No	29	(55%)	24	(45%)		
Weight loss**	0.7–2.5 kg/month	-		-		1.35	0.95-1.91
Postprandial pain	Yes	90	(68%)	42	(32%)	2.31	1.25 -4.26
	No	26	(48%)	28	(52%)		
Exercise related pain	Yes	44	(63%)	26	(37%)	1.03	0.56 - 1.91
	No	72	(62%)	44	(38%)		
Diarrhea	Yes	25	(51%)	24	(49%)	0.53	0.29 - 0.97
	No	91	(66%)	46	(34%)		
Nausea	Yes	46	(61%)	30	(39%)	0.88	0.47 - 1.62
	No	70	(64%)	40	(36%)		
Smoking	Ever smoked	81	(61%)	51	(39%)	0.88	0.48 - 1.63
	Never smoked	34	(64%)	19	(36%)		
Family history CVD	Yes	49	(61%)	31	(39%)	1.08	0.59 - 2.0
	No	57	(63%)	34	(37%)		
CVD	Yes	53	(68%)	25	(32%)	1.51	0.82 - 2.79
	No	63	(58%)	45	(42%)		
Bruit	Yes	32	(78%)	9	(22%)	2.58	1.40 - 4.76
	No	82	(58%)	59	(42%)		
BMI **	19.1 - 25.7 kg/m^2	-		-		0.93	0.59 - 1.47
Classical triad	Yes	19	(79%)	5	(21%)	2.55	0.91 – 7.16
	No	97	(60%)	65	(40%)		
Radiologic evaluation	Stenosis	90	(83%)	19	(17%)	9.29	4.69 - 18.42
	No stenosis	26	(34%)	51	(66%)		
Tonometry	Normal	3	(7%)	43	(93%)	28.56	11.22 -72.76
	Abnormal	107	(87%)	16	(13%)		

CGI = chronic upper gastrointestinal ischemia; BMI = body mass index; CVD = cardiovascular disease; Classical triad = combination of postprandial pain, weight loss and presence of abdominal bruit
* based on 5 completed datasets;
** continuous variable, odds ratios are shown for an increase in values equal to the interquartile range

CGI was diagnosed in 27 (23%) patients. Single vessel stenosis was present in 57 (64%) and multi vessel in 32 (36%) patients diagnosed with occlusive CGI. The characteristics of patients with occlusive and non-occlusive CGI were similar (Table 2). In 2 (7%) patients diagnosed with non-occlusive CGI, clinical significant cardiac dysfunction was diagnosed on additional cardiological evaluation. None of the patients with non-occlusive CGI presented with clinical gastrointestinal bleeding.

Univariable and multivariable analysis

Overall, 1.3% of required data were missing. This in particular pertained to information the timing of weight loss, presence or absence of a family history of cardiovascular disease, body mass index, and tonometry assessment. In patients presenting with clinically suspected "acute-on-chronic" clinical signs of gastrointestinal ischemia, tonometry was not performed; these patients had intervention without delay. The univariable associations of the clinical parameters, radiologic evaluation and tonometry with CGI are shown in Table 3. Postprandial pain (OR 2.3), weight loss (OR 1.6), an abdominal bruit (OR 2.6), a known medical history of cardiovascular disease, (OR ratio 1.5) and presence of the 'classical' triad (OR 2.6), were univariably strongly associated with presence of CGI. However, the combined 'classical' triad was only found in 16% of patients diagnosed with CGI. The multivariable analysis showed that the clinical features postprandial pain, weight loss in kg per month, concomitant cardiovascular disease, and presence of an abdominal bruit were the strongest predictors for the diagnosis CGI. These predictors were included in model A yielding a c-statistic of 0.69. Adding the results of radiologic imaging to this model (model B), improved the discriminative ability to a c-statistic of 0.83. Further addition of tonometry (model C) resulted in a further improvement of discriminative ability of the prediction model to a c-statistic of 0.93 (see Table 4). The ROC-curves related to the three models are shown in Figure 2. The shrinkage factors estimated with bootstrapping were 0.73, 0.91 and 0.92, for model A, model B and model C respectively. The optimism corrected c-statistic after bootstrapping was 0.62, 0.81 and 0.90 for model A, model B and model C, respectively. An alternative model was fitted including the clinical features and results of tonometry, which also resulted in a high discriminative ability of the model (c-statistic of 0.88 after correction for optimism).

Table 4: Multivariable associations between predictors and presence of CGI.

Predictors	Model A			Model B			Model C		
	ß	OR	95 % CI	ß	OR	95 % CI	ß	OR	95 % CI
Postprandial pain	0.89	2.43	1.23 – 4.80	1.02	2.76	1.26 – 6.05	1.45	4.25	1.63 – 11.10
Weight loss (kg/month)	0.17	1.19	0.96 – 1.47	0.17	1.19	0.94 – 1.50	0.29	1.34	0.94 – 1.92
Bruit	0.81	2.24	0.98 – 5.13	0.28	1.33	0.52 – 3.39	0.51	1.67	0.46 – 6.00
CVD	0.46	1.59	0.83 – 3.02	0.47	1.59	0.76 – 3.33	0.11	1.12	0.44 – 2.82
Stenosis				2.21	9.19	4.44 – 19.00	1.65	5.20	2.06 – 13.14
Abnormal tonometry							3.78	43.66	12.52 –152.19
Intercept	-0.66			-1.82			- 4.61		
c- statistic*	**0.62**			**0.81**			**0.90**		

OR = odds ratio; CI = confidence interval; CGI = chronic gastrointestinal ischemia; CVD = cardiovascular disease; ß = regression coefficient;
* after correction for optimism

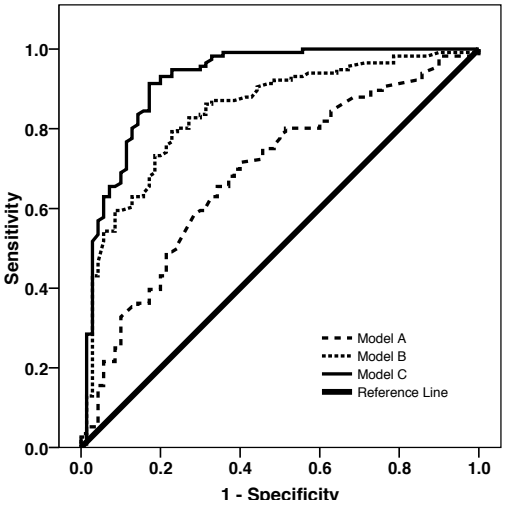


Figure 2 ROC-curves of the three prediction models, sensitivity and specificity at cut-off point 70% is indicated for the three models

We developed a diagnostic model only comprising of the predictors from the 'classical' triad (alternative model A) showing a weak discriminative ability for the diagnosis of CGI (c-statistic 0.63). Besides, we also developed alternative models B and C that included radiologic evaluation as no, single, or multivessel stenoses. The alternative models B and C showed c-statistics of 0.82 and 0.91 after correction for optimism.

DISCUSSION

We assessed the value of medical history, physical examination, vascular and functional assessment for the diagnosis of CGI in a large group of patients suspected for CGI. The diagnostic value of clinical features was limited. Adding radiological evaluation and gastrointestinal tonometry substantially improved the accuracy of diagnosis. This in particular pertained to functional testing by means of gastrointestinal tonometry.

The univariable analysis conducted in our prospective cohort demonstrated that postprandial pain, weight loss in kg per month, an abdominal bruit, the presence of the 'classical' triad, and a known medical history of cardiovascular disease, had strong association with presence of CGI. Earlier studies have shown that postprandial pain and weight loss are the most prevalent symptoms in CGI patients.[1, 3, 7] Hence, symptoms earlier described as 'atypical' for CGI, i.e. exercise related pain, nausea and diarrhoea, are indeed not predictive for the correct diagnosis. However, the overall capacity to correctly predict the presence or absence of CGI on clinical features is

only limited. As earlier described the clinical signs of CGI may vary among patients with CGI. For instance, the 'classical triad of angine abdominal', i.e. a combination of postprandial epigastric pain, loss of weight caused by fear of eating, and an epigastric bruit, is only present in a minority of patients such as in 16% of our cohort.[8, 22] The "classical triad" is strongly predictive of CGI, but the absence of the triad does not rule out CGI. Similarly, a diagnostic model only comprising of the predictors from the 'classical' triad had a weak discriminative ability for the diagnosis of CGI. In daily clinical practice, a history of abdominal angina and physical examination alone cannot be used to diagnose, or rule out CGI.

Postprandial pain is one the most prevalent symptoms in patients with CGI. In daily clinical practice, different types of postprandial can be identified. However, because of the fact that multiple clinical features might represent CGI, we decided to use postprandial pain as a 'simplified' and clear clinical symptom, guided by outcomes of earlier research. Postprandial pain 'in general' is a common symptom, i.e. in functional disorders as gastroparesis, but in these cases this is often not combined with significant weight loss and / or stenosis of at least one of the gastrointestinal arteries. In our opinion, postprandial pain as a "simplified" clinical symptom still represents a strong clinical predictor of CGI. The majority of CGI patients in our cohort are female. This is however in line with earlier reports of larger cohorts of CGI patients which show that between 60 and 70% of patients are female. [3, 6-7, 23]

Radiological evaluation and gastrointestinal tonometry improved the diagnostic value substantially. Especially the addition of gastrointestinal tonometry, i.e. a functional test for mucosal perfusion, increases the ability to discriminate between patients with and without CGI. Moreover, we developed alternative models B and C using the presence of no stenosis, single- or multivessel stenosis of the gastrointestinal arteries after radiologic evaluation. These alternative models seem to have comparable discriminative ability for diagnosis of CGI with original model B and C, where the presence or absence of gastrointestinal arterial stenosis was used as a solely predictor.

The results of our study show that combining clinical features with functional testing by means of gastrointestinal tonometry approximates the diagnostic value of the combination of clinical features, radiological imaging and tonometry (c-statistics of 0.88 and 0.90 in the models, respectively). This finding show that tonometry can be used prior to radiological imaging in patients suspected of CGI The advantage of an initial functional test for mucosal perfusion is that potential complications of radiological imaging, such as contrast allergy, exposure to radiation, and renal toxicity can be avoided in a proportion of patients. In patients with confirmed pathological mucosal perfusion, radiological imaging is needed to distinguish between occlusive and non-occlusive disease to determine accurate treatment. In addition, cardiological (re-) evaluation of patients with the diagnosis non-occlusive CGI

seems important, as in 7% of these patients significant cardiac dysfunction was diagnosed. An earlier published study already showed that gastrointestinal hypoperfusion is one of the first signs related to decreased cardiac output caused by cardiac valve disease and / or heart failure, which can be detected by GI tonometry.[24]

The present study has several limitations. First, the reference diagnosis was not blinded for predictors. In fact, the test results that were used to derive the consensus diagnosis, were also studied for their association with CGI. However, the consensus diagnosis was only used for baseline treatment decisions, whereas the reference diagnosis was defined as persistent clinical response during long-term follow-up after adequate treatment. Moreover, a definite diagnosis of non-CGI was made if a patient was not diagnosed or hospitalized with ischemia related morbidity or mortality after at follow-up. From the 58 patients who had the consensus diagnosis of non-CGI, none of them suffered from ischemia related morbidity or mortality during the follow-up. The consensus diagnosis CGI was made in 128 patients, in 12 patients the consensus diagnosis CGI was changed in non-CGI as the reference diagnosis (false positive result of 9%) as in these patients this diagnosis was not confirmed. These data showing an acceptable low false negative and false positive results, and is comparable with earlier published results using this work-up.[9, 11, 25] Secondly, the number of patients is relatively small for prediction research, although our cohort with 116 CGI patients is the largest CGI cohort reported in literature so far. Against this background, we consider this study a first analysis of diagnostic test performance for CGI. Our model should be validated in other cohorts. Thirdly, most clinicians will only consider CGI after exclusion of a variety of other conditions, and often only if the classical triad of postprandial pain, weight loss, and an abdominal bruit are present. Thus, patients evaluated for CGI generally represent a highly selected population. This also pertains to our patients, although we do have a somewhat different setting. Our medical center is a tertiary referral center for a large region in the Netherlands, covering approximately 4.4 million inhabitants. During the past 6 years we have set up a referral program for patients suspected of CGI. The awareness among referring clinicians and the clinical expertise of our program lower the threshold for referral and CGI evaluation, and thus reduce the selection process prior to referral. A considerable amount of patients with unexplained postprandial and/or abdominal pain, with or without weight loss referred from this region by general practitioners, medical specialists or other departments from the same medical center were considered eligible for evaluation of CGI. Nevertheless, not all patients in our region with these symptoms have been included, some might have been missed, and therefore a selection bias has to be taken into account.

In conclusion, clinical features alone have limited value to correctly diagnose CGI. Radiological imaging of the gastrointestinal arteries and a functional test, such as gastrointestinal tonometry, substantially improve correct diagnosing and allow good to excellent case detection in populations at risk.

REFERENCES

1. Kruger AJ, Walker PJ, Foster WJ, Jenkins JS, Boyne NS, Jenkins J. Open surgery for atherosclerotic chronic mesenteric ischemia. J Vasc Surg 2007;46:941-5.
2. Kolkman JJ, Bargeman M, Huisman AB, Geelkerken RH. Diagnosis and management of splanchnic ischemia. World J Gastroenterol 2008;14:7309-20.
3. Mensink PB, van Petersen AS, Geelkerken RH, Otte JA, Huisman AB, Kolkman JJ. Clinical significance of splanchnic artery stenosis. Br J Surg 2006;93:1377-82.
4. Brandt LJ, Boley SJ. AGA technical review on intestinal ischemia. American Gastrointestinal Association. Gastroenterology 2000;118:954-68.
5. Fioole B, van de Rest HJ, Meijer JR, van Leersum M, van Koeverden S, Moll FL, van den Berg JC, de Vries JP. Percutaneous transluminal angioplasty and stenting as first-choice treatment in patients with chronic mesenteric ischemia. J Vasc Surg 2009.
6. Sarac TP, Altinel O, Kashyap V, Bena J, Lyden S, Sruvastava S, Eagleton M, Clair D. Endovascular treatment of stenotic and occluded visceral arteries for chronic mesenteric ischemia. J Vasc Surg 2008;47:485-491.
7. Oderich GS, Bower TC, Sullivan TM, Bjarnason H, Cha S, Gloviczki P. Open versus endovascular revascularization for chronic mesenteric ischemia: risk-stratified outcomes. J Vasc Surg 2009;49:1472-9 e3.
8. van Bockel JH, Geelkerken RH, Wasser MN. Chronic splanchnic ischaemia. Best Pract Res Clin Gastroenterol 2001;15:99-119.
9. Otte JA, Geelkerken RH, Oostveen E, Mensink PB, Huisman AB, Kolkman JJ. Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2005;3:660-6.
10. Hansen KJ, Wilson DB, Craven TE, Pearce JD, English WP, Edwards MS, Ayerdi J, Burke GL. Mesenteric artery disease in the elderly. J Vasc Surg 2004;40:45-52.
11. Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. Dig Dis Sci 2008;53:133-9.
12. Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Effect of various test meals on gastric and jejunal carbon dioxide: A study in healthy subjects. Scand J Gastroenterol 2006;41:1290-8.
13. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat Med 1991;10:585-98.
14. Schafer JL, Graham JW. Missing data: our view of the state of the art. Psychol Methods 2002;7:147-77.
15. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol 2006;59:1087-91.
16. Team RDC. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing, 2006.
17. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med 1996;15:361-87.
18. Steyerberg EW, Eijkemans MJ, Harrell FE, Jr., Habbema JD. Prognostic modeling with logistic regression analysis: in search of a sensible strategy in small data sets. Med Decis Making 2001;21:45-56.

19. McGinn TG, Guyatt GH, Wyer PC, Naylor CD, Stiell IG, Richardson WS. Users' guides to the medical literature: XXII: how to use articles about clinical decision rules. Evidence-Based Medicine Working Group. Jama 2000;284:79-84.
20. Steyerberg EW, Harrell FE, Jr., Borsboom GJ, Eijkemans MJ, Vergouwe Y, Habbema JD. Internal validation of predictive models: efficiency of some procedures for logistic regression analysis. J Clin Epidemiol 2001;54:774-81.
21. Harrell FE, Jr., Lee KL, Califf RM, Pryor DB, Rosati RA. Regression modelling strategies for improved prognostic prediction. Stat Med 1984;3:143-52.
22. Geelkerken RH, van Bockel JH, de Roos WK, Hermans J, Terpstra JL. Chronic mesenteric vascular syndrome. Results of reconstructive surgery. Arch Surg 1991;126:1101-6.
23. Schermerhorn ML, Giles KA, Hamdan AD, Wyers MC, Pomposelli FB. Mesenteric revascularization: management and outcomes in the United States, 1988-2006. J Vasc Surg 2009;50:341-348 e1.
24. Krack A, Richartz BM, Gastmann A, Greim K, Lotze U, Anker SD, Figulla HR. Studies on intragastric PCO2 at rest and during exercise as a marker of intestinal perfusion in patients with chronic heart failure. Eur J Heart Fail 2004;6:403-7.
25. Otte JA, Geelkerken RH, Huisman AB, Kolkman JJ. What is the best diagnostic approach for chronic gastrointestinal ischemia? Am J Gastroenterol 2007;102:2005-10.

Chapter 3

Endoscopic visible light spectroscopy: a new, minimally invasive technique to diagnose chronic gastrointestinal ischemia

Désirée van Noord[1], Aria Sana[1], David A. Benaron[2], Peter M.T. Pattynama[3], Hence J.M. Verhagen[4], Bettina E. Hansen[1, 5], Ernst J. Kuipers[1,6], Peter B.F. Mensink[1]

Departments of Gastroenterology and Hepatology[1], Intervention Radiology[3], Vascular Surgery[4], Biostatistics[5], Internal Medicine[6], Erasmus MC - University Medical Center, Rotterdam, The Netherlands, Stanford University School of Medicine, Palo Alto, CA, USA[2]

Gastrointestinal Endoscopy 2011; 73 (2): 291-8

ABSTRACT

Background: The diagnosis of chronic gastrointestinal ischemia (CGI) remains a clinical challenge. Currently, there is no single and simple test with a high sensitivity available. Visible light spectroscopy (VLS) is a new technique that non-invasively measures mucosal oxygen saturations during endoscopy.

Objective: The diagnostic accuracy of VLS for detection of ischemia in a large cohort of patients.

Design: Prospective study, adherence to the Standards for reporting of diagnostic accuracy.

Setting: Tertiary referral center.

Patients: Consecutive patients referred for evaluation of possible CGI.

Interventions: Patients underwent VLS next to the standard work-up consisting of evaluation of symptoms, gastrointestinal tonometry and abdominal CT- or MR-angiography.

Main outcome measures: VLS measurements and the diagnosis CGI as established with the standard work-up.

Results: In 16 months, 121 patients were included: 80 in a trainee data set, followed by 41 patients in a validation data set. CGI was diagnosed in 89 (74%) patients. VLS cut-off values were determined based on the diagnosis CGI, and applied in the validation data set and the results compared to the gold standard, resulting in a sensitivity and specificity of VLS of 90% and 60%. Repeated VLS measurements showed improvement in 80% of CGI patients after successful treatment.

Limitations: Single center study, only 43% of patients had repeated VLS measurements after treatment.

Conclusions: VLS during upper endoscopy is a promising, easy to perform, minimally invasive technique to detect mucosal hypoxemia in patients clinically suspected for CGI, showing excellent correlation with the established ischemia work-up.

BACKGROUND AND AIMS

The diagnosis of chronic gastrointestinal ischemia (CGI) remains a challenge in clinical practice. For a long time, CGI was considered to be a very rare disease, only presenting in patients with multiple stenotic abdominal arterial disease. The introduction of gastrointestinal tonometry (TM) as the first functional test and one of the major keys in diagnosing CGI, has changed this view. Several studies with TM have shown that CGI is a clearly identifiable disease identity which can occur both in the presence of multi-vessel as well as single vessel abdominal arterial stenosis (1-3). Currently, a combination of clinical signs, radiological evaluation of abdominal arterial vascular anatomy and a functional test (TM) is the proposed diagnostic work-up in this particular patient group (1, 3, 4). However, TM is only used in a limited number of dedicated centers with a CGI program, which means that the majority of potential CGI patients are still assessed without functional testing. Unfortunately, the wider use of gastrointestinal TM is hampered by its cumbersome and invasive nature.

Visible light spectroscopy (VLS) is a relatively new technique that enables non-invasive measurements of mucosal capillary hemoglobin oxygen saturations during endoscopy (5). The technique uses white light delivered by a fiberoptic probe via the endoscope to directly measure intra-mucosal hemoglobin saturation, relying on the marked difference in absorption spectra of oxygenated and deoxygenated hemoglobin. This saturation reflects the adequacy of mucosal perfusion and should therefore, in theory, be lowered in CGI. VLS could be of great value as a new and less invasive diagnostic tool in patients suspected of CGI, and a recent pilot study using VLS in a few CGI patients showed promising results (6). We therefore prospectively evaluated the diagnostic accuracy of VLS for detection of ischemia in a large cohort of patients clinically suspected for CGI, and evaluated by means of the 'gold standard' proposed diagnostic work up.

METHODS

Consecutive patients which were referred for evaluation of possible CGI to the department of Gastroenterology and Hepatology of a tertiary care center (Erasmus MC - University Medical Center) were asked to participate in the current study and prospectively included after informed consent. In all patients more common causes of upper gastrointestinal symptoms had been previously excluded by upper endoscopy, colonoscopy, abdominal ultrasound and / or CT- or MRA. Suspicion for CGI was defined as fulfilling at least two of the following criteria: 1) presence of postprandial pain, 2) otherwise unexplained weight loss (> 5%), and / or 3) significant stenosis of at least one of the gastrointestinal arteries on previous radiological evaluation. The potential value of VLS as a diagnostic test and the cut-off values

were determined in a trainee data set and these established cut-off values were validated in a validation data set. Mucosal saturation measurements during VLS were compared with the diagnosis of CGI. The study was approved by the Institutional Review Board of the Erasmus MC- University Medical Center. For this diagnostic study, we adhered to the Standards for reporting of diagnostic accuracy (STARD) Initiative (7).

Standard diagnostic work-up

Prolonged (24 hour) gastric and jejunal TM in combination with CTA or MRA to visualize the gastrointestinal arteries (celiac artery (CA), superior mesenteric artery (SMA) and inferior mesenteric artery (IMA)) were performed as standard diagnostic work-up. TM was performed using a standardized protocol, as described previously, enabling mucosal CO_2 measurements both in fasting and postprandial state with gastric and jejunal catheters (1). A significant stenosis of the abdominal arteries was defined as a luminal reduction of >70 %. Medical history, symptoms, physical exam, and the results of all diagnostic procedures except the VLS readings were discussed in a multidisciplinary team consisting of a vascular surgeon, intervention radiologist and gastroenterologist, all specialized in CGI, and a consensus diagnosis was made, classifying individual patients as either having (1) no arterial stenosis, no ischemia, (2) arterial stenosis, no ischemia, (3) non-occlusive mesenteric ischemia (NOMI) (4) occlusive ischemia. The diagnosis NOMI and occlusive gastrointestinal ischemia, codes (3) and (4), were together defined as CGI. The multidisciplinary team was blinded to the results of the VLS measurements, and thus based the consensus diagnosis on the combination medical history, physical examination, angiography, and TM. The team was aware of the fact that each of these parameter could be associated with both false-negative and false–positive findings of TM and for reason based the consensus diagnosis on the total presentation. For the purpose of this study, we used both the baseline consensus working diagnosis, and also the follow-up history into account. Patients with occlusive CGI were offered revascularization of the vascular obstruction by either open surgery or by endovascular stent placement. Patients diagnosed with NOMI were offered medical treatment with vasodilation therapy, following a strict protocol using isosorbidedinitrate or ketanserin tartrate, in a maximum dose of 40 mg od. A definitive diagnosis of CGI was made after persistent relief of symptoms on follow-up after intervention or medical therapy.

Upper endoscopy and visible light spectroscopy measurements

VLS was performed during upper endoscopy, with continuous monitoring of peripheral oxygen saturation and heart rate. In standard fashion, midazolam intravenously (dose 2.5-5 mg), if necessary combined with fentanyl (0.05 mg), was used for conscious sedation. Furthermore, butylscopolamin (20 mg) was administered intravenously before start of VLS measurements to prevent luminal spasms. Peripheral saturation was kept above 94% and oxygen was administered intra-nasally if necessary to maintain this saturation level during

the VLS measurements. Measurements were made using a fiberoptic catheter-based visible light spectroscopy oximeter (T-Stat 303 Microvascular Oximeter, Spectros, Portola Valley, California, USA). This catheter was passed through the accessory channel of the endoscope after irrigation of the target area to remove any bile remnants. The probe was positioned approximately 1 to 5 mm above the mucosa (Figure 1). Similar to peripheral external saturation measurements, the reading showed small rapid variations consistent with true changes in saturation as well as reader variation due to small changes in the position of the probe. For VLS measurements, the probe was positioned close, perpendicular to the mucosa under direct saturation measurement. The actual measurement started once a stable reading was obtained with less than 5% variation in panel read-out. We then averaged three repeated readings per site as actual, most accurate reflection of mucosal saturation at that site. This was repeated at five different locations: descending duodenum, duodenal bulb, antrum, corpus and distal esophagus. The latter five locations were standardized in the way that in every patient the same anatomical locations were used. Subsequently, tonometer catheters were inserted in order to perform TM. The VLS technique measures the percent saturation of hemoglobin in the mucosa, relying on the marked difference in the absorption spectra of oxyhemoglobin and deoxyglobin (6, 8). With a lower total hemoglobin, the balance between oxyhemoglobin and deoxyglobin remains the same. All VLS measurements were performed by the same gastroenterologist (PM) who was kept blinded for the actual readings. The VLS measurements were noted and analysed by a research fellow (DvN). Both, the gastroenterologist and research fellow were unaware of and blinded to radiologic and TM results.

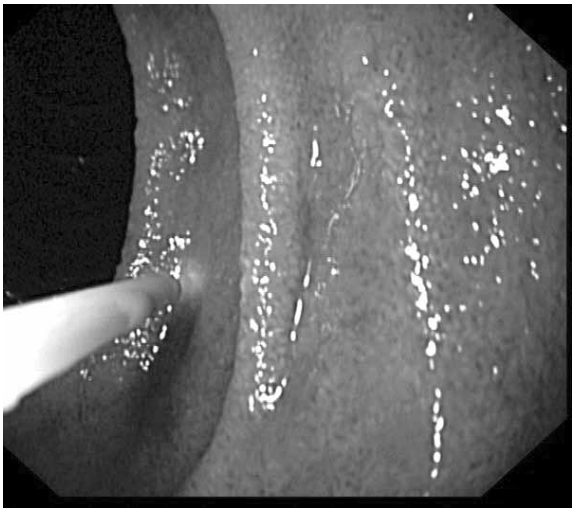


Figure 1. VLS measurements using a fiberoptic catheter-based visible light spectroscopy oximeter. The catheter is passed through the accessory channel of the endoscope and positioned approximately 1 to 5 mm above the mucosa.

Follow-up and repeated VLS measurements

All patients diagnosed with ischemia visited the out-patient clinic at six weeks, three months, six months and one year after treatment for assessment of clinical status and repeated duplex ultrasound scanning of the gastrointestinal arteries. Patients diagnosed as not having ischemia, diagnosis code (1) and (2), had no intervention and were discharged from follow-up. Follow-up of the latter patients was however obtained by means of a survey which was conducted by contacting the primary care or referring physician 12 months after diagnostic evaluation.

All patients treated for CGI were asked to have repeated upper endoscopy with VLS measurements between 6 - 12 months after treatment. The repeated VLS measurements were performed following the same protocol as described earlier. The results of repeated VLS measurements were defined 'normalized' when mucosal saturation(s) were ≥ cut-off levels. The endoscopist (PM) and the research fellow (AS) noting and analyzing the repeated VLS results, were both blinded for the outcome after therapy.

Statistical analysis

In the 'trainee data set' the mean saturations between CGI patients and non-CGI patients and between single and multi-vessel CGI patients were compared with Student T-test. Test performances at different levels were investigated, and for each cut-off, the positive (PPV) and negative predicted value (NPV), as well as the sensitivity and the specificity for diagnosing CGI were calculated and in addition the odds ratio and the c-statistics were estimated. The c-statistics is a measure of discrimination, in this case the ability to distinguish patients with CGI from those without. The c-statistics is in our case equal to the area under the receiver-operating characteristic curve (AUC), a perfect discrimination is indicated by AUC=1 and a poor discrimination is indicated by an AUC equal to or smaller than 0.5. Logistic regression analysis was used to combine these into one useful guiding rule. Saturations were determined per location; patients with missing saturations were excluded from analysis with relation to that location as described in the tables. In case of missing saturation measurements, the impact of missing data was studied comparing the patient characteristics between patients with missing and without missing saturation measurements with either a chi-2 test or Student T-test. Statistical analysis was performed using the SPSS 16.0 program (SPSS Inc. Chicago, IL). A P-value <0.05 was considered statistically significant (all two-tailed). The diagnostic accuracy of the established cut-off values was validated in the 'validation data set' group. The same tests were used as described above.

RESULTS

During a period of 16 months (December 2007- March 2009), 131 patients were referred for evaluation of possible CGI. Ten patients refused informed consent and were therefore excluded from further evaluation. So, 121 patients were included in the present study. The first 80 patients were included in the trainee data set en the next 41 patients in the validation data set. CGI was diagnosed in 58 (73%) patients in the trainee data set and in 31 (76%) patients in the validation data set, see Table 1 for patient characteristics and presenting symptoms. The proportion of patients with ischemia was not significantly different before and after exclusion of patients with missing saturation measurements. No adverse events occurred during both the standard diagnostic work-up and the VLS measurements. In one patient within the trainee data set, VLS measurements were not possible because of agitation during endoscopy.

Endoscopy and VLS measurements

No patients had clear signs of endoscopic abnormalities compatible with acute gastrointestinal ischemia. During VLS measurements, mean peripheral oxygen saturation were comparable in both groups, in both groups 95.9% (ranges 95-100% in the trainee data set and 95-98% in the validation data set). In the total data set, the mean hemoglobin (8.3 mmol/l) in the non-ischemia group did not differ from the mean hemoglobin (7.9 mmol/l) in the ischemia group, p=0.31. The distribution of saturation levels did not diverge from a normal distribution (test for normality p=0.15). The mean saturation in the gastric corpus and antrum, as well as the duodenal bulb, and descending duodenum and the overall mean saturation from all five locations, were significantly decreased in CGI patients as compared to non-CGI patients in the overall patient group (n=121) (Table 2). Odds ratios (95% CI) and c-statistics (95% CI) are presented in Figure 2 and Table 3. Comparing VLS measurements in single and multi-vessel disease in the overall patient group (n=121), multi-vessel patients showed a significantly decreased saturation in the gastric corpus and antrum, as well as the duodenal bulb and the overall mean saturation (Table 4). The results of all patients (n=121) showed that patients without stenosis had significantly higher overall saturation levels (P=0.01), compared to patients with single vessel stenosis or multi-vessel stenosis, irrespective of the diagnosis gastrointestinal ischemia. In the ischemia patients, no significant differences were found comparing mucosal saturation levels in patients without stenosis, i.e. NOMI patients, with the stenotic single- or multi-vessel ischemia patients (P=0.40). A sensitivity analysis including all patients, including those with missing saturation measurements, did not change the estimates substantially.

Trainee data set and validation data set

Based on the significantly decreased saturation values in CGI patients in the trainee data set, cut-off saturation levels for ischemia were calculated for the antrum (63%), duodenal

Table 1. Patient characteristics and presenting symptoms, data given as numbers of patients (percentages) or mean (range).

	Trainee data set n=80	Validation data set N=41
Age (years)	59 (17-86)	61 (21-86)
Gender M/F	30/50	19/22
Postprandial pain	57 (71%)	29 (71%)
Exercise related pain	32 (40%)	17 (42%)
Diarrhea	20 (25%)	8 (20%)
Weight loss	61 (76%)	25 (61%)
Weight loss (kg)	7.5 (1-32)	7.6 (3-30)
BMI (kg/m^2)	22.3 (14.7-35.5)	22.8 (15-37.3)
Abdominal complaints	74 (93%)	41 (100%)
Duration of complaints (months)	25 (1-312)	23 (2-144)
Risk factors for CVD:		
• smoking	30 (38%)	20 (49%)
• other risk factors*	58 (73%)	21 (51%)
Ischemia	58 (73%)	31 (76%)
• Single vessel stenosis:	25 (43%)	20 (65%)
CA/SMA	21/4	16/4
• Multi-vessel stenosis:	21 (36%)	3 (10%)
CA+SMA /CA+IMA /SMA+IMA	13/1/1	0/2/0
CA+SMA+IMA	6	1
• NOMI	12 (21%)	8 (26%)

M=male, F=female, BMI=body mass index, CVD=cardiovascular disease, * including diabetes mellitus, obesity, hypertension, hyperlipidemia, hyperhomocysteinemia and familial history for cardiovascular disease, CA=celiac artery, SMA=superior mesenteric artery, IMA=inferior mesenteric artery, NOMI=non-occlusive mesenteric ischemia

Table 2. VLS mucosal saturation in different locations during upper endoscopy in patients with and without ischemia (mean (SD), in percentages), trainee data set (n=80), validation data set (n=41) and total data set (n=121).

