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Metformin for AAA Growth Inhibition
A randomized controlled trial
MAAAGI-trial

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Signature and date: _____ _____

INVESTIGATOR

I, the undersigned, have read and understand the protocol and agree that it contains all necessary information for conducting the study.

I agree to conduct the study according to this protocol and according to the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice and the applicable national laws and regulations.

Investigator: _____

Signature and date: _____ _____

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this Study Protocol.

Abbreviation or special term	Explanation
AAA	Abdominal Aortic Aneurysm
AE	Adverse Event
eCRF	Electronic Case Report Form
CT	Computed Tomography
DMP	Data Management Plan
DVP	Data Validation Plan
GFR	Glomerular Filtration Rate
ITT	Intention to Treat
PPA	Per Protocol Analysis
Qol	Quality of Life
SmPC	Summary of Product Characteristics
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
US	Ultrasound

1. BACKGROUND, RATIONALE AND OBJECTIVES

1.1 Background

Abdominal aortic aneurysm (AAA) is a major health issue and ruptured AAA a common cause of death in Europe and North America. In Sweden 700-1000 annual deaths are attributed to AAA. To prevent rupture, early detection and preventive surgical repair in selected individuals is recommended. AAA screening programs are already implemented in the UK, Sweden and the USA. Most screening detected AAAs are, however, small (diameter 30-54 mm) and are under surveillance until expansion to the threshold for elective surgical repair; i.e. diameter ≥55 mm for men and ≥50 mm for women. Presently approximately 15,000 men and women are being monitored for small AAAs in Sweden, of whom 70% eventually will require surgical repair. In 2015, ~1200 AAA repairs were performed in Sweden at a cost of ~30 million Euros (Swedvasc 2016). AAA repair is associated with significant mortality (1-5%), perioperative complications (up to 20%), cost (~25,000 Euros/patient) and need for life-long post-operative follow-up, imaging and repeat surgery (in ~20% of cases) (Mani 2009, Mani 2010, Mani 2013).

A key limitation of contemporary treatment strategies of AAA is the lack of therapy directed at small AAA (Wanhainen 2019). Although surgical repair is an effective treatment for large AAA, several large trials (Powell JT 2007, UKSAT 2002, Lederle 2002, Cao 2011) have shown that early surgery of small AAA does not reduce mortality. Given that AAA diameter is the strongest predictor for rupture and the natural course for AAA is continued expansion, a mean to reduce AAA growth rate would be highly beneficial. Commonly used cardiovascular drugs, such as anti-platelet drugs, statins, angiotensin converting enzyme inhibitors and beta-blockers have not been shown to slow AAA growth in cohort studies (Ferguson 2010, Twine 2011). Previous interventional trials with therapy directed at AAA growth reduction include antibiotics, mast cell inhibitors, beta-blockers, platelet inhibitors, and angiotensin converting enzyme blockers, with trials of stem cells and cyclosporine under way. These trials have all been either small, with negative results or had unacceptable side effects (Meijer 2013, Sillesen 2015, PATI 2002, Golledge 2017, Rughani 2012).

Risk factors for AAA often mimic those of arteriosclerosis, with the notable exception of diabetes mellitus. Several large trials have shown that people with diabetes are less likely to develop AAA and when they do; the AAA expands slower and is less likely to rupture (Xiong 2016, Kent 2010, Lederle 1997, Golledge 2008, Thomson 2013, De Rango 2012, Takagi 2016, Theivacumar 2014). However, in 2016 a study of 58 patients with diabetes reported that the drug metformin, the world's most widely used drug for type II diabetes, was associated with reduced AAA growth (Fujimura 2016). Following this, a cohort study of 1.2 million patients with diabetes, reported that metformin prescription was associated with a 36%

reduced risk of developing an AAA (Hsu 2016) and in a cohort of 1755 AAA patients a 36-76% slower AAA growth rate were reported in patients with diabetes and metformin prescription, but not those without (Golledge 2017). A similar finding was made in a cohort of 13,834 American veterans with AAA and diabetes where metformin was independently associated with reduced growth rates of AAA of different sizes (Itoga 2018).

Metformin may reduce AAA growth by inhibiting key pathological mechanisms implicated in AAA, including inflammation and extracellular matrix remodeling. Two different rodent models of AAA have found that metformin may reduce AAA growth in euglycemic animals (Fujimura 2016, Vasamsetti 2015). These studies suggest that metformin may reduce AAA growth by reducing aortic inflammation, elastin degradation, smooth muscle cell depletion and monocyte infiltration independent of glucose levels.

In house data (in manuscript) from a cohort of 526 patents under surveillance for small AAA support these findings with a reduced AAA growth rate and altered cytokine expression in patients prescribed metformin. In a connected experimental animal study, metformin was also shown to inhibit AAA formation, improve endothelial vasomotor function and reduce pro-inflammatory gene expression in euglycemic mice.

