Optimizing diagnosis of splanchnic vein thrombosis with MR Direct Thrombus Imaging

The Rhea-study

Version: 1.7, 2024-03-26

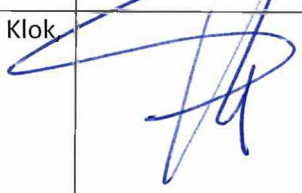
Sponsor trial code: P18.089

ABR research file number: NL65303.058.18

NTR code: 7061

Coordinating investigator:	Drs. S.N.M. ter Haar
Email:	s.n.m.ter_haar@lumc.nl
Telephone:	0031-71-5298096
Address:	Leiden University Medical Center, Department of Thrombosis and Hemostasis, Room C-07-068, Albinusdreef 2, PO Box 9600, 2300 RC, Leiden, The Netherlands
Principal investigators:	Prof. F.A. Klok, Dr. L.J.M. Kroft, Prof. M.V. Huisman, Dr. M.E. Tushuizen, Prof. W. Ageno, Prof. F. Leebeek
Participating centers:	Leiden University Medical Center (Leiden, the Netherlands), Varese University Hospital (Varese, Italy), Erasmus Medisch Centrum (Rotterdam, the Netherlands), Gemelli Hospital (Rome, Italy)
Sponsor:	Leiden University Medical Center
Sponsor address:	Leiden University Medical Center, Albinusdreef 2, PO Box 9600, 2300 RC, Leiden, The Netherlands

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor representative: Prof. dr. H.C.J. Eikenboom		3-4-2024
Principal Investigator: Prof. dr. F.A. Klok, internist		3-4-24

Study synopsis

Title	Optimizing diagnosis of splanchnic vein thrombosis with MR Direct Thrombus Imaging
Short title	Rhea study
Dutch title	De evaluatie van Magnetic Resonance Direct Thrombus Imaging (MRDTI) bij patiënten met een verdenking op buikvene trombose
Sponsor trial code	P18.089
NTR code	7061
ABR research file number	NL65303.058.18
Background	Splanchnic vein thrombosis (SVT) is one of the manifestations of unusual site venous thromboembolism (VTE). SVT includes portal vein thrombosis (PVT), mesenteric vein thrombosis (MVT), splenic vein thrombosis (SpVT) and the Budd-Chiairi syndrome (BCS).[1] There is no validated clinical algorithm for the diagnosis of SVT and there are no specific laboratory tests available to confirm or rule out the disease. Particularly, D-dimer tests do not have a role in the diagnosis of SVT, due to its low specificity and the high percentage of false positive results, especially in patients with cancer, liver cirrhosis or underlying inflammatory conditions, present in more than half of the total SVT population.[2] Thus the diagnosis of SVT relies on imaging tests alone. Whereas Doppler ultrasound is the imaging test of choice for most forms of SVT, its sensitivity is only 90%, as is the sensitivity of CT angiography (CTA).[3] MR angiography (MRA) has been reported to have 90-100% sensitivity for SVT, but this technique is limited by the need to administer a contrast agent. Furthermore, in studies evaluating the accuracy of MRA for the diagnose of SVT,

	gold standard for SVT (surgical validation) was lacking[4, 5] Importantly, many of SVT diagnoses in clinical practice (up to 30%) are incidental findings, i.e. findings on imaging tests of the abdomen performed for another reason than suspected SVT. Whereas the diagnosis of symptomatic SVT is often challenging, the correct diagnosis of acute versus chronic SVT is even more difficult, as neither of the current available imaging tests is helpful in determination of the age and clinical relevance of the thrombus, especially in non-symptomatic patients. Due to this impossibility to determine whether the incidentally observed thrombosis is acute, chronic or even an imaging artefact, the vast majority of patients with incidental SVT are treated with -often lifelong- anticoagulants.[1, 6] It is widely acknowledged that this practice likely results in overdiagnosis and unjust exposure to anticoagulant therapy with associated risk of bleeding. An alternative imaging technique for more accurate diagnosis of SVT is MR Direct Thrombus Imaging (MRDTI). This technique is in an advanced stage of development (Theia study, NCT02262052, supported by TSN grant 2013-02) and is close to implementation in clinical practice. The method is based on the formation of methemoglobin in a fresh thrombus leading to shortening of the T1 signal. It does not require contrast dye. Both the diagnostic accuracy (sensitivity 97-100%, specificity 100%) as well as the inter-observer agreement of MRDTI for first and recurrent DVT of the leg were reported to be excellent (kappa 0.89-0.98). Moreover, it was shown to accurately differentiate acute from chronic thrombosis. There is an unquestionable need for improved diagnostic approaches for (incidental) SVT. We plan to evaluate the MRDTI technique, that has been shown to be accurate in other settings of difficult-to-diagnosis venous

	thrombosis, for the notoriously challenging diagnosis of incidental SVT. This study targets an important unmet need and will provide the basis for precision medicine for patients with SVT in the near future, i.e. the possibility of assessment of the age of the thrombus in patients with incidental SVT, which is of utmost importance for determination of the indication for anticoagulant therapy.
Primary objective	To explore the diagnostic accuracy of MRDTI in the diagnostic management of acute and chronic SVT in a prospective diagnostic proof of concept study
Secondary objectives	1) To optimise MRDTI sequences for imaging SVT. 2) To assess the interobserver agreement of the readers of MRDTI for suspected SVT.
Study design	This study is a prospective diagnostic proof of concept study to explore the diagnostic accuracy of MRDTI in the diagnostic management of acute and chronic SVT. This will be achieved by performing MRDTI scans to adjust and optimize the DTI scan sequence in 3-5 patients with confirmed, acute SVT. If a reproducible clearly positive DTI signal is achieved in all patients, the study can proceed with the inclusion of cohort 1 and 2, i.e. 35 patients with confirmed acute SVT and in 35 patients with confirmed, chronic SVT. All scans will be evaluated post-hoc by expert readers blinded for the final diagnosis. It is predetermined that at least five patients of each SVT site (PVT, SpVT and BCS and at least five patients of each SVT risk factor (oncologic, post-surgical and inflammatory/infectious) will be included. To make sure that cohort 1 is generally similar to cohort 2 frequency matching will be performed, in which all controls will be selected to get the same distribution according to SVT site and risk factor as cases.