		Distal esophagus	Corpus	Antrum	Duodenal bulb	Descending duodenum	Overall
Trainee data set	Ischemia	60.2 (4.0) n=58	62.1 (5.6) n=55	62.9 (5.4)* n=51	58.9 (5.2)* n=52	54.4 (6.7)* n=58	59.8 (3.4)* n=49
	No ischemia	62.3 (5.3) n=19	64.9 (3.4) n=18	65.6 (3.8)* n=20	62.6 (4.4)* n=20	59.2 (4.1)* n=21	62.8 (2.3)* n=16
Validation data set	Ischemia	63.1 (3.8) n=29	62.6 (3.8) n=31	64.2 (3.7) n=31	60.1 (4.2) n=28	53.1 (5.1)* n=31	60.7 (2.4) n=26
	No ischemia	64.3 (5.4) n=10	65.0 (6.3) n=10	66.8 (5.5) n=10	61.1 (6.3) n=9	58.3 (7.1)* n=10	62.9 (5.5) n=9
Total data set	Ischemia	61.2 (4.2) n=87	62.3 (5.0)* n=86	63.4 (4.8)* n=85	59.3 (4.9)* n=80	53.9 (6.2)* n=89	60.1 (3.1)* n=75
	No ischemia	63.0 (5.3) n=29	64.9 (4.5)* n=28	66.0 (4.4)* n=30	62.1 (5.0)* n=29	58.9 (5.2)* n=31	62.8 (3.7)* n=25

SD = standard deviation
*$P < 0.05$

bulb (62%) and descending duodenum (58%) (Figures 3A-C). Measurements were considered positive for ischemia when the measured saturation in the antrum, duodenal bulb or descending duodenum was lower than the cut-off value used in each location. There were no significant differences regarding presenting symptoms of patients between the data sets. If any of the 3 locations yielded a positive test, indicating ischemia, VLS measurements were classified pathological. Overall, pathological VLS measurements were found in 32 (78%) pa-

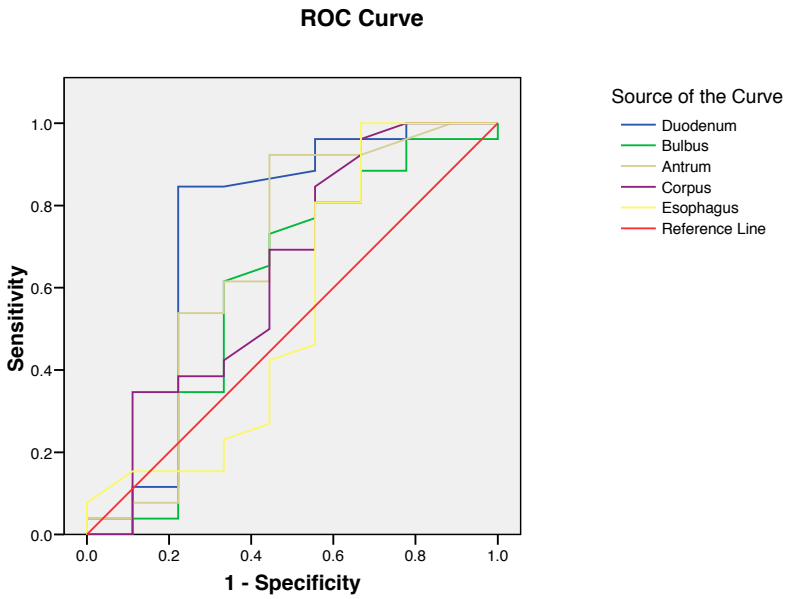


Figure 2. Receiver operating characteristic curve with VLS mucosal saturation in the different measurement locations.

Table 3. Odds for ischemia and c-statistics for ischemia in different locations, trainee data set (n=80), validation data set (n=41) and total data set (n=121).

		Distal esophagus	Corpus	Antrum	Duodenal bulb	Descending duodenum
Trainee data set	Odds ratio (95%CI)	0.89 (0.79-1.02)	0.89 (0.80-1.00)	0.89 (0.79-1.00)	0.84 (0.73-0.96)	0.85 (0.75-0.95)
	c-statistics (95%CI)	0.61 (0.45-0.77)	0.64 (0.51- 0.77)	0.65 (0.51-0.78)	0.70 (0.57-0.84)	0.72 (0.60-0.84)
Validation data set	Odds ratio (95%CI)	1.00 (0.99-1.01)	0.88 (0.75-1.05)	0.84 (0.68-1.03)	0.95 (0.81-1.13)	0.84 (0.71-0.98)
	c-statistics (95%CI)	0.58 (0.34-0.82)	0.64 (0.41-0.86)	0.71 (0.48-0.94)	0.61 (0.36-0.86)	0.77 (0.55-0.99)
Total data set	Odds ratio (95%CI)	0.91 (0.83-1.01)	0.89 (0.81-0.98)	0.88 (0.79-0.97)	0.88 (0.79-0.97)	0.84 (0.77-0.93)
	c-statistics (95%CI)	0.59 (0.46-0.72)	0.65 (0.53-0.76)	0.67 (0.55-0.78)	0.67 (0.55-0.80)	0.75 (0.65-0.85)

tients. Comparing VLS results with the diagnosis of ischemia based on previously established, above-specified criteria, the sensitivity and specificity of VLS measurement were 90% and 60%, with a PPV and NPV of 88% and 67%, respectively.

Follow-up and repeated VLS measurements

After a mean follow-up of 10 (range 1-32) months following intervention, 80 (90%) of the total 89 patients diagnosed with ischemia were free of symptoms. During follow-up, none of the patients with a non-CGI working diagnosis developed ischemia or died of ischemic complications, nor did any of these patients develop progressive symptoms compatible with CGI. Thirty-eight (43%) patients had repeated VLS measurements after treatment. The patient characteristics and clinical outcome after treatment were similar for the patients consenting and refusing repeated VLS measurements. Twenty-nine (76%) patients were free of symptoms, 7 (18%) had persistent symptoms, and 2 (6%) patients had recurrent symptoms

Table 4. VLS mucosal saturation in different locations during upper endoscopy in single- and multi-vessel stenosis patients (mean (SD), in percentages), trainee set (n=80), validation set (n=41) and total data set (n=121).

		Distal esophagus	Corpus	Antrum	Duodenal bulb	Descending duodenum	Overall
Trainee data set	Single vessel	59.2 (4.5) n=28	63.2 (5.2) n=26	65.1 (5.6)* n=25	59.7 (5.5) n=23	56.1 (5.6) n=29	60.6 (3.1) n=20
	Multi-vessel	60.4 (3.9) n=22	60.8 (5.8) n=20	61.4 (4.5)* n=21	57.7 (5.6) n=21	54.0 (7.4) n=22	59.0 (3.5) n=19
Validation data set	Single vessel	62.9 (4.3) n=23	62.7 (4.5) n=24	64.3 (3.6)* n=24	59.5 (4.3) n=24	53.0 (5.4) n=24	60.4 (3.2)* n=23
	Multi-vessel	63.5 (2.4) n=3	61.0 (4.7) n=3	58.9 (6.3)* n=3	58.9 (1.2) n=3	55.1 (5.2) n=3	59.6 (2.0)* n=3
Total data set	Single vessel	60.9 (4.7) n=51	62.9 (4.8)* n=50	64.7 (4.7)* n=49	59.6 (4.9)* n=46	54.7 (5.7) n=53	60.5 (3.1)* n=53
	Multi-vessel	60.8 (3.9) n=25	60.8 (5.6)* n=23	61.1 (4.6)* n=24	57.8 (5.4)* n=23	54.2 (7.1) n=25	58.9 (3.4)* n=25

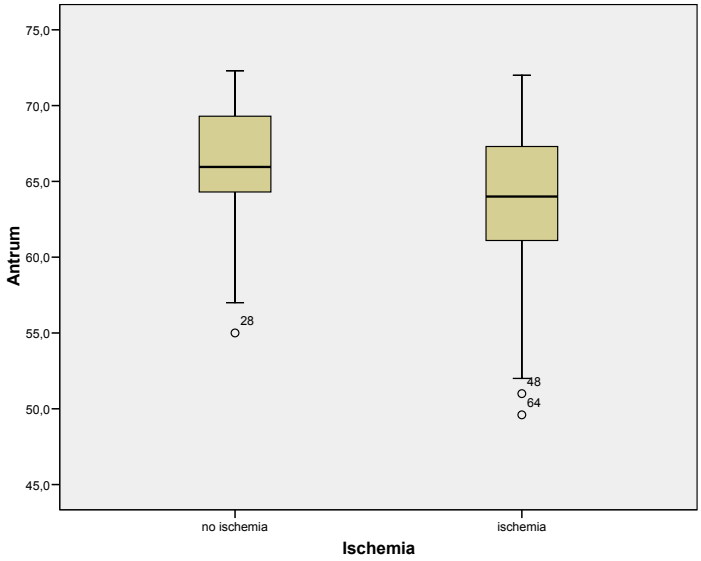
SD = standard deviation
*P < 0.05

after initial clinical success. Among the patients who had become symptom-free, repeated VLS measurements showed normalization in 19 (66%) patients, improvement in 4 (14%), and no change in 6 (20%) patients, compared to 0, 0 and 9 (100%) patients with persistent symptoms, respectively (P = 0.00).

DISCUSSION

Abdominal arterial stenosis is not uncommon and the widespread use of abdominal CT- and MR make clinicians with increasing frequency encounter these lesions. Nevertheless, the demonstration that an arterial stenosis is associated with CGI remains a clinical challenge (9). Single as well as multi-vessel disease often remains asymptomatic, due to the presence of abundant abdominal arterial collateral circulation. Only those patients with significant vascular stenosis in combination with insufficient collateral circulation will develop clinical ischemia (3, 10). Unfortunately, the diagnosis is in these cases often missed due to lack of sensitive diagnostic tests. The diagnosis of CGI and the subsequent decision for intervention can not be based on the results of an individual test. The diagnostic approach in patients referred for evaluation of possible CGI focuses on identification of abdominal arterial stenosis and demonstration of GI mucosal ischemia. At our institution, the current diagnostic strategy is a combination of radiological evaluation of vascular anatomy and functional testing by means of gastrointestinal TM to detect gastrointestinal mucosal ischemia. A diagnosis of presence or absence of CGI is then made in a dedicated multidisciplinary team, based on the combination of symptoms and medical history, and additional test results including gastrointestinal TM. However, despite its good sensitivity for diagnosing mucosal ischemia, TM is an invasive and cumbersome technique which is not generally accepted as regular diagnostic method and also requires 24 hour hospital admission.

The results of the current study show that VLS during upper endoscopy, measuring mucosal oxygenation in a resting situation, can be used as an alternative diagnostic test in this particularly difficult patient group. Our data show a similar or even higher sensitivity, with a lower specific-



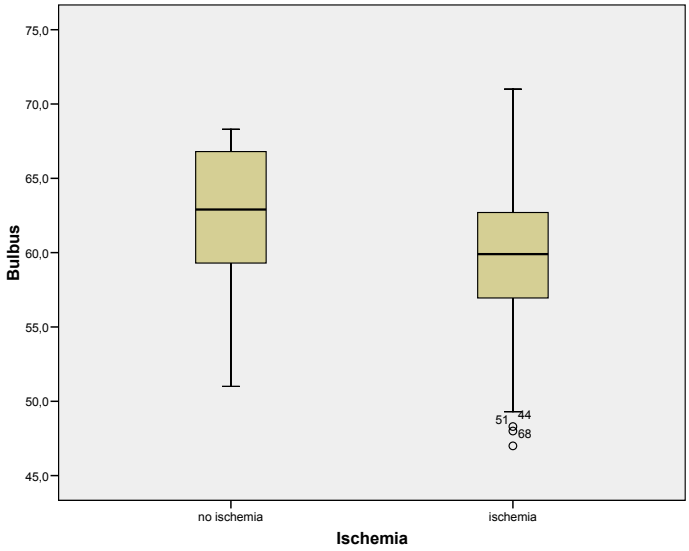


Figure 3. Stem-and-leaf plot for VLS measurements in ischemia and non-ischemia patients. Dots represent outliers.
A antrum (cut-off saturation level 63 %)
B duodenal bulb (cut-off saturation level 62 %)

ity, of VLS as compared to TM (1, 10). We have assessed the VLS performance in several ways: 1) compared the current proposed standard approach 2) by assessing VLS performance in a confirmation cohort, and 3) by showing improved VLS measurements after successful intervention. Normalization of mucosal oxygenation was seen during the repeated VLS measurements in majority of patients after successful treatment. The latter finding underlines even more that

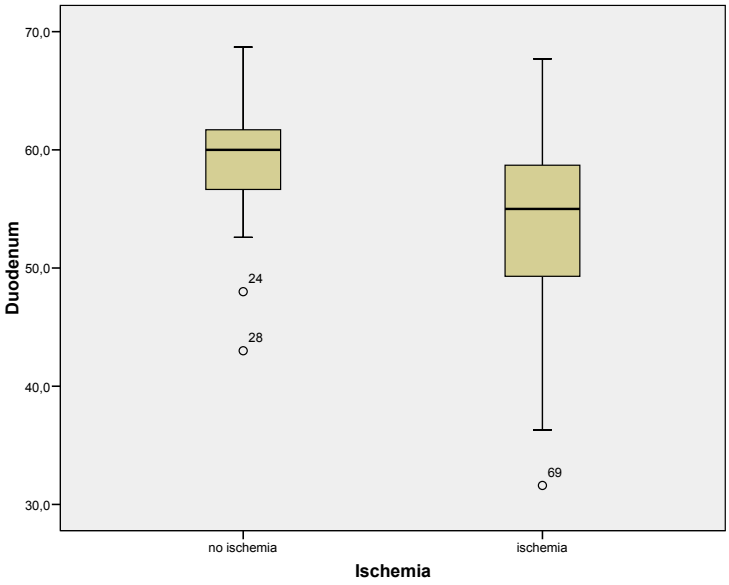


Figure 3 continued.
C descending duodenum (cut-off saturation level 58 %)

lowered VLS measurements are associated with clinically evident mucosal ischemia, which can be reversed by adequate treatment. The fact that VLS is easy to perform could lead to a change in approach in patients clinically suspected for CGI, which is likely to allow more institutions and gastroenterologists to consider and test for this condition. The follow up of patients without established ischemia showed that no patients with CGI had been missed. This is of the utmost clinical importance, as earlier studies have shown that undiagnosed and therefore untreated ischemia correlates with evident morbidity and mortality (3, 4).

In a pilot study using VLS during endoscopy to evaluate the diagnostic value of VLS in CGI patients, Friedland et al. presented three CGI patients with 3-vessel stenosis and substantially decreased mucosal oxygenation from 19 to 50% in the duodenum. Endovascular treatment of the gastrointestinal arteries resulted in improvement of mean mucosal oxygen saturation in these CGI patients from 51 to 64% on repeated VLS measurements. In the same study, 'normal' mucosal hemoglobin oxygen saturation values were presented, obtained from 30 patients. These 'normal' mucosal saturation values as presented in the latter study are similar to our findings in patients with patent gastrointestinal arteries and no CGI, i.e. diagnosis code 1, showing mean mucosal saturation values of 68 (56 – 83) and 64 (56 – 73), respectively in both studies (6).

The present study included 121 patients, clinically suspected of CGI and is therefore the first large cohort study using VLS for the diagnosis of mucosal ischemia. In the current study a significant lower saturation was shown in ischemia patients as compared to non-ischemia

patients in duodenum, antrum, corpus and overall mean saturation (in five locations). Only the distal esophagus did not show decreased saturation measurements in ischemia patients, which can be explained by the fact that the esophagus is not vascularized by one of the abdominal arterial arteries (TC, SMA or IMA). Overall mucosal saturation measurements were also decreased in patients with single vessel stenosis without ischemia, as compared to the 'normal values' presented by Friedland et al (6). We experienced a gradual lowered overall mucosal saturation level comparing the measurements in patients with no abdominal artery stenosis, single vessel and multi-vessel stenotic abdominal arteries irrespective of the diagnosis ischemia. In patients with ischemia, there were no differences in patients with occlusive and non-occlusive (NOMI patients) CGI. Collateral circulation is often affected in this area of the gastrointestinal tract, giving rise to abnormal functional testing which can be used for diagnosis (3). These findings support the theory that VLS indeed measures lowered oxygen saturations, indicating a lowered capacity of collateral abdominal circulation.

A limitation of our study was the fact that the gold standard was based on a multidisciplinary decision on standard work-up consisting of evaluation of symptoms, gastrointestinal TM and abdominal CT- or MR-angiography. To partially overcome this, a definitive diagnosis of CGI was made after persistent relief of symptoms on follow-up after intervention or medical therapy. This was a less-than-ideal gold standard, but currently most reliable way to establish a diagnosis. Another limitation of the present study is the possibility that mucosal ischemia is patchy and could be missed with repeated point measurements. Assessment of the gastroduodenal mucosa by means of a very large number of repeated point measurements might in theory increase the diagnostic yield of VLS, this remains to be established in future research. In the current setting, VLS performed similar to TM in defining patients with or without mucosal ischemia. Furthermore, the possibility remains that ischemia only occurs in response to increased metabolic demand, such as after a meal. All patients were fasting because of the nature of the test as performed during endoscopy. In theory, in patients with less pronounced blood flow impairment, VLS measurements might show low-normal or even normal mucosal saturation measurements in the resting situation. However, we demonstrate with our findings that VLS can also detect the less pronounced musocal blood flow reduction in this range. Postprandial VLS measurements, for example in stomach with jejunal feeding, would be of great interest and is considered for further research. Unfortunately, only in 43% of patients endoscopy and VLS measurements were repeated after treatment. However, the patient characteristics and clinical outcome of treatment were comparable in the patients who had and who did not have repeated measurements, in this way minimizing bias.

In conclusion, VLS measurement of mucosal oxygenation during endoscopy is a promising technique for detection of actual mucosal hypoxemia reflecting ischemia in patients suspected for chronic gastrointestinal ischemia. The technique is easy to perform, can be operated in any endoscopy unit and shows excellent correlation with the established diagnostic methods.

REFERENCES

1. Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. Dig Dis Sci. 2008 Jan;53(1):133-9.
2. van Noord D, Kuipers EJ, Mensink PB. Single vessel abdominal arterial disease. Best Pract Res Clin Gastroenterol. 2009;23(1):49-60.
3. Mensink PB, van Petersen AS, Geelkerken RH, Otte JA, Huisman AB, Kolkman JJ. Clinical significance of splanchnic artery stenosis. Br J Surg. 2006 Nov;93(11):1377-82.
4. Kolkman JJ, Bargeman M, Huisman AB, Geelkerken RH. Diagnosis and management of splanchnic ischemia. World J Gastroenterol. 2008 Dec 28;14(48):7309-20.
5. Benaron DA, Parachikov IH, Cheong WF, Friedland S, Rubinsky BE, Otten DM, et al. Design of a visible-light spectroscopy clinical tissue oximeter. J Biomed Opt. 2005 Jul-Aug;10(4):44005.
6. Friedland S, Benaron D, Coogan S, Sze DY, Soetikno R. Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. Gastrointest Endosc. 2007 Feb;65(2):294-300.
7. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Standards for Reporting of Diagnostic Accuracy. Clin Chem. 2003 Jan;49(1):1-6.
8. Friedland S, Soetikno R, Benaron D. Reflectance spectrophotometry for the assessment of mucosal perfusion in the gastrointestinal tract. Gastrointest Endosc Clin N Am. 2004 Jul;14(3):539-53, ix-x.
9. Hansen KJ, Wilson DB, Craven TE, Pearce JD, English WP, Edwards MS, et al. Mesenteric artery disease in the elderly. J Vasc Surg. 2004 Jul;40(1):45-52.
10. Otte JA, Geelkerken RH, Oostveen E, Mensink PB, Huisman AB, Kolkman JJ. Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol. 2005 Jul;3(7):660-6.

Chapter 4

Visible light spectroscopy in diagnosis of chronic gastrointestinal ischemia: results of a cohort study

A. Sana MD[1], L.M.G. Moons MD PhD[1,4], B. E. Hansen PhD[1,2], P. Dewint MD PhD[1], D. van Noord MD PhD[1] , P.B.F. Mensink MD PhD[1], Ernst J. Kuipers MD PhD[1,3]

Departments of Gastroenterology and Hepatology[1], Center for Medical Decision Sciences[2], Internal Medicine[3], Erasmus MC - University Medical Center, Rotterdam, The Netherlands
Department of Gastroenterology and Hepatology[4], University Medical Center Utrecht, The Netherlands

Submitted

ABSTRACT

Background: Chronic gastrointestinal ischemia (CGI) is more common than previously thought. The aim of this study was to evaluate the treatment response in patients with occlusive CGI who were selected for treatment by means of radiological imaging of the gastrointestinal arteries and visible light spectroscopy (VLS) measurement for detection of mucosal hypoxia. Moreover, we determined predictors of response to treatment in these patients.

Methods: Consecutive patients referred for evaluation of suspected CGI were prospectively included. All patients had an extensive diagnostic work up, including visualization of the gastrointestinal arteries, and assessment of mucosal perfusion by means of visible light spectroscopy (VLS). Treatment response was evaluated in patients with occlusive CGI. Predictors for positive response were assessed using multivariate logistic regression analysis.

Results: From November 2008 to January 2011, 212 were included: occlusive CGI was diagnosed in 107 (50%) patients, 96 (90%) of them were offered treatment. After a median follow-up of 13 months data concerning treatment response were available for 89 (93%) patients: 62 (70%) patients had sustained response during follow-up. Response rate was 64% in patients with single artery disease, and 80% of those with multi-artery disease (P = 0.151). Pre-treatment reported weight loss (OR 1.93), presence of an abdominal bruit (OR 2.36), and a corpus mucosal saturation level < 56% (OR 4.84) were the strongest predictors for a positive treatment response.

Conclusion: Treatment of CGI diagnosed by means of a multimodality approach provides a substantial long-term response rate. Weight loss, abdominal bruit, and low corpus mucosal saturation can be used to determine which patients benefit most from the treatment. This emphasizes the role of multimodality assessment of CGI, including VLS measurement to detect mucosal hypoxia, for optimal selection of patients eligible for treatment.

INTRODUCTION

Chronic abdominal symptoms are quite common, as are vascular stenoses of the gastrointestinal arteries. (1-5) Against this background, and in the absence of a single diagnostic test, the diagnosis of chronic gastrointestinal ischemia (CGI) can be challenging. Current diagnostic approaches include assessment of the medical history, imaging of the gastrointestinal arteries, and assessment of gastrointestinal mucosal perfusion by means of tonometry or visible light spectroscopy (VLS).(6, 7) The latter combined approach aims to make a proper diagnosis, and to select patients who will benefit from treatment.

We previously showed that medical history and physical examination were poor predictors for the presence of CGI. Addition of radiological evaluation and in particular functional testing by means of tonometry substantially improved the accuracy of diagnosis.(8) Despite its additional value, tonometry is not widely used because of its time consuming and invasive nature. VLS has recently been introduced as a new minimally invasive technique to detect mucosal hypoxia in patients clinically suspected of CGI.(9, 10)

VLS enables noninvasive measurements of mucosal capillary hemoglobin oxygen saturation during endoscopy.(9, 11) In a previous study, cut-off values for different locations in the stomach and duodenum were determined.(9) VLS measurement was shown to be a promising technique for detection of mucosal hypoxia in patients suspected of CGI(9), and its excellent correlation with tonometry indicates that VLS can be used instead of tonometry to detect mucosal ischemia.

The aim of this study was to evaluate the response to treatment, including response predictors, in a large, independent population of patients suspected of CGI and assessed by means of vascular imaging and VLS.

METHODS

All consecutive patients with a clinical suspicion of CGI referred to the Erasmus MC were included after informed consent, and prospectively followed. The Institutional Review Boards of the Erasmus Medical Center. The study accorded with the STROBE guidelines for reporting cohort studies. Only patients with written informed consent entered the present study. The Erasmus MC is a tertiary referral center with a catchment area of 4.4 million subjects with a dedicated CGI program, run by the collaborative departments of Gastroenterology and Hepatology, Interventional Radiology, and Vascular Surgery. In all patients, more common causes of upper abdominal complaints had been excluded by upper endoscopy, colonoscopy, and

abdominal ultrasound or abdominal computed tomography. Patients were clinically defined as suspected for CGI if they had unexplained abdominal pain or unexplained weight loss (> 5% of standard weight), and a ≥70% of at least one of the main gastrointestinal arteries on radiological evaluation

Standard diagnostic work-up

All patients underwent a standard work up by means of a thorough medical and physical examination, and an extensive questionnaire concerning clinical complaints and medical and family history. All patients underwent radiological evaluation by means of CT- and MR angiography and/or conventional angiography to visualize the gastrointestinal arteries (celiac artery, superior mesenteric artery and inferior mesenteric artery) in combination with VLS measurement. A significant stenosis of the abdominal arteries was defined as a luminal reduction of >70 %. Mucosal saturation was measured using a fiberoptic catheter-based VLS oximeter (T-Stat 303 Microvascular Oximeter, Spectros, Portola Valley, California, USA) during upper endoscopy as described earlier.(9) This catheter was passed through the accessory channel of the endoscope after irrigation of the target area to remove any bile and food remnants. Peripheral saturation was kept > 94% during the procedure, and oxygen was administered intranasally if necessary. Moreover, butylscopolamin (20 mg) was administered intravenously before start of VLS measurement to prevent luminal spasms. The cut-off values for mucosal hypoxia were < 58% for the descending duodenum, < 62% for the duodenal bulb and < 63% for the gastric antrum, as was determined in our prior study. (9) The mucosal oxygen saturation was determined with repeated assessment in steady state at multiple locations in the descending duodenum, duodenal bulb, the gastric antrum, gastric corpus, and esophagus, and then averaged per site. Mucosal ischemia was defined as detection of mucosal hypoxia at one or more of the above mentioned locations. (9)

Consensus diagnosis of occlusive CGI

Medical history, complaints, and the results of all diagnostic procedures were discussed in a dedicated multidisciplinary team consisting of a vascular surgeon, intervention radiologist and gastroenterologist, all specialized in CGI. The discussion resulted in a final expert-based consensus diagnosis of occlusive CGI or non-CGI. The *consensus diagnosis* of occlusive CGI was made in presence of 1) a clinical suspicion of CGI, 2) a significant stenosis (> 70%) in at least one of the gastrointestinal arteries, and 3) detection of mucosal ischemia by means of VLS measurement. Occlusive CGI was classified as either due to single artery or multi-artery disease depending on the number of arteries involved. A single stenosis in the inferior mesenteric artery was not considered to be associated with CGI. Patients diagnosed with occlusive CGI were offered surgical or endovascular revascularization.

Follow-up and response

All patients diagnosed with CGI were prospectively followed at the out-patient clinic with scheduled visits at six weeks, three months, six months and one year after treatment for assessment of clinical status and repeated duplex ultrasound scanning of the gastrointestinal arteries. After this period, patients were assessed once yearly either at our own out-patient clinic or referred for yearly clinical assessment by their referring physician. The referring physicians were instructed to report recurrent symptoms, and were asked to present and / or confirm follow-up data at the end of the follow-up period. If patients were treated at their referring hospital, follow-up data were obtained in the same manner. Sustained response was defined as self reported complete or > 50% disappearance of postprandial pain, nausea and other major complaints, and persistent weight gain or stabilization during long-term follow-up of at least 6 months after the therapeutic intervention. Loss of response was defined as an initial positive response to treatment, but with loss of this response during follow-up despite patent gastrointestinal arteries evaluated by renewed CTA. Primary non-responders were defined as patients who did not have any symptom improvement despite technically successful revascularization. Patients diagnosed as non-CGI were discharged from regular follow-up. Long term follow-up data of the latter patients was obtained by means of a survey which was conducted by contacting the primary care or referring physician and the patient. The survey focused on current health status, presence of any persisting symptoms, further diagnostic procedures, and events such as hospital admission or death.

Patients diagnosed as non-CGI were discharged from the out-patient-clinic. Follow-up data of these patients were obtained by means of a survey which was conducted by contacting the primary care or referring physician and the patient. The survey focused on current health status, presence of any persisting symptoms, further diagnostic procedures, and events such as hospital admission or death.

Statistical analysis

Patient's characteristics were compared using Student's t-test, Mann-Whitney *U* test or X^2 test. For the univariate analysis the following patients characteristics were studied: age, gender, reported weight loss of any magnitude, weight loss per month (defined as the total amount of weight loss (in kg) a patient had from symptom onset divided by the period (in months) in which the weight loss occurred), postprandial pain, exercise related pain, diarrhea, nausea, smoking, family history of cardiovascular disease, known cardiovascular disease, presence of abdominal bruit, BMI after complaints, the classical triad, results of radiologic evaluation (single or multi-artery stenosis), and VLS measurements in duodenum, duodenal bulb, antrum, corpus, and oesophagus.

With respect to VLS, test performances at different cut-off levels were investigated, and for each cut-off value the c-statistics were estimated. The c-statistics is a measure of discrimination, in this case the ability to distinguish patients who had a persistent positive response to treatment versus those who did not. The c-statistics is equal to the area under the area under the receiver-operating characteristic curve (AUC).

Based on literature and clinical knowledge we used for multivariate analysis the factors with a p-value < 0.20 which were positively associated with positive treatment response. Subsequently, these factors were used to design a multivariate model. For multivariate analysis the following clinical predictors were used: reported weight loss, smoking, presence of an abdominal bruit, single or multi-artery stenosis, and corpus mucosal saturation levels. Akaike's Information Criteria (AIC) was used to compare the goodness of fit between models. The area under the ROC curve (the c-statistics) was calculated to compare discrimination abilities of the models between response and non-response. The model with the lowest AIC and the highest c-statistics was considered the most desirable model. Hereafter non-significant factors were included one-by-one to test for model improvement. In the final model interaction terms were checked.

RESULTS

Patient characteristics

From November 2008 to January 2011, 212 patients were referred for evaluation of suspected CGI and included in the present study (Figure 1; flow chart of the study). The mean age was 59 (range 19 – 87) years and 62 (29%) patients were male; see Table 1. After initial diagnostic work-up, a *consensus diagnosis* of *CGI* was made in 107 (50%) patients: 67 (63%) and 40 (37%) patients with single and multi-artery disease, respectively. Patients diagnosed with occlusive CGI were significantly older and more often female than those diagnosed not having CGI, P = < 0.001 and P = 0.002, respectively. Furthermore, the univariate analysis showed that reported weight loss, postprandial pain, a family history of cardiovascular disease (CVD), presence of classical triad, and an abdominal bruit during physical examination were univariably strongly associated with occlusive CGI; see Table 1.

