The use of xanthine oxidoreductase inhibitors in slowing the progression of renal and cardiovascular disease. A summary of available clinical trials

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Abstract

There is conflicting evidence whether elevated SUA is associated with or causes cardiovascular and renal disease. Identifying trials using xanthine oxidoreductase (XOR) inhibitors to reduce SUA might provide information on the epidemiological link between SUA and cardiovascular and renal disease.

The main objective is to provide a qualitative analysis of the available trials that studied the effect of XOR-inhibitors on cardiovascular and renal morbidity and mortality. EMBASE, MEDLINE and CENTRAL were systematically searched to identify relevant records.Articles in English published in peer reviewed journals reporting clinical trials using XOR-inhibitors on cardiovascular- and renal disease related end-points, including mortality. Studies on gout and surgical patients were excluded.

Titles and abstracts of the identified records were screened for eligibility. Relevant full-text articles were retrieved and main outcomes summarized in a modified PICO-table.

The thesis included 51 studies. Several studies reported reduced left ventricle mass(LVM) in the XOR-inhibitor group vs. placebo. In two studies on patients with congestive heart failure (CHF), assessing worsening of CHF, there was no effect of XOR inhibitors vs. placebo. Some studies reported slower progression of renal disease in the intervention arm. XOR-inhibitors reduced ambulatory blood pressure (BP) in pre-hypertensive and hypertensive adolescents in three small studies. There was a statistically significant effect of XOR-inhibitors on flow-mediated (endothelium dependent) vasodilatation in studies including participants with diabetes type 2, coronary artery disease and chronic kidney disease.

This thesis has identified a knowledge gap regarding the potential utility of SUA lowering drugs, XOR-inhibitors, to reduce the risk of cardiovascular and renal mortality and morbidity. Many small insufficiently powered studies have been conducted so far. There is possibly a potential for treating young subjects with newly developed hypertension with XOR-inhibitors. Well designed studies with clinically relevant end-points are highly called for.

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Preface – the work process

The background for this fifth year thesis is my project at the medical student research program where I have worked at the lab investigating possible mechanistic explanations for the epidemiological association between increasing serum uric acid levels and cardiovascular and renal disease. During search for relevant background literature I often found myself reading expert opinion drawing conclusions from *in vitro* studies, animal models and epidemiological studies. Only occasionally the reviews discussed results from randomised clinical trials. An important enzyme in the uric acid metabolism is the xanthine dehydrogenase (XOR) isoenzymes. We wanted to look further into trials using XOR-inhibitors, which blocks the enzyme and lowers serum uric acid levels and the effect on cardiovascular and kidney related morbidity and mortality.

The work with the thesis have been conducted during the fifth year clinical rotation and in the periods designating to the working with the fifth year thesis.

Outline of the work process:
November 14 – Work on project description
February 15 – Disposition of the thesis and work with the the introduction
August 15 – Pilot searches and final search constructed and conducted
September – October 15 – Titles and abstracts screened, full texts downloaded
November 15 - December 16 – Articles read and key information for tables extracted
January – March 16 – Work on result section and introduction
March – June 16 – Completing the article with discussion, introduction and layout

In order to get the whole width of available trails wide inclusion criteria were used. This resulted in challenges in the data-synthesis when the main results from heterogeneous studies with varying outcomes needed to be presented in a table format. The structure in this thesis is based on the suggested structure in the Cochrane handbook for systematic reviews. However, some deviations were done in order to fit the content of this thesis and that no meta-analysis were conducted. The only major deviation from the project description is that my supervisor challenged me to write the thesis in English instead of Norwegian as planned.

This project has received no external funding and has been conducted with resources available through the UiT – the Arctic University of Norways´s library sevices. The initial literature search was conducted with help from Erik Reierth, first librarian at the Nature and Health science library at UiT – The Arctic University of Norway. Henrik Brovold has planned and conducted the project with important input on method, presentation of results and help with scientific writing from Svetlana Zykova.

Henrik Brovold,

Tromsø 31th may 2016

Introduction

Elevated serum uric acid and the epidemiological link to cardiovascular and renal disease

Hyperuricemia is a common biochemical finding, traditionally linked to monosodium urate (MSU)-deposition diseases, such as gout(1). There is a linear relationship between serum uric acid (SUA) levels and prevalence of gout(2), and recent epidemiological studies have indicated that SUA might also predict future risk of cardiovascular and renal disease.

In the National Health and Nutrition Examination Study (NHANES) 1 Epidemiologic Follow-up Study, rising uric acid was reported to be associated with cardiovascular disease (CVD) and all-cause mortality in both genders(3). A Finnish population-based cohort study of middle-aged men free from cardiovascular disease, cancer, or diabetes at baseline found SUA to be a predictor of cardiovascular death(4). In the AMORIS(5) and Rotterdam(6) studies, uric acid was a risk factor for myocardial infarction and stroke in both genders. However, the Framingham Heart Study reported that SUA was not associated with increased CV risk when drug intake was accounted for(7). Several other studies also failed to find relationship between SUA and cardiovascular risk(8–11). Data from the Tromsø study showed that SUA was associated with all-cause mortality in men and women and increased risk of stroke in men, after adjustment for blood pressure, estimated GFR, urinary albumin/creatinine ratio, drug intake and traditional cardiovascular risk factors(12).

Furthermore, the Tromsø study also showed that increased uric acid levels were associated with a decreased estimated glomerular filtration (eGFR), leading to renal dysfunction and microalbuminuria after seven and thirteen year follow up(13). Another study found that slightly increased uric acid levels were associated with a nearly two-fold risk of incident kidney disease(14). A meta-analysis of observational studies found uric acid to be associated with incident chronic kidney disease (CKD)(15).

Prevalence of hyperuricemia and serum uric acid reference values

The prevalence of hyperuricemia was estimated to be 13.2% (21.2% among men and 5.7% among woman) based on the NHANES 2007-2008 cohort when hyperuricemia was defined as SUA levels > 7.0 mg/dl (>416 μmol/L)(1). The prevalence of hyperuricemia was associated with age, gender, weight and body mass index(1).

The reference values for serum uric acid are based on the normal variations in a healthy population without clinical signs of disease(16). In Norway, the reference values for serum uric acid are 230 - 480 μmol/L in men, 155 - 400 μmol/L in post-menopausal women and 155 - 350 μmol/L in pre-menopausal women(17). There is no consensus on the definition of hyperuricemia and different definitions are used in different publications investigating hyperuricemia. From a biochemical point of view a reference value of 404 to 416 μmol/L (6.8 - 7.0 mg/dl) reflects a theoretical

solubility level of uric acid in biological fluids and this is usually preferred over a statistical definition(18–21). A threshold of 360 μmol/L (6.0 mg/dl) is also suggested as it more accurately identifies a healthy population(16,22).

Current practice in treating hyperuricemia

Currently there is no indication for treating asymptomatic hyperuricemia, unless the patient is considered to be in an immediate risk of gout or other MSU–deposition diseases(18). This consideration is mainly left to the clinician(21). In the American College of Rheumatology Associations 2012 guidelines on gout treatment and hyperuricemia, the consensus is that there is not enough evidence to conclude whether asymptomatic hyperuricemia should be treated or not(19).

If hyperuricemia either directly, or indirectly through its metabolism, is involved in the pathogenesis of cardiovascular and kidney diseases, a reduction of uric acid might be of clinical interest.

Uric acid metabolism, renal excretion and solubility

Uric acid is the end-product in the purine metabolism in humans. Purines are heterocyclic compounds consisting of fused pyrimidine and imidazole rings. They form the basis for the nucleosides guanosine and adenosine, the building blocks for DNA, RNA and other important molecules, such as ATP, ADP, AMP, cyclic AMP, GTP, NADH, and coenzyme A(23). Other purines are hypoxanthine, xanthine and caffeine(24). An excess of purines is eliminated either through the salvage pathway where purine rings are recycled into new molecules, or broken down into hypoxanthine and further to xanthine and uric acid. Both steps are mediated by the enzyme xanthine dehydrogenase (XOR). XOR is the collective term for the two isoenzymes xanthine dehydrogenase(XDH) and xanthine oxidase (XO). XOR is transcribed from a single gene, named xanthine dehydrogenase (XDH). XDH is converted to the isoenzyme XO by oxidation or proteolysis. The conversion of XDH to XO induces the release of reactive oxygen species (ROS) when hypoxanthine is metabolized to uric acid(25).

Uric acid is accumulated in the human body due to either increased production (cell death, catabolism, intake of alcohol, fructose, or purine-rich diet), or decreased elimination as a result of impaired renal function or the use of diuretics(26). Uric acid is normally freely filtered in the kidneys and subjected to both tubular reabsorption and secretion by specific transporter proteins in the renal tubular cells. The renal handling of uric acid is mediated through a complexity of urate transporters, and 90 per cent of filtered uric acid is reabsorbed(27).

In most other species, uric acid is further metabolized by uricase to allantoin and other down-stream products. Humans, together with higher primates and Dalmatian dogs, are therefore unique with uric as the end-product of purine metabolism(25). As a result, humans have uric acid levels close to the saturation limit where it might form insoluble MSU crystals. Uric acid solubility is affected by pH, concentration of

Na^+ ions, proteins and temperature. Assessing the absolute solubility threshold of uric acid in bodily fluids is challenging(28).

Pharmacology of uric acid lowering drugs

There are three principal pharmacodynamic mechanisms in which uric acid lowering drugs work: 1) inhibiting the two isoenzymes xanthine oxidase (XO) and xanthine dehydrogenase (XDH) that convert xanthine and hypoxanthine to uric acid; 2) by increasing net renal excretion of uric acid; 3) by catalysing the breakdown of uric acid to allantoin with the recombinant enzyme uricase. The two first alternatives have traditionally been used to prevent gout and renal stone calculi caused by MSU-crystals. The latter is a newer method used as a part of cancer treatment in order to prevent acute MSU deposition, a part of the tumor-lysis syndrome(29). This thesis will focus on the drugs inhibiting the XOR enzymes.