Study population	*Inclusion criteria* – Patients with confirmed acute SVT; definitions provided in paragraph 4.2 (Cases, group 1) – Patients with confirmed non-symptomatic chronic SVT, defined by incident SVT with chronic thrombi on 2 serial imaging tests with at least 3 months interval (controls, group 2) – Aged 18 years and older – Willing and able to give informed consent *Exclusion criteria* – MRI contra-indication (including but not limited to a cardiac pacemaker or subcutaneous defibrillator; vascular clips in the cerebral vessels; metal splinter in the eye, a hearing aid that cannot be removed; a neurostimulator that cannot be removed; a hydrocephalus pump) – A medical condition, associated illness or co-morbid circumstances that precludes completion of the study procedures (MRI and 90-day follow-up assessment), including but not limited to life-expectancy less than 3 months, inability to lie flat, morbid obesity preventing use of MR and claustrophobia. – Patients with decompensated liver disease with Child-Pugh class C cirrhosis (since MRDTI evaluation will be inadequate in these patients) – Patients with suspected tumour thrombus
Number of subjects	73-75 patients will be enrolled: 3-5 patients for the optimization of the scan sequence, 35 patients with confirmed acute SVT and finally 35 patients with confirmed, non-symptomatic chronic SVT. It is predetermined that at least five

	patients of each SVT site (PVT, SpVT and BCS) and at least five patients of each SVT risk factor (oncologic, post-surgical and inflammatoir/infectious) will be included.
Primary endpoints	The sensitivity and specificity of MRDTI for the diagnosis of acute SVT
Secondary endpoints	The secondary endpoints are 1) optimized MRDTI scan sequences for SVT; 2) the assessment of interobserver agreement between the reviewers.
Study duration and planning	The total duration of this study is expected to be 3 years.
Number of sites	4
Sample size consideration	A sample size of 35 patients in each of groups 1 and 2 was chosen because with this sample size and an expected sensitivity of greater than 90%, the 95% confidence intervals on the point estimates would have a bandwidth of approximately ±15%, ensuring that the point estimate was sufficiently accurate to make decisions about the appropriateness and safety of a future management study. To optimize the MRDTI scan sequences before the start of including patients in group 1 and 2, 3 patients with confirmed SVT will be included. If a reproducible clearly positive DTI signal is achieved in all three to five patients, the study can proceed with the inclusion of cohort 1 and 2. The final sample size is thus 73-75 patients.
Statistical analysis	*Primary analysis* A diagnosis (acute SVT, no signs of acute SVT, or non-diagnostic) for each arm of interest based on an aggregate reading of the MRDTI images will be made by two independent expert readers. A third reader will be involved to resolve any dispute. The sensitivity of MRDTI is determined by calculating the proportion of

	scans that are read as "positive for acute SVT" in patients with a confirmed acute SVT and the specificity is determined by calculating the proportion of scans that are read as "no signs of acute SVT" in patients with chronic SVT. The corresponding exact 95% confidence interval for each of the point estimates will be calculated. In addition to these estimates, sensitivity and specificity estimates with corresponding 95% confidence intervals will be calculated for the initial independent assessment of each of the two readers participating. A point estimate of the sensitivity of >90% will be acceptable for initiating a future management study. *Secondary analyses* For the optimization of the MRDTI scan sequences 3-5 pilot patients will be tested. The final sequences will be used in the 2 study groups when the study PI's all agree that the accuracy and quality of the sequences is adequate For the interobserver agreement between the two reviewers a kappa statistic will be assessed. According to established criteria, a kappa score above 0.8 is considered excellent reliability, a score between 0.6 and 0.8 is considered good reliability, a score between 0.4 and 0.6 is considered moderate reliability and a score below 0.4 is considered poor reliability.

Study schedule

	Visit 0: Clinical assessment	**Visit 1:** Enrolment	**Visit 2:** MRDTI (within 48 hours of visit 1 in acute SVT)	**Visit 3:** 90-day follow-up
Check for inclusion/exclusion criteria		X		
Obtain informed consent		X		
Medical history		X		
Demographic data		X		
Clinical examination		X		
Laboratory test (D-dimer, renal function)		X*		
Doppler ultrasound, CT or MRI of the abdomen	X*			
Treatment decision	X			
MRDTI of abdomen			X	
Recording of				
Death				X
Major bleeding				X
Hospital admission				X
Symptomatic SVT or VTE				X

*Part of clinical practice, no study proceedings

Table of contents

List of abbreviations

AE	Adverse events
BCS	Budd-Chiairi syndrome
CTA	Computed tomography angiography
DUS	Doppler ultrasonography
DVT	Deep vein thrombosis
INR	International normalized ratio
IRB	Institutional Review Board
MRA	Magnetic resonance angiography
MRDTI	Magnetic resonance direct thrombus imaging
MRP	Magnetic resonance portography
MVT	Mesenteric vein thrombosis
PTT	Partial thromboplastin time
PVT	Portal vein thrombosis
SAE	Serious adverse events
SVT	Splanchnic vein thrombosis
SpVT	Splenic vein thrombosis
VTE	Venous thromboembolis

