Early detection of Micro- and Macro-circulation in the ischemic gastrointestinal tract and the diagnosis and treatment of patients with median arcuate ligament syndrome

Ph.D. thesis by

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During my thesis, I was fortunate to marry Lars-Erik, whose presence has been a lifeline. I must emphasize that, during this time, I truly believe that his presence in my life has been vital. He has taken care of me with immense care and made sure I prioritized my well-being by being my chef, driver, sleeping pill, and psychological therapist. As my loving partner, he understood my struggles and encouraged me to stand up for myself in the face of unfairness. His love, patience, encouragement, and endless support have made a significant impact on the completion of this thesis. I am incredibly fortunate to have him as my life partner and I eagerly look forward to sharing the post-graduate chapter of my life with him.

ABBREVIATIONS

α-GST	α-glutathione S-transferase
AGA	American Gastroenterological Association
ALT	Alanine aminotransferase
AMI	Acute mesenteric ischemia
AST	Aspartate aminotransferase
BFR	Blood flow rate
BMT	Best medical treatment
CA	Celiac artery
CI	Confidence interval
CMI	Chronic mesenteric ischemia
CRP	C-reactive protein
CTA	Computer tomography angiogram
CVD	Cardiovascular disease
DSA	Digital subtraction angiography
DUS	Duplex ultrasound
EDV	End diastolic velocity
ESVS	European Society of Vascular Surgery
GALS	Gastroscopy assisted laser Doppler flowmetry and visible light spectroscopy
GET	Gastric exercise tonometry
GI	Gastrointestinal tract
ICU	Intensive care unite
I-FABP	Intestinal fatty acid-binding protein
IMA	Inferior mesenteric artery
IU	International units
LDF	Laser Doppler flowmetry

MAL	Median arcuate ligament
MALS	Median arcuate ligament syndrome
MAP	Mean arterial pressure
MIE	Minimal invasive esophagectomy
MRA	Magnetic resonance imaging angiography
NOMI	Non-occlusive mesenteric ischemia
O2C	Oxygen 2 See
PCO_2	Partial pressure of carbon dioxide
PMAS	Percutaneous mesenteric artery stenting
PSV	Peak systolic velocity
PTA	Percutaneous transluminal angioplasty
rAAA	Rupture of abdominal aorta aneurysm
rHB	Relative hemoglobin amount
ROC	Receiver operating characteristic curve
SD	Standard deviation
SMA	Superior mesenteric artery
SO_2	Oxygen saturation
VLS	Visible light spectroscopy

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THESIS SUMMARY

Mesenteric ischemia is a disorder associated with diminished blood supply to the gastrointestinal tract due to internal or external narrowing of the mesenteric vessels. It may present as an acute or chronic process. The acute form, acute mesenteric ischemia is a serious clinical entity associated with ischemia of the gastrointestinal (GI) tract and gangrene of the bowels which in most cases causes death [1-3]. The chronic form, chronic mesenteric ischemia (CMI), is a more insidious process proceeding over at least three months or years. However, CMI is an impairing state it is immediately not a life-threatening illness, although if left untreated in more than half of the cases develops into the acute phase, which is associated with up to 70% mortality [1, 4, 5].

The most common cause of CMI is arteriosclerosis of the mesenteric vessels, characterized by the classical triad of postprandial abdominal pain, food aversion, and weight loss [6]. A less frequent cause of CMI is external compression of the celiac artery and the celiac ganglion by the median arcuate ligament giving rise to the condition called median arcuate ligament syndrome (MALS). Although the classical triad is described only in 16 - 22% [6] both atherosclerotic CMI and MALS have throughout history remained controversial because of their unspecific wide range of clinical symptoms, unclear findings on the physical examination, lack of specific biomarkers, imaging techniques for early diagnosis and that some have symptom relapse after treatment.

It was previously believed that at least two of the three major mesenteric arteries must be stenotic or occluded to receive treatment and that "single artery stenosis" does not cause problems. As a consequence, there is great doubt in the existence of the disease MALS with "single artery stenosis", and disagreement about its etiology whether it is ischemic or neurological, and therefore excessive skeptics toward surgical treatment on the basis that not all develop symptom relief after surgery. CMI patients suffer for an average of three years before the diagnosis [6, 7]. Considering this wearisome disease that has low prevalence but high risk the guidelines at all times have requested accurate diagnosis early in the course of the disease, we find it crucial to investigate diagnostic tools for early diagnosis of CMI and MALS.

In Paper I, we investigated two different diagnostic tools for examining the mesenteric circulation in patients with atherosclerotic CMI and MALS. We used endoscopic duplex ultrasound, and transabdominal duplex ultrasound (E-DUS and TA-DUS) in detecting stenosis in the celiac artery and superior mesenteric artery. The results were then compared to computer tomography angiogram (CTA) findings, which is the standard diagnostic tool for CMI and MALS. Results showed that E-DUS has higher sensitivity and negative predictive value, but lower specificity than TA-DUS. The study concluded that E-DUS has great potential to be used as an initial screening test for patients suspected of CMI.

In Paper II and Paper III, we investigated the use of laser Doppler flowmetry (LDF) and visible light spectroscopy (VLS) for measuring microcirculation of the GI tract during minimally invasive esophagectomy in patients with esophageal cancer and laparoscopic decompression of coeliac artery in patients with MALS. The results gave significantly lower mixed arterial and venous saturation of hemoglobin levels measured with VLS in patients undergoing minimally invasive esophagectomy and in patients with MALS compared to healthy individuals. This indicates a decrease in tissue saturation. Thus, a significant effect of the combined use of LDF and VLS in detecting ischemic changes in the GI tract was demonstrated in both of our studies. This concludes that the combined use of LDF & VLS offers reliable and prompt feedback regarding the microcirculation of the GI tract. This valuable information assists surgeons in identifying the optimal anastomotic site for patients undergoing surgery for esophageal cancer. In addition, it serves as an early functional diagnostic test for individuals with MALS.

In papers III, IV, and V, we provided evidence that MALS is a medical condition primarily driven by an ischemic factor, and it can be effectively treated with laparoscopic decompression of the celiac artery. The motivation for conducting these studies emerged due to conflicting research, suggesting that MALS is not a vascular disease but rather a neurogenic one, thereby creating doubt on the efficacy of surgical treatment [8]. Moreover, despite the relatively frequent occurrence of a 'J' configuration in CTA scans, signifying external compression of the celiac artery by the median arcuate ligament during expiration (10-24%), it leads to symptomatic manifestations in less than 1% of cases [9].

In Paper IV patients with MALS were treated with laparoscopic decompression of the CA and the results showed symptom relief for up to 90% of the patients with MALS. During the postoperative follow-up, it was observed that 67% of the patients achieved complete relief from symptoms, whereas 23% experienced partial relief within 3-6 months. The use of duplex ultrasound to evaluate the postoperative hemodynamics of CA demonstrated a significant improvement in peak systolic velocity values ($p<0.001$). Furthermore, the patients expressed overall satisfaction with the surgical procedure. The concluding statement in Paper IV emphasizes the significance of considering surgical intervention as a viable option for treating MALS. While the typical diagnostic approach involves excluding alternative causes and consulting a medical team to discuss diagnoses and treatment choices, it remains crucial to recognize the potential advantages associated with surgical treatment. In cases where individuals exhibit clinical symptoms of MALS and other potential diagnoses have been ruled out, and a CTA confirms mesenteric artery stenosis, it's advised to consider laparoscopic decompression of the celiac artery as the best course of treatment.

In Paper V, we identified intestinal ischemic biomarkers in patients with CMI and MALS. We analyzed four biomarkers namely, α-GST, I-FABP, citrulline, and ischemia-modified albumin in both healthy individuals and patients with MALS and CMI. Only α-GST showed statistical significance. The healthy individuals had plasma α-GST levels of 3.3 ng/mL. The plasma α-GST levels were elevated in patients with CMI to 7.8 ng/mL, and in patients with MALS to 8.4 ng/mL. However, after revascularization, the α-GST level returned to normal. The study used a cut-off value of 4 ng/mL for the normal median plasma α-GST level, which showed that the sensitivity and specificity for atherosclerotic CMI and MALS were 93% (95% CI 0.78 to 1.0) and 88% (95% CI 0.69 to 1.1), respectively. The Area Under the Curve was 0.96 ($p<0.0001$) for CMI and 0.85 ($p<0.002$) for MALS.

The observed postoperative clinical improvements, enhancement of hemodynamic values of the celiac artery in Paper III and Paper IV, and normalization of intestinal ischemic biomarkers after treatment in Paper V lend further support to the notion that MALS is an ischemic condition and should be considered for intervention.

THESIS SAMMENDRAG

Mesenteriell iskjemi er en medisinsk tilstand assosiert med nedsatt eller blokkert blodtilførsel til mage- og tarmsystemet på grunn av intern eller ekstern innsnevring av mesenterielle blodkar. Tilstanden kan opptre i en akutt eller kronisk form. Den akutte formen, akutt mesenteriell iskjemi, er en kritisk medisinsk nødsituasjon preget av tarmiskjemi og nekrose i tarmen, ofte med fatalt utfall [1-3]. Den korniske formen, kronisk mesenteriell iskjemi (KMI), derimot, er en langvarig prosess som pågår over minst tre måneder eller til og med flere år. Selv om KMI en hemmende tilstand, er den umiddelbart ikke livstruende. Likevel, hvis den forblir ubehandlet, kan over halvparten av tilfellene progredierer til akutt mesenteriell iskjemi, som er forbundet med en dødelighetsrate på opp til 70% [1, 4, 5].

Den vanligste årsaken til KMI er aterosklerose av mesenterielle kar, karakterisert av den klassiske triaden som består av postprandiale abdominale smerter, mataversjon, og vekttap. En sjeldnere årsak til KMI er ekstern kompresjon av trunkus cøliakus og cøliakus ganglion av median arcuate ligament, noe som fører til tilstanden median arcuate ligament syndrom (MALS). Selv om den klassiske triaden er kun beskrevet i 16 – 22% tilfeller [6], har både atherosklerotiske CMI og MALS historisk sett vært omstridt på grunn av deres uspesifikke spekter av kliniske symptomer, uklare funn ved kliniske undersøkelser, mangel på spesifikke biomarkører, begrensede tidlige sikre diagnostiske metoder og det faktum at noen opplever tilbakevennende symptomer etter behandling.

Tidligere oppfatning har vært at minst to av tre hoved mesenterielle kar må være stenotiske eller okkluderte for at behandlingen skal være nødvendig. Dette førte til betydelig usikkerhet rundt eksistensen av MALS som er en «enkel arterie stenose», samt uenighet om dens etiologi, om den er iskjemisk eller nevrologisk. Som følge av dette var det også overdreven tvil til kirurgisk behandling, med den begrunnelsen at ikke alle opplevde lindring av symptomer etter operasjonen. KMI pasienter gjennomgår i gjennomsnitt tre år med lidelse før de endelig får en riktig diagnose [6, 7]. Med tanke på denne plagsomme sykdommen som har lav forekomst, men høy risiko, har retningslinjene alltid understreket behovet for en presis diagnose tidlig i sykdomsbildsforløpet. Derfor finner vi det helt avgjørende å undersøke diagnostiske metoder for tidlig diagnostisering av både KMI og MALS.

I artikkel I undersøkte vi to ulike diagnostiske verktøy for å vurdere sirkulasjonen til mage- og tynntarmen hos pasienter med atherosklerotisk KMI og MALS. Vi benyttet endoskopisk Duplex ultralyd og transabdominal Duplex ultralyd (E-DUS and TA-DUS) for å påvise stenoser i trunkus cøliakus og arteria mesenterika superior. Hemodynamiske målinger fra E-DUS og TA-DUS ble deretter sammenlignet med funn fra computer tomografisk angiogram (CTA), som er standard diagnostisk metode for KMI og MALS. Resultatene viste at E-DUS hadde høyere sensitivitet og negativ prediktiv verdi sammenlignet med TA-DUS, selv om den viste lavere spesifisitet enn TA-DUS. Konklusjonen i studien antydet at E-DUS har betydelig potensial som en initial screeningstest for pasienter som mistenkes å ha KMI og MALS.

I artikkel II og III undersøkte vi laser Doppler flowmetry (LDF) og visible light spectroscopy (VLS) for måling av mikrosirkulasjon av gastrointestinal traktus under minimalt invasiv øsofagektomi hos pasienter med spiserørskreft og under laparoskopisk dekompresjon av trunkus cøliakus hos pasienter med MALS. VLS avslørte signifikant lavere nivåer av mixed arterial and venous saturation of hemoglobin både hos pasienter som gjennomgikk minimal invasiv esophagektomi og hos pasienter med MALS sammenlignet med friske individer, noe som indikerer iskjemiske forandringer i gastrointestinal traktus. Begge studiene konkluderer at LDF og VLS brukt i kombinasjon gir punktlige og pålitelige målinger av mikrosirkulasjonen av gastrointestinal traktus. Denne verdifulle informasjonen hjelper kirurger med å identifisere det best mulige området med sirkulasjon for anastomose hos pasienter som gjennomgår operasjon for spiserørskreft. I tillegg fungerer det som en tidlig funksjonell diagnostisk test for pasienter med MALS.

I artiklene III, IV og V demonstrerte vi at MALS er en medisinsk tilstand som skyldes iskjemi og som effektivt kan behandles med laparoskopisk dekompresjon av trunkus cøliakus. Motivasjonen for å gjennomføre disse studiene oppsto på grunn av motstridende studier, som antyder at MALS ikke er en vaskulær sykdom, men heller nevrogen tilstand, og dermed skaper tvil om effekten av kirurgisk behandling [8]. Videre, til tross tillegg til det faktum at selv om en "J"-konfigurasjon på CTA, som tegn på ekstern kompresjon av trunkus cøliakus av median arcuate ligament under ekspirasjon, ikke er uvanlig (10-24%), fører den kun til symptomer hos mindre enn 1 % av tilfellene [9].

I artikkel IV ble MALS pasienter behandlet med laparoskopisk dekompresjon av trunkus cøliakus og resultatene viste symptomlindring i opptil 90% av tilfellene. Under den postoperative oppfølgingen ble det observert at 67% av pasientene oppnådde fullstendig lindring av symptomene, mens 23% opplevende delvis lindring i løpet av 3-6 måneder. Ultralyd Duplex av trunkus cøliakus for evaluering av den postoperative hemodynamiske oppfølging viste en signifikant forbedring i Peak systolisk velocity verdier (p<0.001). Pasientene generelt uttrykte tilfredshet med det kirurgiske inngrepet. I konklusjonen har viktigheten av å vurdere kirurgiske inngrep som en mulig behandlingsmetode for MALS fremhevet. Selv om den vanlige diagnostiske og terapeutiske tilnærmingen innebærer eksklusjon av differensielle diagnoser og samkonsultasjon i tverrfaglig team for consensus om diagnostikk og behandling, er det fortsatt avgjørende å gjenkjenne de mulige fordelene forbundet med kirurgisk behandling. Pasienter med kliniske symptomer på MALS hvor andre differensielle diagnoser er utelukket, og en CTA bekrefter stenose i trunkus cøliakus, anbefales det å vurdere laparoskopisk dekompresjon av trunkus cøliakus om den beste behandlingsmetoden.

I Paper V identifiserte vi intestinale iskjemiske biomarkører hos pasienter med KMI og MALS. Vi analyserte fire biomarkører, nemlig α-GST, I-FABP, citrullin og iskemimodifisert albumin, både hos friske individer og pasienter med KMI og MALS. Bare α-GST viste statistisk signifikans. De friske individene hadde plasma α-GST på 3,3 ng/ml. Plasma α-GST-nivåene viste seg til å være forhøyet hos pasienter med KMI til 7,8 ng/ml, og hos pasienter med MALS til 8,4 ng/ml. Etter revaskularisering gikk imidlertid α-GST-nivået tilbake til det normale. Studien brukte en grenseverdi på 4 ng/ml for normal median plasma α-GST nivå, som viste at sensitiviteten og spesifisiteten for atherosklerotisk KMI og MALS var 93 % (95 % CI 0,78 til 1,0) og 88 % (95 % CI 0,69 til 1,1). Area Under the Curve var 0,96 (p<0,0001) for CMI og 0,85 (p<0,002) for MALS.

Den kliniske forbedringene observert og forbedring av hemodynamiske verdier i trunkus cøliakus etter kirurgi i artikkel III og IV, samt normaliseringen av intestinale iskjemiske biomarkører etter behandling i artikkel V, styrker ytterligere hypotesen om at MALS er en iskjemisk tilstand og behandling med intervensjon bør vurderes.

1. INTRODUCTION

1.1 Terminology and Definitions

Bowel ischemia, also known as intestinal ischemia, refers to insufficient blood supply to either the small or large intestine. The primary pathomechanism for this condition is inadequate blood flow that fails to meet the metabolic demands of the intestines due to occlusive or nonocclusive vascular disease. As a result, mucosal injury or full-thickness necrosis may occur. Bowel ischemia can be classified as colonic ischemia or mesenteric ischemia.

Colonic ischemia also named ischemic colitis, is the most common type, accounting for 50 - 60% of GI ischemia, and is ischemia of the large bowels [10]. The three classifications of colonic ischemia: gangrenous, structuring, and transient, defined by Marston et al. in 1966 remain true to this day [11]. The most common symptoms are abdominal pain, nausea, vomiting, and hematochezia, which in severe cases progress to the acute abdomen with peritoneal irritation and sepsis. The infraction of the bowel is a continuous process that develops with local tissue hypoperfusion and hypoxia leading to gangrene of the bowel, sepsis, and death. The onset can be acute or chronic and the etiology occlusive, or non-occlusive. Although the occlusive form is a surgical emergency the non-occlusive form is the most common cause and can be of a transient nature associated with a better prognosis. Because of the difficulty in recognition of the disorder, late diagnosis, and inappropriate treatment, the mortality rate still exceeds 50% [12].

Mesenteric ischemia refers to the malperfusion of the organs depending on the affected mesenteric vessels (arterial inflow or venous return) and whether there is the presence of collateral circulation. The terminology in these last decades has changed from the earlier used term "splanchnic" to the well-established term "mesenteric" vasculature that primarily includes CA, superior mesenteric artery (SMA), and inferior mesenteric artery (IMA) with the following venous drainage parallel to the arterial supply [3]. Arterial obstruction being the main cause of mesenteric ischemia the collateral network interconnecting these vessels plays a major role in ensuring that the loss of a single vessel does not lead to ischemia of the organs.

Mesenteric ischemia in the European Society for Vascular Surgery (ESVS) Guidelines in 2017 is defined by three characteristics [3]:

(i) presence of symptoms (or not)

(ii) clinical presentation: acute, chronic, and acute on chronic ischemia

(iii) vessel involvement (the identification and number of involved arteries, venous obstruction, or external compression).

The narrowing of mesenteric vessels without any noticeable symptoms may not necessarily be considered a syndrome or disease, but rather an incidental finding. It is a commonly observed radiological finding, but only a small proportion of those individuals with such findings have symptoms [6]. Although, in the lack of abundant collateral circulation and increasing age above 65 years, the prevalence of progress to mesenteric ischemia there increases [6, 13]. The disease presents symptoms depending on the onset and the severity of the ischemia. In the acute phase, AMI, acute occlusion can lead to profound ischemia of the organs, resulting in severe abdominal pain, acute malnutrition, intestinal necrosis, and even death [14]. In the chronic phase, CMI, compensatory collateral circulation may take over or develop over time, thus leading to no or fewer symptoms. AMI and CMI can either be occlusive or non-occlusive, either way, if untreated, they can eventuate in life-threatening intestinal necrosis [6].

Early diagnosis is essential for detecting the cause of mesenteric ischemia considering that non-occlusive mesenteric ischemia (NOMI) is often treated with medication and resuscitation while the occlusive type requires surgical intervention (Figure 1).
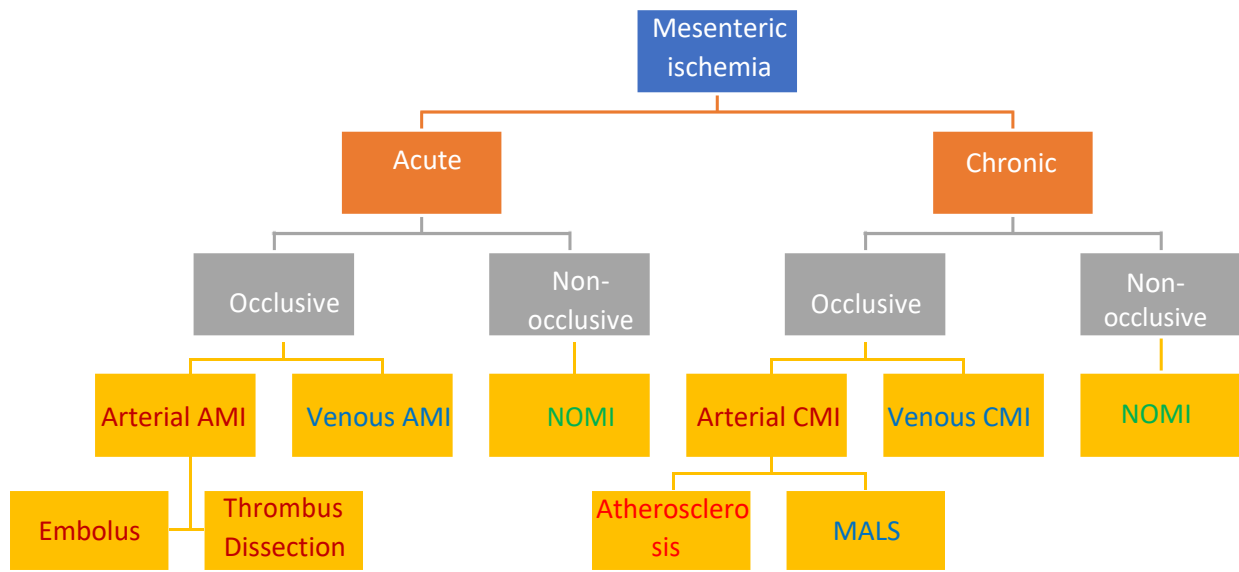
Figure 1. Flowchart of types of mesenteric ischemia. Acute mesenteric ischemia (AMI), Non-occlusive mesenteric ischemia (NOMI), Chronic mesenteric ischemia (CMI), Median arcuate ligament syndrome (MALS).

Acute mesenteric ischemia (AMI) is rare hence a dreadful vascular disease caused by sudden occlusion or reduction of the blood flow in the mesenteric vasculature, causing local hypoxia, resulting in bowel necrosis. There are four etiological forms of AMI: arterial embolism, arterial thrombosis, venous thrombosis, and NOMI. Acute embolic occlusion is the most common etiology, accounting for 40 - 50% of cases (Figure 1) [15]. Thrombosis of atherosclerotic mesenteric arteries constitutes 15 - 25% of the cases, mesenteric venous thrombosis 5 - 15%, and hypoperfusion secondary to systemic hypotension and vasoconstriction (NOMI) compromises 20 - 30% of all cases of AMI [2, 15, 16]. Symptoms of AMI include sudden and severe abdominal pain, accompanied by nausea, vomiting, and diarrhea. Because the symptoms are nonspecific and the physical examination uncertain, a delay in diagnosis can lead to serious complications such as peritonitis and bowel necrosis. It is crucial to diagnose AMI early, as it is a surgical emergency and delayed treatment can result in an early mortality rate of 20 - 80% [4, 5, 17].

In the cases of NOMI, surgery is not necessary, unless bowel necrosis has already occurred [14]. CT angiography is the preferred diagnostic tool and is required for the exclusion of NOMI and the identification of occlusive vessels for emergency treatment to avoid the staggeringly high death rate associated with this entity [2]. It is worth noting that 80% of AMI patients already have pre-existing CMI, which should prompt even earlier diagnosis [14, 18].

Chronic mesenteric ischemia ("abdominal angina" or "intestinal angina") is the chronic form of mesenteric ischemia that in 90% of cases is characterized by the atherosclerosis of CA, SMA, and IMA. A less common form of CMI is median arcuate ligament syndrome (MALS), which occurs when the CA is extrinsically occluded. Other causes of CMI are vasculitis and mesenteric venous thrombosis. The classic symptom of CMI is postprandial abdominal pain, resulting in sitophobia and thereby unintentional weight loss. Other accompanying symptoms may be nausea, vomiting, abdominal bloating, constipation, or diarrhea. Due to the vague nature of these symptoms and clinical findings, the diagnosis is challenging and frequently delayed. Untreated atherosclerotic CMI can progress to AMI, nevertheless, sometimes it occurs as one or more episodes before the major ischemia sets in, known as acute on chronic mesenteric ischemia. [6, 14, 18, 19].

Median arcuate ligament syndrome, also called Celiac artery compression syndrome and Dunbar syndrome, is a symptomatic eccentric compression of the celiac artery (CA) and celiac plexus by the fibers of the median arcuate ligament (MAL) and diaphragmatic crura. Although this radiological finding is seen in up to 7.3% of the healthy population the syndrome represents only those with variable symptoms [6, 20]. These include postprandial abdominal pain, food aversion leading to unintentional weight loss, and other more nonspecific GI symptoms such as nausea, vomiting, diarrhea, or constipation. Etiologies of CMI other than atherosclerosis in the mesenteric arteries and MALS are not covered in this thesis.

1.2 Anatomy and Pathophysiology

The mesenteric vasculature has a wide variety that plays a crucial role in delivering blood to abdominal organs, such as the stomach, liver, spleen, pancreas, and small and large intestines. This circulation is primarily facilitated by three ventral branches of the abdominal aorta: CA, SMA, and IMA (Figure 3). Venous drainage is achieved through the portal vein with the superior mesenteric vein, and inferior mesenteric vein serving as the primary vessels.

Arterial circulation

Knowledge about vascular anatomy is crucial for comprehending the pathophysiology, clinical presentation, and treatment of intestinal ischemia since the affected section of the intestine typically corresponds linearly to the location and extent of vascular occlusion.

The major mesenteric arteries and their branches:
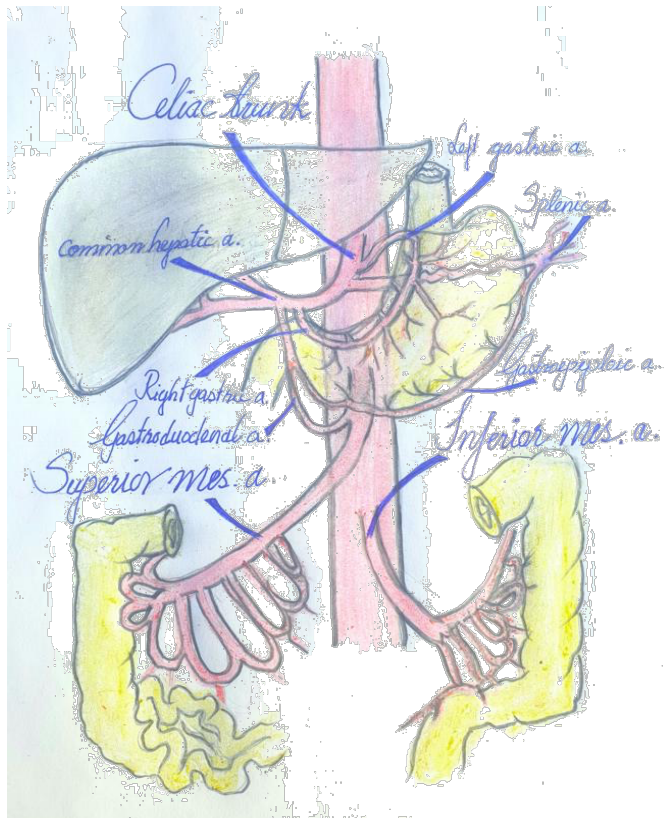
CA is the first and most proximal mesenteric artery, typically originates ventrally from the abdominal aorta at a level between T11 to L1. It enters the abdominal compartment through the aortic hiatus of the diaphragm at the level of T12. The proximal part of the CA, before dividing into splenic left gastric and common hepatic arteries, runs distally at an angle of more than 20° to the aorta. It supplies the liver, spleen, pancreas, and from the distal part of the esophagus to the ampulla of Vater in descending duodenum.

Anatomically low insertion of the diaphragmatic crura arising from L1 – L4 or a high origin of CA might compress CA and the adjacent sympathetic nerve and ganglia by MAL, which is an arch-like tendinous band of fibrous uniting the diaphragmatic crura, causing MALS. This compression increases during expiration. As a result, isolated CA stenosis is much more common than SMA and IMA where the etiology is atherosclerosis or emboli [14].

SMA is the second mesenteric artery that arises approximately 1cm caudally from the abdominal aorta and branches into several arteries, including the middle, right, and ileocolic arteries as well as the jejunal and ileal arteries. This arterial system supplies duodenum distal to ampulla Vater and extends down to the splenic flexure of the large intestine. Due to its angle from the aorta and the high blood flow rate (BFR) than other mesenteric arteries, SMA is particularly susceptible to emboli, accounting for roughly half of the cases of AMI [2]. Of these embolic cases, 39% have the embolus located in the origin, and 16% in the peripheral branches of the SMA [21]. The remaining half of SMA occlusions are typically caused by atherosclerosis, which is the main cause of CMI. Rupture of this atherosclerotic plaque can trigger an acute state, hereby acute on chronic mesenteric ischemia. However, dissection of the SMA, either isolated or concomitant with aortic dissection can also develop into thrombotic AMI [14].

IMA is the third artery in the mesenteric vasculature and originates 3-5 cm above the aortic bifurcation, dividing into the left colic, marginal artery of Drummond, and sigmoidal arteries, which supply the colon from the splenic flexure to the superior portion of the rectum. Isolated IMA occlusion rarely causes mesenteric ischemia.

Collateral circulation

Mesenteric circulation has an extensive collateral blood supply formed by the redundant multiple interconnections between arteries from different parts of the mesenteric circulation. The following collateral network between CM, SMA, and IMA protects guts against ischemia:

CA

                Pancreatoduodenal arcade

SMA

                Marginal artery of Drummond and the Riolan arcade

IMA

                Superior and inferior rectal arteries
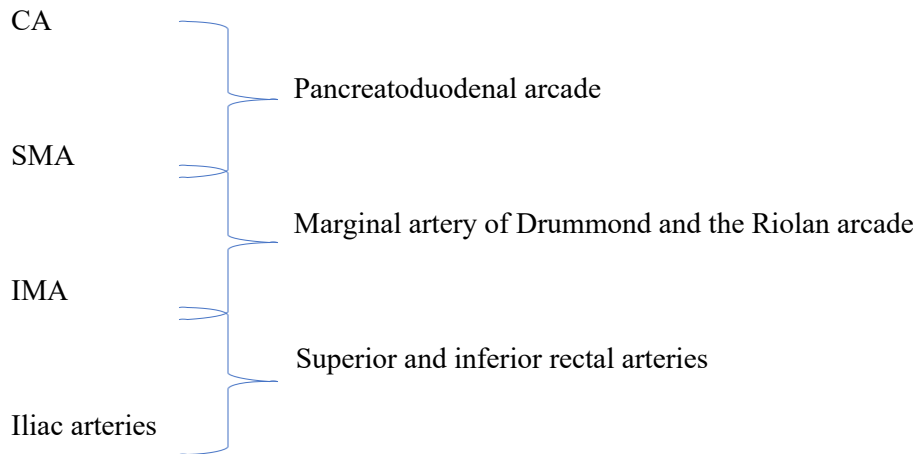
Iliac arteries

The junction of mesenteric arteries known as marginal arteries supply certain regions in the colon called "watershed areas" making them more susceptible to ischemia, especially in the case of NOMI. The watershed area to the neck of the pancreas is supplied by the marginal artery junction of the CA and SMA. The splenic flexure (Griffiths point) watershed area is between SMA and IMA, while the rectosigmoid junction (Sudeks`s point) is the area between IMA and the superior rectal artery. The left colon is more prone to ischemia than the rectum since it receives less blood supply due to its dual vasculature. Approximately 75% of ischemic colitis involves the left colon [22]. The marginal artery is poorly developed in 50% of the population, exposing this group to ischemia even in the case of the disease in one mesenteric artery [23]

Microcirculation

The intestine receives 25% of the cardiac output during fasting, which increases up to 35% after a meal. [1]. During a meal, blood flow to the CA rises, peaking, and after the meal returns to normal levels within 20 minutes, whereas blood flow to the SMA increases after a meal, reaching its peak 30-40 minutes after the meal and returning to baseline within 2-3 hours [24].

The mucosa and submucosa of the gut receive 70% of the mesenteric blood flow. However, the mucosa receives a greater quantity of blood flow compared to the submucosa, hence making it more vulnerable to ischemia. In fact, ischemic changes can be observed at the tip of the villus as early as 3 - 5 minutes after the ischemic accident [25].

The mesenteric microvasculature is a network of blood vessels referred to as the mesenteric bed. It consists of three parallel pathways that serve the muscularis propria, the submucosa, and the mucosa, respectively. These pathways allow for local fluctuations in the blood flow without affecting overall intestinal blood flow. Each pathway is composed of three major components:

Resistance vessels, such as arterioles, have precapillary sphincters that contract or relax, thereby increasing, or decreasing blood flow to the capillaries and influencing the exchange process in the mesenteric bed (Figure 2).

Exchange vessels, such as capillaries, are responsible for the exchange of oxygen, nutrients, and waste products from the arterial circulation into the venous circulation.

Capacitance vessels, such as venules, serve as storage vessels for blood. They contain a smooth muscle layer that can expand and contract, thus maintaining constant blood flow despite the blood circulation being reduced [26]. These venules contain around 80% of the total mesenteric blood flow that by contraction returns blood to the heart [27].
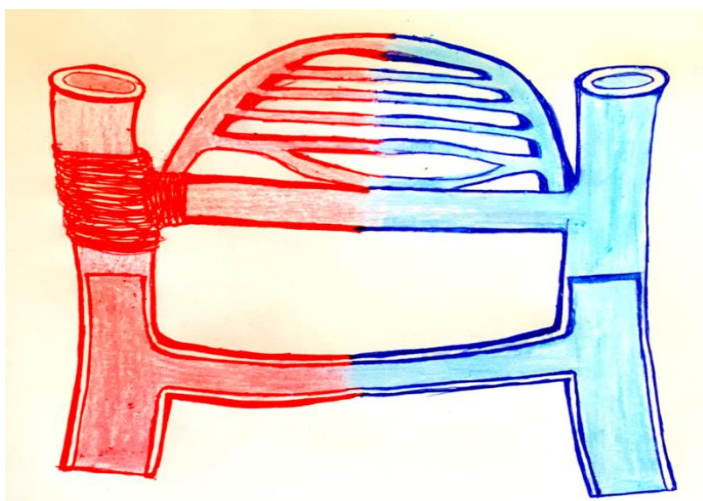
Figure 2. Resistance vessels, which include microscopic arteries and arterioles that regulate blood flow to each circuit and the mesenteric bed.

So, while these different types of vessels serve an important function in the mesenteric vasculature there is a counter-current mechanism in the gut that facilitates the exchange of substances between two adjacent blood vessels, arterioles, and venules, that run parallel to each other. As blood flows through the capillaries in the small intestine, nutrients and water are absorbed into the bloodstream, generating a concentration gradient that drives the movement of these substances from the arterioles into the venules, while waste products move from the venules into the arterioles. This mechanism serves a crucial role in maintaining blood flow and ensuring the efficient function of the digestive system.

During ischemia, the resistance vessels constrict to help maintain blood pressure and redirect blood flow to the areas of the gut that are most in need of oxygen and nutrients. The exchange vessels increase the surface area available for nutrient and oxygen exchange, helping to maintain tissue function. The capacitance vessels release stored oxygen and nutrients to help maintain tissue metabolism in the face of ischemia. The counter-current system as a response to ischemia alters the direction and rate of blood flow to maximize oxygen and nutrient exchange and minimize waste product buildup. Overall, these mechanisms together give the gut the ability to tolerate a 75% reduction in blood flow for 12 hours. Although, irreversible damage to the bowels occurs within 6 hours [28].

Venous drainage

The venous drainage goes parallel to the arterial supply, where the superior mesenteric vein drains the small and large intestines supplied by SMA, and the inferior mesenteric vein drains the transverse and descending part of the colon supplied by IMA. The superior mesenteric vein and inferior mesenteric vein connect with the splenic vein and form the portal vein that passes through the liver and drains into the inferior vena cava, which enters the right atrium in the thorax. Mesenteric venous thrombosis may present with acute or chronic abdominal pain, visceral edema, and portal hypertension. It accounts for 5 - 15% and the most common causes are thrombophilia,

surgery, trauma, portal hypertension, inflammatory bowel disease, and malignancy [29, 30].

Although immediate anticoagulation is the choice of treatment, mesenteric venous thrombosis does

not necessarily cause mesenteric ischemia. Only in a few acute cases, surgical intervention is

necessary to prevent the worsening of intestinal ischemia [30].

1.3 Historical Perspectives of mesenteric ischemia

Knowing the historical perspectives of certain medical conditions is crucial for a

comprehensive understanding of the disorder. Despite the substantial amount of information available

on mesenteric ischemia in the medical records, identifying and treating this illness still remains a

difficult task. Over the course of many centuries, there has been gradual progress in understanding,

diagnosing, and treating this disease. However, despite such progress, the literature contains

prevalent pessimistic views expressed by the practitioners.

HISTORY OF MESENTERIC ISCHEMIA
The Evolution of a Diagnosis and Management

Scott J. Boley, MD, Lawrence J. Brandt, MD,
and Robert J. Sammartano, BS, RPA-C

Occlusion of the mesenteric vessels is apt to be regarded as one of those condition of which . . . the diagnosis is impossible, the prognosis hopeless and the treatment almost useless.

A. J. COKKINIS, 1926[21]

The pessimism expressed by Cokkinis more than 70 years ago concerning mesenteric ischemia is, unfortunately, still shared by many physicians today. Such an attitude partly contributes to, but also results from, the continued poor outcome of most patients with these conditions. However, the progress in our understanding of the pathophysiology, diagnosis, and treatment of acute occlusions of the superior mesen-

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Before 20th century

The first mention of mesenteric ischemia dates back to the late 15th century, but it wasn't until the 19th century that it received medical attention [31]. The first documented case of mesenteric ischemia was reported by Hogdson from Guy´s Hospital in 1815. Many years later in 1843, a German anatomy professor and researcher, F. Tiedemann in his book "Verengung und Schliessung der pulsadern in Krankenheiten", narrowing and closure of the arteries in disease described bowel infarction as a cause of mesenteric occlusion (Figure 3) [32]. By the end of the 19th century, several animal experiments, autopsy studies, and a few dreadful case reports were added to the literature. Renowned and enthusiastic physician R. Virchow, an anthropologist, and biologist famously known in the medical textbooks for the terms Virchow´s node and Virchow`s triad (Figure 4). The latter elucidates the pathomechanism of thromboembolism that remained the main cause of AMI for years to come [33, 34].
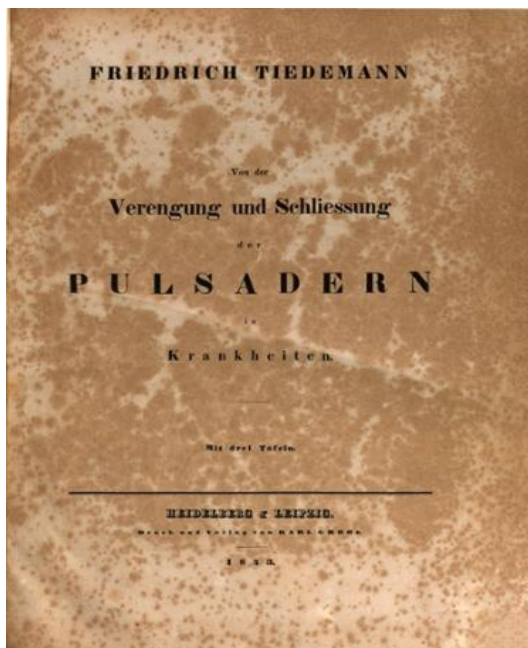
Figure 3. Picture of the original book "The narrowing and closure of the arteries in disease" by Tiedemann, Friedrich (1781 – 1861). "Verengung und Schliessung der pulsadern in Krankenheiten". The figure was adapted with permission from Bayerische StaatsBibliothek for non-commercial use only.