Metformin is a well-established first-line treatment of type II diabetes. It acts primarily by reducing blood glucose levels by inhibiting hepatic glucose production and increase insulin sensitivity. It is cheap, safe and able to reduce micro- and macrovascular complications and overall death (UKPDS 1998, Holman 2008, Griffin 2017). This has prompted several large trials of metformin given to persons without diabetes (DPPRG 2002, Syngelaki 2016) showing that metformin is safe and highly tolerable in variable patient groups.

Metformin is available as a generic drug at low cost. It is generally well tolerated, with few serious side effects. Gastro intestinal problems may occur primarily in the run-in phase. This is usually mitigated by taking metformin with a meal and increasing the dose stepwise over a course of several weeks to the desired level.

As metformin is a generic drug there is no commercial interest in exploring its potential as a drug to reduce AAA growth, necessitating an academically driven trial.

The rationale for this randomised controlled trial is to investigate whether treatment with metformin inhibits growth of small AAAs.

1.2 Objectives

The proposed multi-centre population-based open-label randomized controlled trial with blinded outcome assessment will examine if metformin slows AAA growth in patients with small AAAs who do not have diabetes.

1.2.1 Primary objective

To examine if up to 2g metformin administered daily over a five-year period slows AAA growth measured as computed tomography (CT) imaging assessed AAA diameter in patients with small AAAs who do not have diabetes.

An interim STOP/GO analysis will be performed after two-years of treatment.

1.2.2 Secondary objectives

To examine if metformin limits increase in; a) CT-assessed AAA volume; b) ultrasound assessed AAA diameter; c) improves health-related quality of life; d) reduces the need for surgery (diameter ≥55mm) or rupture; and e) represents a cost-effective treatment to reduce the need for AAA surgery.

1.2.3 Exploratory objectives

To examine; a) if there is a dose or time related response of metformin regarding the primary or secondary endpoints; and b) if metformin favourably modifies circulating inflammation and matrix remodelling biomarkers; or c) affects perivascular adipose tissue.

1.2.4 Safety objective

To determine adverse events; primarily related to known side effects of metformin and possible unexpected effects on AAA, related to metformin treatment after two and five years.

2. STUDY PLAN AND PROCEDURES

2.1 Study design

The MAAAGI-trial is a population-based multi-center, prospective, parallel group, randomized, open label trial with blinded outcome assessment to assess if metformin up to 2g daily over a five-year period will reduce AAA growth in patients who do not have diabetes.

Patients will be recruited from a cohort of patients with diagnosed AAA at the following seven Swedish sites; 1) Uppsala University hospital, Uppsala, 2), Skåne University hospital, Malmö, 3) Gävle county hospital, Gävle, 4) Västerås county hospital, Västerås. 5) Karlstad county hospital, Karlstad 6) Falun county hospital, Falun, and 7) Umeå University Hospital, Umeå.

The patients should have a maximum aortic diameter of 30-49mm for men and 30-44mm for women, not have diabetes and be expected to tolerate metformin.

A total of 500 patients with AAA will be included in the study, 250 in each study arm. Patients will be randomised to metformin or standard care in a 1:1 ratio.

The patients are scheduled for one enrolment visit, eight study visits and ten phone contacts for those randomized to metformin (three to support titration of study drug) and seven phone contacts for those randomized to standard care. The study visits will take place at the vascular laboratory at the respective surgical departments for clinical examination and blood sampling and at the CT units at the radiology departments where CT-imaging will be performed.

After a run in phase to support titration of metformin, visits and phone contacts are scheduled to alternate every three months until 24 months and twice yearly thereafter. CT imaging and AAA US will be performed at baseline, 24 months and end of study, as well as if necessary according to clinical routine. Study drug will start at baseline and continue through completion.

End of study is defined as 1) study completion at 60 months, or 2) discontinuation from study before that for any reason, for example due to AAA repair or patients will.

When all patients have completed the 24-month follow-up (including imaging) an interim analysis will be performed to assess for efficacy and safety; if there is no trend towards a positive effect or signs of a harmful effect of metformin, the study will be stopped at this phase.

The study will be performed in accordance with the ethically acceptable principles that have their origin in the Declaration of Helsinki and according to national legislations and international regulations. In addition, the study will be performed in accordance with this study protocol.