Allopurinol and oxypurinol

Allopurinol and its active metabolite oxypurinol are two purine analogues that inhibit the isoenzymes xanthine oxidoreductase and xanthine oxidase. The normal dose of allopurinol is 200 mg to 300 mg, and the lowest effective dose is 100 mg. Highest recommended dose is 800 mg. Allopurinol is mainly secreted through the kidneys. Allopurinol has a serum half-life between one and two hours, before it is further oxidised to oxypurinol with a half-life of about 15 hours. Allopurinol interacts with mercaptopurine and azathioprine, which require dose adjustments when taken simultaneously. The most commonly reported side effects are gout attacks, gastrointestinal complains and maculopapular rash. From 0.1 to 0.4% of the patients taking allopurinol or oxypurinol develop severe cutaneous adverse reactions (SCARs), which might be life-threatning(29,30).

Febuxostat

Febuxostat is a non-purine inhibitor of both xanthine dehydrogenase and xanthine oxidase. It has a different chemical structure than allopurinol and oxypurinol, and a different binding site on the two XOR isoenzymes. The recommended dose of febuxostat is 40 to 80 mg. The drug has a half-life from five to eight hours. There is a possible interaction between febuxostat and mercaptopurine and azathiopurine. Although febuxostat is metabolized in the liver and excreted through the kidneys, a large part is filtered through the kidneys unchanged. Side effects reported in clinical studies, such as liver function abnormalities, nausea, arthralgia and rash, occurs in about the same rate as in those treated with allopurinol(30).

Possible biological mechanism of the intervention

Although there is an increasing interest for the possible link between hyperuricemia and cardiovascular and kidney morbidity and mortality, there has not been established a clear pathophysiological mechanism from epidemiological, animal and cell studies. Several mechanisms for the association have been proposed and some of them are discussed below(31–34).

Oxidative stress from the xanthine oxidase isoenzyme

Markers of increased oxidative stress are associated with cardiovascular and renal disease(35–38). The XOR isoenzyme xanthine oxidase (XO) is known to produce reactive oxygen species, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2)(25), as a bi-product in the metabolism of hypoxanthine to uric acid. Increased uric acid levels might be a marker of increased reactive oxygen species (ROS) production by the xanthine oxidase enzyme when purines are degraded. This could explain the epidemiological association between uric acid and cardiovascular and renal disease. By blocking this enzyme complex with xanthine oxidase inhibitors, it might be possible to reduce the oxidative stress(39,40). A study by Landmesser et al., 2002, showed increased levels of endothelium bound xanthine oxidase in patients with congestive heart failure (CHF) and impaired flow mediated dilatation (FMD) in the brachial artery. Reduced FMD improved by administrating the XOR inhibitor allopurinol(41). A study on patients with idiopathic dilated cardiomyopathy found improved cardiac efficiency with allopurinol administration(42). The same applied for patients with ischemic CHF given the XOR-inhibitor oxypurinol(43). George et al., 2006, found a significant improvement in FMD in New York Heart assosciation functional class (NYHA) II-III CHF patients treated with allopurinol. This effect was not observed when the uricosuric drug probenicid was used to lower uric acid, indicating that the effect on FMD was not attributed to reduced SUA but is a consequence of blocking the XDH/XO isoenzymes(44).

Effects of soluble uric acid

Another possible explanation for the association between uric acid and cardiovascular and kidney disease is that elevated uric acid level itself has a negative effect on the cardiovascular and renal system. Studies on adipocytes found that soluble uric acid increased oxidative stress by inducing NADPH oxidase(40). Cell studies on human umbilical vein endothelial cells (HUVEC) suggested that uric acid stimulates expression of several components of the renin-angiotensin system (RAS)(45), and may directly contribute to the pathogenesis of hypertension. *In vitro* studies suggested that soluble uric acid reduces the bioavailability of the vasodilator nitrous oxide (NO) in HUVEC and induces production of C-reactive protein (CRP) in both HUVEC and human vascular smooth muscle cells (HVSMC)(46). Inducing hyperuricemia in rats resulted in hypertension, which was attenuated by blocking xanthine oxidase or increasing renal excretion of uric acid(47–49). Soletsky et al., 2012 found that reducing uric acid by allopurinol or probenicid reduced blood pressure in pre-hypertensive adolescents indicating that it is the reduction of uric acid *per se* that causes the reduction in blood pressure observed(50).

The aim of the thesis

Meta-analyses summarize studies by effect size, but this might not always give a good estimate in small and heterogeneous studies(51). Systematic reviews intending to results in a meta-analysis tend use strict inclusion criteria in order to apply meta-analysis statistics, which often results in few included studies. On the other hand,

expert opinion reviews often apply liberal search strategies and use results from epidemiological, animal and cell studies to support their conclusions(52). Before further research is conducted on the possible implications of hyperuricemia and uric acid metabolism in the pathogenesis of cardiovascular disease, it is important to identify what type of trials on humans that already exist and where there is a need for more evidence. This review will present a summary of available studies in a modified PICO-table(53) and will hopefully serve as a bridge between the existing meta-analyses and expert opinions.

Methods

Criteria for considering studies for this review

Inclusion criteria

- Population: Human studies, participants of both sexes and any age except new-borns
- Intervention: Any XOR inhibitor
- Comparator: Other pharmacological intervention or placebo
- Outcomes: Cardiovascular or renal disease related outcome, including mortality
- Study design: Double blinded randomized controlled trials. Open label and non-randomized designs were considered for inclusion when retrieved by the search

Exclusion criteria

- Language: Full-text not available in English
- Non-availability of full-text: Records with no available full-text
- Studies which specifically included patients with gout or other MSU-deposition disease
- Surgical studies, or studies on cardio- and renal protection during surgery or acute disease
- Outcomes related to gout or other crystal deposition disease

Outcomes

Primary outcomes

This review focus on primary end-points related the cardiovascular- and renal system and mortality. The primary end-points stated by the authors in each article will be included. This will also include biochemical and surrogate markers of cardiovascular and renal disease.

Secondary outcomes

Secondary end-points in the identified full-text articles will not be reported in this thesis.

Search methods for identification of studies

Electronic searches

Medline(Ovid), Embase (Ovid) and Cochrane central register of clinical trials (CENTRAL), were used. A pilot search was conducted with a free-text search on: "xanthine oxidase inhibition" in order to identify relevant key words and search terms.

A final refined search was conducted using the following main search concepts: 1) inhibition of XOR; 2) cardiovascular- and renal outcomes together with morbidity and mortality; 3) clinical trials and 4) humans. MESH-terms and EMBASE index words high in the hierarchy were used to give the search high sensitivity. The final search included relevant free text words in the title, abstracts and the authors' own keywords. In Embase, the following limits were used to sort the search: English language, journal article and clinical trial. In Medline, limit to English language was used. See supplementary tables S1, S2 and Figure S1 for a detailed description of the search strategy in the three databases used.

Searching other resources

One article was included from screening the reference lists from four relevant systematic reviews(54–57). www.clinicaltrails.gov was used for the assessment of reporting bias.

Data collection and analysis

Selection of studies

The records were downloaded from Ovid as an electronic reference file and imported to Mendeley Desktop version 1.15.3 (Mendeley group LTD). Mendeley's built-in duplicate system was used to remove duplicates. Henrik Brovold (HB) screened the titles and abstracts. The titles and abstracts excluded were filed in a separate folder in the reference manager. The full-texts were downloaded to the reference manager or ordered through the library service at the UiT – The Arctic University of Norway. Figure 1 (PRISMA diagram) summarizes record selection and flow through the review process.

Data extraction and management

HB extracted the data using a pre-defined table, which included relevant information for this thesis. This information was used to further categorise the included full-text articles into five categories. The outcomes were categorised using the following hierarchy when multiple primary outcomes were reported: 1) Heart related 2) renal related 3) adolescent blood pressure (BP) 4) endothelial function and BP and 5) Biochemical markers.

Assessment of risk of bias in the included studies

Cochrane guidelines recommend a checklist with seven items for assessing the internal validity of RCTs, scoring this as low risk of bias, high risk of bias or unknown risk of bias. The Cochrane collaboration guidelines are generally against scales assigning numeric values to the risk of bias(58). After a pilot using the recommended Cochrane checklist, it was decided that the scoring was too subjective when done by a single investigator, and it was decided to use the Jadad score. Jadad score is a numeric scale based on three questions concerning internal validity. The maximum score is five indicating high internal validity and the lowest is zero indicating low internal validity(59). This score was used as a rough estimate of the studies internal validity and included in the result tables(60).

Data synthesis

The data were summarised in a modified PICO-table including the following information: Last name of the first author, year of publication, study design, number of participants included, description of the population, intervention drug and dose, length of intervention, description of main outcome reported and if the result were significant, Jadad score and adverse effects if they were reported.

Measures of treatment effect

The analysis of this review is based on a qualitative presentation of the content in the identified study. Treatment effect will only be reported as statistically significant on a $p \leq 0.05$ level or not.

Assessment of reporting bias in clinical trials using xanthine oxidase inhibitors

Inspired by an article by Ioannidis, 1998, where a cohort of registered studies with National Institute of Health funding were used to assess reporting bias(61),the web-based clinical trial register (www.clinicaltrials.gov), was used to create a similar cohort of studies on xanthine oxidase inhibition assessing heart and renal related end-points.

Clinicaltrials.gov database was searched on the 17th of July 2015. The search terms used were *oxypurinol OR allopurinol OR febuxostat OR xanthine dehydrogenase OR xanthine oxidoreductase OR xanthine oxidase*. Uric acid was not included because it made the search less specific. The search results were downloaded and converted to an MS excel spreadsheet (Microsoft corporation). The following fields in the database were screened: title, treatment and end-points. The inclusion criteria were the same as used to include studies for this thesis.