Figure 4. Virchow with his son Ernst and daughter Adele. The picture is public domain from an unknown author and adapted from Wikimedia, source: Medizinhistorisches Journal. Band 4, Heft 3/4, Georg Olms, Hildesheim 1969, S. 337

The scientific community before the 20th century believed that the stomach was the only digestive organ in the body. It wasn't until researchers began studying the physiology and function of the digestive system that they discovered the importance of the total alimentary canal, bile, and pancreatic juice [32]. In an animal observational study in 1875, Litten confirmed bowel infarction after ligating the mesenteric vessels [35]. This encouraged others for similar experimental studies thus clarifying the direct connection between bowel infarction and diminished blood supply. Apparently, knowledge of this deadly disease was extremely limited, therefore the focus was only on how to treat catastrophic abdomen with resection of the gangrenous intestines. In 1895 in an article, Elliot stated "'The subject is not mentioned in any modern text-book except Osler's. I have been unable to find even a suggestion that surgery might be useful in such cases. On the contrary, it is distinctly stated by certain authorities that such cases are beyond the help of surgery" [36]. He changed the surgical approach to this disease after performing the first surgery on two patients with AMI by creating 2 stomas after bowel resection and anastomosing them 2 weeks later, a procedure still performed to this day [36].

20th century

Before the 20th century, intestinal resection was the only treatment option; surgeons consequently faced unavoidable massive intestinal resection followed by postoperative deaths and thereby only postmortem material for study [37, 38]. Facing a postoperative state with serious malnutrition, dehydration due to profuse diarrhea, and a high risk of infection and thrombosis the necessity of understanding intensive care became the main agenda. In 1913, Trotter presented 366 cases in his thesis, Embolism and Thrombosis of Mesenteric Vessels, from his institution and the world literature in which arterial occlusions accounted for 53%, venous for 41%, and both for 6% [39]. Arterial embolus was the most common cause, and the preoperative diagnosis was correct in only 3 out of 360 cases. Until the 1950s occlusion of mesenteric veins was still believed to be the main cause of AMI [40].

In 1930, Cokkines famously remarked that "Occlusion of the mesenteric vessels is apt to be regarded as one of those conditions of which… the diagnosis is impossible, the prognosis hopeless, and the treatment almost useless." [37, 40]. The constant dilemma with uncertain etiology, impossible early diagnosis, and treatment with horrible outcomes persisted until Klass in 1951 applied the principles of vascular surgery by performing an embolectomy of SMA without bowel resection [41]. He introduced the concept of revascularizing an ischemic but viable bowel and recommended making a diagnosis before bowel infarction occurs [41]. Until then, the diagnosis was considered "early" if the patient survived after bowel resection. The method of diagnosis was laparotomy and the treatment focused only on survival after bowel resection. As Elliot had stated "The prognosis is very grave", Klass in his brutally honest article, revealed the dreadful outcome of the disease and the constant hopelessness among surgeons: "To add to the picture of gloom, the diagnosis of mesenteric vascular occlusion is rarely made preoperatively" (Figure 5) [41].
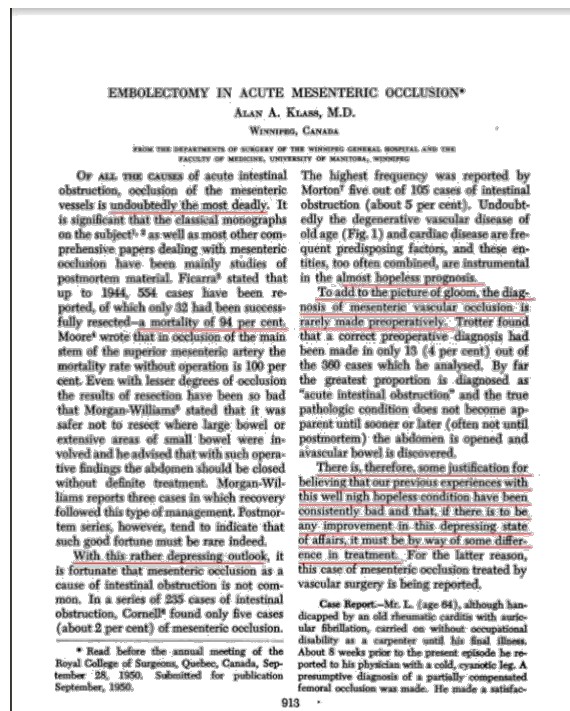
Figure 5. The article's first page by Klass et al. published 28. September 1950. Figure adapted with permission from Annals of Surgery 1951 Nov; 134(5): 913-917.

Shaw and Maynard followed by performing vascular surgery, thus the first thrombendarteriectomy of SMA was carried out in 1958 [41, 42]. Reported survival cases increased, but mortality was still high [37]. The painful death of patients after massive bowel resection was described in the literature. The remaining intestines after massive bowel resection suffered from intestinal motility, slow passage, and severely reduced absorption capacity leading to massive diarrhea, severe malnutrition, and death [43]. The following questions need to be answered "*How much of the bowel is necessary for survival*", "How dispensable is the small bowel" and "*How to preserve nutritional balance after bowel resection*" [44].

Even though South American journals had already reported cases of bowel infarction without evidence of vascular lesions in 1941, Ende is credited for raising awareness of NOMI and the pathomechanism of vasospasm [45-47]. Angiographic examination clarified the term "Nonocclusive mesenteric ischemia" and was established as a criterion to rule out or detect acute mesenteric vessel occlusions [47-50]. This brought scientists closer to understanding the circulation as a whole and the etiology, particularly the pathomechanism of the vessels during and after surgery. It also explained the

fact that high reported cases of mesenteric venous thrombosis in cadavers earlier in the century might have been due to postmortem thrombosis and the lack of knowledge of nonocclusive mesenteric ischemia [37, 40]

In the 1960s, an aggressive approach to mesenteric arterial insufficiency was introduced with Scott J. Boley making notable contributions to the literature [37, 47, 50-53]. Extensive research was conducted to comprehend the pathophysiology of the remaining intestines after revascularization of the mesenteric circulation [37, 51, 53, 54]. At the same time, intensive care medicine was on the rise, thus, uncovering other important factors contributing to this ongoing high mortality rate after surgery. Despite the implementation of enhanced radiological tests and peri- and post-operative care involving antibiotics, anticoagulants, vasodilators, and electrolytes the mortality rate of AMI remained at an alarming 70 - 90% [37]. By the 1980s, newer diagnostic and therapeutic techniques were proposed, potentially increasing the survival rate to 67% [37]. However, an early diagnosis to prevent AMI remains a significant challenge even in the modern era.

Chronic mesenteric ischemia

The term "Chronic mesenteric ischemia" was first presented by Councilman in 1894 [55]. He observed that partial occlusion of the artery in a weak individual caused complete paralysis and consequent distention of the intestine without infraction, suggesting that sufficient blood supply may have remained to preserve the vessel's integrity but not enough for adequate enervation. Therefore, the outcome of occlusion was unpredictable due to variations in allied conditions [36]. This condition is characterized by chronic occlusion of the vessels supplying the intestines, leading to abdominal pain and malnutrition. Schnitzer et al in 1901 further defined abdominal pain as a long-standing postprandial pain in the abdomen due to chronic intestinal ischemia [56]. He compared the symptoms to claudication in the lower extremities during exercise. Thus, Goodman termed it "abdominal angina" in 1918 and Mikkelsen "intestinal angina" in 1957 [57, 58]. In 1936, Dunphy et al described CMI symptoms as postprandial pain, weight loss, and altered intestinal motility [59].

Furthermore, Mikkelson et al. noted in their abstract that CMI can progress to AMI; "The term intestinal angina most closely identifies the syndrome that may, for a period of months to years, precede complete mesenteric arterial occlusion."[57]. Despite the definition of CMI as patients with longstanding symptoms and a high risk of developing AMI, the diagnosis remained controversial throughout the 19th century. The argument used by Klein in 1921 was that some patients had stenotic or occluded mesenteric vessels at the autopsy without any abdominal pain [60]. After an extensive review, he concluded that sudden or gradual occlusion of SMA could result in 3 possible outcomes (Figure 6):

1. *Complete establishment of a competent collateral circulation which will (a) persist effectively throughout the patient's life; or (b) subsequently break down, usually because of aggravated disease of the heart or the blood vessels.*
2. *Intestinal obstruction without infarction owing to a blood supply sufficient for the parts' life but not for function.*
3. *Intestinal infarction, with the injury varying through all stages from a moderate lesion of the mucosa to pronounced necrosis of all intestinal walls.*
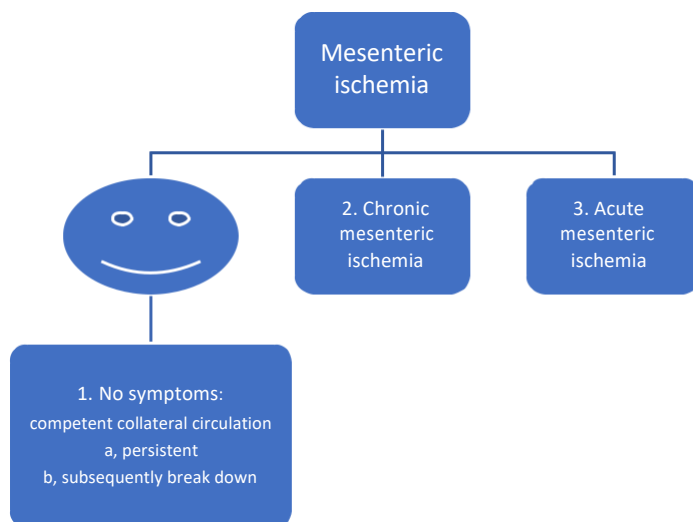
Figure 6. A schematic diagram assembling the description of mesenteric ischemia by Klein et al.

Klein's conclusions remain valid to this day. The treatment of CMI has been mainly surgical with either endarterectomy, excision, or bypass with graft. Mor et al. in 1962 performed bypass surgery with a Dacron graft from the infrarenal aorta to SMA [61]. Furrer et al. in 1980 achieved their first successful endovascular balloon dilatation of CA and SMA, while Lindblad et al. in 1996

suggested endovascularly stent implantation in occluded SMA for a more durable [62, 63]. Regardless of the great advances in treatment, there is still no effective prevention and diagnosis for CMI.

Median Arcuate Ligament Syndrome

The first source of discovery of compression of the CA by the diaphragmatic crura was reported by Lipshutz et al. in 1917 during an anatomical study of the mesenteric vasculature in cadavers[64]. Subsequent studies by Harjola in 1963 and Dunbar in 1965 observed symptomatic relief after surgical decompression of the median arcuate ligament in patients with a rare obstruction of the CA associated with postprandial abdominal pain, weight loss, and vomiting [65]. Thus, this clinic-radiological syndrome was dubbed by others as "Dunbar syndrome". Further case reports of surgical decompression with symptom relief followed until the 1970s, including a case report by Synder and Mahoney hence the name "Snyder-Mahoney" syndrome" [66]. Harjola et al. in 1968 agreed with the majority in their argument that pain in MALS is ischemic in nature; "the pain is ischemic in character" and that "the association of pain with ingestion suggests an ischemic cause". They further stated that it is unclear if the margin of the aortic hiatus causes neurogenic stimuli in the celiac ganglion, as the pain associated with the syndrome should be continuous. The splitting of the ganglion may not have the same effect as gangliectomy, and pain during ingestion suggests an ischemic cause due to stenosis of the celiac axis [67]. He appointed the syndrome as "Celiac axis syndrome" further designated as Celiac artery compression syndrome (CACS). In the early 1970s, the surgical approach became foggy by the fact that some authors experienced unsatisfactory results in long-term follow-up studies of patients treated with decompression of CA[68]. Discouraged by these results further publications of long-term studies in the 1980s showed the positive outcome of surgically treated MALS patients. Roayaie et al. introduced the laparoscopic approach to the management of MALS at the start of this century [69].

1.4 Epidemiology

The accurate worldwide prevalence of mesenteric ischemia is poorly defined due to few population-based studies, and smaller case series and autopsy data. The methodology differences between studies further limit direct comparison.

The Swedish autopsy registry has been a valuable source for population-based studies on the incidence of mesenteric ischemia. One study conducted in Malmö, Sweden between 1970 and 1982 on a population of 264, 000 –230,000 with an autopsy rate of 87% revealed an overall incidence rate of 12.9/100,000 person-years for AMI [70, 71]. The most common cause of mesenteric ischemia was arterial thromboembolism accounting for 68% of the acute cases, followed by 16% thrombosis, and 16% NOMI [21].

An executive finding was that SMA occlusion was 1.5 times more common than the rupture of an abdominal aorta aneurysm (rAAA) [21]. The in-hospital mortality rate of AMI was 93%, and clinical suspicion of intestinal ischemia before surgery or death was present only in 33% of cases [70, 71]. Another study conducted by Alcosta et al. on the incidence of AMI from in-patients and the same autopsy registry in Malmö from 1987 to 1996 and 2000 to 2006 with a population of 267,000 inhabitants showed contradictory results [18]. Although the incidence of mesenteric ischemia increased with age, the overall incidence rate appeared to decrease from 8.4 / 100 000 person-years to 5.4/ 100 000 person-years and the mortality rate from 87% to 25%. However, the incidence should have increased due to the increased proportion of octogenarians, comorbidities, and early diagnosis with modern diagnostic tools. These contradictory results are explained by a lower autopsy rate of 25% and the early discovery of AMI with better treatment options. The study also found that the incidence rates for both men and women increased equally and exponentially with age, and CMI symptoms were present in 73% of patients before the onset of AMI [70].

In Maryland, United States, 0.07% admitted to the hospital had AMI [72]. Although the mortality rate of 24% is remarkably low compared to other epidemiological studies. Beaulieu et al. conducted in approximately 1000 hospitals in the United States from a national survey of

inpatients (Nationwide inpatient sample) and reported similar results of AMI accounting for 0.06% of all hospital admissions.

In Japan, the incidence of atherosclerosis is lower therefore the incidence of AMI was 0.012% of total admitted patients in 26 national hospitals [73]. Although the in-hospital mortality for SMA occlusion was 57%, for mesenteric venous thrombosis 38%, and for NOMI 25%. Huerta et al estimated the overall incidence of AMI from the national general practitioner database in the United Kingdom to be 0.63 / 100 00 person-years [74].

In Finland between 2009 and 2013 in a retrospective observational study, Kärkkäinen et al presented an incidence rate of AMI of 7.3 / 100 000 person-years. The study findings showed a higher incidence of AMI than rAAA but also the fact that AMI is a more probable cause of acute abdomen in patients above 75 years old than acute appendicitis [75]. The death rate among the elderly was 1.8 times higher in AMI survivors than those with acute cholecystitis [75].

In recent years various epidemiological studies on mesenteric ischemia have been contributed to the literature. An annual incidence of AMI in a current retrospective, population-based study from Estonia was 8.7 /100 000 and 1-year mortality was 74% [76]. A similar longitudinal cohort study in Scotland recorded all patients dying from a vascular disorder of the intestines over 36 years. The death rates had nearly doubled during this time and the females to male ratio was 2:1, thus implicating the increase in reporting likely due to the acknowledgment of the disease, and mainly the increase in the elderly population with an increased prevalence of comorbidities, the latter, and social deprivation applies mostly to women [77]. The incidence of mesenteric ischemia is underestimated and underreported because of the large burden of this disease on the community facilities and is mostly managed at non-tertiary hospitals thus omitting non-hospitalized patients.

Terlow et al. published the incidence of CMI and reported causes in the Dutch population in 2020. CMI incidence was 9.2 /100 000 person-years [78]. The incidence of the compression of the CA is reported by Saleem et al. to 2 / 100 000 population. Compression of the coeliac artery is not as infrequent finding as one may think. This finding is reported to be in 10 - 24% of the population. Although patients with MALS are usually young women between 30 – and 50 years and are four times more affected than males [79].

1.5 Risk Factors

Chronic mesenteric ischemia increases the risk of developing acute mesenteric ischemia [1, 14, 80]. The chronic arterial narrowing or blockage associated with this condition can make the intestines more vulnerable to acute events such as thrombosis, embolism, or sudden arterial occlusion. Subsequently, these acute events can further compromise the blood supply to the intestines, leading to ischemia and potential tissue damage. The main cause of CMI is atherosclerosis [81]. Atherosclerosis is a complex and multifactorial disease that involves the accumulation of lipid-rich plaques within the walls of arteries, leading to progressive narrowing and hardening of the vessels. Several risk factors have been identified that contribute to the development and progression of atherosclerosis, including:

- Age: As people age, their risk of developing atherosclerosis increases.
- Gender: Men are at a higher risk of developing atherosclerosis than premenopausal women, but the risk becomes similar after menopause.
- Genetics: Certain genetic factors can increase the risk of atherosclerosis, such as familial hypercholesterolemia.
- High blood pressure: Uncontrolled high blood pressure can damage the lining of blood vessels, leading to the formation of plaques.
- High cholesterol: Elevated levels of low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol can contribute to the development of atherosclerosis.
- Smoking: Smoking increases oxidative stress and inflammation, both of which can damage blood vessel walls and promote atherosclerosis.
- Diabetes: People with diabetes are at an increased risk of developing atherosclerosis due to high levels of blood glucose and associated metabolic abnormalities.
- Obesity: Obesity can contribute to the development of atherosclerosis through various mechanisms, including inflammation and insulin resistance.
- Physical inactivity: Lack of regular physical activity can lead to weight gain, high blood pressure, and other risk factors for atherosclerosis.

- Diet: A diet high in saturated and trans fats, cholesterol, and refined carbohydrates can contribute to the development of atherosclerosis.

These risk factors can interact with each other, further increasing the risk of developing atherosclerosis. Thus, lifestyle modifications and medications that target these risk factors are important for preventing and managing atherosclerosis.

The pathomechanism of atherosclerosis involves the damage to the accumulation of Low-density Lipoproteins (LDL) in the tunica intima, forming a fatty streak. Leukocytes and smooth muscle cells migrate into the intima, forming foam cells, which degenerate the extracellular matrix and further destroy the inner cell wall. These foam cells eventually die creating a fibrous plaque that is hardened by calcium salts and bulges into the lumen of the artery. The endothelial cells over the plaque are compromised, leading to the formation of a thrombus. The plaque can rupture, or the thrombus can migrate as emboli, causing acute occlusion of the mesenteric artery. In roughly half of AMI cases, the cause is an embolus originating from the heart, mainly due to atrial fibrillation or recent myocardial infarction [14, 15]. Although anticoagulation and statin use has increased in recent decades, atherosclerosis, atrial fibrillation, emboli, and thrombus formation are more common in the increasing elderly population in the Western world, putting them at high risk for developing mesenteric ischemia [70, 75]. While most patients with CMI are women, those with MALS tend to be younger and female, with a female-to-male sex ratio of 2:1 to 3:1 [82].

1.6 Diagnosis

1.6.1 Clinical Features

Diagnoses of mesenteric ischemia, regardless of its type, whether it is acute or chronic, arterial or venous, occlusive or non-occlusive, are difficult to achieve in time. It requires a process of elimination to reach the diagnosis, making it crucial to gather a thorough natural history as the first important step to guide us in the right direction:

1. Age. Gender. Family history (diabetes, hyperlipidemia, malignancies).

2. Previous history: cardiovascular diseases (atrial fibrillation, aortic valve stenosis, coronary artery diseases, heart attack) general atherosclerosis, peripheral arterial disease, hypercoagulable background, abdominal surgeries, and inflammatory diseases (lupus, vasculitis). These coexisting conditions are the risk factors but also should be identified and managed since they may limit the treatment options.

   Other diagnoses that should be excluded are ulcers, liver-pancreas-gallbladder disease (hepatitis, pancreatitis, cholecystolithiasis/-cystitis) renal diseases, and malignancies.

3. Detailed anamnesis of the type and onset of the symptoms;

   o Acute onset as in AMI or the acute on CMI presenting frequently with acute abdominal pain "out of proportion" attendant with/without peritonitis.

   o Chronic symptoms for at least 3 months with historical features and classic triad associated with CMI:

     • "Mesenteric angina" is abdominal pain, commonly 30-60 min after eating [83]. The symptom debut may vary in the case of acute on chronic mesenteric ischemia.

     • Fear of food and consequently weight loss

     • Nausea, diarrhea, or constipation.

Oderich et al. discovered abdominal pain in 96% of patients undergoing mesenteric revascularization, only 74% had postprandial pain, 45% had a fear of food and 84% had weight loss [84]. Considering the obesity epidemic in the Western world CMI patients no longer remain underweight or cachectic but in a series 35% of the CMI patients undergoing revascularization were overweight or obese [85]. Hence this classic triad is only presented in 22% of CMI cases, and the clinical signs and symptoms are often unspecific, a higher clinical suspicion is mandatory to stay on track [6].

Clinical examinations are often normal in CMI and in the early stages of the AMI, therefore physical examination is habitually inconclusive. Generalized abdominal pain with no specific localization or mid-abdominal, described as crampy or dull with normal bowel sounds and soft abdomen with little or no tenderness. Severe abdominal pain disproportional to clinical findings is common in AMI and it should be noted that physical examination does not always reveal bowel

necrosis. In the presence of peritonitis, bowel ischemia is already irreversible, and the situation is grave.

Additional diagnostic tools are necessary for further investigation:

1.6.2 Biochemistry

Raised lactate, leukocytosis, neutrophilia, and rising creatinine and general classical lever markers might enlighten us about the severity and the progression of the disease but show poor diagnostic accuracy for intestinal ischemia. There are no available specific biomarkers that are approved for CMI in clinical practice. [1, 86].

1.6.3 Imaging

1. Duplex Ultrasonography (DUS)

DUS is commonly used in mesenteric ischemic centers as a diagnostic tool to evaluate the severity of stenosis in the mesenteric arteries including CA and SMA and in some centers, the IMA[82]. It is a low-cost tool with a sensitivity and specificity of up to 85% to 90% [81, 87, 88]. Nevertheless, with the expertise of a skilled technologist, DUS is an effective and reliable examination tool, except in patients with obesity, bowel gas, and heavily calcified vessels. Therefore, patients are required to fast for 6-8 hours before the DUS examination to minimize the amount of bowel gas. DUS provides an overview of the vascular structure and identifies stenosis and post-stenotic dilatation in the mesenteric arteries. Pulsed Doppler is then used to measure the peak systolic velocity (PSV), and end-diastolic velocity (EDV) to determine the stenotic grad in the inspiratory and expiratory phases. The clinical use of DUS has been increasingly recommended in the guidelines as an initial investigation tool and as a screening test for the diagnosis of CMI, rather than just for follow-up purposes [3, 6, 82, 89, 90].

2. Computer Tomographic Angiography (CTA)

The three-dimensional multiplane imaging provided by the new thin-section multidetector CTA scanner offers excellent spatial resolution, allowing for detailed information on the vasculature and the visualization of abdominal organs. This excludes other potential causes of abdominal pain. CTA with intravenous contrast material in arterial and venous phases (thin axial images 1 to 3mm) provides a detailed anatomy of the mesenteric vessels, including collateral circulation, anatomical variations, origin and length of vessels, location of lesions, type of stenosis (emboli, thrombus, atherosclerosis), and extent of the stenosis. Stenosis with a reduction in lumen >50% or 70% is defined as hemodynamically significant. CTA, in addition, can identify findings such as thickening of the bowel wall, bowel dilatation, ascites, mesenteric fat stranding, pneumatosis intestinalis, portal venous gas, free intraabdominal gas, and solid organ infarction are achieved indicating peritonitis and bowel necrosis. Therefore, CTA not only aids in the diagnosis of mesenteric ischemia but also contributes to treatment planning. With a diagnostic accuracy ranging from 95% to 100%, it remains the gold-standard method of imaging in our center and is recommended as a first-line study for the diagnosis of mesenteric ischemia [82, 91, 92]. In MALS patients, 3-D imaging is necessary to demonstrate the transient compression seen only during deep expiration. The demonstration of focal narrowing of CA with a hooked shape appearance may not appear on axial images only. [9].

3. Contrast-enhanced Magnetic Resonance Angiography (C-MRA)

MRA has been proposed as a viable alternative to the detection of CA and SMA stenosis in young patients or those with kidney failure due to its major improvements and clear benefits in the absence of radiation exposure and contrast-induced nephropathy. Nevertheless, MRA has certain limitations that prevent it from being superior to CTA. For instance, it is contraindicated in patients with pacemakers, and claustrophobia, and the examination's long duration may lead to artifacts during of patient's movements, respiration, and patient movements, which may negatively affect the quality of the image. Moreover, MRA is unsuitable for evaluating mesenteric stents.

DSA, historically gold-standard imaging, has largely been replaced by CTA and MRA due to its invasive nature character and potential complications such as bleeding, embolus, and infections [91]. However, DSA remains the most useful technique when diagnosis and percutaneous intervention are both required simultaneously. Its invasive nature and ability to visualize the mesenteric vessels from different planes and projections give an optimal view of the extensive vessel calcification, metallic artifacts, and prior stents. In case of disputed stenosis, DUS provides hemodynamic data by measuring the intraluminal pressure proximal and distal to the stenosis. A trans-stenotic pressure gradient > 10 mmHg in SMA reveals hemodynamic significance [84].

1.6.4 Endoscopic Ultrasonography

Endoscopic ultrasound is a minimally invasive procedure where an ultrasound probe at the tip of a thin flexible tube is inserted through the mouth into the upper GI tract for direct vitalization of the GI mucosa and assessing diseases intraluminal and of the nearby vessels and organs.

1.6.5 Functional Tests

Intraoperative bowel viability in mesenteric ischemia has been assessed with various techniques, including gastrointestinal tonometry, gastric exercise tonometry, pulse oximetry, fluorescein, indocyanine Green, Laser Doppler Flowmetry (LDF), and Visible Light Spectroscopy (VLS) [24]. However, LDF and VLS have never been used simultaneously in patients with CMI.

Laser Doppler Flowmetry and Visible Light Spectroscopy

The O2C system utilizes both continuous wave laser light (500 - 630 nm) and white light (830 nm) that are transmitted through an optical fiber to the tissue. Fibers on the probe placed on the tissue surface collect the scattered light. Due to its shorter wavelength, white light can penetrate deeper into the tissue compared to laser light.

In VLS, the absorption and scattering of white light in biological tissues enable the measurement of hemoglobin saturation and concentration. This is possible because the absorbance spectra of oxygenated and deoxygenated hemoglobin exhibit a marked difference.

For LDF measurements, the O2C system detects reflected laser light from moving red blood cells in the tissue. The Doppler shift of this reflected light is generated by the velocity of the erythrocytes and is detected by a photodetector in the instrument. The electrical signal generated by the photodetector is used to calculate the flow, which is expressed as red blood cell flux in mL/min/100-gram tissue.

1.6.6. Biomarkers

Various biomarkers have been evaluated to diagnose mesenteric ischemia. These biomarkers are released from damaged enterocytes as a result of ischemia and encompass markers such as - glutathione S-transferase (-GST), intestinal fatty acid-binding protein (I-FABP), citrulline, ischemic-modified albumin, and other similar markers [93-97].

- α-GST is a low molecular weight detoxifying iso-enzyme that is particularly concentrated in intestinal mucosa and hepatocytes and has a half-life of fewer than 60 minutes.

- I-FABP, on the other hand, is a cytosolic enterocyte protein found in epithelium cells of the small bowel that is released during dying enterocytes and is a sensitive and specific biomarker for AMI. Furthermore, elevated plasma levels of I-FABP have also been found in patients with CMI.

- Citrulline is a non-protein amino acid that is abundantly synthesized by the enterocytes in the small intestine, and it is a suitable biomarker of enterocyte function. Its plasma levels depend on gut synthesis and renal elimination, thus decrease in conditions such as mucositis and intestinal ischemia.

- Ischemic-modified albumin is altered by human serum albumin as the cause of ischemia and is increased in the plasma of patients with AMI.

Currently, clinical and laboratory investigations of these mesenteric ischemia biomarkers are exclusively conducted in patients with AMI. Plasma levels of these biomarkers are influenced by liver and renal diseases hence failing in diagnosing specifically AMI. Furthermore, none of these

biomarkers have shown sufficient sensitivity to be used as a screening test and therefore not appropriate for routine clinical use. Accordingly, there is still a requirement for serological indicators for the diagnosis of AMI and CMI that exhibit high sensitivity and specificity.

1.7 Treatment

Treatment goals for patients with CMI are to relieve pain, restore normal weight, and improve the overall quality of life. However, the most important and urgent treatment goal is to prevent the disease from progressing to AMI and bowel infarction. Since the majority of CMI patients have atherosclerosis antiplatelet therapy and statins are recommended. The treatment is revascularization, either endovascular or open. Endovascular treatment since introduced in the 1980s has increasingly replaced open surgery and is now recommended as the initial treatment for CMI [98, 99]. Although endovascular revascularization has lower mortality and morbidity rates, it has a higher incidence of restenosis requiring interventions and recurrent symptoms. Open surgical revascularization is reserved for cases where endovascular treatment is not possible mostly due to severe occlusions where there is a need for a bypass [6]. Although, it remains uncertain whether there are any variations in long-term survival between these two treatments [84, 99]. As most comprehensive studies are limited by reselection bias and long-term follow-up, the quality of the supporting data is still restricted.

The ESVS guidelines still suggest that revascularization should be considered only for patients who develop symptoms of CMI [6]. SMA with the largest surface area, the highest blood flow after a meal, and the extent distribution area is the most important mesenteric vessel for primary revascularization.

2. AIMS OF THESIS

The overall aim of this thesis was to investigate the macro and microcirculation, in addition to the level of serum biomarkers in the hypoperfused GI tract and treatment effects in patients with MALS. To impede this, five clinical prospective studies were conducted:

- In Paper I, the main objective was firstly to compare the diagnostic accuracy of two ultrasound modalities, EUS and TA-DUS, with the gold standard CTA in identifying stenosis in CA and SMA in patients with CMI. Secondly, to determine whether EUS has better diagnostic accuracy than TA-DUS for early detection of CMI.

- In Paper II, the main aim was to provide real-time microcirculation data of the stomach and gastric tube with the combined use of LDF and VLS during different phases of minimally invasive esophagectomy (MIE).

- In Paper III, the main objective was twofold. Firstly, to evaluate the transmucosal microcirculation of the stomach and duodenum with gastroscopy-assisted LDF and VLS (GALS) in patients with MALS, before and after the decompression of the CA. Secondly, to assess the health-related quality of life (QoL) in MALS patients after surgery.

- In Paper IV, the main objective was to present the findings of 10 years of experience with laparoscopic decompression of CA in patients with MALS and to investigate the clinical results after surgery.

  In Paper V, we investigated the accuracy of ischemia biomarkers for the diagnosis of atherosclerotic CMI and MALS.

3. MATERIAL AND METHODS

3.1 Study Design

- Paper I, an observational prospective blind comparison study, was conducted to investigate the diagnostic efficacy of EUS and TA-DUS compared to a gold standard test, CTA. It is presented as a validation study examining the potential usefulness of EUS in the early diagnosis of patients with CMI and MALS.

- Paper II, an analytical and observational prospective cohort pilot study designed to evaluate the feasibility and validity of the combined LDF and VLS in patients undergoing minimal invasive esophagectomy (MIE).

- Paper III, an analytical and observational prospective comparative cohort study conducted to measure microcirculation before and after treatment in patients with MALS and compare it to healthy individuals. The endpoint was to measure the health outcomes of patients with MALS after the decompression of CA, using a descriptive system, Quality of life (QoL).

- In Paper IV, the study design was a cohort, an observational and descriptive study in which MALS patients are selected, treated, and presented as prospective follow-up trials.

- In Paper V, an experimental case-control study for the investigations of the accuracy of ischemic biomarkers in the diagnosis of CMI and MALS.

3.2 Study Method

This thesis consists of five studies: One on esophagus cancer (Paper II), two on MALS (Paper III and IV), and two on atherosclerotic CMI and MALS (Paper I and V). In the pilot study (Paper II) we recruited a total of 10 patients at the Regional Center for cancer esophageal treatment at the Department of Gastrointestinal Surgery in Oslo University Hospital, Ullevål. They met the inclusion criteria of being capable of giving informed consent, potentially curable, receiving neoadjuvant radio-chemotherapy, and being scheduled for MIE.

The patients in the rest of the study groups, Paper I, III, IV, and V, were referred either from primary health care or other hospitals to the Department of Vascular Surgery at Oslo University Hospital, Ullevål, and Aker. The department is the only center in Norway with a dedicated group of physicians, physiologists, interventionists, and researchers for the diagnosis, treatment, and follow-up of patients with atherosclerotic CMI and MALS. All patients referred to this center with suspected CMI, including MALS, have undergone an extensive GI workup (TA-DUS, CT abdomen/pelvis, and endoscopy), to exclude other common differential diagnoses of upper abdominal pain, such as gastritis, peptic ulcers, cholelithiasis, chronic pancreatitis, and colorectal malignancy. The inclusion criteria for these patients were the presence of at least two of the three symptoms of the CMI classical triad, namely postprandial pain in the epigastrium, food aversion, and weight loss, as well as other associated autonomic symptoms such as nausea, vomiting, palpitation, sweating, nervosity, diarrhea, or constipation.

All patients underwent a detailed clinical examination followed by diagnostic imaging with TA-DUS and CT angiography. Each patient is investigated with a CTA mesenteric- protocol (3D software with Dual-phase imaging inspiration and in deep expiration phase). A confirmed CTA stenosis of mesenteric vessels ≥50% was considered hemodynamically significant. A TA-DUS was performed by a highly experienced physiologist for initial diagnosis and then follow-up trials. Hemodynamic velocities PSV ≥275 cm/s in SMA and ≥200 cm/s in CA were considered clinically significant. Once patients were suspected of having CMI or MALS based on their symptoms and clinical examination, and other potential diagnoses were ruled out, positive radiological findings were verified using TA-DUS and CTA. The findings were then discussed in a multidisciplinary panel consisting of vascular surgeons and interventional radiologists to determine the best course of direct patient management, which could include PTA or surgery. All surgical procedures were performed by the same surgeon. Despite treatment or not, all patients are followed up with TA-DUS and clinical examinations at 2–3 months, 6 months, 12 months, and yearly thereafter. Table 1 in this thesis presents the inclusion and exclusion criteria for all five papers.

Table 1 on the next page illustrates the inclusion and exclusion criteria of Papers I, II, III, IV, and V.

Abbreviations: AMI: acute mesenteric ischemia. CMI: chronic mesenteric ischemia, GI: gastrointestinal, CTA: computer tomographic angiography, TA-DUS: transabdominal Duplex ultrasound, SMA: superior mesenteric artery, CA: celiac artery, IMA: Inferior mesenteric artery, E-DUS: endoscopic Duplex ultrasound, LDF&VLS: laser Doppler flowmetry and visible light spectroscopy, GALS; Gastroscopy assisted laser Doppler flowmetry and Visible light spectroscopy, PTA: percutaneous transluminal angioplasty, MALS: median arcuate ligament syndrome.

Study population	n=50	n=10	n=47	n=52	n=74
Exclusion criteria	-Unable to obtain consent. -AMI -Unwilling or unable to perform E-DUS	-Unable to obtain consent	-Unable to obtain consent. -AMI -Atherosclerotic CMI -Unwilling or unable to perform E-DUS and operation	-Unable to obtain consent. -AMI -Atherosclerotic CMI -Rejected operation	-Unable to obtain consent. -AMI -Patients without CMI symptoms
Inclusion criteria Study population	CMI and MALS	Esophagus cancer	MALS	MALS	CMI and MALS
Exclusion of differential diagnosis	GI-workup	GI-workup	GI-workup	GI-workup	GI-workup
Symptoms	2 of the following symptoms	Symptoms related to esophageal cancer	2 of the following symptoms	2 of the following symptoms	2 of the following symptoms
Clinical examination	Nonspecific findings	Cancer related	Nonspecific findings	Nonspecific findings	Nonspecific findings
CTA	SMA, CA and or IMA stenosis ≥50%		CA 50% stenosis	CA 50% stenosis	SMA, CA and or IMA stenosis ≥50%
TA-DUS	PSV for CA ≥200 cm/s and for SMA ≥275 cm/s		PSV for CA ≥200 cm/s	PSV for CA ≥200 cm/s	PSV for CA ≥200 cm/s and for SMA ≥275 cm/s
Multidisciplinary panel	Consensus	Consensus	Consensus	Consensus	Consensus
Follow-up	Clinical and TA-DUS at 3, 6, 12 months, and yearly thereafter	Clinical and CTA	Clinical and TA-DUS at 3, 6, 12 months, and yearly thereafter	2-3 months, 6 months, 12 months, and yearly thereafter	2-3 months, 6 months, 12 months, and yearly thereafter
Other examinations and evaluations	E-DUS	Laparoscopic LDF&VLS	GALS Quality of Life (QoL)	Primary & secondary endpoints	Biomarkers
Treatment/Intervention	PTA or surgery	MIE	Surgery: Laparoscopic decompression of CA	Surgery: Laparoscopic decompression of CA	PTA or surgery
Control group	No	No	Group 1: upper abdominal pain Group 2: CMI	No	Health individuals N=16

3.3 Study Population

Paper 1 included a total of 50 patients with CMI, out of which 36 had atherosclerotic CMI and the remaining 14 patients had MALS. These patients were divided into two groups, Group A (untreated) and Group B (treated), and their results were compared. The degree of CTA stenosis was calculated with the following formula: % stenosis = (1 -[narrowest lumen diameter/diameter normal distal artery]) x 100. All patients were required to fast overnight before undergoing TA-DUS and E-DUS. Prior to E-DUS, patients received conscious sedation with midazolam (mean 3.35 mg) and alfentanil (mean 0.77 μg). The procedure was performed by the same experienced endoscopist.

In Papers II and III the same functional test, combined LDF & VLS, was utilized. However, in Paper II, transserosal microcirculatory measurements were taken, whereas, in Paper III, transmucosal measurements were collected. Paper II is a pilot study of 10 consecutive patients, while Paper III involved 15 MALS patients compared to 32 healthy individuals and 38 CMI patients.

In Paper IV, fifty-two consecutive patients diagnosed with MALS were treated with laparoscopic decompression of CA.

In Paper V, blood samples were collected from 74 individuals, including 44 CMI patients, 14 MALS, and 16 healthy individuals as a control group. A total of 58 patients were treated, including 30 CMI patients and 14 MALS patients. The study analyzed four intestinal ischemic biomarkers: α-glutathione S-transferase (α-GST), protein (I-FABP), ischemia-modified albumin, and citrulline. Additional plasma levels of p-amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), and creatinine were also measured.

3.4 Demographic Information

Patient demographic data in all studies was obtained from customized registration forms filled by patients and from their electronic medical records.

3.5 Intervention

- In Paper I, CMI and MALS patients examined with E-DUS and TA-DUS were divided into two groups: treated and untreated. A total of 21 patients received treatment, 14 of them underwent PTA + stent in one or more mesenteric arteries, while the remaining 5 patients were treated surgically with laparoscopic decompression and 2 with aortomesenteric bypass.

- In Paper II, all 10 patients in the pilot study were examined with combined LDF & VLS during MIE.

- In Papers I, III, IV, and V, all MALS patients were treated with laparoscopic decompression of CA, while atherosclerotic CMI patients were treated either with PTA + stent or surgery, such as laparoscopic or open aortomesenteric or aortosplenic bypass.

3.5.1 Serum Ischemic Biomarkers

In Paper V, venous blood samples were collected from all CMI (Group A) and MALS (Group B) patients in at least 6 hours of fasting before and 3 months after treatment. Blood samples were also collected from healthy individuals (Group C). The plasma samples in Ethylenediaminetetraacetic acid (EDTA) tubes were within 30 minutes centrifuged for 15 minutes at four °C, 3000 rpm. EDTA was according to protocol frozen in Nunc polypropylene vials and stored at −80 °C until analysis at the Hormone Laboratory at Oslo University Hospital, Aker, Norway. The laboratory technician was blinded for the study groups. The ELISA kits for I-FABP, citrulline, and ischemia-modified albumin had test detection ranges of 0.16–10 ng/mL, 5.0–100 nmol/mL, and 7.8–500 ng/ml. The ELISA kits for citrulline (MBS723693), I-FABP (MBS2507811), and ischemia-modified albumin (MBS760561) were provided by MyBioSource, San Diego, CA, USA. Although, the kit for α-GST had a detection range of 0.156 ng/mL-10 ng/mL., and the ELISA kit for α-GST(CSB-E08906h) was provided by Cusabio Technology, Houston, TX, USA. The intra-assay and inter-assay coefficient of variation (CV) for ELISA kits for Citrulline, IFABP, ischemia-modified albumin, and α-GST were <10% and <12%,

<6.3% and 6%, <8% and <10%, <8% and <10%. The assay procedures accompanying the ELISA kits were followed. The results of optic density measurements were corrected for the dilution factor to obtain the exact plasma concentration of the biomarker. In addition, plasma levels of p-amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), and creatinine were determined. AMY-P, ASTLP, ALTLP, ALP2, CREP2, and CRPL3 kits were utilized, and samples were analyzed on Cobas 6000.