	Enrolment	Baseline/ Visit 1	Phone 2*	Phone 3*	Phone 4*	Phone 5	Visit 6	Phone 7	Visit 8	Phone 9	Visit 10	Phone 11	Visit 12	Phone 13	Visit 14	Phone 15	Visit 16	Phone 17	Completion
Visit window		*Day 0*[1]	*2 weeks*	*4 weeks*	*6 weeks*	*3 months*	*6 months*	*9 months*	*12 months*	*15 months*	*18 months*	*21 months*	*24 months*	*30 months*	*36 months*	*42 months*	*48 months*	*54 months*	*60 months*
Informed consent	x																		
In- & exclusion criteria	x																		
Demographics[2]	x																		
Smoking status	x						x		x		x		x		x		x		x
Medical history	x																		
Current medication	x								x				x		x		x		x
Physical examination[3]	x												x						x
Vital signs[4]	x						x		x		x		x		x		x		x
QoL questionnaires[5]	x												x						x
CT scan		x											x						x
Blood samples[6]	x								x				x		x		x		x
Randomisation		x																	
Ultrasound[7]	x												x						x
Drug prescription*		x					x		x		x		x		x		x		
Drug accountability*			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Target dose of study drug*		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
AE registration	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SAE registration	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 1. Overview of study activities. Phone 2-5 are to be performed +/- 5 days from target date and the remaining activities +/- 4 weeks from target date.

1 Within four weeks of enrollment
2 Demographics includes date of birth, sex and ethnicity
3 Physical examination includes cardio-pulmonary status and general appearance
4 Vital signs include blood pressure, heart rate, height (only at enrollment) and body weight
5 EQ-5D and RAND-36
6 Safety lab at all sites and blood samples for biobank at Uppsala and Malmö
7 Final Ultrasound +/- one week from CT scan
* For those randomized to metformin only, see item 4.1.1 below

3. PATIENT SELECTION CRITERIA

Patients under surveillance for small AAA are identified through local registers at respective site. Those with the most recently examined AAA are screened for eligibility. All patients scheduled for enrolment visit are accounted for.

3.1 Inclusion criteria

For inclusion in the study patients should fulfil the following criteria:

1. Provision of written informed consent.
2. Male and female patients.
3. Age 50-80 years.
4. Documented AAA Ø 30-49 mm for men and 30-44 mm for women.
5. Fasting p-glucose <7.0 mmol/L (WHO 1999). Fasting is defined as no caloric intake for ≥8 h.

3.2 Exclusion criteria

Patients should not enter the study if any of the following exclusion criteria are fulfilled:

1. Short expected survival.
2. History of current or previous diabetes mellitus.
3. Current or previous use of metformin.
4. Not expected to tolerate metformin.
5. Contraindications to metformin treatment according to SmPC:
    a Renal failure with glomerular filtration rate (GFR) <45ml/min according to the revised Lund-Malmö formula.
    b Hypersensitivity to metformin or any of the excipients included in the tablet.
    c Acute metabolic acidosis.
    d Diabetic pre-coma.
    e Acute conditions with the potential to alter renal function such as; dehydration, severe infection or shock

f Acute or chronic disease which may cause tissue hypoxia such as; decompensated heart failure, respiratory failure, recent myocardial infarction or shock

g Hepatic insufficiency, acute alcohol intoxication, alcoholism

6. Known or suspected connective tissue disorder (Marfans syndrome, etc), infected or inflammatory aneurysm, aneurysm development after aortic dissection or previous surgery of the infrarenal aorta.
7. Enrolment in either another investigational drug or medical device study or another investigational study of an approved drug or medical device within 30 days prior to enrolment of the current study.
8. If, in the opinion of the investigator, it is not in the patient's medical interest to participate in the study or the patient is unlikely to be able to comply with the study protocol.
9. Pregnancy. Women of childbearing potential are only included after a confirmed menstrual period and a negative highly sensitive urine or serum pregnancy test as well as willingness to comply with highly effective anti-contraception throughout the study period, see 5.3.

4. STUDY CONDUCT

4.1 Treatment

The treatment allocation in this study will consist of;

- Study Arm 1; Metformin tablets taken orally with target dose of 2g daily
- Study Arm 2; Standard care as described in current AAA guidelines (Wanhainen 2019)

Standard care includes help with smoking cessation if applicable; encouragement of physical activity and a healthy diet; blood pressure control; statin and anti-platelet therapy treatment if the patient have clinical manifestations of atherosclerotic disease. Local guidelines dictate which classes of drugs are recommended and who is responsible for treatment and follow up. The study participants allocated to metformin receive standard care plus metformin treatment.

Patients will be encouraged to take metformin every day at similar times, preferably with a meal to minimize side effects.

Metformin is titrated stepwise to avoid gastrointestinal side effects. Starting dose is one tablet of metformin 500mg day 1-14, two tablets day 15-28, three tablets day 29-42 and four tablets daily from day 43 till the remainder of the study. If a patient misses one dose he/she is

encouraged to take one extra tablet with the following dose. No more than one dose should be compensated for if missed.

If a patient has severe side effects in the run-in phase, titration to target dose will be slowed. If a patient has severe side effects at the target dose, the target dose will be reduced to a level at which the side effects are tolerable and stay at this level for the remainder of the study. Target dose and compliance to this dose is recorded at each contact.