Treatment and follow-up

Consensus CGI was defined in 107 patients and 96 (90%) patients received revascularization; see Figure 1. The remaining 11 (10%) did not receive treatment due to either refusal (n = 5), or presence of severe co-morbidity (n = 6). Follow-up data were available for 93/96 patients (97%) with a median follow-up of 13 (interquartile range (IQR) 7 – 19) months. Four patients presented with an acute-on-chronic gastrointestinal ischemia, and died shortly after treat-

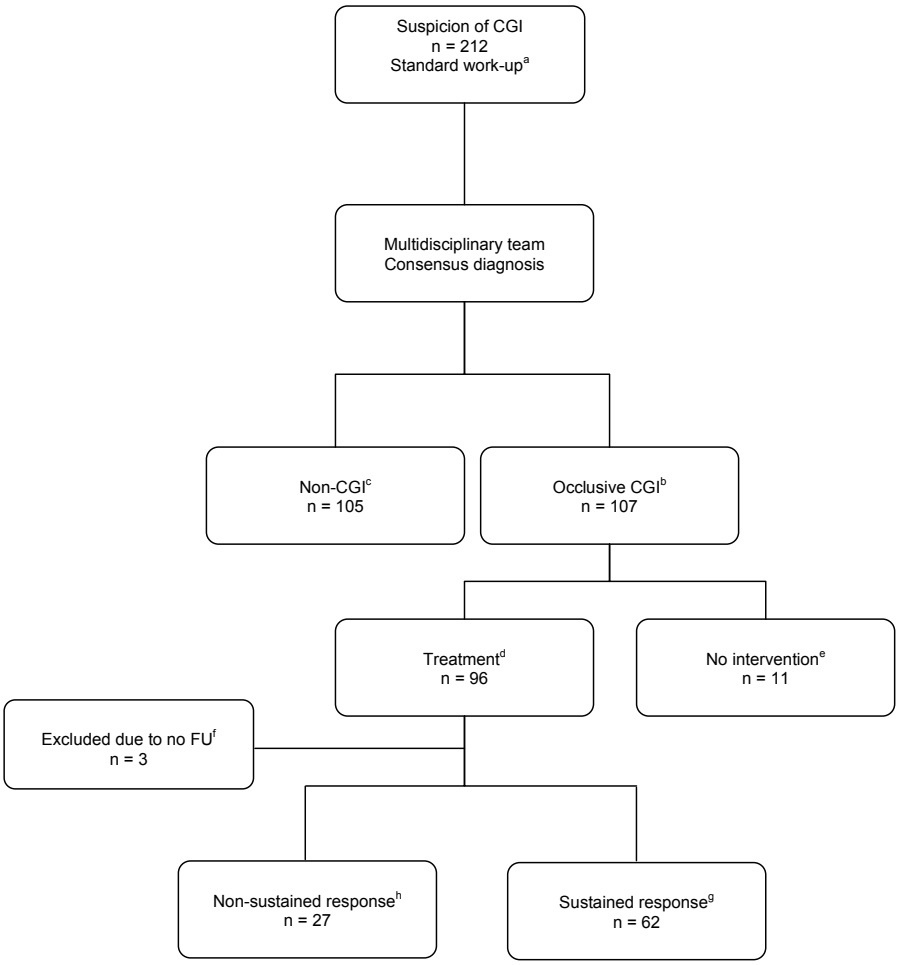


Figure 1: Flow-chart of the study
CGI = Chronic gastrointestinal ischemie; FU = follow-up
a Standard work-up: history taking, radiological imaging (CTA or MR angiography or digital subtraction angiography)
b 107 (50%) with occlusive
c 105 (50%) with non CGI
d Occlusive CGI: intervention: n = 96; stent placement (n = 59), surgery (n = 23); percutaneous transluminal angiography angioplasty (n = 6), anticoagulative agent (n=1)
e No intervention: refusal of patient (n =6), conservative treatment because patients were not eligible for treatment due to co-morbidity (n = 5).
f Sustained response: complete or > 50% disappearance of postprandial pain, nausea and other major complaints presented at the time of the analysis, and weight gain or stabilization after therapeutic intervention
g Non-sustained response (n = 26); primary non-responders (n = 19), loss of response (n = 7): initially responded positive to treatment, but symptoms recurred despite technical successful intervention
h Lost during follow-up (n =3), deceased shortly after intervention due to acute-on-chronic gastrointestinal ischemia (n = 4)

Table 1: Patient characteristics patients with occlusive CGI, and non-CGI

Patient characteristics	Total group		Occlusive CGI		Non-CGI		P-value
	n = 212		n = 107		n = 105		
Age (years)*	59	(19 - 87)	63	(20-87)	55	(19 - 82)	<0.001
Male gender	62	(29)	21	(20)	41	(39)	0.002
Period complaints (months)**	16	(8-36)	13	(7-27)	18	(11-36)	0.158
Abdominal pain	198	(94)	101	(95)	97	(92)	0.381
Reporting weight loss	123	(58)	71	(66)	52	(50)	0.013
Weight loss (kg/month)**	1.5	(0.8-3.0)	1.5	(0.8-3.9)	1.3	(0.6-2.5)	0.111
Postprandial pain	144	(68)	81	(76)	63	(60)	0.014
Exercise related pain	61	(29)	26	(24)	35	(33)	0.146
Nausea	78	(37)	39	(36)	39	(37)	0.917
Diarrhea	40	(19)	17	(16)	23	(22)	0.263
Smoking	130	(62)	70	(65)	60	(58)	0.248
Family history CVD	96	(47)	56	(55)	40	(39)	0.021
CVD	110	(52)	62	(58)	48	(46)	0.075
Ulcus	7	(3)	5	(5)	2	(2)	0.259
Abdominal bruit	37	(18)	30	(28)	7	(7)	<0.001
BMI (kg/m^2)**	23.2	(20.1-26.7)	22.5	(19.6-25.7)	23.5	(20.3 -28.0)	0.065
Classical triad	19	(9)	17	(16)	2	(2)	<0.001
Single artery	118	(56)	67	(63)	51	(49)	-
Multi-artery	44	(21)	40	(37)	4	(4)	<0.001
*Laboratory evaluation***							
GFR (ml/min.)	78	(62-90)	73	(60-90)	83	(65-90)	0.017
Albumin	44	(42-47)	44	(41-47)	45	(43-47)	0.025
Lactate	1.7	(1.3-2.0)	1.6	(1.3-2.0)	1.7	(1.3-2.0)	0.857
CRP	2.0	(1.0-5.0)	2.0	(1.0-5.0)	2.0	(1.0-4.0)	0.780
D-dimeer	0.5	(0.3-0.9)	0.6	(0.4-1.1)	0.3	(0.2-0.6)	<0.001
Anti-trypsin	16	(8-29)	14	(7-28)	17	(9-32)	0.255

CGI = chronic gastrointestinal ischemia; CVD = cardiovascular disease; * values shown in mean (range); ** values shown in median (IQR)

ment due to the sequelae of transmural necrosis. These patients were not included in the analysis due to lack of follow-up data. Thus, long-term follow-up data were available for 89 patients, of which 59 (66%) patients had single artery disease, and 30 (34%) had multi-artery disease. In the 89 patients, endovascular revascularization was performed in 65 patients, surgical revascularization in 23 patients, and one patient was treated with anticoagulative agent (acenocoumarol) because the cause of the stenosis was neither atherosclerosis nor vasculitis. Endovascular therapy was performed by means of stent placement in 59 patients, and transluminal angioplasty in six patients. In one patient it was uncertain whether the stenosis in the celiac trunk was secondary to atherosclerosis or a celiac artery compression syndrome (CACS), and initially this patient was treated with endovascular stent placement. Within three months after intervention this patient presented with recurrent complaints, and digital subtraction angiography (DSA) showed a fractured stent. The patient was then offered

Table 2: Patient characteristics responders and non-responders with occlusive disease

Patient characteristics	Total group		Responder		Non responder		P-value
	n = 89		n = 62		n = 27		
Age (years)*	63	(20 - 87)	64	(20-87)	62	(31-86)	0.532
Male gender	20	(23)	14	(23)	6	(22)	0.970
Period complaints (months)**	14	(7-32)	14	(7-36)	14	(9-24)	0.989
Abdominal pain	84	(94)	59	(95)	25	(93)	0.637
Reporting weight loss	59	(66)	44	(71)	15	(56)	0.157
Weight loss (kg)**	8	(4-15)	8	(4-17)	8	(6-13)	0.950
Weight loss (kg/month)**	1.5	(0.8-4.0)	1.5	(0.8-4.8)	1.3	(0.7-3.5)	0.485
Postprandial pain	63	(71)	44	(71)	19	(70)	0.955
Exercise related pain	24	(27)	15	(24)	9	(33)	0.372
Nausea	33	(37)	24	(39)	9	(33)	0.629
Diarrhea	16	(18)	11	(18)	5	(19)	0.930
Smoking	59	(66)	44	(71)	15	(56)	0.157
Family history CVD	44	(52)	28	(48)	16	(64)	0.165
CVD	52	(58)	38	(61)	14	(52)	0.406
Ulcus	4	(5)	4	(7)	0	(0)	0.310
Abdominal bruit	28	(32)	23	(37)	5	(19)	0.083
BMI (kg/m^2)**	22.7	(19.4-25.6)	23.1	(18.8-25.9)	22.4	(19.5-25.0)	0.867
Classical triad	15	(17)	11	(18)	4	(15)	0.735
Single artery	59	(66)	38	(61)	21	(78)	-
Multi-artery	30	(34)	24	(39)	6	(22)	0.130
*Laboratory evaluation***							
GFR (ml/min.)	75	(60-90)	73	(52-90)	78	(66-89)	0.344
Albumin	44	(41-47)	44	(40-46)	44	(42-49)	0.135
Lactate	1.6	(1.3-2.0)	1.7	(1.3-2.1)	1.5	(1.3-2.0)	0.673
CRP	2	(1.0-5.0)	2.0	(1.0-6.0)	1.0	(1.0-3.0)	0.022
D-dimeer	0.6	(0.3-1.1)	0.6	(0.4-1.2)	0.4	(0.3-1.1)	0.252
Anti-trypsin	13	(7-28)	12	(6-28)	18	(10-28)	0.796

CGI = chronic gastrointestinal ischemia; CVD = cardiovascular disease; * values shown in mean (range); ** values shown in median (IQR)

surgical cleavage of the ligament, but the surgical revascularization did not lead to recurrent relief of symptoms. Restenosis occurred in 11 patients: six suffered from in–stent restenosis, of whom five had again symptom improvement after an additional revascularization procedure, while one of them died because of acute-on-chronic gastrointestinal ischemia due to in-stent restenosis, one patient suffered from occlusion of a surgical bypass, and died due to acute-on-chronic gastrointestinal ischemia, and four patients suffered from stent fracture, in one of them followed by CACS release as mentioned above.

Morbidity and mortality

Complications due to revascularization procedures occurred in 20 out of 89 (23%) (Table 3). Of the 89 patients, eleven (12%) died during follow-up: due to in-stent-restenosis and occlusion

of surgical bypass leading to death due to acute-on-chronic gastrointestinal ischemia (n=2), due to pulmonary carcinoma (n = 6), severe COPD and pneumonia (n = 1), neuroendocrin tumor (n =1), and unknown cause (n =1).

Clinical success rate

In this section we present the positive response rate of patients with clinical suspicion of CGI who were selected for treatment by means of a standard diagnostic work-up including imaging of the gastrointestinal arteries and VLS measurement. Sixty-two (70%) of 89 treated patients had a sustained response during follow-up. The clinical response rate was 38 / 59 (64%) in patients with single artery disease, and 24 / 30 (80%) in those with multi-artery disease (P = 0.151). Twenty-seven (30%) patients did not have a sustained response: 20 (74%) primary non-responders, and seven (26%) secondary non-responders, i.e. patients who developed loss of response.

VLS measurements

The mean mucosal saturations in the esophagus, gastric corpus, antrum, duodenal bulb, and descending duodenum were significantly lower in patients with occlusive CGI compared to non-CGI; see Table 4. In responders the median mucosal saturation level in the gastric corpus was significantly lower than in non-responders; see Table 4. Using a mucosal saturation cut-off value of 56% the area under the curve for distinction of treatment responders was 0.69 (95% confidence interval 0.57-0.81).

Predictors of positive treatment response

The difference in characteristics of responders and non-responders of patients treated for occlusive CGI are shown in Table 2. We used the following parameters for multivariate logistic regression: reported weight loss (OR 1.96, 95% CI 0.77-4.99), abdominal bruit (OR 2.60, 95% CI 0.86-7.79), smoking (OR 1.96, 95% CI 0.77-4.99), single- or multi-artery disease (OR 2.21, 95% CI 0.78-6.26), and corpus measurement < 56% (OR 5.52, 95% CI 1.19-25.72). Multivariate logistic regression showed that the model based on reported weight loss (OR 1.93, 95% CI 0.72-5.17), presence of abdominal bruit (OR 2.36, 95% CI 0.75-7.36), and low baseline corpus mucosal saturation (OR 4.84, 95% CI 1.02-22.95) had the lowest AIC (104.63). These parameters were the strongest predictors for a positive treatment response. Other models which were tested included a combination of the variables weight loss, smoking, bruit, single or multi-artery disease, and corpus saturation < 56% and the AIC's ranged from 105.00 to 110.04). The chance of a positive response with different combination of the predictors is shown in Table 5. In patients without weight loss, without abdominal bruit, and with a corpus saturation > 56%, the response rate was shown to be less than 50%. In patients with any one of the predictors present, the chance of response ranged between 65% and 80%, and the chance of response was > 85% in patients with all three predictors present.

Table 3: Complications of revascularization

Complication	Endovascular	Surgical
	n = 65	n = 23
Access site		
Hematoma	8	-
Nerve damage	1	-
Thrombosis	1	-
AV malformation	1	-
Pseudoaneurysm	1	1
Stenosis a. brachialis	2	
Cardiovascular		
Stroke	1	-
Atrioventricular block	-	1
Abdominal		
Ileus	-	1
Splenic infarction	-	1
Respiratory		
Respiratory failure	-	1

Table 4: VLS mucosal saturation in different locations during upper endoscopy

Patient characteristics	Descending duodenum		Duodenal bulb		Antrum		Corpus		Distal esophagus	
Total group, n = 212										
Occlusive CGI n = 107	51	(5.7)*	57	(6.4)*	62	(5.3)*	59	(6.2)*	62	(4.1)*
Non-CGI n = 105	59	(5.5)*	63	(4.7)*	65	(4.3)*	64	(5.3)*	65	(4.6)*
Treated patients, n = 89										
Responders n = 62	50	(6.3)	56	(6.7)	62	(5.3)	59	(6.7)**	62	(4.2)
Non-responders n = 27	51	(5.5)	58	(5.5)	63	(4.7)	62	(4.8)**	63	(3.7)

Values are shown in mean (SD). * $P < 0.001$; ** $P = 0.05$

Follow-up of non-CGI patients

Follow-up in patients who were diagnosed as not having CGI showed symptom resolution in 28 (26%), either spontaneously (n=19), or after cholecystectomy (n =2), after polypectomy in colon (n =1), after treatment with proton pump inhibitor (n =2), eradication of *Helicobacter pylori* (n =1), after treatment of ascites as complication of liver cirrhosis (n =1), correction of hyperthyroidia (n = 1), and nephrectomy for renal cell carcinoma (n =1). None of the non-CGI patients with the consensus diagnosis was during a median follow-up of 23 (IQR 14-29) months diagnosed with ischemia-related morbidity or mortality.

Table 5: Chance of response including weight loss, bruit, corpus measurement < 56% as predictors

Chance of response		Weight loss	Bruit	Corpus measurement < 56%
< 50%	n = 18	Absent	Absent	Absent
65% - 80%	n = 6	Absent	Present	Absent
	n = 2	Absent	Absent	Present
	n = 31	Present	Absent	Absent
> 85%	n = 13	Present	Present	Absent
	n = 4	Absent	Present	Present
	n = 10	Present	Absent	Present
	n = 5	Present	Present	Present

DISCUSSION

We evaluated the treatment response in a large cohort of patients clinically suspected for CGI, who were selected for treatment based on a standardized diagnostic work-up including radiological imaging and VLS measurements. A sustained response was reached in 70% of patients, after a follow-up period of 13 (interquartile range (IQR) 7 – 19) months. Pre-treatment weight loss, presence of an abdominal bruit, and corpus mucosal saturation below 56% were the strongest predictors of a positive response to treatment. Presence of ≥ 2 predictors was associated with a response rate of over 85%, whereas the absence of all three predictors was associated with a treatment response below 50%.

The prevalence of abdominal arterial stenoses in the asymptomatic population ranges from 0.1%-10%, (2) (4, 12), depending on the age, (1) ethnic background, (12) and the presence of vascular stenosis elsewhere in the body. (13, 14) In patients with abdominal arterial stenosis clinical significant gastrointestinal ischemia is in majority of cases prevented by the often abundant abdominal arterial collateral circulation. Detecting one or multiple artery stenosis in patients with abdominal symptoms may thus be merely a coincidental finding, and identifying those patients who will benefit from revascularization of a single artery stenosis remains a clinical challenge. Careful patient selection is the key to success of revascularization treatment in this patient population. It was recently shown that patient selection based on symptoms only is highly inaccurate, as the presenting symptoms are aspecific for CGI,(8) and only a minority of patients has the classic triad of symptoms such as postprandial pain, weight loss and an abdominal bruit. (15). The addition of mucosal perfusion measurements to the diagnostic work-up in patients suspected for CGI, has been shown to be pivotal to achieve acceptable diagnostic accuracy rates. The latter resulting in higher success rates

after revascularization, especially in the even more challenging, and often disputed, group of patients with single abdominal arterial stenosis. (6, 8, 15). We previously assessed the use of VLS for detection of mucosal hypoxia , with a direct comparative study with the current 'gold standard' tonometry (9,15-17). Thresholds for normal saturations in the duodenum, duodenal bulb, and antrum were established.(9) The presented study is so far the largest cohort using VLS measurement for assessing mucosal hypoxia in diagnosis of this group of patients. The mean saturations of duodenum, duodenal bulb, antrum, corpus and oesophagus were significantly lower in CGI patients than in non-CGI patients. The measured differences were in line with the earlier reports, thereby confirming the discriminative value of VLS. This study shows that including VLS measurements, as a direct measurement of mucosal hypoxemia, in the diagnostic work-up leads to an overall acceptable diagnostic yield and response rate. The latter being comparable to the diagnostic yield and response rate achieved by tonometry (6, 15). However, the use of tonometry in daily clinical practice is hampered by its time consuming nature, and additional burden for the patients. VLS measurement is less invasive, less time consuming than tonometry, and is easily accessible in every modern endoscopy unit. This may lower the threshold for physicians to analyse patients with chronic unexplained abdominal pain and an abdominal arterial stenosis for the presence of CGI

Furthermore, we determined predictors for a positive treatment response. After multivariate analysis, a corpus saturation level below 56% was one of the strongest predictors of a positive treatment response. The other two predictors were abdominal bruit, and reported weight loss of any magnitude. An abdominal bruit and weight loss, were already identified as being associated with a consensus diagnosis of CGI in earlier reports. (8, 15) Based on these three predictors, a model was made showing that the chance of a positive treatment response can be stratified to the number of predictors present. Especially the presence of $^{3}2$ predictors or the absence of any predictor was of discriminative value with a > 85% versus a < 50% response rate respectively. These predictors can support the selection of patients for treatment, especially in patients with considerable comorbidity or single artery disease.

This study has several limitations. Firstly, the median follow-up period was only13 (IQR 7 – 19) months, to be defined as mid-term follow-up. Long-term follow-up data are needed to evaluate if the one-year response rate will be maintained. Secondly, the majority of patients who were diagnosed as non-CGI did not have an intervention. Ideally, all patients should have been treated to correlate treatment response to baseline VLS measurements and other parameters. However, this approach was considered ethically inacceptable due to the results of earlier studies and potential complications. Furthermore, follow-up showed that none of the patients with the consensus diagnosis of non-CGI developed any signs of ischemia-related morbidity or mortality during follow-up. This implies that the consensus diagnosis of non-CGI was correct in the majority of patients. Thirdly, patients were referred to a tertiary care centre

with a special interest for vascular disorders of the gastrointestinal tract. The consensus diagnosis CGI was reached in half of the studied patient population. Given the potential for referral bias, the results must be interpreted with caution and may not be extrapolated to all general gastroenterology practices, as test characteristics may be different when applied to a population with a low prevalence of CGI

In conclusion, this study shows that a multi-diagnostic work-up, including VLS measurement as direct mucosal hypoxemia test, provides an acceptable diagnostic yield and response rate. This emphasizes the role of a direct mucosal hypoxemia measurement for assessment of CGI and selection of patients for treatment. Moreover, reported weight loss, presence of an abdominal bruit, and a lowered corpus mucosal oxygen saturation with VLS are the strongest predictors of a positive response to restoring vascular patency. These parameters can potentially be used to determine which patients benefit most from treatment.

REFERENCES

1. Roobottom CA, Dubbins PA. Significant disease of the celiac and superior mesenteric arteries in asymptomatic patients: predictive value of Doppler sonography. AJR Am J Roentgenol 1993;161:985-8.
2. Bron KM, Redman HC. Splanchnic artery stenosis and occlusion. Incidence; arteriographic and clinical manifestations. Radiology 1969;92:323-8.
3. Derrick JR, Pollard HS, Moore RM. The pattern of arteriosclerotic narrowing of the celiac and superior mesenteric arteries. Ann Surg 1959;149:684-9.
4. Hansen KJ, Wilson DB, Craven TE, et al. Mesenteric artery disease in the elderly. J Vasc Surg 2004;40:45-52.
5. Jarvinen O, Laurikka J, Sisto T, et al. Atherosclerosis of the visceral arteries. Vasa 1995;24:9-14.
6. Otte JA, Geelkerken RH, Oostveen E, et al. Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2005;3:660-6.
7. Mensink PB, Moons LM, Kuipers EJ. Chronic gastrointestinal ischaemia: shifting paradigms. Gut 2011;60:722-37.
8. Sana A, Vergouwe Y, van Noord D, et al. Radiological imaging and gastrointestinal tonometry add value in diagnosis of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2011;9:234-41.
9. Van Noord D, Sana A, Benaron DA, et al. Endoscopic visible light spectroscopy: a new, minimally invasive technique to diagnose chronic GI ischemia. Gastrointest Endosc 2011;73:291-8.
10. Friedland S, Benaron D, Coogan S, et al. Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. Gastrointest Endosc 2007;65:294-300.
11. Benaron DA, Parachikov IH, Cheong WF, et al. Design of a visible-light spectroscopy clinical tissue oximeter. J Biomed Opt 2005;10:44005.
12. Park CM, Chung JW, Kim HB, et al. Celiac axis stenosis: incidence and etiologies in asymptomatic individuals. Korean J Radiol 2001;2:8-13.
13. Valentine RJ, Martin JD, Myers SI, et al. Asymptomatic celiac and superior mesenteric artery stenoses are more prevalent among patients with unsuspected renal artery stenoses. J Vasc Surg 1991;14:195-9.
14. Glockner JF. Incidental findings on renal MR angiography. AJR Am J Roentgenol 2007;189:693-700.
15. Mensink PB, van Petersen AS, Geelkerken RH, et al. Clinical significance of splanchnic artery stenosis. Br J Surg 2006;93:1377-82.
16. Otte JA, Geelkerken RH, Huisman AB, et al. What is the best diagnostic approach for chronic gastrointestinal ischemia? Am J Gastroenterol 2007;102:2005-10.
17. Boley SJ, Brandt LJ, Veith FJ, et al. A new provocative test for chronic mesenteric ischemia. Am J Gastroenterol 1991;86:888-91.

Chapter 5

Patients with Chronic Gastrointestinal Ischemia have a Higher Cardiovascular Disease Risk and Mortality

Aria Sana MD[1], Désirée van Noord PhD[1], Peter B.F. Mensink PhD[1], Stephanie Kooij MSc[2], Kim van Dijk MSc[2], Bert Bravenboer PhD[3], Aloysius G. Lieverse PhD[4], Eric J.G. Sijbrands PhD[2] Janneke G. Langendonk PhD[2] , Ernst J. Kuipers PhD[1,2]

Departments of Gastroenterology and Hepatology[1], Internal Medicine[2], Erasmus Medical Centre, Rotterdam, Department of Internal Medicine, Catharina Ziekenhuis[3], Eindhoven, Department of Internal Medicine, Maxima Medisch Centrum[4], Eindhoven, The Netherlands

Atherosclerosis 2012, in press

ABSTRACT

Objectives: We determined the prevalence of classical risk factors for atherosclerosis and mortality risk in patients with CGI.

Methods: A case-control study was conducted. Patients referred with suspected CGI underwent a standard work-up including risk factors for atherosclerosis, radiological imaging of abdominal vessels and tonometry. Cases were patients with confirmed atherosclerotic CGI. Controls were healthy subjects previously not known with CGI. The mortality risk was calculated as standardized mortality ratio derived from observed mortality, and was estimated with ten-year risk of death using SCORE and PREDICT.

Results: Between 2006 and 2009, 195 patients were evaluated for suspected CGI. After a median follow-up of 19 months, atherosclerotic CGI was diagnosed in 68 patients. Controls consisted of 132 subjects. Female gender, diabetes, hypercholesterolemia, a personal and family history of cardiovascular disease (CVD), and current smoking are highly associated with CGI. After adjustment, female gender (OR 2.14 95 % CI 1.05-4.36), diabetes (OR 5.59, 95% CI 1.95-16.01), current smoking (OR 5.78, 95% CI 2.27-14.72), and history of CVD (OR 21.61, 95% CI 8.40-55.55) remained significant. CGI patients > 55 years had a higher median ten-year risk of death (15% vs. 5%, P = 0.001) compared to controls. During follow-up of 116 person-years, standardized mortality rate was higher in CGI patients (3.55; 95% CI 1.70-6.52).

Conclusions: Patients with atherosclerotic CGI have an increased estimated CVD risk, and severe excess mortality. Secondary cardiovascular prevention therapy should be advocated in patients with CGI.

INTRODUCTION

Chronic gastrointestinal ischemia (CGI) is in the majority of cases caused by narrowing of the gastrointestinal arteries, with atherosclerosis being the most common underlying cause. [1-2] Subsequent cardiovascular disease (CVD) preventive therapy is not considered standard care.

The conventional risk factors for atherosclerosis are: male gender, age, diabetes mellitus, smoking, hypertension, hypercholesterolemia, medical history of previous CVD, positive family history of (premature) CVD, obesity, and physical inactivity.[3-4] Any ischemic event is a strong independent risk factor for a new cardiovascular event.[5-8] [9-11]

Although CGI is considered a manifestation of atherosclerosis, studies focused on a cardiovascular work-up of classical risk factors for atherosclerosis in patients with CGI are missing. Previous case series have reported inconsistent findings. A wide range of distribution of classical risk factors for atherosclerosis has been reported in the baseline characteristics of case series: a 10-93% prevalence of hypercholesterolemia, 64-92% of hypertension, and 36-72% of previous coronary artery disease.[1, 12-18] The primary objective of this case-control study was to determine the contribution of classical atherosclerotic risk factors to atherosclerotic CGI, and the secondary objective was to assess the mortality risk in treated patients.

METHODS

A case-control study was conducted. The Institutional Review Boards of the Erasmus Medical Center and the Eindhoven hospitals approved the study. Only patients with written informed consent entered the present study. Control subjects from the DiaGene Study population served as controls.[19] DiaGene is a case-control study, including patients with diabetes (cases) and subjects without a previous history of diabetes (controls). The DiaGene is conducted in three general hospitals in Eindhoven, The Netherlands, Controls were recruited via an advertisement in different local news papers. Subjects could participate in the study if they were not known with diabetes. Upon registration and after giving their written informed consent, they received an invite to one of the participating medical centers. They completed a questionnaire concerning cardiovascular risk assessment, medical and family history for CVD, medication use, and smoking. Fasting blood samples were drawn and body mass index and blood pressure were determined. After obtaining all the information concerning cardiovascular risk assessment, there was no follow-up of the controls. In the current study, we included the first 132 consecutive subjects who responded for the DiaGene study as controls, i.e. subjects without diabetes. None of these controls were diagnosed with CGI.

Case selection

Consecutive patients with suspected CGI referred to our tertiary center for evaluation were considered for inclusion. Patients with intravascular atherosclerotic CGI were included as cases. The full description of the selection of the cases is described below.

Diagnostic work-up including cardiovascular diagnostic work up

All patients referred with suspected CGI underwent a thorough medical and physical examination, including ECG and with extensive questioning concerning cardiovascular risk assessment, clinical complaints, weight before complaints, medical and family history for CVD using a structured questionnaire. During physical examination, height and weight were measured. From all patients referred with suspected CGI, venous blood samples were obtained after 9-hours fasting for measurement of serum cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and blood glucose. Documented coronary artery disease included at least one of the following criteria: history of cardiac angina, myocardial infarction (MI), percutaneous coronary intervention (PCI), or coronary artery bypass graft surgery (CABG). Documented cerebrovascular accident included a diagnosis of transient ischemic attack (TIA) or ischemic stroke. Documented peripheral arterial disease consisted of intermittent claudication previous treatment such as angioplasty, endovascular stent placement, peripheral arterial bypass graft or atherectomy. Hypercholesterolemia was considered present when subjects reported hypercholesterolemia, and/or used a statin in the absence of CVD, and/or when LDL-cholesterol or total cholesterol was above the 95th percentile for age and gender. Hypertension was defined as known with hypertension or using anti-hypertensive medication or a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg. Diabetes mellitus was defined according to WHO criteria or use of blood glucose lowering agents.

Next to thorough medical and physical examination, all patients suspected for CGI underwent radiological evaluation by means of abdominal CT- or MR angiography, and/or conventional angiography to visualize the gastrointestinal arteries (celiac artery, superior mesenteric artery and inferior mesenteric artery) in combination with functional testing for mucosal perfusion by means of a 24-h gastric and jejunal tonometry as described before, [2, 20-21]

Consensus diagnosis and definite diagnosis of CGI

Medical history, complaints, and the results of all diagnostic procedures were discussed in a dedicated multidisciplinary team consisting of a vascular surgeon, intervention radiologist and gastroenterologist, all specialized in CGI. [21] The discussion resulted in a final expert-based *consensus diagnosis* of CGI or non-CGI. The *consensus diagnosis* of CGI was made in presence of 1) a clinical suspicion of CGI, 2) a significant stenosis (> 70%) in at least one of the gastrointestinal arteries, and 3) detection of mucosal ischemia by means of 24-h gastric and jejunal tonometry. Patients with a consensus diagnosis CGI were classified as hav-

ing either occlusive or non-occlusive CGI. Non-occlusive CGI, also called i.e non-occlusive mesenteric ischemia (NOMI), was defined as gastrointestinal ischemia in the absence of significant vascular stenosis. Patients with occlusive disease were classified as either having an intravascular stenosis, or extravascular compression due to the celiac-artery compression syndrome (CACS) (see Figure 1). Patients diagnosed with NOMI, vascular arteritis and CACS were excluded from the present analyses. All remaining patients with CGI were diagnosed

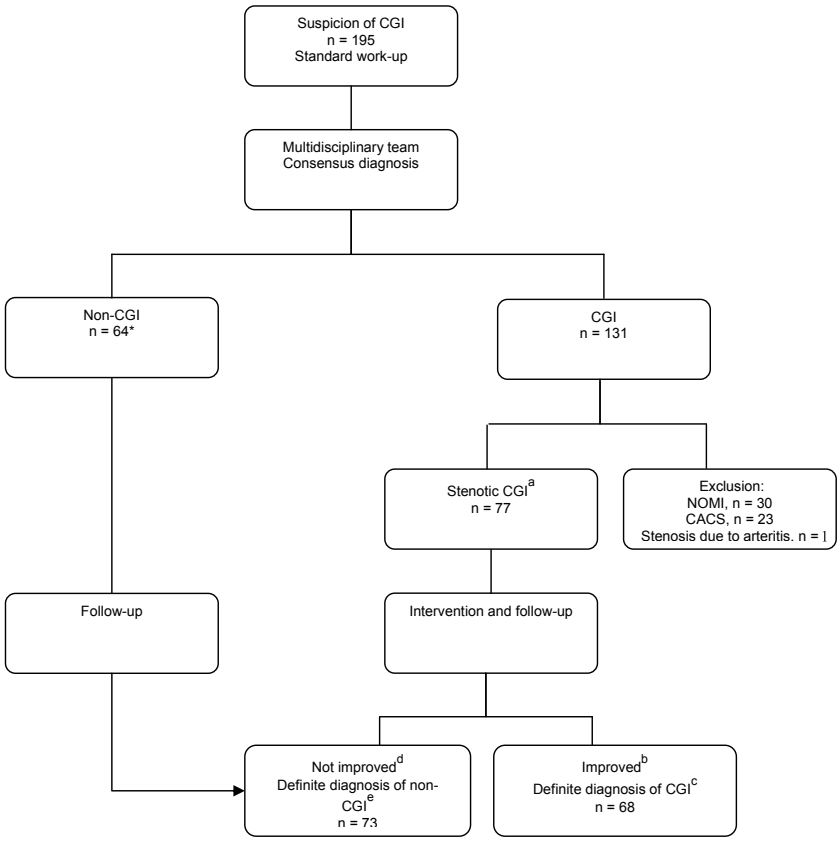


Figure 1: Flow-chart of the study
CGI = chronic gastrointestinal ischemia ; NOMI = non-occlusive mesenteric ischemia ; CACS= celiac artery compression syndrome ; CAC = celiac artery compression without ischemia
[a] Stenotic CGI = CGI in presence of intravascular atherosclerotic stenosis; treated with endovascular stent placement or surgery
[b] Symptoms improvement
[c] A definite diagnosis of CGI was made if a patient was free of symptoms after adequate therapy for at least 12 months of follow-up;
[d] Symptoms unchanged despite adequate treatment, n = 9
[e] A definite diagnosis of non-CGI was made if a patient was not diagnosed or hospitalized with ischemia related morbidity or mortality after follow-up and if symptoms did not improve despite adequate treatment. * CAC, n = 7

with intravascular stenotic or atherosclerotic CGI. These patients were considered eligible for endovascular or surgical revascularization. All treated patients were prospectively followed at the out-patient clinic with regular visits. [21] The *definite diagnosis* of atherosclerotic CGI was made after persistent relief of symptoms during long-term follow-up of at least 12 months after initiation of therapy.[21] Patients with *definite atherosclerotic CGI* were included as cases in the current study.