3.5.2 Endoscopic Ultrasonography

Endoscopic ultrasound (EUS) of the mesenteric arteries is a minimally invasive diagnostic method. It is a procedure that combines two imaging techniques - endoscopy and ultrasound - to obtain detailed images of the mesenteric arteries. During an EUS procedure, a flexible endoscope with an ultrasound probe attached to its tip is inserted through the patient's mouth and guided down into the stomach or duodenum, depending on the area being examined. The ultrasound probe emits sound waves that bounce back from the surrounding tissues, creating high-resolution images of the mesenteric arteries on a monitor. EUS of the mesenteric arteries is considered minimally invasive because it does not require any surgical incisions or general anesthesia. However, it is still an invasive procedure in the sense that it involves inserting a device into the body and can cause some discomfort or minor complications, such as bleeding or infection. Overall, EUS of the mesenteric arteries is considered a safe and effective diagnostic tool for evaluating blood flow to the abdominal organs and diagnosing conditions such as CMI and MALS.

3.5.3 Laser Doppler Flowmetry and Visible Light Spectroscopy

A 2.6 mm microprobe with combined LDF & VLS modalities (O2C; LEA Medizintechnik, Germany) was placed on specific measurement points based on the arterial supply of stomach and duodenum in order to measure various parameters simultaneously; blood flow, velocity, mixed arterial and venous saturation of hemoglobin (StO_2), and the amount of hemoglobin per tissue volume (rHb).

In Paper II, during the surgical procedure of MIE, a laparoscopic trocar was used to channel the O2C microprobe and placed on predefined points on the serosa of the stomach and gastric tube (Figures 7A and B). Before each recording, an ambient light correction was performed automatically to maintain illumination of the examined area and allow for visual control of the O2C microprobe throughout the examination. Baseline measurements were taken from predefined anatomical sites on the greater curvature's anterior surface before establishing pneumoperitoneum with CO_2 and before any intraperitoneal dissection. Measurements were then repeated at the same anatomical sites after gastric tube formation and subsequently after construction of the TGEA, with approximately 3-4 cm between each measuring site. In all patients, the gastroesophageal anastomosis was constructed at site M7, and a marking suture was placed at the gastric incisura towards the level of site M3 to identify the site after the gastric pull-up and anastomosis. All measurements were performed under stable hemodynamic conditions without the administration of vasopressor medications, and the systemic oxygen saturation was maintained above 97%.

In Paper III, during gastroscopy, the O2C microprobe was passed through a working channel of an Olympus Flexible Gastroscope and placed on the mucosa of the stomach and duodenum for the measurements (Figure 8). Unstable or fluctuating recordings were repeated. To ensure accurate measurements, the absorption spectra of oxyhemoglobin were kept well above 50% of the arbitrary unit (AU) scale using the graphical picture provided by the LCD monitor of the O2C unit (Figure 7B). The measurement protocol involved taking 5 seconds of continuous measurements at each anatomical position, resulting in approximately 200 measurements at each anatomical site. The system provided real-time quantitative measurement and stored the raw data for later analysis.
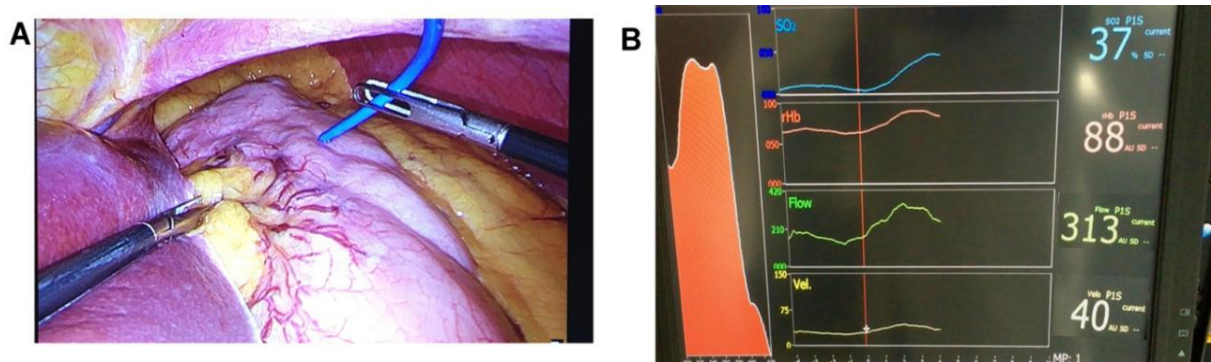
*Figure 7 **(A–B).** Transserosal microcirculation recordings: (A) microprobe on ventricle surface (B) LCD monitor with the real-time absorption spectrum of oxyhemoglobin (red), and graphic presentation of StO2, relative hemoglobin, flow and velocity, and the mean numerical values. Figure adapted from Berge et al.*

*Figure 8: An O2C LM-10 microprobe (O2C, LEA Medizintechnik GmbH) (blue) seen through a gastroscope (left). The microprobe is passed through the working channel of the gastroscope and contains flexible glass fiber optics emitting white light, and a laser beam and is connected to a machine that allows for simultaneous use of laser Doppler flowmetry and visible light spectroscopy. The black orifice at the bottom of the image is the pylorus. Right: Distal end of the LM-10 microprobe. **Figure adapted from Berge et al. 2020?***

3.5.4 Endovascular Treatment

All patients were provided with information about surgical alternatives and the potential risk factors. After giving consent to the endovascular treatment (ET) for CMI the procedure was performed percutaneously in the angiography suite. An interventional radiologist performed ET on patients using local anesthesia. Depending on the angle between the visceral arteries and the aorta, percutaneous transfemoral or transbrachial access was utilized. A 4-French sheath was inserted via the Seldinger technique into the femoral or brachial artery, and an aortography was conducted to locate the ostium of the target vessel. Bare-metal stents mounted on balloons were employed. A long sheath (5- to 8-French, depending on the stent type and diameter) was introduced into either the celiac trunk or the superior mesenteric artery, and a 0.035″ stiff guidewire was placed into a peripheral branch of the artery. After assessing the diameter and length of the stent in the peri-interventional angiography, it was positioned and released into the vessel. Intravenous heparin (22,000 IU/24hr) was consistently administered through the same catheter to prevent peri-catheter thrombosis and complications arising from distal embolization.

3.5.5 Thoracolaparoscopic Minimal Invasive Esophagectomy

All patients underwent a standard thoracolaparoscopic, "Ivor-Lewis" type minimally invasive esophagectomy under general anesthesia by the same team of surgeons, who were blinded to the perioperative microcirculation measurements. The procedure involved the complete mobilization of the stomach, dissection of the short gastric arteries and left gastric artery, and preparation of the gastric tube after regional lymphadenectomy. A multi-step thoracolaparoscopic subtotal resection of the esophagus with two-field lymphadenectomy was performed, followed by anastomosis of the gastric conduit to the proximal residual esophagus at the carina level using a circular staple device. The introduction site for the circular stapler was closed with a linear stapler and oversewn. Thoracic drains were placed near the anastomosis and diaphragmatic hiatus, and a decompressing nasogastric tube was inserted. The patients were extubated in the operating theater and postoperatively, a mean systemic arterial pressure of 65 mmHg or higher was targeted, with vasopressors administered if needed. Patients were monitored for three days in the postoperative surveillance department and underwent routine upper endoscopy and CT with oral contrast of the esophagus on the third postoperative day. Complications were reported and graded according to the Clavien-Dindo classification of surgical complications, as recommended by the Esophagectomy Complications Consensus Group [100].

3.5.6 Laparoscopic Surgery

The Laparoscopic Decompression Technique

The surgical procedure was conducted with the patient under general anesthesia in a supine position on a split-leg operation table. The operator was positioned between the patient's legs, with one assistant on each side of the table. Trocars were placed in the positions shown in Figure 9, with a 30° video laparoscope used through one of the trocars. To achieve good exposure of the lesser omentum, the left liver lobe was elevated with a Nathanson liver retractor placed through a small incision distally and to the left side of the xiphisternum. The gastro-hepatic ligament was incised along the upper border of the pancreas. If required, a slight distal retraction of the duodenum and pancreas

by the assistant was used to provide good exposure to the CA while the patient was positioned in a reverse Trendelenburg. Care was taken to keep a safe distance from the left gastric artery, and in a few cases, also from the aberrant left hepatic artery. The common hepatic artery was carefully approached along the upper border of the pancreas and followed centrally towards the CA bifurcation. Ultracision Harmonic ACE+ and monopolar electro-cautery hook were alternately used to dissect the CA from any nerve or fibrous tissue on its cranial surface. The left gastric artery and the inferior phrenic arteries were respected and preserved. The right diaphragmatic crus and median arcuate ligament were divided, and dissection was continued 1–2 cm cranially on the aorta, proximal to the origin of the CA. The decompression of the CA was considered accomplished only when it was completely skeletonized on its cranial surface (Figure 10). The fascia at the 12 mm trocar position was closed with Polysorb braided sutures, and the skin incisions were closed with intracutaneous sutures. No wound drain was used. The patients were allowed to take oral food on the same day after surgery were fully mobilized on the first postoperative day and were normally discharged after a median hospital stay of 2 days (range 2–3 days).

Figure 9. Trocar position on the abdominal wall for transperitoneal laparoscopic decompression of the celiac artery in a patient with median arcuate ligament syndrome. Figure adapted from Kazmi et al. [101]
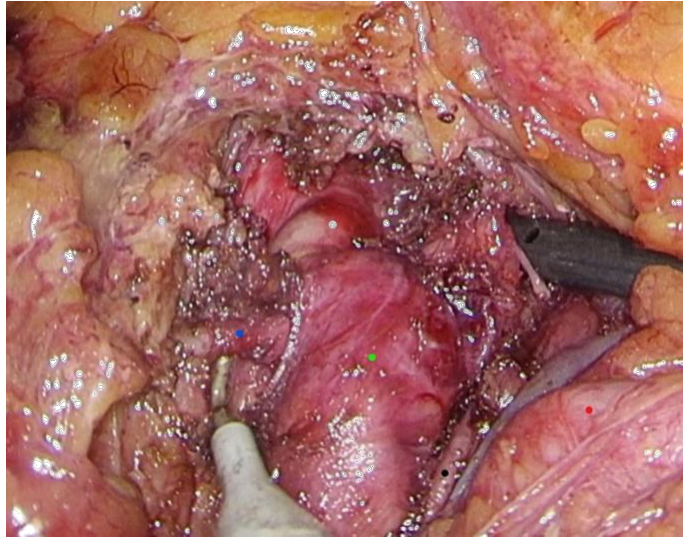
Figure 10. Transperitoneal laparoscopic celiac artery (CA) decompression for median arcuate ligament syndrome. The white dot represents the decompressed segment of CA originating from the aorta, and the green dot represents the post-stenotic dilated segment of the CA. The blue dot represents the right inferior phrenic artery. Black and red dots represent the left gastric artery and splenic artery, respectively. Figure adapted from Kazmi et al. [101]

The Laparoscopic Aortomesenteric Bypass

Patients were under general anesthesia and prophylactic antibiotics, Cephalothin 2 g, were positioned in a supine position on a split-leg table with the surgeon standing between the legs, a surgical nurse on the right side, and an assistant on each side of the operating table. Under direct visualization, the first trochar was placed through the umbilicus, and pneumoperitoneum with 12 mmHg continuous pressure was achieved. 1-2 fan retractors (Covidien Endo Retract II, Ethicon Endo-Surgery, Cincinnati, OH, USA) were used to hold the small intestine on the right side of the abdominal cavity. The ligament of Treitz had to be divided and the duodenum mobilized to reveal the abdominal aorta and the SMA. The overlying peritoneum over the abdominal aorta and iliac arteries was dissected, and SMA was identified in the area just below the conjunction of the superior and inferior mesenteric veins. The ligament of Treitz was divided and the duodenum mobilized to dissect free an adequate length of the SMA. In one case, the right iliac artery, and in another, the left graft limb of a previously laparoscopically operated aortobifemoral bypass, was dissected for anastomosis. A ring-enforced 8mm PTFE was introduced through a 12 mm trochar and prepared adequate length

for the anastomosis. Intravenous 5000IU Heparin was administered and left circulated for at least 2 minutes. A laparoscopic aortic clamp through a trochar was carefully placed on the infrarenal aorta parallel to the abdominal aorta. Aortotomy and arteriotomy on SMA and End-side anastomoses were made between the infrarenal aorta (in one case right iliac artery and in another case left graft limb) and PTFE graft and the between SMA and PTFE graft. The anastomoses were sewn with 2 hemi-circular 6-0 polypropylene sutures of 12 - 15 cm and secured with Teflon pledget (BD, BARD peripheral vascular, AZ, USA). Before the last sutures backflow was confirmed and the graft was flushed thoroughly with a Heparin-saline solution. Grafts were routinely covered with peritoneum and or omentum to avoid direct contact with the nearby intestines. The fascia was closed with absorbable monofilaments and the skin with metal clips.

3.5.7 Open Surgery

Patients are in the supine position under general anesthesia and intravenously prophylactic antibiotics with Cephalothin 2 g every 90 minutes with a total of 4 doses. A midline laparotomy and the lateralization of the small bowels were performed to visualize the infrarenal abdominal aorta. The infrarenal aorta and SMA distal to the stenosis were freely dissected and prepared for clamping and rubber bands. Intravenous 5000 IU Heparin was administered and left circulated for at least 2 minutes. Arteriotomy on the aorta and then SMA is performed. An 8 mm PTFE graft in its right length was anastomosed proximally to the aorta and distally to SMA in an end-to-side method. Sutured in a hanging-loop technique with Optilene 4-0 (B. Braun, Hessen, Germany). Prior to the last sutures, the graft was checked for backflow and the graft was flushed thoroughly with a Heparin-Saline solution. The graft patency was evaluated perioperatively by measuring the velocities and flow in the graft and distal to the distal-anastomosis with Duplex ultrasound duplex and flowmetry (VeriQC machine that is capable of both transit-time flow measurement and ultrasound evaluation). The graft was always covered with peritoneum or omentum and the fascia closed with Polydioxanone (absorbable synthetic monofilament) in continuous closure.

3.5 Outcome Measures

- Paper I: The velocity measured (PSV and EDV) using E-US and TA-DUS were compared with the CTA stenosis grad to determine the diagnostic efficacy of E-DUS for stenosis in the SMA and CA in patients with CMI and MALS

- Paper II: Tissue perfusion was quantified by measuring transserosal blood flow (flux), velocity, StO_2, and rHb in three phases during MIE. Furthermore, the patients were monitored for up to 18 months for anastomotic leaks (using the Clavien-Dindo classification of surgical complications) and mortality.

- Paper III: Transmucosal blood flow (flux), velocity, StO_2, and rHb were measured in patients with MALS before and after the laparoscopic decompression and compared to patients with CMI and a control group with healthy individuals. The clinical outcomes before and after the treatment were measured with Health-related quality of life (QoL) using EQ-5D.

- Paper IV: Fifty-two MALS patients who underwent laparoscopic decompression of CA were followed up with TA-DUS and clinical examination at 2-3 months, 6 months, 12 months, and yearly thereafter to collect follow-up data on
  - The primary endpoint was the relief of at least one of the MALS symptoms.
  - The secondary endpoint was morbidity, symptom recurrence, and reintervention.

- Paper V: the study compared intestinal ischemic biomarkers in patients with CMI and MALS before and after treatment with those of healthy individuals to aid in diagnosis.

Quality of life

In Paper III, "health-related quality of life" was used to define our study's treatment effects and clinical outcomes. A valid questionnaire EuroQol (EQ-5D-5L) in the Norwegian language was completed by patients at baseline and 12 months after treatment. Quality of life is a multidimensional concept used to describe and the value health-related quality of life (physical, emotional, and social

health status) of an individual by filling out a questionnaire. It's a quantitative measure of the health outcomes that reflect the patient`s assessment.

EQ-5D-5L is a widely used versatile descriptive system, it is a self-assessed, health-related, quality of life questionnaire for the evaluation of the generic quality of life in Europe. It measures the QoL by asking 5 questions: morbidity, self-care, usual activities, pain/discomfort, and anxiety/depression. Each of these 5 questions is subdivided into 5 levels of perceived problems (1 indicating no problems and 5 indicating extreme problems). The second part of EQ-5D-5L is a vertical visual analog scale (EQ VAS), where the endpoints are labeled from 0, "the worst imaginable health state" to 100, "the best imaginable health state".

3.6 Statistical Methods

Paper I

The study analyzed continuous data using median and interquartile range (IQR) and categorical data using numbers and percentages. Cross-tabulation was used to compare the diagnostic results of the two different ultrasound techniques with the results of CTA, which is considered the gold standard for mesenteric artery stenosis.

PSV velocities of ≥200 cm/s for CA and ≥275 cm/s for SMA were used to compare flow velocities with CTA-verified stenosis ≥50% and ≥70% separately.

Cross-tabulation determined the diagnostic accuracy of ultrasound techniques compared to CTA by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA).

Receiver operating characteristic curves (ROC) analysis was performed and the area under the curve (AUC) was estimated. ROC analysis is a statistical method that plots the true positive rate (sensitivity) against the false positive rate (1-specificity) for different cut-off points of a diagnostic test. The resulting curve can then be used to determine the area under the curve (AUC), which provides a measure of the test`s overall accuracy. An AUC of 0.50 indicates a test with no diagnostic accuracy (i.e., a coin flip), while an AUC of 1.0 indicates a perfect diagnostic test. The AUC was used

to interpret the accuracy of the ultrasound techniques as fail, poor, fair, good, or excellent, based on the range of values obtained. Historically, ROC analysis was first developed during World War II to measure radar operators' performance in distinguishing between real and false signals. In the 1950s and 1960s, the method was applied to medical diagnostic tests, and in the 1970s and 1980s, it became widely used in signal detection theory and machine learning. Today, ROC analysis is used in many fields, including medicine, engineering, and finance, to evaluate the performance of binary classifiers and identify optimal cutoff points for decision-making [102]

The data analysis was conducted using IBM SPSS Statistics Version 27.

Paper II

Descriptive data analysis was conducted to evaluate the results of the surgical intervention or treatment. The data were presented as either a median (range) or a mean (standard deviation), depending on the distribution of the data.

Paired sample t-test was used to determine the statistical significance of any changes observed between groups or within the same group. The paired sample t-test is a statistical method used to compare two related groups or evaluate changes within the same group before and after treatment. It is useful when working with paired data or samples that are related in some way, such as before-and-after measurements of treatment on the same group of individuals. The test is used to determine whether the mean difference between the two related groups is statistically significant or simply due to chance.

A P-value of less than 0.05 was considered statistically significant, indicating that the observed differences were not due to chance and were likely to be the result of the intervention or treatment. Statistical analyses were performed using SPSS version 25 (IBM SPSS Statistics).

Paper III

The statistical analysis of the data in this study employed several techniques. The presentation of normally distributed data was done through mean values with standard deviations or median values

with range, as appropriate. The independent Student's t-test was used for continuous outcome variables, while Fisher's exact test was employed for categorical data. The Wilcoxon signed ranks test was also utilized to investigate changes after intervention.

To determine statistical significance, a significance level of 5% ($p < 0.05$) was set. The sample size was calculated by using G*power Version 3.1.9.6 (Franz Faul Universität, Kiel, Germany) based on a previous study on CMI. With a study power of 80%, a sample size of 10 patients was calculated.

The study explored test performance at different cut-off levels using an ROC curve, and sensitivity and specificity were calculated for diagnosing MALS. Statistical analysis was conducted using IBM SPSS Statistics version 25 (IBM Corp. Armonk, NY).

Paper IV

The study presented the average values and standard deviations for normalized data. Percentages were used to show proportions. To determine if there were any changes after surgery, the Mann-Whitney test was used. Statistical significance was considered at 5% ($p<0.05$). The Mann-Whitney test, also known as the Wilcoxon rank-sum test or the Wilcoxon-Mann-Whitney test, is a non-parametric statistical test used to compare the distribution of two independent groups. It is used when the data does not follow a normal distribution, or when the assumption of equal variances is violated in a parametric test like the independent t-test.

In the Mann-Whitney test, the data from both groups are combined and ranked in order from the lowest to the highest value. The rank sums are then calculated for each group and the test statistic U is calculated based on the rank sums. The U value represents the probability of randomly selecting a value from one group that is greater than a value from the other group.

The null hypothesis of the Mann-Whitney test is that there is no difference between the two groups being compared. If the calculated U value is less than the critical value from the Mann-Whitney U distribution, then the null hypothesis is rejected, and it is concluded that there is a significant difference between the two groups. The significance level is typically set at 5% ($p<0.05$).

Paper V

The study utilized various statistical techniques to analyze the collected data. The continuous data were presented as the median and interquartile ranges, and the Mann-Whitney U-test was used to analyze them. The categorical data, on the other hand, were presented as proportions and percentages. A p-value of less than 0.05 was considered statistically significant. Contingency tables were utilized to analyze the categorical data and calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA). To estimate the diagnostic accuracy of the biomarkers, the study used a Receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was calculated. The data analysis was performed using SigmaPlot version 14.0 (Systat Software, San Jose, CA, USA).

3. ETHICAL CONSIDERATION

Comprehensive information regarding the studies was provided to the participants through both written materials and oral communication. Only individuals with adequate cognitive abilities were considered for inclusion in the study. The participants were explicitly informed about their right to withdraw from the study at any point, without any impact on their future treatment or care. Following the provision of information, all participants provided written consent, indicating their willingness to participate in the study. To ensure ethical practice, therapy decisions were not influenced by the results of the unvalidated diagnostic tests, and the patients received surgical treatments uninfluenced by the study involvement. Most patients considered the additional follow-up in the outpatient clinic and gastroenterology laboratory to be an added service rather than a burden. Privacy and confidentiality of participants' data must be protected, and their autonomy and right to withdraw from the study should be respected. The study adhered to the principles outlined in the Helsinki Declaration to ensure ethical conduct and safeguard the rights and welfare of the participants.

In Papers I, III, IV, and V the research protocol underwent approval by the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (REK Sør-Øst B 2016/682) and was subsequently registered in the ClinicalTrials.gov Protocol Registration and Results System (NCT02914912). In Paper II, the research protocol received

approval from the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (approval number 2018/500/REK sør-øst A) and was registered on ClinicalTrials.gov (ClinicalTrials.gov ID NCT03724162).

In Paper I patients underwent investigations using both TA-DUS and E-DUS, which were conducted prior to the revascularization procedures and within a period of 1-6 months afterward. At our center, it is customary to perform TA-DUS examinations before and after treatment and during the follow-up phase. The patients conveyed their satisfaction with these additional examinations, expressing their appreciation for the noninvasive nature of the TA-DUS diagnostic procedures and the improved monitoring and control they afford. E-DUS on the other hand is an invasive method. An upper endoscopy was conducted in Paper I and Paper III to examine the circulation in the stomach and duodenum. Upper endoscopy is a standard procedure for patients with upper abdominal complaints and is essential for diagnosing and ruling out common causes of abdominal pain. However, since many patients had already undergone an endoscopy before being referred to our center, a second endoscopy was necessary before the operation, followed by a third postoperatively. Although patients experienced some discomfort during the procedure, the risk of complications was low.

In Papers II, III, and IV, several ethical concerns were raised. Innovations in surgery are often the driving force behind advancements, leading to improved patient care and decreased morbidity and mortality rates. For instance, in the case of CMI, endovascular therapy is now recommended over open surgery due to reduced perioperative morbidity. However, the primary ethical dilemma associated with novel surgical techniques is the uncertainty surrounding their risks and benefits for patients. When implementing a new procedure, the true risks may not be fully understood at the time, and complications such as postoperative infections and bleeding tend to occur shortly after the intervention. These immediate complications are properly documented and attributed to the procedure, but there is a possibility of other complications emerging months or even years later that may not be directly linked to the intervention.

All patients involved in the study provided informed consent for both their participation in the study and the surgical procedure, which is standard for elective surgeries. However, it should be noted that not all risks and benefits associated with the procedure were fully known at the time, and thus, they

were explained in more general terms. This included common surgical complications such as infections, bleeding, postoperative pain, graft thrombosis, and cardiovascular incidents. Patients were presented with the option of the traditional procedure (open surgical bypass) or no surgery, considering the elective nature of the treatment. However, it is important to acknowledge that the consent process, based on shared decision-making between the physician and patients, might have been influenced by the innovators' potential optimism bias. The information provided was primarily based on our expectations that the procedure would lead to better patient outcomes, and patients likely anticipated similar benefits from the new technique.

For any surgical innovation, it is crucial to maintain detailed records of patient outcomes. While publication bias often leads to the concealment of less successful techniques, it is important to publish and share information regarding both successful and unsuccessful procedures. The safety of laparoscopic techniques in vascular surgery has been extensively studied through the Norwegian Laparoscopic Aortic Surgery Trial (NLAST) conducted at our institution and collaborating institutions before including patients. The laparoscopic mesenteric bypass procedures in this study were performed by Kazmi, a highly experienced surgeon in laparoscopic vascular surgery, with several publications on laparoscopic aortobifemoral bypasses as part of NLAST over the past 15 years.

The novel aspect of this study was the application of laparoscopic vascular surgery to a new indication, CMI, including the first laparoscopic bypass to the splenic artery, as far as our knowledge extends. Participating in the study did not compromise the patients' legal rights in any way in case of complications.

All patients willingly sought surgical treatment for their condition and had the option to withdraw their consent at any time without providing a reason. Efforts were made to address any practical issues and retain the patient if a request to withdraw from the trial arose. If retention was not possible, the patient would have been treated according to center guidelines.

In paper V patients were fully informed about the nature of the invasive procedure, including its purpose, potential risks, benefits, and any alternatives. This includes using appropriate anesthetics, ensuring aseptic techniques to prevent infections, and monitoring patients during and after the procedure.

4. RESULTS OF PAPERS

4.1 Paper I

In Paper I, we investigated the validity of E-DUS as an early diagnostic tool for mesenteric ischemia in patients with CMI and MALS by comparing it with the clinically recommended TA-DUS and the gold standard, CTA. Typically, patients with CMI and MALS undergo numerous exclusionary examinations, including TA-DUS, and endoscopy, which are performed early during the investigation. Combining endoscopy with ultrasound duplex provides a reasonable approach to investigating the diagnostic potential of E-DUS for detecting CA and SMA stenosis. We considered CTA-verified stenosis of $\geq 50\%$ and $\geq 70\%$ in CA and SMA, respectively, and ultrasound velocities of PSV ≥ 200 cm/s for CA and ≥ 275 cm/s for SMA are significant stenosis. Although, patients treated with PTA with stents have reported having persistently high PSV beyond 335 cm/s despite asymptomatic angiographic stenosis of <20% [103]. Thus, ultrasound velocities may vary in patients who have undergone prior treatment. Therefore, we divided our study population into two groups based on treatment and compared their results.

In total, 50 CMI patients (36 atherosclerotic CMI and 14 MALS) were examined in 12 months. Twenty-nine patients with no prior treatment (Group A) and twenty-one patients had previously been treated (Group B) but had either residual symptoms or relapse (Figure 11).

The main findings were a higher sensitivity for E-DUS than TA-DUS for identifying both $\geq 50\%$ and $\geq 70\%$ stenosis in CA and SMA. Although, the specificity for E-DUS was particularly lower in CA than for TA-DUS (Table 2).

- In Group A (Untreated); The sensitivity of E-DUS for diagnosing both $\geq 50\%$ and $\geq 70\%$ stenosis was slightly better than TA-DUS.

- In Group B (treated), E-DUS had higher sensitivity than TA-DUS for both $\geq 50\%$ and $\geq 70\%$ stenosis in both mesenteric arteries.

ROC curve presented better AUC values for E-DUS for both CA (0,79 for 70% stenosis and 0.70 for 50% stenosis) and SMA (0,75 for 70% stenosis and 0.79 for 50% stenosis) than TA-DUS

(Figure 12). However, AUC for E-DUS for ≥50% stenosis was higher for SMA than TA-DUS but for CA the AUC was lower than TA-DUS (Figure 12).

Conclusion: E-DUS is a valid examination test for early diagnosis of CMI in patients undergoing endoscopic investigation for upper abdominal pain.
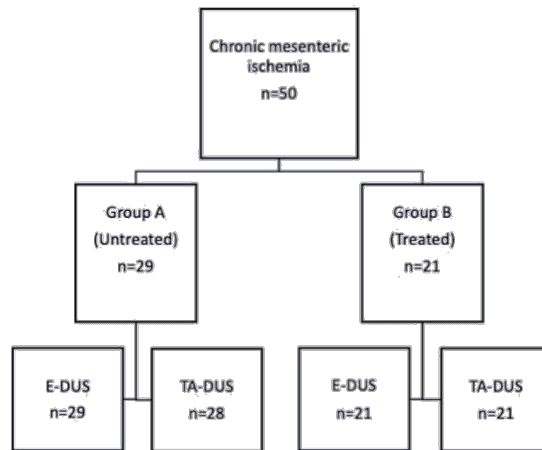
Figure 11. Patient flow chart in 50 patients with chronic mesenteric ischemia (CMI) investigation with transabdominal duplex ultrasound (TA-DUS), endoscopic duplex ultrasound (E-DUS), and computed tomography angiography (CTA) Figure adapted from Safi et al. [89]

| | | CTA ≥70% | | | | CTA ≥50% | | | |
| | | CA | | SMA | | CA | | SMA | |
		E-DUS	TA-DUS	E-DUS	TA-DUS	E-DUS	TA-DUS	E-DUS	TA-DUS
Sensitivity	Total	91%	81%	100%	92%	78%	57%	67%	56%
	Group A	100%	82%	100%	100%	90%	80%	59%	64%
	Group B	82%	80%	100%	83%	73%	64%	83%	50%
Specificity	Total	37%	72%	78%	88%	30%	67%	90%	95%
	Group A	30%	60%	86%	84%	33%	45%	100%	92%
	Group B	50%	90%	67%	93%	50%	100%	89%	100%
PPV	Total	55%	71%	62%	75%	82%	86%	90%	93%
	Group A	50%	60%	70%	70%	75%	67%	100%	90%
	Group B	64%	89%	55%	83%	79%	100%	90%	100%
NPV	Total	83%	81%	100%	97%	25%	27%	69%	65%
	Group A	100%	82%	100%	100%	60%	63%	63%	69%
	Group B	72%	82%	100%	93%	43%	55%	80%	60%
OA	Total	62%	76%	84%	89%	68%	57%	78%	74%
	Group A	59%	78%	90%	88%	72%	65%	76%	77%
	Group B	67%	85%	76%	90%	67%	75%	86%	72%

Abbreviations: E-DUS, endoscopic duplex ultrasound; TA-DUS, transabdominal duplex ultrasound; CTA, computed tomography angiography; CA, celiac artery; SMA, superior mesenteric artery; PPV, positive predictive value; NPV, negative predictive value; OA, overall accuracy.

Table 2 Results of the Validity Assessment of Peak Systolic Velocities Measured with Duplex Ultrasound (for the Detection of ≥50% and ≥70% CTA-Verified Stenosis in All Patients (n=50), Treatment-Naive Patients (Group A; n=29) and Patients After Treatment (Group B; n=21). PSV Cut-Offs: ≥200 cm/s for Celiac Artery and ≥275 cm/s for the Superior Mesenteric Artery (SMA) Source: Safi et al. [89]
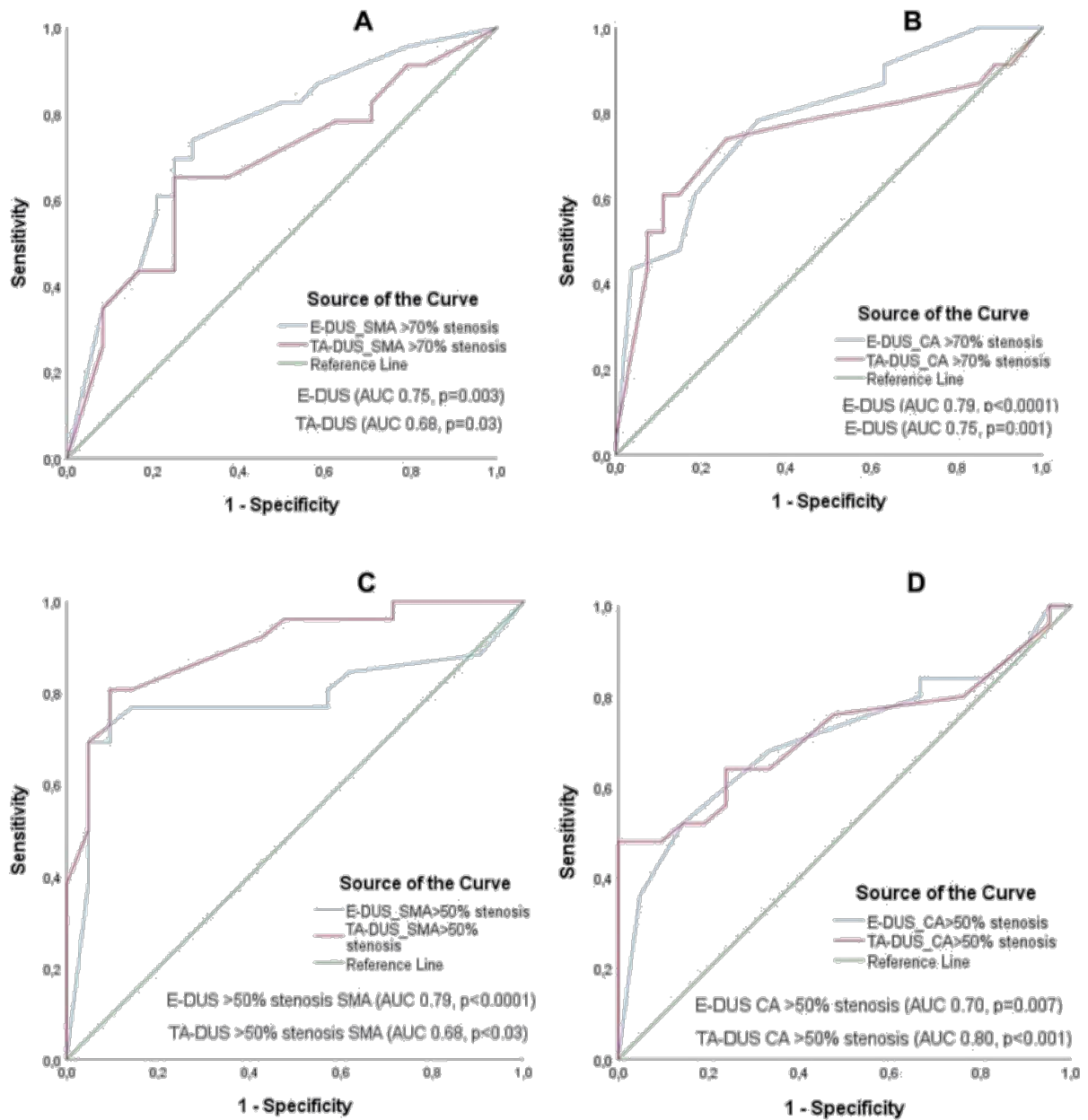
Figure 12 (A–D) ROC curve analysis of endoscopic duplex ultrasound (E-DUS) and transabdominal duplex ultrasound (TA-DUS) peak systolic velocities of ≥200 cm/s for celiac artery (CA) and ≥275 cm/s for superior mesenteric artery (SMA) with computed tomographic angiogram (CTA)-verified stenosis of ≥50% and ≥70% in patients with chronic mesenteric ischemia. (A) Sensitivity and false-positive rate (1-specificity) in ≥70% stenosis in SMA; (B) ≥70% stenosis in CA; (C) ≥50% stenosis in SMA; (D) ≥50% stenosis in CA. Figure adapted from Safi et al. [89]

4.2 Paper II

Ischemia at the anastomotic site during MIE plays a major role in thoracic gastroesophageal anastomosis leaks (TGEA leaks). However, the hypoperfused site of the gastric tube may not be visually apparent to the surgeon. Therefore, an intraoperative GI viability assessment technique that can detect the microcirculatory changes in the GI tract can guide the surgeon in selecting the most perfused site for the anastomosis. In this pilot study conducted in a single esophageal cancer center, we examined 10 patients undergoing MIE with combined LDF & VLS. LDF provides velocity and flow (red blood cell flux), whereas VLS provides StO2 and rHb values. Combined LDF & VLS probe was channeled through the laparoscopic trocar and placed transserosal on the stomach and gastric tube to achieve real-time measurements of the microcirculation in 3 different phases of MIE. Figure 13 illustrates these 3 phases: baseline, gastric tube construction, and after gastroesophageal anastomosis.

The main finding was the statistically significant reduction in StO2, which decreases by 16% from baseline to gastric tube formation, and 42% after anastomosis (P < 0.001). Among the ten patients studied, three (30%) developed TGEA leaks. They had a lower StO2 at baseline compared to those without leaks, as well as a further reduction after the anastomosis. The rHb concentration exhibits a significant increase in the cranial part of the gastric tube that is most prone to ischemia as illustrated in Figure 14. Patients with leaks had higher rHb concentration (61%) than those without leaks (17%). While the mean change in velocity was similar in patients with or without leaks, those with leaks had a 36% increase in mean tissue blood flow after anastomosis, whereas those without leaks had a 26% increase. All patients were followed up for 16 months.

Conclusion: LDF & VLS are valuable tools during esophagectomy to detect tissue ischemia in the gastric tube, guiding the surgeon to avoid the most hypoperfused area for anastomosis formation.
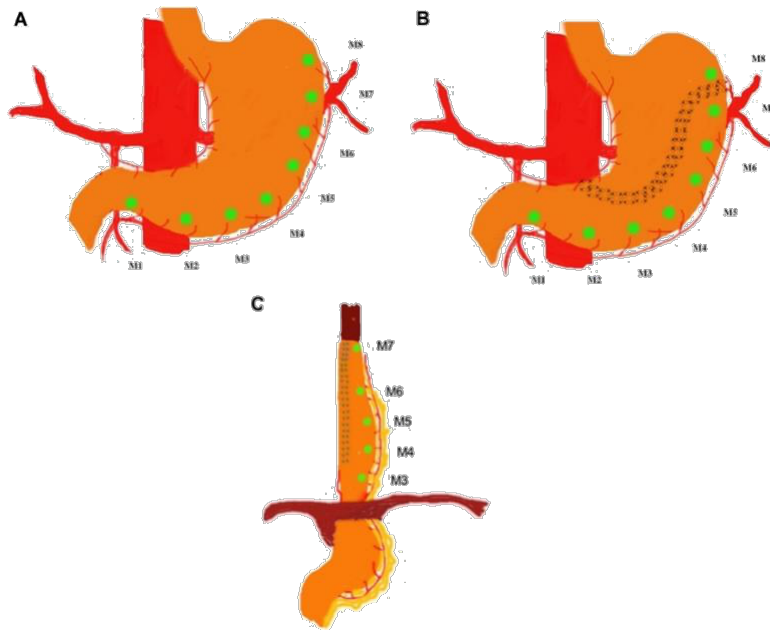
Figure 13. Adapted from Safi et al.[104] A– Measuring points M1 to M8 (green dots) at baseline, after gastric tube construction, and gastroesophageal anastomosis
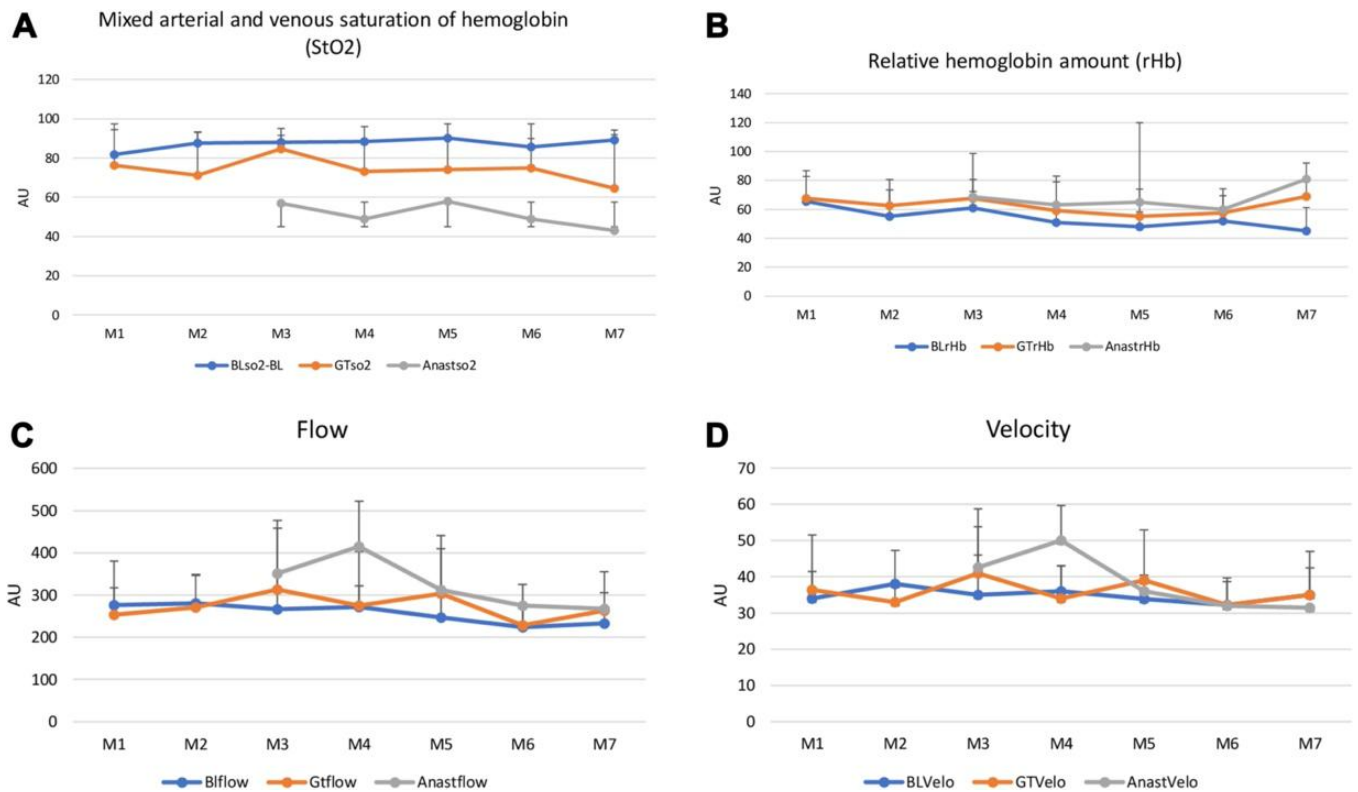
Figure 14. (A–D). adapted from Safi et al.[104] Mean values with a standard deviation of intraoperative transserosal microcirculation of stomach and gastric tube in patients with esophageal cancer. AU, arbitrary units; M1-M7, measuring sites.