1. Introduction and hypothesis

Compared to lower extremity deep vein thrombosis (DVT) and pulmonary embolism (PE), very little research has focused on the diagnostic management of splanchnic vein thrombosis (SVT).[3] The tests that are currently used to diagnose SVT, doppler ultrasound (DUS) and computed tomography angiography (CTA), have a number of limitations, particularly related to patient safety. For example, CT angiography involves the use of ionizing radiations and iodinated contrast agent, with potential renal toxicity or allergic reactions. DUS has the advantage of being relatively inexpensive and widely available. It is however operator-dependent and the accuracy can be limited by body habitus and the presence of intestinal gas.[3] Importantly, whereas the diagnosis of symptomatic SVT is often challenging, the correct diagnosis of acute versus chronic SVT is even more difficult, as neither of the current available imaging tests is helpful in determination of the age of the thrombus or its clinical relevance. Due to this impossibility to determine whether the incidentally observed thrombosis is acute, chronic or even an imaging artefact, the vast majority of patients with incidental SVT are treated with -often lifelong- anticoagulants. It is widely acknowledged that this practice likely results in overdiagnosis and unjust exposure to anticoagulant therapy with associated risk of bleeding. In the proposed study we will evaluate whether magnetic resonance direct thrombus imaging (MRDTI) is a valid, reliable, safe and effective alternative for the diagnosis of acute and chronic SVT. Our primary hypothesis is that MRDTI will demonstrate acceptable sensitivity and specificity for the diagnosis of acute SVT and the differentiation between acute and chronic SVT.

2. Background and rationale

2.1 Epidemiology of splanchnic vein thrombosis

Splanchnic vein thrombosis (SVT) includes portal vein thrombosis (PVT), mesenteric vein thrombosis (MVT), splenic vein thrombosis (SpVT) and the Budd-Chiari syndrome (BCS). SVT is underdiagnosed,

given the heterogeneous clinical presentations and the non-negligible rate of asymptomatic incidental findings.[1] In a retrospective review of computed tomography (CT) scans of the abdomen performed for reasons other than the search for suspected SVT, the prevalence of unsuspected abdominal vein thrombosis was 1.7%.[7] Furthermore, 18-30% of the patients with SVT are incidentally detected.[8, 9]

PVT is the most frequent manifestation of SVT, with a yearly incidence of 3.8 cases in males and 1.7 cases in females per 100.000 individuals.[1] BCS is the least frequent manifestation of SVT with a gender-specific incidence of 2.0 cases and 2.2 cases per million individuals, respectively.[1] MVT has an incidence of 2.7 per 100,000 persons per year, with a peak incidence in the age category 70–79 years.[3] There are no precise data available on the incidence of SpVT, other than a 10% prevalence among patients with pancreatitis.[3]

The diagnosis of SVT is often difficult to establish due to the anatomical heterogeneity, with different clinical manifestations according to the site of thrombosis.[3] Abdominal pain is the most frequent symptom and is reported in 60% of the patients with MVT or BCS, and in 40% of the patients with PVT. Hypersplenism, thrombocytopenia, ascites and portal hypertension are frequently reported in PVT and BCS. Other nonspecific symptoms include fever, nausea or vomiting, diarrhea and anorexia.[9]

In at least one-third of the patients with SVT a precipitating risk factor is identified, which include liver cirrhosis, inflammatory, infectious and autoimmune diseases, abdominal surgery, malignancy, inherited and acquired thrombophilia, pregnancy-post-partum periods, hormonal therapy, JAK2 positive myeloproliferative neoplasms (MPN) and paroxysmal nocturnal hemoglobinuria (PNH).[10] The leading cause of BCS is myeloproliferative neoplasm (MPN). In 50% of the patients with BCS a MPN is reported.[11] In patients with PVT, abdominal cancer (gastrointestinal, pancreatic and hepatobiliary system) and livercirrhosis were the most commonly reported acquired risk factors. Isolated SpVT is most commonly associated with underlying acute pancreatitis.[12]

2.2 Available Methods for the Diagnosis of splanchnic vein thrombosis

2.2.1. Biomarkers

There is no clinical algorithm for the diagnosis of SVT and there are no specific laboratory tests. D-dimer does not have a role in the diagnosis, due to its low specificity and the high percentage of false positive results, especially in patients with cancer, liver cirrhosis or underlying inflammatory conditions, present in more than half of the total SVT population.[2] Liver function tests are usually normal or may be elevated in case of an underlying hepatic disease and thus non diagnostic for SVT. Mildly prolonged international normalized ratio (INR) and partial thromboplastin time (PTT) can be seen in patients with PVT in absence of clinical evidence of liver diseases.[3] The diagnosis of SVT thus relies on different imaging tests.

2.2.2. Doppler ultrasonography

Doppler ultrasound (DUS) is the current imaging of choice for the diagnosis of PVT and BCS. Previous studies evaluating the accuracy of DUS for the diagnosis of PVT compared to angiographic or surgical-pathologic findings, found a sensitivity of 89-93% and specificity of 92-99%.[13, 14]

In BCS, DUS is usually the first choice imaging, since it is very accurate when performed by an experienced technician.[1] In a small study, a sensitivity of DUS for the diagnosis of BCS of 87,5% was found. The diagnosis of BCS was confirmed by histopathologic findings or by cavography with hepatic vein catheterisation.[15] However in a recent study, contrast enhanced US is found to be more accurate than colour DUS for the diagnosis of PVT and BCS in patients with cancer, with a sensitivity and specificity of >90% and 100%, respectively.[16] For the diagnosis of MVT DUS showed good specificity of 100% at cost of low sensitivity of 79-90%, due to operator-dependent differences. Overlying bowel gas can hinder the visualization of mesenteric veins and make it impossible to visualize the smaller mesenteric vessels.[5] DUS is usually a first-line non-invasive diagnostic test in

SpVT. However, the location of the splenic vein is a disadvantage for DUS, since collateral veins near the splenic hilum can be confused with the splenic vein.