Estimation of ten years risk of death due to cardiovascular disease

The ten-year mortality risk was estimated in patients without a history of cardiovascular event, with the SCORE risk system, and for patients with previous MI and CABG the PREDICT risk score was used. The PREDICT risk score has been developed for estimating the 6-year mortality risk after hospitalization for acute MI or unstable angina. It is based on factors such as age, ECG at the time of hospitalization, comorbidities and renal function. There is no risk score for estimating the ten-year risk of death due to cardiovascular event for patients with peripheral artery disease or with a cerebrovascular event in the past. We estimated the ten-year mortality in patients with a history of stroke or TIA by using the risk of death at ten years for these patients as described in the DUTCH transient ischemic Trial.[22]

Baseline measurements

Venous blood was sampled after 9-hours fasting for measurement of serum cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and blood glucose. Kidney function was categorized according to classification established by the National Foundation Kidney Disease Outcomes Quality Initiative. [23]

Ten year mortality risk and absolute mortality

For the secondary objective we estimated the mortality risk in patients with atherosclerotic CGI by estimating the ten-year risk of death due to CVD as well as calculating the all cause mortality as standardized mortality ratio.[24] We calculated the expected mortality during follow-up using age and sex specific mortality rates of the Dutch population. The ten-year risk of death due to CVD was determined for cases and controls by means of the SCORE risk chart.[25] For patients with a history of a cardiac event, in particular myocardial infarction or CABG, the PREDICT risk score[26] was used to estimate the ten-year risk of death due to CVD. This risk score system was originally developed for estimating the 6-year mortality risk after hospitalization for acute MI or unstable angina. The latter score system is based on factors such as age, ECG at the time of hospitalization, comorbidities and renal function. We estimated the ten-year mortality risk by deducing it from the 6-year mortality. The PREDICT project showed that cancer mortality increased to 15% in 2-6 years. Subsequently, for estimating the ten-year mortality risk due to CVD alone, we corrected the calculated ten-year mortality risk for the 15% of estimated mortality risk due to cancer related mortality. Currently, there

is no risk score for estimating the ten-year risk of mortality due to stroke. A recent cohort study reported the survival of stroke patients in the DUTCH transient ischemic attack Trial. [22] The cumulative risk of death at ten years was 47% for patients who presented with stroke at baseline and 34% for those with TIA. We used these estimations for ten-year mortality in patients with a history of stroke or TIA. Thus, the median (IQR) estimated ten-year risk of death due to a cardiovascular event was assessed using the SCORE and PREDICT risk scores, while the mortality risk in patients with a history of a cerebrovascular event were based on data from the DUTCH transient ischemic attack trial.[22]

Statistical analyses

Continuous data were described as mean (range), and median (interquartile range (IQR)) in case of skewed data, and percentages were given for categorical data. Mean values between patients with atherosclerotic CGI and controls were compared with student's T-test or Mann Whitney U-test and categorical data were compared with Chi-square test or Fisher's exact test. All cause mortality was calculated as standardized mortality ratio as described previously.[24] We calculated the 95 percent confidence interval of the standardized mortality ratio assuming a Poisson distribution of the observed number of deaths and using exact limits. A two-sided p-value below 0.05 was considered significant. Missing data of each variable were not included in the analysis for that specific variable. Crude and adjusted OR's and their 95 % confidence interval were estimated using logistic regression models.

RESULTS

Between January 2006 and September 2009, a total of 195 patients were referred for evaluation of CGI; see Figure 1. A consensus diagnosis of CGI was reached in 131 cases (67% of all referred); 77 (59%) had atherosclerotic CGI and were offered revascularization, After a median follow-up of 19 (interquartile range 9.50-27.50) months the definite diagnosis of atherosclerotic CGI was confirmed in 68 patients, i.e. these patients improved after treatment, and these were used as cases in this study; see Figure 1. The remaining nine patients did not have symptom improvement after revascularization and therefore were not classified as having a definite diagnosis of atherosclerotic CGI. They were excluded from cases. From 68 patients who underwent revascularization: 59 (87%) had percutaneous revascularization, eight (12%) had surgical revascularization, and one (1%) patient died due to acute-on-chronic gastrointestinal ischemia before treatment. Autopsy results showed extensive gastrointestinal arterial atherosclerotic disease. There were no differences in age, gender, history of cardiovascular disease, and risk factors for atherosclerosis between patients who underwent percutaneous revascularization or surgery. Thirty-six (53%) patients had single vessel stenosis, and 32 (47%) patients had multi-vessel stenosis. The controls consisted of 132

subjects. Patients' and controls' characteristics are listed in table 1. Overall, 1.7% and 0.7% of required data concerning CGI cases and controls, respectively, were missing. This in particular pertained to systolic and diastolic blood pressure levels and glucose, total cholesterol, LDL-, HDL- cholesterol, and triglyceride levels.

Table 1 Crude OR's in patients with atherosclerotic CGI and controls

Patient characteristics	CGI n = 68		Controls n = 132		Crude OR	
Age[a]	67	(57-75)	66	(63-72)	0.98	(0.94-1,00)
Female Gender	47	(69%)	63	(%)	2.45	(1.32-4.55)**
Diabetes	14	(21%)	7	(5%)	4.63	(1.77-12.11)**
Hypercholesterolemia	26	(38%)	32	(24%)	1.94	(1.03-3.64)*
Hypertension	46	(68%)	81	(61%)	1.32	(0.71-2.44)
Smoking						
Never	13	(19%)	52	(39%)	1.00	Reference
Former	25	(37%)	17	(13%)	1.91	(0.90-4.02)
Current	30	(44%)	63	(48%)	5.88	(2.48-14.00)***
CVD	35	(52%)	7	(5%)	18.94	(7.72-46.47)***
Family history of CVD	35	(52%)	43	(33%)	2.20	(1.21-4.00)**
Cholesterol lowering agents	32	(47%)	23	(17%)	4.21	(2.19-8.11)***
Anti-hypertensive agents	36	(53%)	39	(30%)	2.68	(1.46-4.92)***
Current BMI (kg/m^2)[a]	21.9	(19.3-24.3)	25.8	(24.1-28.1)	0.76	(0.69-0.84)**
Kidney function[b]						
Normal or mildly decreased	53	(78%)	119	(90%)	Reference	
Low	15	(22%)	13	(10%)	2.59	(1.15-5.83)*
Total cholesterol (mmol/l)	4.3	(3.6-5.0)	5.2	(4.7-5.9)	0.43	(0.31-0.61)***
LDL-cholesterol (mmol/l)	2.2	(1.8-2.8)	3.5	(3.0-4.0))	0.27	(0.17-0.42)***

CVD = cardiovascular disease; BMI= body mass index; OR = odds ratio; OR is presented with 95% CI
Data are presented as mean (range), unless otherwise specified; [a] Median (IQR 25-75); OR are reported with 95 %CI * = P-value < 0.05; ** = P-value < 0.01; *** = P-value < 0.001; [b] Normal or mildly decreased = GFR 60 - > 90 ml/min/ 1·73m^2, Low = GFR < 90 ml/min/ 1·73m^2 Family history of premature cardiovascular disease is defined as cardiovascular disease in a first degree relative younger than 60 years

Univariate and multivariate analysis

The healthy subjects of the DiaGene Study served as a control population in the current study. As mentioned in the Methods section, fasting blood samples were drawn from all controls after recruiting. Five percent of these controls were diagnosed with *de novo* diabetes mellitus at inclusion. Diabetes, current smoking, history of CVD, hypercholesterolemia, family history of CVD, the use of cholesterol lowering agents, and anti-hypertensive agents were positively associated with atherosclerotic CGI. Females are more likely than males to have atherosclerotic CGI. The crude univariate analyses of the distribution of classical risk factors in patients with atherosclerotic CGI and controls are presented in Table 1. The odds ratio's (OR) remained significant for female gender, diabetes and current smoking after further adjustment of the selected risk factors for gender, diabetes, hypercholesterolemia, current smoking, family history of CVD, and kidney function. (see Table 2). The OR for CVD was attenuated after adjustment for gender (OR 21.61, 95% confidence interval 8.40-55.55).

Atherosclerotic CGI cases had a median weight loss prior to referral of 9 kg (IQR 6-12 kg). The median period of weight loss was six (IQR 3.8-12.0) months. The BMI of patients with atherosclerotic CGI was median (IQR) 24.9 (22.2-28.2) (kg/m^2) prior to the start of abdominal complaints, and it decreased to median 21.9 (19.3-24.3) (kg/m^2) at the time of the analysis. LDL cholesterol levels were lower in patients with atherosclerotic CGI compared to controls (see Table 1). Similarly, patients with atherosclerotic CGI had lower systolic blood pressure levels than in the controls, although well within the normal range (data not shown). There was no difference in HDL-cholesterol, triglycerides and glucose between patients with atherosclerotic CGI and controls (data not shown).

There was no difference in the prevalence of risk factors for atherosclerosis between atherosclerotic CGI cases with single- versus multi-vessel stenosis (data not shown). The prevalence of risk factors between patients with atherosclerotic CGI and a history of CVD, and those without a history of CVD is presented in Table 3. Comparing the distribution of risk factors between male and female with atherosclerotic CGI, female had a significant lower BMI than male (median 21.1, IQR 17.6-23.3 vs. 23.5, IQR 21.1-27.2, $P < 0.05$), respectively. There were no other gender differences concerning the prevalence of risk factors.

Mortality risk

The data for estimating the death risk were available in 63 (93%) out of 68 CGI cases, and 124 (94%) out of 132 controls. The ten-year mortality risk due to cardiovascular disease was higher in CGI cases than controls, median (IQR) 8 % (2-28) versus 5% (4-8), $P = 0.06$, respectively. The median (IQR) ten-year mortality risk in CGI cases >55 years was significantly higher than in controls, 15% (5-37) versus 5% (4-8), respectively; $P = 0.001$.

Table 2: Multivariate analysis adjusted for selected risk factors

Patient characteristics	OR[a]	95% CI
Female gender	2.14	(1.05-4.36)*
Diabetes	5.59	(1.95-16.01)**
Hypercholesterolemia	1.81	(0.88-3.74)
Hypertension	1.20	(0.58-2.45)
Smoking		
Never	1.00	Reference
Former	1.71	(0.76-3.88)
Current	5.78	(2.27-14.72)**
Family history of CVD	1.94	(0.98-3.86)
Kidney function		
Normal or mildly decreased	1.00	Reference
Low	2.52	(0.98 -6.49)

CVD = cardiovascular disease; OR = odds ratio; CI = confidence interval
[a] Adjusted for gender, diabetes, hypercholesterolemia, hypertension, current smoker, family history of CVD, and kidney function * = P-value < 0.05; ** P-value < 0.001; Family history of premature cardiovascular disease is defined as cardiovascular disease in a first degree relative younger than 60 years

Table 3: Patient characteristics and risk factors for atherosclerosis in atherosclerotic CGI patients with and without history of CVD

Patient characteristics	With history of CVD		Without history of CVD	
	n = 35		n = 33	
Age, years	70	(62-77)*	65	(51-73)
Male gender	14	(40%)	7	(21%)
Diabetes	12	(34%)†	2	(6%)
Hypercholesterolemia	18	(51%)*	8	(24%)
Hypertension	27	(77%)	19	(58%)
Smoking				
Never	5	(14%)	8	(24%)
Former	14	(40%)	11	(33%)
Current	16	(46%)	14	(43%)
Family history of premature CVD	20	(57%)	15	(46%)
Cholesterol lowering agent	23	(66%)†	9	(27%)
Anti-hypertension agents	24	(69%)†	12	(36%)
Current BMI (kg/m^2)	22.6	(20.3-267)	21.3	(18.0-23.3)
Total cholesterol (mmol/l)	4.3	(3.0-4.8)	4.4	(3.6-5.1)
LDL-cholesterol (mmol/l)	2.1	(1.7-2.8)	2.4	(1.9-3.9)

CVD = cardiovascular disease; BMI= body mass index; Data are presented as median (IQR 25-75), unless otherwise specified; P-values are for controls vs. atherosclerotic CGI; * p < 0.05 † p < 0.01; Family history of premature cardiovascular disease is defined as cardiovascular disease in a first degree relative younger than 60 years

During a mean follow-up of 7.4 (range 0 – 31) months., a total of 10 deaths were observed, this was equal to 116 person-years in the CGI group. Three deaths took place within 3 months after the visit to our clinic as a result of acute gastrointestinal ischemia (percutaneous revascularization =1, surgery = 1, and death before treatment = 1). The remaining seven patients (percutaneous revascularization n = 6, surgery n = 1) died due to cancer (carcinoma of the sinus piriformis (n = 1), adenocarcinoma of the small intestine (n =1), and lung cancer (n =1)), fatal MI (n =1), terminal heart failure (n =2), and from an unknown cause (n =1). The all cause mortality relative to the Dutch population standardized for age and calendar period was 3.55 (95 percent confidence interval, 1.70 to 6.52, P = 0.001). After excluding 3 deaths that took place within 3 months after the visit to our clinic as a result of acute gastrointestinal ischemia, we observed 7 deaths in 116 person years: the SMR of these patients with atherosclerotic CGI was 2.48 (95 percent confidence interval, 1.01- 5.11, P = 0. 025). This severe excess mortality resulted of three patients dying from cancer (carcinoma of the sinus piriformis, adenocarcinoma of the small intestine, and lung cancer), one patient having a fatal MI, two having terminal heart failure, and one dying from an unknown cause.

DISCUSSION

Our study firstly showed that atherosclerotic CGI is associated with classical risk factors for atherosclerosis. Current smoking, diabetes, hypercholesterolemia, history and family history of CVD, and use of cholesterol lowering agents and anti-hypertensive agents are more prevalent in patients with atherosclerotic CGI. After adjusting for multiple risk factors diabetes, current smoking, and previous CVD were significantly associated with atherosclerotic CGI. Female preponderance is confirmed and remains striking. Secondly, our study shows that patients over 55 years of age with atherosclerotic CGI have a high risk of ten-year cardiovascular mortality. This was confirmed during follow-up by severe excess mortality. Patients with atherosclerotic CGI are at risk for CVD, and this advocates the strong need to consider secondary preventive therapy in patients with atherosclerotic CGI.

These results are even more striking considering the fact that most patients lost a substantial amount of weight before the baseline measurement. The measured levels of cholesterol, glucose and blood pressure in patients with atherosclerotic CGI were nearly normal. This difference can partly be explained by marked weight loss in most patients with CGI,[1, 27] as weight loss is the initial treatment option for hypercholesterolemia, hypertension and hyperglycaemia. Moreover, the use of cholesterol lowering agents and anti-hypertensive agents were significantly higher in patients with atherosclerotic CGI comparing to controls. Fifty-two percent of patients with atherosclerotic CGI had known CVD, and therefore they were already treated with cholesterol lowering agents and anti-hypertensive agents. Therefore, weight

loss and higher use of statins in CGI patients, resulted in lower LDL cholesterol levels (see Table 1). Similarly, the weight loss and higher use of anti-hypertensive drugs in CGI patients resulted in lower systolic blood pressure levels than in the controls, although well within the normal range (data not shown).

Previous case series comparing endovascular or surgical treatment for CGI, have reported a wide range of prevalences of risk factors for atherosclerosis. [1, 12-18] The prevalences of risk factors for atherosclerosis has been reported in the baseline characteristics of case series: a 10-93% prevalence of hypercholesterolemia, 64-92% of hypertension, and 36-72% of previous coronary artery disease.[1, 12-18] This may have been due to several factors. Firstly, except for one study,[16] patients with CACS were not excluded from the analysis of data in these study cohorts. Secondly, all studies so far were uncontrolled. We evaluated the risk factors for atherosclerosis systematically at the time of analysis in our study, including medical and family history, and laboratory evaluation. Moreover, we used an age-matched control group to compare the prevalence of risk factors for atherosclerosis.

The healthy control subjects of the DiaGene Study served as a control population in the current study. The controls were selected on absence of previous diabetes, yet 5% was diagnosed with diabetes de novo at inclusion. This DiaGene cohort is comparable in age, BMI, and hypertension to two other population based studies in the Netherlands: the ERGO study [28]which consists of residents of a suburb in Rotterdam, and The Hoorn Study[29] which is a separate cohort of older Dutch subjects. As such, the DiaGene cohort represents the age-matched general Dutch population.

A remarkable predominance of female gender in patients with atherosclerotic CGI was observed. This is in line with earlier reports of larger cohorts of CGI patients.[1, 12, 14, 18] Earlier case series reveal high prevalence CACS in women,[30-32] however we excluded patients with CACS in our study. The female preponderance with comparable CVD risk factors, suggests a female specific anatomical or other susceptibility for ischemia due to intestinal vascular narrowing. Possible explanations for the female preponderance in CGI patients could be that women with chronic abdominal pain are more often referred to medical specialists, or have less potential for collateral circulation and therefore are more prone to develop CGI.

Patients with atherosclerotic CGI had a marked excess mortality during follow-up. The severe excess mortality resulted from CVD and cancer, both related to risk factors for atherosclerosis such as smoking. This observed mortality was in line with the estimated risk scores. The ten-year risk of death in all patients was estimated by combining the SCORE, the PREDICT score, and the DUTCH transient ischemic Trial. For the PREDICT score, missing ECG data at the time of the primary CGI event can have resulted in an underestimation of 10 year mortality risk and

thus have led to a risk underestimation in the cases. By combining the different risk scores, we have estimated that patients with atherosclerotic CGI are at higher risk of cardiovascular mortality than controls. Taken together, the risk scores seem to have produced valid high risk estimations, as illustrated by the observed excess mortality.

This study has several limitations. First, our study is a single center study. Second, patients evaluated for CGI usually represent a highly selected population. Likely, patients who had more severe atherosclerotic disease were more likely to be referred for evaluation. And most clinicians will only consider CGI after exclusion of a variety of other conditions. Our department has a different setting. During the past 6 years we have set up a referral program for patients suspected of CGI. The awareness among referring clinicians and the clinical expertise of our program lowers the threshold for referral and CGI evaluation, and thus reduces the selection process prior to referral. Third, it is a relatively small group for evaluating risk factors for atherosclerosis, the cases included 68 patients with atherosclerotic CGI. Larger studies are therefore needed to confirm these results. And fourth, the observational nature of the study is a limitation, but the incidence of CGI is so low that decades of patient inclusion would be required for a sufficiently powered randomized trial.

Conclusions

Patients with atherosclerotic CGI have a significant risk for cardiovascular mortality, resulting from more prevalent classical risk factors for atherosclerosis. Based on these results and previously reported cases series, we would advocate CVD preventive therapy consisting of personalized life-style optimalization, statins, antihypertensives on indication, and after successful revascularization and recovery of intestinal mucosal low dose aspirin should be considered. Future research, most likely observational, is needed to observe a positive effect of the secondary prevention therapy in these patients.

REFERENCES

1 Mensink PB, van Petersen AS, Geelkerken RH, Otte JA, Huisman AB, Kolkman JJ. Clinical significance of splanchnic artery stenosis. Br J Surg 2006;93:1377-82.

2 Mensink PB, Moons LM, Kuipers EJ. Chronic gastrointestinal ischaemia: shifting paradigms. Gut 2011;60:722-37.

3 Expert Panel on Detection EaToHBCiA. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;285:2486-97.

4 Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, American Heart A, National Heart LaBI. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.

5 A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. Lancet 1996;348:1329-39.

6 Vliegenthart R, Hollander M, Breteler MM, van der Kuip DA, Hofman A, Oudkerk M, Witteman JC. Stroke is associated with coronary calcification as detected by electron-beam CT: the Rotterdam Coronary Calcification Study. Stroke 2002;33:462-5.

7 Cotter G, Cannon CP, McCabe CH, et al. Prior peripheral arterial disease and cerebrovascular disease are independent predictors of adverse outcome in patients with acute coronary syndromes: are we doing enough? Results from the Orbofiban in Patients with Unstable Coronary Syndromes-Thrombolysis In Myocardial Infarction (OPUS-TIMI) 16 study. Am Heart J 2003;145:622-7.

8 Bhatt DL, Peterson ED, Harrington RA, et al. Prior polyvascular disease: risk factor for adverse ischaemic outcomes in acute coronary syndromes. Eur Heart J 2009;30:1195-202.

9 Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. N Engl J Med 1992;326:381-6.

10 Wijnhoud AD, Maasland L, Lingsma HF, Steyerberg EW, Koudstaal PJ, Dippel DW. Prediction of major vascular events in patients with transient ischemic attack or ischemic stroke: a comparison of 7 models. Stroke 2010;41:2178-85.

11 Verouden NJ, Haeck JD, Kuijt WJ, et al. Early ST-segment recovery after primary percutaneous coronary intervention accurately predicts long-term prognosis after acute myocardial infarction. Am Heart J 2010;159:1005-11.

12 Kasirajan K, O'Hara PJ, Gray BH, et al. Chronic mesenteric ischemia: open surgery versus percutaneous angioplasty and stenting. J Vasc Surg 2001;33:63-71.

13 Silva JA, White CJ, Collins TJ, Jenkins JS, Andry ME, Reilly JP, Ramee SR. Endovascular therapy for chronic mesenteric ischemia. J Am Coll Cardiol 2006;47:944-50.

14 Mell MW, Acher CW, Hoch JR, Tefera G, Turnipseed WD. Outcomes after endarterectomy for chronic mesenteric ischemia. J Vasc Surg 2008;48:1132-8.

15 Fioole B, van de Rest HJ, Meijer JR, et al. Percutaneous transluminal angioplasty and stenting as first-choice treatment in patients with chronic mesenteric ischemia. J Vasc Surg 2010;51:386-91.

16 Kruger AJ, Walker PJ, Foster WJ, Jenkins JS, Boyne NS, Jenkins J. Open surgery for atherosclerotic chronic mesenteric ischemia. J Vasc Surg 2007;46:941-5.

17 Oderich GS, Bower TC, Sullivan TM, Bjarnason H, Cha S, Gloviczki P. Open versus endovascular revascularization for chronic mesenteric ischemia: risk-stratified outcomes. J Vasc Surg 2009;49:1472-9 e3.

18 Sarac TP, Altinel O, Kashyap V, et al. Endovascular treatment of stenotic and occluded visceral arteries for chronic mesenteric ischemia. J Vasc Surg 2008;47:485-91.
19 Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010;42:579-89.
20 Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. Dig Dis Sci 2008;53:133-9.
21 Sana A, Vergouwe Y, van Noord D, et al. Radiological imaging and gastrointestinal tonometry add value in diagnosis of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2011;9:234-41.
22 van Wijk I, Kappelle LJ, van Gijn J, et al. Long-term survival and vascular event risk after transient ischaemic attack or minor ischaemic stroke: a cohort study. Lancet 2005;365:2098-104.
23 Levey AS, Coresh J, Balk E, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 2003;139:137-47.
24 Sijbrands EJ, Westendorp RG, Defesche JC, de Meier PH, Smelt AH, Kastelein JJ. Mortality over two centuries in large pedigree with familial hypercholesterolaemia: family tree mortality study. BMJ 2001;322:1019-23.
25 Conroy RM, Pyorala K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003;24:987-1003.
26 Jacobs DR, Jr., Kroenke C, Crow R, Deshpande M, Gu DF, Gatewood L, Blackburn H. PREDICT: A simple risk score for clinical severity and long-term prognosis after hospitalization for acute myocardial infarction or unstable angina: the Minnesota heart survey. Circulation 1999;100:599-607.
27 Brandt LJ, Boley SJ. AGA technical review on intestinal ischemia. American Gastrointestinal Association. Gastroenterology 2000;118:954-68.
28 Gloviczki P, Duncan AA. Treatment of celiac artery compression syndrome: does it really exist? Perspect Vasc Surg Endovasc Ther 2007;19:259-63.
29 Grotemeyer D, Duran M, Iskandar F, Blondin D, Nguyen K, Sandmann W. Median arcuate ligament syndrome: vascular surgical therapy and follow-up of 18 patients. Langenbecks Arch Surg 2009;394:1085-92.
30 Mensink PB, van Petersen AS, Kolkman JJ, Otte JA, Huisman AB, Geelkerken RH. Gastric exercise tonometry: the key investigation in patients with suspected celiac artery compression syndrome. J Vasc Surg 2006;44:277-81.

Chapter 6

Revascularization of a Single Gastrointestinal Artery Stenosis should be considered in Patients with Unexplained Refractory Chronic Gastrointestinal Symptoms

Leon M.G. Moons[1], Aria Sana[1], Sjoerd F.M. Jenniskens[2], Adriaan Moelker[2], Pieter Dewint[1], Désirée van Noord[1], Hence J.M. Verhagen[3], Peter B.F. Mensink[1], Ernst J. Kuipers[1], Ellen V. Rouwet[3]

Departments of Gastroenterology and Hepatology, department of interventional Radiology, and department of Vascular Surgery, Erasmus MC - University Medical Center, Rotterdam, the Netherlands

Submitted

ABSTRACT

Objective: A proportion of patients with unexplained gastrointestinal symptoms may suffer from chronic gastrointestinal ischemia (CGI) secondary to a single gastrointestinal artery stenosis. We therefore evaluated the success rates of revascularization of either the celiac or superior mesenteric artery in patients with unexplained gastrointestinal symptoms and confirmed mucosal ischemia.

Methods: In this prospective cohort study, all patients with unexplained gastrointestinal symptoms referred to our hospital for analysis of CGI, had a standard work-up consisting of history taking and physical examination, imaging of gastrointestinal arteries, and evaluation for mucosal ischemia with either tonometry or visible light spectroscopy. A consensus diagnosis of CGI was made after a multidisciplinary assessment of the results by gastroenterologists, vascular surgeons, and radiologists. Patients with a consensus diagnosis of CGI were offered endovascular or surgical revascularization, as appropriate. Clinical response was defined as >50% relief of symptoms experienced by the patient.

Results: A consensus diagnosis of CGI was made in 71/103 patients. Follow-up after revascularization was available in 68 patients (median follow-up 19 ± 13 months). The response rate to revascularization was 46/68 (67%). A non-H. pylori, non-NSAID related gastric ulcer, and a symptom pattern compatible with dyspepsia were identified as predictors of a positive response to revascularization. An IBS symptom pattern and BMI > 30 were negative predictors.

Conclusion: Unexplained gastrointestinal symptoms should be evaluated for CGI, and those with a single gastrointestinal artery stenosis and confirmed mucosal ischemia may greatly benefit from revascularization.

INTRODUCTION

Gastrointestinal symptoms are common in the general population. Although the vast majority of patients do not seek medical evaluation for their symptoms, a considerable group of patients however do so, utilizing a significant proportion of health care resources. Failure to respond to empirical therapy, or the presence of alarm features such as unexplained weight loss, nocturnal symptoms, suggestive family history, or age, help in selecting patients for additional investigations such as blood and stool tests, endoscopy with or without biopsies, or additional imaging[1, 2, 3]. Following the proposed diagnostic algorithms[1, 2, 3], a majority of patients is diagnosed with a functional gastrointestinal syndrome such as functional dyspepsia or irritable bowel syndrome. Functional disorders are defined by the presence of a specific pattern of symptoms attributed to a specific region of the gastrointestinal tract, in the absence of any structural, systemic, or metabolic disease likely to explain the symptoms. In daily practice, managing patients with investigated functional disorders who are refractory to the initial management strategies can be challenging.

Not uncommonly, an isolated stenosis of the celiac artery (CA) or the superior mesenteric artery (SMA) is detected in patients with gastrointestinal symptoms. This finding presents a clinical challenge, for the current paradigm is that a single stenosis of the CA or the SMA is unlikely to cause chronic gastrointestinal ischemia given the abundant collateral circulation of the splanchnic vascular bed. Furthermore, stenoses of a single gastrointestinal artery are highly prevalent in the general -asymptomatic- population, ranging from 10-20%. [4, 5, 6, 7, 8, 9] The celiac artery is by far the most frequently affected artery, being the site of a single artery stenosis in 80%.[4] Currently, a single artery stenosis is generally not considered to be a structural disease likely to explain the gastrointestinal symptoms. Hence, revascularization procedures are usually not offered to patients with unexplained abdominal symptoms and a single artery stenosis.

This concept is currently being challenged, given the promising clinical success rates that have been reported in some studies for celiac artery revascularization in patients with the celiac artery compression syndrome (CACS), i.e. external compression of the celiac artery by the median arcuate ligament. The considerable variation in the reported success rates of revascularization in patients with CACS may be greatly related to patient selection for treatment. [10, 11, 12, 13] Indeed, the symptoms of chronic gastrointestinal ischemia are aspecific and no single symptom can predict who will respond to revascularization. [14] However, it has recently been shown that assessment of mucosal ischemia with a functional test, being either tonometry or visible light spectroscopy, significantly enhances the diagnostic accuracy of detecting gastrointestinal ischemia and, hence, increases the clinical success rate of revascularization. [15, 16, 17]

Based on these new insights, we propose that patients with confirmed mucosal ischemia may benefit from revascularization of a single gastrointestinal artery stenosis. We therefore evaluated the clinical success rates to revascularization of a stenosis of either the celiac artery or superior mesenteric artery in patients with unexplained refractory gastrointestinal symptoms.

METHODS

Study population

Patients referred to our tertiary care center in a period from January 2006 to October 2010 for analysis of chronic gastrointestinal ischemia based on a single artery stenosis in either the CA or the SMA were prospectively included in this study. Approval for the study was obtained from the Medical Ethical Committee of the Erasmus MC University Medical Center Rotterdam, The Netherlands.

Baseline evaluation

At baseline, all patients had a standardized diagnostic work-up consisting of history taking and physical examination, imaging of the gastrointestinal arteries with either CT- or MR-angiography and/or conventional catheter angiography, and testing for mucosal ischemia with either 24-hour tonometry or visible light spectroscopy [14, 15, 16]. An upper endoscopy was performed in all patients with standard biopsies of the duodenum, gastric antrum, and corpus. Biopsies from the gastric corpus and antrum were used to detect *Helicobacter pylori*. Patient demographics analysis included age, gender, BMI at presentation, baseline and follow-up symptoms such as pain (abdominal, postprandial and/or exercise-related), bowel dysfunction (diarrhea or constipation), weight loss (in kg), bruit, ulcer observed at upper endoscopy, past medical history, and risk factors for atherosclerosis (current or past tobacco smoking, diabetes, hypertension, dyslipidemia, and positive family history for cardiovascular disease). Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic pressure >90 mmHg. Dyslipidemia was defined as LDL-C >4.2 mmol/L or HDL-C <0.9 mmol/L. Additional blood tests included thyroid stimulating hormone (TSH), C-reactive protein, lactate, calcium, complete blood count, electrolytes, and liver functions tests.

Diagnosis

During a multidisciplinary assessment of the diagnostic results by a gastroenterologist, vascular surgeon and radiologist, a consensus diagnosis of chronic gastrointestinal ischemia was made if i) the presence of a significant (≥50%) single artery stenosis of either the CA or the SMA was confirmed, ii) the patient had at least one of the following symptoms: (postprandial) abdominal pain, weight loss, diarrhoea, and iii) mucosal ischemia was detected by

24-hour tonometry or visible light spectroscopy. In patients with CA stenosis, the diagnosis of CACS was established if CTA demonstrated focal narrowing of the proximal celiac artery with poststenotic dilatation and indentation on the superior aspect of the celiac artery, creating a hook-shaped contour of the celiac artery. The characteristic kinking in the absence of calcifications distinguishes this condition from other causes of celiac artery stenosis such as atherosclerosis[19]. Since these imaging features are exaggerated on expiration, an additional catheter angiography of the celiac artery in inspiration and expiration was performed in unclear cases. Only patients with a consensus diagnosis of chronic gastrointestinal ischemia were treated by either surgical or endovascular revascularization. Patients not meeting the criteria for the diagnosis of chronic gastrointestinal ischemia were sent back to the referring physician.

Treatment

Patients with atherosclerotic stenosis of the CA or SMA were primarily treated by percutaneous transluminal angioplasty (PTA) with endovascular stent placement (Palmaz Blue, Cordis Cooperation). Recurrent in-stent stenoses were treated by PTA. Patients with recurrent restenoses or stenoses deemed unfit for primary endovascular revascularization (e.g., occlusion) were treated by bypass surgery. The majority of patients diagnosed with CACS were treated by surgical division of the median arcuate ligament to decompress the celiac artery. A small number of patients with CACS were treated by PTA and stenting of the CA. Procedural details collected were the specific vessel treated, type of treatment, access location, as well as procedure-related morbidity and mortality.