4.3 Paper III

MALS is considered controversial mainly for two reasons. Firstly, it is unclear whether the etiology of the condition is ischemic or neurological. Secondly, it is uncertain whether surgery is necessary, as some patients continue to experience symptoms even after the procedure. In order to address these issues, in Paper III, we utilized GALS to evaluate the microcirculatory changes in patients with MALS (n = 15) before and after treatment and compared the results to healthy individuals (n = 38) and patients with CMI (n = 32) (Figure 15 and 16). The effectiveness of treatment was then evaluated through three methods:

1. Clinically at 1-, 3-, 6-, and 12-months

2. Re-examination with GALS after three months of treatment (Figure 15)

3. Pre- and postoperative measurement of health-related quality of life (QoL) assessed with Euroqol, EQ-5D-5L

The main findings indicated that StO_2 was the most effective variable for identifying ischemia. Preoperative transmucosal StO_2 was significantly ($p = 0.02$) lower in patients with MALS (StO_2 76 6) than the healthy individuals (StO_2 81 4). After surgery, we observed a significant improvement in StO_2 ($SO2$ 81±3.7, p=0.05). Of the patients who showed clinical improvement after laparoscopic decompression, 11 (92%) out of 15 had a confirmed diagnosis of MALS with clinical improvement. Follow-up lasted a median of 18 months (4-24 months), during which four of the five dimensions investigated with EQ-5D-5L showed improvement, and a statistically significant improvement in the patient's self-perceived health status was recorded ($p = 0.02$).

Conclusion: The fact that MALS patients had lower StO_2 compared to healthy individuals supports a possible ischemic etiology. This theory is further supported by the 3 main findings after surgery: the increase in StO2, clinical improvement higher QoL score Hence, GALS has the potential to be used as a diagnostic tool, and surgery should be considered in the treatment of patients with MALS.
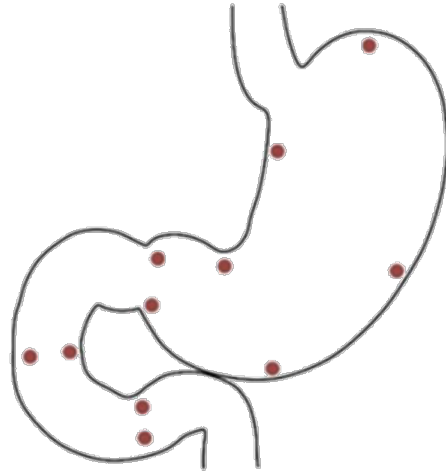
Figure 15. Measurement points in the stomach and duodenum in the study of perioperative microcirculatory changes in patients with MALS. Abbreviation: MALS, median arcuate ligament syndrome. Source: Berge et al. [7]
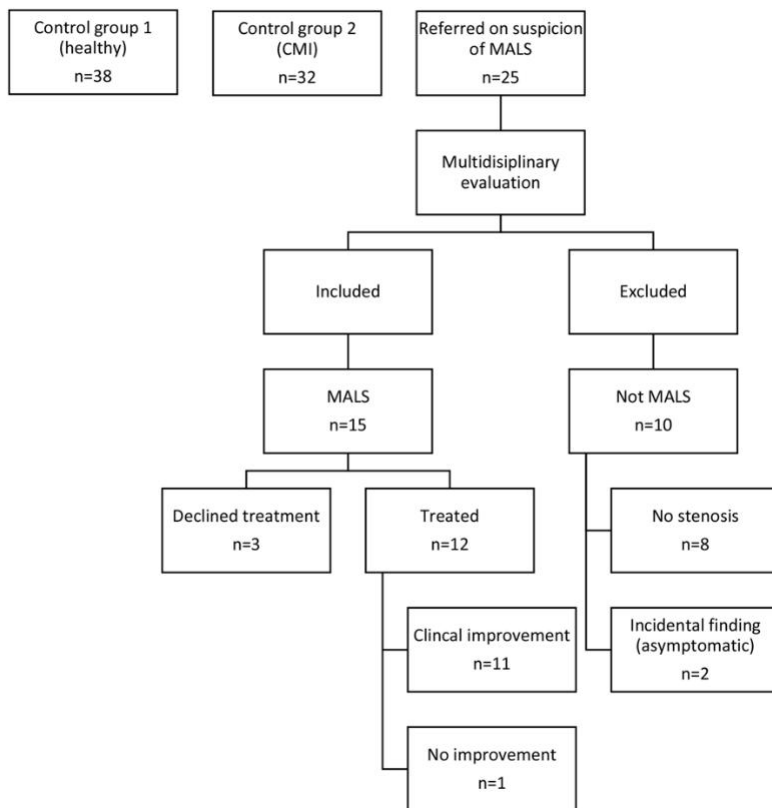
Figure 16. Flow chart of the inclusion process and outcomes in the study of perioperative microcirculatory changes in patients with MALS. Abbreviations: MALS, median arcuate ligament syndrome; CMI, chronic mesenteric ischemia. Source: Berge et al. [7]

4.4 Paper IV

In paper IV we aimed to contribute to the existing knowledge on the controversial disorder of MALS by presenting our 10 years of experience with laparoscopic decompression of CA (Figures 9 and 10). At a single center, 57 consecutive patients were diagnosed with MALS through a detailed medical history, careful clinical examination, and extensive excluding GI examination. The diagnosis was confirmed with CTA stenosis 50% of CA, and TA-DUS showing a PSV 2.0 m/s in CA. After consensus at the multidisciplinary panel, a total of 52 patients underwent CA decompression, 51 laparoscopically, and one open decompression. All patients were prospectively followed up after surgery and followed up with CTA, TA-DUS, and clinical examination at 3, 6, 12 months, and annually thereafter. The study´s primary endpoint was relief from at least one MALS symptom, and the secondary endpoint included morbidity, symptoms recurrence, and reintervention.

The main finding in the study´s primary endpoint showed that 90% of patients with MALS experienced symptom relief after surgical treatment. Within 3-6 months after surgery, 35 patients (67%) had complete symptom relief, and 23 patients (23%) had partial relief. Postoperative Duplex ultrasound also revealed significant ($p < 0.001$) improvement in PSV values compared to the values before treatment (Figure 17). Nevertheless, 5 patients (10%) did not experience any improvement in their preoperative symptoms at 3–6 months follow-up

The main findings in the study´s secondary endpoint were that 5 patients (10%) experienced operative complications, 4 patients required reintervention (3 PTA with stent, and 1 open surgery). Only 2 patients (4%) had symptom recurrence and 2 patients (4%) died of other causes (such as cancer) during the study period.

Conclusion: Our 10 years' experience shows a persistent clinical improvement in most of the MALS patients treated with laparoscopic decompression of CA.
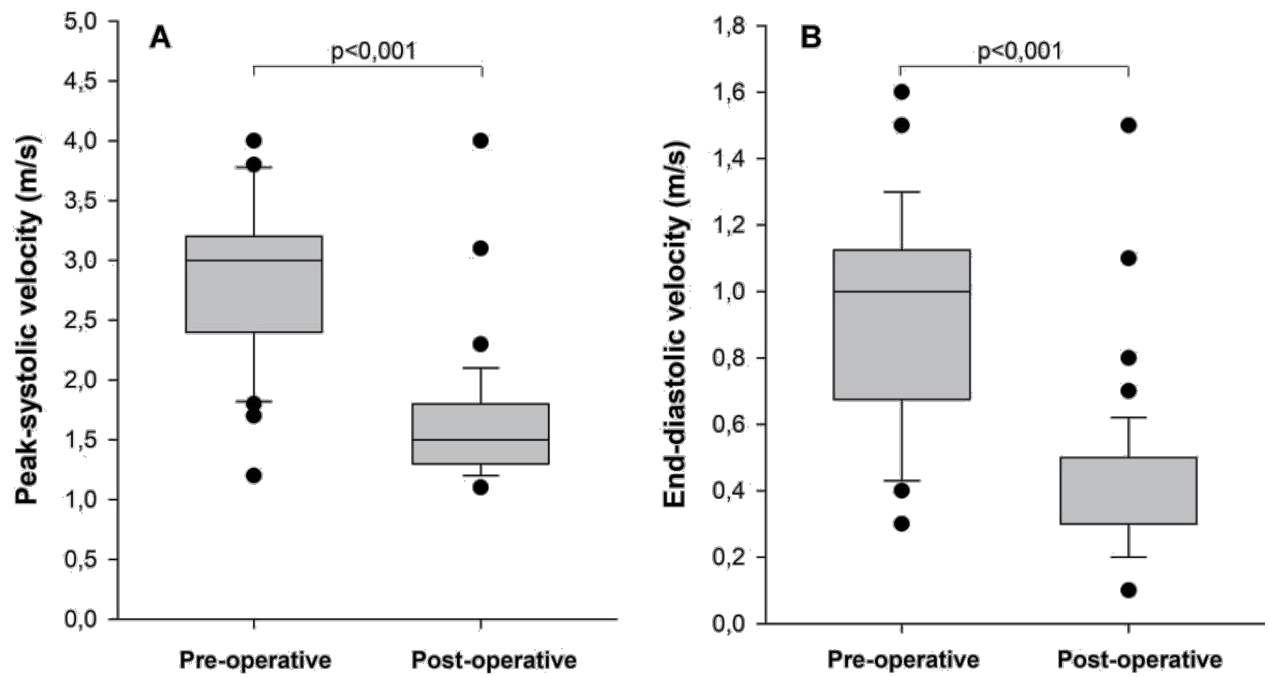
Figure 17. Changes in the transabdominal duplex ultrasound flow velocities before and after the laparoscopic decompression of the CA in 52 patients with median arcuate ligament syndrome. (A) Peak systolic velocity (PSV); (B) end-diastolic velocity (EDV). Boxes represent the interquartile range with the median as a horizontal line compared with the Mann–Whitney U-test. Source: Kazmi et al. [101]

4.5 Paper V

CMI is an underdiagnosed and undertreated condition. It is usually caused by atherosclerosis in the mesenteric vessels and more rarely by MALS. The etiology of MALS has been a topic of great disagreement for years and its existence is still a matter of constant debate. In paper V we aimed to identify potential plasma biomarkers that could indicate mesenteric ischemia in patients with CMI and MALS.

We analyzed four potential biomarkers, including plasma α-glutathione S-transferase (α-GST), intestinal fatty acid-binding protein (I-FABP), citrulline, and ischemia-modified albumin in 58 consecutive patients with CMI (group A, n =44) and MALS (group B, n=14) before and after revascularization, and compared them with healthy individuals (group C, n=16) (Figure 18).

The main finding was that plasma α-GST levels were significantly increased in the patients with CMI (7.8 ng/mL, $p<0.001$) and MALS (8.4 ng/mL, $p<0.001$) compared to control group C (3.3 ng/ml) (Figure 19). The median plasma α-GST threshold of 4 ng/ml as normal levels for the diagnosis of atherosclerotic CMI and MALS showed a sensitivity of 93% and 86%, and a specificity of 86% and 88%, respectively. The AUC for ROC curves was 0.96 ($p<0.0001$) for CMI and 0.85 ($p<0.002$) for MALS (Figure 20). There was no difference in the other biomarkers between the patient groups and the healthy group, C.

Conclusion: Plasma α-GST levels are elevated in both CMI and MALS patients. Elevated plasma levels of α-GST suggest ischemia as an etiology of MALS.
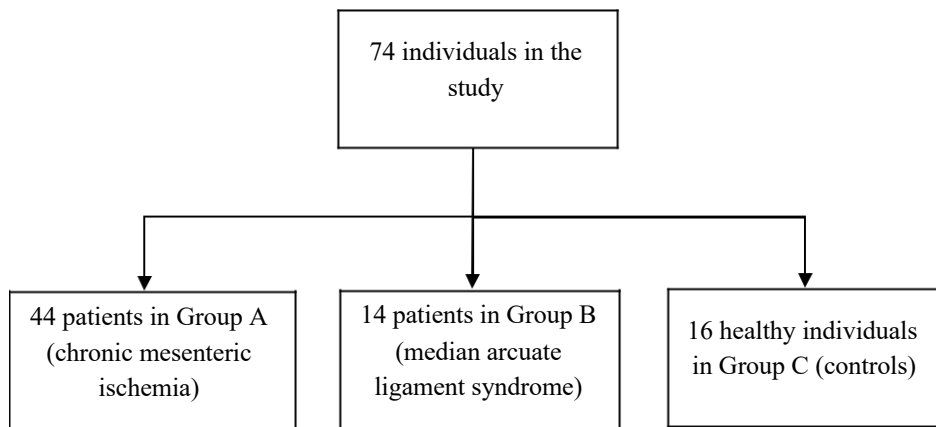
Figure 18. Flow chart of study individuals. Source Kazmi et al. [105]
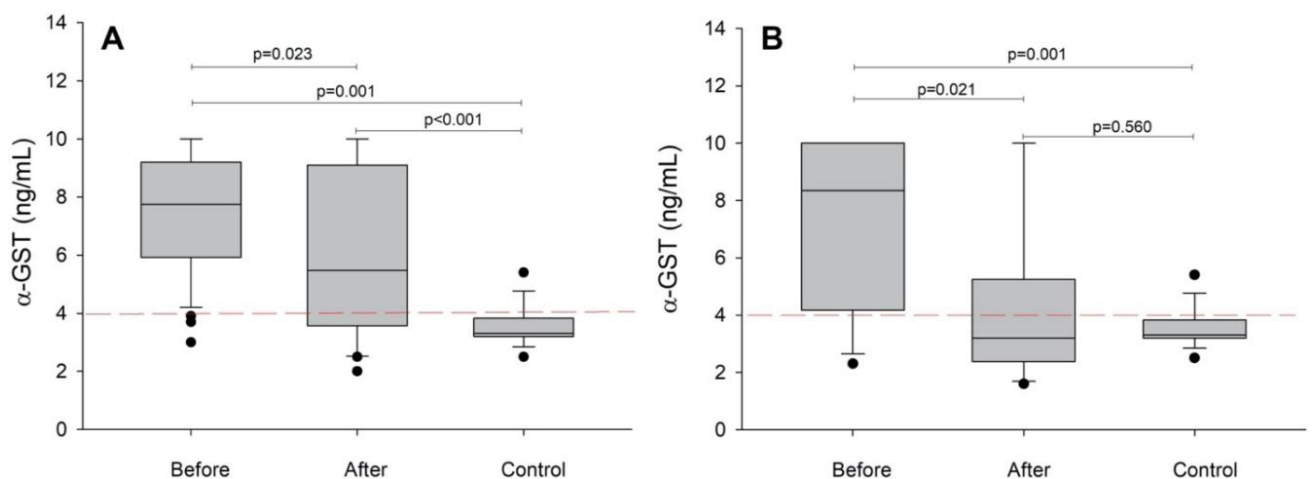
Figure 19. (A) Median plasma α-glutathione S transferase (α-GST) levels in the patients with chronic mesenteric ischemia due to atherosclerosis (Group A, n=30), and (B) median arcuate ligament syndrome (Group B, n=14), before and after the surgical or endovascular intervention, compared with healthy individuals (Group C, n=16).

Boxplot represents the 10th, 25th, 50th, 75th, and 90th percentile, with the black horizontal line representing the median. Filled black dots represent outliers. The dotted horizontal line in red represents the upper limits of normal α-GST plasma level. Mann–Whitney U-test is used to test differences between the groups. Source Kazmi et al. [105]
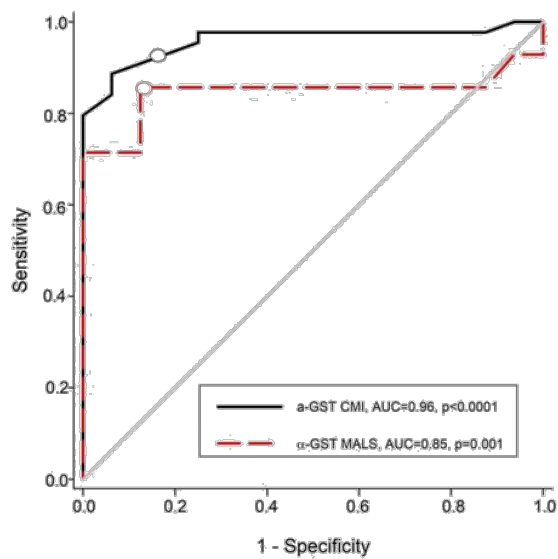
Figure 20 Receiver operating characteristic (ROC) curve and area under the curve (AUC) of median plasma α-GST levels in the patients with chronic mesenteric ischemia (CMI, n=44) due to atherosclerosis and median arcuate ligament syndrome (MALS, n=14). Black (O) and red (O) represent the threshold values for the corresponding sensitivity and specificity. The upper limit of 4 ng/mL was used as a cut-off for α-GST levels. The Grey 45° diagonal line represents random chance (AUC=0.5). Source Kazmi et al. [105]

5. DISCUSSION

5.1 Macro-circulatory diagnostic tools for early detection of atherosclerotic CMI and MALS

In paper I, investigating the microcirculation in patients with CMI (atherosclerotic CMI and MALS) we found that E-DUS is more sensitive than TA-DUS in identifying stenosis of 70% and 50% in CA and SMA, with better NPV in 70% stenosis. A similar finding was presented by Noh et al., although in contrast, a study by Almansa et al. showed higher specificity for E-DUS, our current study revealed that E-DUS had higher sensitivity but lower specificity than TA-DUS, the latter especially for CA (37% vs 72%) [106, 107]. When compared to CA, E-DUS and TA-DUS both demonstrated high specificity for SMA stenosis (70% and 50%).

E-DUS showed increased sensitivity in the total group (CA 9% and SMA 100% in stenosis 70% and CA 78% and SMA 67% in stenosis 50%) and in the treatment-naïve group A (CA 100%, SMA 100% in stenosis 70% and CA 90% and SMA 59%in stenosis 50%). The higher sensitivity of E-DUS suggests that it can effectively identify patients who are suspected of having CMI at an early stage, making it a preferred diagnostic tool. In addition, the higher NPV (CA 83%, SMA 100%) in E-DUS for stenosis 70% indicates that CMI can be ruled out in patients experiencing upper abdominal pain.

Previously treated CMI patients, in fact, have shown a 33% incidence of re-stenosis in their stented mesenteric arteries, and persistently high PSV readings have been reported in those arteries, exceeding 335 cm/s (REF). Although influences the diagnostic accuracy of duplex examination in prior-treated patients, therefore duplex is recommended as a screening test and as a follow-up test to detect stent stenosis or bypass graft failure in patients with CMI [108]. In our study, TA-DUS showed better specificity and lower sensitivity than E-DUS in prior-treated patients.

ROC curve analysis for E-DUS presented better AUC values for both CA and SMA with ≥70% stenosis than TA-DUS (Figure 12). Although, in CA 50% stenosis TA-DUS had better AUC than E-DUS (0.80 vs 0.70), respectively.

High sensitivity but low specificity for E-DUS compared to TA-DUS indicates that E-DUS can detect CMI patients, but the probability of false positive tests might be high. This being said, E-DUS is a great diagnostic tool that should be utilized in patients undergoing endoscopic investigation for upper abdominal pain with a susception of CMI. The probability of this test being true positive might be high but due to lower specificity, the test may be positive in patients with upper abdominal pain not consistent with CMI. This type I error (false positive) can be adjusted by further examination with CTA that anyhow most patients with CMI to verify the diagnosis. A study evaluated the diagnostic role of TA-DUS and CTA in 60 patients with MALS and demonstrated that patients positively screened with TA-DUS had focal narrowing of the AC TA-DUS screening focal narrowing of the CA [109]. EUS of the mesenteric arteries is considered minimally invasive because it does not require any surgical incisions or general anesthesia. It is important to note that even though EUS is generally considered safe, there is a small risk of complications associated with any invasive medical procedure. These risks may include bleeding, infection, or perforation of the digestive tract. Therefore, patients should be made aware of the potential risks associated with the procedure before undergoing it.

From the historical perspective, it is interesting to see that Dr. Moneta 2007 emphasized the fact that TA-DUS was underutilized as a screening test, and strongly suggested that patients with false positive tests should undergo a confirmatory CTA [108]. This should be considered for E-DUS as an initial screening test for patients suspected of CMI.

5.2 Microcirculatory diagnostic tools for the detection of ischemia in the GI tract with

functional test: Visible light spectroscopy and laser Doppler flowmetry.

To our knowledge, Paper II, and Paper III are the first studies to present data on the simultaneous use of LDF & VLS for detecting ischemia by measuring microcirculation of the GI tract during MIE (Paper II) in patients with esophageal cancer and MALS patients undergoing laparoscopic decompression of CA (Paper III). LFD & VLS in both studies provided a real-time microcirculatory status of the GI tract; transserosal in MIE (Paper II), and transmucosal in MALS (Paper III). Measurements of StO2 and (rHb) are achieved with VLS, while blood flow (flux) and velocity are measured with LDF.

Patients with esophagus cancer (Paper II) and patients with MALS (Paper III) utilizing VLS revealed statistically significantly lower StO_2 compared to controls. Friedland et al. in a pilot study, and Van Noord et al in a large cohort study described a similar significant decrease in mucosal saturation in patients with CMI [110, 111]

In Paper III, preoperative measurements from MALS patients (n=15) compared with healthy individuals (Control group 1(CG1) n=38) and with CMI patients (Control group 2(CG2) n=32) showed a significantly lower baseline StO2 (76 6) in MALS patients than healthy individuals (CG1, 81 4, p=0.02), although StO2 in patients with CMI was, however, significantly lower (CG2, 67 9%) than in both healthy individuals (p<0.001) and MALS patients (p=0.004). After laparoscopic decompression that was performed on 12 patients with MALS, their StO2 values normalized, and 92% (11 out of 12) reported either complete or partial symptom improvement, with no change in saturation observed in the remaining patients who did not respond clinically (Figure 21).

MALS patients compared with healthy individuals did not reveal any significant difference in rHb, flow, and velocity. However, after laparoscopic decompression, rHb and flow increased, while velocity showed a slight decline. This contrasts with the findings of Berge et al in our previous combined LDF and VLS validation study, where a significant reduction in all 4 variables (StO_2, rHb, flow, and

velocity) was observed in CMI patients (n=40) compared to a control group (n=38). In that study, StO_2 increased by 73%, and 32 out of 40 CMI patients (97%) experienced symptom relief after either endovascular or open surgery. Atherosclerotic CMI patients due to older age and cardiovascular diseases appear to have more significantly impaired circulation than MALS patients that are younger and otherwise healthier.
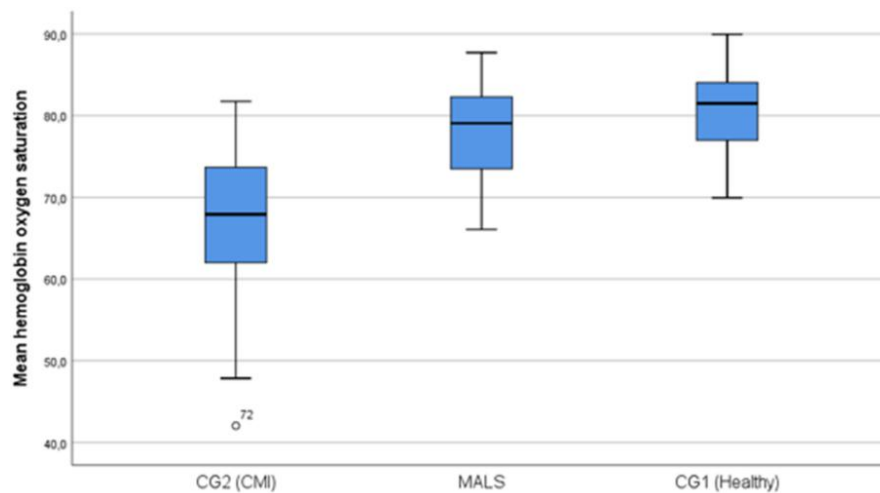
Figure 21. Boxplot of preoperative combined arterial and venous oxygen saturation in CG1 (n=38), CG2 (CMI, n=32), and patients with MALS (n=11). The thick black line represents the median, the blue box represents the 25–75th percentile and the bars are minimum and maximum points (excluding outliers).
Abbreviations: CG1, control group 1; CG2, control group 2; CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome. Source: Berge et al. [7]

In paper II, an ischemic event can occur during MIE for patients with esophagus cancer. The blood supply to the wall of the gastric tube has distinctive anatomical characteristics, notably that the uppermost 20% of the gastric tube receives blood exclusively through a microscopic meshwork of capillaries and arterioles. This makes it highly vulnerable to ischemia when undergoing gastric tube formation and even more so during manipulation, tension, and strangulation during gastric pull-up for the construction of gastroesophageal anastomosis (Figure 13).

All patients undergoing MIE exhibited a decrease in StO_2 at all measuring sites in the gastric tube, as compared to the baseline measurements, however, this reduction was statistically significant at sites M4 (p=0.04), M5, and M7 (p=0.03) (Figure 14). After the construction of TGEA, the StO2 reduction became

more pronounced (p < 0.001) at all measurement sites compared to the baseline measurements. The mean StO2 was reduced by 16% (range 4% - 28%) from baseline to gastric tube formation and by 42% (range, 35% - 52%) after the anastomosis. The concentration of rHb was significantly increased at the most cranial site M7 (p=0.04) after gastric tube formation. Local blood flow (flux) and velocity increased in all patients although, significantly only at site M4 (p=0.009 and p=0.04).

A lower mean StO$_2$ was observed at baseline in the three patients with anastomotic leaks. This reduction increased significantly after the construction of the anastomosis. Additionally, mean rHb presented a 61% increase in patients with leaks compared to 17% without leaks. An increase in tissue blood flow (flux) was found at the anastomotic site, with a larger increase in patients with leaks (39%) than in those without leaks (26%).

The microcirculatory notable changes detected at site M4 suggest that this region may be susceptible to ischemia during MIE due to its location at the intersection of the right and left gastroepiploic arteries. Additionally, the observed significant decrease in StO$_2$ and an increase in rHb at M7, particularly in patients with anastomotic leaks, indicates the presence of microcirculatory ischemic changes in the uppermost 20% of the gastric tube, which is the area most vulnerable to ischemia. These changes were effectively detected using LDF and VLS.

Although both Paper II and Paper III demonstrated a significant decrease in StO2 during ischemia, there were distinct differences in the observed changes in rHb. In patients with MALS, rHb remained unaltered when compared to healthy individuals; however, it increased following revascularization. Conversely, in patients undergoing MIE, rHb showed a significant increase after the ischemic event caused by the formation of gastric tube and subsequent anastomosis, particularly in most hypo perfused site M7. The suggested reason behind this is venous congestion and tissue edema. The sudden traumatic ischemia in patients with esophageal cancer leads to the denudation of endothelial cells in the upper two-thirds of the villi within an hour of ischemia, whereas the crypts remain relatively intact. Robinson et al. observed the destruction of mature enterocytes at the villus tip of a dog while crypts in the intestines remained intact after an hour of ischemia [25]. A recent study by Hoe et al described

histopathological changes during ischemia and reperfusion of the intestinal wall; 1st hour starts in the mucosa with loss of endothelial cells and connective tissue, and after 2nd hour the mucosal glands and the crypts are still intact and continue to secrete sodium, chloride, and water, but the destroyed endothelial cells can no longer reabsorb the fluid, leading to tissue edema. After 4th hour of ischemia and 4 hours of reperfusion, large areas with complete mucosa and submucosal necrosis were observed with edema and dilated vessels[112]. This corroborates the finding of Gerau et al. who discovered, a significant decrease in StO2 in addition to an increase in rHb in the gastric tube of patients with leaks [113]. Additionally, Buise et al. observed that patients developed venous congestion after esophagectomy [114]. Murakami et al. performed microvascular anastomosis on the neck and demonstrated that both arterial circulation and venous congestion were relieved after such a vascular anastomosis [115, 116].

Due to the lack of oxygen in the blood during ischemia, StO2 decreases and rHb increases. Upon reperfusion, excess of oxygenated blood StO2 increases but at the same time, the edema and venous congestion caused by the ischemic episode maintain the continuous increase in the local blood flow and rHb as compensatory physiological responses to ischemia. This venous congestion is further enhanced by the ligation of the veins during MIE, particularly in those with leaks. Additionally, the countercurrent mechanism of shunting blood flow from the mucosa to the serosal layer during an ischemic insult may have contributed to the increase in local blood flow measured with transserosal LDF in our study cohort.

The reason rHb, flow, and velocity were normal in patients with MALS and CMI may be due to its rather chronic progression. The GI tract in patients with MALS has adapted to this chronic but intermittent ischemic state by preserving the integrity of the resting intestine despite reduced blood flow. The altered eating habits are a result of insufficient digestive capacity. In addition, there may be histopathological alterations such as damage and regeneration of endothelial cells (with or without inflammation), villi atrophy, and establishment of a protective preconditioning state that is thought to affect the permeability and transport channels, hence cell damage and local tissue edema [117]. It is interesting to know that the protective effect of ischemic preconditioning is suppressed by

hypercholesterolemia, hyperglycemia, and hypertension, as in the case of CMI patients with atherosclerosis.

Numerous animal studies have investigated histochemical changes resulting from acute intestinal, but little is known about such changes in the case of CMI. Therefore, additional functional and histochemical studies are needed to improve our understanding of the underlying pathomechanisms.

Despite improvements in surgical techniques, the reported incidence of 22% TGEA leaks, and gastric tube necrosis remain life-threatening complications of MIE. A subjective visual and tactile evaluation of the gastric tube for TGEA formation by the surgeon is currently the only available method, which, apparently has low accuracy. Unfortunately, there are no reliable functional tests to manage this condition. The same applies to MALS which has long been considered a controversial disorder, and confirming a diagnosis and selecting appropriate treatment remains challenging to this day. Although several functional tests have been studied, none of them have yet been validated for clinical use.

Both our studies demonstrated the noteworthy usefulness of combined LDF & VLS in identifying ischemic changes within the GI tract. This technique provides dependable real-time information on the microcirculation of the GI tract, enabling surgeons to identify and select the optimal anastomotic site and serving as an early functional test for diagnosing MALS.

5.3 Median arcuate ligament syndrome: an incidental finding or an actual medical concern

In papers III, IV, and V, we demonstrated that MALS is indeed a medical condition most probably with an ischemic etiology that can effectively be treated with laparoscopic decompression of CA.

MALS is a subject of controversy due to several reasons. Firstly, a "hook" or "J" configuration on CTA or ultrasound indicating extrinsic compression of the CA by MAL is a common finding, although only a few develop symptoms [9]. A retrospective study by Hoe et al found CT evidence of external compression of the CA in 87% of asymptomatic patients [118].

Secondly, it was previously believed that mesenteric ischemia symptoms occur only when two out of three mesenteric arteries are affected. Consequently, there is a significant disagreement regarding whether MALS patients with "single artery stenosis" should receive surgical treatment or not.

Thirdly, "the "neurogenic theory" of MALS is another source of controversy, suggesting that compression of the celiac ganglion and neuropathy is the primary etiology and that MALS is not a vascular disease [119].

Fourthly, some studies reported unsatisfactory results in long-term follow-up of patients treated with decompression of CA creating doubt about surgical treatment.

In the literature, the gold standard diagnostic method for CMI is the resolution of symptoms after vascular treatment [6]. In paper IV, laparoscopic decompression of CA resulted in symptom relief for 90% of consecutive MALS patients (47 out of 51). In the postoperative follow-up, 67% of patients experienced complete symptom relief, while 23% experienced partial relief within 3-6 months. The postoperative hemodynamics of CA with duplex ultrasound showed a significant improvement in PVS values ($p<0.001$) and patients showed overall satisfaction with the surgery.

After the introduction of laparoscopic decompression of CA by Roayaie et al., numerous case reports and series have been published, showcasing a wide range of symptom relief following CA decompression. In line with previous studies, our patients experienced similar symptom relief rates. Reilly et al found 70-90% improvement, Roseborough et al. reported a rate of 93.3%, and Jimenez et al. reported a 75% improvement after laparotomy and 90% after laparoscopy [120-122]. However, some studies reported lower rates of symptom relief, such as Cienfuegos et al reported 69 %, Khrucharoen et al reported 83% clinical improvement in their review article, Mann et al 78%, and Chen et al., in a recent study, reported 40% complete relief and 38% partial relief within a year [123-126].

Notably, a recent retrospective review by Charles DeCarlo et al from a multicenter, international database of MALS patients who underwent CA decompression found 58,8% complete and 24,4 partial symptom relief in a median of 1,59 years of follow-up time. The study also found that treatment failure was least associated with laparoscopic surgery, with 3-year freedom from a treatment failure rate of 62.4

for laparoscopy [127]. Our study however showed a decline in operation time and hospital stay, which is consistent with findings from studies conducted in the last decade [69, 121, 125].

Extern compression of CA findings on CT without symptoms is not a syndrome but a phenomenon. In Paper IV, 21 out of 78 patients with extern compression of CA identified on CTA were asymptomatic. MALS treatment should be implemented in patients with the presence of clinical symptoms of MALS and a CTA finding in both respiratory phases. Whereas 13 - 50% of healthy individuals may exhibit isolated compression of CA during expiration, which could be clinically insignificant [9]. Hence, evaluation of true compression can be done during the end-inspiratory phase The other fact is that MALS is a diagnosis of exclusion, other differential diagnoses should have been excluded and the patient discussed it with a multidisciplinary team before surgical treatment.

Ischemia was detected with functional tests conducted in Paper III, as well as in earlier studies by Berge et al. and Menisk et al. [7, 128, 129]. The clinical improvement observed and the postoperative increase in PSV in CA in Paper III and Paper IV after treatment further substantiate the ischemic nature of the intestine in patients with "single artery disease" as in MALS [69]. This fact is reinforced by our findings in Paper V, in which we demonstrated that the ischemic biomarker, α-GST, is significantly higher in patients with MALS compared to healthy individuals and they decreased to normal levels after revascularization.

From the 4 intestinal ischemic biomarkers (α-GST, I-FABP, citrulline, and ischemia-modified albumin) we studied, however, only α-GST showed statistical significance. In a systemic review by Didrikx et al. Alfa-GST and I-FABP showed the best diagnostic accuracy in patients with AMI [97]. On the other hand, Treskes et al in their systemic review of ischemic biomarkers in AMI found ischemia-modified albumin and I-FABP to best performance. Certuline was also a promising marker with high reported specificity. However, it's important to interpret their results with caution due to small and heterogeneous patient population studies.

The level of α-GST in healthy individuals was 3.3 ng/mL. In CMI patients it increased to 7,8 ng/mL while in MALS patients it increased to 8.4 ng/mL. After revascularization, α-GST

returned to normal (Figure 19). By using a cut-off value of 4 ng/ml for normal median plasma α-GST level, we found that the sensitivity and specificity for atherosclerotic CMI and MALS were 93% (95% CI 0.78 1.0) and 88% (95% CI 0.69 1.1), respectively. The AUC was 0.96 (p<0.0001) for CMI and 0.85 (p<0.002) for MALS (Figure 20).

The boxplot diagram in our research illustrates a widespread atherosclerotic CMI after treatment. This may be explained by that atherosclerotic CMI is a multivessel disease and after treatment of one vessel, the patients may still have ischemic tendencies. Other factors such as time for blood sampling or the presence of diseases such as diabetes mellitus may also affect the spread.

Due to the controversy and doubt surrounding the origins and presence of MALS findings in Paper III (GALS in CMI and MALS), Paper IV (Lap. Decompression in MALS), and Paper V (ischemic biomarkers in CMI and MALS) showed evidence of ischemia in patients with MALS with the help of serum biomarkers, functional test and the resolution of symptoms after surgical treatment.

6. CONCLUSION

Mesenteric ischemia is a severe disorder that can manifest as either acute or chronic mesenteric ischemia. Chronic mesenteric ischemia is a gradually developing condition that can progress to the acute phase if left untreated, which can be fatal. Therefore, it is crucial to make an accurate and early diagnosis of CMI and MALS. Different diagnostic tools have been researched to improve early diagnosis, and the combined use of Laser Doppler flowmetry and visible light spectroscopy has proven to be a reliable method to detect ischemic changes in the gastrointestinal tract. This can help surgeons identify the best anastomotic site and act as an early functional diagnostic test for MALS.

In one of our studies, we found elevated levels of plasma α-GST in patients with CMI of atherosclerotic origin as well as in those with MALS. This finding provides evidence that MALS can be categorized as an ischemic disorder. Therefore, measuring plasma α-GST levels can be a valuable diagnostic tool for identifying patients with MALS and CMI of atherosclerotic origin.

Laparoscopic decompression of the celiac artery has shown to be an effective treatment for MALS with a high rate of symptom relief. However, it is essential to consider and discuss potential alternative diagnoses with a team of medical experts before deciding on a course of treatment. In summary, the early detection and proper management of mesenteric ischemia are critical for the successful treatment of patients with MALS.

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Errata List

Doctoral Candidate: Natkai Safi

Title of thesis: Early detection of Micro- and Macro-circulation in the ischemic gastrointestinal tract and the diagnosis and treatment of patients with median arcuate ligament syndrome

Types of error corrected:
Correction of language
Change of page layout or text format

Page	Line	Error	Original Text	Type of correction
Cover page	Last	Layout	UiO and OUS Logo on next page	Both logos moved to the Cover Page
2nd page	Title	Language	Innholdsfortegnelse	Table of content
Page 4	1 and 22	Text format	Increased indent	Increased outdent
Page 6	5 and 12	Text format	Line space	Equal line space
Page 17	19	Layout	Figure title and figure on different pages	Figure title closet to the figure
Page 21	Last	Layout	Figure text is on next page	Figure text to figure
Page 23	Last	Layout	Missing figure	Missing figures insert
Page 25	15	Layout	Title in a different page from the text	Title moved to the same page as text
Page 33	9,10,12,14, 16,18,20, 22,24	Text format	Missing bullet points	Added bullet points
Page 35	1,6,8,9,11, 12, 14, 15	Text format	Section title with missing numbering and bullet point	Added numbering and bullet points
Page 36	10	Text format	Section title with missing numbering	Added numbering
Page 37	1 and 19	Text format	Section title with missing & incorrect sub numbering	Added and corrected numbering
Page 38	1	Text format	Section title with missing numbering	Added numbering and moved closer to text
Page 39	14,16,19, 22	Text format	Missing bullet points	Added bullet points
Page 45	Table 1	Layout	Last row on next page	Whole table om same page
Page 49	Figure 7	Text format	Figure and figure text on different pages	Figure and figure text on same page

Paper 1

ORIGINAL RESEARCH

Early Identification of Chronic Mesenteric Ischemia with Endoscopic Duplex Ultrasound

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Introduction: Due to diagnostic delay, chronic mesenteric ischemia (CMI) is underdiagnosed. We assumed that the patients suspected of CMI of the atherosclerotic origin or median arcuate ligament syndrome (MALS) could be identified earlier with endoscopic duplex ultrasound (E-DUS).