2.2.3. CT angiography

In the diagnosis of PVT CT angiography compared to intraoperative findings as gold standard, showed a sensitivity of 90% and a specificity of 99%.[13] Furthermore, CT and MR are superior to DUS for providing information about the cause of thrombosis, such as infection, inflammation and cancer.[1]

CT and MR are confirmatory diagnostic tests when DUS is non diagnostic in patients with a clinical suspicion of BCS.[17] Furthermore, CT and MR are superior for assessing the liver perfusion and liver parenchyma and showing indirect signs of BCS (hypertrophy of caudate lobe) and causes for secondary BCS.[18]

For the diagnosis of MVT, CTA and MRA are considered the diagnostic tests of choice. They are more sensitive than DUS, since visualization of mesenteric veins with DUS is often very challenging.[17] Furthermore, the advantage of CT over DUS is the ability to visualise the mesentery and the bowel walls, since thickening of the bowel wall is the most common sign of intestinal infarction due to MVT.[19] Previous small studies evaluating diagnostic accuracy of MVT compared to angiography, intraoperative findings or autopsy found a sensitivity and specificity of 91-91% and 94-100%, respectively.[20, 21]

SpVT is most commonly diagnosed by abdominal contrast-enhanced CT, since SpVT is usually an incidental finding in the evaluation of patients with complicated pancreatic disorders or pre-operative patients with chronic pancreatitis, the two most common situations that accompany SpVT.[22] For the diagnosis of SpVT, dynamic contrast-enhanced CT showed a sensitivity of 71% compared to angiography.[3]

2.2.4. MR imaging

In the diagnosis of PVT, contrast enhanced MRA is able to evaluate the patency of the portal venous system and flow direction. In a previous study evaluating the diagnostic accuracy of MR portography (MRP) versus DUS, MRP was more accurate in the diagnosis of PVT with a sensitivity and specificity of 90% and 99%, respectively.[4] In another small study evaluating contrast enhanced MRA compared to reference standard, defined as surgical evaluation in the majority of patients and other imaging tests (DUS or CT) in the remaining, had a sensitivity of 100% and specificity of 98%.[5]

In the diagnosis of BCS, MR angiography is better for the visualisation of liver perfusion and liver parenchym compared to DUS.[3] MR is usually performed when CTA is contra-indicated in patients with suspected MVT. Small studies evaluating the diagnostic accuracy of MR found a sensitivity and specificity close to 100% for the detection of MVT confirmed by surgical procedures, DUS or CT scan.[5, 23] Conventional angiography is rarely performed nowadays, because of its invasive nature. In patients with high clinical suspicion of SVT and non-diagnostic CT or MR findings, or in patients in need of endovascular therapeutic procedures, angiography may still be considered.[19]

2.3 Acute versus chronic SVT

Acute SVT has a poor short- and long-term prognosis [24] and a worse survival rate than usual site VTE (lower extremity deep vein thrombosis and/or pulmonary embolism).[9] Therefore, a timely diagnosis is crucial, in order to establish the appropriate anticoagulant treatment. Every manifestation of SVT has a risk of potentially life-threatening thrombotic complications, such as the development of bowel ischemia and the risk of hemorrhagic complications.[1] BCS has a high mortality rate of 10% at 6 months, 13% at 1 year and 18% at 2 years.[25] In one-third of the patients with acute MVT intestinal ischemia is reported and has a mortality rate of 20% at 30 days.[26] Usually, depending on the site of thrombosis, anticoagulant treatment should be started as soon as

possible after diagnosis to prevent progressive and/or recurrent thrombosis. The 2012 guidelines of the American College of Chest Physicians (ACCP) indeed recommend anticoagulation in all patients with symptomatic SVT.[27]

Importantly, since many patients with SVT are at high risk of bleeding, some SVT patients are not treated with anticoagulants. In one study, 25% of the patients with acute PVT or SpVT suffered gastrointestinal bleeding shortly after initiation of anticoagulant therapy, mainly from gastroeosophageal varices due to portal hypertension.[9] As a consequence, the ACCP guidelines suggest no anticoagulation in patients with asymptomatic, incidentally detected SVT.[27] However, it is often challenging to diagnose acute SVT as neither of the current available imaging tests is helpful in determination of the age of the thrombus or its clinical relevance. A clear differentiation between acute or chronic thrombotic is therefore often impossible. With an ever increasing incidence of incidentally detected SVT due to the extensive use of CT imaging, especially in patients with liver cirrhosis or solid cancer, the lack of a decisive diagnostic test to differentiate acute from chronic SVT is clearly an unmet clinical need.[3] This illustrates the relevance of the current research proposal.

2.4 Magnetic Resonance Direct Thrombus Imaging as a Promising Alternative

2.4.1. MRDTI for the lower extremity

Magnetic Resonance Direct Thrombus Imaging (MRDTI) has been shown to be a highly accurate diagnostic method for a first deep vein thrombosis of the legs.[28, 29] The method is based on measurement of the T1 signal which shortens as a result of the formation of methemoglobin in a fresh thrombus.[30] This technique does not require the administration of gadolinium contrast and the acquisition time is short, making it a safe and patient-friendly test. MRDTI was found to have a high sensitivity of 95% and specificity of 91% for a first venography proven symptomatic DVT of the legs [31]. Moreover, interobserver agreement was excellent with a kappa-statistic of 0.89-0.98. A second study found even higher diagnostic accuracy with a sensitivity of 95% and a specificity of

100% [32]. Moreover, the positive signal disappeared in all patients after a period of six months.[32] In the subsequently performed Return-study, it was shown that MRDTI differentiated accurately between patients with confirmed recurrent ipsilateral DVT and those with asymptomatic residual intravascular clots and a normal D-dimer level with a sensitivity of 95% (95%CI 83-99) and a specificity of 100% (95%CI 92-100). This study additionally confirmed the high interobserver agreement with a kappa-value of 0.98.[28]

2.4.2. MRDTI for splanchnic vein thrombosis

Only few reports are available on non-contrast enhanced MRDTI for diagnosis of SVT. In one small study evaluating non-contrast and gadolinium enhanced MR imaging for diagnosis of BCS results suggested that MR imaging may be a useful diagnostic modality for detection and classification (acute versus subacute/chronic) of BCS, but correlation with the gold standard (histopathologic findings) was missing.[33] Moreover, two small and outdated studies suggested that in acute SVT and PVT, the clot may be detected inside the lumen of the veins as isointense material on T1-weighted images and hyperintens material on T2-weighted images.[34, 35] However, in both studies the gold standard for SVT/PVT diagnosis was lacking and the differentiation between acute versus chronic thrombus was not validated.