A study was considered completed when it had the status completed in the register or it had passed the pre-specified study completion date in the clinicaltrials.gov register and this had not been updated. When a study completion date was not specified in clinicaltrials.gov database and the date for the end of the study was not available in the corresponding published article, primary completion date from the

clinicaltrials.gov was used. If it was not possible to retrieve a completion date, the study was excluded from the analysis.

Studies not considered completed prior to the inclusion date July 17th, were excluded from further analysis. Studies were the protocol had been withdrawn before enrolment of participants, were not included in the analysis. These studies had the status *withdrawn* in the dataset. Studies with the status *terminated* were included because they had started enrolling participants before termination.

The study was considered unpublished if it was not published before the inclusion date of 17th July 2015. This was judged by the following method:

1. Search in PubMed and Google scholar on the NTC-number
2. Search on study acronym where available
3. Search on the name of the listed principal investigator
4. Search on responsible institution
5. Search on alternative study-ID

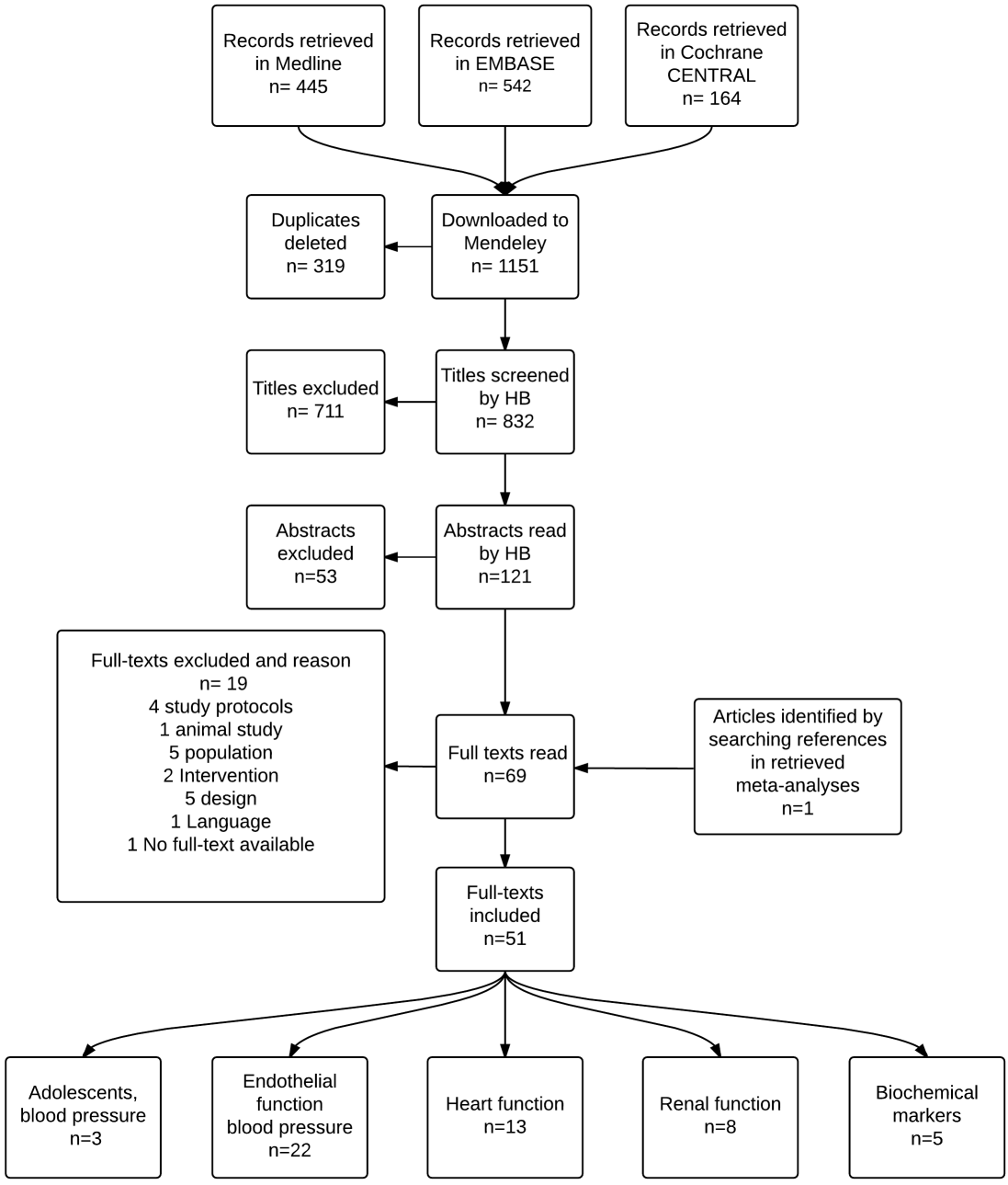
SPSS for Mac version 21 (IBM) was used to make a Kaplan-Meyer plot with a one-minus survival function. A published study was considered as a ”failure” to be unpublished. The follow-up time was from the retrieved study completion date to July 17th 2015.

Results

Results of the search and studies included and excluded

A total of 1151 available records from the three databases were downloaded to the reference manager Mendeley Desktop version 1.15.3. In total, 319 duplicates were removed using Mendeleys automatic function for identifying duplicates. The remaining 832 titles were duplicated and filed in separate folders in the reference manager. Titles were screened by HB. This left 121 abstracts that were read by HB. The full text articles that were available (n= 68) were retrieved. In addition, one study was identified by screening the included studies in four relevant systematic reviews(54,57,62,63). After reading the full texts, 51 articles fulfilled the inclusion criteria, 18 articles were excluded and one article was not available as full-text.

Figure 1: Modified PRISMA diagram showing the search results and a brief description of the process of including full-text articles and categorizing them into five categories.



Medline, EMBASE and Cochrane´s CENTRAL were searched in order to identify records of RCTs using XOR inhibitors to treat chronic cardiovascular and renal disease. Surgical studies and studies in acute medical conditions were not included. Studies in gout population or in gout end-points were not-included. (Details of the search is described in the appendix Table S1, S2 and Figure S1 Reasons for exclusion is specified in the appendix (Table S3).

Excluded studies

As shown in Table 1, a total of 782 studies were excluded after the duplicates were removed. Of these, 18 were removed after the full-texts were downloaded or retrieved from the library and read. One study was not available in full text and therefore excluded(64). Table S3 in the appendix gives a brief description of the reason for the exclusion. Four articles were excluded because they described study protocols (65–68), five were excluded because the study population included gout patients and therefore did not fulfil the pre-defined inclusion criteria(69–73). Two studies used an intervention that did not fulfil the inclusion criteria(74,75). Five studies had a study design that was not included in the pre-specified criteria(76–80). One study was an animal study(81) and one study was not available in English(82).

Risk of bias in included studies

Table 1: Showing Jadad score in the five different groups the retrieved full-texts were categorized into.

End points	**N (studies)**	**Median Jadad score (25^{th} -75^{th} percentile)**	
Heart function	13	4.5	(2.8-5.0)
Renal function	7	2.0	(2.0- 3.5)
Adolescents BP	3	4.0	(3.0-4.5)
Endothelial & BP	22	3.0	(2.0-4.0)
Biochemical markers	5	2.0	(2.0-3.0)

Median Jadad score with 25^{th} to 75^{th} interquartile range.
BP indicating blood pressure.

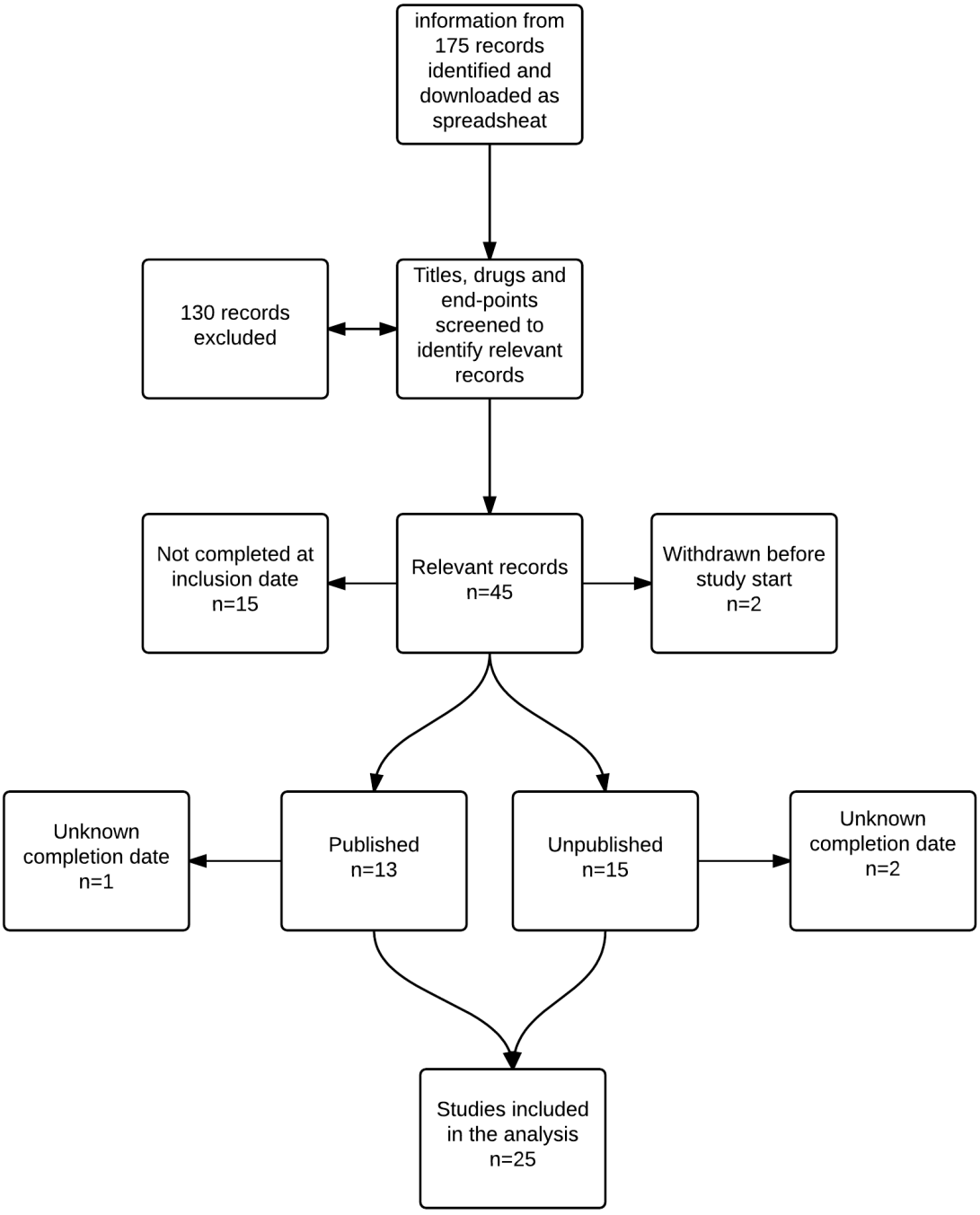
The heart function and adolescents blood pressure (BP) group had the highest median Jadad score with 4.5 and 4.0 respectively (Table 1). This indicates better internal validity in these categories than in the endothelial & BP category with a median of 3.0 and the renal function and biochemical markers with a median Jadad score of 2.0.