Patients and Methods: Fifty CMI patients with CTA-verified stenosis of either ≥50% and ≥70% of celiac artery (CA) and superior mesenteric artery (SMA) were examined with E-DUS and transabdominal duplex ultrasound (TA-DUS). Peak systolic velocities (PSV) of >200cm/s and >275cm/s for CA and SMA, respectively, were compared with CTA. Subgroup analysis was performed for the patients with (n=21) and without (n=29) prior revascularization treatment of CMI. The diagnostic ability of E-DUS and TA-DUS was tested with crosstabulation analysis. Receiver operating characteristics (ROC) curve analysis was performed, and the area under the curve (AUC) was calculated to investigate the test accuracy.

Results: In the patients with ≥70% stenosis, E-DUS had higher sensitivity than TA-DUS (91% vs 81% for CA and 100% vs 92% for SMA). AUC for SMA ≥70% in E-DUS was 0.75 and with TA-DUS 0.68. The sensitivity of E-DUS for CTA-verified stenosis ≥70% for CA was 100% in the patients without prior treatment. E-DUS demonstrated higher sensitivity than TA-DUS for both arteries with stenosis ≥50% and ≥70% in the treatment-naive patients.

Conclusion: E-DUS is equally valid as TA-DUS for the investigation of CMI patients and should be used as an initial diagnostic tool for patients suspected of CMI.

Keywords: chronic mesenteric ischemia, intestinal ischemia, acute mesenteric ischemia, duplex ultrasound, computed tomography angiography, MALS

Background

Chronic mesenteric ischemia (CMI) is a relatively rare disorder and, if left untreated, can progress to acute mesenteric ischemia (AMI), which is a life-threatening condition with high mortality rates (50–70%).[1] Asymptomatic CMI has 5-year mortality of up to 40%, and it may be even higher (86%) if all three mesenteric arteries are affected.[2] Atherosclerosis of the mesenteric arteries is the most common cause of CMI.[3,4] Another cause of CMI, especially in a relatively younger population, is median arcuate ligament syndrome (MALS).[5]

The typical clinical presentation is abdominal pain with postprandial worsening resulting from persistent intestinal hypoperfusion due to insufficient blood supply during increased metabolic demand after eating.[6] Changes in the eating pattern, ie, avoiding large meal portions, usually lead to undesirable weight loss in these patients. Other complaints may follow, such as diarrhea or constipation, nausea, vomiting, and in severe cases of ischemia, worsening abdominal pain even during exercise and activity.[7] However, these symptoms are poorly related to CMI.[8,9]

To date, no biomarker with sufficient sensitivity or specificity has been identified for routine clinical investigation of CMI.[3,4] Catheter-based angiography as the gold standard of CMI investigation has been replaced by computed

tomography angiography (CTA), which has a sensitivity of 100% and a specificity of 95–100%.[4,10,11] In case of contraindications to CTA, contrast-enhanced magnetic resonance angiography may be an alternative.[4,11,12]

The CMI patients must be followed since the reported incidence of restenosis of the endoprosthesis is as high as 33%, and the mortality after acute occlusion of the stent be 50%.[13–15] The guidelines recommend a transabdominal duplex ultrasound (TA-DUS) as an adjunct to the initial investigation of the patients with CMI as well as for the follow-up.[3,4,16,17] Validation studies in the 1990s compared duplex ultrasound (DUS) flow velocities with digital subtraction angiography (DSA)-verified stenosis of the mesenteric arteries, and a wide range of cut-offs for velocities were reported and used in the different DUS criteria for significant mesenteric artery stenoses. DUS is operator-dependent, and the visualization of the mesenteric arteries can be challenging in some patients.[13,14,18] Furthermore, it has been reported that the patients after revascularization and particularly after stenting of the mesenteric arteries, can still have persistently higher peak systolic velocity (PSV) beyond 335 cm/s despite asymptomatic angiographic stenosis of <20% of the stented superior mesenteric artery (SMA).[19]

Recently, endoscopic ultrasound (E-DUS) has been evaluated for the investigation of CMI.[20] This modality may have a role in the early diagnosis of patients with CMI since the endoscopic examination is frequently performed as an initial investigation procedure in patients with upper abdominal pain.[20] However, the diagnostic potential of E-DUS in patients with CMI has not yet been fully elucidated. In the present study, we investigate CMI patients with both E-DUS and TA-DUS to determine their diagnostic accuracy for celiac artery (CA) and SMA stenosis. We hypothesized that E-DUS is superior to TA-DUS in the early detection of CMI.

Patients and Methods

This study is a single-center study performed at the Department for Vascular Surgery at Oslo University Hospital. From December 2017 until December 2018, patients with postprandial abdominal pain, changes in food intake pattern, weight loss, and CTA-verified stenosis of the mesenteric arteries were prospectively included in the study. The patients were investigated with both TA-DUS and E-DUS. They were divided into Group A (treatment-naive; n = 29) and Group B (prior treatment, but with relapse or residual symptoms; n = 21). Table 1 illustrates the patients' characteristics and the

Table 1 Baseline Characteristics and Comorbidities in Fifty Patients with Chronic Mesenteric Ischemia, Caused by Either Atherosclerosis or Median Arcuate Ligament Syndrome

Variables	n=50
Median age, years (IQR)	73 (58)
Gender (male: female)	24:26
Comorbidity	
Ischemic heart disease	23 (46%)
Atrialr fibi llat ion	7 (14%)
Stroke	10 (20%)
Hypertension	24 (48%)
COPD	15 (30%)
Diabetes mellitus	8 (16%)
Smoking	40 (80%)
Median body mass index (IQR)	20 (23)
Hyperlipidemia	31 (62%)
Postprandial pain	50 (100%)
Gastroscopy prior to DUSexaminations	48 (96%)
Median duration of symptoms before DUS examinations (years, IQR)	3.4 (2)
Median arcuate ligament syndrome	14 (28%)
Atherosclerosis of mesenteric arteries	36 (72%)

Abbreviations: COPD, chronic obstructive pulmonary disease; IQR, interquartile range; DUS, duplex ultrasound.

clinical presentation. The patients in Group B were previously treated for atherosclerotic changes in the mesenteric arteries in 16 cases and MALS in 5 patients. Despite prior endovascular or surgical treatment, the patients still had a symptom or had a relapse of symptoms of CMI. The investigations were performed 1–6 months after the revascularization procedures.

The patients with MALS had a ≥50% stenosis of the CA on CTA. The CTA was taken in the deep expiration phase. The patients with atherosclerosis had CTA-verified stenosis or occlusion in either one or both, CA and SMA. CTA changes of IMA were also registered. Multi-sliced CTA (64 row-multidetector, Siemens Medical Systems; Forchheim, Germany) of the abdominal aorta and the mesenteric arteries was performed, and the scans were examined in multiple plans. A lumen diameter reduction of ≥50% in the mesenteric arteries was considered a positive test. Grading of the stenosis in each artery was done with the following formula: % stenosis = (1 -[narrowest lumen diameter/diameter normal distal artery]) x 100.[21]

Transabdominal Ultrasound

TA-DUS was performed with a GE Vivid E95 ultrasound scanner and a GE C1-6 curve array probe (GE Healthcare, Chicago, IL, USA) by the same experienced operator (JH). Conventional B-mode and color Doppler were performed to evaluate the vascular status, identifying stenosis and post-stenotic turbulence. Pulsed Doppler was used to measure peak systolic velocity (PSV) and end-diastolic velocity (EDV) of the mesenteric arteries in the inspiratory and expiratory phases. Harmonic imaging was utilized to minimize artifacts. Every effort was made to keep the insonation angle <60°. The patients were in the overnight fasting state, and the procedure was performed in the morning.

Endoscopic Ultrasound

E-DUS combines endoscopy and duplex ultrasound to obtain detailed images beyond the innermost lining of the digestive tract. The procedure was performed with a Hitachi Aloka ProSound F75 and an Olympus GF-UCT180 curved linear array ultrasonic video scope (180° ultrasound field of view). All E-DUS examinations were performed by the same experienced endoscopist (KÅ) at the Endoscopy Laboratory of the Department of Gastroenterology, Oslo University Hospital. Standards for the E-DUS procedure were followed.[22,23] All patients were in at least 6 hours of fasting state before the examination. All procedures were performed under conscious sedation with midazolam (mean 3.35 mg) and alfentanil (mean 0.77 μg). SaO_2 was kept above 95% during the procedure. The patients were carefully monitored for any hemodynamic changes. The video scope was placed in the upper part of the stomach along the lesser curvature and a longitudinal view of the aorta was obtained to identify the origin of the CA and SMA. None of the patients developed complications related to the endoscopy.

Definitions and Measurements

Our main aim was to investigate the two ultrasound modalities' ability to identify the patients with CTA-verified≥50% stenosis and ≥70% stenosis of both CA and SMA in the study population. In addition, we aimed to determine if the diagnostic ability of either of the duplex ultrasound modalities was better in the patients in Group B than in Group A.

We used PSV criteria for CA ≥200 cm/s and SMA ≥275 cm/s as definitions of significant stenoses and compare these velocities with the CTA findings of ≥50% and ≥70% stenosis.[16,24,25]

We compared EDV ≥55 cm/s in CA and ≥45 cm/s in SMA corresponding to CTA verified ≥50% stenoses.[25] Occluded arteries identified with DUS were considered among the patients with ≥70% stenosis.

The duplex ultrasound operators of TA-DUS and E-DUS were blinded to the CTA findings and each other's DUS findings. Only TA-DUS was performed during the follow-ups. All enrolled patients were followed-up at 3, 6, 12 months, and yearly after that.

Statistical Analysis

Continuous data are presented with median and interquartile range (IQR) and categorical data with numbers and percentages. Cross-tabulation was performed for the calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA) of E-DUS and TA-DUS. PSV velocities of ≥200 cm/s for

CA and ≥275 cm/s for SMA were used. Flow velocities were compared with CTA-verified stenosis ≥50% and ≥70% separately. Receiver operating characteristic curves (ROC) analysis was performed, and the area under the curve (AUC) was estimated. AUC was interpreted as 0.50–0.60, fail; 0.60–0.70, poor; 0.70–0.80, fair; 0.80–0.90, good; 0.90–1.0, excellent.[26] Data analysis was performed with IBM SPSS Statistics Version 27 (IBM Corp., USA).

Ethical Statement

The database for patients with chronic mesenteric ischemia was approved in 2016 by the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (REK Sør-Øst B 2016/682). It is also registered in ClinicalTrails.org Protocol Registration and Results System (NCT02914912). The study was conducted per the Declaration of Helsinki. All patients gave informed, written consent prior to the study commencement.

Results

A total of 50 patients were included in the study period. The median age of included patients was 71 years (IQR 58), and 26 (52%) were females (Table 1). The median duration of symptoms was 3.4 years (IQR 2). All patients in the study were investigated with E-DUS; however, one of these patients died before TA-DUS, and in another three (6%), an acoustic window for reliable measurements of flow velocities could not be obtained. Figure 1 illustrates the patient's flow.

CTA Findings

Based on CTA and clinical findings, 23 (46%) patients had CTA-verified atherosclerotic stenosis in all three mesenteric vessels. Fourteen (28%) patients had MALS with single artery stenosis of the CA. The remaining thirteen (26%) patients had atherosclerosis of CA and SMA. In total, 36 patients (66%) had CMI due to atherosclerosis in CA and SMA, and among these three patients (6%) also had MALS. Five patients (10%) had a total occlusion of CA, and SMA was occluded in eight patients (16%)e.

In five patients with prior laparoscopic decompression operation for MALS, CTA demonstrated between 50% and 70% stenoses. In three patients treated with the stent of the CA for atherosclerotic stenosis, two had ≥70% stenosis on CTA, and one had <50% stenosis. Nine patients had a stent in SMA, two had on CTA ≥70% stenoses, six patients had stenosis between 50% and 70%, and one patient had <50% stenosis. Two patients had stents in both mesenteric arteries, one of them had stenosis between 50% and 70% in both arteries, and the other had stenosis between 50% and 70% only in the CA, but ≥70% SMA stenosis on the CTA. In one patient with an aortomesenteric bypass to SMA, CTA
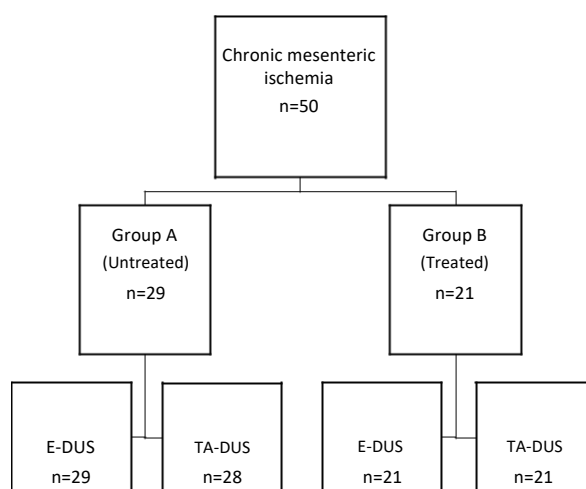
Figure 1 Patient flow in 50 patients with chronic mesenteric ischemia (CMI) was investigated with transabdominal duplex ultrasound (TA-DUS), endoscopic duplex ultrasound (E-DUS), and computed tomography angiography (CTA).

Table 2 Results of the Validity Assessment of Duplex Ultrasound Determined Peak Systolic Velocities of ≥200 cm/s for Celiac Artery and ≥275 cm/s for the Superior Mesenteric Artery (SMA) for the Detection of ≥50%and ≥70%Computed Tomography Angiography-Verified Stenosis

		CTA ≥70%		CTA ≥50%	
		CA	SMA	CA	SMA
		PSV	PSV	PSV	PSV
		≥200 cm/s	≥275 cm/s	≥200 cm/s	≥ 275 cm/s
Sensitivity	E-DUS	91%	100%	78%	68%
	TA-DUS	81%	92%	57%	58%
Specificity	E-DUS	37%	75%	30%	91%
	TA-DUS	72%	88%	67%	95%
PPV	E-DUS	55%	62%	82%	90%
	TA-DUS	71%	75%	86%	93%
NPV	E-DUS	83%	100%	25%	69%
	TA-DUS	81%	97%	27%	65%
OA	E-DUS	62%	84%	68%	78%
	TA-DUS	76%	89%	57%	74%

Abbreviations: E-DUS, endoscopic duplex ultrasound; TA-DUS, transabdominal duplex ultrasound; PPV, positive predictive value; NPV, negative predictive value; CTA, computed tomography angiography; CA, celiac artery; SMA, superior mesenteric artery; PSV, peak systolic velocity; OA, overall accuracy.

demonstrated occluded SMA and ≥70% stenosis of CA. The patient had previously unsuccessful treatment with a stent in SMA and CA before the bypass operation. In another patient with a bypass to the splenic artery, CTA demonstrated a stenosis grade of ≥70% in the gastrosplenic trunk and <50% in the SMA.

DUS Findings

The sensitivity of E-DUS for the identification of ≥70% CTA-verified stenosis in the whole group (n=50) was higher than for TA-DUS, 91%, 95% CI 0.91 0.91 and likelihood ratio positive (LR+) 1.5 vs 81%, 95% CI 0.81 0.81 and LR+ 2.9) for CA. The sensitivity of E-DUS was 100%, 95% CI 1 1, LR+ 4.6 vs 92%, 95% CI 0.92 0.92 and LH+ 7.8 in SMA for TA-DUS.

For ≥50% stenosis of CA, E-DUS showed a sensitivity of 78%, specificity of 30% and LH+ 1.1. Sensitivity for TA-DUS was 58%, specificity of 67% and LH+ 1.7. Whereas, for ≥50% stenosis of SMA E-DUS had a sensitivity of 68%, specificity of 91% and LH+ 7.5. TA-DUS had a sensitivity of 57%, specificity of 95% and LR+ 12. The results of cross-tabulation for the whole study population are summarized in the Table 2.

E-DUS had a better NPV than TA-DUS in patients with ≥70% stenosis for CA (83% versus 81%) and SMA (100% versus 93%) (Table 2).

In Group A, the sensitivity of E-DUS and TA-DUS for diagnosing ≥70% stenosis of both arteries was similar (Table 3). Both ultrasound modalities had an NPV of 100% for SMA stenosis of ≥70%.

For a ≥50% stenosis of CA, the sensitivity of E-DUS and TA-DUS was 90% and 80%, respectively. However, both modalities had similar and low specificity, NPV, and PPV values. For ≥50% of SMA, the PPV of E-DUS and TA-DUS was 100% and 90%, respectively (Table 3).

In Group B, E-DUS had higher sensitivity than TA-DUS for both stenosis grades in both mesenteric arteries (Table 3). Also, the NPV of E-DUS was higher than for TA-DUS, particularly for a ≥70% stenosis of SMA. NPV for both modalities were low in the patients with ≥50% stenosis of CA (Table 3).

Selected results from the present study and similar studies are summarized in Table 4. EDV did not show as high sensitivity and specificity as the PSV for identifying neither ≥50% nor ≥70% of the CA or SMA in both duplex ultrasound modalities.

Table 3 Results of the Validity Assessment of Peak Systolic Velocities Measured with Duplex Ultrasound (for the Detection of ≥50% and ≥70% CTA-Verified Stenosis in All Patients (n=50), Treatment-Naive Patients (Group A; n=29) and Patients After Treatment (Group B; n=21). PSV Cut-Offs: ≥200 cm/s for Celiac Artery and ≥275 cm/s for the Superior Mesenteric Artery (SMA)

		CTA ≥70%				CTA ≥50%			
		CA		SMA		CA		SMA	
		E-DUS	TA-DUS	E-DUS	TA-DUS	E-DUS	TA-DUS	E-DUS	TA-DUS
Sensitivity	Total	91%	81%	100%	92%	78%	57%	67%	56%
	Group A	100%	82%	100%	100%	90%	80%	59%	64%
	Group B	82%	80%	100%	83%	73%	64%	83%	50%
Specificty	Total	37%	72%	78%	88%	30%	67%	90%	95%
	Group A	30%	60%	86%	84%	33%	45%	100%	92%
	Group B	50%	90%	67%	93%	50%	100%	89%	100%
PPV	Total	55%	71%	62%	75%	82%	86%	90%	93%
	Group A	50%	60%	70%	70%	75%	67%	100%	90%
	Group B	64%	89%	55%	83%	79%	100%	90%	100%
NPV	Total	83%	81%	100%	97%	25%	27%	69%	65%
	Group A	100%	82%	100%	100%	60%	63%	63%	69%
	Group B	72%	82%	100%	93%	43%	55%	80%	60%
OA	Total	62%	76%	84%	89%	68%	57%	78%	74%
	Group A	59%	78%	90%	88%	72%	65%	76%	77%
	Group B	67%	85%	76%	90%	67%	75%	86%	72%

Abbreviations: E-DUS, endoscopic duplex ultrasound; TA-DUS, transabdominal duplex ultrasound; CTA, computed tomography angiography; CA, celiac artery; SMA, superior mesenteric artery; PPV, positive predictive value; NPV, negative predictive value; OA, overall accuracy.

ROC Curve Analysis

ROC curve analysis estimated an AUC of 0.75 (p = 0.001, CI 95% 0.61 0.88) for E-DUS for a ≥70% stenosis of the SMA, and 0.68 (p =0.03, CI 95% 0.52 0.83) for TA-DUS (Figure 2A). For the PSV criterium of ≥275 cm/s, the sensitivity was 0.70 and the false-positive rate (1-specificity) was 0.25 for E-DUS. TA-DUS had a sensitivity of 44% and a false-positive rate of 0.23.

ROC curve analysis of E-DUS and TA-DUS for ≥70% stenosis of the CA had an AUC of 0.79 (p<0.0001, 95% CI 0.66 0.91) and 0.75 (p= 0.001, 95% CI 0.60 0.90), respectively (Figure 2B). For the PSV criterium of ≥200 cm/s with E-DUS, the sensitivity was 0.92, and the false-positive rate was 0.64. In TA-DUS, the test sensitivity was 0.72, and the false-positive rate was 0.24.

For a ≥50% stenosis of SMA, with a PSV of ≥275 cm/s, ROC curve analysis of E-DUS and TA-DUS demonstrated an AUC of 0.79 (p<0.0001, 95% CI 0.66 0.92) and 0.68 (p <0.03, 95% CI 0.52 0.83), respectively (Figure 2C). For a PSV of 275 cm/s, E-DUS had a sensitivity of 0.65 and a false-positive rate of 0.21. In TA-DUS, the AUC sensitivity was 0.44, and the false-positive rate was 0.26.

For CTA-verified ≥50% stenosis of CA and a PSV criterium of ≥200 cm/s, ROC curve analysis of E-DUS and TA-DUS showed an AUC of 0.70 (p=0.007, 95% CI 0.56 0.87) for the former, and 0.80 (p<0.0001, 95% CI 0.64 0.97) for the latter DUS modality (Figure 2D). For E-DUS, the sensitivity was 0.80 with a false-positive rate of 0.56. In TA-DUS, AUC sensitivity was 0.52 with a false-positive rate of 0.22.

Discussion

The present study demonstrates a higher sensitivity for E-DUS than TA-DUS for identifying both ≥50% and ≥70% stenosis in CA and SMA. In addition, the NPV of E-DUS was better than TA-DUS in patients with ≥70% stenosis for both CA and SMA.

Similarly, in a previous study by Noh et al, a higher E-DUS sensitivity was found for stenoses in CA or SMA than TA-DUS.[27] In a study by Almansa et al (2011), E-DUS had a sensitivity of 63% but a high specificity of 84% (Table 4).[20] In contrast to Almansa et al, our study showed a lower specificity for E-DUS than TA-DUS, particularly for CA (37% vs

Table 4 Published Results of the Validation Studies for the Peak Systolic Velocities (PSVs), End Diastolic Velocities(EDVs) and Digital Subtraction Angiography (DSA)d Verified Stenosis of the Celiac Artery (CA) and the Superior Mesenteric Artery (SMA). The Results of the Validation of the Present Study in Fifty Chronic Mesenteric Ischemia Patients with CTA Verified Stenosis of CA and SMA

| | | PSV | | | | EDV | | | |
| | | CA | | SMA | | CA | | SMA | |
		>70%	>50%	>70%	>50%	>70%	>50%	>70%	>50%
Moneta 1993		>200 cm/s		>275 cm/s					
TA-DUS	Sens	87%		92%					
	Spec	80%		96%					
	OA	82%		96%					
AbuRahma 2012		>320 cm/s	>240 cm/s	>400 cm/s	>295 cm/s	100 cm/s	40 cm/s	70 cm/s	45 cm/s
TA-DUS	Sens	80%	87%	72%	87%	58%	84%	65%	79%
	Spec	89%	83%	93%	89%	91%	48%	95%	79%
Van Pettersen 2013	*	> 280 cm/s	> 268 cm/s	> 268 cm/s	> 220 cm/s	>57 cm/s	> 64 cm/s	> 101 cm/s	> 62 cm/s
TA-DUS	Sens	66%	66%	75%	84%	83%	78%	74%	75%
	Spec	77%	80%	86%	76%	56%	65%	96%	94%
	§	> 272 cm/s	> 243 cm/s	> 205 cm/s	> 277 cm/s	>84 cm/s	> 83 cm/s	> 52 cm/s	> 52 cm/s
	Sens	72%	68%	78%	68%	66%	53%	78%	76%
	Spec	77%	71%	84%	93%	81%	81%	93%	93%
Almansa 2011		> 200 cm/s		> 275 cm/s					
TA-DUS	Sens	80%		80%					
	Spec	78%		78%					
	NPV	97%		97%					
E-DUS	Sens	63%		63%					
	Spec	84%		84%					
	NPV	94%		94%					
Present study 2021		> 200 cm/s	> 200cm/s	> 275 cm/s	> 275 cm/s	> 55 cm/s	> 55cm/s	> 45 cm/s	> 45cm/s
TA-DUS	Sens	81%	57%	92%	56%	65%	46%	62%	42%
	Spec	72%	67%	88%	95%	73%	67%	85%	90%
	NPV	81%	27%	97%	65%	73%	23%	85%	56%
	OA	76%	57%	89%	74%	70%	50%	79%	64%
E-DUS	Sens	91%	78%	100%	67%	55%	68%	85%	71%
	Spec	37%	30%	78%	90%	65%	70%	51%	59%
	NPV	83%	25%	100%	69%	56%	35%	90%	62%
	OA	62%	68%	84%	78%	60%	68%	60%	66%

Note: *Flow velocities during expiration; §Flow velocities during inspiration.

abbreviations: E-DUS, endoscopic duplex ultrasound; TA-DUS, transabdominal duplex ultrasound; Sens, sensitivity; i Spec, specificity; NPV, negative predictive value; OA, overall accuracy.
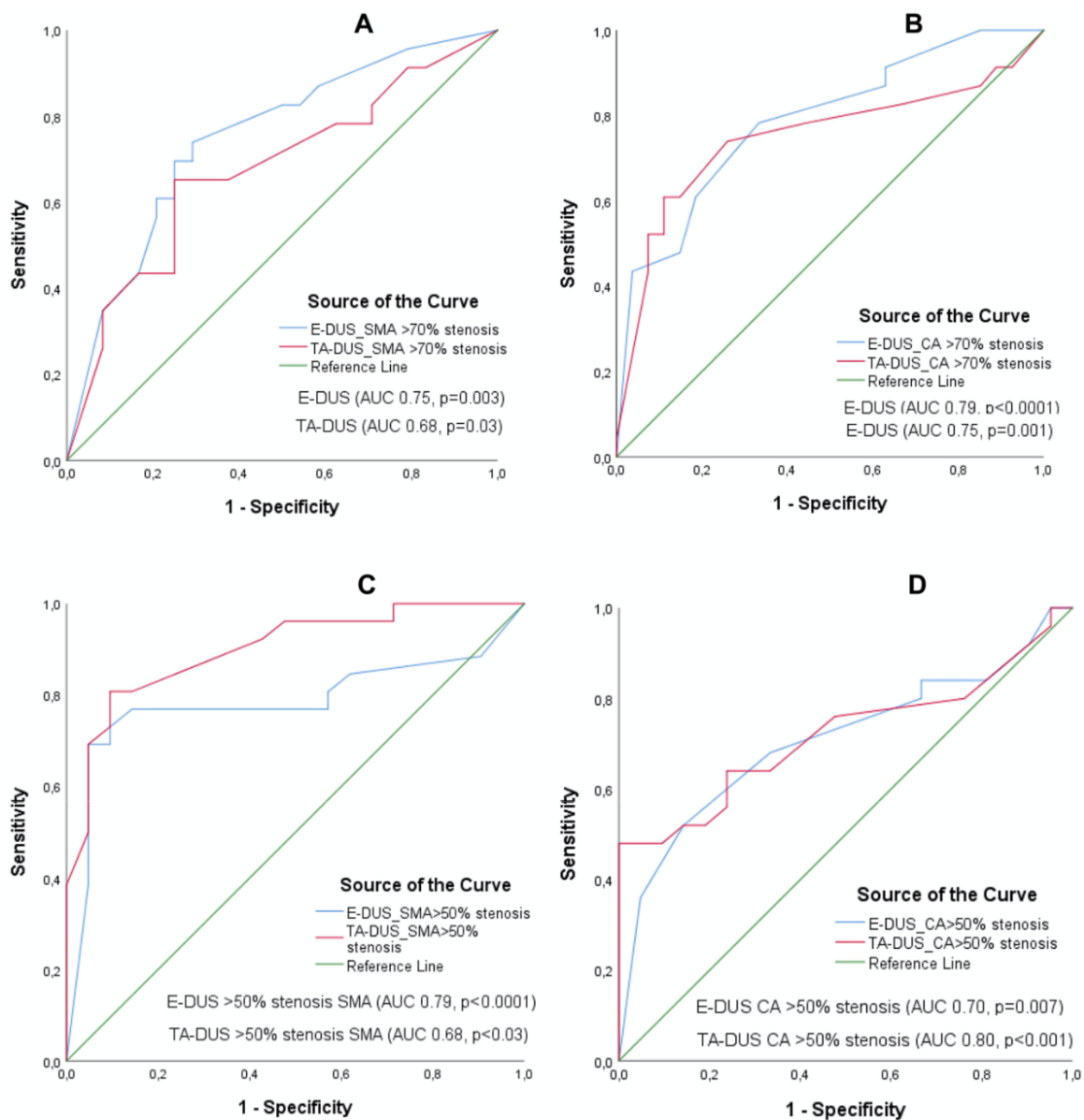
Figure 2 (**A** - **D**) ROC curve analysis of the ability of endoscopic duplex ultrasound (E-DUS) and transabdominal duplex ultrasound (TA-DUS) peak systolic velocities of ≥200 cm/s for celiac artery (CA) and ≥275 cm/s for superior mesenteric artery (SMA) to detect computed tomographic angiogram (CTA)-verified stenosis of ≥50%and ≥70%int fifty patients with chronic mesenteric ischemia. (**A**) Sensitivity and false-positive rate (1-specificity) in ≥70%stenosis in SMA; (**B**) ≥70%stenosis in CA; (**C**) ≥50% stenosis in SMA; (**D**) ≥50%stenosis in CA.

72%) (Table 4). However, for CTA-verified stenosis ≥50% for SMA, our study demonstrated excellent specificity in E-DUS and TA-DUS (90% vs 95%).

In the treatment-naïve patients (Group A) with CMI, E-DUS had excellent sensitivity (100%) and NPV (100%) for both arteries with CTA-verified stenosis ≥70%, irrespective of etiology, atherosclerosis, or MALS. Therefore, a negative E-DUS can probably exclude CMI in these patients. These findings suggest that E-DUS is an excellent initial diagnostic test for the diagnosis of CMI in patients undergoing endoscopic investigation for upper abdominal pain. Similarly, in

patients with CTA-verified≥50% stenosis of the CA and SMA, the sensitivity and PPV of E-DUS are better than TA-DUS.

In the patients with prior treatment (Group B), the sensitivity of E-DUS was better than for TA-DUS in both arteries and with both CTA-verified stenosis grades of ≥50% and ≥70%. However, the specificity was insufficient (50%) for diagnosing CA stenosis of either ≥50% or ≥70%. Although E-DUS has adequate sensitivity to diagnose the mesenteric artery stenosis in patients who underwent a prior treatment for CMI (Group B), the modality lacks specificity to exclude the healthy patients. This lack of specificity is due to type-I error and has also been observed in CMI patients after TA-DUS investigation.[13,19] However, E-DUS has a better NPV than TA-DUS exclusively in patients treated for stenosis or occlusion in the SMA.

Most CMI patients undergo gastroscopy in the initial work-up of chronic upper abdominal pain. Therefore, it may be relevant to investigate mesenteric artery stenosis with E-DUS during endoscopy in patients with CMI suspicion. Additionally, E-DUS has been presented as superior to TA-DUS in detecting other diseases of the persistent upper abdominal pain.[28,29]

AUC of 0.79 for E-DUS was acceptable for identifying CTA-verified ≥50% stenosis of SMA. However, in the case of TA-DUS, a lower criterion for PSV could have provided better sensitivity without increasing the false-positive rate (eg., PSV 175 cm/s, the sensitivity of 73%, and false-positive rate of 22%). E-DUS, PSV ≥200 cm/s, had the best combination of sensitivity and false-positive rate. In CA stenosis ≥50%, both E-DUS, and TA-DUS had acceptable AUC (0.7 and 0.8, respectively), but with low specificity and high false-positive rates, particularly in E-DUS (60%).

The limitation of our study is the small size of the study population. However, compared to previously published studies on the use of E-DUS, our study population only includes patients with CTA-verified CMI. Nevertheless, the results of our study should be verified in a larger cohort of patients with CMI. In future studies, a more comprehensive range of PSVs should be investigated for their potential to correctly identifying the stenosis grades in the CA and SMA. Since CMI is an uncommon disorder, symptom debut to its diagnosis is prolonged; E-DUS can identify patients with CMI at the time of the initial endoscopic investigation.[2] This method can also be used to perform the transmucosal microcirculation assessment with laser Doppler flowmetry and visible light spectrometry, which may increase the probability of a correct diagnosis of CMI.[5] Due to the limitations in the specificity of E-DUS, the patients should be further investigated with CTA for the confirmation of the CMI diagnosis.

Conclusion

E-DUS is as valid as TA-DUS for the investigation of patients with CMI. It might be of more value than TA-DUS as an initial diagnostic tool for its potential to identify CMI earlier and prevent patient suffering and even mortality.

Data Sharing Statement

Request for identified data sharing can be directed to the prime investigator of the study, Kazmi, SSH MD, Ph.D., syekaz@ous-hf.no.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this study.

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Paper 2

Laser Doppler Flowmetry and Visible Light Spectroscopy of the Gastric Tube During Minimally Invasive Esophagectomy

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Introduction: Ischemia is considered as the main reason for thoracic gastroesophageal anastomotic leaks after esophagectomy. Microcirculatory monitoring with laser Doppler flowmetry and visible light spectroscopy may provide valuable intraoperative real-time information about the gastric tube's tissue perfusion and circulation.

Patients and Methods: Ten patients with esophageal cancer operated with minimally invasive esophagectomy participated in this single-center, prospective, observational pilot study. A single probe with laser Doppler flowmetry and visible light spectroscopy was used to perform transserosal microcirculation assessment of the gastric tube at predefined anatomical sites during different operation phases. Group comparison and changes were evaluated using the paired sample t-test.

Results: A reduction in StO2 was found at all measuring sites after the gastric tube formation compared with the baseline measurements. The mean StO2 reduction from baseline to gastric tube formation and after anastomosis was 16% (range 4%–28%) and 42% (range, 35%–52%), respectively. A statistically significant increase in the rHb concentration, representing venous congestion, was detected at the most cranial part of the gastric tube ($P = 0.04$). Three patients developed anastomotic leaks.

Conclusion: Intraoperative real-time laser Doppler flowmetry and visible light spectroscopy are feasible and may provide insight to microcirculatory changes in the gastric tube and at the anastomotic site. Patients with anastomotic leaks seem to have critical local tissue StO2 reduction and venous congestion that should be further evaluated in studies with larger sample sizes.

Keywords: esophagectomy, gastric tube circulation, gastroesophageal anastomosis complications

Introduction

Anastomotic leak is a severe complication after thoracic gastroesophageal anastomosis (TGEA) and is typically reported at incidences of 10–20%.[1-3] Although multifactorial, ischemia at the anastomotic site plays a central role in TGEA leaks.[4] The cranial 20% of the gastric tube is vascularized by an intramural vascular network and may be prone to the development of ischemia by manipulation, tension, and strangulation during gastric pull-up and anastomosis construction.[3,5] The ischemic changes may not be visually apparent during the surgical procedure, and constructing the anastomosis at a hypoperfused site may result in an anastomotic leak. Intraoperative information about the gastric tube's microcirculatory status may guide the surgeon to define the

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anastomotic site with the best possible microcirculation. Despite the introduction of numerous intraoperative bowel viability assessment techniques, only a few techniques are feasible for intraoperative assessment of the gastric tube during esophagectomy.[6,7] In addition to laser Doppler flowmetry (LDF), which is one of the methods most extensively investigated, visible light spectroscopy (VLS) has emerged as a promising method for the detection of the microcirculatory changes in the gastrointestinal tract.[4,8–10]

The main aim of our pilot study was to evaluate the feasibility of combined use of LDF and VLS for transserosal microcirculation assessment of the stomach and the gastric tube during different phases of minimally invasive esophagectomy.

Patients and Methods

During seven months from July 2018 to January 2019, patients with esophageal cancer, scheduled for minimally invasive esophagectomy at Oslo University Hospital, Ullevål, were considered for this pilot study enrollment, at the discretion of the attending surgeon and from the availability of the principal investigator at the time of surgery. The institution is a regional center for esophageal cancer's surgical treatment, with an annual volume of about 50 patients operated. Inclusion criteria for study enrollment included patients with the capacity to give informed consent and a potentially curable distal esophageal cancer. All patients received neoadjuvant radiochemotherapy with 41.1 Gy and Carboplatin and paclitaxel according to the CROSS radio-chemotherapy regime.[11]

During surgery, a 2.6 mm microprobe with combined LDF and VLS modalities (O2C; LEA Medizintechnik, Germany), was channeled through a laparoscopic trocar to quantify transserosal, blood flow, velocity, mixed arterial and venous saturation of hemoglobin (StO2), and the amount of hemoglobin per tissue volume (rHb).

The O2C transmits continuous wave laser light (500–630 nm) and white light (830 nm) through an optical fiber to the tissue, where it is scattered and collected with fibers of the probe placed on the tissue surface.[12] The white light tends to penetrate deeper into the tissue than the laser light due to its shorter wavelength.[13] In VLS, the principle of absorbance and scattering of white light in biological tissues, gives a marked difference in absorption spectra of oxygenated and deoxygenated hemoglobin thereby, directly measures hemoglobin saturation and concentration.[8,13,14]

For LDF measurements, reflected laser light from the moving red blood cells in the tissue generates a Doppler shift. The frequency of this reflected light is dependent upon the velocity of the cells (erythrocytes) and is detected by a photodetector within the instrument and transformed into an electrical signal. The LDF produces a value referred to as a flow (red blood cell flux) expressed as mL/min/100-gram tissue.

Measurements were repeated in case of unstable or fluctuating recordings. The graphical picture provided by the LCD monitor of the O2C unit, help to keep the absorption spectra of oxyhemoglobin well above 50% of the arbitrary unit (AU) scale during examinations (Figure 1A and B).[15] This step allowed us to perform the repeated transserosal measurements in all subjects without applying unnecessary pressure on the serosa. Before each recording, an ambient light correction was performed automatically, which allowed keeping the examined area illuminated and maintaining the O2C microprobe's visual control throughout the examination. A standard measurement protocol of 5 seconds of continuous measurements at each anatomical position resulted in approximately 200 measurements at each
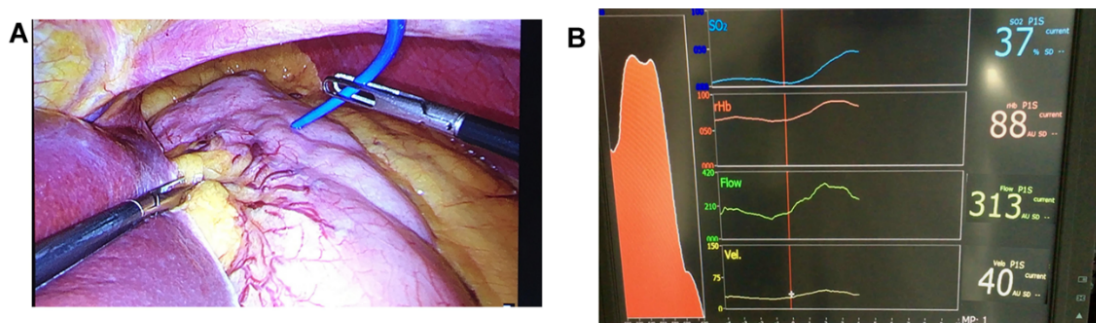
Figure I (A–B). Transserosal microcirculation recordings: (A) microprobe on ventricle surface (B) LCD monitor with the real-time absorption spectrum of oxyhemoglobin (red), and graphic presentation of StO2, relative hemoglobin, flow and velocity and the mean numerical values.

anatomical site. The system provided a real-time quantitative measurement and stored the raw data for later analysis.

After establishing pneumoperitoneum with CO2, and before any intraperitoneal dissection, baseline measurements were recorded from predefined anatomical sites on the greater curvature's anterior surface. Measurements were repeated at the same anatomical sites after gastric tube formation and subsequently, after the construction of the TGEA (Figure 2A–C). The distance between each measuring site was approximately 3–4 cm apart. In all patients, the gastroesophageal anastomosis was constructed at the site M7. A marking suture was placed at the gastric incisura towards the level of site M3, to identify the site after the gastric pull-up and anastomosis. All measurements were performed under stable hemodynamic conditions. No vasopressor medications were administered

during the measurements, and the systemic oxygen saturation was kept > 97%.