With MRDTI shown to be a valuable diagnostic test for arm vein thrombosis and sinus thrombosis in recent studies, the investigators hypothesize that MRDTI will also proof to be an accurate diagnostic test for acute SVT and for the distinction between acute and chronic SVT since formation of methemoglobin in a thrombus is common to all venous thromboses.[36, 37]

3. Objectives

3.1 Primary objective

The primary objective of this study is to explore the diagnostic accuracy of MRDTI in the diagnostic management of acute and chronic SVT in a prospective diagnostic proof of concept study.

3.2 Secondary objectives

The secondary objectives of this study are:

1) To optimise MRDTI sequences for imaging of SVT

2) To assess the interobserver agreement of the readers of MRDTI for suspected SVT

4. Methods

4.1 Study design

This study is a prospective diagnostic proof of concept study to explore the diagnostic accuracy of MRDTI in the diagnostic management of acute and chronic SVT. This will be achieved by performing MRDTI scans to adjust and optimize the DTI scan sequence in 3-5 patients with confirmed, symptomatic SVT. If a reproducible clearly positive DTI signal is achieved in all patients, the study can proceed with the inclusion of cohort 1 and 2, i.e. 35 patients with confirmed acute SVT (cases) and in 35 patients with confirmed, non-symptomatic chronic SVT (controls). All scans will be evaluated post-hoc by expert readers blinded for the final diagnosis. It is predetermined that at least five patients of each SVT site (PVT, SpVT and BCS) and at least five patients of each SVT risk factor (oncologic, post-surgical and inflammatoir/infectious) will be included. To make sure that cohort 1 is generally similar

to cohort 2 frequency matching will be performed, in which all controls will be selected to get the same distribution according to SVT site and risk factor as cases.

4.2 Definition of SVT

4.2.1. Acute Splanchnic vein thrombosis

Acute splanchnic vein thrombosis is defined as acute symptomatic or acute incidental asymptomatic thrombosis in the mesenteric, splenic, portal or hepatic veins.

Acute symptomatic thrombosis refers to acute onset (< 2 week existent) of symptoms characteristic for SVT thrombosis (including but not limited to abdominal pain) with SVT confirmed with DUS, CTA or MRA, dependent on the anatomical location. Acute incidental asymptomatic thrombosis refers to an incidentally detected SVT with DUS, CTA or MRA, absent on previous diagnostic tests performed in the last 2 weeks before the new incidental finding, in patients without symptoms characteristic of SVT.

The diagnosis of SVT can be made with DUS, CTA and MRA. The diagnosis SVT is defined as the absence of flow in the involved vein (mesenteric, splenic, portal or hepatic vein) as well as the hyperechogenic thrombus inside the lumen seen on DUS. Acute SVT on CTA is defined as a non-enhancing filling defect in the lumen of the involved vein and possibly increased enhancement of hepatic ischemic areas during the arterial phase and decreased enhancement in portal phase. At MR imaging, acute SVT is isointense on T1-weighted images and hyperintense on T2-weighted images.[1]

DUS is the current first line imaging test of choice for the diagnosis of PVT and BCS. However, when DUS is inconclusive MRA/MRP or CTA serves as reference test for the diagnosis of both PVT and BCS. For the diagnosis of SpVT, DUS is the first choice imaging test, however in a non-diagnostic test result, contrast enhanced CT will serve as the reference test.

4.2.2. Chronic Splanchnic vein thrombosis

Chronic Splanchnic vein thrombosis is defined by incident SVT with chronic thrombi on 2 serial imaging tests with an at least 3 months interval.

4.3. MRDTI

MRDTI will be performed as described before with a 1.5 or 3.0 Tesla unit using a T1-weighted magnetisation prepared three-dimensional gradient-echo sequence. The sequence includes a water-only excitation radiofrequency pulse to abolish the fat signal, and the effective inversion time is chosen to nullify the blood signal. Imaging will be performed of the affected veins in two imaging blocks with a total acquisition time of 8-12 minutes by using a 55-cm body coil.

4.4. Therapeutic management

This is not a management study: before inclusion in the study, a final diagnosis and management plan, i.e. initiation of anticoagulant treatment or not, is made and discussed with the patients. The MRDTI findings are not used for this decision process. When venous thrombosis at any anatomical site is diagnosed during follow-up, treatment with therapeutically dosed anticoagulants will be initiated without delay, according to current guidelines.

5. Subjects

5.1 Population base

Consecutive patients with confirmed acute symptomatic SVT, who fulfil all the inclusion criteria and meet none of the exclusion criteria, are eligible for inclusion, as are patients with confirmed chronic

SVT. It is planned to enrol 73-75 subjects in the Rhea study (see paragraph 8.1 for detailed sample size considerations).

5.2 Inclusion criteria

1. Patients with confirmed acute SVT; definitions provided in paragraph 4.2 (Cases, group 1)
2. Patients with confirmed non-symptomatic chronic SVT defined by incident SVT with chronic thrombi on 2 serial imaging tests with at least 3 months interval (controls, group 2)
3. Aged 18 years and older
4. Willing and able to give informed consent

5.3 Exclusion criteria

1. MRI contra-indication (including but not limited to a cardiac pacemaker or subcutaneous defibrillator; vascular clips in the cerebral vessels; metal splinter in the eye, a hearing aid that cannot be removed; a neurostimulator that cannot be removed; a hydrocephalus pump)
2. A medical condition, associated illness or co-morbid circumstances that precludes completion of the study procedures (MRI and 90-day follow-up assessment), including but not limited to life-expectancy less than 3 months, inability to lie flat, morbid obesity preventing use of MR and claustrophobia.
3. Patients with decompensated liver disease with Child-Pugh class C cirrhosis (since MRDTI evaluation will be inadequate in these patients)
4. Patients with suspected tumour thrombus

6. Study endpoints

6.1. Primary endpoint

The primary endpoint of this study is the sensitivity and specificity of MRDTI for the diagnosis of acute and chronic SVT.