Table 2. Identity of investigational product(s)

Investigational product	Dosage form and strength	Study arm and time point
Metformin	Tablet 500mg, 1 daily	Study arm 1, Day 0-14
Metformin	Tablet 500mg, 2 daily	Study arm 1, Day 15-28
Metformin	Tablet 500mg, 3 daily	Study arm 1, Day 29-42
Metformin	Tablet 500mg, 4 daily	Study arm 1, Day 43-completion

Target dose of metformin 2g is chosen to reflect doses given in observational studies. It is predicted to be a sufficient dose to translate to a meaningful biologic effect whilst still safe and with acceptable side effects. The option to reduce the dose is expected to reduce drop-out rate. Once the final target dose is reached a different metformin tablet strength may be used for convenience, i e 1g rather than 500mg tablets.

4.1.1 Labelling

The study medication will be prescribed by the investigator and retrieved by the patient at the pharmacy. The prescription note will state that the drug is used for study purpose. The patients are being reimbursed for their study drug costs.

4.1.2 Procedures for randomisation

On signing the Informed Consent the patients will be enrolled (enrolment = the process of allocating a unique identifier to a patient on entry into a clinical study, after obtaining written Informed Consent) and identified with a unique enrolment code (E-code) of 4 digits where the first digit is the site number and the three following the patient number. Uppsala=1, Malmö=2, Gävle=3, Västerås=4, Karlstad=5, Falun=6, and Umeå=7.

Enrolment will take place before treatment randomisation (randomisation = the assignment of patients to treatment in a clinical study according to a randomisation schedule generated by a random process) to assure allocation concealment. A randomisation list will be generated using a computerized procedure, by sorting 275 treatment allocations in each arm in random order using Excel (Microsoft, WA, USA). A total of 550 treatment allocations will be generated to accommodate possible erroneous entries to the database. Patients will be randomised strictly sequentially as they are eligible for randomisation. Simple randomization will be used without stratification given the large number of subjects.

The patients will be randomized on a 1:1 schedule to; metformin tablets in incremental doses to 2g daily *or* standard care.

If a patient discontinues from the study, the patient number will not be reused, and the patient will not be allowed to re-enter the study.

4.1.3 Compliance

The patient is asked to note missed doses of medicine for any reason. The patient brings used bottles of metformin and receipts from the pharmacy at the study visit and remaining doses are counted to calculate compliance. Three consecutive days or more of missed medication are noted separately.

4.1.4 Concomitant treatment(s)

Medication, which is considered necessary for the patient's safety and well-being, may be given at the discretion of the investigator(s).

All medication is allowed, except for antidiabetic agents. If the need for antidiabetic medication should arise, the patient is discontinued from the study.

The study protocol includes patient history and measures of cardiovascular function such as blood pressure, heart rate and cardiac and respiratory auscultation. If un- or undertreated diseases or risk factors are discovered, treatment will be initiated as appropriate or the patient is referred to relevant care. Active smokers are encouraged to smoking cessation.

It is likely that CT imaging will generate accidental findings in some patients. These findings will be dealt with according to clinical praxis at respective site. It is the responsibility of the investigator at respective site to make sure relevant care is provided for patients with accidental findings on imaging, laboratory tests or physical examination.

Concomitant medications will be recorded in the medication list in the eCRF.

4.2 Withdrawal from study

Patients may be discontinued from the study at any time, at the discretion of the investigator.

Patients are free to discontinue their participation in the study at any time for any reason.

Patients who discontinue from the study should always be asked about the reason(s) for the discontinuation (and informed about their right not to disclose their reason for doing so) and the presence of any adverse events (AEs). If possible, they should always be seen and assessed by an investigator and asked to perform completion outcome measures, including CT, US and QoL questionnaires. A pre-operative CT may be used for follow up should indication to operate the aorta arise. Adverse events should be followed up.

Study treatment should be stopped in the following cases: development of diabetes, persistent renal failure with GFR<45ml/min, severely deteriorating health, aortic surgery or onset of exclusion criteria.

If the study medication is stopped, patients will be asked to remain in the study and follow the other procedures according to the protocol. All randomised patients remain in the group they were originally allocated to for the purpose of intention-to-treat (ITT) analyses.

Patients who discontinue the study will not be replaced.

Sponsor has to be informed if a patient is withdrawn by any reason before study end. The "End of study" form must be filled out by the investigator and the reason should be stated.

The study number will not be reused if a patient is withdrawn.

5. COLLECTION AND RECORDING OF STUDY VARIABLES

The patients must sign informed consent before any study specific investigations are performed. The investigator will ensure that all data collected in the study, are recorded in a timely manner according to any instructions provided. Electronic Case Report Forms (eCRF) will be used in this study (See Section 7.2).