Operative Technique

A standard thoracolaparoscopic, "Ivor-Lewis" type minimal invasive esophagectomy was performed in all patients.[16] This approach included complete mobilization of the stomach, dissection of the short gastric arteries and the left gastric artery, and gastric tube preparation after regional lymphadenectomy. A multi-step thoracolaparoscopic subtotal resection of the esophagus with two-field lymphadenectomy was performed. The gastric conduit was anastomosed to the proximal residual esophagus at the carina level by a circular staple device. The introduction site for the circular stapler was closed using a linear stapler and oversewn.
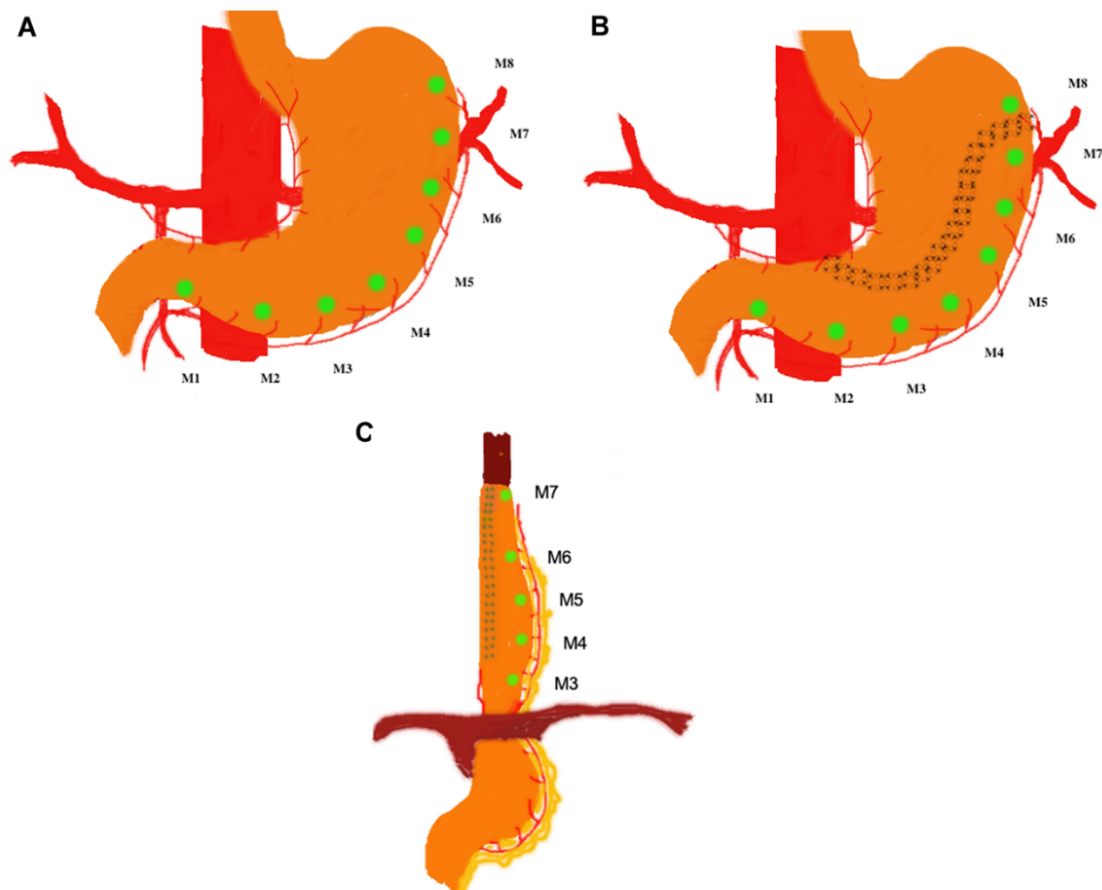
Figure 2 (A–C). Measuring points M1 to M8 (green dots) at baseline, after gastric tube construction, and gastroesophageal anastomosis.

All surgical procedures were performed under general anesthesia with the same team of surgeons who were blinded to the results of the perioperative microcirculation measurements. Thoracic drains were placed close to the anastomosis and the diaphragmatic hiatus. A decompressing nasogastric tube was positioned, and the patients were extubated in the operating theatre. Postoperatively, a mean systemic arterial pressure of 65 mmHg or higher was targeted, and vasopressors were administered if needed. All patients stayed at the postoperative surveillance department for three days and were routinely examined on the third postoperative day with upper endoscopy and computed tomography (CT) with oral contrast of the esophagus. Complications are reported on and graded according to Clavien-Dindo classification of surgical complications as recommended by the Esophagectomy Complications Consensus Group.[17]

The study protocol was approved by the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (approval number 2018/500/REK sør-øst A) and registered in Clinicaltrials. gov (ClinicalTrials.gov ID NCT03724162). The study conforms to the provisions of the Declaration of Helsinki. Informed written consent was obtained from all patients included in the study.

Statistical Analysis

A descriptive data analysis was performed. Data are presented as median (range) or mean (standard deviation) dependent on data distribution. Group comparison and mean changes were evaluated using the paired sample *t*-test. A *P*-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 25 (IBM SPSS Statistics).

Results

During a period of seven months, ten patients with esophageal cancer were included. Patient characteristics are given in Table 1. There was no 30-day and 90-day mortality. During a median follow-up period of 16 months (range, 12–18 months), one patient died. Three patients (30%) had an anastomotic leak type I and Grade II surgical complication (Table 1). These three leakages were identified by standard upper endoscopy on the third postoperative day and confirmed with a CT with oral contrast on postoperative day six. In one patient, the leakage was treated solely with antibiotics. In the other two

Table I Baseline Characteristics of Patients (n=10)

Variables				
Median age, years	59 (47–83)			
Gender (male: female)	3:7			
Comorbidity				
Ischemic heart disease	0			
Atrial fibrillation	1			
Stroke	0			
Hypertension	5			
Pulmonary disease	5			
Hypercholesterolemia	2			
BMI, median	24.8 (21.6–31.1)			
GERD	7			
Smoking	2			
Histology				
AC/SCC	8/2			
Tumor location (Distal Esophagus/Cardia)	9/1			
Clinical cancer staging				
Stage I	1			
Stage II	3			
Stage III	5			
Stage IV	1			
Type of operation				
Ivor-Lewis MIE	10			
Anastomotic leak				
Definition: Type 1	3			
Clavien–Dindo classification of surgical complications	I	II	IIIa	IIIb
Anastomotic leak	0	3	0	0
Pneumonia	0	3	0	0
Pleural effusion	0	2	8	0
Atrial fibrillation	0	4	0	0
Pulmonary embolism	0	1	0	0
[a]Median ICU stay, days	4 (4–10)			
[b]Median ICU stay, days	4 (4–12)			
[a]Median hospital stay, days	25 (25–34)			
[b]Median hospital stay, days	17 (7–39)			
Mortality				
30 days and 90 days	0			

Abbreviations: AC, adenocarcinoma; ASA, American Society of Anesthesiologists; BMI, body mass index; GERD, gastroesophageal reflux disease; ICU, intensive care unit; SCC, squamous cell carcinoma; [a]With leaks; [b]Without leaks.

patients, antibiotics were administered, and the Jackson-Prat drain kept for an extended period of 5–7 postoperative days.

Intraoperative transserosal microcirculation assessment was successfully performed in all patients. The median recording time required for the measurements was 6 minutes (range, 3–8 minutes).

When analyzing the whole study group, a reduction in StO2 was found at all measuring sites in the gastric tube as compared with the baseline measurements. The reduction was statistically significant at the sites M4 ($P = 0.04$), M5, and M7 ($P = 0.03$). The reduction in StO2 became amplified ($P < 0.001$) after the construction of TGEA at all measuring sites as compared with the baseline measurements (Figure 3A). The mean StO2 reduction from baseline to gastric tube formation and after anastomosis was 16% (range 4% - 28%) and 42% (range, 35% - 52%), respectively. A statistically significant increase in the rHb concentration was detected at the most cranial part of the gastric tube, site M7 ($P = 0.04$) (Figure 3B).

The mean LDF measurements of the whole study group showed a statistically significant increase in the local blood flow at the site M4 ($P = 0.009$) after TGEA (Figure 3C). A similar change in velocity measurements was observed in the gastric tube after TGEA ($P = 0.004$) (Figure 3D).

In the three patients with leaks, the mean StO2 reduction after anastomosis, as compared with the baseline StO2, was 49% (range, 25% - 69%), while in patients without leaks, it was 39% (range, 32% - 46%). After anastomosis, rHb increased from baseline to 61% (range, 33% - 147%), and 17% (range, 0–38%), respectively in the patients with and without leaks. Although, the mean change in velocity was similar in patients with or without leaks, respectively 12% (range, −13% - 41%) and 11% (range, −14% - 44%), the mean tissue blood flow after anastomosis was increased by 36% (range, −5% - 57%) and 26% (range, −4% - 51%) respectively. This increase was statistically significant only at M4 in patients without leaks ($P = 0.02$). Table 2 gives a detailed account of microcirculation assessment results in patients with and without anastomotic leaks.

Discussion

This is the first study to present data on the simultaneous use of LDF and VLS for assessment of the gastric tube microcirculation in patients with esophageal cancer undergoing minimally invasive surgery. The O2C technology utilized, provides a real-time information to the surgeons
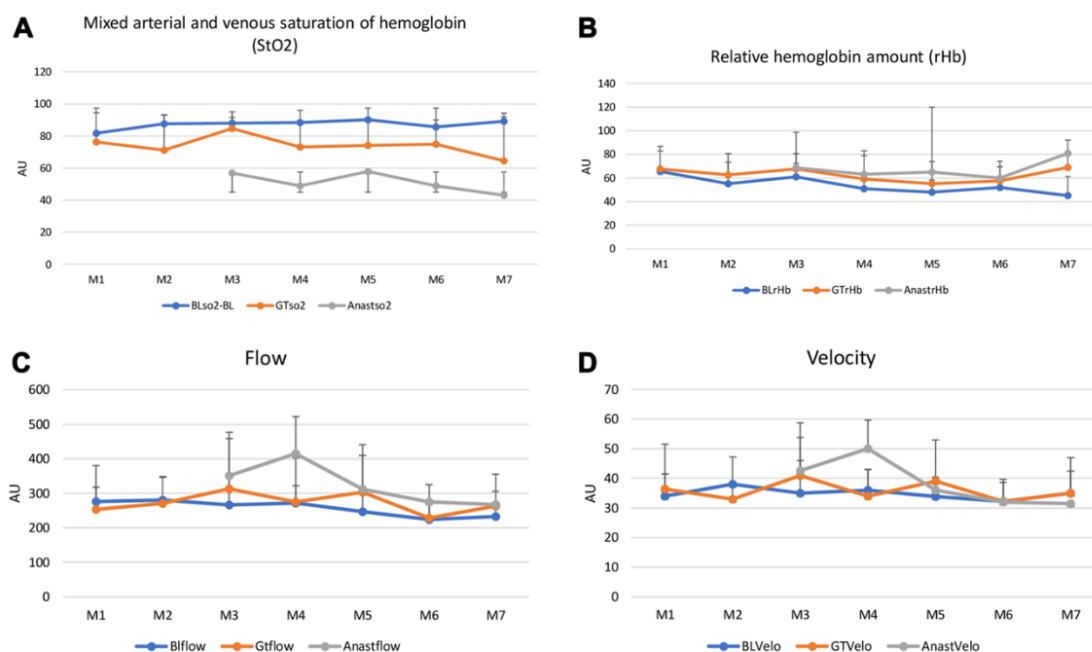

Figure 3 (**A–D**). Mean values with standard deviation of intraoperative transserosal microcirculation of stomach and gastric tube in patients with esophageal cancer. AU, arbitrary units; M1-M7, measuring sites.

Table 2 Results of Intraoperative Transserosal Microcirculation Assessment of Gastric Tube and Thoracic Gastroesophageal Anastomosis with Laser Doppler Flowmetry and Visible Light Spectroscopy in Patients with Esophageal Cancer

Oxygen saturation (AU) (Mean ± SD)			ªMean diff. in %	P	Relative Hemoglobin (AU) (Mean ± SD)			ªMean diff. in %	P
	Baseline	Gastric tube	Anastomosis			Baseline	Gastric tube	Anastomosis		
M1[b]	69 ± 15	72 ± 27				80 ± 20	78 ± 13			
M1[c]	87 ± 9	78 ± 22				60 ± 15	63 ± 22			
M2[b]	88 ± 7	76 ± 33				76 ± 14	58 ± 16			
M2[c]	88 ± 6	69 ± 21				46 ± 14	65 ± 21			
M3[b]	84 ± 7	90 ± 1	63 ± 28	25	.26	60 ± 16	81 ± 11	85 ± 35	42	.2
M3[c]	90 ± 8	83 ± 8	54 ± 16	40	.005	62 ± 11	62 ± 11	62 ± 23	0	.2
M4[b]	82 ± 12	74 ± 21	44 ± 39	46	.16	58 ± 15	77 ± 11	81 ± 21	40	.3
M4[c]	91 ± 4	73 ± 16	51 ± 11	44	.005	48 ± 13	51 ± 20	55 ± 27	15	.5
M5[b]	93 ± 2	67 ± 13	55 ± 3	41	.005	56 ± 16	69 ± 19	81 ± 10	46	.1
M5[c]	89 ± 9	77 ± 17	60 ± 16	33	.005	45 ± 7	9 ± 19	58 ± 36	29	.4
M6[b]	85 ± 12	81 ± 16	26 ± 12	69	.01	63 ± 31	62 ± 22	84 ± 6	33	.4
M6[c]	86 ± 14	73 ± 16	59 ± 13	32	.005	48 ± 11	56 ± 17	49 ± 16	3	.8
M7[b]	87 ± 7	85 ± 14	30 ± 14	66	.005	58 ± 24	47 ± 9	143 ± 77	15	.2
M7[c]	90 ± 5	56 ± 29	49 ± 29	46	.01	40 ± 11	78 ± 23	55 ± 19	38	.1

Flow (AU) (Mean ± SD)			ªMean diff. in %	P	Velocity (AU) (Mean ± SD)			ªMean diff. in %	P
	Baseline	Gastric tube	Anastomosis			Baseline	Gastric tube	Anastomosis		
M1[b]	373 ± 139	290 ± 53				34 ± 11	34 ± 8			
M1[c]	235 ± 69	237 ± 70				34 ± 6	38 ± 19			
M2[b]	354 ± 90	313 ± 140				44 ± 14	32 ± 2			
M2[c]	249 ± 29	252 ± 49				35 ± 7	34 ± 5			
M3[b]	307 ± 130	414 ± 251	292 ± 16	-5	.9	32 ± 12	50 ± 28	34 ± 6	5	.9
M3[c]	249 ± 65	270 ± 82	376 ± 183	51	.9	36 ± 12	38 ± 14	46 ± 15	28	.005
M4[b]	305 ± 70	402 ± 190	477 ± 135	57	.3	36 ± 14	43 ± 12	45 ± 5	25	.4
M4[c]	258 ± 41	221 ± 63	389 ± 117	51	.02	36 ± 4	30 ± 6	52 ± 9	47	.005
M5[b]	294 ± 80	431 ± 219	427 ± 157	45	.1	32 ± 3	43 ± 12	45 ± 10	39	.2
M5[c]	227 ± 43	249 ± 66	262 ± 95	15	.4	34 ± 6	36 ± 7	32 ± 11	6	.7
M6[b]	263 ± 63	282 ± 173	346 ± 152	32	.5	31 ± 11	31 ± 6	27 ± 3	13	.6
M6[c]	208 ± 30	206 ± 59	244 ± 89	17	.4	33 ± 5	33 ± 9	34 ± 12	3	.7
M7[b]	266 ± 98	332 ± 157	403 ± 79	52	.2	35 ± 17	41 ± 12	35 ± 5	0	1
M7[c]	219 ± 70	233 ± 51	210 ± 27	-4	.8	35 ± 12	32 ± 5	30 ± 4	14	.4

Notes: P values, paired sample t test. ªPercent changes comparison between Baseline and Anastomosis. [b]With anastomotic leaks and [c]without leaks.
Abbreviation: AU, arbitrary units.

during surgery. We found a significant reduction in the tissue StO2 in the gastric tube. The tissue StO2 deteriorated further, and a statistically significant reduction in the transversal StO2 after anastomosis was observed in all patients, compared to baseline values. In this pilot study, the patients with leaks had a lower mean StO2 at baseline compared with the patients without leaks, and they also had a more reduction in the mean StO2 after anastomosis.

The mean proportional increase in rHb in the patients with leaks was higher (61%) than those without leaks (17%). This increase in rHb was most evident in the most cranial part of the patients with leaks. This is an important observation as the increase in rHb represents venous congestion that may impact tissue StO2. Gerau et al also found that in addition to significantly reduced StO2, there was an increase in the rHb in the gastric tube of patients with leaks.[18] Furthermore, Buise et al found that the patients developed venous congestion after esophagectomy.[19] Murakami et al performed microvascular anastomosis on the neck and showed that both the arterial circulation and venous congestion were relieved after such vascular anastomosis.[20,21] However, in contradiction to Buise et al, they did not find reduced StO2. In the present study, we also found an increase in the mean

tissue blood flow from baseline to the anastomosis in patients with leaks (39%). This increase in tissue blood flow is of smaller magnitude in the patients without leaks (26%). Thus, the combined use of LDF and VLS enables substantiated information regarding blood circulation at the anastomotic site for the surgeon during surgery and may also further enlighten pathophysiological changes induced in the gastric tube.

Based on the present findings, patients with leaks may have more ischemic changes, aggravated by the venous congestion as represented by rHb increase. The increase in local blood flow is probably a compensatory physiological response to ischemia that may contribute to venous congestion due to reduced venous drainage caused by surgical trauma to the veins during esophagectomy and gastric tube formation. Extrinsic compression at the esophageal hiatus may also obstruct venous drainage and could have caused venous congestion in the patients with leaks.[21] Manipulation and axial tension in the tube may also play a part in venous drainage.[4] The physiological counter-current mechanism of shunting blood flow from the mucosa to the serosal layer during an ischemic insult may also have contributed in the increase in local blood flow measured with transserosal LDF in our study cohort.[22] To avoid vascular injury, a fair distance from the left gastroepiploic artery was maintained during free dissection of the greater omentum. Although the diaphragmatic hiatus's opening was wide, this could still have been a potential source of external compression on the venous drainage, secondary to expected postoperative edema.

The advantages of LDF and VLS are the quickness of measurements, low invasiveness, and high reproducibility.[6] VLS is validated for the investigation of chronic mesenteric ischemia.[14] Many studies have utilized LDF and VLS to assess the microcirculation in gastric tube. However, most of these studies of the microcirculatory assessment of gastric tube have been conducted on with the patients with anastomosis constructed on the neck. Most of the studies of the microcirculatory assessment of the gastric tubes are initial experiences and feasibility studies. Furthermore, either LDF or light spectrophotometry has been utilized. Only two studies incorporated both LDF and VLS.[15,23] The former was the investigation of transmucosal microcirculation in patients with non-specific abdominal pain, and the latter included patients with chronic mesenteric ischemia and a control group with non-specific abdominal pain. Other studies such as Pham et al had no real-time measurements,

while Wang et al excluded patients with anastomotic leaks.[24,25]

Although the assessment of ischemia can be identified as a common aim in these studies, the studies differ in patient demographics and the different measurement units. It is, therefore, hard to draw conclusions and to standardize these methods for routine clinical use. Interestingly, many of the available studies could not provide intraoperative real-time information about microcirculation.[26,27] In contrast, the present study confirms the feasibility of the combined use of LDF and VLS intraoperatively, as made possible by the O2C technology.

Although studies have shown adverse effects of both radio- and chemotherapy on the tissue, the neoadjuvant radio-chemotherapy is the current standard of treatment of esophageal cancer before radical surgical resection for most patients.[11] Furthermore, the clinical results of major studies have so far not demonstrated more anastomotic leakages.[28] A major limitation of our pilot study is the small sample size. The study was not designed and powered to investigate anastomosis leaks in general. Therefore, statistical evaluations should be interpreted cautiously. Anastomotic leaks were identified early due to an aggressive diagnostic approach, including routine gastroscopy at day three and liberal use of early CT with oral contrast. The standardized surgical technique applied represents a strength of the study. The study results hold promise for future appropriately powered studies to provide intraoperative useful cut-off values for StO2, rHb, local tissue blood flow, and velocity. The results of more extensive studies may be used to standardize microcirculation assessment modalities for regular clinical use.

Conclusion

Concomitant intraoperative transserosal LDF and VLS may help identify local ischemia in the gastric tube during esophagectomy. Patients with anastomotic leaks seems to have a profound local tissue StO2 reduction, which is further aggravated by the development of venous congestion.

Data Sharing Statement

Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures), will be made available and shared with investigators whose proposed use of the data has been approved, but an independent review committee identified for this purpose. Proposals should be directed to associate professor Syed Sajid Hussain Kazmi MD Ph.D.

sshkazmi@gmail.com, project leader. To gain access, data requesters will need to sign a data access agreement.

Acknowledgment

Disclosure

The authors have nothing to disclose.

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Paper 3

ORIGINAL RESEARCH

Perioperative Microcirculatory Changes Detected with Gastroscopy Assisted Laser Doppler Flowmetry and Visible Light Spectroscopy in Patients with Median Arcuate Ligament Syndrome

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Purpose: Physiological tests may aid in diagnosing median arcuate ligament syndrome (MALS). MALS is a symptomatic compression of the celiac artery causing symptoms similar to chronic mesenteric ischemia (CMI) of atherosclerotic etiology. Simultaneous use of visible light spectroscopy (VLS) and laser doppler flowmetry (LDF) during upper endoscopy may detect microcirculatory changes in these patients.

Patients and Methods: In a single-center, prospective comparative cohort, 25 patients were evaluated for MALS. Patients with a consensus diagnosis of MALS (n−15) underwent a gastroscopy assisted, transmucosal microcirculatory assessment with LDF and VLS. Results were compared to individuals with normal intestinal circulation (n=38) evaluated with duplex ultrasonography, and to patients with chronic mesenteric ischemia (n=32). Treatment response was evaluated clinically at 1, 3, 6, and 12 months, and with ultrasound, VLS and LDF at three months. Health-related quality of life (QoL) was assessed with Euroqol (EQ-5D-5L), preoperatively, and 12 months postoperatively.

Results: Preoperative mean transmucosal oxygen saturation was significantly lower in patients with MALS (SO_2 76±6), as compared to healthy individuals (SO_2 81=4), p−0.02. An overall significant improvement in SO_2 after surgical decompression of the celiac artery was found (SO_2 81⊥3.7, p=0.05). Eleven (92%) patients with clinical improvement after laparoscopic decompression had a definitive diagnosis of MALS. Median follow-up was 18 months (4–24 months). Four of the five dimensions investigated with EQ-5D-5L improved.

Conclusion: VLS detected a significantly lower baseline transmucosal SO_2 in patients with MALS as compared to control subjects with normal intestinal circulation. An improvement in SO_2 after laparoscopic decompression was found, supporting a possible ischemic etiology in our patient population.

Keywords: mesenteric ischemia, functional test, endoscopy, vascular surgery, abdominal pain

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Introduction

Median arcuate ligament syndrome (MALS) is caused by compression of the celiac artery (CA) by the median arcuate ligament and was first described anatomically by Lipschutz in 1917.[1] The median arcuate ligament is a fibrous band that crosses over the aorta and connects the right and left crura of the diaphragm. The anatomical variant with compression of the CA due to either a high origin of the artery or a low insertion of the crura is present in 10–24% of the population.[2,3] This is the most

A common cause of single-vessel stenosis in the mesenteric arteries. Although this external compression is usually asymptomatic, it can, in some patients, cause postprandial epigastric pain or nausea, weight loss, and an epigastric bruit.[2,4]

The diagnosis is controversial, mainly because the pathophysiology of MALS remains unclear. The prevailing theory is that insufficient amounts of blood pass through the compressed CA in times of increased metabolic demand, leading to ischemia.[4,5] Another theory is that a neuropathic compression of the vessel may lead to irritation of sympathetic pain fibers causing pain, much like in the case of other nerve compression diseases, such as a carpal tunnel.[6]

The clinical presentation of MALS is variable.[4] The diagnosis depends on the exclusion of other possible causes of abdominal pain, and patients often undergo extensive examinations. Upper endoscopy is a standard initial investigation in patients with postprandial upper abdominal complaints.[4,7] Using gastric exercise tonometry (GET) as a functional test, one study has demonstrated ischemia in patients with MALS.[8] The test is, however, somewhat cumbersome, the required equipment is no longer available, and alternative functional tests hence need to be explored.

Gastroscopy assisted laser Doppler flowmetry (LDF) and visible light spectroscopy (VLS) (GALS) have successfully been utilized to examine transmucosal microcirculatory changes in the gastrointestinal tract both in healthy individuals and patients with chronic mesenteric ischemia (CMI) due to atherosclerosis of the mesenteric arteries.[9,10] Even in the case of a single vessel pathology, GALS has been able to detect ischemic changes in the stomach and duodenum.[10,11]

Traditionally, the treatment of MALS has been an open surgical release of the compression on the celiac artery, but for the last decade, a laparoscopic approach has been utilized.[12,13]

This study aimed to examine the transmucosal micro-circulation of the stomach and duodenum in patients with MALS utilizing GALS before and after the laparoscopic decompression of the CA.

We hypothesized that patients with MALS have reduced microcirculation in the stomach as compared to subjects with healthy intestinal circulation and that the microcirculation and symptoms improve in patients after the surgical procedure. Furthermore, we hypothesized that

the patient's health-related quality of life (QoL) improved after surgery.

Patients and Methods

The study was designed as a single-center prospective comparative cohort. It was conducted at Oslo University Hospital during a 36 month period from September 2016 to September 2019. Patients included were referred from either primary health care or other hospitals. The suspicion of MALS was based on symptomatology, CT angiography findings ($\geq 50\%$ stenosis in deep expiration phase (Figure 1)), and exclusion of common differential diagnoses such as ulcer disease, cholelithiasis, pancreatitis, inflammatory bowel disease, and malignancy. All patients were subjected to a detailed clinical examination and CT angiography before discussion in a multidisciplinary panel comprising of vascular surgeons and interventional radiologists. Patients were required to have at least two of the three following criteria; postprandial pain, unintentional weight loss, and changes in habits of food intake (reduced amount and/or increased frequency of meals, the exact frequency and composition of each meal was not recorded). CT angiography in deep inspiration and deep expiration phase was undertaken. A reconstruction in axial, sagittal, and coronal planes was examined to confirm.

Figure 1 CTA sagittal plane of a patient with MALS taken in deep expiration. An external compression of the celiac artery and a normal anatomy of the superior mesenteric artery is demonstrated.
Abbreviations: MALS, median arcuate ligament syndrome; CTA, computed tomography angiography.

MALS. A consensus diagnosis of MALS was made based on the above.

In patients with a consensus diagnosis of MALS, a transmucosal microcirculatory investigation with GALS was performed. The investigation was repeated after surgical treatment, and results were compared to data from individuals with healthy intestinal circulation (Control Group 1 (CG1) n=38). The individuals in CG1 were recruited from a list of patients awaiting upper endoscopy due to dyspepsia, control of Barrett's esophagus, or control after ulcer disease. None of these had postprandial abdominal pain or weight loss. A transabdominal duplex ultrasound (Vivid E95, General Electric Healthcare, Chicago, IL) with a curvilinear probe C1–6 was performed after a minimum of six hours of fasting to evaluate the patency of the intestinal arteries in these control individuals. An experienced specialist in ultrasonography performed all examinations. Significant stenosis (>70%) was defined as a peak systolic velocity of the superior mesenteric artery (SMA) ≥275 cm/s and CA ≥200 cm/s.[14]

Additionally, data from MALS patients were compared to previously published data on patients with chronic mesenteric ischemia (CMI), (Control Group 2 (CG2), n=32) due to atherosclerosis.[10] In this previous study, the inclusion criterion was stenosis ≥70% or occlusion of ≥1 mesenteric artery on CT angiography in addition to symptom relief after intervention with percutaneous transluminal angioplasty with or without stenting or aortomesenteric bypass.

Symptom relief was defined as either a complete or partial disappearance of symptoms. A definitive diagnosis of MALS was thereby based on a complete or partial relief of symptoms after an intervention.

Measurements

All patients were investigated with upper endoscopy at the gastric laboratory before and three months after the laparoscopic surgical treatment. All endoscopies were performed by the same team of examiners, comprising an experienced gastroenterologist and two vascular surgeons. The patients were examined in a fasting state and a left, lateral decubitus position. Peripheral oxygen saturation was monitored in all patients receiving benzodiazepines or analgesics and kept >95%. Air insufflation was kept at a minimum, and no spasmolytic agent was administered.

The measurement points were chosen based on the arterial supply of the stomach and duodenum by the branches of the CA and superior mesenteric artery (SMA)
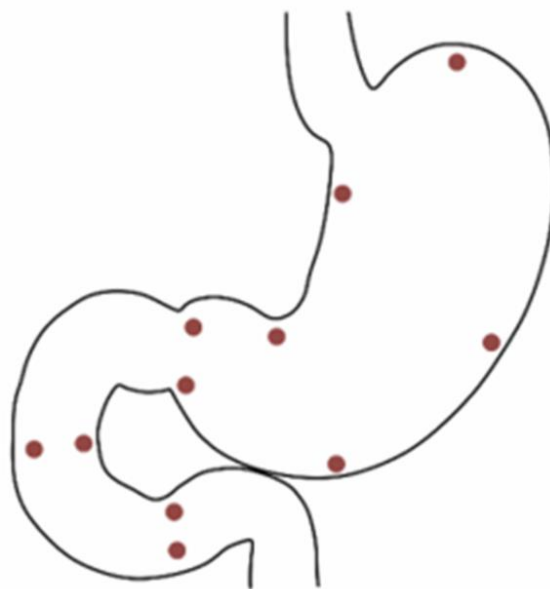
Figure 2 Measurement points in the stomach and duodenum in the study of perioperative microcirculatory changes in patients with MALS.
Abbreviation: MALS, median arcuate ligament syndrome.

(Figure 2). A mean of the four measurements in the duodenum (descending and horizontal part), the four measurements at the lesser curvature and pylorus, and three measurements at the greater curvature were calculated.

A 2.6 mm "Oxygen 2 See" (O2C) microprobe, LM-10 (O2C, LEA Medizintechnik, Giessen, Germany), was passed through the working channel of an Olympusx flexible gastroscope. The O2C utilizes both LDF and VLS to make simultaneous measurements of flow (i.e., red blood cell flux), velocity, combined venous and arterial saturation of capillary hemoglobin (μHb_{SO2}) and a relative hemoglobin-amount (μHb_{con}).[15]

The machine detected movement and pressure artifacts, and visually assessed unstable, or fluctuating recordings were discarded, and the measurements were automatically or manually repeated.

The study patients were followed up at 1 (clinical evaluation), 3 (upper endoscopy with microcirculatory measurements, clinical evaluation, duplex ultrasound), 6 (clinical evaluation), 12 (clinical evaluation, QoL assessment) months, and yearly (clinical evaluation) thereafter.

Health-Related Quality of Life

At inclusion and 12 months postoperatively, the patient's completed a validated and Norwegian-translated questionnaire.

EuroQol (EQ-5D-5L).[16] EQ-5D provides a descriptive profile and a single index value for health status based on five dimensions; mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each of the five dimensions is divided into five levels of perceived problems from 1 (indicating no problems) to 5 (indicating extreme problems). The second part of the EQ-5D-5L is a visual analog scale from 0 (worst imaginable health state) to 100 (best imaginable health state).

Operative Technique

All patients were treated laparoscopically, and the same vascular surgeon performed all procedures. The patients were under general anesthesia and in a supine position with the surgeon placed between the legs of the patient. A 12 mm trocar was placed under visual guidance between the xiphoid process and the umbilicus. The abdominal cavity was insufflated with CO_2, and three additional trocars (5 mm) were placed, one on the right side, and two on the left side of the patient's abdomen. The left liver lobe was elevated using Nathanson's liver retractor (Cook Medical, Bloomington, Indiana, United States). The omental bursa was opened through the division of the hepatogastric ligament. The common hepatic artery or splenic artery was identified and followed proximally to the celiac trunk bifurcation. The CA was dissected free using a monopolar hook until the median arcuate ligament was identified. The left gastric artery and the small diaphragmatic branches from the CA were preserved. The median arcuate ligament was divided with either the monopolar hook or Ultracision Harmonic (Ethicon Inc., Somerville, New Jersey, United States). Care was taken to clear the cranial surface of the CA and its origin from the aorta, from any fibrous, muscular, or nervous tissue. The aorta was free-dissected 2–3 cm cranially and on both sides of the origin of the celiac artery. After removing the Nathanson's liver retractor, exsufflation was done, and trocars were removed. Patients were discharged after a median hospital stay of 2 days (range 2–3 days).

Ethics and Trial Registration

Informed written consent was obtained from all patients and control subjects. The study protocol was approved by the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (REK sør-øst B 2016/682) and registered in the ClinicalTrials.gov Protocol Registration and Results System (NCT02914912). The study was conducted in accordance with the Declaration of Helsinki.

Statistics

Normally distributed data are presented as mean values with standard deviations or median values with range unless otherwise stated. For continuous outcome variables, the independent Student's t-test was applied, and Fisher's exact test was applied to categorical data. Wilcoxon signed ranks test was used to investigate the change after intervention. The statistical significance was set at 5% ($p < 0.05$).

A power analysis was performed based on the results from our previous study on CMI with a mean oxygen saturation of 81±4% in the individuals with healthy intestinal circulation and an anticipated saturation of 76% in patients with MALS (CMI 67±9%). With the study power set at 80%, a sample size of 10 patients was calculated.

Test performance at different cut-off levels was explored using the receiver operating characteristics (ROC) curve, and sensitivity and specificity for diagnosing MALS were calculated.

Statistical analysis was performed using IBM SPSS Statistics version 25 (IBM Corp. Armonk, NY).

Results

From September 2016 to September 2019, a total of 25 patients were referred from primary health care and from other hospitals to the Department of Vascular Surgery, Oslo University Hospital, for the evaluation of MALS (Figure 3). Of these, eight were excluded based on ≤50% stenosis on CTA in the deep expiration phase. Two had ≥50% stenosis, but no symptoms, and the findings were regarded as incidental. Fifteen patients were hence included in this study, and based on the standard diagnostic workup, they were diagnosed with MALS and offered surgical treatment. Laparoscopic decompression was the treatment of choice in all patients; however, three patients chose to decline surgery and are being followed-up in the out-patient clinic. Twelve patients underwent a successful laparoscopic decompression of the CA. Of these, 11 reported clinical improvement on follow-up. These 11 patients have been considered as having a confirmed diagnosis of MALS and are included in the statistical analysis. Patient characteristics are presented in Table 1.

All patients with a confirmed diagnosis of MALS described postprandial abdominal pain upon referral, nine (82%) of them reported pain debuting within 30 minutes
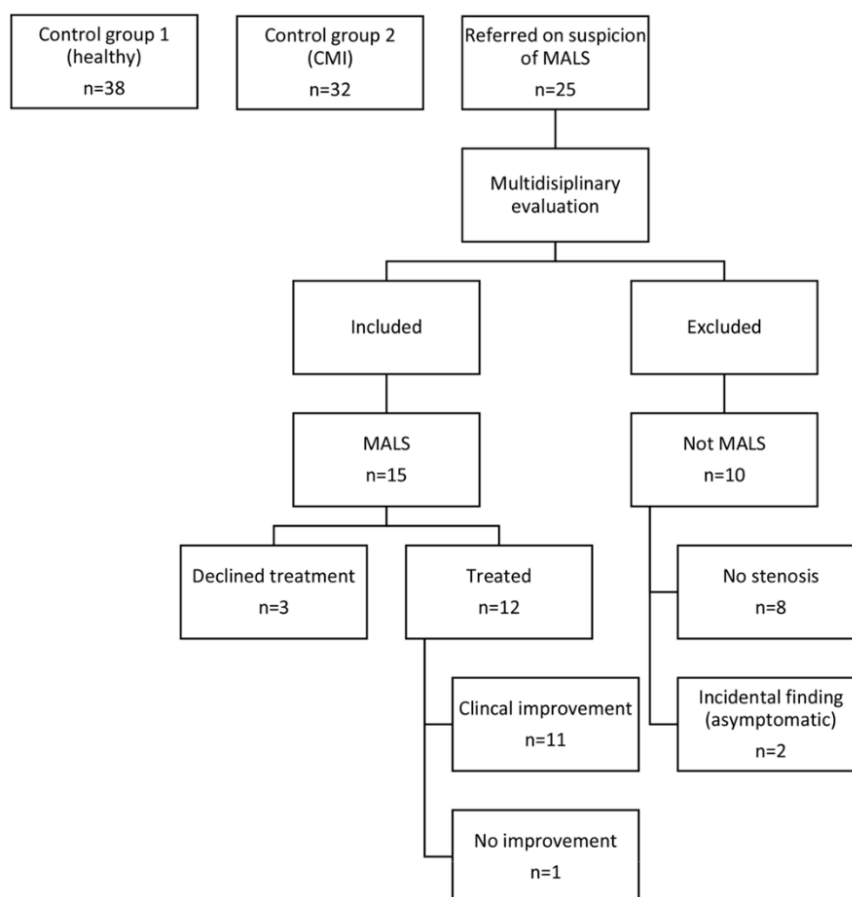
Figure 3 Flow chart of the inclusion process and outcomes in the study of perioperative microcirculatory changes in patients with MALS.
Abbreviations: MALS, median arcuate ligament syndrome; CMI, chronic mesenteric ischemia.

a meal, two (18%) experienced pain 30–60 minutes after a meal. The pain lasted for more than 60 minutes in six (55%) of these patients. A mean weight loss of 5kg (±7kg) was reported in seven patients (64%). The time from debut to diagnosis varied, with a median of 96 months (16–600). The baseline characteristics of CG1 and CG2 are presented in Table 2.

GALS could be performed in all patients with MALS. All endoscopies were negative with regard to the concomitant disease. The mean total examination time was 12 minutes (±6 minutes). Ten patients (91%) agreed to go through a second upper endoscopy three months after surgical intervention. One successfully treated patient with symptom relief refused a second upper endoscopy due to discomfort during the endoscopic procedure.

The preoperative mean transmucosal oxygen saturation was significantly lower in patients with MALS (SO_2 76

±6%), as compared to healthy individuals (CG1, SO_2 81 ±4%, p=0.02, Table 3, Figure 4). There was no statistically significant difference in relative Hb amount, flow, or velocity between the groups. The results of the preoperative measurements in CMI patients (CG2, SO_2 67±9%) were, however, significantly lower than in both healthy individuals (CG1) and MALS patients (p<0.001, p=0.004 respectively).

Five patients with MALS (45%) had oxygen saturation (SO_2) below the cut-off of 78% from our study on CMI.[10] Using ROC analysis, we found the sensitivity for identifying an individual with MALS was 54% and specificity of 66% with SO_2 cut-off of 78% (Figure 5). A cut-off of 80% increases the sensitivity to 82% with a specificity of 60%.

After a median follow up of 18 months (4–24 months), eleven (92%) of the 12 patients treated with laparoscopic

Table 1 Patient Characteristics in the Study on Visible Light Spectroscopy and Laser Doppler Flowmetry During Upper Endoscopy in Patients with Median Arcuate Ligament Syndrome

Patient Characteristics	(n=11)
Reporting weight loss	7 (64)
Weight loss (kg)[a]	5±7
Abdominal pain	11 (100)
Debut <30 minutes after a meal	9 (82)
Debut >30 minutes after a meal	2 (18)
Duration of abdominal pain <30 minutes	2 (18)
Duration of abdominal pain 30- 60 minutes	3 (27)
Duration of abdominal pain >60 minutes	6 (55)
Diarrhea/obstipation/nausea	9 (81)
Changes in eating habits	8 (73)
Abdominal bruit	8 (73)
Time from debut until diagnosis (months)[b]	96 (16- 600)

Notes: Values are presented as [a] mean±SD, [b] median (range), or absolute numbers with percentages

decompression reported a complete or partial improvement in their symptoms compared to pre-intervention status. A partial recurrence of preoperative complaints was reported in four patients (36%). However, they still reported improvement in their symptoms compared with the preoperative status. All of these had an open CA on follow-up duplex ultrasound.

At three months, a second upper endoscopy was performed. An overall significant improvement in SO_2 after surgical decompression of the CA was found (preoperative SO_2 76±6, postoperative SO_2 81±3.7, p=0.05). On an individual basis, an improvement in transmucosal SO_2 was found in eight (72%) of the MALS patients post-operatively (Figure 6). Two patients had no change in saturation (including one patient without symptom relief). Both rHb and flow increased after intervention, whereas velocity declined (Table 4). With regards to velocity, there was an on average higher pre-interventional velocity in MALS patients compared to controls (CG1 and CG2) (Table 3). A reduction in velocity was observed postoperatively, however, not being statistically significant.

A normalization of peak systolic velocity on duplex ultrasound was found postoperatively in ten patients (Table 5). One had an open CA, but PSV>2,0m/s postoperatively.