6.2. Secondary endpoints

The secondary endpoints of this study are:

1) optimized MRDTI scan sequences for SVT;

2) the assessment of interobserver agreement between the reviewers.

7. Data analysis

7.1 Sample size calculation

A sample size of 35 patients in each of groups 1 and 2 was chosen because with this sample size and an expected sensitivity of greater than 90%, the 95% confidence intervals on the point estimates would have a bandwidth of approximately ±15%, ensuring that the point estimate was sufficiently accurate to make decisions about the appropriateness and safety of a future management study. To optimize the MRDTI scan sequences before the start of including patients in group 1 and 2, 3-5 patients with confirmed SVT will be included. If a reproducible clearly positive DTI signal is achieved in all three patients, the study can proceed with the inclusion of cohort 1 and 2. The final sample size is thus 73-75 patients. It is predetermined that at least five patients of each group (PVT, SpVT and BCS) will be included.

We expect that the required sample size is met within a time frame of three years. This expectation is based on the actual number of cases of acute SVT being diagnosed in the two study sites and considering that the number of chronic SVT diagnosis exceeds that of acute SVT. The Varese University Hospital is a tertiary referral center for SVT, and the LUMC is home to both a large oncology as well as organ transplantation departments where the diagnosis of SVT is made frequently.

7.2 Data analysis

7.2.1. Primary endpoint

A diagnosis (acute SVT, no signs of acute SVT, or non-diagnostic) for each arm of interest based on an aggregate reading of the MRDTI images will be made by two independent expert readers. A third reader will be involved to resolve any dispute. The sensitivity of MRDTI is determined by calculating the proportion of scans that are read as "positive for acute SVT" in patients with a confirmed acute SVT and the specificity is determined by calculating the proportion of scans that are read as "no signs of acute SVT" in patients with chronic SVT. The corresponding exact 95% confidence interval for each of the point estimates will be calculated. In addition to these estimates, sensitivity and specificity estimates with corresponding 95% confidence intervals will be calculated for the initial independent assessment of each of the two readers participating. A point estimate of the sensitivity of >90% will be acceptable for initiating a future management study.

7.2.2. Secondary analyses

For the optimization of the MRDTI scan sequences 3 pilot patients will be tested. The final sequences will be used in the 2 study groups when the study PI's all agree that the accuracy and quality of the sequences is adequate.

For the interobserver agreement between the reviewers a kappa statistic will be assessed. According to established criteria, a kappa score above 0.8 is considered excellent reliability, a score between 0.6 and 0.8 is considered good reliability, a score between 0.4 and 0.6 is considered moderate reliability and a score below 0.4 is considered poor reliability.

7.3 Data management

7.3.1. Data collection

Data will be collected, used and stored, which concerns data such as name, address, date of birth and medical information. Diligent efforts will be made to ensure the study data are stored securely and confidential information is protected. The handling of personal data will comply with the General Data Protection Regulation (GDPR).

All study participants will receive a study number which is a unique identifier (not based on patient initials and birth date). The key to the code will be safely stored in the local research institute and safeguarded by the principal investigator. The unique studynumber will be used on the CRF and with the MR images stored in the radiology database. The data that will be sent to the sponsor will only contain the code and not names or other data that can identify study participants. All electronic data and records will be saved under their unique study number and stored in a secured file on the computer. Access to study files and electronic records will be restricted to authorized study personnel. The local investigators are responsible for ensuring that all sections of the eCRF are completed correctly, and that entries can be verified against source data.

7.3.2. Data handling

The electronic data entry system allows the principle investigators to control the entry process with the help of the built-in review functions. Any missing data or inconsistencies will be reported back to

the respective site and clarified by the responsible investigator. The principle investigators are authorized, in the case of discrepancies or correction of data errors, to directly contact the responsible person at the study site. After completion of data entry and if no further corrections are to be made in the database, the access rights will be withdrawn, and the database will be declared closed and used for statistical analyses.

7.3.3. Storage and Archiving of Data

The principle investigators will archive all study data (subject identification code list, source data, and investigator's files) and relevant correspondence in the Investigator Site File and this is archived in a closed storage cabinet. Only the principle investigator has access to the subject identification code list and source data. The monitor which has been hired by the sponsor of the study and the Healthcare and Youth Inspectorate also have access to the data in case of safety reviewing. The Investigator Site File, all source data, and other pertinent documents will be archived for 15 years at the research location.

7.3.4. Withdrawing consent

Study participants can withdraw their consent to the use of the personal data at any time. The study data collected until the moment of the withdraw can be used in the study.

7.3.5. Processing data

The principle investigator and coordinating investigator of the study in the LUMC are responsible for the processing of personal data of the study participants. For questions about rights concerning processing data study participants can contact the principal and coordinating investigators or Data Protection Officer of the LUMC or the Dutch Data Protection Authority.

8. Ethical considerations

8.1 Regulatory statement

The study protocol and consent forms will be submitted to the Research Ethics Committee. Patient recruitment will not commence before formal approval has been granted. The study will be conducted according to the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice and Hong Kong, Somerset West and Edinburgh) and in accordance with the Guidelines for Good Clinical Practice (CPMP/ICH/135/95 - 17th July 1996). The study will be carried out in keeping with local legal and regulatory requirements.

8.2 Inclusion and consent

Before being admitted to the study, the subject must consent to participate after being fully informed about the nature, scope, and possible consequences of participation. The consent documents must be in a language understandable to the subject and must specify who informed the subject. After reading the informed consent document, the subject must give consent in writing. The subject's consent must be confirmed by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussions. A copy of the signed informed consent document must be given to the subject. The original signed consent document will be retained by the investigator.