Assessment of selective reporting

A total of 175 studies fulfilling the pre-specified inclusion criteria, were found searching www.clinicaltrials.gov. The records retrieved is a separate dataset of records not necessary corresponding to the studies included included in the main analysis in this thesis. Forty-five studies were judged as relevant. Of these 15 were not identified as *completed* by the inclusion date July 17^{th} 2015, and excluded from further analysis. Two studies were withdrawn prior to the planned start date of the study and were excluded from further analysis. The final analysis included 25 studies. For further details, see Figure 2. Due to the wide inclusion criteria, the studies included in this analysis were heterogeneous with respect to population, numbers,

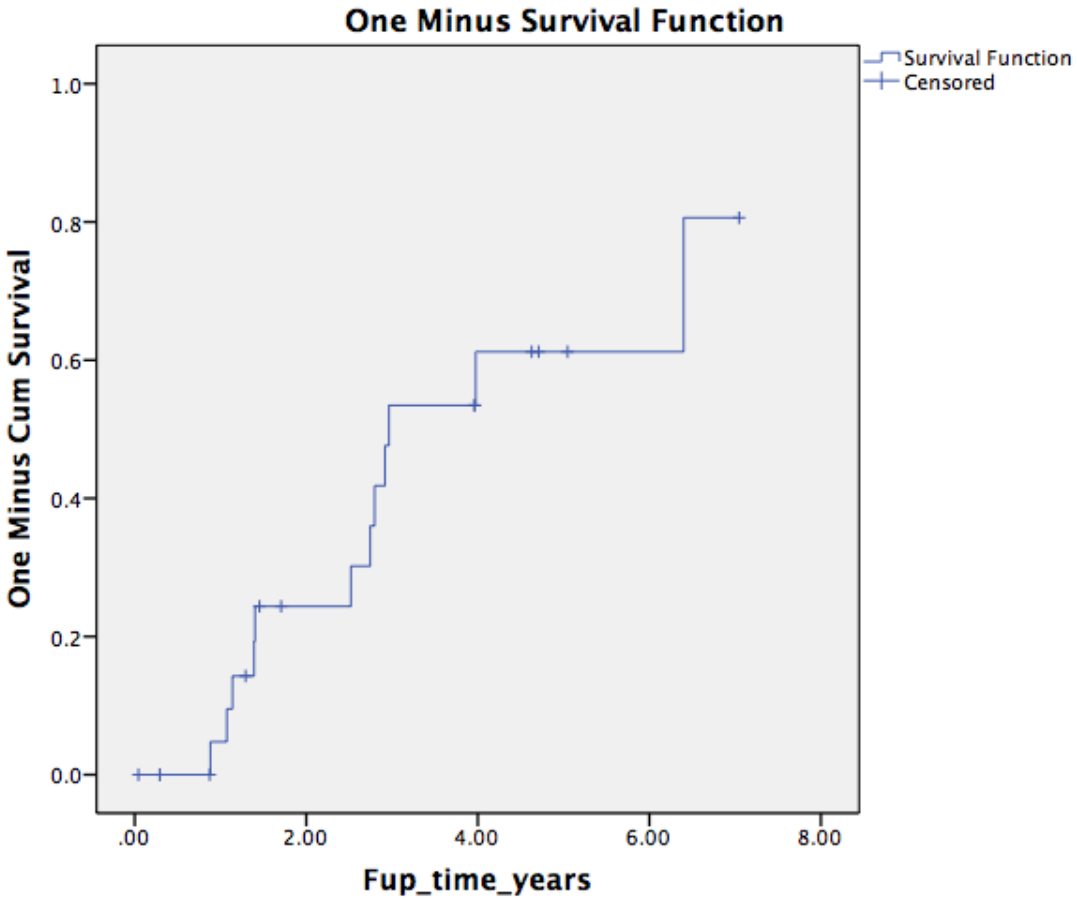
and outcomes, however, all studied XOR-inhibitors effect on mortality, cardiovascular and renal disease related end points.

Figure 2: Modified PRISMA diagram showing the Identification of the studies included in the analysis of selective reporting.



www.clinicaltrials.gov were searched in order to identify records of RCTs using XOR inhibitors to treat chronic cardiovascular and renal disease. Surgical studies and studies in acute medical conditions were not included. Studies in gout population or in gout end-points were not-included. A, total of 175 records were identified. Finally, 25 studies were included in the analysis.

Figure 3. *A* Kaplan-Meier plot showing the cumulative proportion of studies on XOR-inhibition registered on clincaltrials.gov and published within 8-years after the initially reported study completion date.



The inclusion criteria for the studies were: studies on XOR-inhibitors with end-points on chronic cardiovascular and renal disease. (See detailed inclusion criteria in methods section) Y-axis shows One minus survival function. X-axis is time in years from study completion date reported in clinicaltrials.gov to the date the study is available. Studies not published before July 17th were censored.

Of the 25 studies included in this analysis six had at least partial funding from the pharmacology industry and the remaining 19 had no reports of industrial funding. The results in Table 3 indicate that three years after the reported study *completion date* over 50% of the studies reported to the clincaltrials.gov are published. After four years approximately 60% of the studies are published. According to Figure 3, approximately 80% of the studies are published seven years after reported completion date in clinicaltrials.gov. This means that as much as 15 to 20% of the RCTs on this intervention are either published many years after the study is completed, never completed, or completed but never published.

Effect of interventions

The following section will provide a narrative of the main results from the studies included in this thesis. The individual studies are summarized in a modified PICO tables at the end-of this section. (Table 2, Table 3, Table 4, Table 5 and Table 6).

Studies assessing heart-related outcomes

Overall, 13 studies were categorised in the heart related end-point group (Table 2). Eleven of the studies were double blinded RCTs and two studies were non-randomised intervention studies. The two largest studies Givertz et al., 2015 and Hare et al, 2008 both with Jadad of 5, included approximately 60% of the total study population in this outcome category(83,84). Eight studies are conducted in CHF patients with a NYHA II-IV functional class, three in Coronary artery disease (CAD) patients, one in CKD, stage III patients and one in Diabetes Mellitus type 2 (DM 2) patients with left ventricle hypertrophy (LVH). The median Jadad score is 4.5.

The two largest studies by Givertz et al, 2015 and Hare et al., 2008 with 253 and 405 participants respectively, used a cardiovascular composite end-point (CCE). The CCE consisted of death, hospitalization, emergency department visit or heart failure clinic visit for worsening heart failure (HF) or change of medication for worsening HF(83,84). The two studies both included CHF NYHA III-IV patients. Givertz et al., 2015 only included hyperuricemic patients with SUA > 565 μmol/L. In both studies CCE was non-significant in the intervention group compared to placebo after 24 weeks on XOR inhibitors(83,84). A small study by Nasr et al., 2010 used a composite end point consisting of global left cardiac function, heart failure morbidity and/or mortality in NYHA III-IV patients and reported no significant effect of 300 mg allopurinol for 36 weeks against placebo(85).

Cingolani et al., 2006 found no statistically significant effect of 600 mg oxypurinol on echocardiography measured left ventricle ejection fraction (LVEF) after one-month treatment in NYHA II-III patients. There was no effect of the intervention on a functional six-minute walk test (6MWT) in the same study(86). One non-randomised study found significant improvement of LVEF measured by cardiac magnetic resonance imaging (CMRI) 5.2±0.9 hours after a single infusion of 400 mg oxypurinol(43). Three studies assessed the effect of XOR-inhibition on left ventricular mass (LVM) or left ventricular mass index (LVMI), by CMRI(87–89). The three CMRI studies had a similar design, and follow-up of nine months. The study in DM2 patients with LVH found a significant reduction in LVM compared to placebo(87), the study on CAD patients could not demonstrate any significant difference(88). The study in a CKD stage III population, with a daily dose of 300 mg allopurinol found a significant reduction in LVM(89).

Noman et al. 2010 reported a significant improvement in time to ST-segment depression on ECG, exercise time and time to chest pain in Bruce protocol stress test in CAD patients with stable angina in a double blind crossover RCT comparing allopurinol 600 mg daily vs. placebo for six weeks before crossover(90).