5.1 Study measurements

5.1.1 Clinical data

At the enrolment visit the patients will undergo a clinical examination which consists of; recording of demographic data (date of birth, sex and ethnicity), medical history and concomitant diseases, smoking status, heredity of AAA, current medication, recording of weight & height, heart rate & blood pressure, cardio-pulmonary status and general appearance.

At all points of contact with phone or visit at the clinic any AE and SAE are recorded, target dose of study drug is determined and compliance is asked for. Discontinuation (temporarily or permanent) of study medication ≥ 3 consecutive days will be recorded.

At visits medical events and any changes in smoking status will be noted and vital signs and weight is recorded. Compliance to study drug is noted and remaining tablets are counted. At completion visit and at 24 months, the above plus a physical examination is performed. Changes in current medication are recorded yearly.

At enrolment visit, at 24 months and completion quality of life will be recorded with EQ-5D and RAND -36, see appendix 1.

At enrolment blood samples will be taken for fP-Glc and P-Creatinine, analysed at accredited hospital laboratory. P-Creatinine will be tested for safety every 12 months. A 24 months S-B12 is controlled for possible metformin induced deficiency. In Uppsala and Malmö venous blood samples will be taken at baseline, 24 months and completion for scientific reasons.

5.1.2 Imaging

At baseline, 24 months and completion, the aorta will be examined by non-contrast enhanced CT with 1 mm slices or thinner from the jugulum to the groins.

At enrolment, 24 months and completion the abdominal aorta will be examined by US. The assessment of abdominal aortic diameter with US will be done by the local US technician, using the standardized technique measuring the largest anterior-posterior diameter from leading edge to leading edge perpendicular to the blood flow in the aneurysm. Inclusion criteria will be based on US determined diameter.

If a subject withdraws from the study due to operative need of the aorta, the preoperative CT will be used for analysis in the study. The assessment of aortic diameter on CT will be done using outer edge to outer edge perpendicular to blood flow. Volumetric AAA analysis of CT images will be performed using manual drawing of the vessel wall contours in axial slices of the entire abdominal aortic as well as semiautomatic measures in 3mensio, Pie Medical, Maastricht. Analysis will start at the slice just below the most distal renal artery excluding accessory renal arteries and end at the slice just above the aortic bifurcation. Measures of perivascular adipose tissue will be made adjacent to the aorta.

All CT assessment (diameter and volume) will be done at a core lab at the Department of Radiology, Uppsala university hospital, Uppsala. The CT identification is the four digits enrolment code. The reviewer will be blinded regarding treatment allocation.

CT images will also be reviewed by local radiologists according to GCP at respective hospital. Accidental findings of unrelated pathology on the CT scan will be dealt with as appropriate by investigator at respective site.

5.1.3 Telephone contacts

The patients allocated to metformin will initially be contacted by the study nurse every two weeks in the run in phase. Thereafter phone contacts are every six months the first two years and yearly thereafter. The purpose for these calls is to support the patients in the run in phase for those allocated to metformin treatment, record drug accountability and if any AE or SAE had occurred since last visit and if previous AE or SAE are resolved.

5.2 Safety

A Summary of product characteristics (SmPC) including safety data of the study drug is stored at respective site in the investigators brochure.

5.2.1 Definition of adverse events

An AE is any unwanted medical occurrence that does not necessarily have to have a causal relationship with the investigational product. An AE can be any unfavourable, unintended clinical sign, symptom, medical complaint or clinically relevant change in laboratory variables or clinical tests. Accidents, operations not pre-planned or deterioration in concurrent illness are also considered as AEs. Known gastrointestinal side-effects of metformin are actively asked for and noted separately.

5.2.1 Assessment of intensity

The Investigator should rate the intensity of any AE as follows:

Mild: The AE does not interfere with the patient's usual function.

Moderate: The AE interferes to some extent with the patient's usual function.

Severe: The AE interferes significantly with the patient's usual function.

When an AE occurs, the information must be recorded at the AE page. Every AE must be followed up and at study end if the AE still occurs, note in the e-CRF, AE outcome – not resolved.

5.2.2 Assessment of causality

The investigator shall judge whether, in his/her opinion; the AE is potentially associated with the study drug. When stating the causality, the following nomenclature should be used:

Unrelated:	There is little or no chance that the study treatment caused the AE.
Possibly related:	The association of the AE with the study treatment is unknown, however, the AE is not clearly due to another condition.
Probably related:	A reasonable temporal association exists between the AE and the study treatment. Based on the Investigator's clinical experience, the association of the AE with the study treatment seems likely.

5.2.3 Definitions of serious adverse event

A serious adverse event is an AE occurring during any study phase (i.e., run-in, treatment, washout or follow-up), that fulfils one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s).

5.2.4 Recording of adverse events

AE + SAE will continuously be recorded from enrolment at all points of contact thru to completion at 24 months and documented in the eCRF and AE and SAE log.