Follow-up

The effects of treatment were evaluated at 3-month intervals using a standard questionnaire. If symptoms recurred, the date of recurrence was registered. Stent patency was evaluated with abdominal ultrasound 3-6 months following the primary procedure. Patients who presented with recurrent symptoms during follow-up were evaluated with CTA to assess the patency of the stent or bypass.

Outcome measures

The primary outcome measure was response of dyspeptic symptoms to revascularization, defined as at least 50% relief of presenting symptoms as experienced by the patient. Patients were classified as responders, primary non-responders, or loss of responders. Responders were defined as either a sustained positive response to treatment or if symptom recurrence was relieved after treatment of an observed restenosis. Primary non-responders were defined as patients who did not respond to revascularization in spite of adequate patency of the treated artery at follow-up. Loss of response was defined as the recurrence of symptoms in spite of patency of the stent or bypass at follow-up. Other outcome measures were interven-

tion-related morbidity and mortality, primary and secondary patency of stent or bypass, and symptom free survival.

Statistical analysis

Depending on the distributional properties, outcome measures were expressed as means ±SD. Outcome of therapy was analysed with the student's t-test. Differences in baseline characteristics were determined by the χ^2 test. Univariate analysis for calculation of predictors of response was performed with logistic regression. Statistical significance was defined as $p < 0.05$.

RESULTS

Patient characteristics

A total of 103 patients with unexplained gastrointestinal symptoms and a single artery stenosis were analysed. All patients underwent an upper endoscopy during the standard work-up prior to the measurement of mucosal ischemia with VLS or tonometry. A non-H. pylori, non-NSAID ulcer was observed in four patients (4%). Eight patients were referred for remaining symptoms after *Helicobacter pylori* eradication. Biopsies taken at upper endoscopy during work-up showed H. pylori in only one of these patients. Seventy-six percent of the patients used or have used a PPI for a minimal period of 2 weeks. NSAIDs were used by 37% of patients. A colonoscopy was performed in 56/103 (54%) ranging from 43% in patients with dyspeptic symptoms to 69% in patients with symptoms compatible with IBS. Blood and stool tests were unrevealing. Abdominal imaging consisting of CT/MRI was performed in 82% of cases. Analysis of all previous investigations excluded more common disorders as the cause of the gastrointestinal symptoms.

Of the 103 patients, 93 presented with a CA stenosis and 10 with an SMA stenosis. The etiology of the CA stenosis was atherosclerosis in 53 patients (57%), celiac artery compression syndrome (CACS) in 36 patients (39%), Takayasu's arteritis in one patient (1%), fibromuscular dysplasia in one patient (1%), and unknown in two patients (2%). All cases of SMA stenosis were atherosclerotic. A consensus diagnosis of chronic gastrointestinal ischemia was reached in 73/103 patients (71%). With regard to the stenotic lesions, 37/54 patients (69%) presenting with an atherosclerotic CA stenosis, 24/35 patients (69%) with CACS, 4/4 patients (100%) with a CA stenosis of other etiology, and 8/10 patients (80%) with an SMA stenosis were diagnosed with chronic gastrointestinal ischemia.

Primary treatment

A total of 69/73 (95%) patients diagnosed with ischemia were treated. Follow-up was available for 68 patients. The characteristics of these patients are provided in Table 1. Of the four patients who were not treated, three patients refused treatment, and one had an underlying condition which was treated first with good effect on the symptoms (Waldenström's disease). Of the 35 patients with an atherosclerotic CA stenosis, 33 (94%) patients were treated with CA stenting and two (6%) patients received an antegrade aortohepatic bypass after a technically unsuccessful endovascular procedure. Twenty-two patients diagnosed with CACS were treated by surgical division of the median arcuate ligament to decompress the celiac artery.

Table 1. Baseline characteristics of patients diagnosed with chronic gastrointestinal ischemia

	All patients diagnosed with CGI receiving revascularization treatment (n=68)		All non-CGI patients (n=54)[#]	Responders versus all non-CGI patients	
	Responders (n=46)	Non-responders (n=22)		*p*-value	OR (95%CI)
Patient characteristics					
Age (y)	57 ± 17	57 ± 15	58 ± 17	0.893	1.00 (0.98-1.02)
Female	57%	73%	67%	0.385	1.43 (0.64-3.20)
Hypertension	27%	18%	37%	0.215	0.58 (0.25-1.37)
Smoking	51%	41%	41%	0.299	1.52 (0.69-3.34)
Dyslipidemia	24%	27%	41%	0.195	0.91 (0.65-1.26)
Diabetes	6.5%	4.5%	4%	0.334	2.36 (0.41-13.52)
BMI (kg/m^2)	22 ± 3.4	23 ± 4.6	24 ± 5.0	0.519	0.90 (0.82-1.0)
Obesity (BMI >30)	2%	5%	17%	0.015*	0.11 (0.01-0.89)
CVD	44%	32%	39%	0.775	1.12 (0.51-2.48)
Family history of CVD	44%	68%	57%	0.282	1.07 (0.71-1.61)
Presenting symptoms					
Abdominal pain	91%	100%	94%	0.580	0.65 (0.14-3.05)
Postprandial pain	72%	86%	72%	0.877	0.93 (0.39-2.21)
Exercise related pain	48%	55%	37%	0.368	1.44 (0.65-3.18)
Nausea	46%	36%	43%	0.742	1.14 (0.52-2.50)
Diarrhea	22%	27%	26%	0.545	0.75 (0.30-1.90)
Constipation	9%	18%	17%	0.216	0.46 (0.13-1.59)
Reporting weight loss	63%	68%	59%	0.738	1.15 (0.52-2.54)
Weight loss (kg)	7 ± 7.2	7 ± 6.6	6 ± 6.0	0.501	1.02 (0.96-1.08)
Abdominal bruit	22%	9%	13%	0.265	1.8 (0.63-5.22)
Classic triad of CGI	16%	9%	7%	0.228	2.19 (0.60-8.00)
Gastric ulcer	9%	0%	0%	0.028*	2.26 (1.81-2.83)

Data are presented as percentages or as mean ± SD. Differences were determined by the χ^2 test and univariate odd ratio's were calculated with logistic regression. Statistical significance was defined as $p < 0.05$ (*). Hypertension was defined as systolic blood pressure > 140 mmHg and/or diastolic pressure > 90 mmHg. Dyslipidemia was defined as LDL > 4.2 mmol/L and/or HDL < 0.9 mmol/L. CVD = cardiovascular disease; CGI = chronic gastrointestinal ischemia; non-CGI patients were those who did not receive the consensus diagnosis of CGI or those patients who received the consensus diagnosis of CGI but who had no response to revascularization treatment

The patient with CA stenosis due to fibromuscular dysplasia was treated with PTA only. The patient with Takayasu's arteritis was treated with CA stenting. Of the two patients with a CA stenosis of unknown etiology, one was treated with PTA and the other with CA stenting. All patients with an SMA stenosis were treated with SMA stenting. The overall technical success rates for endovascular therapy or surgical revascularization were 95% and 100%, respectively. As mentioned, the two patients with an unsuccessful endovascular procedure received an antegrade aortohepatic bypass.

Clinical success

Of the 68 revascularized patients with follow-up, 53 (78%) responded after primary treatment (median follow-up of 19 ± 13 months). However, during follow-up 12 patients developed recurrent symptoms, lowering the primary clinical success rate to 60%. Of the patients with a loss of response, 7/12 showed a restenosis, which was treated in five patients (3 surgical bypasses and 2 CA stents). This second attempt of revascularization was clinically successful in three patients. Fifteen patients did not have instant symptom relief to the first treatment. Six of these patients had a persistent stenosis at follow-up, which was treated in five patients (2 CA stents, 2 bypasses, and 1 CACS release). This treatment was clinically successful in an additional two patients. The overall secondary success rate was 46/68 (68%).

The clinical success rates according to the site of stenosis are provided in Table 2. The success rate for revascularization of the SMA was slightly, though not significantly, higher than the success rate for revascularization of the CA (75% vs 67%, OR 1.8 95%CI 0.5-6.8; p=0.636). There was no difference in clinical success rates between treatment of an atherosclerotic CA stenosis or CACS (both 68%). If a second procedure was attempted in case of a restenosis, the clinical response rate was 5/10 (50%). Success of revascularization was not dependent on the use of PPI, NSAIDs or H. pylori status.

Table 2. Clinical success rates for single gastrointestinal artery revascularization

Location	Clinical success rate
Celiac artery	
Atherosclerosis	23/34 (68%)
Celiac artery compression syndrome	15/22 (68%)
Non-specified	2/4 (50%)
Overall	40/60 (67%)
Superior mesenteric artery	6/8 (75%)
Overall	46/68 (67%)

Patency

After a median follow-up of 19 ± 13 months, restenosis occurred in 13 patients (22%) after CA revascularization, whereas none was observed after SMA revascularization. Restenosis was accompanied by stent fracture (n=6; 18% of all primary stenting procedures), intimal hyperplasia/thrombus (n=3), a remaining CA stenosis after CACS release (n=2), bypass graft thrombosis (n=1), or following PTA without stent placement (n=1). In 3/6 patients stent fracture in the CA was due to previously unrecognised CACS. The overall restenosis rate was 29% after primary endovascular revascularization, and 13% after primary surgical revascularization.

A total of 11 secondary procedures were performed for recurrent symptomatic stenosis of the CA. Five of the six patients with CA stent fracture were treated by aortohepatic bypass surgery. The three patients with in-stent restenosis due to intimal hyperplasia/thrombus were treated by in-stent PTA. The two patients with persistent CA stenosis after CACS release were treated by CA stenting. Thrombosis of one of the two primary aortohepatic bypass grafts required repeated thrombolysis and PTA of a stenosis at the distal anastomosis. The overall primary and secondary patency rates were 80% and 91%, respectively (median follow-up of 17 ± 14 months).

Complications

Complications were reported in 26/68 (38%) of treated patients (Table 3). There was no procedure related mortality (death within 30 days of the procedure). During follow-up six patients died due to metastasized pulmonary carcinoma (n=4), stroke (n=1), or old age (88 yrs; n=1). The brachial artery approach (n=32) for endovascular therapy was associated with a higher complication rate as compared to the femoral artery approach (n=13; 44% vs 15%, OR 4.3 95%CI 0.8- 22.7; *p*=0.085) and included two stroke.

DISCUSSION

This study demonstrates that patients with otherwise unexplained gastrointestinal symptoms, a significant stenosis of either the celiac artery or the superior mesenteric artery, and confirmed mucosal ischemia may greatly benefit from revascularization of a single gastrointestinal artery. These findings offer new opportunities to treat this challenging patient population.

Gastrointestinal ischemia as a consequence of stenosis of a single gastrointestinal artery is considered to be rather uncommon. Furthermore, the prevalence of a single artery stenosis in the asymptomatic population ranges from 10-20%, [6, 7, 8, 20] depending on the age,[4] ethnic background,[6] and the presence of vascular stenosis elsewhere in the body. [21, 22]

Table 3. Complications of gastrointestinal artery revascularization

Complication	Endovascular (n=43)	Surgical (n=36)
Respiratory		
Pneumonia	1	2
Pneumothorax	-	1
Cardiovascular		
Stroke	2	-
Atrial fibrillation	-	1
Abdominal		
Ileus	-	2
Bowel perforation	-	1
Post-operative bleeding	-	1
Splenic infarction	1	
Access site		
Hematoma	8	
Dissection	1	
Pseudoaneurysm	2	
Thrombosis	2	
AV-fistula	1	
Nerve damage	2	
Allergic reaction	1	1
Gastroesophageal reflux		1

Detecting a single artery stenosis in patients with abdominal symptoms may thus be merely a coincidental finding, and identifying those patients who will benefit from revascularization of a single artery stenosis remains a clinical challenge. [11, 13, 23] Careful patient selection is the key to success of revascularization treatment in this patient population. Although high success rates may be achieved if only patients with the classic triad of symptoms, i.e. postprandial pain, bruit, and severe weight loss, are revascularized, [12] the prevalence of this classic triad in the present study was only 16% in responders and 9% in non-responders. Thus, in line with previous findings [16], the majority of patients who respond to revascularization lack the supposedly classic triad of symptoms. Therefore, selection of patients for revascularization solely based on symptoms results in under- or overtreatment.

With a lack of clinical, endoscopic[24], and radiologic predictors of successful outcome of revascularization of single artery stenoses, and the absence of a discriminative biochemical test, functional tests for mucosal ischemia are pivotal in the diagnostic work-up of patients suspected of chronic gastrointestinal ischemia. The use of functional tests such as tonometry or visible light spectroscopy substantially improves the ability to discriminate between patients who respond to revascularization treatment and those who do not [14, 15, 16, 25]. Gastrointestinal tonometry appears to be an accurate functional test to detect gastrointestinal ischemia, with a sensitivity and specificity of 78% and 92%, respectively [16, 25, 26]. An alternative for tonometry is visible light spectroscopy, with a 90% sensitivity and a 60% specificity for detecting mucosal ischemia [15, 27]. As compared with tonometry, visible light

spectroscopy is less invasive, less time-consuming, and therefore more patient friendly. In our study, treatment success of patients selected with a positive tonometry test was slightly, though not significantly, higher than for a positive spectroscopy test (73% vs 60%). This may be related to the higher specificity of tonometry as compared to spectroscopy for the detection of mucosal ischemia[15, 25].

In the present study, a functional test for mucosal ischemia was part of the standard diagnostic work-up for all patients presenting with abdominal symptoms and a single gastrointestinal artery stenosis. Interestingly, using this approach, selection of patients for revascularization based on a positive test for mucosal ischemia resulted in relief of symptoms in two-thirds of patients. This challenges the current belief that single artery stenoses cannot be symptomatic and should not be revascularized.

It is unknown whether the non-responding patients have symptoms attributable to other gastrointestinal disorders, or whether the mucosal ischemia in these patients is not resolved by revascularization of a macrovascular stenosis. In previous studies we have shown that the functional test improves in 80-100% of patients who responded to revascularization, whereas no improvement in muocal oxygen saturation was observed in patients who did not experience relief of symptoms despite patent arteries.[15, 17] Mucosal ischemia may persist in a subset of patients after successful revascularization, due to a coinciding pathophysiologic mechanism, such as microcirculatory insufficiency. However, strong evidence to substantiate this hypothesis is currently lacking.

Goals of treatment are symptom relief and restoration of normal weight and nutritional status. In contrast to chronic gastrointestinal ischemia based on stenosis of $^{3}2$ gastrointestinal arteries, single artery stenosis is not associated with the progression to acute gastrointestinal ischemia.[28] Hence, the burden of symptoms must be weighed against the risks of therapeutic intervention. Technical success, patency, and complication rates reported in the current study were comparable to those previously reported.[16, 29] Although both the surgical and the endovascular revascularization procedures carried no mortality in this study, the overall morbidity rate was 38%. The majority of the complications were access-related and resolved without persistent consequences, but 4 patients (6%) developed irreversible neurological damage. The brachial artery approach is considered to be an anatomical advantageous route to the origins of the gastrointestinal arteries. However, the brachial approach was associated with more, albeit not significantly, severe complications than the femoral approach in patients undergoing endovascular revascularization. Given these potential complications of revascularization procedures, conservative measures such as frequent small meals, use of PPI, and analgesics, are advised as the first management option for patients with only mild

symptoms of chronic gastrointestinal ischemia based on a single artery stenosis, whereas revascularization procedures should be reserved for the patients with a high disease burden.

Earlier reports on single artery stenoses have mainly focussed on the celiac artery compression syndrome (CACS). This syndrome is characterized by external compression of the celiac artery at the level of the diaphragm by the median arcuate ligament or celiac plexus, limiting blood flow particularly during expiration. [30] For CACS, there is still a debate whether the pain is of neurogenic or vascular origin. Some consider the pain originating from compression of the periarterial celiac nerve plexus, in which case patients may benefit from the neurolysis and transection of the periaortic ganglionic tissue during open CACS release.[31, 32] However, the similarities in success rates for retroperitoneal endoscopic release of CACS [33], in which most of the celiac plexus remains intact, for open surgical CACS release, and for endovascular revascularization of atherosclerotic CA stenoses, supports the vascular origin of the symptoms.

This study has several limitations. First, it was conducted in a tertiary care centre for vascular disorders of the gastrointestinal tract. The consensus diagnosis of chronic gastrointestinal ischemia was reached in 71% of the studied patient population. Given the potential for referral bias, the results must be interpreted with caution and may not be extrapolated to all general gastroenterology practices. Second, a placebo effect of single artery revascularization cannot be ruled out in this prospective cohort study. However, the success percentage of 67% is much higher than the placebo effect observed in controlled studies of functional gastrointestinal disorders.[34, 35]

In summary, the findings of the present study show that patients with unexplained refractory gastrointestinal symptoms should be evaluated for gastrointestinal ischemia, and that those with a single gastrointestinal artery stenosis and confirmed mucosal ischemia may greatly benefit from revascularization.

REFERENCES

1 Tack J, Talley NJ. Gastroduodenal disorders. *Am J Gastroenterol* 2010;105:757-63.
2 Spiller RC, Thompson WG. Bowel disorders. *Am J Gastroenterol* 2010;105:775-85.
3 Sperber AD, Drossman DA. Functional abdominal pain syndrome: constant or frequently recurring abdominal pain. *Am J Gastroenterol* 2010;105:770-4.
4 Roobottom CA, Dubbins PA. Significant disease of the celiac and superior mesenteric arteries in asymptomatic patients: predictive value of Doppler sonography. *AJR Am J Roentgenol* 1993;161:985-8.
5 Jarvinen O, Laurikka J, Sisto T, *et al.* Atherosclerosis of the visceral arteries. *Vasa* 1995;24:9-14.
6 Park CM, Chung JW, Kim HB, *et al.* Celiac axis stenosis: incidence and etiologies in asymptomatic individuals. *Korean J Radiol* 2001;2:8-13.
7 Bron KM, Redman HC. Splanchnic artery stenosis and occlusion. Incidence; arteriographic and clinical manifestations. *Radiology* 1969;92:323-8.
8 Hansen KJ, Wilson DB, Craven TE, *et al.* Mesenteric artery disease in the elderly. *J Vasc Surg* 2004;40:45-52.
9 Derrick JR, Pollard HS, Moore RM. The pattern of arteriosclerotic narrowing of the celiac and superior mesenteric arteries. *Ann Surg* 1959;149:684-9.
10 Lord RS, Tracy GD. Coeliac artery compression. *Br J Surg* 1980;67:590-3.
11 Geelkerken RH, van Bockel JH, de Roos WK, *et al.* Coeliac artery compression syndrome: the effect of decompression. *Br J Surg* 1990;77:807-9.
12 Reilly LM, Ammar AD, Stoney RJ, *et al.* Late results following operative repair for celiac artery compression syndrome. *J Vasc Surg* 1985;2:79-91.
13 Evans WE. Long-term evaluation of the celiac band syndrome. *Surgery* 1974;76:867-71.
14 Sana A, Vergouwe Y, van Noord D, *et al.* Radiological imaging and gastrointestinal tonometry add value in diagnosis of chronic gastrointestinal ischemia. *Clin Gastroenterol Hepatol* 2011;9:234-41.
15 Van Noord D, Sana A, Benaron DA, *et al.* Endoscopic visible light spectroscopy: a new, minimally invasive technique to diagnose chronic GI ischemia. *Gastrointest Endosc* 2011;73:291-8.
16 Mensink PB, van Petersen AS, Geelkerken RH, *et al.* Clinical significance of splanchnic artery stenosis. *Br J Surg* 2006;93:1377-82.
17 Mensink PB, van Petersen AS, Kolkman JJ, *et al.* Gastric exercise tonometry: the key investigation in patients with suspected celiac artery compression syndrome. *J Vasc Surg* 2006;44:277-81.
18 Drossman DA, Corazziari E, Delvaux M, *et al.* Rome III: The Functional Gastrointestinal Disorders. In: McLean VA, ed.: Degnon Associates 2006:885-93.
19 Horton KM, Talamini MA, Fishman EK. Median arcuate ligament syndrome: evaluation with CT angiography. *Radiographics* 2005;25:1177-82.
20 Levin DC, Baltaxe HA. High incidence of celiac axis narrowing in asymptomatic individuals. *Am J Roentgenol Radium Ther Nucl Med* 1972;116:426-9.
21 Valentine RJ, Martin JD, Myers SI, *et al.* Asymptomatic celiac and superior mesenteric artery stenoses are more prevalent among patients with unsuspected renal artery stenoses. *J Vasc Surg* 1991;14:195-9.
22 Glockner JF. Incidental findings on renal MR angiography. *AJR Am J Roentgenol* 2007;189:693-700.
23 Mihas AA, Laws HL, Jander HP. Surgical treatment of the celiac axis compression syndrome. *Am J Surg* 1977;133:688-91.
24 Van Noord D, Biermann K, Moons LM, *et al.* Histological changes in patients with chronic upper gastrointestinal ischaemia. *Histopathology* 2010;57:615-21.

25 Otte JA, Geelkerken RH, Oostveen E, *et al.* Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. *Clin Gastroenterol Hepatol* 2005;3:660-6.
26 Otte JA, Huisman AB, Geelkerken RH, *et al.* Jejunal tonometry for the diagnosis of gastrointestinal ischemia. Feasibility, normal values and comparison of jejunal with gastric tonometry exercise testing. *Eur J Gastroenterol Hepatol* 2008;20:62-7.
27 Friedland S, Benaron D, Coogan S, *et al.* Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. *Gastrointest Endosc* 2007;65:294-300.
28 Wilson DB, Mostafavi K, Craven TE, *et al.* Clinical course of mesenteric artery stenosis in elderly americans. *Arch Intern M*ed 2006;166:2095-100.
29 Oderich GS, Bower TC, Sullivan TM, *et al.* Open versus endovascular revascularization for chronic mesenteric ischemia: risk-stratified outcomes. *J Vasc Surg* 2009;49:1472-9 e3.
30 Loukas M, Pinyard J, Vaid S, *et al.* Clinical anatomy of celiac artery compression syndrome: a review. *Clin Anat* 2007;20:612-7.
31 Harjola PT. A Rare Obstruction of the Coeliac Artery. Report of a Case. *Ann Chir Gynaecol Fenn* 1963;**52**:547-50.
32 Snyder MA, Mahoney EB, Rob CG. Symptomatic celiac artery stenosis due to constriction by the neurofibrous tissue of the celiac ganglion. *Surgery* 1967;61:372-6.
33 van Petersen AS, Vriens BH, Huisman AB, *et al.* Retroperitoneal endoscopic release in the management of celiac artery compression syndrome. *J Vasc Surg* 2009;50:140-7.
34 Talley NJ, Tack J, Ptak T, *et al.* Itopride in functional dyspepsia: results of two phase III multicentre, randomised, double-blind, placebo-controlled trials. *Gut* 2008;57:740-6.
35 Wong WM, Wong BC, Hung WK, *et al.* Double blind, randomised, placebo controlled study of four weeks of lansoprazole for the treatment of functional dyspepsia in Chinese patients. *Gut* 2002;51:502-6.

Chapter 7

Vasodilation therapy for patients with non-occlusive chronic gastrointestinal ischemia: a prospective pilot-study

A. Sana MD[1], L.M.G. Moons MD PhD[1], E.V. Rouwet MD PhD[2], T.W. Galema MD PhD[3], A. Moelker MD PhD[4] D. Van Noord MD PhD[1], P.B.F. Mensink MD PhD[1], Ernst J. Kuipers MD PhD[1,5]

Departments of Gastroenterology and Hepatology[1], Vascular Surgery[2], Cardiology[3], Interventional Radiology[4], Internal Medicine[5], Erasmus MC - University Medical Center, Rotterdam, The Netherlands

Provisionally accepted in World Journal of Gastroenterology 2012

ABSTRACT

Aim: To evaluate therapeutic effect of vasodilating medication in patients with mucosal ischemia and patent gastrointestinal arteries (non-occlusive chronic gastrointestinal ischemia).

Methods: Patients referred to our tertiary unit for evaluation of CGI were prospectively included. Patients were diagnosed with non-occlusive CGI if both symptoms and functional testing were compatible with CGI, but gastrointestinal artery stenoses were absent. Patients were treated with escalating doses of isosorbide dinitrate (ISD; 20 or 40 mg od), followed by ketanserin (KTS; 20 or 40 mg od) if ISD was not successful or tolerated. Clinical response was defined as complete or partial relief of predominant presenting symptoms.

Results: Over a period of 3.5 years, 353 patients were referred with suspected CGI. Non-occlusive CGI was diagnosed in 47 (13%) patients: 17 (36%) males, mean age 57 (29-77) years. Vasodilation therapy was advised in 39 (83%) patients. One patient refused using ISD. Sustained response was achieved in 5 (13%) patients treated with ISD (median follow-up 29 months). Of 33 non-responders to ISD, 27 (82%) were treated with KTS. One patient was lost during follow-up. Two (8%) of them had a sustained response (median follow-up 11 months) after KTS. Two other patients had repeated positive response after reintroducing ISD. Per protocol analysis showed an overall therapeutic response of (9/31) 29%.

Conclusion: Vasodilating medication is of benefit to one third of patients with non-occlusive CGI. RCT's are needed to optimize effectiveness of vasodilating agents, and predict therapeutic response.

INTRODUCTION

In recent years chronic gastrointestinal ischemia (CGI) has gained recognition as one of the causes of otherwise unexplained chronic abdominal pain[1]. Ischemia is the result of diminished oxygen supply to target organs due to obstructive disease of the arteries and veins as well as due to systemic low flow state, anemia or pulmonary disease. Intestinal ischemia is caused by insufficient blood flow to the stomach and intestines, which in most cases is caused by significant atherosclerotic narrowing of the main gastrointestinal arterial branches.[1-4] However, the introduction of functional tests to evaluate mucosal perfusion, such as gastrointestinal tonometry[2,4] and visible light spectroscopy[5-6], has shown that the spectrum of CGI may also include patients without macrovascular pathology, i.e. non-occlusive CGI.

Non-occlusive mesenteric ischemia (NOMI) is a common phenomenon encountered in ICU patients, which is thought to be caused by prolonged splanchnic hypoperfusion. The exact mechanisms leading to this condition are not clearly understood, and it is observed that many different pathologic conditions can be complicated by NOMI i.e. abdominal or cardiac surgery, sepsis, cardiac failure, renal disease, and patients being dialyzed, abdominal compartment syndrome, or gastrointestinal arterial spasm secondary to medication[7-12]. NOMI is the cause of acute mesenteric ischemia in 20% to 30% of cases and is associated with high mortality rates[10].

The detection of mucosal ischemia with functional tests in patients with chronic abdominal pain in the absence of macrovascular pathology suggests that there may also be a chronic state of splanchnic hypoperfusion, analogous to NOMI in the critical care setting. Several conditions and pathophysiologic mechanisms may account for non-occlusive CGI. In a series of patients with non-occlusive CGI, vasospasm of the gastrointestinal arteries was observed during angiography[2].Furthermore, in patients with chronic heart failure, splanchnic hypoperfusion has been found at low levels of exercise[13]. In theory, patients with non-occlusive CGI could benefit from vasodilating medication[14].

Currently, there are no published data on this particular group of patients. Hence, the aim of this study was to assess the effectiveness of vasodilating medication in patients diagnosed with non-occlusive CGI.

METHODS

Population

The study was performed within a tertiary care program for diagnosis and treatment of CGI at a university referral center. All data of consecutive patients referred for evaluation of CGI were prospectively included after informed consent. The Institutional Review Board of the Erasmus MC- University Medical Center approved the study.

Standard diagnostic work-up

In all patients the more common causes of upper gastrointestinal complaints had been excluded by upper endoscopy, colonoscopy, and abdominal ultrasound or computed tomography. All patients evaluated for suspected CGI underwent a standard work-up by means of a thorough medical and physical examination, and an extensive questionnaire on complaints and medical and family history. Visualization of the gastrointestinal arteries (celiac artery, superior mesenteric artery and inferior mesenteric artery) was performed in all patients by means of CT or MR angiography, and / or conventional angiography.

Detection of mucosal ischemia

Mucosal perfusion was assessed by means of 24-hour gastric and jejunal tonometry or visible light spectroscopy. Tonometry was performed according to a standardized protocol for mucosal CO_2 measurements with gastric and jejunal catheters both in fasting and postprandial state, as described previously[4,15]. A positive (abnormal) tonometry test was defined as: 1) a pathologic response after 3 or more meals, or 2) a combination of one or two pathologic responses after meals combined with a median PCO_2 > 8.0 kPa in between meals.

As per march 2009 tonometry was replaced by visible light spectroscopy measurements as the functional diagnostic test in the standard work-up of all patients, because of the non-invasiveness of visible light spectroscopy measurements and comparable sensitivity as tonometry[6]. Mucosal saturation was measured using a fiberoptic catheter-based visible light spectroscopy oximeter (T-Stat 303 Microvascular Oximeter, Spectros, Portola Valley, California, USA) during upper endoscopy[6]. This catheter was passed through the accessory channel of the endoscope after irrigation of the target area to remove any bile and food remnants. [5,16] The recently validated cut-off values of 58% for the descending duodenum, 62% for the duodenal bulb and 63% for the gastric antrum were used for the detection of mucosal ischemia[6]. Measurements were considered to be positive for ischemia when the measured saturation in the antrum, duodenal bulb, or descending duodenum was lower than the cut-off value used in each location[6].

Diagnosis of non-occlusive ischemia

During a multidisciplinary assessment of the diagnostic results by gastroenterologists, vascular surgeons and interventional radiologists, a consensus diagnosis of non-occlusive CGI was based on: 1) presence of symptoms suggestive of CGI, 2) absence of macrovascular pathology

at gastrointestinal artery imaging, and 3) detection of mucosal ischemia with functional testing (positive tonometry or visible light spectroscopy), and 4) adequate exclusion of other causes of abdominal pain. Absence of macrovascular pathology was defined as three completely patent gastrointestinal arteries, and absence or minor atherosclerotic changes of the arteries on CT- or MR angiography. If, after re-evaluation of the imaging, suspicion persisted of even non-significant atherosclerotic narrowing in any gastrointestinal artery, a conventional digital subtraction angiography of the gastrointestinal arteries was performed to exclude stenosis.

Vasodilating medication regimen for non-occlusive CGI

Patients with a consensus diagnosis of non-occlusive CGI were offered medical treatment with vasodilating medication consisting of isosorbide dinitrate (ISD) 20 or 40 mg od as the first-line therapy. If there were side effects or no clinical improvement after four weeks the first-line therapy was ceased and replaced by the second-line treatment. As second-line treatment ISD was replaced by ketanserin (KTS) 20 mg or 40 mg od. If the symptoms persisted after a second period of four weeks the patients was classified as non-responder.

Follow-up

All patients diagnosed with non-occlusive CGI were assessed on an outpatient basis four weeks after the start of vasodilating medication, after any change in medication, and at 3-month intervals thereafter. Clinical response was defined as complete or partial relief of the major presenting symptoms, i.e. pain relief, weight gain or weight stabilization, and relief of diarrhoea or nausea. Symptoms and any side effects of vasodilating medication were noted. The clinical response of each patient to the vasodilating medication was independently reviewed by three reviewers (AS, LM, and ER). In case of disagreement, the particular case was re-evaluated until a consensus was reached.

Cardiological assessment

During standard work-up, all patients underwent an electrocardiogram (ECG). Patients diagnosed with non-occlusive CGI, who did not have a cardiological assessment including Doppler echocardiography in the previous 6 months, were referred for evaluation of cardiac function. All data on cardiac function, i.e. ECG and Doppler echocardiography outcomes, were reviewed by an independent cardiologist (TG).

Statistical analysis

Continuous data were expressed as mean, standard deviation, and range; categorical data were expressed as percentages. Categorical data of responders and non-responders to vasodilating medication were compared using the Fisher's exact test. A p-value of $\alpha = 0.05$ (all-two sided) was considered statistically significant. The overall treatment response was assessed using per protocol analysis. The effect was evaluated in patients who actually followed through the treatment algorithm and from whom the follow-up data were available.

RESULTS

From October 2006 to April 2010, 353 patients were referred for evaluation of suspected CGI. Macrovascular pathology was absent in 101 patients. Non-occlusive CGI was diagnosed in 47 patients: 13% of total group, 47% of patients with normal gastrointestinal arteries.