Studies reporting renal related outcomes

The results discussed here are found in Table 3. The participants included had varying degree of renal disease from no renal disease to end-stage kidney disease. Three studies used SUA above a specified threshold as an inclusion criteria(91–93). All studies compared allopurinol against placebo. The allopurinol dose ranged from 100 to 300 mg daily in all studies except Rosenfeld et al., 1974, which used a target level of uric acid to direct the therapy(94). The follow-up period ranged from three to 40 months in these. Goicoechea et al., 2015 followed a cohort from an earlier RCT for a total of 84 months(95). Siu et al., 2006 and Goicoechea et al., 2010 used a composite end-point assessing worsening of kidney disease as an endpoint(93,96). In total, three studies used eGFR as an end-point and none utilized measured renal clearance. The median Jadad score is 2.0.

Siu et al., 2006 and Goicoechea et al., 2010 both with a Jadad score of 4.0, and 12 and 24 months follow-up respectively, reported a significant improvement of the kidney composite end-point in the allopurinol group compared to placebo(93,96). Goicoechea et al., 2010 also reported a significantly reduced risk of cardiovascular events and reduced frequency of hospital admissions in the allopurinol treatment group(96). In the follow-up study of this cohort, the effects on the renal composite end-point and cardiovascular mortality were still significant 84 months post allocation to treatment. The total mortality rate was similar between the treatment group and the placebo group(97). Estimated GFR was used as a main outcome in three studies. Rosenfeld et al., 1974 reported no effect in the intervention group after 30-40 months of treatment(94). Kanbay et al., 2007 and Kanbay et al., 2011 studies, with an intervention lasting three and seven months respectively, reported significant difference in eGFR in the treatment group compared to placebo(92,98). Momeni et al., 2010 reported that allopurinol significantly reduced proteinuria in patients with diabetic nephropathy compared to placebo(99). Kanbay et al., 2007 reported no effect on proteinuria in the allopurinol group compared to placebo(98).

Studies reporting blood pressure in adolescents as outcome

The studies assessing ambulatory blood pressure in adolescents are found in Table 4. One study is open label and two are double blinded RCTs. A total of 134 participants were included. The included participants were defined as pre-hypertensive or stage 1 hypertensive. In two of the studies, SUA levels over a defined threshold were used as an inclusion criteria. The interventions used are allopurinol 400 mg daily in the Feig et al., 2008 study, allopurinol 200 mg daily vs. probenicid 500 mg daily vs, placebo in the Soletsky et al., 2012 study. The study by Assadi et al., 2014 used combination of allopurinol 300 mg plus the angiotensinogen converting enzyme (ACE) inhibitor enalapril 20 mg daily vs. enalapril 20 mg daily. All studies reported statistically significant reduction in ambulatory blood pressure. Feig et al. reported significant reduction in both ambulatory and 24-hour blood pressure(100). Interestingly, in the Soletsky et al., 2012 study the use of the uricosuric drug probenicid and xanthine oxidase inhibitor allopurinol reduced blood pressure when compared to placebo indicating that lowering SUA reduced hypertension in adolescents. There was no significant difference between the two drug intervention

groups(50). Assadi et al., 2014 reported a statistically significant reduction in ambulatory blood pressure when allopurinol 300 mg daily was added to 20 mg enalapril(101).

Studies reporting measures of endothelial function and vascular function

There are 22 studies in this category (Table 5). Six studies had double blind parallel group RCT design, ten studies had double blind crossover RCT design, and the remaining studies had a non-randomised open label design. The median number of participants included in a study was 20, ranging from 9 to 100 participants. The studies had heterogeneous populations with five studies on (CHF), three studies on cerebrovascular disease, three on DM2, three on CAD, two on smokers and six studies with participants from other populations. Twenty of 22 studies compared XOR-inhibitors against placebo, one study allopurinol against losartan and placebo and one study allopurinol plus atorvastatin vs. atorvastatin alone. The median follow-up was four weeks ranging from single infusion studies with less than one-day follow-up to 52 weeks' follow-up post randomisation. The median Jadad score was 3.0 with the 25^{th} and 75^{th} percentile from 2.0 to 4.0 respectively. Xanthine oxidase inhibitors effect on flow mediated vasodilatation was investigated in 15 of 22 studies included in this category.

Thirteen studies reported that use of XOR inhibitors led to statistically significant increase in FMD. Three studies including hypercholesterolemic patients reported no statistically significant difference(102–104). Five studies reported EDV as a primary outcome. Four of these reported a beneficial effect of XOR-inhibition on EDV(44,105–107), while one study reported no statistically significant effect(104). One study with an open label design with single infusion of oxypurinol reported significant effect only in the group including hypercholesterolemic patients but not in the healthy controls or hypertensive patients(108). Five studies reported endothelium-independent vasodilatation (EIV) as a main outcome. Four of these found no statistically significant beneficial effect(104,106,108,109). One study found a statistically significant effect in smokers after a single dose of 600 mg allopurinol. Augmentation index (AiX) a measurement of arterial stiffness by pulse contour analysis was measured in four studies. In three studies with a follow up from 8 weeks to 12 months and allopurinol 300 to 600 mg daily significantly improved AiX compared to placebo(55,110,111). In patients with subcortical stroke treated with allopurinol 300 mg daily for three months there was no significant effect of the intervention(112).

Studies reporting biochemical outcomes

There were five double blinded RCTs studying biochemical outcomes (Table 6). In total, 203 patients were included. Three studies included participants with NYHA II-III CHF, one included participants with CKD and one was conducted in healthy athletes. All studies used allopurinol 300 mg daily as the intervention. Allopurinol was compared against placebo in two studies and against rosuvastatin and placebo in two studies. One study compared allopurinol against rosuvastatin. The outcomes

assessed in these studies were biochemical markers of disease. The median Jadad score in these studies was two.

Bowden et al., 2013 found reduced total cholesterol and ApoB in the treatment group however the other outcomes LDL, triglycerides ,HDL, total cholesterol/HDL also listed as primary outcomes were not significant(113). The three studies comparing the effect of allopurinol against rosuvastatin on circulatory levels of myeloperoxidase (MPO)(114), matrix metalloproteases and tissue inhibitors of metalloproteinases(115), as well as level of circulating endothelial progenitor cells (EPC)(116) found a statistically significant effect in the rosuvastatin group compared to the allopurinol or placebo groups. In the comparison of allopurinol against placebo there was no statistically significant effects reported.

Adverse reactions

Of the 51 studies included in this thesis, 22 reported none adverse events related to the study drugs and 19 studies did not report on adverse effects. Adverse events related to treatment with the XOR inhibitors were reported in 10 studies. One study reported GI-symptoms in two of the patients receiving allopurinol who discontinued the study(96). The most common symptoms were rashes. There were reported none fatal events related to the intervention drugs. Table 2, Table 3, Table 4, Table 5 & Table 6 provide details of adverse reactions reported.

Table 2. PICO table of studies identified using XOR inhibitors as intervention assessing heart related outcomes

Author	Year	Study design	N	Population	Intervention and follow up time		Primary outcomes	$p \leq 0.05$	Jadad-score	Adverse effects
Givertz, M(83)	2015	RCT, double blind	253	NYHA III-IV, with SUA > 565 μmol/L	Allopurinol, 600 mg/d	24 weeks	↑CCE*	**X**	**5**	15% in allopurinol group experienced skin and rash complications vs. 6% in the placebo group
Hare, J(84)	2008	RCT, double blind	405	NYHA III-IV	Oxypurinol 600 mg/d,	24 weeks	↑CCE*	**X**	**5**	None related to study drug
Nasr, G(85)	2010	RCT, double blind	59	NYHA III-IV	Allopurinol 300 mg/d,	36 weeks	↑Composite end-point**	**X**	**2**	N.A
Cingolani, H(86)	2006	RCT, double blind	60	NYHA II-III	Oxypurinol 600 mg/d,	1 month	↑LVEF ↑6MWT	**X** **X**	**3**	None related to study drug
Baldus, S(43)	2006	Non random-ised inter-vention	20	CAD, NYHA III-IV	Oxypurinol, 400 mg single infusion		↑LVEF	√	**0**	None related to study drug

Rekhraj, S(88)	2013	RCT, double blind	66	CAD with LVH on ECG	Allopurinol 600 mg/d	9 months	↓LVM ↓LVMI	√ **X**	**5**	One patient developed a rash in the Allopurinol group
Szwejkowski, B(87)	2013	RCT, double blind	66	DM2, with LVH on Echo	Allopurinol 600 mg/d	9 months	↓LVM ↓LVMI	√ √	**5**	None related to study drug
Kao, M(89)	2011	RCT, double blind	67	CKD, stage III	Allopurinol 300 mg/d,	9 months	↓LVMI	√	**4**	Two with rash and one with arthralgia in Allopurinol group
Noman, A(90)	2010	RCT, double blind crossover	65	CAD, stable angina	allopurinol 600 mg/d	6 weeks	↑Time to ST depression, ↑Exercise time ↑Time to chest pain	√ √ √	**5**	None related to study drug
Gavin, A(117)	2005	RCT, double blind crossover	50	NYHA II-III	Allopurinol 300 mg/d	12 weeks	↑mBruce-test Time to ex-haustion ↑6MWT	 **X** **X**	**3**	Two in the allopurinol group developed rash, five in the placebo-group withdrew for other reasons

Cappola, T(118)	2001	Non, random-ised inter-vention	9	Dilated cardiomyopathy, EF 30% NYHA II-III	Allopurinol infusion 0,5-1,5 mg/min		↓MvO_2 ↑SW	√ √	**0**	N.A
Shehab, A(119)	2001	RCT, double blind crossover	19	NYHA II-IV	Allopurinol 300 mg/d	2 months	↑Autonomic function	**X**	**4**	None related to study drug
Hirsch, G(120)	2012	RCT, double blind	16	NYHA II-III	Allopurinol 300 mg, single infusion		↑ATP/pCR ratio	√	**2**	N.A

*CCE sequential rules based on: death; hospitalization, emergency department visit or emergent clinic visit for worsening HF; medication change for worsening HF(121)
**composite end point comprising global left cardiac function as well as heart failure morbidity and mortality.(85)

Jadad-score is a numeric score from 0 to 5. A Jadad score of 5 is the highest score indicating good internal validity. Outcomes where there is a significant difference between the intervention group and control group on a *$p \leq 0,05$ is indicated with* **√.** Conversely, non-significant outcomes are indicated with **X**. N.A indicates not applicable.