5.2.5 Follow-up of unresolved adverse events

For all AEs, the patient will be followed until either the AE has ceased or until the patient is under professional medical care and a potential causality between the study treatment and the AE has been assessed.

5.2.6 Reporting of serious adverse events

All SAEs will be documented. The investigator is responsible for informing the Regional Ethical Review Board, and/or the Regulatory Authority of the SAE as per local requirements.

The Sponsor is responsible for SAEs reporting to the authorities and SUSARS (Suspected Unexpected Serious Adverse Events) reporting. A SUSAR will be reported to authorities if the SAE is assessed by the Sponsor to be a SUSAR, i.e. the event is related to the study drug and not described in the product specification. The CIOMS form will be used with assistance from the Swedish medical products agency.

A yearly safety report is submitted to the regional ethical review board and medical products agency.

5.2.7 Temporary withdrawal

The patient will be informed to temporarily withdraw the study drug 48h following intra venous contrast media injection, in case of vomiting, diarrhoea, fever, reduced fluid intake or suspect or manifest dehydration or lactic acidosis according to clinical routine for metformin treatment. The study drug is continued following resolution of the risk for dehydration and/or temporary renal impairment. See table 2

5.2.8 Procedures in case of medical emergency

The Investigators should ensure that there are procedures and expertise available to cope with emergencies during the study. If an emergency occurs, please notify the Sponsor:

Name: Anders Wanhainen

Telephone number: 018-6114623, 073-3993906

5.2.9 Laboratory safety assessment

At enrolment and every 12 months P-Creatinine will be analysed. Metformin may induce vitamin B12 deficiency after prolonged treatment and S-B12 is therefore assessed at 24 months. The blood volume will not exceed 40 mL at each visit, including concurrent blood samples for scientific reasons. Extra blood panels will be drawn at the discretion of the

investigator if there is concern for primarily renal or hepatic impairment due to alterations in clinical status or medication. Elevated fP-Glucose or P-Creatinine discovered at inclusion visit or throughout the study will be dealt with according to clinical praxis. Typically by referral to the patients family doctor.

The analyses will be performed at the local hospital laboratories and follow the Swedish biobank law for collecting blood samples in a clinical study. Tubes will be labelled and handled according to local routines and will be discarded after analysis.

Blood samples will be taken for scientific reasons in Uppsala and Malmö. Samples are centrifuged, frozen and stored for subsequent analysis in accordance with the Swedish biobank law for collecting blood samples in a clinical study.

5.3 Pregnancy

A limited amount of data from the use of metformin in pregnant women does not indicate an increased risk of congenital abnormalities. Risks concerning pregnancy within the study are chiefly due to radiation exposure from repeat CT scans. Study participants which are women of childbearing potential are required to use birth control methods that can achieve a failure rate of less than 1% per year when used consistently and correctly. This includes; combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation, progestogen-only hormonal contraception associated with inhibition of ovulation intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner and sexual abstinence. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. After enrolment into the study, pregnancy test for women with childbearing potential will be performed at clinical suspicion only. In case of pregnancy the study participant will be withdrawn from the study and, if possible, be seen and assessed by an investigator and asked to perform completion outcome measures except CT scan due to radiation exposure.

5.4 Insurance

All patients in the trial are covered by the patient insurance according to the Patient Injury Act.

6. ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the protocol, applicable regulatory requirements, GCP and the ethical principles of the Declaration of Helsinki as adopted by the 18th World Medical Assembly in Helsinki, Finland, in 1964 and subsequent versions.

6.1 Ethics and regulatory review

It is the responsibility of the Sponsor to obtain approval of the study protocol/protocol amendments, the patient information and the Informed Consent from the ethical review board and regulatory authority before enrolment of any patient into the study.

The written approval from the ethical review board should be dated and have an attached list of those persons (with names and positions) present at the meeting.

The Sponsor shall report all serious and unexpected AEs to the ethical review board. If a study stops prematurely at a study centre for any reason, the ethical review board must be informed. At the end of the study, the Sponsor should notify the ethical review board.

The Sponsor should file all correspondence with the ethical review board.

6.2 Informed consent

It is the responsibility of the investigator to provide each patient with full and adequate verbal and written information about the objectives, procedures and possible risks and benefits of the study. All patients should be given the opportunity to ask questions about the study and should be given sufficient time to decide whether to participate in the study. The written patient information must not be changed without prior discussion with the Sponsor.

The patients will be notified of their voluntary participation and of their freedom to withdraw from the study at any time and without giving any reason. Patients must also be informed that withdrawing from the study will not affect their future medical care, treatment or benefits to which the patient is otherwise entitled.

The Investigator is responsible for obtaining written Informed Consent from all patients prior to enrolment in the study.