The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained. All participants will be told that participation is voluntary and they are free to leave the study at any time. Because the timeframe for patient recruitment and imaging are critical to the success of the study, research personnel will maintain regular communication with staff in clinical areas where eligible patients are likely to be recruited. After they

have given written acknowledgement of informed consent to participate, a medical screening will take place. Through patient interview and review of medical records, it will be confirmed that they satisfy all inclusion criteria and do not meet any of the exclusion criteria.

8.3 Confidentiality

The name of the subjects and other confidential information are subject to medical professional secrecy. For the primary and secondary analysis, only anonymized information that is included in the standardized paper CRF forms can be used. This information is depersonalized and stored under an individual identification code that is assigned to each patient at inclusion after signing informed consent. The study patients will declare in the written consent to release the investigator from the medical professional secrecy to allow identification of subject's name and/or inspection of original data for monitoring purposes by health authorities and authorized persons (monitors).

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified. Study findings stored on a computer will be kept safe in accordance with local data protection laws and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of data legislation will be fulfilled in its entirety.

8.4 Responsibilities of principal investigators

The principal investigators will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, and their study-related duties and functions. The principal investigators will maintain a list of sub-investigators and other appropriately qualified persons to whom they have delegated significant study-related duties.

8.5 Approval of Study Protocol

Before the start of the study, the study protocol, informed consent document, and any other appropriate documents will be submitted to the independent ethics committee/institutional review board of the LUMC in Leiden. The document of final approval by the independent ethics committee should mention the title of the study, the study code, all study sites, the documents they reviewed, and the date of decision. Before the first subject is enrolled in the study, all ethical and legal requirements must be met. The investigator must keep a record of all communications with the independent ethics committee and the competent authorities.

8.6 Ongoing information for independent ethics committee

8.6.1. Amendments

The competent authorities including the independent ethics committee must be informed of all subsequent protocol amendments and administrative changes, in accordance with the respective local legal requirements. Amendments must be evaluated to determine whether formal approval should be sought and whether the informed consent document should also be revised. The independent ethics committee must be informed of all subsequent protocol amendments which require formal approval in accordance with the legal requirements.

8.6.2. Annual progress report

The coordinating investigator will submit a summary of the progress of the study to the accredited institutional review board (IRB) once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the study, serious adverse events/ serious adverse reactions, other problems, and amendments.

8.6.3. End of study report

The investigator will notify the accredited IRB of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last follow-up visit. In case the study is ended prematurely, the investigator will notify the accredited IRB within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited IRB.

8.6.4. Public disclosure and publication policy

The results of this study will be disclosed unreservedly according to the Central Committee on Research Involving Human Subjects (CCMO) statement on publication policy (http://www.ccmo.nl/attachments/files/ccmo-statement-publicatiebeleid-3-02-en.pdf).

8.6.5. Monitoring and Quality Assurance

Monitoring in all sites in the Netherlands will be executed by (internal) monitors of the LUMC according to the monitor plan.

8.7 Insurance

The sponsor has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical

Research in Humans of 2015).. This insurance provides cover for damage to research subjects through injury or death caused by the study:

1) € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2) € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3) € 7.500.000,-- (i.e. seven million five hundred thousend Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9. Safety reporting

9.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

9.2 Adverse events

Adverse events are defined as any suspected recurrent venous thromboembolism occurring to a subject during the study. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.3 Serious adverse events

A serious adverse event is an objectively proven (fatal) PE or death. The investigator will report all (fatal) PEs and deaths to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the (fatal) PEs and deaths through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for the (fatal) PE and deaths followed by a period of maximum of 8 days to complete the initial preliminary report.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

10. Agreements

10.1 Financing of the study

This study is supported by an unrestricted grant from the International Society on Thrombosis and Haemostasis (ISTH).

10.2 Publication

Any publication of the results, either in part or in total (articles in journals or newspapers, oral presentation, etc.) by the investigators or their representatives, shall require the approval of the

principal investigators. It is planned to publish the results of the study as an original article in an appropriate medical journal as well as to present the results at international congresses. The choice of the journal for the publication will be made by the principal investigators in agreement with the co-authors. Besides the principal investigators, further authors of this article must meet the following criteria:

- Substantial contribution to the recruitment of subjects, i.e. inclusion of at least 5 study subjects;
- Substantial contribution to the interpretation of the data;
- Substantial contribution to drafting the article or revising it for intellectual content