Characteristics of patients diagnosed with non-occlusive CGI

The mean age was 57 (range 29 – 77) years, with 17 (36%) males. The most commonly reported complaints were abdominal pain, postprandial pain, and weight loss (Table 1). Smoking was reported in 21 (45%) patients and 26 (55%) patients reported having a positive family history of cardiovascular disease (CVD). Twenty patients (43%) were known with CVD, and another five patients (11%) reported having cardiac disease, including heart failure (n = 3), aortic valve disease (n = 1), and arrhythmia (n =1). Twelve out of 47 (26%) patients with the consensus diagnosis of non-occlusive CGI were younger than 50 years. Only 2 of these patients (4% of the total group) had a history of CVD and one patient (2% of the total group) had DM. The median MDRD GFR was 83 (interquartile range 70-90) ml/min/ 1·73m^2.

Vasodilating medication: effect and side-effects

First-line vasodilation therapy was offered to 39/47 (83%) patients, and 38/39 (97%) patients actually initiated first-line treatment, because one patient refused medication because of earlier reported multiple allergies to medication. Eight patients had other treatment (see Table 2). All 38 patients received ISD as first-line treatment. Ten out of 38 (26%) patients initially responded to the first-line medication, but this medication was stopped in five patients due to side effects (n = 3) or temporary response (n = 2). First-line medication produced a sustained response in 5/38 (13%) patients with a median follow-up of 29 (interquartile range (IQR) 21- 39) months (see Figure 1).

Twenty-seven out of 33 (82%) eligible patients received second-line treatment. In six patients second-line treatment was not initiated (see Figure 1 and Table 3). Follow-up data were available for 26/27 (96%) patients (Figure 1), as one patient was lost during follow-up. After a median follow-up of 11 (IQR range 5 -18) months, 4/26 (15%) had a sustained response, while 22/26 (85%) did not have any symptom improvement. Two of these four patients initially responded to ISD, but had recurrence of symptoms after 9 and 12 months, respectively. Both of these patients switched to a second-line treatment with KTS, but without clinical response. Eventually, in these two patients reintroduction of ISD resulted in a positive sustained response at mean follow-up of 9 months. Thus, of the 26 patients who received second- line treatment and from whom follow-up data were available, two (8%) of them had positive clinical response to KTS, and another two had repeated positive response after reintroducing ISD.

Table 1: Baseline characteristics of patients diagnosed with non-occlusive CGI

Patient characteristics	Non-occlusive CGI	
	n = 47	
Age (years)*	57	(29-77)
Male gender	17	(36%)
Reporting weight loss	30	(64%)
Weight loss (kg/month)**	1.5	(0.5-2.8)
Abdominal pain	44	(94%)
Postprandial pain	35	(75%)
Exercise related pain	16	(34%)
Nausea	17	(36%)
Diarrhea	11	(23%)
Smoking	21	(45%)
Family history CVD	26	(55%)
Cardiovascular disease	20	(43%)
DM	9	(19%)

* mean age (range); ** median (IQR range); CGI = chronic gastrointestinal ischemia; CVD = cardiovascular disease; DM = diabetes mellitus

Table 2: Characteristics of patients not receiving vasodilating agents

Category	Total	Other treatment	Follow-up
	n = 8		
Cardiac dysfunction	n = 4	Pericarditis constrictiva, n =1	Symptoms resolved greatly after treatment
		Symptoms caused by forward failure, n =3	Already received maximum treatment
Pneumonia	n = 1		Symptoms resolved after treatment
Side effect of other drug	n = 1	Digoxin use after heart transplantation	Refused cooperation
Exercise related	n = 2	Symptoms occurred after extensive exercise	Symptoms under control with sufficient fluid intake before and during exercise

Taken together, 39 patients were advised to use ISD. Using per protocol analysis 8 patients were excluded from the analysis; one patient refused ISD due to earlier reported allergy, one patient who received second-line treatment was lost during follow-up, and six patients did not receive second-line treatment. Thus, 31 patients actually received first and second-line treatment and complete follow-up data was available in these patients (see Figure 1). Per protocol analysis shows that 9/31 (29%) patients had sustained response after first or second-line treatment (see Figure 1). There were no statistically significant difference in baseline characteristics between the responders and non-responders. Furthermore, treatment outcome was not related to the functional test to detect mucosal ischemia: 5/19 patients (26%) assessed by tonometry and 4/20 (20%) patients selected with visible light spectroscopy had a sustained response to vasodilating agents (P = 0.35).

Side effects of vasodilation therapy were reported in 15/38 (39%) patients on ISD, and 4/27 (15%) patients who received KTS (see Table 4). The most common side effects reported were

headache (53% vs. 50%) and dizziness (20% vs. 25%) in patients ISD and KTS, respectively. In total 3/38 (8%) of patients who used ISD stopped medication because of moderate to severe side effects and refused to use second-line treatment (see Table 2).

Cardiological assessment and other treatments

Eight out of 47 patients (17%) with non-occlusive CGI received other treatment than vasodilating agents (Table 3). The multidisciplinary team did not advise vasodilating agents in these patients because their complaints were thought to be caused by cardiac dysfunction, lung dysfunction due to pneumonia, or side effects of other medication. Moreover, in two patients the complaints of non-occlusive CGI occurred after extensive exercise.

All patients underwent ECG as part of the standard diagnostic work-up. In 22/50 patients diagnosed with non-occlusive CGI, additional cardiological assessment was performed. No differences were observed in the prevalence of arrhythmia, myocardial infarction, left ventricular dysfunction, or valve dysfunction between the responders to vasodilating medication and the non-responders (data not shown).

DISCUSSION

In this study we aimed to assess the effectiveness of vasodilating medication in patients diagnosed with non-occlusive CGI. Treatment of 39 patients with an escalating dose of vasodilating medication resulted in symptom relief in almost one-third of patients. Additional evaluation of cardiac function did not reveal a decreased cardiac output as the cause of splanchnic hypoperfusion.

Since recent years the use of a functional test for detecting mucosal ischemia is gaining more and more territory in the diagnostic work-up of patients suspected with CGI. Functional testing by means of tonometry or visible light spectroscopy improves the selection of patients with actual CGI and those are likely to benefit from the revascularization [2,6,17-18]. We have identified a subgroup of patients who presents with symptoms suggestive of CGI, who show mucosal ischemia on functional testing, but have patent gastrointestinal arteries on imaging. This entity is called non-occlusive CGI, and this is observed in 13-20% of patients analyzed for CGI [2,6,14]. It is suggested that these patients might benefit from vasodilating medication[14].

To our knowledge, only one other study described the effects of vasodilating agents, including, nitrates, ketanserin, nicorandil and doxazosin, in patients with non-occlusive CGI[14]. In this prior study, 63% of patients experienced a >50% pain reduction in response to at least one vasodilating drug. By comparison, in the present study a sustained response to

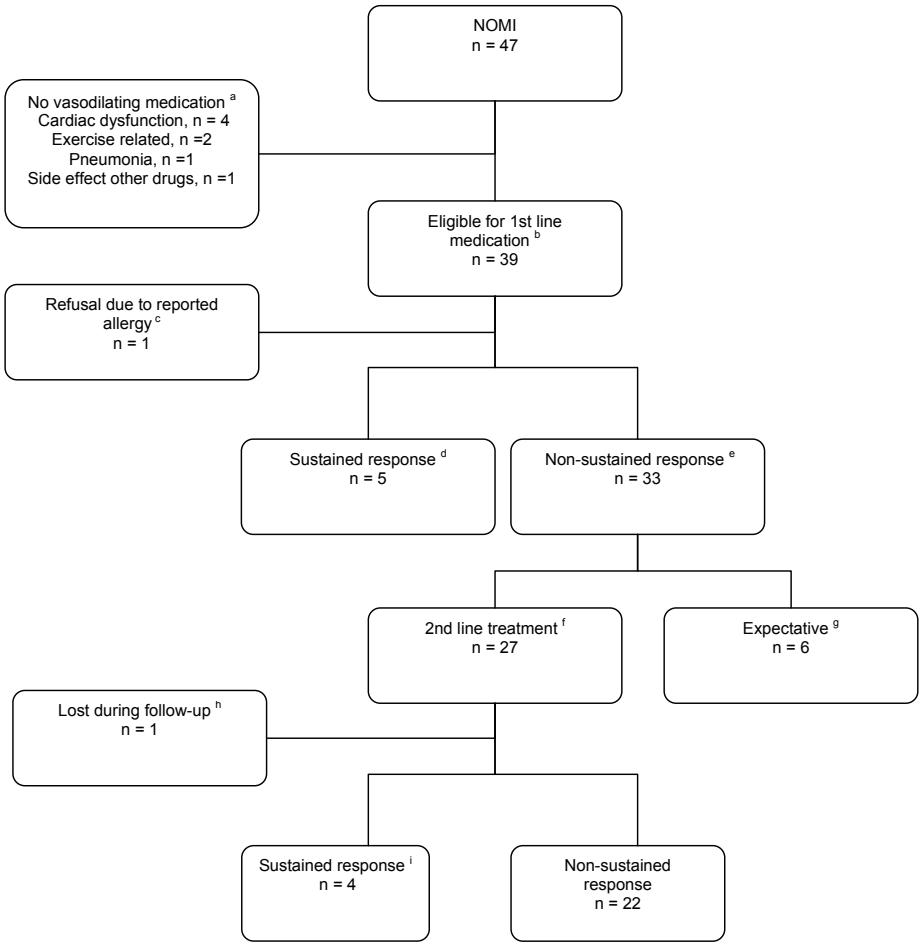


Figure 1: Flow-chart of response to medication

[a]: Symptoms improved after treatment for pericarditis constructiva (n=1), caused by forward failure, and already received maximum therapy (n=3). Symptoms occurred after extensive exercise (n=2). Abdominal symptoms improved after treatment for pneumonia (n = 1). Abdominal symptoms developed due to use of digoxin after heart transplantation (n=1).

[b]: Isosorbide dinitrate n = 39

[c]: One patient refused medication because of earlier reported multiple allergies to medication, and was excluded from further analysis.

[d]: Sustained response = pain free and/or weight stabilization/weight gain or free from other major complaints such as nausea and/or diarrhea

[e]: Change of medication despite initial symptom improvement due to side effects (n = 3), temporary response (n=2), and non responders after first-line treatment (n = 28)

[f]: Ketanserin (n = 27)

[g]: Symptom improvement after coronary artery bypass graft (n=1),symptoms improved after treatment of liver abscess (n = 1). Spontaneous resolvement (n = 1). Refusing or not eligible for second line treatment (n = 3).

[h]: One patient was lost in follow-up and is excluded from further analysis.

[i]: Sustained response after ketanserin (n = 2). Second-line treatment without positive response, isosorbide dinitrate was reintroduced and patients had repeated positive response (n = 2)

Table 3: Characteristics of six patients not receiving second-line treatment

Category	Total	Other treatment	Follow-up
	n = 6		
Refusal	n = 3	Due to side effects after using ISD	-
Other diagnosis	n = 2	Patient underwent CABG, n= 1	Symptoms resolved
		Treated for liver abscess, n =1	Symptoms resolved
Spontaneous resolvement	n =1	Nausea was major symptom and resolved spontaneously	-

CABG = coronary artery bypass grafting, ISD = isosorbide-dinitrate

Table 4: Side effects of vasodilating agents

Side effects	Isosorbide dinitrate n = 15		Ketanserin n = 4	
Headache	8	(53%)	2	(50%)
Dizziness	3	(20%)	1	(25%)
Headache and dizziness	3	(20%)	1	(25%)
Nausea	1	(7%)	0	-

vasodilating medication was obtained in 29% of cases. This discrepancy in success rates may relate to differences in study methods. First, in the current study, the clinical response to treatment was evaluated independently by three reviewers, two of them were not involved in the treatment of these patients. Second, in our study patients were selected with either tonometry or visible light spectroscopy, whereas all patients were selected with tonometry in the prior one. Although a positive tonometry test was associated with a higher response rate than a positive visible light spectroscopy measurement (26% vs 20%), this difference was not significant and also not approaching a response rate of >60%. Furthermore, differences in the causes of splanchnic hypoperfusion may underlie the observed discrepancies in therapy success. In the previous mentioned study, other vasodilators, i.e., nicorandil and doxazosin, were added to the treatment with KTS and ISD. This however increased the response rate by only 14%, and is, hence, unlikely to have a major contribution to the treatment algorithm. Unfortunately, based on the data in the current study, no predictors of a positive clinical response to vasodilating medication could be defined.

Patients using ISD reported twice as many side effects as patients who used KTS. Overall, the side effects of both drugs were well tolerated and only 8% of patients who used ISD refused to switch to KTS as second-line treatment due to side effects from using ISD.

As mentioned before, non-occlusive CGI is a relatively unknown disorder and its etiology is incompletely understood. A chronic state of splanchnic hypoperfusion due to various pathological conditions is thought to be present in this group of patients. Analogous to causes of

acute NOMI it has been suggested that splanchnic hypoperfusion in non-occlusive CGI may be related to a decrease in cardiac output. Patients with chronic heart failure have been shown to display a rise in intragastric PCO_2 concentration on tonometry during low-level exercise, indicating gastrointestinal hypoperfusion [13]. To investigate a potential relationship between non-occlusive CGI and heart failure, 22 patients diagnosed with non-occlusive CGI in the current study underwent additional cardiac analysis using echocardiography. None of these patients had a history of heart failure, arrhythmia or valvular disease. The three patients with a history of heart failure in the current cohort were not treated with vasodilating medication. Cardiac analysis did not reveal heart failure in any of the patients with non-occlusive CGI. As the chances of having a significantly lowered cardiac output in the absence of exertional dyspnea, normal ECG and echocardiography, were considered to be very low, no invasive testing was performed to measure the cardiac output. Furthermore, there were no differences in left ventricular function between responders to vasodilating medication and non-responders. These data suggest that although patients with chronic heart failure may exhibit splanchnic hypoperfusion, non-occlusive CGI is not necessarily related to a decrease in cardiac output.

One of the limitations of this study is the lack of a placebo control group. Placebo effects for treatment of patients with chronic abdominal pain may be as high as 30-40%[19-20]. Furthermore, since the functional test for mucosal ischemia was not repeated during follow-up, it is unknown whether mucosal perfusion improved in the patients that reported symptom relief in response to vasodilating medication. Therefore, it can not be ruled out that the observed treatment response is a placebo effect.

In conclusion, non-occlusive CGI is a diagnosis of exclusion, based on the presence of mucosal ischemia in patients with symptoms of gastrointestinal ischemia without gastrointestinal artery stenoses. One third of these patients have sustained relief of symptoms in response to vasodilating medication after a median follow-up of almost two and a half years. The side effects of vasodilating medication are generally well tolerated, and without medication these patients would still suffer from chronic abdominal pain. However, a randomized placebo-controlled trial is needed to exclude a potential placebo effect and to assess whether vasodilating medication actually improves mucosal perfusion in patients with non-occlusive CGI.

REFERENCES

1 Mensink PB, Moons LM, Kuipers EJ: Chronic gastrointestinal ischaemia: Shifting paradigms. Gut 2011;60:722-737. PMid:21115543 doi:10.1136/gut.2009.199695

2 Otte JA, Geelkerken RH, Oostveen E, Mensink PB, Huisman AB, Kolkman JJ: Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2005;3:660-666. PMID:16206498 doi:10.1016/S1542-3565(05)00155-2

3 Oderich GS, Bower TC, Sullivan TM, Bjarnason H, Cha S, Gloviczki P: Open versus endovascular revascularization for chronic mesenteric ischemia: Risk-stratified outcomes. J Vasc Surg 2009;49:1472-1479 e1473. PMID:19497510

4 Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ: Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. Dig Dis Sci 2008;53:133-139. PMid:17530402 DOI: 10.1007/s10620-007-9833-1

5 Friedland S, Benaron D, Coogan S, Sze DY, Soetikno R: Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. Gastrointest Endosc 2007;65:294-300. PMid:17137857 doi:10.1016/j.gie.2010.10.025

6 Van Noord D, Sana A, Benaron DA, et al. Endoscopic visible light spectroscopy: A new, minimally invasive technique to diagnose chronic gi ischemia. Gastrointest Endosc 2011;73:291-298. PMid:21168842 doi:10.1016/j.gie.2010.10.025

7 Kozuch PL, Brandt LJ: Review article: Diagnosis and management of mesenteric ischaemia with an emphasis on pharmacotherapy. Aliment Pharmacol Ther 2005;21:201-215. PMid:15691294 DOI: 10.1111/j.1365-2036.2005.02269.x

8 Archodovassilis F, Lagoudiannakis EE, Tsekouras DK, et al. Nonocclusive mesenteric ischemia: A lethal complication in peritoneal dialysis patients. Perit Dial Int 2007;27:136-141. PMid:17299146

9 Ori Y, Chagnac A, Schwartz A, et al. Non-occlusive mesenteric ischemia in chronically dialyzed patients: A disease with multiple risk factors. Nephron Clin Pract 2005;101:c87-93. PMid:15956804 DOI: 10.1159/000086346

10 Trompeter M, Brazda T, Remy CT, Vestring T, Reimer P: Non-occlusive mesenteric ischemia: Etiology, diagnosis, and interventional therapy. Eur Radiol 2002;12:1179-1187. PMid:11976865 DOI: 10.1007/s00330-001-1220-2

11 Kolkman JJ, Mensink PB: Non-occlusive mesenteric ischaemia: A common disorder in gastroenterology and intensive care. Best Pract Res Clin Gastroenterol 2003;17:457-473. PMID:12763507 doi:10.1016/S1521-6918(03)00021-0

12 Mitsuyoshi A, Obama K, Shinkura N, Ito T, Zaima M: Survival in nonocclusive mesenteric ischemia: Early diagnosis by multidetector row computed tomography and early treatment with continuous intravenous high-dose prostaglandin e(1). Ann Surg 2007;246:229-235. PMid:17667501 doi: 10.1097/01.sla.0000263157.59422.76

13 Krack A, Richartz BM, Gastmann A, et al. Studies on intragastric pco2 at rest and during exercise as a marker of intestinal perfusion in patients with chronic heart failure. Eur J Heart Fail 2004;6:403-407. PMid:15182764 DOI: 10.1016/j.ejheart.2004.03.002

14 M. Bigirwamungu-Bargeman RHG, A.B. Huisman, J.J. Kolkman: Abdominal migraine, a new and treatable disorder mimicking functional dyspepsia. 2009 [Abstract].

15 Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ: Effect of various test meals on gastric and jejunal carbon dioxide: A study in healthy subjects. Scand J Gastroenterol 2006;41:1290-1298. PMid:17060122 doi:10.1080/00365520600670059

16 Friedland S, Soetikno R, Benaron D: Reflectance spectrophotometry for the assessment of mucosal perfusion in the gastrointestinal tract. Gastrointest Endosc Clin N Am 2004;14:539-553, ix-x. PMid:15261201 doi:10.1016/j.giec.2004.03.011

17 Mensink PB, van Petersen AS, Geelkerken RH, Otte JA, Huisman AB, Kolkman JJ: Clinical significance of splanchnic artery stenosis. Br J Surg 2006;93:1377-1382. PMid:17022013 DOI: 10.1002/bjs.5481

18 Sana A, Vergouwe Y, van Noord D, et al. Radiological imaging and gastrointestinal tonometry add value in diagnosis of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2011;9:234-241. PMid:21115136 doi:10.1016/j.cgh.2010.11.006

19 Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med 2011;364:22-32. PMid:21208106 DOI: 10.1056/NEJMoa1004409

20 Patel SM, Stason WB, Legedza A, et al. The placebo effect in irritable bowel syndrome trials: A meta-analysis. Neurogastroenterol Motil 2005;17:332-340. PMid:15916620 DOI: 10.1111/j.1365-2982.2005.00650.x

Chapter 8

Hypoxia-inducible factor 1 alpha expression as marker for intestinal inflammation and ischemia

A. Sana MD[1], K. Biermann[2], R. Smits PhD[1], P. Van der Groep[5] P.B.F. Mensink MD PhD[1], P. Van Diest MD PhD[5], Ernst J. Kuipers MD PhD[1,4], L.M.G. Moons MD PhD[1,5]

Departments of Gastroenterology and Hepatology[1], Pathology[2], Internal Medicine[3], Erasmus MC - University Medical Center, Rotterdam, Department of Pathology[4], Utrecht University Medical Center, Utrecht[5], The Netherlands

Submitted

ABSTRACT

Aim: The aim of our study was to compare the expression of HIF-1 alpha in normal colon, ischemic colitis, as well as in Crohn's disease (CD), ulcerative colitis (UC), and infectious colitis. Furthermore, we wanted to investigate if there is a correlation between the degree of inflammation and the expression level of HIF-1 alpha.

Methods: Biopsies of 30 subjects with a normal colon, and of 33 patients with various types of colitis (9 pts with ischemic colitis, 9 with CD, 10 with UC, and 5 with infectious colitis) were collected for immunohistochemistry. Immunohistochemical staining was evaluated both on the basis of the localization and intensity of staining. Differences in scores between diagnostic groups were assessed using Fisher's Exact, Kruskal-wallis rank-sum, and Spearman tests.

Results: Expression of HIF-1 alpha was only seen in 37% samples of normal colon, but HIF-1 alpha was expressed in the majority of samples of various types of colitis. Expression was mainly detected at the bottom of the crypts in the positive normal colon samples, whereas the colitis group predominantly showed expression throughout the crypt and/or at the epithelial surface. The staining intensity in normal colon was weak in most samples, while the various colitis groups predominantly showed a moderate to strong staining. There was a moderately strong correlation between the degree of inflammation seen on histopahtology and the intensity of HIF-1 alpha expression (Spearman correlation 0.578).

Conclusion: HIF-1 alpha is not only expressed in ischemic colitis, but also in patients with various inflammatory disorders. This implies that HIF1-alpha expression is a sign of inflammation, and can not differentiate between ischemia and other sources of inflammation. The intensity of expression seems to correlate with the degree of inflammation seen on histopahtology.

INTRODUCTION

Ischemic colitis (IC) is the most frequently encounterend ischemic injury to the gastrointestinal tract, especially in the elderly population. The clinical presentation is dependent on the predominant clinical pattern, but the typical presentation is an acute onset of mild abdominal pain over the affected segment of the colon with passage of blood mixed with stools. Ischemic colitis accounts for 1-3 per 1000 hospitalizations per year, and is supposed to be missed more often due to a low clinical index of suspicion [2], and the mild and transient nature of ischemic colitis in the majority of patients.[2, 3]

Colonoscopy is the preferred diagnostic tool, with biopsies to confirm the diagnosis. However, making the histological diagnosis ischemic colitis is difficult, as clear pathognomonic features as ghost cells and mucosal infarction are absent in the majority of cases. [2] Several reports have introduced the entity probable ischemic colitis for those cases where the clinic presentation and endoscopic appearance favour ischemic colitis, but histology did not confirm the diagnosis. The proportion of probable ischemic colitis varies between studies between 20-40%[2, 4, 5] New biomarkers may therefore support the histological diagnosis of ischemic colitis. A potential marker may be hypoxia inducible factor 1 alpha (HIF-1 alpha). HIF-1 is a heterodimeric transcriptional factor which consists of a HIF-1 alpha and a HIF-1 beta subunit.[6] The HIF-1 beta subunit is expressed constitutively, while expression of HIF-1 alpha subunit is dependent on oxygen tension. In the presence of adequate oxygen supply, HIF-1 alpha protein is degraded by the ubiquitin-proteasome pathway. [7, 8] Hypoxia inhibits this process and leads to enhanced concentration of HIF-1 alpha and subsequent binding to HIF-1 beta.[8] This will lead to formation of the HIF-1 complex which regulates the expression of a variety of genes responsible for angiogenesis, glycolysis, and the inhibition of apoptosis.

Okuda, et al.[9] showed that overexpression of HIF-1 alpha occurred in patients with ischemic colitis, which disappeared with resolution of the inflammation in the colon. However, besides its role in hypoxia, HIF-1α has also been associated with carcinogenesis and inflammation of the colon [ref], and HIF-1α expression may therefore be expressed in more disease entities than ischemic colitis only. The differential diagnosis of an elderly patient with abdominal pain, and rectal blood loss consists of ischemic colitis, infectious colitis and inflammatory bowel disease (IBD) in the majority of cases[10] We therefore tested, whether HIF-1α has indeed an added value in discriminating between these diseases at histology.

Material and methods

Patients and controls

HIF-1α expression was analysed in 62 colon samples of 44 patients. These consisted nine biopsies of nine patients with histological confirmed ischemic colitis, 18 biopsies of 18 patients with a known IBD (9 M Crohn and 9 ulcerative colitis), five biopsies of five patients with an infectious colitis, and 30 biopsies of 12 controls. The patients with ischemic colitis IBD, and infectious colitis were selected from a hospital database. The patients with ischemic colitis, 90% left sided, were selected when the clinical presentation and course of the disease, the endoscopic appearance indicated ischemic colitis, and the original pathology report confirmed ischemic colitis at histology. The biopsies of patients with ulcerative colitis were collected at endoscopy performance due to complaints (n=5) and surveillance (n=3) or were resection specimen (n =1). Tissue samples of patients with Crohn's disease were collected at the time of endoscopy due to complaints (n=4) and surveillance (n=4), or during surgery (n=1). Infectious colitis was confirmed by means of stool culture and histology confirmation: clostridium difficile (n=3), campylobacter jejuni (n=1), and E-coli (n=1). From controls, tissue samples of ascending and descending colon, and rectum were collected during regular double-balloon enteroscopy, while endoscopy and histopathologic findings showed no abnormalities in the intestines and colon. The controls underwent the double balloon enteroscopy due to gastrointestinal tract bleeding (n=6) and unexplained abdominal complaints (n=6). The Institutional Review Board of the Erasmus MC- University Medical Center approved the study.

Endoscopy

We re-evaluated the endoscopy reports of patients with the different forms of colitis to assess the degree of inflammation. The degree of inflammation was scored in patients with ulcerative colitis using the Mayo Endoscopic Scoring of Ulcerative Colitis (schroeder NEJM 1987) and in patients with Corhn's disease using the SES-CD scoring (Sostegni APTT 2003). Because there is not a well defined scoring systeem for ischemic colitis and infectious colitis we used the criteria in the above mentioned scoringsystems to evaluate the endoscopic degree of inflammation in these patients groups: no sign of inflammation was scored as 0, erythema, decreased vascular pattern as 1, severe edema and ulcers as 2, and transmural necrosis was scored as 3.

Histology

All Haematoxylin & Eosin staining of the selected patients were re-analyzed to confirm the original diagnosis. The presence and degree of inflammation was scored as no inflammation, weak, medium and strong inflammation.

Immunohistochemistry

Formaldehyde-fixed and paraffin-embedded tissue samples were cut into 5 µm thick paraffin slides. Slides were deparaffinized with xylene and serial ethanol dilutions. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 15 minutes. Antigen retrieval was achieved by microwave treatment (200 W for 10 minutes in Tris EDTA, pH 9.0), followed by cooling. Slides were blocked in protein block. The slides were overnight incubated with a monoclonal mouse antibody against human HIF-1α (1: 200; Clone 610959 BD Transductions Laboratories™, stad, land), GLUT (XX) and CAIX (X). Binding of the primary antibody was visualized with the Novocastra™ Novolink™ Polymer Detection System (Novocastra Laboratories Ltd, New Castle United Kingdom). Finally, peroxidase activity was developed with DAB and counterstained with hematoxylin. The slides were washed between each step in PBS. All slides were dehydrated in ethanol dilutions and xylene, before they were mounted. Positive controls were tissues from mammacarcinoma and negative controls were achieved by omitting the primary antibody. All tissue samples were stained for GLUT-1 and CA IX.

Evaluation of the intensity of the HIF-1α staining

Two authors scored all slides (KB, AS) blinded. Immunohistochemical staining was evaluated on the basis of localization of stained nuclei in the crypt. The intensity of staining was scored as negative, weak, medium or strong.

Statistics

Differences in scores between diagnostic groups were assessed using Fisher's Exact, Kruskal-wallis rank-sum, and Spearman tests. P-values < 0.05 were considered to be significant.

RESULTS

Patient characteristics

The patient's characteristics of the normal subjects and patients with different diagnoses are summarized in Table 1.

Endoscopy and histology

Table 1 describes the endoscopy and histology findings of the controls and patients with different forms of colitis. Using the collected biopsies we aimed to investigate the expression of HIF-1 alpha, as a marker of hypoxia, and its downstream targets CA IX and GLUT-1 in ischemic colitis, Crohn's disease, ulcerative colitis and infectious colitis. In addition, we assessed the correlation between the degree of inflammation and the expression level of HIF-1 alpha.

Table 1: Patient characteristics according to different diagnosis

Patients characteristics	Normal		IBD				Ischemic colitis		Infectious colitis	
			CD		UC					
	n = 12		n = 9		n =9		n = 9		n = 5	
Median age in years (IQR range)	52	(43-60)	38	(34 -56)	36	(25-49)	75	(60-77)	66	(41-77)
Gender										
Male	6	(50%)	2	(22%)	7	(78%)	4	(44%)	3	(60%)
Female	6	(50%)	7	(88%)	2	(22%)	5	(66%)	2	(40%)
Histopathology										
Inflammatory activity		-								
Inactive			3	(33%)	3	(33%)	0	-	0	-
Weak			1	(11%)	0	-	1	(11%)	1	(20%)
Moderate			3	(33%)	2	(22%)	4	(44%)	0	-
Strong			2	(22%)	4	(44%)	4	(44%)	4	(80%)
Endoscopy										
Inflammatory activity		-								
Inactive disease			3	(33%)	2	(22%)	0	-	0	-
Mild disease			2	(22%)	1	(11%)	2	(22%)	2	(40%)
Moderate disease			3	(33%)	1	(11%)	6	(67%)	0	-
Severe disease			1	(11%)	5	(56%)	1	(11%)	3	(60%)

IQR = Interquartile range; IBD = inflammatory bowel disease; CD = Crohn's disease; UC = Ulcerative colitis; Endoscopic activity was scored: in ulcerative colitis using Mayo Endoscopic Scoring of Ulcerative Colitis, in Crohn's disease by SES-CD scoring; in ischemic colitis and infectious colitis using scoring system based on the later two scoring systems

HIF-1 alpha expression in normal and ischemic colitis

HIF-1 alpha expression was found in 11 out of 30 (37%) biopsies of the normal colon. The expression of HIF-1 alpha did not differ significantly in different regions of the colon: in ascending colon 4 (40%) biopsies showed HIF-1 alpha expression, compared to 5 (50%) of biopsies in descending colon, and 2 (20%) in rectum. In contrary to normal colon, HIF-1 alpha was seen in all biopsies (100%) of ischemic colitis patients, P = 0.001. After we determined the presence of HIF-1 alpha, we investigated the localization of HIF-1 alpha in the crypt. In the majority (73%) of the samples of normal colon HIF-1 alpha was expressed at the bottom of the crypt, and in the interstitial cells. Whereas, the ischemic colitis group predominantly (78%) showed expression throughout the crypt, and interstitial cells. Subsequently, we looked at the staining intensity in both groups; the staining was weak in most samples of normal colon (82%) with only few samples (18%) showing moderate staining. In contrast, the ischemic colitis group showed mostly a moderate (78%) to strong staining (22%). After we showed that HIF-1 alpha is significantly more often expressed in ischemic colitis than in normal colon, we aimed to investigate whether HIF-1 alpha is also expressed in other forms of colitis

HIF-1α expression in other forms of colitis

Firstly, we investigated the expression of HIF-1 alpha in nine biopsies of patients with Crohn's disease and in nine biopsies of patients with ulcerative colitis and compared it with expres-

sion of HIF-1 alpha in ischemic colitis. HIF-1 alpha was expressed in the majority of biopsies of patients with IBD, namely in almost 70%. HIF-1 alpha expression was seen throughout the crypt or at the surface of epithelium. In almost 60% of the biopsies of patients with IBD, the intensity of HIF-1 alpha expression was moderate, this resembles to ischemic colitis where almost 80% was moderately stained. In the remaining biopsies of IBD patients, HIF-1 alpha was expressed weakly in 33% and strong in seven percent. When we compared the biopsies of Crohn's disease with ulcerative colitis then the majority of biopsies of patients with ulcerative colitis showed expression through out the crypt comparing to patients with Crohn's disease (80% vs. 43%, respectively). The intensity of staining was in most of the biopsies of ulcerative colitis moderate comparing to biopsies of patients with Crohn's disease (80% vs. 43%, respectively). Secondly, we also evaluated the expression of HIF-1 alpha in five biopsies of patients with infectious colitis as well. HIF-1 alpha was also expressed in all biopsies (100%) of patients with infectious colitis. HIF-1 alpha was localized throughout the crypt in 40% of the biopsies, vs. 78% in ischemic colitis, and in the remaining 60% of biopsies with infectious colitis HIF-1 alpha was expressed at the bottom of the crypt, vs. 22% in ischemic colitis patients.