The patients will consent to:

- Participating in the study.
- Personnel concerned at regulatory authorities to gain full access to hospital records, to control the data collected in the study.
- Recording, collection and processing of data and storing of data in a database.
- Collection of blood for analysis and storage in a biobank.
- Possible transfer of information from the study to countries outside the European Union (EU).

It should be clearly stated that the data will not identify any patient taking part in the study, in accordance with the EU General Data Protection Regulation (GDPR).

A copy of the patient information and the Informed Consent form should be given to the patient. The Investigator (or the designated representative) who gave the verbal and written information to the patient shall sign the Informed Consent form. The Investigator should file the signed Informed Consent forms in the Investigator's File for possible future audits and inspections.

It is suitable to notify the patient's family doctor of the patient's consent to participate in the study.

6.3 Risks – Benefits

The uncertain knowledge state supporting the study-hypothesis emphasizes the importance of a risk assessment for each patient. A risk determination of potential risks in this study has been made. These risks together with the actions to minimize these risks are compiled below:

RISKS	ACTION
Suspected drug related adverse event, such as gastrointestinal events	If not tolerated, treatment with study drug should be reduced in dose or stopped, temporarily or permanent, and appropriate medical action should be initiated according to the nature and severity of the AE.
Patient require surgery or intravenous contrast media during study	Temporary discontinuation of the study drug according to clinical praxis for metformin. Typically 48 h after intravenous contrast media, in the perioperative phase and during fasting.
Patient develops suspect or manifest dehydration during study	If risk of dehydration for any reason the study drug is temporarily discontinued. This typically includes but is not limited to fever, serious infection, vomiting, diarrhoea and reduced fluid intake.
Patient develops suspect or manifest hypoxia during study	If risk of hypoxia for any reason the study drug is temporarily discontinued. This typically includes but is not limited to, sepsis, chock, myocardial infarction and decompensated heart failure.

Patient requiring continuous treatment with drugs that may temporarily affect renal function, such as NSAIDs	Study drug should be discontinued during treatment if there is evidence of renal impairment
Patient develops B12 deficiency secondary to metformin treatment	S-B12 levels are controlled every 24 months and replacement therapy is initiated if indicated
BENEFITS	
Other than obtaining an extensive medical evaluation and close monitoring of the AAA, no clear benefit was identified for the patients who participate in the study.	

Table 2, possible risks and planned action

7. DATA MANAGEMENT

All data management will be handled by the investigator(s).

7.1 Monitoring of the study

In accordance with the principles of Good Clinical Practice (ICH GCP), monitoring of the study will be arranged by the Sponsor/PI. Three research nurses with formal training in monitoring according to ICH-GCP will monitor the study. During the study, the Monitor will have regular contacts with the study sites, including visits to ensure that the study is conducted and documented properly in compliance with the protocol, GCP and applicable regulatory requirements.

The extent of monitoring is described in a monitoring plan. The Monitor will ensure that accountability of investigational products is performed and will review source documents for verification of consistency with the data recorded in the eCRFs. The Monitor will also provide information and support to the investigators. The monitor will have access to all data in the eCRF, whereas investigators at each site only have access to data from their own site. The PI may access the data at all time.

Questionnaires, lab data, medical records and consent forms are source data stored in the investigator file as well as data entered directly in the eCRF and imaging.

The study centre may also be patent to quality assurance audit by the authorities. The investigators and other responsible personnel must be available during the monitoring visits and inspections and should devote sufficient time to these processes.

The investigators should provide curriculum vitae (CV) to be responsible for the study, as well as all personnel at the delegation list. All investigators and other responsible personnel should be listed together with their function in the study on the signature and delegation list.

7.2 Data management

Anonymised clinical data will be entered in an eCRF created in RedCap, provided by Uppsala University.

The investigator is responsible for ensuring the accuracy, completeness and timeliness of the data recorded in the eCRFs. The monitor will check the accuracy and completeness of the data reported in the eCRF's on a regular basis and before the database is locked.

The eCRF will be in accordance with the study specific Data Validation Plan (DVP). All inconsistencies detected during this procedure will be resolved through queries being issued to the investigational site. Any corrections made to entered data will be audited.

Data entered to the eCRF will be anonymised. Records of which e-code each patient is allocated will be kept locally at each site in a secure location.

All imagining will be reviewed anonymised by an operator blinded to treatment allocation.

8. STATISTICAL METHODS

All statistical analyses will be performed by the investigator(s).

8.1 Analysis set

All patients for whom a signed informed consent was obtained and an eCRF was started will be accounted for.

The Safety Analysis Set will include all patients who received at least one dose of study drug.

An intention-to-treat (ITT) analysis will include all randomised patients who received at least one dose of study drug and for whom at least one post dose observation for an efficacy endpoint is recorded.