EQ5D-5L was completed pre- and postoperatively by 9 of the 11 successfully treated patients. The visual analog scale showed an overall improvement from a score of 44 preoperatively to 62 postoperatively. Improvement after surgery was recorded in all patients except one. Four of the five dimensions investigated with EQ-5D-5L

Table 2 Baseline Characteristics of Patients with MALS and Control Subjects with Normal Intestinal Circulation (CG1), and Control Subjects with Chronic Mesenteric Ischemia (CMI, CG2)

Variables	MALS	CG1 (Healt hy)	p-value	CG2 (CMI)	p-value
	n=11	n=38		n=32	
Age (years)	45 (24- 72)	60 (20- 82)	0.01	73 (53- 89)	<0.001
Height (cm)	172±6	169±9	0.22	168±9	0.20
Weight (kg)	62±9	74±16	0.02	64±16	0.89
BMI	21±2	26±5	0.001	22±5	0.43
Female	7 (63)	21(55)	0.73	20(63)	0.52
Smoking history	8(73)	22 (58)	0.49	29(91)	0.16
Ischemic heart disease	0(0)	7 (18)	0.33	13(41)	0.02
Atrialr fibi llat ion	0(0)	1 (3)	1	5(16)	0.30
Stroke/TIA	0(0)	3 (8)	1	5(16)	0.30
Diabetes mellitus	0(0)	5 (13)	0.57	12(38)	0.02
Hypertension	1(9)	10 (26)	0.41	22(69)	0.001
Lung disease	2(18)	6 (16)	1	7(22)	1
Intermittent claudication	0(0)	0(0)	-	12(38)	0.02
Medication					
Single platelet	0(0)	8 (21)	0.41	23 (72)	<0.001
Double platelet	0(0)	1 (3)	0.49	7(22)	0.40
Statin	0(0)	10 (26)	0.15	28(88)	<0.001

Note: Values are Presented as Mean (SD) or Median (Range) or Absolute Numbers with Percentages.
Abbreviations: MALS, median arcuate ligament syndrome; CG1, control group 1; CG2, control group 2.

Table 3 Mean Values of Combined Arterial and Venous Oxygen Saturation (SO2), Relative Hemoglobin Amount (rHb), Flow and Velocity for the Three Examined Areas in the Stomach and Duodenum, and a Mean of All Areas in Patients with MALS, CG1 and CG2. SO2 in Percent. Relative Hemoglobin Amount, Flow, and Velocity in Arbitrary Units

Variables		Duodenum	p-value	Pylorus and Lesser Curvature	p-value	Greater Curvature	p-value	Total All Areas	p-value
		Mean±SD		Mean±SD		Mean±SD		Mean ±SD	
SO_2	MALS(n=11)	67±8		83±8		78±11		76±6	
	CG1 (n=38)	75±8	0.006	84±6	0.76	82±6	0.13	81±4	0.02
	CG2 (CMI) (n=32)	57±14	0.02	74±10	0.01	70±12	0.07	67±9	0.004
rHb	MALS	82±9		82±6		87±5		84±5	
	CG1	87±7	0.09	83±8	0.85	86±8	0.76	85±5	0.45
	CG2(CMI)	76±8	0.02	74±9	0.006	78±9	0.004	76±6	0.001
Flow	MALS	308±66		306±86		277±55		297±50	
	CG1	316±64	0.72	311±72	0.84	317±98	0.21	314±57	0.37
	CG2(CMI)	285±61	0.29	260±65	0.06	240±65	0.10	262±52	0.06
Velocity	MALS	39±4		38±5		39±5		39±4	
	CG1	40±4	0.36	37±3	0.47	38±5	0.59	38±3	0.90
	CG2(CMI)	39±4	0.82	33±4	0.005	34±4	0.006	35±3	0.01

Abbreviations: MALS, median arcuate ligament syndrome; CG1, control group 1 (normal intestinal circulation); CG2, control group 2; CMI, chronic mesenteric ischemia.

improved. One dimension (self-care) was not affected prior to intervention and remained unchanged.

Discussion

To our knowledge, this is the first study to investigate the transmucosal microcirculation in patients with MALS using a gastroscopy assisted LDF and VLS. The results

of this study supports the hypothesis that MALS has an ischemic etiology. We observed a significantly lower transmucosal oxygen saturation in patients with MALS compared to healthy individuals at baseline. Further, a borderline significant increase in transmucosal oxygen saturation was observed after the intervention. In addition, an increase in rHb and flow after the laparoscopic surgical
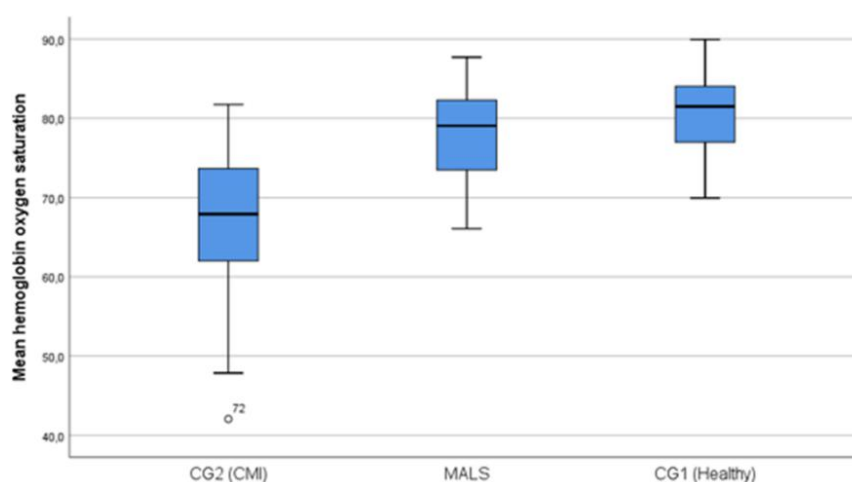
Figure 4 Box-plot of preoperative combined arterial and venous oxygen saturation in CG1 (n=38), CG2 (CMI, n=32), and patients with MALS(n=11). The thick black line represents the median, the blue box represents the 25- 75th percentile, and the bars are minimum and maximum points (excluding outliers).
Abbreviations: CG1, control group 1; CG2, control group 2; CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome.
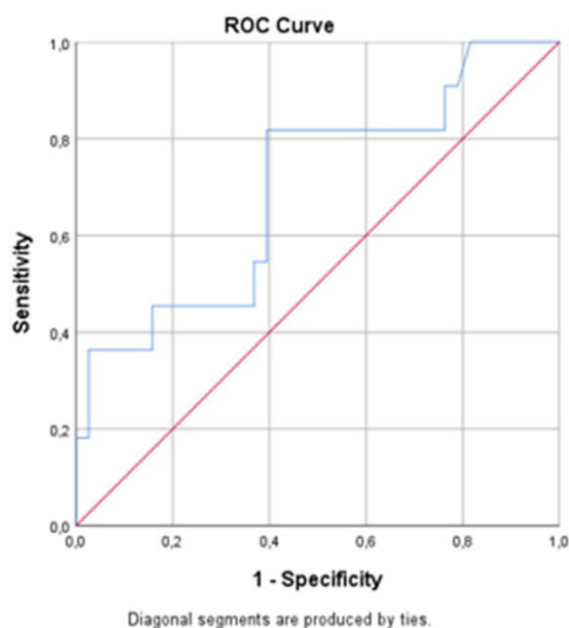
Figure 5 ROC curve of a mean of all measurements of saturation in both the stomach and duodenum in patients with MALS(n=11) compared to CG1 (n=38). **Abbreviations:** ROC, receiver operated characteristics; MALS, median arcuate ligament syndrome; CG1, control group 1; AUC, area under the curve; CMI, chronic mesenteric ischemia.

intervention suggests an improvement in the local perfusion.

Two main hypotheses are postulated to explain the pathophysiology of MALS.[5] The prevailing theory is that the symptoms are a result of ischemia due to insufficient oxygen delivery, either by means of direct compression of the vessel or due to a neuropathic component leading to

Table 4 Combined Arterial and Venous Oxygen Saturation (SO2), Relative Hemoglobin Amount (rHb), Flow and Velocity Before and After Laparoscopic Decompression of the Celiac Artery in Patients with Median Arcuate Ligament Syndrome

Variables (n=11)		Mean±SD	p-value
SO$_2$	Preoperative Postoperative	76.6±6.2 81.0±3.7	0.05
rHb	Preoperative Postoperative	83.8±4.7 85.9±3.5	0.28
Flow	Preoperative Postoperative	299.9±49.8 313.45±52.6	0.65
Velocity	Preoperative Postoperative	38.47±4.2 37.4±4.1	0.35

splanchnic vasoconstriction. The reduction in blood flow through the CA could cause a steal effect from the SMA, leading to small bowel ischemia. The other theory is that a neuropathic compression of the vessel may lead to inflammation and irritation of sympathetic pain fibers causing pain, much like in the case of other nerve compression diseases, such as carpal tunnel.[6] The celiac plexus is a hub for abdominal visceral afferent nerve fibers with pain sensation. Blocking the transmission of pain sensation through this hub has been performed with the injection of anesthetic agents for relief of intractable pain associated with malignant disease and chronic pancreatitis. Limited data on its efficacy are available. One review reports a favorable outcome of the procedure with pain relief in 72% (celiac plexus neurolysis) of the patients with
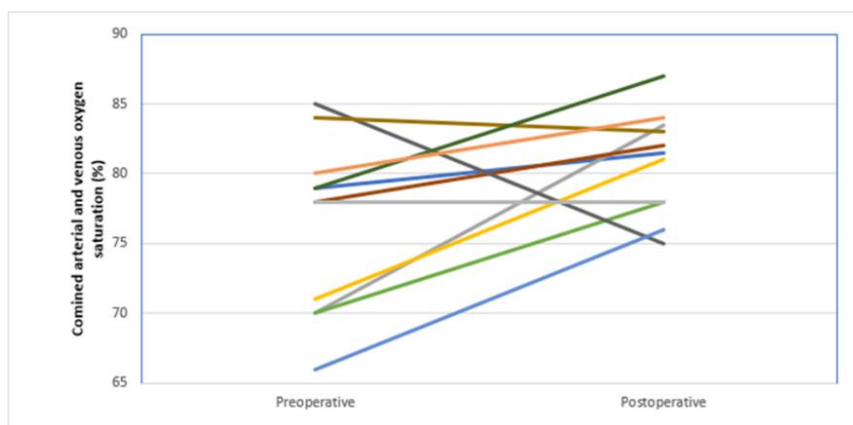

Figure 6 Combined arterial and venous oxygen saturation (%), before and after (n=11, p=0.05) laparoscopic decompression of the CA in patients with MALS. Each line represents one patient.
Abbreviations: MALS, median arcuate ligament syndrome; CA, celiac artery.

Table 5 Pre-and Post-Intervention Demographics and Characteristics in Patients with Median Arcuate Ligament Syndrome

Patient Number	Gender	Age (Years)	BMI (kg/m²)	Preoperative Ultrasound	Preoperative CTA	Preoperative Saturation(%)	Treatment	Conversion	Post operative Ultrasound (m/s)	Postoperative Saturation (%)	Effect of Treatment
1	Female	28	19.	Not performed	CA stenosis	082.	Laparoscopic decompression	No	71.	082.	Yes
02	Male	69	23.	1,7 m/s	CA stenosis	473.	Laparoscopic decompression	No	03.	183.	Yes
53	Female	22	22.	2,5 m/s	CA stenosis	773.	Laparoscopic decompression	No	71.	081.	Yes
4	Female	36	19.5	Not performed	CA stenosis	076.	Laparoscopic decompression	No	91.	Not willing	Yes
35	Female	60	21.	0 m/s	CA occlusion	071.	Laparoscopic decompression	No	51.	777.	Yes
08	Female	40	20.	1. m/s	CA stenosis	777.	Laparoscopic decompression	No	Not performed	382.	Yes
77	Male	46	22.	Technical difficulties	CA stenosis	684.	Laparoscopic decompression	No	81.	274.	Yes
6 0	Male	24	21.2.	m/s	CA stenosis	883.	Laparoscopic decompression	No	81.	84.	Yes
8 9	Female	45	19.2.	m/s	CA stenosis	479.	Laparoscopic decompression	No	02.	087.	Yes
10	Female	62	16.4.	m/s	CA stenosis	90			1.5	75.8	Yes
11	Male	65	22.	Not performed	CA stenosis	6			1.5	84.27	Yes
12	Female	65	23.	Not performed	CA stenosis	2	Laparoscopic decompression No80 Laparoscopic decompression No 678 Laparoscopic decompression No 66		1.5	78.2	No

Abbreviations: CA, celiac artery; CTA, CT angiography; BMI, body mass index.

pancreatic cancer and 51% (celiac plexus block) of the patients with chronic pancreatitis.[17]

The excellent collateral circulation of the bowel and the cyclic change in the rate of stenosis of the celiac artery should intuitively permit an adequate supply to the micro-circulation in a resting, fasting state. To unveil significant ischemic changes to the microcirculation in patients diagnosed with MALS was not anticipated.

In our previous study, CMI patients had significantly reduced microcirculation compared to control individuals, suggesting that VLS could play an essential part in the diagnostic process. The sensitivity of VLS (SO_2+rHb) for diagnosing CMI was 97% and specificity 79%. VLS appeared to be a more sensitive diagnostic test for ischemia than LDF. As ischemic changes could be masked by the intermittent nature of the flow reduction in patients with MALS, LDF variables could possibly become more apparent in periods of increased metabolic demand, such as after a meal or during physical exercise with a redistribution of blood flow to skeletal muscles. In the study with GET on patients with MALS, physical exercise is used as a stress test and resulted in the detection of ischemic changes in these patients. However, in a recently published study with VLS after luminal feeding in patients with CMI, mucosal oxygen saturation increased in both healthy individuals and CMI patients, and the provocation provided no discriminative ability toward the diagnosis of CMI.[18] This study had limitations, as natural digestion is merely approximated, and measurements were performed after an expected peak in postprandial hyperemia. Only one study has previously, successfully, utilized a functional test to evaluate the microcirculation in these patients, where an increase in pCO_2 was found in patients with MALS when applying GET.[8] GET is a rather cumbersome technique and is no longer available on the market.

In our study, the transmucosal oxygen saturation increased, and the velocity declined after decompression of the CA in eight of eleven patients with symptom relief (one patient declined the second upper endoscopy). No change in saturation was detected in the patient without clinical response.

Both LDF (velocity) and VLS (saturation) were able to detect changes after intervention. Although SO_2 seems to be the best variable for the detection of ischemia in the microcirculation in these patients, a combination of the two methods allows a more detailed understanding of the physiological changes in the microcirculation before and after surgical intervention.

The improvement in the transmucosal saturation in the patients with symptomatic improvement may help to conclude a successful laparoscopic decompression of the CA. In our study, we were not able to reproduce the diagnostic accuracy of VLS during upper endoscopy with regards to CMI. With a cut-off of 80% SO_2, the sensitivity is 82% but according to our results, sensitivity and specificity are still too low for this test to be utilized for patient selection.

A common view is that stenosis of a single mesenteric artery is insufficient to lead to mesenteric ischemia. However, several recent studies, including our own on CMI, suggest that ischemia can develop even with single-vessel pathology.[10,19,20]

Follow-up with duplex ultrasound after laparoscopic decompression shows normalization of velocities in all controlled patients except one.

Although we presented a material over 36 months from Norway's largest hospital, a relatively small number of patients were included. MALS patients were significantly younger than healthy individuals (CG1) (p=0.01) and had a lower BMI. In comparison to CMI patients (CG2), MALS patients had a significantly lower prevalence of hypertension, diabetes mellitus, intermittent claudication, and ischemic heart disease. Furthermore, patients with MALS and CMI were examined with CT angiography, and healthy controls were examined with color duplex ultrasound.

Laparoscopic decompression for MALS was first reported in 2000 and is now the treatment of choice in this patient group.[4,21,22] The procedure requires a small operating field and is well fitted for laparoscopic surgery. The approach used in our institution has proven to be safe and to date without complications. None of the patients in this study have reported any adverse events or worsening of their preoperative symptoms.

In total, 92% of the treated patients reported symptom relief, suggesting that our patient selection was adequate. A correlation between the severity of a stenosis and outcome after surgical decompression has been described, with better clinical outcomes found in patients with ≥70% stenosis.[23] Our patients had ≥50% stenosis. However, as this is an exclusion diagnosis, a test with higher specificity is warranted to identify patients who might benefit from surgery.

Conclusion

Gastroscopy-assisted VLS may detect perioperative changes in the transmucosal SO_2 in patients with MALS. Laparoscopic decompression is a safe and effective treatment for patients with MALS. Improvement in transmucosal SO_2

after laparoscopic decompression may support a possible ischemic etiology in patients with MALS.

Data Sharing Statement

Individual participant data that underlie the results reported in this article, after identification (text, tables, and figures), will be made available after the ongoing study on chronic mesenteric ischemia at Oslo University Hospital, in 2022 and be available for three years. In addition to this, the study protocol will be available. Data will be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose. Proposals should be directed to M.D. Ph.D. Syed Sajid Hussain Kazmi, syekaz@ous-hf.no, project leader. To gain access, data requestors will need to sign a data access agreement.

Author Contributions

All authors, STB, NS, AWM, JOS, JH, and SSHK made a substantial contribution to the conception and design, acquisition of data, analysis, and interpretation of data; and took part in drafting the article and revising it critically. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Paper 4

ORIGINAL RESEARCH

Laparoscopic Surgery for Median Arcuate Ligament Syndrome (MALS): A Prospective Cohort of 52 Patients

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Background: The selection of patients with MALS for surgical treatment depends upon the reliability of the symptom interpretation and the diagnostic work-up. We aimed to follow up the results of the laparoscopic decompression of the patients with MALS.

Patients and Methods: In a single-center, 52 consecutive MALS patients were followed-up, prospectively, after transperitoneal laparoscopic decompression. MALS was diagnosed with a computed tomography angiography (CTA) verified stenosis, ≥50% of the celiac artery (CA), and with duplex ultrasound, a peak systolic velocity (PSV) ≥2.0 m/s. Postoperative, CTA, and duplex ultrasound were performed, and the patients were followed-up at 3, 6, 12 months, and yearly after that.

Results: Mean age of the patients was 47 ±21 years, and 65% were females. The patients had a mean weight loss of 8.4 ±7.2 kg. Fifty-one patients had the laparoscopic operation with a mean operation time of 102 ± 28 minutes. Forty-seven patients (90%) achieved relief from the symptoms either completely (67%) or partially (23%) at 3–6 months of follow-up. Significant improvement in postoperative PSV was found compared to the preoperative values, p<0.001. Five patients (10%) with no immediate effect of the operation, but two of them became free from symptoms during the mean study follow-up of 2.4 ± 2 years. Five patients (10%) had operative complications, including one trocar injury to the liver, one pneumothorax, and three cases of bleeding from the branches of CA. Two patients died of cancer disease during the study period. Only two patients (4%) had symptoms relapse, both later treated successfully.

Conclusion: Laparoscopic transperitoneal decompression provides most of the patients a persistent relief from MALS symptoms.

Keywords: median arcuate ligament syndrome, postprandial pain, laparoscopy, mesenteric ischemia

Background

MALS is a disorder known to be due to external compression of the CA by the fibrotic preaortic celiac ganglionic tissue and the median arcuate ligament.[1–3] External compression of the CA is a common radiological finding; however, it is asymptomatic in most patients.[4] The incidence of MALS has been estimated to be about 1 2/100,000 patient years.[5,6]

Symptoms of MALS are postprandial abdominal pain, nausea, vomiting, changes in eating habits, and ultimately, weight-loss. Many other more common diseases share these symptoms, and hence the diagnosis is often delayed. Abundant case reports have been published about MALS, but few large case series.[7–9] Since 2000, laparoscopic surgery has been incorporated as a preferred treatment of patients with MALS.[10] Different laparoscopic approaches have been applied to release the median arcuate ligament and dissect CA free from any constricting connective tissue.[11–14] Although there is a potential of operative injuries, the risk of complications is reported to be low during laparoscopic surgery for MALS.[7,11,12]

We present the results of our ten years of experience with MALS patients, with an aim to add to the collective evidence about its existence and increase our understanding of this long-debated and disputed disease.

Materials and Methods

The patients were referred to the Department of Vascular Surgery at the Oslo University Hospital from either primary health care or other departments or hospitals. The department is the only center in Norway with a dedicated group of physicians to investigate and treat patients with MALS. In addition, all MALS patients, despite treatment, are regularly followed-up with the circulation laboratory.

From March 2011 to August 2021, seventy-eight patients suspected of chronic mesenteric ischemia (CMI) were identified to have external compression of the CA on computed tomography angiography (CTA). Further investigation showed that twenty-one (27%) had an asymptomatic CA compression.[4] A total of fifty-seven patients were diagnosed as suffering from MALS and therefore eligible for laparoscopic surgery. Figure 1 illustrates the inclusion of the patients in this study.

A detailed medical history was taken, and a careful clinical examination was performed by a dedicated team of physicians at the Department of Vascular Surgery, Oslo University Hospital.

Symptoms of MALS were defined as postprandial pain in the epigastrium and upper abdomen, changes in food intake patterns (smaller food portions with increased frequency), and weight-loss. Other symptoms of autonomic characteristics like nausea, vomiting, diarrhea, constipation, palpitation, sweating, clammy skin, and nervosity were also registered. All patients included in the study had an extensive gastrointestinal investigation to exclude hepatic, pancreatic, and other gastrointestinal diseases prior to the inclusion. The patients were examined with duplex ultrasound and CTA before the surgery and were followed postoperatively, with duplex ultrasound and clinical examination, at 2–3 months, 6 months, 12 months, and yearly, thereafter.
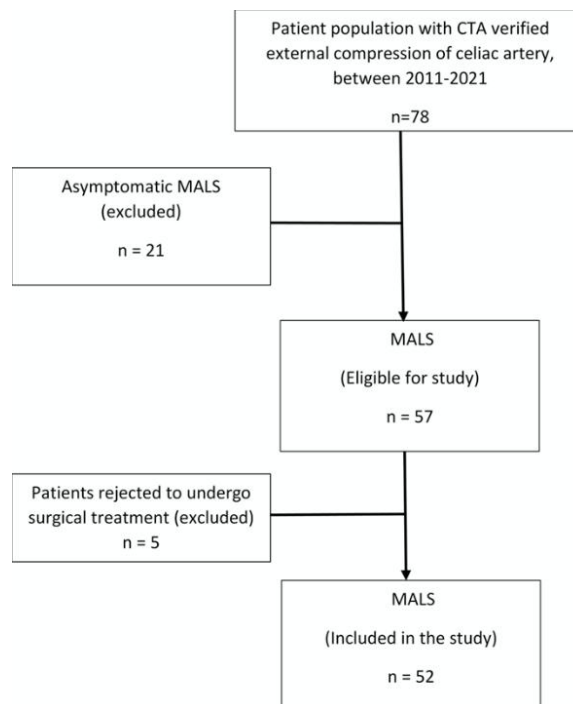

Figure 1 Flow chart of patients included in the study.
Abbreviations: CTA, computed tomography angiography; MALS, median arcuate ligament syndrome.

The patients with the symptoms of MALS and CTA verified stenosis ≥50% of the lumen diameter of the CA, or with a peak, systolic velocity (PSV) ≥2.0 m/s were diagnosed as MALS. The findings and the clinical status were discussed in a multiple-disciplinary team, comprising of vascular surgeons, intervention radiologists, gastroenterologists, and ultra-sound technicians before a decision for surgical treatment was made.

The study's primary endpoint was the relief from at least one of the MALS symptoms. Secondary endpoints were morbidity, recurrence of symptoms, and reinterventions.

Duplex Ultrasound

A dedicated, experienced physiologist (JH) performed a transabdominal duplex ultrasound, using a curvilinear transducer (C1-6) with Vivid E 95 scanner (General Electric Healthcare, Chicago, IL) on all the patients before and after the surgical treatment. All patients were examined after a minimum of six hours of fasting. Peak systolic velocity (PSV) of ≥ 2.0 m/s was considered as significant stenosis of the CA.[15] End diastolic velocity (EDV), flow profile, and the effect of respiration on the flow profile were also registered.

Computed Tomography Angiography (CTA)

Multi-sliced CTA (64 row-multidetector, Siemens Medical Systems; Forchheim, Germany) was obtained in deep inspiration and expiration of the abdominal aorta and the mesenteric arteries.[16] The scans were scrutinized in multiple plans. CA stenosis of ≥50% lumen reduction was considered significant stenosis (Figure 2).

Laparoscopic Decompression Technique

The operation was performed under general anesthesia and with the patient in the supine on a split-leg operation table. The operator is positioned between the legs and one assistant is on both sides of the table. Figure 3 shows the positions of the
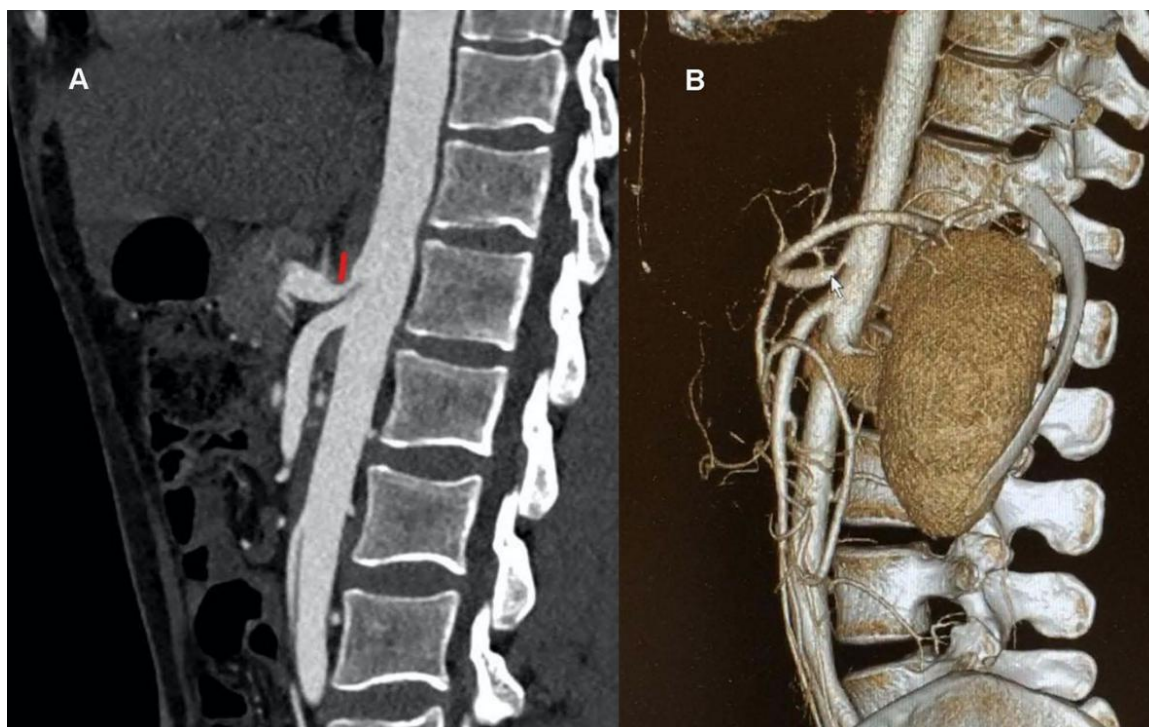

Figure 2 (**A**) Computed tomography angiography, sagittal view of aorta and celiac artery (CA) in a patient with MALS; redline points at the CA artery stenosis caused by the median arcuate ligament. (**B**) Three-dimension reconstruction of the CA stenosis due to external compression. The arrow points at the CA stenosis and the post-stenotic dilatation.

Figure 3 Trocar position on the abdominal wall for transperitoneal laparoscopic decompression of the celiac artery in a patient with median arcuate ligament syndrome.

trocars. A 30° video laparoscope (Olympus Corporation, Tokyo, Japan) was used through this trocar. The left liver lobe was elevated with Nathanson liver retractor (Cook Medical, Bloomington, Indiana, United States), placed through a small incision distally and to the left side of xiphisternum to achieve a good exposure of the lesser omentum. The liver retractor was held with an autostatic instrument stabilizer, ENDOBOY (ASFS Medic's, Niort, France). The gastro-hepatic ligament was incised along the upper border of the pancreas.

The patient was positioned in a reverse Trendelenburg, and if required, a slight distal retraction of the duodenum and pancreas by the assistant provided good exposure of the CA. The common hepatic artery was carefully approached along the upper border of the pancreas and followed centrally towards the CA bifurcation. Care was taken to keep a safe distance from the left gastric artery, and in a few cases (n = 3), also from the aberrant left hepatic artery. Ultracision Harmonic ACE+ (Ethicon Inc., Somerville, New Jersey, United States) and monopolar electro-cautery hook were alternately used to dissect celiac artery from any nerve or fibrous tissue on its cranial surface. The left gastric artery and the inferior phrenic arteries were respected and preserved. The right diaphragmatic crus and median arcuate ligament were divided, and dissection was continued 1–2 cm cranially on the aorta, proximal to the origin of CA. The decompression of the CA was considered accomplished only when the CA was completely skeletonized on its cranial surface (Figure 4). Fascia at the 12 mm trocar position was closed with Polysorb braided sutures (Medtronic), and the skin incisions were closed with intracutaneous sutures. No wound drain was used. The patients were allowed to take oral food on the same day after surgery. They were mobilized fully on the first postoperative day.

Ethics and Trial Registration

The study complies with the Declaration of Helsinki. The patients were prospectively registered in the database for chronic mesenteric ischemia since 2016, approved by the Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway (REK sør-øst B 2016/682). It was also registered in ClinicalTrails.org Protocol Registration and Results System (NCT02914912). Informed written consent was obtained from the patients for the treatment.
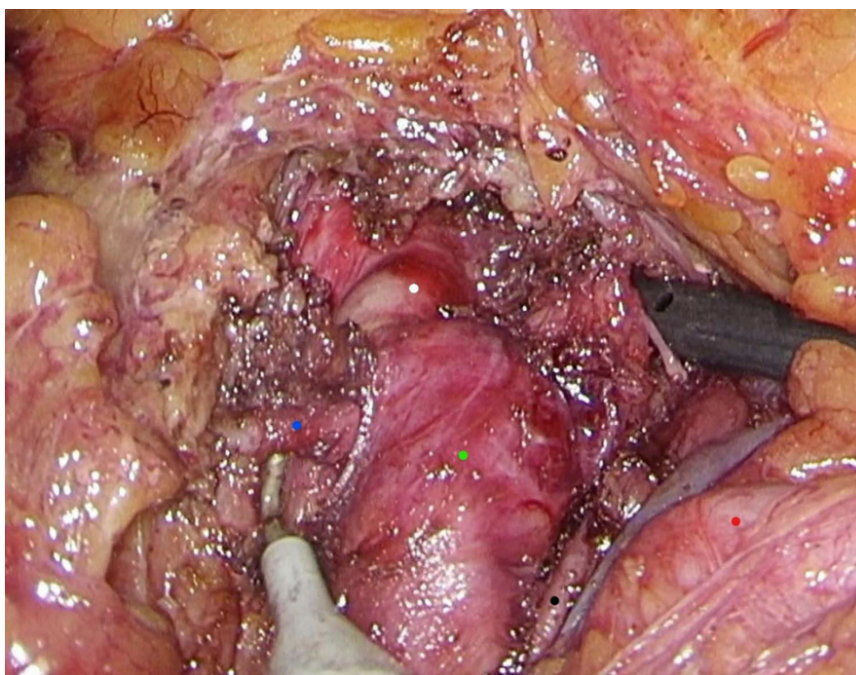
Figure 4 Transperitoneal laparoscopic celiac artery (CA) decompression for median arcuate ligament syndrome. The white dot represents the decompressed segment of CA originating from the aorta, and the green dot represents the post-stenotic dilated segment of the CA. The blue dot represents the right inferior phrenic artery. Black and red dots represent the left gastric artery and splenic artery, respectively.

Statistics

Normalized data are presented as mean values with standard deviations. Proportions are given in percentage. Changes after surgical intervention are investigated with the Mann–Whitney test. The statistical significance was set at 5% ($p < 0.05$).

Results

During ten years, fifty- two patients, with a mean age of 47 ± 21 years, underwent surgical treatment for MALS. All operations were performed by one surgeon (SSHK). Eighteen (35%) patients also suffered from depression and anxiety, and thirty-three (65%) used analgesics. Table 1 illustrates the coexisting diseases, and Table 2 summarizes patients' demographics, clinical findings, and investigation results.

The typical symptoms were postprandial abdominal pain, changes in eating patterns, and weight loss in fifty- one patients (98%). However, sixteen patients (31%) also had various autonomic symptoms like nausea, diarrhea, obstipation, palpitation, nervosity, chest pain, and clammy skin. Thirty-five (67%) patients also had abdominal pain during activity and exercise. Twenty-two (42%) patients had constant abdominal pain with postprandial aggravation. The patients in the study had a mean BMI of 21.6 ± 3.5 kg/m^2 and had a mean weight loss of 8.4 ± 7.2 kg. The mean duration of symptoms before their first evaluation with the vascular surgery department was 4.7 ± 3.5 years.

All patients were investigated with CTA. Four (8%) had the common hepatic artery origin from the superior mesenteric artery (SMA). One patient (patient 11) treated with open surgical decompression of the CA has had multiple laparotomies after colon cancer surgery and had a double-barrel colostomy due to enterocutaneous fistula. CTA disclosed external compression of both CA and SMA from a very hypertrophied diaphragmatic crux. After decompression, the patient underwent a successful reversal of the colostomy. The patient died of cancer disease two years after the decompression of the mesenteric arteries. Another patient (patient 13) also died of cancer two years after the laparoscopic decompression. After eight months, this patient had a relapse of postprandial abdominal pain and duplex ultrasound

Table 1 Comorbidities in the Patients Diagnosed with MALS

Comorbidities	Number of Patients 52 (%)
Heart disease	12 (23%)
Hypertension	20 (29%)
Dyslipidemia	17 (33%)
CVD	1 (2%)
Renal disease	3 (6%)
PAD	2 (4%)
Diabetes mellitus	5 (10%)
Gastritis/ peptic ulcer	22 (42%)
Prior intestinal/colon surgery	3 (6%)
Depression/anxiety	18 (35%)
COPD	8 (15%)
IBD	4 (8%)

Abbreviations: CVD, cerebrovascular disease; PAD, peripheral arterial disease; COPD, chronic obstructive pulmonary disease; IBD, irritable bowel disease.

confirmed PSV of 3.0 m/s; CTA confirmed stenosis of CA (although better lumen diameter than before laparoscopic decompression). The patient received a stent in the CA and again got relief from symptoms.

One patient (patient 51) included in his study was previously treated with a stent in CA for atherosclerotic stenosis, with an excellent response on symptoms. However, within three months, the patient experienced a relapse of symptoms. Investigation with duplex ultrasound showed a significant increase in PSV to 4.0 m/s, and CTA demonstrated a stent fracture in the CA due to a previously overlooked external compression from the median arcuate ligament. The patient underwent laparoscopic decompression and realignment of the fractured stent the day after surgery. PSV normalized after the treatment, and at three months follow-up, the patient was without any symptoms and had gained weight.

The mean operation time for the study population was 102 ±28 minutes. Perioperative findings and results are summarized in Table 3. Forty-seven (90%) patients achieved an either complete or partial improvement of their symptoms at follow-up time-points of 3–6 months. Thirty-five (67%) patients had a complete recovery from their symptoms, and another twelve (23%) patients had partial improvement of their symptoms. The patients in this study have been followed for a mean period of 2.4 ±2 years. Mean weight gain was 5 ±4.4 kg at one-year follow-up after the surgery.

Five patients (10%) had no improvement in their preoperative symptoms at 3–6 months of follow-up. One of these was lost to follow-up. Another had postoperative PSV of 4.0 m/s and on CTA although a better postoperative CA lumen diameter, but still >50% stenosis in the CA. This patient had known occlusion in the SMA from before. The patient is scheduled to undergo endovascular CA and SMA occlusion treatment. In another patient, both CTA and DUS at follow-up do not show any significant residual stenosis of the CA. The patient had microscopic hematuria and is under further investigation for superior mesenteric artery syndrome and Nut-Cracker syndrome. Two patients were referred to the pain clinic, and at 2–3 years follow-up, they no longer had abdominal pain, did not use analgesics, and could eat regular food.

Five patients (10%) had complications during the surgery. In one patient (patient 44) with a body height of 155 cm, the left liver lobe was perforated during 12 mm trocar insertion. However, there was no serious operative bleeding, and the patient recovered without any early or late sequelae. Two patients (patients 1 and 43) had bleeding from the inferior phrenic artery. In one of these patients (patient 1), a laparoscopic suturing for hemostasis led to persistent stenosis of the CA, confirmed on a postoperative CTA. However, the lumen diameter of CA was still better than before the decompression, and the patient achieved symptom relief; therefore, no intervention was done to improve the lumen diameter. In the other patient, an endoscopic clip was used for hemostasis. One patient (patient 5) had an injury to the left gastric artery, which had to be ligated with endo-clips. The patient had a complete remission of her symptoms through a follow-up period of 5 years. In one patient (patient 12), the tip of the liver retractor perforated the diaphragm and caused pneumothorax. The laparoscopic decompression could be completed after the patient received a thoracic pleural drain.

Table 2 Patient Characteristics, Symptoms, and Investigations

Patient/ Year	Sex/ Age	BMI	W t Loss (kg)	Duration (Years)	Symptoms			Autonomic Symptoms	Preoperative PSV/ EDV m /s	Smoking
					PPP	Meal	Activity			
1/2011	F/17	23	2	6	Yes	Yes	Yes	Yes	3.0/0.6	No
2/2011	F/17	19	7	3	Yes	Yes	Yes	Yes	3.3/0.7	No
3/2012	M/24	20	2	3	Yes	Yes	Yes	Yes	3.4/1.2	No
4/2012	M/25	23	16	2	Yes	Yes	Yes	Yes	3.7/1.0	No
5/2012	F/79	18	10	2	Yes	Yes	Yes	No	2.0/0.6	Yes
6/2013	M/16	20	1	6	Yes	No	Yes	Yes	1.9/0.6	No
7/2013	F/51	21	15	4	Yes	Yes	Yes	No	§	No
8/2014	F/28	23	3	4	Yes	Yes	Yes	No	**	No
9/2014	M/64	20	10	8	Yes	Yes	No	No	2.3/1.1	No
10/2015	F/41	16	5	3	Yes	Yes	Yes	No	2.6/0.8	No
11/2015	F/63	15	15	5	Yes	Yes	No	No	**	No
12/2015	F/65	19	1	5	Yes	Yes	Yes	No	3.0/1.0	No
13/2016	F/56	20	1	5	Yes	Yes	Yes	No	**	No
14/2017	F/28	18	4	2	Yes	Yes	No	No	3.2/1.0	No
15/2017	M/21	19	7	1.5	Yes	Yes	Yes	Yes	2.0/0.6	Yes
16/2017	F/22	24	2	11	Yes	Yes	Yes	No	2.5/1.0	No
17/2017	F/60	21	5	10	Yes	Yes	Yes	No	**	Yes
18/2017	M/46	23	1	11	Yes	Yes	No	No	**	No
19/2017	M/45	20	7	1	Yes	Yes	Yes	Yes	2.6/0.8	No
20/2018	M/70	24	0	5	Yes	Yes	Yes	No	1.7/0.4	No
21/2018	M/29	31	27	2	Yes	Yes	Yes	Yes	2.2/0.6	Yes
22/2018	F/40	22	30	6	Yes	Yes	No	No	1.8/0.3	Yes
23/2019	F/26	19	7	8	Yes	Yes	Yes	No	2.8/1.0	No
24/2019	F/23	19	30	1	Yes	Yes	Yes	Yes	§	No
25/2019	F/45	20	3	20	Yes	Yes	Yes	No	2.9/1.0	Yes
26/2019	M/13	19	1	0.5	Yes	Yes	Yes	No	3.1/1.1	No
27/2019	M/22	18	10	1	Yes	Yes	Yes	No	**	No
28/2019	F/64	24	18	1	Yes	Yes	No	Yes	3.0/1.0	Yes
29/2019	F/62	17	9	5	Yes	Yes	N	No	4.0/1.6	Yes
30/2019	M/66	23	20	8	Yes	Yes	No	No	3.0/1.0	No
31/2020	F/80	23	8	3	Yes	Yes	No	No	2.7/1.0	Yes
32/2020	F/70	23	7	5	Yes	Yes	Yes	Yes	3.2/0.8	Yes
33/2020	F/32	17	9	2	Yes	Yes	Yes	No	3.0/1.0	No
34/2020	F/34	23	11	5	Yes	Yes	Yes	Yes	3.6/1.2	Yes
35/2020	M/36	28	0	3	Yes	Yes	Yes	Yes	2.8/1.5	No
36/2020	F/57	33	9	3	Yes	Yes	No	No	3.8/1.3	Yes
37/2020	F/31	22	2	10	Yes	Yes	Yes	No	3.0/1.1	Yes
38/2020	F/32	26	3	8	Yes	Yes	Yes	Yes	2.7/1.2	No
39/2020	M/69	22	5	4	Yes	Yes	No	No	**	No
40/2020	M/73	26	1	5	Yes	Yes	No	Yes	§	Yes
41/2020	M/75	25	7	6	Yes	Yes	Yes	Yes	3.5/1.2	No
42/2020	F/38	21	10	2	Yes	Yes	Yes	No	2.5/1.0	No
43/2021	M/50	25	15	0.5	Yes	Yes	No	No	**	No
44/2021	F/69	18	15	5	Yes	Yes	Yes	No	3.2/1.1	No
45/2021	F/80	22	1	1	Yes	Yes	No	Yes	3.2/1.2	No
46/2021	F/69	22	7	0.5	Yes	Yes	Yes	No	4.0/1.0	No
47/2021	F/77	19	10	7	Yes	Yes	No	No	3.2/1.3	Yes
48/2021	F/15	25	10	5	Yes	Yes	Yes	No	2.5/1.0	No

(*Continued*)

Table 2 (Continued).