We have shown that HIF-1 alpha is not only overexpressed in ischemic colitis, but HIF-1 alpha expression is also present in other forms of colitis such as IBD and infectious colitis. HIF-1 alpha is expressed throughout or at the surface of the crypt in the majority of the samples of these forms of colitis. The intensity of the expression is in the majority of samples moderate to strong. However, in contrast to ischemic colitis and infectious colitis five biopsies of patients with IBD did not show HIF-1 alpha expression; four of them were surveillance biopsies. Moreover, the intensity of expression of HIF-1 alpha differed between the various types of colitis, as well as between Crohn's disease and infectious colitis. The question rose if intensity of HIF-1 alpha expression is correlated to the intensity of inflammation.

Inflammation and HIF-1 alpha expression

Endoscopy

Comparing the endoscopic finding with the expression of HIF-1 alpha, we saw that HIF-1 alpha was absent in the majority of biopsies (80%) without any sign of inflammation. HIF-1 alpha was overexpressed in any degree of inflammation, from weak to strong degree of inflammation, ranging from 86% to 100%. There was a weak correlation between the degree of inflammation as seen during endoscopy and the intensity of HIF-1 alpha expression (Spearman correlation 0.351). HIF-1 alpha is overexpressed in any degree of inflammation seen during endoscopy.

Histology

HIF-1 alpha expression was detected in almost all biopsies (93%) with any degree of inflammation. There was a moderately strong correlation between the degree of inflammation and the intensity of HIF-1 alpha expression (Spearman correlation 0.578). Thus, these results have shown that histology findings have a stronger correlation with the intensity of HIF-1 alpha staining than the endoscopy findings.

Expression of downstream targets CA-IX and GLUT-1

CA-IX and GLUT-1 are known downstream markers of HIF-1 alpha[ref]. Therefore, we evaluated the presence of these downstream targets in 20 samples: six in normal colon, four with Crohn's disease, four with ulcerative colitis, three with ischemic colitis, and three with infectious colitis. HIF-1 alpha expression was present in the majority (70%) of the biopsies. CA-IX was only detected in a small number of biopsies (n=4) which were HIF-1 alpha positive, and in three of these biopsies there was co-localization with HIF-1 alpha expression: one in ulcerative colitis, and two in infectious colitis. GLUT-1 was positive in less than half of 20 biopsies, which were also showed HIF-1 alpha expression. On the other hand CA-IX and GLUT-1 were also positive in biopsies which were HIF-1 alpha negative. CA-IX and GLUT-1 were not always expressed in presence of HIF-1 alpha in the several biopsies of the normal colon and various forms of colitis. The presence of these downstream targets in biopsies without expression of HIF-1 alpha suggests that other factors than HIF-1 alpha. Further research is needed to investigate which downstream targets are activated by HIF-1 alpha expression in colitis.

DISCUSSION

Diagnosis of ischemic colitis is challenging, because endoscopic and histology findings are not specific enough to differentiate between ischemic colitis and other forms of colitis. This leads us to search for a biomarker, which could help us to differentiate between various forms of ischemic colitis in order to recognize the patients with ischemic colitis in a early stage to initiate the optimal therapy. Therefore, this study was performed to evaluate whether HIF-1 alpha is overexpressed in ischemic colitis. Our results showed that HIF-1 alpha is indeed overexpressed in ischemic colitis. Subsequently, we investigated whether HIF-1 alpha expression is present in other forms of colitis such as IBD and infectious colitis. Thus, HIF-1 alpha can not differentiate between ischemic colitis and other forms of colitis. Furthermore, there was a moderately strong correlation between the degree of inflammation seen on histology findings and the intensity of HIF-1 alpha expression.

HIF-1 is a transcriptional factor, which is induced by hypoxia, and consists of two subunits of which HIF-1 alpha is dependant on oxygen tension. Under normoxic conditions HIF-1 alpha

is highly unstable, while in hypoxia its formation is significantly enhanced. [6-8] In our study HIF-1 alpha was expressed in a minority of samples of normal colon. Earlier studies showed different results concerning expression of HIF-1 alpha in normal colon tissue, ranging from no expression to 90% expression in samples of normal colon.[9, 11-13] In the current study HIF-1 alpha expression was seen in most of the samples of the samples of the colitis group, ranging from 50% to 100% of samples. An earlier study investigated HIF-1 alpha expression in tissue samples of normal colon and samples of patients with active ulcerative colitis and Crohn's disease. [13] HIF-1 alpha was unreactive in normal colonic tissue, while in ulcerative colitis and Crohn's disease HIF-1 alpha was expressed focally in epithelial cells, stromal fibroblasts, and myocytes, in nuclei as well as in cytoplasm. Previously, in a study with 13 patients with ischemic colitis, HIF-1 alpha was seen in the colonic lesions of these patients. [9] Weak HIF-1 alpha products were also reported in the normal colonic tissue. This corresponds with our results where the staining intensity in normal colon was weak in most of the samples of normal colon. In our study HIF-1 alpha was expressed in normal colon predominantly at the bottom of the crypt. This is in contrast with earlier report, which demonstrated that HIF-1 alpha was expressed at the surface epithelium, possibly due to the anaerobic environment in the lumen of the colon leading to a relatively hypoxic state of surface epithelium.[12] Future studies are needed to evaluate the function of the HIF-1 alpha expression at the bottom of the crypts of the normal colon.

Our results showed that HIF-1 alpha is not only present in ischemic colitis, but also in IBD and infectious colitis. It seems that HIF-1 alpha is rather a sign of inflammation and ischemia than of ischemia alone. Studies with mouse model IBD showed the HIF-1 alpha is induced in inflamed lesions.[14] Multiple factors predispose the inflamed intestinal epithelial cells to decrease oxygen supply resulting in hypoxia, such as vasculitis, vasoconstriction, and edema. [15] Moreover, inflammatory cells, which are present at the site of inflammation, enhance locally the oxygen consumption leading to hypoxic conditions. [15] HIF-1 alpha expression was induced after stimulation by cytokines IL-1ß and TNF-α in rheumatoid synovial fibroblasts.[16] On the other hand it is assumed that hypoxia alpha may directly or indirectly stimulate the expression of the chemokine IL-8. It is hypothesized that hypoxia and inflammation both influence each other. [14] These features support our finding of HIF-1 alpha expression in patients with IBD and infectious colitis as well as in ischemic colitis.

HIF-1 alpha expression activates several target genes involved in glycolysis and angiogenesis, including glucose transporter 1 (GLUT-1) which improves glucose uptake. [12] Tumor-associated transmembrane carbonic anhydrase IX (CA-IX) is strongly inducible by hypoxia in a variety of tumors such as head and neck, nasopharyngeal carcinoma, breast, and ovary. [17, 18] In a study of patients with normal colon, colorectal adenoma's and adenocarcinoma's, expression of GLUT-1 was closely correlated with HIF-1 alpha. CA-IX was present at the bottom of the nor-

mal colon tissue, and did not co-localize with HIF-1 alpha expression. CA-IX co-localized with HIF-1 alpha expression in adenoma's and adenocarcinoma's. In our study, CA-IX and GLUT-1 were expressed in samples with HIF-1 alpha expression as well as in samples without positive HIF-1 alpha expression, and the expression of CA-IX was only in few samples co-localized with HIF-1 alpha expression. This indicates that CA-IX and GLUT-1 are activated in these samples of colitis patients probably by factors other than HIF-1 alpha.

One of the limitations of this study is that expression of HIF-1 alpha was investigated in a small number of biopsies with various forms of colitis. However, HIF-1 alpha was significantly overexpressed in colitis, even in this relatively small number of biopsies. Secondly, we only used biopsies from active ischemic colitis to assess the presence of HIF-1 alpha. It could be interesting to investigate the expression of HIF-1 alpha after the episode of ischemic colitis when the symptoms resolved and at endoscopy the signs of inflammation were decreased or totally resolved. Thirdly, CA-IX and GLUT-1 did not seem to be activated by HIF-1 alpha in the investigated biopsies. Future studies are needed to investigate other downstream targets to assess which ones are stimulated during inflammation by HIF-1 alpha.

In conclusion, this study showed that HIF-1 alpha is not only expressed in ischemic colitis, but also in patients with various inflammatory disorders. The intensity of expression correlates with the degree of inflammation. This implies that HIF1-alpha expression is a sign of inflammation, and can not differentiate between ischemia and other causes of inflammation. Future studies are needed to identify new markers ,which are able to support the diagnosis ischemic colitis at histology.

REFERENCES

1. Brandt LJ, Boley SJ: Colonic ischemia, Surg Clin North Am 1992, 72:203-229
2. Montoro MA, Brandt LJ, Santolaria S, Gomollon F, Sanchez Puertolas B, Vera J, Bujanda L, Cosme A, Cabriada JL, Duran M, Mata L, Santamaria A, Cena G, Blas JM, Ponce J, Ponce M, Rodrigo L, Ortiz J, Munoz C, Arozena G, Ginard D, Lopez-Serrano A, Castro M, Sans M, Campo R, Casalots A, Orive V, Loizate A, Tito L, Portabella E, Otazua P, Calvo M, Botella MT, Thomson C, Mundi JL, Quintero E, Nicolas D, Borda F, Martinez B, Gisbert JP, Chaparro M, Jimenez Bernado A, Gomez-Camacho F, Cerezo A, Casal Nunez E, Workgroup for the Study of Ischaemic Colitis of the Spanish Gastroenterological A: Clinical patterns and outcomes of ischaemic colitis: results of the Working Group for the Study of Ischaemic Colitis in Spain (CIE study), Scand J Gastroenterol 2011, 46:236-246
3. Sotiriadis J, Brandt LJ, Behin DS, Southern WN: Ischemic colitis has a worse prognosis when isolated to the right side of the colon, Am J Gastroenterol 2007, 102:2247-2252
4. Assadian A, Senekowitsch C, Assadian O, Hartleb H, Hagmuller GW: Diagnostic accuracy of sigmoidoscopy compared with histology for ischemic colitis after aortic aneurysm repair, Vascular 2008, 16:243-247
5. Moons LMG, Van Noord, D, Biermann, K, et al. : Histology in patients clinically suspect for ischemic colitis: normal findings do not exclude the diagnosis, Gastroenterology 2009, 136:639
6. Wang GL, Jiang BH, Rue EA, Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension, Proc Natl Acad Sci U S A 1995, 92:5510-5514
7. Salceda S, Caro J: Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes, J Biol Chem 1997, 272:22642-22647
8. Huang LE, Gu J, Schau M, Bunn HF: Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway, Proc Natl Acad Sci U S A 1998, 95:7987-7992
9. Okuda T, Azuma T, Ohtani M, Masaki R, Ito Y, Yamazaki Y, Ito S, Kuriyama M: Hypoxia-inducible factor 1 alpha and vascular endothelial growth factor overexpression in ischemic colitis, World J Gastroenterol 2005, 11:1535-1539
10. Brandt LJ: Bloody diarrhea in an elderly patient, Gastroenterology 2005, 128:157-163
11. Talks KL, Turley H, Gatter KC, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages, Am J Pathol 2000, 157:411-421
12. Greijer AE, Delis-van Diemen PM, Fijneman RJ, Giles RH, Voest EE, van Hinsbergh VW, Meijer GA: Presence of HIF-1 and related genes in normal mucosa, adenomas and carcinomas of the colorectum, Virchows Arch 2008, 452:535-544
13. Giatromanolaki A, Sivridis E, Maltezos E, Papazoglou D, Simopoulos C, Gatter KC, Harris AL, Koukourakis MI: Hypoxia inducible factor 1alpha and 2alpha overexpression in inflammatory bowel disease, J Clin Pathol 2003, 56:209-213
14. Karhausen J, Furuta GT, Tomaszewski JE, Johnson RS, Colgan SP, Haase VH: Epithelial hypoxia-inducible factor-1 is protective in murine experimental colitis, J Clin Invest 2004, 114:1098-1106
15. Colgan SP, Taylor CT: Hypoxia: an alarm signal during intestinal inflammation, Nat Rev Gastroenterol Hepatol 2010, 7:281-287
16. Westra J, Brouwer E, Bos R, Posthumus MD, Doornbos-van der Meer B, Kallenberg CG, Limburg PC: Regulation of cytokine-induced HIF-1alpha expression in rheumatoid synovial fibroblasts, Ann N Y Acad Sci 2007, 1108:340-348

17. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL: Hypoxia-inducible expression of tumor-associated carbonic anhydrases, Cancer Res 2000, 60:7075-7083
18. Hui EP, Chan AT, Pezzella F, Turley H, To KF, Poon TC, Zee B, Mo F, Teo PM, Huang DP, Gatter KC, Johnson PJ, Harris AL: Coexpression of hypoxia-inducible factors 1alpha and 2alpha, carbonic anhydrase IX, and vascular endothelial growth factor in nasopharyngeal carcinoma and relationship to survival, Clin Cancer Res 2002, 8:2595-2604

Chapter 9

General discussion and future directions

INTRODUCTION

Chronic gastrointestinal ischemia (CGI) has for long been thought of as a rare disease. This condition was previously only considered in patients with unexplained abdominal pain in the presence of stenotic disease of two or more gastrointestinal arteries. The introduction of functional testing has played a pivotal role in the diagnosis of CGI and has changed the above-mentioned concept of this disease. Functional testing has shown that CGI is more common than previously thought because it can also be caused by single vessel disease, as further confirmed by studies which showed that a majority of patients with single vessel disease had sustained response after adequate treatment. Moreover, functional testing helps to select those patients for treatment who are most likely to benefit from it. Gastrointestinal tonometry was introduced in the 1980's to detect mucosal ischemia. Studies showed that tonometry is an accurate tool to detect mucosal ischemia in patients clinically suspected of CGI. In this thesis we first assessed the additional diagnostic value of functional testing, i.e. tonometry in diagnosis of CGI. However, because of its invasive nature, the use of tonometry for diagnostic work-up of patients suspected of CGI has stayed limited to a few referral centers. Recently, a new minimally invasive technique, visible light spectroscopy (VLS), has been introduced to detect ischemia in patients with possible CGI. We determined the diagnostic accuracy of VLS to detect ischemia in patients clinically suspected of CGI. Then we evaluated the response to treatment in a large cohort of patients with possible CGI who were selected for treatment by means of a diagnostic work-up including radiological imaging, and VLS. Furthermore, in this thesis we assessed the risk factors of atherosclerosis in patients with stenotic disease of the gastrointestinal arteries, and evaluated the response to vasodilating medication in patients with non-occlusive CGI.

Chapter 1 discusses the aims and the outline of this thesis. The diagnosis of CGI remains a clinical challenge. The combination of postprandial pain, weight loss and an abdominal bruit is known as the 'classical' triad of CGI, but recent studies in larger cohorts of CGI patients have shown that this triad is only present in around 20% of CGI patients.[1-4] In addition to these clinical signs, there is no single test which is sensitive and specific enough to diagnose CGI. The current diagnostic approach in patients clinically suspected of CGI therefore includes a thorough medical and physical examination, imaging of the gastrointestinal arteries and a functional test to assess mucosal perfusion.[5-6] In **chapter 2,** we assessed the diagnostic value of clinical features, visualization of the gastrointestinal arteries, and evaluation of mucosal perfusion in patients clinically suspected of CGI. A multivariable model solely based on clinical features showed limited discriminative ability for the presence or absence of CGI. Addition of radiological imaging of the gastrointestinal arteries to the prediction model improved the discriminative ability substantially, and adding tonometry to the prediction model further improved the discriminative ability of the model.

Several studies with tonometry have shown that tonometry is an accurate functional test to detect mucosal ischemia.[7-8] Nevertheless, tonometry is not widely used because of its time consuming and invasive nature. VLS is a relatively new technique which enables to detect mucosal ischemia during upper endoscopy.[9] In **chapter 3** we prospectively evaluated the diagnostic accuracy of VLS in large cohort of patients clinically suspected of CGI. VLS during endoscopy was shown to be a promising, minimally invasive technique to detect ischemia in this group of challenging patients, with a sensitivity, and specificity of 90%, and 60%, respectively. Repeated VLS measurements showed improvement in 80% of CGI patients after clinically successful therapy.

In **chapter 4** we evaluated the treatment response in a large cohort of patients clinically suspected of CGI, who were selected for treatment by means of a diagnostic work-up including radiological imaging and VLS. Two-hundred-and-twelve patients referred for suspicion of CGI were prospectively included and followed. Treatment response was evaluated in patients with occlusive CGI. Occlusive CGI was diagnosed in 107 (50%) patients. Ninety-six (90%) of them were offered treatment. After a median follow-up of 13 months data concerning treatment response were available in 89 patients. Sixty-three (71%) patients had sustained response during follow-up. Response rate was 64% in patients with single vessel disease, and 83% in those with multi-vessel disease. Moreover, we determined predictors of positive response to treatment in these patients. Reported weight loss (OR 1.93), abdominal bruit (OR 2.36), and corpus saturation < 56% (OR 4.84) were the strongest predictors for a positive treatment response. When one of these parameters was present, the response rate was less than 50%, and the presence of two or three of these parameters predicted a response rate of > 85%. The classical risk factors for atherosclerosis such as male gender, age, diabetes mellitus, smoking, hypertension, hypercholesterolemia, positive family history of cardiovascular disease (CVD), obesity, and physical inactivity are well defined. [10-11] Any ischemic event is a strong risk factor for a new cardiovascular event. [12] [13-18] Although CGI is considered a manifestation of atherosclerosis, studies aiming at classical risk factors for atherosclerosis in patients with CGI are scarce. In **chapter 5** we presented the results of a case-control study which was designed to determine the contribution of classical atherosclerotic risk factors to atherosclerotic CGI, and the mortality risk in treated patients. Cases were patients with confirmed atherosclerotic CGI. Control subjects from the DiaGene Study population served as controls.[19]. They were randomly selected, and previously not diagnosed with CGI or diabetes. In 3 years, 195 patients were evaluated for suspected CGI. After a median follow-up of 19 months, atherosclerotic CGI was diagnosed in 68 patients. Controls consisted of 132 subjects. Female gender, diabetes, hypercholesterolemia, a personal and family history of CVD, and current smoking were highly associated with CGI. After adjustment for several factors, female gender (OR 2.14), diabetes (OR 5.59), current smoking (OR 5.78), and history of CVD (OR 21.61) remained significantly associated with CGI. CGI patients > 55 years had a higher median ten-year risk of death (15% vs. 5%, P = 0.001) compared to controls. During follow-up of 116 person-years, standardized mortality rate was higher in CGI patients (3.55).

For a long time it was thought that CGI only affects people with stenosis of at least two of the three gastrointestinal arteries, because it was assumed that a single stenosis of the celiac artery or the superior mesenteric artery is unlikely to cause CGI because of the abundant collateral circulation of the splanchnic vascular bed. Introduction of functional testing has shown that a majority of patients with single vessel stenosis and mucosal ischemia can benefit from treatment. In **chapter 6** we evaluated the clinical success rate to revascularization of a stenosis of either the celiac artery or superior mesenteric artery in patients with unexplained refractory gastrointestinal symptoms. A consensus diagnosis of CGI was made in 71/103 patients. Follow-up after revascularization was available in 68 patients (median follow-up 19 ± 13 months). The response rate to revascularization was 46/68 (67%). CGI is in the majority of cases caused by narrowing of the gastrointestinal arteries, with atherosclerosis being the most common underlying cause.[6, 8]

The introduction of functional tests to detect mucosal ischemia, such as gastrointestinal tonometry [7, 20] and visible light spectroscopy [9, 21], has shown that the spectrum of CGI may also include patients without macrovascular pathology, i.e. non-occlusive CGI. It is suggested that non-occlusive CGI results from a chronic state of splanchnic hypoperfusion. In a series of patients with non-occlusive CGI, vasospasm of the gastrointestinal arteries was observed during angiography. [20] Furthermore, in patients with chronic heart failure, splanchnic hypoperfusion has been found at low levels of exercise.[22] In theory, patients with non-occlusive CGI could benefit from vasodilating medication.[23] In **chapter 7** we assessed the effectiveness of vasodilating medication in patients diagnosed with non-occlusive CGI. Patients were diagnosed with non-occlusive CGI if both symptoms and functional testing were compatible with CGI, but gastrointestinal artery stenoses were absent. Patients were treated with escalating doses of isosorbide dinitrate (first line treatment) (ISD; 20 or 40 mg od), followed by ketanserin (second line treatment) (KTS; 20 or 40 mg od) if ISD was not successful or tolerated. Over a period of 3.5 years, 353 patients were referred with suspected CGI. Non-occlusive CGI was diagnosed in 47 (13%) patients. Vasodilation therapy was advised in 39 (83%) patients. One patient refused using ISD. Sustained response was achieved in 5 (13%) patients treated with ISD (median follow-up 29 months). Of 33 non-responders to ISD, 27 (82%) were treated with KTS. One patient was lost during follow-up. Two (8%) of them had a sustained response (median follow-up 11 months) after KTS. Two other patients had repeated positive response after reintroducing ISD. Thus, 31 patients actually received first and second-line treatment and complete follow-up data was available in these patients. Per protocol analysis showed that 9/31 (29%) patients had sustained response after first or second-line treatment.

Ischemic colitis accounts for 50-60% of all forms of gastrointestinal ischemia[24]. Endoscopy is often the first choice diagnostic approach in patients clinically suspected of ischemic colitis, since mucosal damage can be seen in ischemic regions and tissue samples can be taken

for histopathological analyses. However, endoscopic and histopathological findings often show nonspecific abnormalities[25-26], making it difficult to diagnose ischemic colitis. New techniques were needed to optimize diagnosis of ischemic colitis. A potential marker may be hypoxia inducible factor 1 alpha (HIF-1 alpha). HIF-1 alpha is a transcriptional factor which is induced by hypoxia.In **chapter 8** we compared the expression of HIF-1 alpha in normal colon, ischemic colitis, as well as in Crohn's disease (CD), ulcerative colitis (UC), and infectious colitis. Furthermore, we investigated if there is a correlation between the degree of inflammation and the expression level of HIF-1 alpha. Biopsies of 30 subjects with a normal colon, and of 33 patients with various types of colitis (9 pts with ischemic colitis, 9 with CD, 10 with UC, and 5 with infectious colitis) were collected for immunohistochemistry to determine the expression of HIF-1 alpha. HIF-1 alpha was not only expressed in ischemic colitis, but also in patients with various inflammatory disorders, including CD, UC, and infectious colitis. Expression was mainly detected at the bottom of the crypts in the positive normal colon samples, whereas the colitis group predominantly showed expression throughout the crypt and/or at the epithelial surface. The staining intensity in normal colon was weak in most samples. In contrast, the various colitis groups predominantly showed a moderate to strong staining. There was a moderately strong correlation between the degree of inflammation seen on histopathology and the intensity of HIF-1 alpha expression (Spearman correlation 0.578).

CONCLUSIONS AND FUTURE DIRECTIONS

The proposed diagnostic approach in patients clinically suspected of CGI includes radiological imaging of the gastrointestinal arteries and functional testing to assess mucosal ischemia. The studies in this thesis has shown that radiological imaging of the gastrointestinal arteries and functional testing substantially improve the diagnosis of CGI, and the strongest diagnostic contribution comes from assessment of mucosal ischemia. VLS measurement during upper endoscopy is a promising, easy to perform, minimally invasive technique to detect mucosal ischemia in patients clinically suspected of CGI. Results of a large cohort showed that a multi-diagnostic, including VLS as a functional test, and -disciplinary approach for suspected CGI patients, provides a high response rate on mid-long term follow-up. Moreover, unexplained gastrointestinal symptoms should be evaluated for CGI, and those with a single gastrointestinal artery stenosis and confirmed mucosal ischemia may greatly benefit from revascularization. This emphasizes the role of functional testing for assessment of CGI and selection of patients for treatment, as well as the major impact of treatment in both single and multi-vessel CGI. Furthermore, results showed that patients with atherosclerotic CGI have an increased estimated CVD risk, and severe excess mortality. Secondary cardiovascular prevention therapy should be advocated in patients with CGI. Patients with patent gastrointestinal arteries, but the presence of mucosal ischemia, so called non-occlusive CGI, can be treated with vasodilating medication.

However, this is only beneficial to one third of these patients. HIF-1 alpha can not differentiate between ischemia and other sources of inflammation, because it is expressed in patients with ischemic colitis as well as in other forms of colitis including Crohn's disease, ulcerative colitis, and infectious colitis, indicating that HIF-1 alpha is a sign of inflammation.

Future research should focus on improvement of diagnosis of CGI and better selection of those patients who would benefit most from revascularization. VLS measurement had a high sensitivity, but a relatively low specificity, future research can validate the established cut-off values and new cut-off values can be determined to enhance the specificity of VLS measurement. VLS measurements are conducted during upper endoscopy in a fasting state. It could be argued that mucosal ischemia occurs when metabolic demand exceeds the oxygen supply like during postprandial state. For this reason future research could focus on VLS measurement with jejunal feeding to determine the additional impact of feeding on accuracy of VLS measurement. Not all patients who were treated had repeated VLS measurements after treatment. A cohort study with repeated VLS measurements in all patients would determine the role of repeated measurements in follow-up of treated patients with CGI. Abdominal duplex ultrasound has an acceptable sensitivity and specificity for detection of stenosis in gastrointestinal arteries. Flow measurements in celiac artery and superior mesenteric artery by means of duplex ultrasound measurements at the time of analysis for CGI and at fixed time during follow-up can show how flow measurements in arteries can be used to detect restenosis in these arteries. This could lead to the approach that patients with recurrent symptoms could first have abdominal duplex ultrasound and only in case of suspicion of restenosis patients are then referred for radiological imaging such as CTA. Treatment of single vessel disease has been a challenging area for a long time. Studies have reported clinical success in patients with single vessel CGI. However, a randomized controlled trial including intervention by means of endovascular treatment and a sham procedure will present solid evidence for the proportion of patients who would benefit from treatment of a single vessel disease. Patients with the diagnosis of non-CGI have either, no stenosis and no ischemia, or have stenosis, but no ischemia. Thus, patients who have stenosis, but functional testing does not reveal mucosal ischemia are not selected for treatment. However, it remains unclear what proportion of these patients would have responded positively to treatment if they are treated despite normal measurements at functional testing. Ideally, all patients clinically suspected of CGI with stenosis in one or more of the gastrointestinal arteries, should be treated to evaluate the response in this group. However, because of the presence of asymptomatic stenosis, a certain research would encounter ethical concerns. Currently, follow-up of patients with non-CGI consists of a survey including questions concerning questions about ischemia related morbidity or mortality during follow-up and other diagnosis. So far, this seems the best available manner to evaluate either the consensus diagnosis of non-CGI was correct or not.

REFERENCES

1. Brandt LJ, Boley SJ. AGA technical review on intestinal ischemia. American Gastrointestinal Association. Gastroenterology 2000;118:954-68.
2. Fioole B, van de Rest HJ, Meijer JR, van Leersum M, van Koeverden S, Moll FL, van den Berg JC, de Vries JP. Percutaneous transluminal angioplasty and stenting as first-choice treatment in patients with chronic mesenteric ischemia. J Vasc Surg 2009.
3. Sarac TP, Altinel O, Kashyap V, Bena J, Lyden S, Sruvastava S, Eagleton M, Clair D. Endovascular treatment of stenotic and occluded visceral arteries for chronic mesenteric ischemia. J Vasc Surg 2008;47:485-491.
4. Oderich GS, Bower TC, Sullivan TM, Bjarnason H, Cha S, Gloviczki P. Open versus endovascular revascularization for chronic mesenteric ischemia: risk-stratified outcomes. J Vasc Surg 2009;49:1472-9 e3.
5. Otte JA, Geelkerken RH, Huisman AB, Kolkman JJ. What is the best diagnostic approach for chronic gastrointestinal ischemia? Am J Gastroenterol 2007;102:2005-10.
6. Mensink PB, Moons LM, Kuipers EJ. Chronic gastrointestinal ischaemia: shifting paradigms. Gut 2011;60:722-37.
7. Mensink PB, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. Dig Dis Sci 2008;53:133-9.
8. Mensink PB, van Petersen AS, Geelkerken RH, Otte JA, Huisman AB, Kolkman JJ. Clinical significance of splanchnic artery stenosis. Br J Surg 2006;93:1377-82.
9. Friedland S, Benaron D, Coogan S, Sze DY, Soetikno R. Diagnosis of chronic mesenteric ischemia by visible light spectroscopy during endoscopy. Gastrointest Endosc 2007;65:294-300.
10. Expert Panel on Detection EaToHBCiA. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;285:2486-97.
11. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C, American Heart A, National Heart LaBI. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
12. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. Lancet 1996;348:1329-39.
13. Cotter G, Cannon CP, McCabe CH, Michowitz Y, Kaluski E, Charlesworth A, Milo O, Bentley J, Blatt A, Krakover R, Zimlichman R, Reisin L, Marmor A, Lewis B, Vered Z, Caspi A, Braunwald E. Prior peripheral arterial disease and cerebrovascular disease are independent predictors of adverse outcome in patients with acute coronary syndromes: are we doing enough? Results from the Orbofiban in Patients with Unstable Coronary Syndromes-Thrombolysis In Myocardial Infarction (OPUS-TIMI) 16 study. Am Heart J 2003;145:622-7.
14. Vliegenthart R, Hollander M, Breteler MM, van der Kuip DA, Hofman A, Oudkerk M, Witteman JC. Stroke is associated with coronary calcification as detected by electron-beam CT: the Rotterdam Coronary Calcification Study. Stroke 2002;33:462-5.
15. Bhatt DL, Peterson ED, Harrington RA, Ou FS, Cannon CP, Gibson CM, Kleiman NS, Brindis RG, Peacock WF, Brener SJ, Menon V, Smith SC, Jr., Pollack CV, Jr., Gibler WB, Ohman EM, Roe MT. Prior polyvascular disease: risk factor for adverse ischaemic outcomes in acute coronary syndromes. Eur Heart J 2009;30:1195-202.

16. Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. N Engl J Med 1992;326:381-6.
17. Verouden NJ, Haeck JD, Kuijt WJ, Koch KT, Henriques JP, Baan J, Vis MM, Piek JJ, Tijssen JG, de Winter RJ. Early ST-segment recovery after primary percutaneous coronary intervention accurately predicts long-term prognosis after acute myocardial infarction. Am Heart J 2010;159:1005-11.
18. Wijnhoud AD, Maasland L, Lingsma HF, Steyerberg EW, Koudstaal PJ, Dippel DW. Prediction of major vascular events in patients with transient ischemic attack or ischemic stroke: a comparison of 7 models. Stroke 2010;41:2178-85.
19. Voight BF, Scott LJ, Steinthorsdottir V, Morris AP, Dina C, Welch RP, Zeggini E, Huth C, Aulchenko YS, Thorleifsson G, McCulloch LJ, Ferreira T, Grallert H, Amin N, Wu G, Willer CJ, Raychaudhuri S, McCarroll SA, Langenberg C, Hofmann OM, Dupuis J, Qi L, Segre AV, van Hoek M, Navarro P, Ardlie K, Balkau B, Benediktsson R, Bennett AJ, Blagieva R, Boerwinkle E, Bonnycastle LL, Bengtsson Bostrom K, Bravenboer B, Bumpstead S, Burtt NP, Charpentier G, Chines PS, Cornelis M, Couper DJ, Crawford G, Doney AS, Elliott KS, Elliott AL, Erdos MR, Fox CS, Franklin CS, Ganser M, Gieger C, Grarup N, Green T, Griffin S, Groves CJ, Guiducci C, Hadjadj S, Hassanali N, Herder C, Isomaa B, Jackson AU, Johnson PR, Jorgensen T, Kao WH, Klopp N, Kong A, Kraft P, Kuusisto J, Lauritzen T, Li M, Lieverse A, Lindgren CM, Lyssenko V, Marre M, Meitinger T, Midthjell K, Morken MA, Narisu N, Nilsson P, Owen KR, Payne F, Perry JR, Petersen AK, Platou C, Proenca C, Prokopenko I, Rathmann W, Rayner NW, Robertson NR, Rocheleau G, Roden M, Sampson MJ, Saxena R, Shields BM, Shrader P, Sigurdsson G, Sparso T, Strassburger K, Stringham HM, Sun Q, Swift AJ, Thorand B, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet 2010;42:579-89.
20. Otte JA, Geelkerken RH, Oostveen E, Mensink PB, Huisman AB, Kolkman JJ. Clinical impact of gastric exercise tonometry on diagnosis and management of chronic gastrointestinal ischemia. Clin Gastroenterol Hepatol 2005;3:660-6.
21. Van Noord D, Sana A, Benaron DA, Pattynama PM, Verhagen HJ, Hansen BE, Kuipers EJ, Mensink PB. Endoscopic visible light spectroscopy: a new, minimally invasive technique to diagnose chronic GI ischemia. Gastrointest Endosc 2011;73:291-8.
22. Krack A, Richartz BM, Gastmann A, Greim K, Lotze U, Anker SD, Figulla HR. Studies on intragastric PCO2 at rest and during exercise as a marker of intestinal perfusion in patients with chronic heart failure. Eur J Heart Fail 2004;6:403-7.
23. M. Bigirwamungu-Bargeman RHG, A.B. Huisman, J.J. Kolkman. Abdominal Migraine, a new and treatable disorder mimicking functional dyspepsia. 2009.
24. Theodoropoulou A, Koutroubakis IE. Ischemic colitis: clinical practice in diagnosis and treatment. World J Gastroenterol 2008;14:7302-8.
25. Brandt LJ, Boley SJ. Colonic ischemia. Surg Clin North Am 1992;72:203-29.
26. Gandhi SK, Hanson MM, Vernava AM, Kaminski DL, Longo WE. Ischemic colitis. Dis Colon Rectum 1996;39:88-100.