The per-protocol analysis (PPA) consists of patients who sufficiently complied with the protocol. Inclusion in the PPA will require at least the following:

- An at least 80% compliance with the intended use will be accepted as per protocol.
- No major protocol deviations.

- Measure of primary efficacy endpoint at 60 months after baseline, and at 24 months for the interim analysis.

All efficacy analyses will be performed as both ITT and PPA. The ITT analyses is to be considered as main and the PPA as supportive.

8.2 Outcome variables

8.2.1 Primary variable

Efficacy in this study will be assessed by difference of AAA diameter determined by CT after 24 and 60 months vs at baseline, expressed as mm/year. The primary outcome is effect on AAA growth rate following 60 months metformin treatment vs standard care.

8.2.2 Secondary variables

Secondary efficacy variables are; 1) difference in log-transformed AAA-volume determined by CT at 24 and 60 months vs at baseline (Log-transformation is undertaken because AAA volumes appear to follow a right-skewed distribution based on in house data), 2) difference in AAA-diameter determined by US at 24 and 60 months vs at baseline, 3) difference in circulating inflammation and matrix remodelling biomarkers assessed by established assays, 4) difference in quality of life score at 24 and 60 months vs at baseline, 5) difference in need for AAA surgery and aneurysm rupture at 24 and 60 months and 6) cost effectiveness of metformin treatment 2 g daily to reduce need for prophylactic surgery of AAA.

8.2.3 Safety variables

Safety variables include differences in drug related AE and GFR.

8.3 Statistical analysis

The primary efficacy end-point is the between groups difference in mean annual AAA growth over 60 months. AAA growth will be analysed using an analysis of covariance (ANCOVA) model where AAA growth at 60 months is the dependent variable and treatment group and baseline AAA is included as independent variables. The results will be presented as the estimated group difference with 95 % confidence intervals at 60 months.

Similar methods will be used to assess growth as log transformed CT-volume and ultrasound diameter. Analysis of other outcomes: 1) Laboratory data and perivascular adipose tissue are exploratory endpoints and will be reported with descriptive statistics and evaluated with appropriate inferential statistics, 2) Patient reported outcome (QoL questionnaires) will be analysed using non-parametric test (Wilcoxson rank-sum test), 3) Pre-defined clinical events,

such as AAA-repair, rupture and death, are expected to be few, and the presentations will use descriptive statistics, 4) Economic analysis: Will be performed adopting a health system perspective. The cost-effectiveness analysis will produce an incremental cost effectiveness ratio as an estimated cost per surgical procedure avoided.

Handling of missing values: The primary analyses will be performed among patients with a measured value at the specific time point. However, as the number of missing values might be substantial several sensitivity analyses of the primary analysis will be performed:

- Multiple imputation (with different assumptions)
  - Assume that drop-outs respond according to randomized group
  - Assume that drop-outs respond according to control group
- Fixed imputation depending on reason for drop-out
  - For clinical events, such as AAA-repair, rupture and death, impute worst possible value.
  - For other events impute using a maximum likelihood function

8.4 Sample size estimation

Based on in house data and original data from an Australian study of the relationship between metformin and AAA growth (Golledge 2017) it is conservatively estimated that metformin treatment will lead to a 30% reduction in annual AAA growth. This is regarded as a meaningful change which is likely to translate to an important reduction in requirement for AAA repair. MRI data from the Uppsala group show a mean growth rate of 3mm/year, SD 2.17. Thus needing 184 patients for 80% power at a 30% reduced growth rate. CT data will likely show 10-15% larger SD, adding a 30% dropout rate over five years we approximate a need to recruit 500 patients, 250 in each arm, for 80% power at 2-tailed α 0.05.

9. CRITERIA FOR TERMINATION OF THE STUDY

The Sponsor reserves the right to discontinue the study prior to inclusion of the intended number of subjects but intends to exercise this right only for valid scientific or administrative reasons. After such a decision, all delivered unused investigational products and other study related materials must be collected without delay and all eCRFs must be completed as far as possible.

The study could be prematurely discontinued in the following cases (examples):

- Unexpectedly high proportion of drug related AEs.
- New findings about the investigational product(s) that changes the benefit/risk ratio.
- Study protocol is difficult to cope with.
- Recruitment of eligible subjects is far too low.
- Unacceptable low investigator or subject compliance.
- Critical change in personnel, administrative or scientific standards at the at the study centre.

10. INTERIM ANALYSIS

When all patients have completed the 24-month follow up (including imaging), an interim analysis will be performed where the primary endpoint, as well as log transformed CT-volume and ultrasound diameter will be analysed using ANCOVA. If there is no trend towards a protective effect (all three p-values for treatment effect >0.2), or a significant harmful effect on AAA growth or AAA related events, the study will be terminated and all on-going metformin treatment stopped.

The decision how to proceed with the study following the interim analysis will be at the discretion of the sponsor.

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