11. Reference List

1. Riva, N. and W. Ageno, *Approach to thrombosis at unusual sites: Splanchnic and cerebral vein thrombosis.* Vasc Med, 2017. **22**(6): p. 529-540.
2. Dai, J., et al., *Association between D-dimer level and portal venous system thrombosis in liver cirrhosis: a retrospective observational study.* Int J Clin Exp Med, 2015. **8**(9): p. 15296-301.
3. Riva, N. and W. Ageno, *Clinical manifestations and imaging tools in the diagnosis of splanchnic and cerebral vein thromboses.* Thromb Res, 2017.
4. Cakmak, O., et al., *Role of contrast-enhanced 3D magnetic resonance portography in evaluating portal venous system compared with color Doppler ultrasonography.* Abdom Imaging, 2008. **33**(1): p. 65-71.
5. Kreft, B., et al., *Detection of thrombosis in the portal venous system: comparison of contrast-enhanced MR angiography with intraarterial digital subtraction angiography.* Radiology, 2000. **216**(1): p. 86-92.
6. Riva, N., et al., *Clinical history and antithrombotic treatment of incidentally detected splanchnic vein thrombosis: a multicentre, international prospective registry.* Lancet Haematol, 2016. **3**(6): p. e267-75.
7. Ageno, W., et al., *Incidental diagnosis of a deep vein thrombosis in consecutive patients undergoing a computed tomography scan of the abdomen: a retrospective cohort study.* J Thromb Haemost, 2012. **10**(1): p. 158-60.
8. Amitrano, L., et al., *Prognostic factors in noncirrhotic patients with splanchnic vein thromboses.* Am J Gastroenterol, 2007. **102**(11): p. 2464-70.
9. Thatipelli, M.R., et al., *Survival and recurrence in patients with splanchnic vein thromboses.* Clin Gastroenterol Hepatol, 2010. **8**(2): p. 200-5.
10. Klute, K., et al., *Clinical presentations, risk factors, treatment and outcomes in patients with splanchnic vein thrombosis: a single-center experience.* J Thromb Thrombolysis, 2016. **42**(2): p. 267-71.
11. De Stefano, V. and I. Martinelli, *Splanchnic vein thrombosis: clinical presentation, risk factors and treatment.* Intern Emerg Med, 2010. **5**(6): p. 487-94.
12. Riva, N., et al., *Clinical approach to splanchnic vein thrombosis: risk factors and treatment.* Thromb Res, 2012. **130 Suppl 1**: p. S1-3.
13. Bach, A.M., et al., *Portal vein evaluation with US: comparison to angiography combined with CT arterial portography.* Radiology, 1996. **201**(1): p. 149-54.
14. Tessler, F.N., et al., *Diagnosis of portal vein thrombosis: value of color Doppler imaging.* AJR Am J Roentgenol, 1991. **157**(2): p. 293-6.
15. Bolondi, L., et al., *Diagnosis of Budd-Chiari syndrome by pulsed Doppler ultrasound.* Gastroenterology, 1991. **100**(5 Pt 1): p. 1324-31.
16. Rossi, S., et al., *Contrast-enhanced versus conventional and color Doppler sonography for the detection of thrombosis of the portal and hepatic venous systems.* AJR Am J Roentgenol, 2006. **186**(3): p. 763-73.
17. L.D. DeLeve, D.C.V., G. Garcia-Tsao, *American Association for the Study Liver Diseases, Vascular disorders of the liver.* Hepatology 2009. **49 (5)**(1729–1764.).
18. Hoekstra, J. and H.L. Janssen, *Vascular liver disorders (I): diagnosis, treatment and prognosis of Budd-Chiari syndrome.* Neth J Med, 2008. **66**(8): p. 334-9.
19. Bradbury, M.S., et al., *Mesenteric venous thrombosis: diagnosis and noninvasive imaging.* Radiographics, 2002. **22**(3): p. 527-41.
20. Harward, T.R., et al., *Mesenteric venous thrombosis.* J Vasc Surg, 1989. **9**(2): p. 328-33.
21. Acosta, S., A. Alhadad, and O. Ekberg, *Findings in multi-detector row CT with portal phase enhancement in patients with mesenteric venous thrombosis.* Emerg Radiol, 2009. **16**(6): p. 477-82.

22. Sakorafas, G.H. and A.G. Tsiotou, *Splenic-vein thrombosis complicating chronic pancreatitis.* Scand J Gastroenterol, 1999. **34**(12): p. 1171-7.
23. Harnik, I.G. and L.J. Brandt, *Mesenteric venous thrombosis.* Vasc Med, 2010. **15**(5): p. 407-18.
24. Sogaard, K.K., et al., *Survival after splanchnic vein thrombosis: A 20-year nationwide cohort study.* Thromb Res, 2016. **141**: p. 1-7.
25. Darwish Murad, S., et al., *Etiology, management, and outcome of the Budd-Chiari syndrome.* Ann Intern Med, 2009. **151**(3): p. 167-75.
26. Acosta, S., et al., *Epidemiology, risk and prognostic factors in mesenteric venous thrombosis.* Br J Surg, 2008. **95**(10): p. 1245-51.
27. Kearon, C., et al., *Antithrombotic therapy for VTE disease: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines.* Chest, 2012. **141**(2 Suppl): p. e419S-e496S.
28. Tan, M., et al., *Magnetic resonance direct thrombus imaging differentiates acute recurrent ipsilateral deep vein thrombosis from residual thrombosis.* Blood, 2014. **124**(4): p. 623-7.
29. Klok, F.A., M. Tan, and M.V. Huisman, *Letter by Klok et al Regarding Article, "18F-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Enables the Detection of Recurrent Same-Site Deep Vein Thrombosis by Illuminating Recently Formed, Neutrophil-Rich Thrombus".* Circulation, 2015. **131**(24): p. e530.
30. Saha, P., et al., *Magnetic resonance T1 relaxation time of venous thrombus is determined by iron processing and predicts susceptibility to lysis.* Circulation, 2013. **128**(7): p. 729-736.
31. Fraser, D.G., et al., *Diagnosis of lower-limb deep venous thrombosis: a prospective blinded study of magnetic resonance direct thrombus imaging.* Ann.Intern.Med, 2002. **136**(2): p. 89-98.
32. Westerbeek, R.E., et al., *Magnetic resonance direct thrombus imaging of the evolution of acute deep vein thrombosis of the leg.* J Thromb.Haemost., 2008. **6**(7): p. 1087-1092.
33. Noone, T.C., et al., *Budd-Chiari syndrome: spectrum of appearances of acute, subacute, and chronic disease with magnetic resonance imaging.* J Magn Reson Imaging, 2000. **11**(1): p. 44-50.
34. Haddad, M.C., et al., *MR, CT, and ultrasonography of splanchnic venous thrombosis.* Gastrointest Radiol, 1992. **17**(1): p. 34-40.
35. Zirinsky, K., et al., *MR imaging of portal venous thrombosis: correlation with CT and sonography.* AJR Am J Roentgenol, 1988. **150**(2): p. 283-8.
36. Yang, Q., et al., *Early Detection and Quantification of Cerebral Venous Thrombosis by Magnetic Resonance Black-Blood Thrombus Imaging.* Stroke, 2016. **47**(2): p. 404-9.
37. Dronkers, C.E.A., et al., *Diagnosing upper extremity deep vein thrombosis with non-contrast-enhanced Magnetic Resonance Direct Thrombus Imaging: A pilot study.* Thromb Res, 2018. **163**: p. 47-50.