Summary

SUMMARY

Chapter 1 presents the aims and outline of this thesis.

Chapter 2 shows the results of prospective cohort study with the aim to assess the diagnostic value of clinical features, visualization of the gastrointestinal arteries, and evaluation of mucosal perfusion in patients clinically suspected of CGI. 186 patients referred for suspicion of CGI were included, and underwent a thorough medical and physical examination, radiological imaging of the gastrointestinal arteries, and tonometry. 116 (62%) patients were diagnosed with CGI. A multivariable model solely based on clinical features showed limited discriminative ability for the presence or absence of CGI (c-statistic, 0.62). Adding radiologic imaging to the prediction model improved the discriminative ability substantially (c-statistic, 0.81), and adding tonometry to the prediction model further improved the discriminative ability of the model (c-statistic, 0.90).

Chapter 3 describes the diagnostic accuracy of VLS in large cohort of patients clinically suspected of CGI. In 16 months 121 consecutive patients were included: 80 patients in a trainee data set, followed by 41 in a validation data set. CGI was diagnosed in 89 (74%) patients. VLS cut-off values were determined based on the diagnosis of CGI, and applied in the validation data set, and the results compared to the golden standard. VLS measurement had a sensitivity, and specificity of 90%, and 60%, respectively. Repeated VLS measurements showed improvement in 80% of CGI patients after clinically successful therapy.

Chapter 4 evaluates the treatment response in a large cohort of patients clinically suspected of CGI, who were selected for treatment with diagnostics including radiological imaging, and VLS, and in addition we determined predictors of positive response to treatment in these patients. 212 patients referred for suspicion of CGI were prospectively included and followed. Occlusive CGI was diagnosed in 107 (50%) patients. 96 (90%) were offered treatment. After a median follow-up of 13 months data concerning treatment response were available in 89 patients. 63 patients (71%) patients had sustained response during follow-up. Response rate was 64% in patients with single vessel disease, and 83% of those with multi-vessel disease ($P = 0.054$). Weight loss (OR 1.93), abdominal bruit (OR 2.36), and corpus saturation $< 56\%$ (OR 4.84) were the strongest predictors for a positive treatment response. The presence of all these three parameters predicts a response rate of up to 85%.

Chapter 5 describes the results of a case-control study, which was designed to determine the contribution of classical atherosclerotic risk factors to atherosclerotic CGI, and the mortality risk in treated patients. Patients referred with suspected CGI underwent a standard work-up including cardiovascular risk factors, radiological imaging of abdominal vessels and tonom-

etry. Cases were patients with confirmed atherosclerotic CGI. Controls were healthy subjects previously not known with CGI. In 3 years, 195 patients were evaluated for suspected CGI. After a median follow-up of 19 months, atherosclerotic CGI was diagnosed in 68 patients. Controls consisted of 132 subjects. Female gender, hypercholesterolemia, a personal and family history of cardiovascular disease (CVD), and smoking were highly associated with CGI. After adjustment female gender (OR 0.45 95 % CI 0.22-0.93), current smoking (OR 5.81, 95% CI 2.28-14.80), and history of CVD (OR 21.61, 95% CI 8.40-55.55) remained significant. CGI patients > 55 years had a higher median ten-year risk of death (15% vs. 5%, P = 0.001) compared to controls. During a follow-up of 116 person-years, the standardized mortality rate was higher in CGI patients (3.55; 95% confidence interval 1.70-6.52).

Chapter 6 describes the success rates of revascularization of either the celiac or superior mesenteric artery in patients with unexplained gastrointestinal symptoms and confirmed mucosal ischemia. A consensus diagnosis of single vessel CGI was made in 71/103 patients. Follow-up after revascularization was available in 68 patients (median follow-up 19 months). The response rate to revascularization was 46/68 (67%).

Chapter 7 describes the effectiveness of vasodilating medication in patients diagnosed with non-occlusive CGI in a prospective cohort-study. Patients were diagnosed with non-occlusive CGI if both symptoms and functional testing were compatible with CGI, but gastrointestinal artery stenoses were absent. Patients were treated with escalating doses of isosorbide dinitrate (first line treatment) (ISD; 20 or 40 mg od), followed by ketanserin (second line treatment) (KTS; 20 or 40 mg od) if ISD was not successful or tolerated. In 3.5 years, 353 patients were referred with suspected CGI. Non-occlusive CGI was diagnosed in 47 (13%) patients. Vasodilation therapy was advised in 39 (83%) patients. One patient refused using ISD. Sustained response was achieved in 5 (13%) patients treated with ISD (median follow-up 29 months). Of 33 non-responders to ISD, 27 (82%) were treated with KTS. One patient was lost during follow-up. Two (8%) of them had a sustained response (median follow-up 11 months) after KTS. Two other patients had repeated positive response after reintroducing ISD. Per protocol analysis showed an overall therapeutic response of (9/31) 29%.

Chapter 8 shows the results of the comparison of the expression of HIF-1 alpha in normal colon, ischemic colitis, as well as in Crohn's disease (CD), ulcerative colitis (UC), and infectious colitis. Biopsies of 30 subjects with a normal colon, and of 33 patients with various types of colitis (9 pts with ischemic colitis, 9 with CD, 10 with UC, and 5 with infectious colitis) were collected for immunohistochemistry to determine the expression of HIF-1 alpha. Expression of HIF-1 alpha was only seen in 37% samples of normal colon, but HIF-1 alpha was expressed in the majority of samples of various types of colitis. Expression was mainly detected at the bottom of the crypts in the positive normal colon samples, whereas the colitis group

predominantly showed expression throughout the crypt and/or at the epithelial surface. The staining intensity in normal colon was weak in most samples, while the various colitis groups predominantly showed a moderate to strong staining. There was a moderately strong correlation between the degree of inflammation seen on histopahtology and the intensity of HIF-1 alpha expression (Spearman correlation 0.578).

Chapter 9 discusses the major findings of this thesis and directions for future reseach.

Summary in Dutch

SAMENVATTING

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

Hoofdstuk 2 presenteert de resultaten van een prospectieve studie met als doel om de diagnostische waarde van klinische kenmerken, afbeelding van abdominale arteriën en tonometrie aan te tonen in de diagnostiek van mucosale ischemie in patiënten die klinisch verdacht worden van chronische maagdarm ischemie (CGI). Er werden 186 patiënten, die verwezen werden met verdenking op CGI, geïncludeerd in deze studie. Bij al deze patiënten is er een standaard work-up verricht bestaande uit een uitvoerige anamnese, lichamelijk onderzoek, radiologische afbeelding van abdominale arteriën en tonometrie. 116 (62%) patienten werden gediagnosticeerd met CGI. Een multivariabel model waarin alleen de klinische kenmerken zijn meegenomen voor de voorspelling van CGI liet een beperkt onderscheidende vermogen zien voor de aan- of afwezigheid van CGI (c-statistiek, 0.62). Toevoeging van radiologische afbeelding aan het predictie model verbeterde het onderscheidend vermogen aanzienlijk (c-statistiek, 0.81), en toevoeging van tonometrie aan het predictie model zorgde voor een verdere verbetering van het onderscheidend vermogen van het model (c-statistiek, 0.90).

Hoofdstuk 3 beschrijft de diagnostische accuratesse van visible light spectroscopy (VLS) in een cohort van patiënten met klinische verdenking op CGI. In 16 maanden werden 121 opeenvolgende patiënten geïncludeerd: 80 patiënten in een "trainee data" set, gevolgd door 41 patiënten in een "validatie data" set. De diagnose CGI werd gesteld bij 89 (74%) patiënten. VLS afkappunten werden bepaald op basis van de diagnose CGI en vervolgens toegepast in de "validatie data" set. De resultaten werden vergeleken met de gouden standaard, namelijk de definitieve diagnose CGI na langdurige follow-up. VLS meting liet een sensitiviteit en specificiteit zien van respectievelijk 90% en 60% voor de diagnose van CGI. Mucosale saturaties verbeterden in 23 (80%) van de patiënten die een herhaalde VLS meting ondergingen na klinisch succesvolle behandeling.

Hoofdstuk 4 evalueert de respons op behandeling in een cohort van patiënten met klinische verdenking op CGI die middels radiologische afbeelding van de abdominale arteriën en VLS meting werden geselecteerd om behandeld te worden. Daarnaast is er ook gekeken of er voorspellers bepaald konden worden die een positieve respons in deze patiënten voorspellen. Er werden 212 patiënten met een verdenking op CGI prospectief geïncludeerd en gevolgd. De diagnose occlusieve CGI werd gesteld bij 107 (50%) patiënten. 96 (90%) van deze 107 patiënten kregen de behandeling aangeboden. Data betreffende follow-up was beschikbaar in 89 patienten na een mediane follow-up duur van 13 maanden. 63 patiënten (71%) waren klachtenvrij. Respons op behandeling was 64% in patiënten met éénvatslijden en 83% in patiënten met

meervatslijden (P = 0.054). Gewichtsverlies (OR 2.16, 95% BI 0.80-5.89), abdominale souffle (OR 3.15, 95% BI 0.93-10.67) en saturatie in corpus < 56% (OR 4.40, 95% BI 0.92-21.08) waren de sterkste voorspellers van een positief respons. Indien al deze drie voorspellers aanwezig zijn bij een patiënt die behandeld wordt, dan is er een kans op 85% op een positieve respons.

Hoofdstuk 5 geeft de resultaten weer van een case-control studie met het doel om te bepalen of de klassieke risicofactoren van atherosclerose geassocieerd zijn met CGI op basis van atherosclerose, en wat de risico op sterfte is in deze groep patiënten. Patiënten die werden verwezen met mogelijk CGI ondergingen een standaard work-up bestaande uit het in kaart brengen van cardiovasculaire risicofactoren, radiologische afbeelding van de abdominale arteriën en tonometrie. De cases waren patiënten met bevestigd CGI en de controles waren gezonde deelnemers die in het verleden niet gediagnosticeerd waren met CGI. In 3 jaar werden 195 patiënten met verdenking op mogelijk CGI geïncludeerd. Na een follow-up duur van 19 maanden werden 68 patiënten gediagnosticeerd met atherosclerotische CGI. De controles bestonden uit 132 deelnemers. Het vrouwelijk geslacht, hypercholesterolemie, hart-en vaatziekten (HVZ) in de voorgeschiedenis en bij eerstegraads familieleden en roken waren positief geassocieerd met het ontwikkelen van CGI. Na correctie bleven het vrouwelijk geslacht (OR 0.45 95 % BI 0.22-0.93), roken (OR 5.81, 95% BI 2.28-14.80), en een voorgeschiedenis met HVZ (OR 21.61, 95% BI 8.40-55.55) significant geassocieerd met het ontwikkelen van CGI. CGI patiënten ouder dan 55 jaar hadden een hogere mediane 10-jaars risico op sterfte in vergelijking met de controles (15% vs. 5%, P = 0.001). Na een follow-up van 116 persoonsjaren was de standaard mortaliteitsratio hoger in CGI patiënten (3.55; 95% BI 1.70-6.52).

Hoofdstuk 6 beschrijft een studie met het succes percentage van revascularisatie van de truncus coelicus of arteria mesenterica superior in patiënten met onverklaarbare gastrointestinale symptomen en bevestigd mucosale ischemie. Een consensus diagnose van CGI op basis van éénvatslijden werd gesteld bij 71 van de 103 patiënten. Follow-up na revascularisatie was beschikbaar in 68 patiënten (mediane follow-up 19 maanden). Het respons percentage na revascularisatie was 67% (46/68).

Hoofdstuk 7 geeft een prospectieve cohort studie weer waarin het effect van vaatverwijdende medicatie bij patiënten met niet-occlusieve CGI wordt beschreven. Patiënten werden gediagnosticeerd met niet-occlusieve CGI als patiënten op basis van symptomen verdacht werden op CGI en als mucosale ischemie aanwezig was, in afwezigheid van stenose van abdominale arteriën. Patiënten werden behandeld met oplopende dosering van isosorbidedinitraat (eerste lijnsbehandeling) (ISD; 20 or 40 mg dd), gevolgd door ketanserin (tweede lijnsbehandeling) (KTS; 20 or 40 mg dd) als ISD niet tot klachtenverbetering leidde of in geval van bijwerkingen. In 3.5 jaar werden 353 patiënten verwezen met een klinische

verdenking op CGI. De diagnose niet-occlusieve CGI werd gesteld bij 47 (13%) patiënten. Vaatverwijdende medicatie werd geadviseerd aan 39 (83%) van de patiënten.
Één patiënt (2%) weigerde om ISD te gebruiken. Blijvende respons werd bereikt in 5 (13%) patiënten die behandeld werden met ISD (mediane follow-up 29 maanden). Van de 33 niet-responders, werden 27 (82%) behandeld met KTS. Follow-up data was beschikbaar in 26 patiënten. Twee patiënten (8%) hadden een blijvende respons na het gebruik van KTS (mediane follow-up duur 11 maanden). Twee andere patiënten ondervonden in eerste instantie een positief effect door ISD en na enige tijd verdween dit effect. Na een periode gestopt te zijn met ISD, werd het middel opnieuw gebruikt door patiënten waarna het tot klachtenverbetering leidde. Bij analyse per protocol werd een respons gezien van 29% (9/31).

Hoofdstuk 8 laat de resultaten zien van een studie waarin een vergelijking is gemaakt van de expressie van HIF-1 alpha in het normale colon, ischemische colitis, ziekte van Crohn, colitis ulcerosa en infectieuze colitis. Biopten van 30 deelnemers met een normale colon en 33 patiënten met verschillende typen colitis (9 patiënten met ischemische colitis, 10 met colitis ulcerosa en 5 met infectieuze colitis) werden verzameld voor immunohistochemische kleuring. Expressie van HIF-1 alpha werd gezien in 37% van de biopten van het normale colon en in bijna 80% van de biopten van de patiënten met colitis. In de biopten van het normale colon kwam HIF-1 alpha met name tot expressie in het diepe gedeelte van de crypte, terwijl in de biopten van de groep met colitis de HIF-1 alpha met name tot expressie kwam alleen in het bovenste gedeelte van de crypte of zowel in het bovenste als het onderste gedeelte van de crypte. Er was een matig sterke correlatie tussen de mate van ontsteking gezien bij histopathologisch onderzoek en de intensiteit van HIF-1 alpha expressie (Spearman correlatie 0.578).

Hoofdstuk 9 beschrijft de belangrijkste bevindingen van dit proefschrift en aanwijzingen voor toekomstig onderzoek.

List of publications

LIST OF PUBLICATIONS

1. **A. Sana,** Y. vergouwe, D. Van Noord, L.M.G. Moons, P.M.T Pattynama, H.J.M Verhagen, E.J. Kuipers, P.B.F. Mensink. Diagnosing chronic gastrointestinal ischemia: value of clinical features, radiological imaging, and gastrointestinal tonometry. Clinical Gastroenterology and Hepatology 2011;9:234-241

2. D. Van Noord, **A. Sana,** D.A. Benaron, p.M.T. Pattynama, H.J.M. Verhagen, B.E. Hansen, E.J. Kuipers, P.B.F. Mensink. Endoscopic visible light spectroscopy: a newminimally invasive technique to diagnose chronic gastrointestinal ischemia. Gastrointestinal Endoscopy 2011;73:291-8

3. **A. Sana,** L.M.G. Moons, E.V. Rouwet, T.W. Galema, A. Moelker, D. Van Noord, P. B.F. Mensink, E. J. Kuipers.Vasodilation therapy for patients with non-occlusive chronic gastrointestinal ischemia: a prospective pilot-study. *Provisionally accepted in World Journal of Gastroenterology*

4. **A. Sana,** D. Van Noord, P.B.F. Mensink, S.Kooij, K. Van Dijk, B. Bravenboer, A.G. Lieverse, E.J.G. Sijbrands, J. G. Langendonk, E. J. Kuipers. Cardiovascular Disease and Mortality in Chronic Gastrointestinal Ischemia. *Atherosclerosis 2012, in press*

5. **A. Sana,** L.M.G. Moos, B.E. Hansen, P. DeWint, D. Van Noord, P.B.F. Mensink, E.J. Kuipers. Visible light spectroscopy in diagnosis of chronic gastrointestinal ischemia: a cohort study. *Submitted.*

6. **A. Sana,** L.M.G. Moons, K. Biermann, R. Smits, P.B.F. Mensink, P. Van Diest, E.J. Kuipers. Hypoxia inducible factor 1 alpha expression as marker of intestinal inflammation and ischemia. *Submitted.*

7. L.M.G. Moons, **A. Sana,** S.F.M. Jenniskens, A. Moelker, P. DeWint, D. Van Noord, H.J.M. Verhagen, P.B.F. Mensink, E.J. Kuipers, E.V. Rouwet. Revascularization of a single gastrointestinal artery stenosis should be considered in patients with unexplained refractory chronic gastrointestinal symptoms. *Submitted.*

PhD portfolio

PHD PORTFOLIO

Oral Presentations

2009

Risk factors for abdominal arterial atherosclerosis
Dutch Society of Gastroenterology, Veldhoven the Netherlands

2010

Diagnosing chronic upper gastrointestinal ischemia: predictive value of symptoms, radiological imaging, and gastrointestinal tonometry
Dutch Society of Gastroenterology, Veldhoven the Netherlands

2011

Clinical effect of vasodilating agents in patients diagnosed with non-occlusive mesenteric ischemia
Dutch Society of Gastroenterology, Veldhoven the Netherlands

Chronic gastrointestinal ischemia due to atherosclerotic narrowing is related to risk factors for cardiovascular disease
Dutch Society of Gastroenterology, Veldhoven the Netherlands

2012

Visible light spectroscopy in diagnosis of chronic gastrointestinal ischemia: a cohort study
Dutch Society of Gastroenterology, Veldhoven the Netherlands

Poster Presentations

2010

Risk factors for abdominal arterial atherosclerosis
Digestive Disease Week, New Orleans, USA.

Diagnosing chronic upper gastrointestinal ischemia: predictive value of symptoms, radiological imaging, and gastrointestinal tonometry
Digestive Disease Week, New Orleans, USA.

2011

Chronic gastrointestinal ischemia due to atherosclerotic narrowing is related to risk factors for cardiovascular disease
Digestive Disease Week, Chicago, USA.

2012

Predictors for positive response in patients treated for chronic gastrointestinal ischemia
Digestive Disease Week, San Diego, USA.

Visible light spectroscopy in diagnosis of chronic gastrointestinal ischemia: results of a cohort study
Digestive Disease Week, San Diego, USA.

Courses

Principles of research in medicine - NIHES, Erasmus MC, Rotterdam, the Netherlands
Introduction to data analysis - NIHES, Erasmus MC, Rotterdam, the Netherlands
Regression analysis - NIHES, Erasmus MC, Rotterdam, the Netherlands
Biomedical English writing and communication - Erasmus MC, Rotterdam, the Netherlands

Tutoring

Supervising medical students in extracurricular research activities

Membership

2009 Dutch Society of Gastroenterology

Acknowledgements

DANKWOORD

Vanaf maart 2009 heb ik gewerkt aan mijn promotieonderzoek. In de afgelopen drie jaar ben veel indrukwekkende, interessante en lieve mensen tegengekomen die mij hebben geholpen om dit proefschrift tot stand te brengen.

Mijn promotor, Prof. Dr. E.J. Kuipers, wil ik hartelijk bedanken voor de begeleiding tijdens mijn promotieonderzoek. Uw enthousiasme, kritische blik en heldere inzichten hebben mij al deze jaren geleid bij het uitvoeren van mijn onderzoek. De overleggen met u zijn verhelderend en motiverend geweest voor mij. Hartelijk dank voor uw steun en begrip en ik vond het een eer met u samengewerkt te hebben.

Dr. P.B.F. Mensink, beste Peter, als co-promotor heb je mij geleid met mijn eerste stappen in het uitvoeren van promotieonderzoek. Ik heb me altijd gelukkig geprezen om door jou begeleid te worden. Je bent altijd duidelijk en weet ingewikkelde materie op een simpele manier uit te leggen. Wat ik ook enorm bewonder is dat je me altijd hebt geleerd om het groter geheel te zien en om niet te vergeten om naast het werk ook van mijn leven te genieten. Je hebt altijd voor me klaargestaan, zelfs toen je in Australië woonde.

Dr. L.M.G. Moons, beste Leon, na het vertrek van Peter naar Australië heb jij op een uitstekende manier mijn begeleiding als co-promotor overgenomen. Jouw enthousiasme en kritische blik hebben mij geholpen om mijn eigen kritische blik verder te ontwikkelen. Ik waardeer het enorm dat je me altijd hebt gemotiveerd om het beste uit een project te halen. Hartelijk dank voor je tijd en hulp. Zelfs tijdens je werk in het UMC Utrecht heb je me je hulp aangeboden en mij van waardevolle wetenschappelijke tips voorzien.

Dr. R.A. de Man, ik wil u hartelijk bedanken dat u mij de kans hebt gegeven om me te specialiseren als een maag-, darm- en leverarts.

Dr. P. DeWint, beste Pieter, bedankt voor je ondersteuning en inbreng binnen mijn promotieonderzoek. Ik vond het erg prettig om met je samen te werken. Dr. E.T.T.L. Tjwa, beste Eric, ik heb je in eerste instantie leren kennen als mijn kamergenoot op het MDL lab en later heb ik het genoegen gehad om met je samen te werken. Bedankt dat je altijd voor mij hebt klaar gestaan en me adviezen gegeven op het gebied van wetenschap en carrière.

Tevens wil ik heel graag de leden van de leescommissie bedanken voor hun moeite en tijd om mijn proefschrift te beoordelen en hartelijk dank aan alle leden die de moeite hebben genomen om in de grote commissie deel te nemen.

Dr. E.F.H. van Bommel en dr. R. Beukers en alle andere internisten en maag-, darm- en leverartsen van het Albert Schweitzerziekenhuis wil ik bedanken voor hun begeleiding bij mijn vooropleiding interne geneeskunde en een deel van mijn opleiding tot maag-, darm- en leverarts. Tevens wil ik al mijn collega's van de interne geneeskunde en maag-, darm- en leverziekten bedanken voor een prettige werksfeer.

Dr. M.J.M. Smits, beste Ron, hartelijk dank voor je hulp en adviezen rondom het basaal wetenschappelijk onderzoek. Dr. K. Biermann, beste Katharina, ik ben je dankbaar voor al je ondersteuning op de afdeling pathologie.

Beste Bettina Hansen, bedankt dat ik bij je kon aankloppen met mijn statistiek vragen en daar ben ik erg blij om.

Ik wil graag het multidisciplinaire team van maagdarm ischemie, leden van de vakgroepen interventie radiologie en vaatchirurgie, bedanken voor hun prettige en leerzame samenwerking.

Bovendien wil ik alle co-auteurs bedanken voor hun ondersteuning en moeite om mooie manuscripten tot stand te brengen. In het bijzonder, dr. J.G. Langendonk van de interne geneeskunde, beste Janneke, ontzettend bedankt voor alle tijd en moeite die je in ons project hebt gestoken. Je stond altijd klaar en nam ruim de tijd om te overleggen. Dat waardeer ik enorm.

Zonder de bereidheid en vrijwilligheid van patiënten zouden de projecten niet tot stand zijn komen, daarom wil ik alle patiënten hartelijk bedanken voor hun medewerking.

Beste Désirée, heel erg bedankt voor je hulp en de prettige samenwerking als mijn voorgangster op dit project. Beste Jehan, ik was blij verrast toen ik hoorde dat jij mijn opvolgster gaat worden. Ik wens je het allerbeste met het voortzetten van het onderzoeksproject.

Beste Wendy en Bernadette, als secretaresses van de afdeling maag-, darm- en leverziekten hebben jullie mij altijd ondersteund en begeleid bij de procedure van het promotietraject. Ontzettend bedankt lieve dames.

Beste Minou Mangroo, lieve Minou, je hebt me altijd erg goed geholpen met de logistiek van patiëntenzorg. Ik heb ook altijd genoten van onze niet-werk gerelateerde gesprekken. Daarnaast wil ik de andere lieve dames en heer, Nayla, Nermin, Lakshmie, Wilma, Ellen en Ronald, van de polikliniek maag-, darm- en leverziekten bedanken voor hun ondersteuning. Lieve Nayla, Nermin en Lakshmie, ik mis onze gesprekken en gezelligheid.

Ik wil graag alle medewerkers van de afdeling endoscopie inclusief de lieve dames van de balie en verpleegafdeling maag-, darm- en leverziekten bedanken voor hun hulp met de patiëntenzorg. In het bijzonder wil ik Marieke, Audery, Janiska, Deborah, Michel, Behka en Sandra bedanken voor de prettige samenwerking tijdens het VLS programma.

Ik wil graag alle arts-assistenten van maag-, darm- en leverziekten bedanken voor hun hulp en gezelligheid tijdens mijn onderzoek en in het bijzonder wil ik Huseyin Aktas bedanken, die me altijd als zijn "zusje" zag, en bereid was om mij van waardevolle adviezen te voorzien. Lieve Marion Kerkhof, ik ben erg blij om samen met jou in Dordrecht te werken. Beste Lieke en Hajo, ik vond het zeer prettig om met jullie samengewerkt te hebben op de polikliniek van maagdarm ischemie.

Tijdens mijn promotieonderzoek heb ik vaker van werkplaats gewisseld, met als voordeel dat ik ook op het MDL heb gewerkt. Ik wil graag mijn lieve kamergenoten, Nadine, Colin, Bisheng, Eric, Farbod en Mark, bedanken voor de gezelligheid. Daarnaast wil ik alle andere medewerkers op het MDL lab bedanken voor hun gezelligheid en samenwerking. Lieve Suomi, ik ben blij dat ik een lieve vriendin zoals jij heb ontmoet. Ik wens je het allerbeste en ik weet zeker dat je een hele goede chirurg gaat worden. Beste Waqar, erg bedankt dat je me hebt geholpen met het maken van figuren.

Ik wil graag mijn collega arts-onderzoekers, Angela, Robert, Vincent R, Vincent de J, Jerome, Nicoline, Esther, Margot, Aafke, Jildou, Jorie, Lisanne, Susanne, Leonie, Caroline, Femme, Pauline, Edith, Willem Pieter, Vera, Veerle, Daphne, Atija, Ludi, Ad, Milan, Else-Mariëtte, Roeland en alle andere onderzoekers bedanken voor de gezelligheid tijdens de congressen en de lunchen. Lieve Özlem, ik ben erg blij dat jij mijn collega bent geworden in Dordrecht. Lieve Edmée, we hebben altijd over alles met elkaar kunnen praten en met elkaar kunnen delen en het is me een waar genoegen om jou ontmoet te hebben.

Lieve roomies, Lauran, Celine, Florine en Wesley, met jullie heb ik lief en leed gedeeld. Ontzettend bedankt voor de gezelligheid. Florine, je wordt zeker een mooie bruid en ik wens je nog vele jaren van geluk met jouw Michel. Lieve Lauran, vanaf de eerste dag op het werk was jij mijn maatje. We hebben samen veel gelachen en mooie discussies. Ik wens je veel liefde met jouw Margareta en wens je veel succes met het afronden van jouw proefschrift. Bedankt dat je altijd voor me klaar hebt gestaan. Lieve Celine, samen hebben wij veel gelachen en soms ook gehuild. Je bent een mooi en lief persoon en ontzettend bedankt dat je altijd voor me hebt klaar gestaan. Ik wens je ontzettend veel succes in je carrière en met het afronden van jouw promotieonderzoek. Je bent een doorzetter, dus je komt er wel.

Lieve Renate, wij begonnen op dezelfde dag met ons promotieonderzoek en vanaf die dag zijn wij maatjes. Jij hebt mij altijd gesteund en begrepen en daarom vond ik het een eer dat jij mijn paranimf wilde zijn. Ik wens je een super mooie toekomst met jouw Robert en jullie kleine wonder Julia.

Ik wil heel graag mijn vrienden en vriendinnen bedanken voor de gezellige en mooie tijden naast het werk. Lieve Lieke, bedankt voor je support al die tijd. Beste Meelad, bedankt voor je kritische noten en veel succes met jouw mooie carrière. Beste Achid, bedankt voor het ontwerpen van mijn mooie omslag. Ik ben er erg blij mee. Beste mevrouw Azzat Ahanger, ik wil u van harte bedanken voor uw hulp bij het vertalen van het stukje in Dari.

Mijn lieve zusje, Roya, ik vind het een eer dat jij mijn paranimf wilde zijn. Je staat altijd voor me klaar en heb jouw steun in alles. Ik prijs me ontzettend gelukkig dat ik jou, een lief en mooi persoon, als zusje heb en ik hoop dat we altijd aan elkaars zijden zullen staan.

Lieve papa en mama, Nasrin, Mark, Nil, Lilian, Yama, Fadua, Maya, Sahel, Shakib, Sevilay, Lina en Farzad, ik wil jullie ontzettend bedanken voor jullie steun en liefde in al deze jaren. Zonder jullie zou ik niet zo ver zijn gekomen en ik ben blij om zulke prachtige mensen in mijn leven te hebben. Lieve papa en mama van Mojtaba, Farwah, Eskander en de rest, een lievere schoonfamilie had ik me niet kunnen wensen. Ontzettend bedankt voor jullie mooi cadeau en steun.

And last but not least, mijn liefste Mojtaba. Vanaf het moment dat ik jou heb leren kennen heb je mij gesteund, vooral in de meest drukke periode van mijn onderzoek waarbij ik het moest combineren met het werken in de kliniek. Jouw liefde en luisterend oor hebben mij enorm geholpen in het volbrengen van dit proefschrift. Ik kijk uit naar jouw mooi proefschrift en naar alle mooie en liefdevolle momenten wat ons te wachten staat in de toekomst. Er is er maar één van jou!

Curriculum vitae

CURRICULUM VITAE

Aria Sana werd geboren op 10 april 1983 te Herat, Afghanistan. In 1996 is zij met haar ouders, drie broers en zusje naar Nederland gekomen. In 2002 behaalde zij het eindexamen VWO aan het Katholieke Scholengemeenschap Etten-Leur. In 2002 begon zij aan haar studie geneeskunde aan de Radboud Universiteit Nijmegen. In 2006 heeft zij wetenschappelijk onderzoek gedaan in Indonesië, getiteld "Nutritional status of HIV patients in Bandung, West Java, Indonesia", onder begeleiding van Prof. Dr. J.M. Tolboom, Dr. M.A. Dijkhuizen en Dr. F.T. Wieringa. Tevens heeft zij haar oudste co-schap in Managua, Nicaragua gelopen. Vervolgens heeft zij haar co-schappen afgesloten met een keuze co-schap op de afdeling maag-, darm- en leverziekten te Canisius Wilhelmina Ziekenhuis waar haar enthousiasme voor dit vakgebied groeide. Na het behalen van het arts-examen in december 2008 heeft zij van januari tot februari 2009 als arts-assistent op de afdeling interne geneeskunde van het Jeroen Bosch Ziekenhuis te Den Bosch gewerkt. In maart 2009 kon zij beginnen als arts-onderzoeker op de afdeling maag-, darm- en leverziekten van het Erasmus MC te Rotterdam. Zij begon onder begeleiding van Prof. Dr. E.J. Kuipers en Dr. P.B.F. Mensink aan het onderzoek naar de verbetering van de diagnostiek en behandeling van chronische maagdarm ischemie. Na het vertrek van P.B.F. Mensink naar Australië werd zij begeleid door Dr. L.M.G. Moons. In april 2012 is zij begonnen aan haar opleiding tot maag-, darm- en leverarts (opleider Dr. R. A. de Man). Haar vooropleiding interne geneeskunde (opleider Dr. E.F.H van Bommel) en een deel van de opleiding tot maag-, darm- en leverziekten (opleider Dr. R. Beukers) wordt gevolgd in het Albert Schweitzer Ziekenhuis in Dordrecht.