6MWT indicates six minute walk test; ATP/pCR ratio adeonsine triphosphate/phospocreatinine ratio; CCE cardiovascular composite end-point; CAD coronary artery disease; CHF congestive heart disease; CKD chronic kidney disease; DM2 diabetes mellitus 2; EF ejection fraction; LVH left ventricle hypertrophy; LVEF left ventricle ejection fraction; LVM left ventricle mass; LVMI left ventricle mass index; NYHA New York Heart association functional class; MvO2 myocardial O_2 consumption; SUA serum uric acid; SW (myocardial) stroke work

Table 3. PICO table of studies identified using XOR inhibitors as intervention assessing renal related outcomes

Author	Year	Study design	N	Population	Intervention and follow up time		Primary outcomes	*p ≤ 0,05*	Jadad-score	Adverse effects
Goicoechea, M(95)	2015	RCT, follow up*	113	eGFR < 60 ml/m2/min	Allopurinol 100 mg/d	84 months	↑eGFR ↓Cardio-vascular events ↓Mortality	√ √ X	N.A	None related to study drug
Goicoechea, M(96)	2010	RCT, double blind	113	eGFR < 60 ml/m2/min	Allopurinol 100 mg/d	24 months	↑eGFR ↓Cardio-vascular events ↓Hospital admissions	√ √ √	**4**	Two with GI symptoms in allopurinol group stopped treatment
Siu, Y(93)	2006	RCT, double blind	54	CKD, SUA > 450 μmol/L	Allopurinol 100-200 mg/d	1 year	↑Composite kidney end-point**	√	**4**	One skin rash in allopurinol group
Kanbay, M(92)	2011	RCT, double blind	72	SUA > 416 μmol/L	Allopurinol 300 mg/d	7 months	↑eGFR ↑FMD% ↓Ambulatory BP	√ √ √	**2**	None related to study drug
Kanbay, M(98)	2007	Open label	48	SUA > 416 μmol/L	Allopurinol 300 mg/d	3 months	↑eGFR ↓Protein-uria ↓ambulatory BP	√ X √	**0**	One urticarial rash in the allopurinol group

Rosenfeld, JB(94)	1974	RCT, single blind	117	Patients stratified in five groups after BP and eGFR	Allopurinol to target SUA level	30-40 months	↓Creatinine ↑eGFR ↓Ambula-tory BP	**X** **X** **X**	**2**	N.A
Momeni, A(99)	2010	RCT, double blind	44	Diabetic nephropathy > Proteinuria 500 mg/24 hours	Allopurinol 100 mg/d	4 months	↓Proteinuria	√	**2**	None related to study drug
Jalalzadeh, M(91)	2012	RCT, single blind, crossover	55	Hypertensive hyperuricemic hemodialysis patients (men SUA 387 μmol/L women SUA 327 μmol/L)	Allopurinol 100 mg/d	3 months	↓Ambulatory BP	√	**3**	N.A

*Post hoc analysis of the Goicoechea, 2011 study.
**1) < 40% increase in Cr levels, 2) >40% increase in Cr 3) End-stage renal disase (ESRD) 4) Death

Jadad-score is a numeric score from 0 to 5. A Jadad score of 5 is the highest score indicating good internal validity. Outcomes where there is a significant difference between the intervention group and control group on a *$p \leq 0,05$ is indicated with* **√.** Conversely, non-significant outcomes are marked with **X**. N.A indicates not applicable.

CKD indicates chronic kidney disease; eGFR estimated glomerular function; BP blood pressure; FMD flow mediated vasodilatation; SUA serum uric acid

Table 4. PICO table of studies identified using XOR inhibitors as intervention assessing blood pressure in adolescents as primary outcome

Author	Year	Study design	N	Population	Intervention and follow up time		Primary outcomes	*p ≤ 0,05*	Jadad-score	Adverse effects
Feig, D(100)	2008	RCT, double blind crossover	30	Adolescents 11-17 years stage 1 hypertension	Allopurinol 400 mg/d,	4 weeks	Ambulatory BP 24h BP	√ √	**4**	None related to study drug
Soletsky, B(50)	2012	RCT, double blind	60	Adolescents 11-17 years, prehypertension SUA > 300 μmol/L	Placebo vs allopurinol 200 mg/d vs probenicid 500 mg/d,	8 weeks	Significant reduction in BP in the treatment groups vs placebo. N.S between the two treatment groups		**5**	One hypersensitivity reaction in the allopurinol group 1 of 20 participants
Assadi, A(101)	2014	RCT, open label	52	Adolescents 12-19years, Prehypertension SUA > 327 μmol/L	Enalapril 20 mg/d vs enalapril 20 mg + allopurinol 300 mg/	8 weeks	Ambulatory BP in + allopurinol group	√	**2**	N.A

Jadad-score is a numeric score from 0 to 5. A Jadad score of 5 is the highest score indicating good internal validity. Outcomes where there is a significant difference between the intervention group and control group on a *p ≤ 0,05 is indicated with* **√.** Conversely, non-significant outcomes are marked with **X**. N.A indicates not applicable.

BP indicates blood pressure; SUA serum uric acid; N.S non significant

Table 5. PICO table of studies identified using XOR inhibitors as intervention assessing endothelial function, blood pressure or/and other measures of vascular function as outcome.

Author	Year	Study design	N	Population	Intervention and follow up time		Primary outcomes	*p ≤ 0,05*	Jadad-score	Adverse effects
B, Yelken(122)	2012	Open label, crossover	19	Hyperuricemic, CKD III-IV	Allopurinol 150 mg/d	8 weeks	↑FMD	√	**0**	N.A
Dogan, A(107)	2011	RCT, single blind	100	DM2	Allopurinol 900 mg/d	12 weeks	↑FMD ↑EDV	√ √	**1**	None related to study drug
Rajendra, S(111)	2011	RCT, double blind crossover	90	CAD	Allopurinol 600 mg/d	8 weeks	↑FMD ↑AiX ↓vascular oxidative stress	√ √ √	**5**	None related to study drug
Greig, D(102)	2011	RCT, double blind	74	CHF, NYHA II-IV	Atorvastatin 20mg/d + placebo vs. atorvastatin 20 mg/d + allopurinol 300 mg/d,	4 weeks	↑FMD ↑6MWT	**X** **X**	**5**	None related to study drug
Haehling von, S(123)	2010	RCT, double blind	17	CHF, NYHAII-III vs. healthy controls	Allopurinol 300 mg/d	1 week	↑FMD ↑post-ischemic blood flow	√ √	**3**	None related to study drug
Yiginier, O(124)	2008	RCT, double blind	57	Metabolic syndrome	Allopurinol 300 mg/d	1 month	↑FMD ↓MDA ↓MPO-activity ↓hsCRP	√ √ √ **X**	**3**	N.A

							↓Fibrinogen	X		
Landmesser, U(125)	2007	Open label, experimental	24	CAD	Losartan 50 mg/d (n=10) vs allopurinol 300 mg/d (n=9), placebo (n=5)	4 weeks	↑FMD, ↓XO-activity allo-purinol & losartan vs placebo	√ √	**0**	None related to study drug
George, J(44)	2006	RCT, double blind crossover	30	CHF, NYHA II-III	Allopurinol 300 mg/d & 600 mg/d vs placebo	1 month	↑FMD**	√	**3**	One patient with rash in allopurinol 300 mg group
Eskurza, I(103)	2006	RCT, double blind crossover	18	Sedentary healthy adults divided into young 21-34y and old 55-71y	Allopurinol, 600 mg, single dose		↑FMD	**X**	**2**	N.A
El Solh, A(126)	2006	RCT, double blind crossover	12	Sleep apnoea	Allopurinol 300mg/d	2 weeks	↑ FMD ↓MDA	√ √	**4**	None related to study drug
Baldus, S(127)	2005	Open label, Single group	18	CAD	Oxypurinol 200 mg, single infusion		↑FMD ↑Coronary artery endothelial function	√ √	**0**	None related to study drug
Guthikonda, S(128)	2004	RCT, single blind crossover	12	Smokers	Allopurinol 600 mg, single dose		↑FMD*	√	**2**	N.A

Guthikonda, S(105)	2003	RCT single blind crossover	28	Smokers and healthy age matched controls	Allopurinol 600 mg, single dose		↑FMD* ↑EDV* ↑EIV*	√ √ √	**3**	N.A
Farquharson(106)	2002	RCT, double blind crossover	11	CHF, NYHA II-III	Allopurinol 300 mg/d	1 month	↑FMD ↑EDV ↑EIV	√ √ **X**	**2**	N.A
Doehner, W(129)	2002	RCT, crossover, double blind	15	CHF, NYHA II-III, SUA > 400 uM	Allourinol 300 mg/d	1 week	↑FMD in the leg	√	**3**	None related to study drug
Butler, R(109)	2000	RCT, crossover with age matched control group	11 DM2 & 12 healthy controls	DM2, mild HT vs age-matched controls.	Allopurinol 300 mg/d vs placebo	1 month	↑EDV ↑EIV	√ **X**	**3**	N.A
O´Driscoli, JG(104)	1999	RCT, double blind crossover	9	s-cholesterol 6-10 mmol/L.	Allopurinol 300 mg/d	4 weeks	↑FMD ↑EDV ↑EIV	**X** **X** **X**	**3**	N.A
Cardillo, C(108)	1997	Open label	60	20 Healthy Controls 20 Hyper-cholesterol-emic 20 Hypertensive	Oxypurinol infusion, 300 µg/min		Significant ↑EDV in the hyper-cholesterolemic patients, none in HT or controls ↑EDV, no effect in either group		**0**	N.A