Patient/ Year	Sex/ Age	BMI	Wt Loss (kg)	Duration (Years)	Symptoms			Autonomic Symptoms	Preoperative PSV/ EDV m/s	Smoking
					PPP	Meal	Act ivity			
49/2021	F/67	21	3	2	Yes	Yes	Yes	No	2.0/0.8	No
50/2021	F/39	21	10	5	Yes	Yes	No	No	1.8/0.3	Yes
51/2021	M/45	23	7	7	Yes	Yes	No	No	1.2/0.5	Yes
52/2021	M/58	26	3	3	Yes	Yes	Yes	No	2.2/1.0	Yes

Notes: §Not performed; **Either not visualized CA (Celiac artery) or unreliable PSV/EDV.
Abbreviations: BMI, Body mass index; Wt, Weight; PPP, Postprandial pain; PSV, Peak systolic velocity; EDV, End diastolic velocity.

In one patient (patient 11) with external compression of both CA and SMA, a stent was placed in SMA to ensure patency and sufficient circulation to the intestine for a successful colostomy reversal. In another patient (patient 47), a stent in CA was placed a day after laparoscopic decompression. SMA had a long occlusion in this patient, and the CA was extremely gracile despite laparoscopic decompression.

One patient (patient 35) with partial improvement in symptoms developed severe symptoms and had multiple hospital admissions one year post-operatively. Despite follow-up with a pain clinic, the patient had an extremely high daily use of opioids and other analgesics. Based on CTA findings of residual stenosis, severe symptoms of abdominal pain, and steadily increasing use of analgesics, the patients underwent re-exploration of CA with open surgery. A fibrous scar, possibly compressing the CA from lateral sides, was the only finding during the operation. No external compression on the cranial surface of the CA was found. Histopathology of the biopsy tissue confirmed scar tissue and excluded any neurinoma. The patient has been followed in one year and partially improved his symptoms.

Preoperative duplex ultrasound with PSV measurement in CA was successfully performed in thirty-nine patients (78%). Mean preoperative PSV was 2.8 ±0.6 m/s. In eight patients (16%), the CA could not be visualized, or a reliable flow measurement could not be performed. Two of these eight patients had near occlusion of the CA. In three patients (6%), a preoperative duplex ultrasound and velocity measurements were not performed. Postoperative duplex ultrasound with PSV could be performed in 48 patients (96%). Median postoperative PSV was 1.6 ±0.5 m/s. One patient was lost to follow-up, and CA could not be visualized properly in another. Comparisons of the pre-and postoperative duplex ultrasound flow velocities showed a statistically significant improvement in PSV and EDV after the surgical treatment (p< 0.001) (Figure 5).

All patients with either complete or partial decompression gave positive feedback about the effect of the operative treatment. In the patients' opinion, MALS decompression had been helpful, and they recommended this treatment to the other patients suffering from MALS.

Discussion

This study demonstrates symptom relief achieved in 90% of patients with MALS operated with laparoscopic decompression. The symptom relief was either complete (67%) or partial (23%) within 3–6 months after laparoscopic decompression of the CA. The operation time is shorter than the earlier reports.[9,11] The majority of the patients could be fully mobilized and discharged from the hospital the day after surgery. Only a few patients (n=2) had recurrent symptoms requiring intervention during a mean follow-up period of 2.4 years. This finding confirms that the patients in this study seem to have a persistent relief from the symptoms after laparoscopic surgery. Furthermore, it is interesting to observe that even some of the patients (n = 2) who did not have any effect of the surgical treatment at 3–6 months of follow-up time point did achieve relief from any symptoms of MALS later time-point.

The patients in our study group had a mean duration of symptoms for 4.7 years prior to the referral to our Vascular Surgery Department. The doctors' poor understanding of this disease may contribute to the delay in its diagnosis and treatment. In 33% of patients, symptoms of autonomic activation were found in addition to the typical

Table 3 Postoperative Patients' Characteristics and Clinical Data

Patient	OT (m in)	Operative Complication	Hosp Stay	Symptoms Relief			Adjut ant PTA/Stent / Reoperation	Postop PSV/ EDV cm/s	Follow-Up Years	Postop CTA
				a	b	c				
1	195	Bleeding IPA	3	Yes				2.1/0.6	10	Yes
2	87		3		Yes			1.8/0.4	2	Yes
3	80		2			Yes		1.5/0.3	5	Yes
4	79		6	Yes				1.7/0.5	9	Yes
5	83	Injury to left gastric artery	4	Yes				**	5	Yes
6	142		3	Yes				1.5/0.2	5	No
7	100		4	Yes				1.6/0.4	2	Yes
8	110		3	Yes				1.3/0.3	7	No
9	85		2	Yes				1.4/0.3	3	No
10	120		5	Yes				1.3/0.3	5	No
11	83		4	Yes			Stent SMA	1.1/0.2	3	Yes
12	143	Pneumothorax	4	Yes				1.2/0.3	5	No
13	93		3	Yes			Stent TC	3.0/1.0	2	Yes
14	127		5	Yes				1.7/0.4	3	No
15	84		3		Yes			1.5/0.4	3	Yes
16	101		3	Yes				1.4/0.5	3	No
17	126		2		Yes			1.5/0.3	3	Yes
18	118		2		Yes			1.8/0.5	2	Yes
19	121		2		Yes			1.5/0.3	3	Yes
20	68		4	Yes				1.3/0.2	2	Yes
21	125		4		Yes			1.2/0.3	3	Yes
22	102		2	Yes				1.2/0.1	3	No
23	78		4		Yes			1.8/0.3	2	Yes
24	107		6			Yes		§	0.25	No
25	95		2	Yes				1.5/0.5	2	No
26	105		2	Yes				1.6/0.4	2	No
27	96		1	Yes				1.4/0.3	2	No
28	91		2		Yes			2.1/0.8	2	Yes
29	81		3		Yes			1.4/0.4	2	No
30	166		2	Yes				1.5/0.3	2	No
31	77		2			Yes		1.8/0.5	2	Yes
32	96		2	Yes				1.5/0.5	2	No
33	130		4			Yes		1.8/0.6	2	Yes
34	105		4	Yes				1.5/0.4	1	No
35	125		3		Yes		Laparotomy	1.3/03	1	Yes
36	91		2		Yes			1.5/0.4	1	No
37	61		3		Yes			1.8/0.3	1	No
38	82		2	Yes				2.3/0.7	1	No
39	150		2	Yes				1.5/0.4	1	No
40	91		2	Yes				1.2/0.1	1	No
41	111		2	Yes				1.3/0.1	1	No
42	86		4	Yes				1.5/0.3	1.5	Yes
43	163	Bleeding IPA	2			Yes		4.0/1.5	0.5	Yes
44	84	Liver injury	2	Yes				1.5/0.3	1	No
45	101		2	Yes				2.0/0.3	1	No
46	49		2	Yes				1.2/0.2	1	No
47	58		3	Yes			Stent CA	1.4/0.3	0.5	No
48	90		4	Yes				1.3/0.2	0.5	No

(*Continued*)

Table 3 (Continued).

Patient	OT (min)	Operative Complication	Hosp Stay	Symptom s Relief			Adjutant PTA/Stent/ Reoperation	Postop PSV/ EDV cm /s	Follow- Up Years	Postop CTA
				a	b	c				
49	76		5	Yes				1.4/0.1	0.5	No
50	102		2	Yes			Stent CA	1.1/0.2	0.25	No
51	90		2	Yes				1.4/0.2	0.25	No
52	100		2	Yes				1.3/0.3	0.25	No

Notes: [a]Complete relief, [b]Partial relief, [c]No effect, **Could not be visualized properly with duplex ultrasound, [§]Lost to follow-up.
Abbreviations: OT, operation time; Hosp, hospital; IPA, inferior phrenic artery; PTA, percutaneous transluminal angioplasty; PSV, peak systolic velocity; EDV, end diastolic velocity.

symptoms of MALS. This clinical picture could have contributed to the delay in referral and diagnosis. The patients experienced that when the doctors had difficulty interpreting their symptoms, they were assigned a psychiatric diagnosis. Thirty-five percent of the patients in this study had depression and anxiety diagnoses.[8] Careful history taking during the investigation revealed that these psychiatric diagnoses were assigned to them after the start of their symptoms.

Laparoscopic decompression of the CA and a surgical dissection until completely skeletonized CA is a minimally invasive surgical technique for treating MALS.[12] However, the patients are also at risk of surgical complications with this technique.[7,9,11] Injury to the left liver lobe could have been avoided by placing the trocar under direct vision through the abdominal wall, especially in a patient with short height. Placement of the trocar through the ventral abdominal wall for a 30° endoscope, below the level of the CA origin from the aorta, may not provide a good vision of the artery, mainly where it lies compressed behind the median arcuate ligament. If the trocar is not placed high enough, the upper margin of the pancreas may hamper the surgical field. Alternatively, a 50° laparoscope or traction of the left gastric artery and
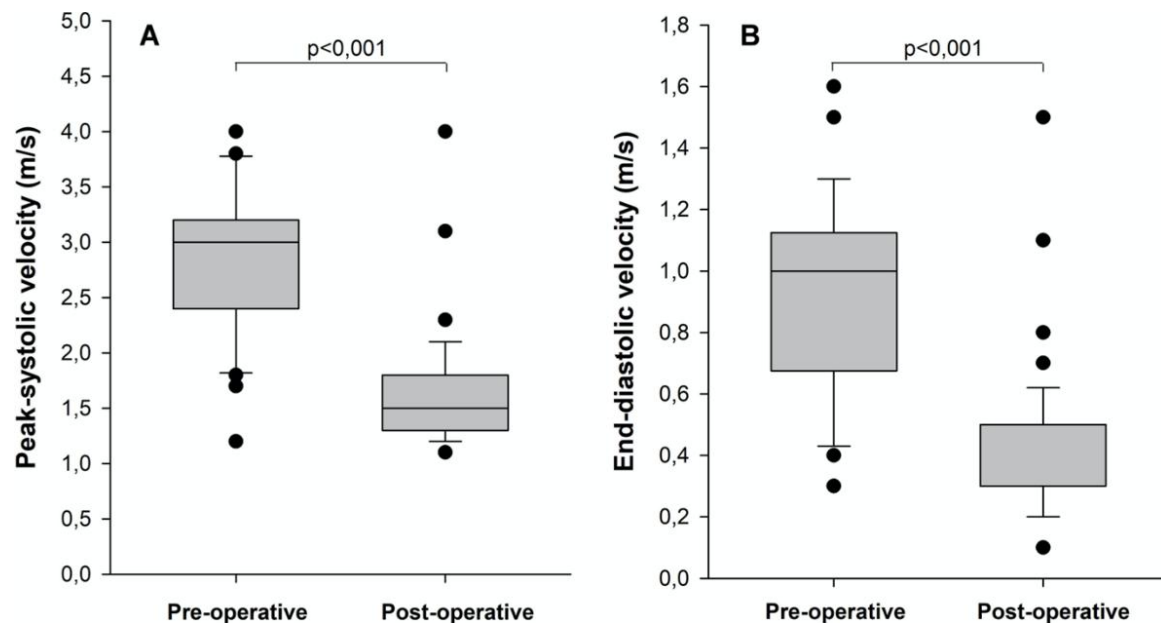

Figure 5 Changes in the transabdominal duplex ultrasound flow velocities before and after the laparoscopic decompression of the CA in 52 patients with median arcuate ligament syndrome. (**A**) Peak systolic velocity (PSV); (**B**) end-diastolic velocity (EDV). Boxes represent the interquartile range with the median as a horizontal line compared with the Mann- Whitney *U*-test.

common hepatic artery with tape can be used to facilitate the free dissection of the CA.[13] CTA scans may help to plan the optimal trocar position for the laparoscope.

In the patient with the injury to the left gastric artery, hemostasis was achieved with surgical clips. The patient still had complete recovery despite occlusion of the left gastric artery and gained weight during the follow-up. It was probably due to the incremental improvement in circulation through the splenic artery and the gastroepiploic artery arcade.

Through a physiological test, gastric exercise tonometry, it has been shown that MALS has an ischemic origin.[17] However, the instruments for conduction of this test have not been available to be used in our study population. In a previous study, we have found reduced preoperative circulation with the help of gastroscopy-assisted transmucosal laser Doppler flowmetry and visible light spectroscopy in patients with MALS compared to a control group of healthy individuals.[18] It was a small study population, and the transmucosal microcirculatory assessment was performed in a fasting state. The increase in the transmucosal circulation could partially have occurred due to the division of the nerve fibers overlying the ventral surface of the celiac artery. However, during the laparoscopic decompression, the segment of CA underlying the median arcuate ligament had relatively sparsely crossed by any nerve fibers. In a study by Weber et al, a strong correlation between the celiac plexus block and a positive response to operation has been shown in the patients with MALS.[19] Although 20% of patients had no pain relief after celiac plexus block, but still had a positive response to a subsequent surgery. They found that 14% had an unsuccessful effect of surgery despite a positive effect of preoperative celiac plexus block. Previously, neurolysis with phenol or alcohol in otherwise healthy patients has resulted in complications as serious as permanent paraplegia.[20–22] In the present study, all patients with partial effect and in need of opioid analgesics, particularly those with no effect of the operation, were referred to the pain clinic.

We have been restrictive about the adjuvant endovascular treatment of the patients having the partial effect of the laparoscopic decompression. In the multi-disciplinary team, it was concluded that the clinical consequences of restenosis or occlusion of the stent are probably more severe; therefore, we chose to follow these patients.[23,24] Postoperative CA lumen diameter, although better than the diameter before the operation, was still around 50% in some cases. However, PSV in most of these patients was reduced below 2.0 m/s. Later in the follow-up, most of these patients had a stable clinical picture, ie, better than before surgery, or even complete relief from the symptoms. Previously, in a small study on 13 patients, a retrospective analysis showed better outcomes and results for patients with >70 stenoses of the CA. However, the study was underpowered to find that difference besides two of three patients with CTA-verified stenosis grade 50–70%, remained symptom-free during a median follow-up period of 117 months.[25]

Despite controversies about its pathophysiology, most of the patients with MALS seem to have a good effect of laparoscopic decompression.[26] Laparoscopic surgical technique will continue to be regarded as one of the most minimally invasive surgical procedures for treating MALS. The patients with MALS should preferably be investigated and follow up with the dedicated departments.

Conclusion

Laparoscopic decompression of the celiac artery seems to provide persistent relief from symptoms in most patients with median arcuate ligament syndrome.

Data Sharing Statement

Detailed individual participant data that underlie the results reported in the article is given in Tables 2 and 3. Further de-identified data can be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose. The proposal should be directed to the project leader, Syed Sajid Hussain Kazmi, syekaz@ous-hf.no. Data requestors will need to sign a data access agreement to gain access.

Author Contributions

All authors made a significant contribution to the work reported, whether that is the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing

the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Paper 5

ORIGINAL RESEARCH

Plasma α-Glutathione S-Transferase in Patients with Chronic Mesenteric Ischemia and Median Arcuate Ligament Syndrome

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Background: Chronic mesenteric ischemia (CMI) due to either atherosclerosis of the mesenteric arteries or median arcuate ligament syndrome (MALS) is an underdiagnosed entity. The etiology of MALS and its existence have been debated and questioned. We aimed to identify plasma biomarkers indicating mesenteric ischemia in patients with CMI and MALS.

Methods: Plasma α-glutathione S-transferase (α-GST), intestinal fatty acid-binding protein (I-FABP), citrulline, and ischemia modified albumin (IMA) were analyzed in fifty-eight patients with CMI (Group A, n=44) and MALS (Group B, n=14) before and after revascularization. The plasma levels of these potential biomarkers were compared with those of healthy individuals (Group C, n=16). Group comparison was performed with the Mann–Whitney U-test. Cross-tabulation and its derivatives were obtained. Receiver operating characteristic (ROC) curves and area under the curve (AUC) were calculated.

Results: Plasma levels of α-GST were significantly raised in the patients with CMI (7.8 ng/mL, p<0.001) and MALS (8.4 ng/mL, p<0.001), as compared with the control Group C (3.3 ng/mL). The threshold for normal median plasma α-GST levels of 4 ng/mL yielded a sensitivity of 93% and 86%, specificity of 86% and 88%, respectively, for the diagnosis of CMI due to atherosclerosis and MALS. AUC of ROC curves was 0.96 (p<0.0001) for CMI and 0.85 (p<0.002) for MALS. The patient groups did not differ from the healthy controls in any other biomarkers.

Conclusion: Plasma α-GST levels are elevated in CMI and MALS patients. Elevated plasma levels of α-GST suggest ischemia as the etiology of MALS.

Keywords: biomarker, chronic mesenteric ischemia, intestinal ischemia, median arcuate ligament syndrome, α-GST

Introduction

The clinical presentation of chronic mesenteric ischemia (CMI), described as abdominal pain with postprandial worsening, results from intestinal hypoperfusion due to insufficient blood supply during times of increased intestinal metabolic demand.[1,2] The resulting food aversion may lead to weight loss in these patients. However, these symptoms are not specific to CMI.[3–5] Particularly, in patients with median arcuate ligament syndrome (MALS), which is a condition with stenosis of a single mesenteric artery caused by an external compression from the median arcuate ligament, it has long been debated whether the symptoms are due to intestinal ischemia or merely a neurogenic disorder.[6–8]

The diagnosis of CMI and MALS is usually consensus-based. It relies on the exclusion of alternative diagnoses and the verification of stenosis or occlusion of one or more mesenteric arteries on computed tomography angiography (CTA) and duplex ultrasound (DUS).[9]

A vast array of biomarkers for diagnosing mesenteric ischemia have previously been evaluated. These biomarkers are released from enterocytes injured by ischemia, ie, α-glutathione S-transferase (α-GST), intestinal fatty acid-binding

protein (I-FABP), and citrulline.[10–14] α GST is a light molecular weight iso-enzyme, particularly in high concentrations in hepatocytes. It has a half-life of less than 60 minutes.[15–17] IFABP is a cytosolic enterocyte protein released by the dying mature enterocytes. It is a biomarker of high sensitivity and specificity for acute mesenteric ischemia.[18] Furthermore, increased plasma levels of IFABP have also been found in patients with chronic mesenteric ischemia.[19] Citrulline is a non-protein amino acid synthesized abundantly by the small intestine's enterocytes. It is thought to be a suitable biomarker of enterocyte function, and its plasma levels are expected to decrease in conditions like mucositis and intestinal ischemia.[12,13] In addition, ischemia modified albumin (IMA) is elevated in plasma from patients with acute mesenteric ischemia.[20] Currently, clinical and laboratory investigations of these mesenteric ischemia biomarkers are exclusively performed in patients with acute mesenteric ischemia. Still, none of these has shown adequate sensitivity to be used as a screening test for acute mesenteric ischemia, and consequently, they are not suitable for routine clinical practice.[21–23] Besides, these biomarkers have pathological plasma levels in the case of liver and renal diseases.[12,24,25] On the other hand, studies investigating CMI patients for ischemia biomarkers are lacking.[22]

We aimed to investigate ischemia biomarkers (plasma α-GST, I-FABP, Citrulline, and IMA) in patients with CMI due to atherosclerotic changes or MALS. We hypothesized that patients with CMI have increased plasma levels of mesenteric ischemia biomarkers compared to healthy individuals.

Methods

Between 2016 and 2020, patients with a consensus diagnosis of CMI were included in this study at the Department of Vascular Surgery, Oslo University Hospital. The included patients had postprandial abdominal pain, changes in food intake patterns, and weight loss. All patients with CMI had an extensive gastrointestinal workup before inclusion in the study to exclude other more common causes of abdominal pain, food aversion, and weight loss. Based on the CTA and DUS findings, the patients were divided into Group A (atherosclerotic CMI) and Group B (MALS). A group of healthy individuals (Group C) served as the control group (Figure 1). The healthy Group C individuals did not use any medicines and had no known systemic disease.

An experienced physiologist in all patients performed Transabdominal DUS. The patients were investigated in at least 6 hours of fasting state. A peak systolic velocity (PSV) of ≥200 cm/s in the celiac artery or ≥275 cm/s in the superior mesenteric artery was considered clinically significant. [26,27] DUS was repeated at follow-up at 3, 6, 12 months, and yearly after that.

The patients were investigated with 1 mm thickness, multidetector CTA (64 row-multidetector, Siemens Medical Systems: Florsheim, Germany). The scans were examined in multiple plains and confirmed ≥50% stenosis of the celiac and/or superior mesenteric artery. In the patients with MALS, a CTA acquired in the deep expiration phase confirmed ≥50% stenosis of the celiac artery lumen caused by an external compression from the median arcuate ligament. The patients with CTA and/or DUS verified ≥50% of the celiac artery and/ or superior mesenteric artery but without symptoms of chronic mesenteric ischemia were excluded. Also, patients with abdominal pain but with ≤50% stenosis of the celiac artery and/or superior mesenteric artery were not included in the study.
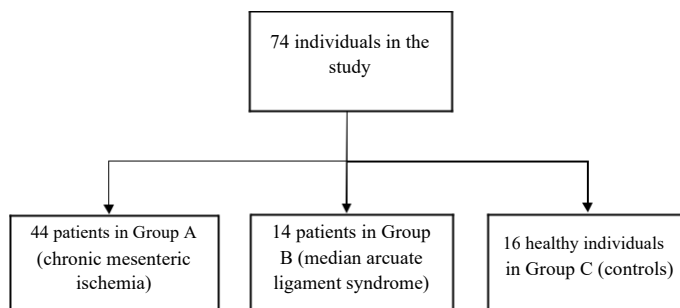

Figure 1 Flow chart of study individuals.

Blood Samples

Blood samples were drawn from the median cubital vein, with the patients in at least 6 hours of fasting. Ethylenediaminetetraacetic acid (EDTA) tubes were placed on ice before sampling and centrifuged for 15 minutes at four °C, 3000 rpm, within 30 minutes after sampling. Plasma samples were frozen in Nunc polypropylene vials and stored at −80 °C until analysis. In Group A and B patients, venous blood samples were taken before and three months after surgical or radiological revascularization. Blood samples were drawn only once from the healthy controls in Group C.

The blood samples were analyzed at the Hormone Laboratory at Oslo University Hospital, Aker, Norway. The ELISA kits for I-FABP, citrulline, and IMA had test detection ranges of 0.16–10 ng/mL, 5.0–100 nmol/mL, and 7.8–500 ng/mL, respectively. At the same time, the kit for α-GST had a detection range of 0.156 ng/mL-10 ng/mL. The ELISA kits for citrulline (MBS723693), I-FABP (MBS2507811), and IMA (MBS760561) were provided by MyBioSource, San Diego, CA, USA, and the ELISA kit for α-GST(CSB-E08906h), by Cusabio Technology, Houston, TX, USA. The intra-assay and inter-assay coefficient of variation (CV) for ELISA kits were <10% and <12%, <6.3% and 6%, <8% and <10%, <8% and <10%, respectively, for Citrulline, IFABP, IMA, and α-GST. The assay procedures accompanying the ELISA kits were followed. The results of optic density measurements were corrected for the dilution factor to obtain the exact plasma concentration of the biomarker. In addition, plasma levels of p-amylase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), C-reactive protein (CRP), and creatinine were determined. AMY-P, ASTLP, ALTLP, ALP2, CREP2, and CRPL3 kits were utilized, and samples were analyzed on Cobas 6000. The laboratory technician was blinded for the study groups.

Statistical Analysis

Continuous data are presented as median and interquartile ranges and analyzed with the Mann–Whitney U-test. Categorical data are presented as proportions and percentages. A p-value of <0.05 was considered statistically significant. Contingency tables were used to analyze categorical data and calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy (OA). The diagnostic performance of the biomarkers was analyzed with a Receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was calculated to estimate the diagnostic accuracy.[28–30] Sigma Plot version 14.0 (Systat Software, San Jose, CA, USA) was used for data analysis.

The study complies with the Declaration of Helsinki. The Regional Committees for Medical and Health Research Ethics in the South-Eastern region of Norway approved the study (REK Sør-Øst B 2016/682-1). It was also registered on www.clinicaltrials.gov (NCT02914912). All study participants provided informed written consent.

Results

The study included fifty- eight patients consecutively and divided into Group A (CMI, n=44) and Group B (MALS, n=14). Thirty-six (62%) were females. Sixteen healthy individuals constituted the controls in Group C. Thirty patients in Group A and all in Group B underwent revascularization. Patients' demographics are presented in Table 1. Fourteen patients in Group A did not receive revascularization. Nine of these patients had extensive atherosclerotic changes in the affected artery, CA (n=4) or SMA (n=3), or both arteries (n=2). Three patients refused to have any surgical or interventional treatment, and two patients were still waiting for treatment.

Plasma Levels of α-GST

Median plasma α-GST levels were significantly raised in both Group A (7.8 ng/mL, p<0.001) and Group B (8.4 ng/mL, p<0.001) compared with Group C (3.3 ng/mL). After revascularization in thirty patients in Group A, a statistically significant reduction in the plasma α-GST levels was found (p=0.023). However, the median plasma levels of α-GST in the patients in a Group A were still significantly higher than in the control group (5.5 ng/mL vs 3.3 ng/mL, p=0.001). In the patients with MALS (Group B), postoperative median plasma α-GST levels were no longer statistically different from the control group. Figure 2 illustrates the plasma α-GST levels in all three study groups.

Table 1 Patient Demographics

	Group A (CMI) n=44	Group B (MALS) n=14	Group C (control) n=16
Age (years)	70 (13) p<0.001*	45 (36) *0.358	45 (21)
Female	26 (59%)	10 (71%)	6 (38%)
BMI (kg/m^2)	20 (3.5) *0.999	21.5 (3.3) *0.935	23.5 (2.3)
Weightloss (kg)	9 (9.3)	7 (6.75)	0
Symptom duration (years)	3 (3)	5 (6.5)	-
CVD	19 (43%)	2 (14%)	0
Hypertension	28 (63%)	4 (29%)	1
Hypercholesterolemia	32 (73%)	3 (21%)	2
Smoking	25 (57%)	7 (50%)	0
COPD	10 (22%)	0	0
Diabetes mellitusi	11 (25%)	1 (7%)	0
Renal insuffiency	5 (11%)	0	0
Gastritis	24 (55%)	6 (43%)	0

Notes: *Mann- Whitney U-test comparison with the control group C. Data is present as median (IQR) and n (%). **Abbreviations**: CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome; IQR, interquartile range; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease.

By using >4 ng/mL as an upper threshold for normal plasma α-GST levels, the cross-tabulation analysis showed a sensitivity and specificity of 93% (95% CI 0.78 1.0) and 88% (95% CI 0.69 1.1), respectively, for the diagnosis of CMI. The test had a type I error of 12.5% and a type II error of 6.8%. A PPV of 95% and an NPV of 82% for a 73% prevalence of CMI in the study population were calculated. The overall accuracy of the test was 92%. Chi-Square test showed 2-sided p value <0.001. (Table 2)

In the patients with MALS, a sensitivity of 86% (95% CI 0.65 1.1) and a specificity of 88% (95% CI 0.69 1.1) were calculated. Overall accuracy was 87%, and the Chi-Square test showed a 2-sided p-value <0.001. (Table 2)

The patients with CMI in Group A had an AUC of 0.96 (p<0.000, 95% CI 0.91 1.0), whereas the patients with MALS in Group B had an AUC of 0.85 (p=0.001, 95% CI 0.66 1.0) (Figure 3).
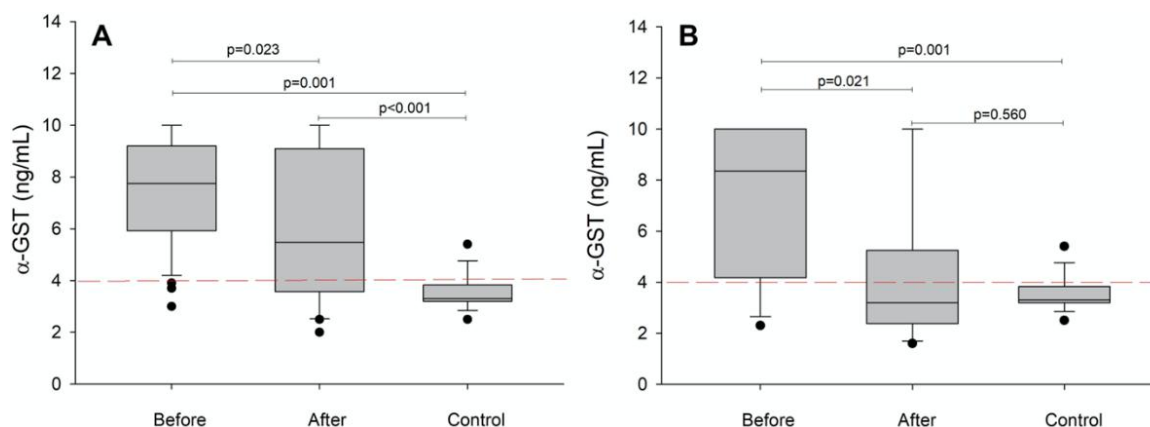

Figure 2 (**A**) Median plasma α-glutathione S transferase (α-GST) levels in the patients with chronic mesenteric ischemia due to atherosclerosis (Group A, n=30), and (**B**) median arcuate ligament syndrome (Group B, n=14), before and after the surgical or endovascular intervention, compared with healthy individuals (Group C, n=16). Boxplot represents the 10th, 25th, 50th, 75th, and 90th percentile, with the black horizontal line representing the median. Filled black dots represent outliers. The dotted horizontal line in red represents the upper limits of normal α-GST plasma level. Mann- Whitney U-test is used to test differences between the groups.

Table 2 Analysis of Plasma α-GST >4 ng/mL as the Cut-off for the Diagnosis of Patients with CMI of Atherosclerotic Origin and MALS

CMI				
Plasmaα-GST Cut-off >4 ng/mL	Positive	Negative	PPV	NPV
Positive	27	2	93%	
Negative	3	14		82%
Sensitivity	90%			OA 89%
Specificty		88%		
MALS				
Positive	12	2	86%	
Negative	2	14		88%
Sensitivity	86%			OA 87%
Specificty		88%		

Abbreviations: α-GST, α-glutathione S-transferase; CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome; PPV, positive predictive value; NPV, negative predictive value; OA, overall accuracy.

Plasma Levels of the Other Biomarkers

Plasma citrulline levels were not different in the patient groups from the controls. Neither I-FABP nor human plasma IMA was elevated in the blood samples from either group at any time point compared with the control Group C (Table 3).

None of the study patients had a hepatic or pancreatic disease, and only five patients in Group A (CMI) (3 females and two males) had >90 μmol/mL serum creatinine. Also, the median plasma levels of amylase, AST, ALT, and CRP in the patient groups were within the normal range (Table 4).
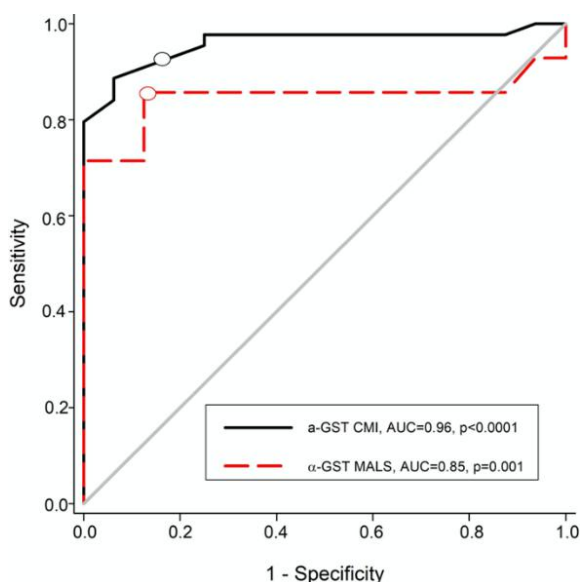

Figure 3 Receiver operating characteristic (ROC) curve and area under the curve (AUC) of median plasma α-GST levels in the patients with chronic mesenteric ischemia (CMI, n=44) due to atherosclerosis and median arcuate ligament syndrome (MALS, n=14). Black (O) and red (O) represent the threshold values for the corresponding sensitivity and specificity. The upper limit of 4 ng/mL was used as a cut-off for α-GST levels. The Grey 45° diagonal line represents random chance (AUC=0.5).

Table 3 Median Plasma Levels of Candidate Mesenteric Ischemia Biomarkers in Patients with CMI Due to Atherosclerosis (Group A), and MALS(Group B), Compared with Healthy Individuals in Group C

	Test Detection Range	Group A		Group B		Group C
		Before	After	Before	After	
α-GST (IQR)	0.156 ng/mL - 10 ng/mL	7.8 (3.2) *p <0.001*	5.5 (5.3) *p = 0.001*	8.4 (5.2) *p = 0.001*	3.2 (1.9) *p = 0.064	3.3 (0.5)
I-FABP (IQR)	0.16- 10 ng/mL	0.16 (0.1) *p = 0.327	0.16 (0) *p = 0.068	0.16 (0.1) *p = 0.907	0.16 (0.3) *p = 0.504	0.16 (0.1)
Citrulline (IQR)	5.0- 100 nmol/mL	2.7 (1.1) p = 0.039	3.2 (1.1) p = 0.503	2.9 (1.1) *p = 0.429	3.1 (0.8) *p = 0.244	3.4 (0.1)
IMA (IQR)	7.8- 500 ng/mL	23.4 (0) *p = 1.0	23.4 (0) *p = 1.0	23.4 (0) *p = 0.385	23.4 (0) *p = 1.0	23.4 (0)

Notes: *Mann- Whitney *U*-test comparison with Group C.
Abbreviations: CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome; α-GST, α- glutathione S transferase; IQR, interquartile range; I-FABP, intestinal fatty acid binding protein; IMA, ischemia-modified albumin.

Table 4 Baseline Plasma Levels of Pancreas Amylase, AST, ALT, CRP, and Creatinine in Patients with CMI (Group a, n=44), MALS(Group B, n=14), and Healthy Individuals (Group C, n=16)

Plasma Marker	Reference Range	Group A Median (IQR)	Group B Median (IQR)	Group C Median (IQR)
p-Amylase	10- 65 U/L	24 (17)	28 (2)	34 (2)
AST	10- 35 U/L	24 (12)	22 (6)	24 (8)
ALT	10- 45 U/L	20 (10)	14 (10)	20 (6)
CRP	< 0.1 mg/L	2 (11)	0.9 (2)	1.9 (9)
Creatinine	45- 105 µmol/L	70 (26)	69 (19)	78 (18)

Abbreviations: CMI, chronic mesenteric ischemia; MALS, median arcuate ligament syndrome; IQR, interquartile range; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, c-reactive protein.

Discussion

This study demonstrates significantly raised plasma levels of α-GST in patients with CMI of atherosclerotic origin and with MALS. The threshold value of 4 ng/mL α-GST demonstrated very high sensitivity (90% and 86%) and PPV (95% and 86%), respectively, for the CMI patients with atherosclerotic lesions and MALS. The ROC curve for this cut-off value yields an excellent AUC of 0.96 and 0.85 in the patients with CMI and MALS, respectively.

To our knowledge, raised plasma α-GST levels have previously not been demonstrated in patients with CMI. In addition, this is the first time a mesenteric ischemia-specific marker from the mucosal enterocytes has been identified for patients with MALS. The etiology of MALS has long been disputed and debated in the medical literature, and even its existence has been questioned.[6,31,32] Identifying raised plasma levels of an ischemia biomarker in patients with MALS before surgery, followed by a decrease in the postoperative plasma levels, strongly supports the ischemic etiology in MALS.[7] These current findings also support previously reported findings of impaired microcirculation in the mucosal lining of the stomach and duodenum detected with laser Doppler flowmetry and visible light spectroscopy in patients with CMI and MALS.[8,33]

All study patients with CMI of atherosclerotic origin had multiple mesenteric artery lesions. However, the patients with MALS had only stenosis, caused by external compression of the celiac artery by the median arcuate ligament. The finding of raised plasma levels of α-GST in the patients with MALS in this study disagrees with the notion that there should be changes in at least two mesenteric arteries to develop chronic mesenteric ischemia.[34]

This study found the best combination of sensitivity and false-positive rate for the plasma α-GST threshold of 4 ng/ mL, which corresponds with earlier findings by Delaney et al in patients with acute mesenteric ischemia.[10]

Although dα-GST has been identified as an intestinal ischemia marker with good sensitivity and specificity in patients with acute mesenteric ischemia, later studies and meta-analyses have recommended IFABP as a biomarker with higher sensitivity and specificity than α-GST.[21] Due to the small size of the study populations and thereby some uncertainty about the findings the guidelines have not included routine use of these biomarkers in patients with acute mesenteric

In a previous study, elevated plasma I-FABP was found in 22 patients with CMI, at baseline and after gastric exercise tonometry. Furthermore, tonometry after a test meal provocation demonstrated significantly increased plasma I-FABP levels in 17 patients with CMI compared to the controls.[19] The authors did not suggest any cut-off for abnormal plasma I-FABP levels. In our study population, plasma I-FABP levels were either below or within the detection range and did not differ significantly between the patients and the control group. The blood samples in our study were taken in a fasting state. Our study identified plasma α-GST as a biomarker that seems easily detectable in patients with CMI and MALS without needing any provocation test before blood sampling.

After revascularization, CMI patients may develop reperfusion injury to the mucosal tissue, and, in severe cases, it may progress to reperfusion syndrome.[35,36] The resulting inflammatory changes are self-limiting within several days after revascularization.[36] The patients with CMI of atherosclerotic origin and MALS had a statistically significant reduction in plasma α-GST three months after revascularization. Although the second blood samples were drawn three months after the revascularization procedures the plasma levels were still significantly higher in the patients in Group A than in the control group. In the patients with MALS (single artery stenosis), α-GST levels were no longer different from the controls. Still, raised plasma levels of α-GST in Group A patients with atherosclerotic mesenteric artery disease could be due to revascularization performed on the only single artery in most of the patients in this Group A. The persistent stenosis or occlusion of the other mesenteric artery could have played a role in the persistently raised plasma α-GST levels irrespective of the time-point of the blood sampling.

Plasma levels of p-amylase, AST, ALT, CRP, and Creatinine were within the normal reference range, suggesting that the raised levels of plasma α-GST are due to intestinal ischemia in the patients with CMI and MALS I in this study.

This study is a single-center study, and except for laboratory technicians, the rest of the study group was not blinded to the patients and the healthy controls. Another study limitation was that the optimal size for the study and adequate power was not determined at the start of the study. Future studies about the plasma biomarker for CMI and MALS may also include a control group of patients with abdominal pain and weight loss caused by other diseases. The studies should be designed with a study population that also considers the impact of reperfusion symptoms and the impact of single or multiple artery revascularizations on the plasma levels of α-GST in patients with CMI.

Conclusion

Patients with CMI of atherosclerotic origin and MALS have raised levels of plasma α-GST. Elevated plasma α-GST suggests MALS as an ischemic disorder.

Data Sharing Statement

De-identified data can be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for this purpose. The proposal should be directed to the project leader, Syed Sajid Hussain Kazmi, sshkazmi@gmail.com. Data requestors will need to sign a data access agreement to gain access.

Disclosure

Dr Asle Wilhelm Medhus reports grants from Takeda, outside the submitted work. The authors report no other conflicts s of interest in this work.

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