Higgins, P(55)	2014	RCT, double blind	80	Recent stroke or TIA	Allopurinol 300 mg/d	12 months	↓CBP ↑AIx ↓CIMT	√ √ √	**5**	Three patients in the placebo group discontinued treatment
Dawson, J(112)	2009	RCT, double blind	50	Recent subcortical stroke	Allopurinol 300 mg/d	3 months	↑CVR ↑AIx ↑PWV.	**X** **X** **X**	**4**	None related to study drug
Dawson, J(130)	2009	RCT, crossover, double blind	14	DM2, HbA1C > 9,0%	Allopurinol 300 mg/d,	2 weeks	↓ICA flow w/NMMA infusion	√	**3**	N.A
Khan, F(110)	2008	RCT, double blind	30	Stroke survivors with SUA > 380 mmol/L	Allopurinol 300 mg/d	8 weeks	↑AiX	√	**5**	None related to study drug

*Only the results for the smokers included in the table. The healthy controls had no change in endothelial function, when receiving the intervention drug allopurinol.
**in the Allopurinol 600 mg/daily group

Jadad-score is a numeric score from 0 to 5. A Jadad score of 5 is the highest score indicating good internal validity. Outcomes where there is a significant difference between the intervention group and control group on a *$p \leq 0,05$ is indicated with* **√.** Conversely, non-significant outcomes are marked with **X**. N.A indicates not applicable.

6MWT indicates six-minute walk test; AiX augmentation index; CAD coronary artery disease; CBP central blood pressure; CIMT carotid intima media thickness; CHF congestive heart disease; CKD chronic kidney disease;CVR central vascular resistance; EDV endothelium dependent vasodilatation; EIV endothelium independent vasodilatation; FMD flow mediated vasodilatation; hsCRP high sensitive C-reactive protein; ICA-flow intracereberal artery flow; MDA malonaldidehyde; MPO myeloperoxidase; NMMA NG-monomethyl-L-arginine; NYHA New York Heart association functional class; PWV pulse wave velocity; SUA serum uric acid; CKD chronic kidney disease; TIA transient ischemic attack

Table 6. PICO table of studies identified using XOR inhibitors as intervention assessing biochemical markers as outcome

Author	Year	Study design	N	Population	Intervention and follow up time		Primary outcomes	$p \leq 0,05$	Jadad-score	Adverse effects
Sanchis-Gomar, F(131)	2013	RCT, double blind	12	Healthy athletes	Single dose allopurinol 300 mg before a fotball match		Post match vs pre match: Copeptin, MR-Pro-ADM, GDF15 PIGF, sVEGFR1/sFLT-1	 √ X X X X	**3**	None related to study drug
Bowden, R.G(113)	2013	RCT, double blind	24	CKD	Allopurinol 300 mg,	8 weeks	↓Total-Cholesterol ↓ApoB ↓LDL ↓Triglycerides ↑HDL ↓total cholesterol/HDL	√ √ X X X X	**3**	None related to study drug
Tousoulis, D(115)	2010	RCT, double blind	42	CHF, NYHA II-III	Placebo vs. rosuvastatin 10 mg/d vs. allopurinol 300 mg/d	1 month	↓MMP-2 ↓MMP9, TIMP-1 & TIMP2 in the Rosuvastatin group, no change in allopurinol or placebo group		**2**	N.A
Andreou, I(114)	2010	RCT, double blind	60	CHF, NYHA II-III	Placebo vs. rosuvastatin 10 mg/d vs. allopurinol 300 mg/d	1 month	Rosuvastatin significantly ↓MPO No effect in the allopurinol group		**2**	N.A

Tousoulis, D(116)	2011	RCT, double blind	65	CHF, NYHA II-III	Allopurinol 300 mg/d vs. rosuvastatin 10 mg/d,	1 month	↑EPC significantly increased in the Rosuvastatin group, none in the two others	**2**	N.A

Jadad-score is a numeric score from 0 to 5. A Jadad score of 5 is the highest score indicating good internal validity. Outcomes where there is a significant difference between the intervention group and control group on a *$p \leq 0{,}05$ is indicated with* **√.** Conversely, non-significant outcomes are marked with **X**. N.A indicates not applicable and refers to studies not reporting adverse effects.

ApoB indicates apolipoprotein B; CHF congestive heart failure; CKD chronic kidney disease; EPG endothelial progenitor cells; HDL high density lipoprotein; hsCRP high sensitivity C-reactive protein; LDL low density lipoprotein; GDF15 growth differentiation factor 15; MMP-2 matrix metalloprotease 2; MMP-9 matrix metalloprotease 9; MPO myeloperoxidase; NYHA New York Heart assosciation functional class; MR- pro ADM midregional part of proadrenomedullin; PIGF placental growth factor; TIMP-1 tissue inhibitor of metalloproteases 1; TIMP-2 tissue inhibitor of metalloproteases 2; VEGFR-1/sFLT-1 serum vascular endothelial growth factor receptor-1; VEGF vascular endothelial growth factor

Discussion

Summary of main results

There is lacking evidence to consider treating asymptomatic hyperuricemic patients in order to reduce cardiovascular and renal morbidity and mortality. XOR inhibition had no effect on a CCE in the two larger studies completed with a follow up of 24 weeks(84,132). There is some evidence that long-term treatment with allopurinol reduces LVM and LVMI after long-term treatment(87–89).

Two well-conducted, medium sized, studies with a Jadad score of 4 and follow-up for one and two years reported that XOR inhibition slows the progression of renal disease(93,96). This is further supported by studies showing beneficial effects of allopurinol on eGFR in patients with eGFR < 60 ml/min and patients with CKD and uric acid > 450 μmol/L(133,134). A major limitation to all included studies assessing renal function is that they do not use measured GFR as an end-point(135).

In pre-hypertensive and stage 1 hypertensive adolescents the available evidence shows a reduction in ambulatory BP when treated with XOR-inhibitors. In one study, XOR-inhibitors had an additive effect combined with an ACE-inhibitor. Compared to placebo, the uricosuric drug probenecid and XOR inhibitors had a similar effect on reducing ambulatory blood pressure. A study from 2006 in NYHA II-III patients assessed the effect of reducing uric acid with probenecid vs. allopurinol on FMD. In this study allopurinol improved FMD compared to placebo, but there was no such effect when probenecid was used to lower serum uric acid(44).

In small studies with short follow-up, allopurinol in doses from 300 mg daily to 900 mg daily had a statistically significant effect on FMD in 13 of 17 studies identified assessing FMD as a primary outcome. This effect was probably mediated by improved endothelial-dependent vasodilatation. There is conflicting evidence regarding the effect of XOR inhibition on endothelium-independent vasodilatation. However, the majority of studies indicates that XOR inhibitors work by improving endothelial-dependent vasodilatation(55,56).

There are only five studies done on biochemical outcomes. They have an overall low Jadad score and it is hard to estimate the clinical significance of the findings. Three of the studies compared the statin rosuvastatin against allopurinol and concluded that allopurinol had no effect on the outcomes assessed(114–116). The study done on football players were different than the others by including healthy athletes who received a single dose of allopurinol before a football match(131). The study by Bowden et al., 2011 have some significant results, however, they need to be verified in independent trials(113).

There were patient-reported adverse effects related to the use of allopurinol, which mainly consisted of skin rashes, a well-known side-effect of allopurinol. There were no reports of severe cutaneous reactions (SCARs), a potentially lethal condition

induced by allopurinol, oxypurinol and febuxostat(29). None of the studies reported deaths or non-cutaneous severe reactions related to use of XOR inhibitors.

Overall completeness and applicability of the evidence

The studies by Givertz et al., 2015 and Hare et al.,2008 were done on a population of CHF NYHA III-IV patients with clinically relevant hard end-points . The main limitation of these studies is the relatively short follow-up of 24 weeks(84,132). Further, the use of a cardiovascular composite end-point in order to improve the power of a study might mask clinically relevant end-points. Composite end-points combining surrogate end-points and outcomes with different severity might distort the true clinical relevance of an intervention because the least severe outcome is often more likely to happen. The use of hospitalization as an endpoint may not be correct because participants in a clinical trial might be more likely to be admitted to a hospital than non-participants(136).

The studies assessing LVM by CMRI and echocardiography have long follow-ups and are interesting from a scientific point of view. The clinical applicability of these results is unclear as the effects measured are small even though they are statistically significant(87–89). The results from the renal outcomes are interesting from a clinical point of view if XOR inhibitors can halt progression of renal disease. There is a need for larger and better-controlled studies, ideally with measured glomerular filtration rate(GFR), to assess if this could be an effective treatment for slowing progression of renal disease. The studies on ambulatory blood pressure done on adolescents might have a future application in the clinic. The main concern here is safety. Allopurinol is estimated to cause SCARs in 0.1% to 0.4% of patients(29). In adolescents, a life-style intervention is probably more adequate than using XOR inhibitors or uricosuric drugs for treating early onset hypertension. In the study by Soletsky et al., 2012 there was one adverse reaction reported in the allopurinol group consisting of 20 participants(137).

Endothelial dysfunction is considered a surrogate marker of cardiovascular risk(138). The longest follow up in studies reporting significant improvement in FMD was seven months(133). This indicates that longer studies might be needed to observe the long term effect. Reduced blood pressure is a logical positive effect of improved endothelial function. Unfortunately, blood pressure measurements were not reported in all the studies and very few reported it as a primary outcome.

Quality of evidence

Overall the quality of evidence is not convincing. The studies with renal related outcomes (Table 3) and the studies with biochemical outcomes (Table 6), with 13 of the 51 included studies, have the lowest Jadad score with a median of 2.0. The studies assessing endothelial function, blood pressure or/and other measures of vascular function as outcome (Table 5) included in this thesis have a median Jadad score of 3.0 indicating slightly better internal validity. However, in this group most studies are small RCT´s with a median number of participants of only 20. Another limitation in the endothelial category is the relatively short follow-up. The assessed

internal validity is best in the heart related category (Table 2) with a high median Jadad score of 4.5 and two large well-conducted studies with clinically relevant end-points and the highest external validity of all trials included in the review(83,84).

The three studies done on ambulatory blood pressure in an adolescent population have two adequately blinded studies(50,100) and one study is open label(101). The main limitation there is the short follow-up and low total number of participants. This means that studies including more participants and with longer follow-up are needed before it is possible to conclude that XOR-inhibitors reduces ambulatory blood pressure in adolescents.

Randomised clinical trials with low internal validity - in this review represented by low Jadad score - are at high risk of bias(58). The majority of studies had relatively short follow up, from four weeks to three months, which is too short to pick up long-term effects of an intervention. Further, most of the studies included in this review use surrogate markers of disease. This is known to be problematic for assessing the real life effect of an intervention(139).The use of surrogate end-points weaken the external validity of the interventions that are studied(136).

Results from other reviews

Allopurinols effect on eGFR is assessed in a systematic review by Fleeman et al., 2014 who found few RCTs reporting measures of renal function and none of the included studies reported allocation concealment which is an important aspect of internal validity(54). Overall there is no significant effect of allopurinol on eGFR except for one trial with 24 months follow up(96).

A meta-analysis of ambulatory blood pressure including eleven studies found a statistically significant effect on both systolic and diastolic blood pressure. The overall effect size was small with -3.33 mmHg 95% CI (-5.25 to - 1.25) mmHg on systolic blood and -1.29 mmHg 95% CI (-2.48 to -0.10) on diastolic blood pressure favouring XOR-inhibitors. This effect is probably driven by the studies on adolescent summarised in Table 4 in this thesis and a study of Siu et al in a CKD population(57). The meta-analysis by Fleeman et al., 2014 found no effect on systolic and diastolic blood pressure(54).

A systematic review by Higgins et al., 2012 found improvement in FMD in a meta-analysis of five studies assessing XOR-inhibitors' effect on endothelial function. They also found reduced malonaldidehyde (MDA) levels and improvement in endothelium dependent vasodilatation(62). These findings are supported by a review done by Feig et al, 2014 which had the same results in a meta-analysis of 11 studies. Thus it seems that allopurinol improves flow mediated vasodilatation, this is mediated by an endothelium dependent mechanism. There is no significant effect of allopurinol on endothelium independent vasodilatation(56).

Strengths and limitations of this thesis

The main objective of this thesis was to identify and describe the studies examining the effect of XOR-inhibition on cardiovascular and renal end-points. The desired outcome was a broader and possibly less biased overview of the field than an expert opinion reviews mixing cell, animal and epidemiological studies, but at the same time not as narrow-focused as systematic reviews with meta-analyses done on specific end-points and only including few studies. A secondary objective was to use the clinicaltrials.gov register to assess reporting bias which might have distorted the available evidence on the effect of XOR-inhibition on cardiovascular and renal outcomes.

This thesis provides a comprehensive analysis of studies with an experimental design performed on human participants in order to assess the effect of XOR-inhibitors on cardiovascular and renal outcomes. The strength of including many studies done on different end-points with different populations is also the thesis´s main weakness. The heterogeneity in included studies makes it difficult to precisely report all aspects of the studies conducted without going into greater details of each.

The decision of only studying the main outcomes reported by the authors of the article weakens some aspects of this work because information from other reported end-points is not included in this thesis. A solution to this problem would be reporting secondary end-points we found relevant. In our opinion, this would lead to less objectivity in the selection process and reporting of results. The use of a singe investigator to screen the search and assess eligibility of the studies is another limitation weakening this thesis.

The use of clinicaltrials.gov data provides a new opportunity for assessing publication bias. The data were made available by introducing mandatory registration of all clinical intervention studies on human subjects(140) prior to study commencement. Publically available registers will hopefully provide more transparent and correct reporting of clinical trials. The cohort of registered clinical trials in this thesis included 25 studies in the final analysis. If more studies were retrieved it would have been possible to conduct Cox-regression analysis with publication as outcome with factors like number of included participants, drug studied and source of funding. However the number of studies included were to few to do a cox-regression on multiple factors(141). A simpler statistical analysis would be to stratify the cohort on industrial vs non-industrial funding and do a log-rank test. The cohort only included six industrial funded studies compared against 19 non-industrial funded studies. The power was considered to be insufficient to do this analysis and restricted the analysis to the Kaplan-Meyer plot shown in Figure 3. Ten of the 25 studies are censored before five years follow up, this adds insecurity to our attempt to estimate publication bias and the results should be interpreted with care.

Conclusion and directions for future research

The study on Allopurinol as a potential anti-anginal drug by Noman et al., 2010 is intriguing. This effect might be attributed to less increase in systolic blood pressure during the stress test. This is the only study that included testing blood pressure

during exercise stress(90). Inhibition of XOR and improvement in flow-mediated vasodilation might have an impact on blood pressure during exercise that is worth further investigation.

Using XOR inhibitors to slow progression of chronic renal disease should be further explored in larger studies with stricter design and longer follow up in order to examine if there is a clinical relevant effect of adding uric acid to hyperuricemic CKD patients without signs or symptoms of gout.

An adequately powered RCT with a factorial design in young participants with recently discovered hypertension and high normal to increased SUA could be a possible way to further investigate the effect of reducing uric acid in pre-hypertensive and hypertensive adolescents. By using a factorial design, it is possible to test multiple hypotheses simultaneously. A possible design could be randomizing to the following: first allocation; ACE/ATII-inhibitor or placebo and second allocation; uricosuric drug or XOR inhibitor. The primary outcome could be ambulatory blood pressure and secondary outcomes FMD and increase in blood pressure during stress-test. A design like this could clarify whether there is an additional effect of lowering uric acid compared to established blood pressure treatment in newly diagnosed hypertensive subjects and if this effect is mediated by reducing uric acid levels or by inhibiting XOR.

Appendix

Table S1. Describtion of Ovid MEDLINE search used in this thesis

#	Searches	Results	Search Type
1	xanthine oxidase.ab,hw,kf,ti,nm.	11112	Advanced
2	xanthine dehydrogenase.ab,hw,kf,ti,nm.	1518	Advanced
3	allopurinol.ab,hw,kf,ti,nm.	9031	Advanced
4	ox?purinol.ab,hw,kf,ti,nm.	652	Advanced
5	febuxostat.ab,hw,kf,ti,nm.	368	Advanced
6	"xanthine oxidase inhibitor*".ab,hw,kf,ti,nm.	916	Advanced
7	exp Xanthine Oxidase/	6794	Advanced
8	exp Xanthine Dehydrogenase/	1033	Advanced
9	exp Allopurinol/	6864	Advanced
10	exp Oxypurinol/	408	Advanced
11	CKD.ab,hw,kf,ti,nm.	15042	Advanced
12	Chronic kidney disease*.ab,hw,kf,ti,nm.	25292	Advanced
13	Heart failure.ab,hw,kf,ti,nm.	150863	Advanced
14	CHF.ab,hw,kf,ti,nm.	11230	Advanced
15	LVEF.ab,hw,kf,ti,nm.	8530	Advanced
16	"Endothel*".ab,hw,kf,ti,nm.	322718	Advanced
17	Vasodilation.ab,hw,kf,ti,nm.	39852	Advanced
18	Creatinine clearance.ab,hw,kf,ti,nm.	16006	Advanced
19	renal function.ab,hw,kf,ti,nm.	64833	Advanced
20	kidney function.ab,hw,kf,ti,nm.	33445	Advanced
21	hypertension.ab,hw,kf,ti,nm.	396177	Advanced
22	mortality.ab,hw,kf,ti,nm.	557807	Advanced
23	death.ab,hw,kf,ti,nm.	597745	Advanced
24	morbidity.ab,hw,kf,ti,nm.	280779	Advanced
25	cardiac failure.ab,hw,kf,ti,nm.	10538	Advanced
26	"blood pressure".ab,hw,kf,ti,nm.	382539	Advanced
27	exp Cardiovascular System/	1063055	Advanced
28	exp Cardiovascular Diseases/	1985701	Advanced
29	exp Cardiovascular Physiological Phenomena/	830059	Advanced
30	exp Kidney/	311567	Advanced
31	exp Kidney Diseases/	436422	Advanced
32	exp Kidney Failure, Chronic/	82937	Advanced
33	exp Heart Failure/	93302	Advanced
34	exp Ventricular Dysfunction/	28584	Advanced
35	exp Clinical Trial/	837943	Advanced
36	exp Comparative Study/	1733132	Advanced
37	*Uric Acid/ai, bl, ph [Antagonists & Inhibitors, Blood, Physiology]	4992	Advanced
38	*Hyperuricemia/bl, mo, pa, pp, th [Blood, Mortality, Pathology, Physiopathology, Therapy]	252	Advanced
39	exp humans/	14300939	Advanced
40	or/1-10	19010	Advanced

41	or/11-34	4463296	Advanced
42	35 and 39 and 40 and 41	303	Advanced
43	37 or 38 or 40	23546	Advanced
44	35 and 39 and 41 and 43	480	Advanced
45	limit 44 to english language	445	Advanced

Table describing the search conducted 20.08.15 in Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) 1946 to Present used to identify records used in this thesis.

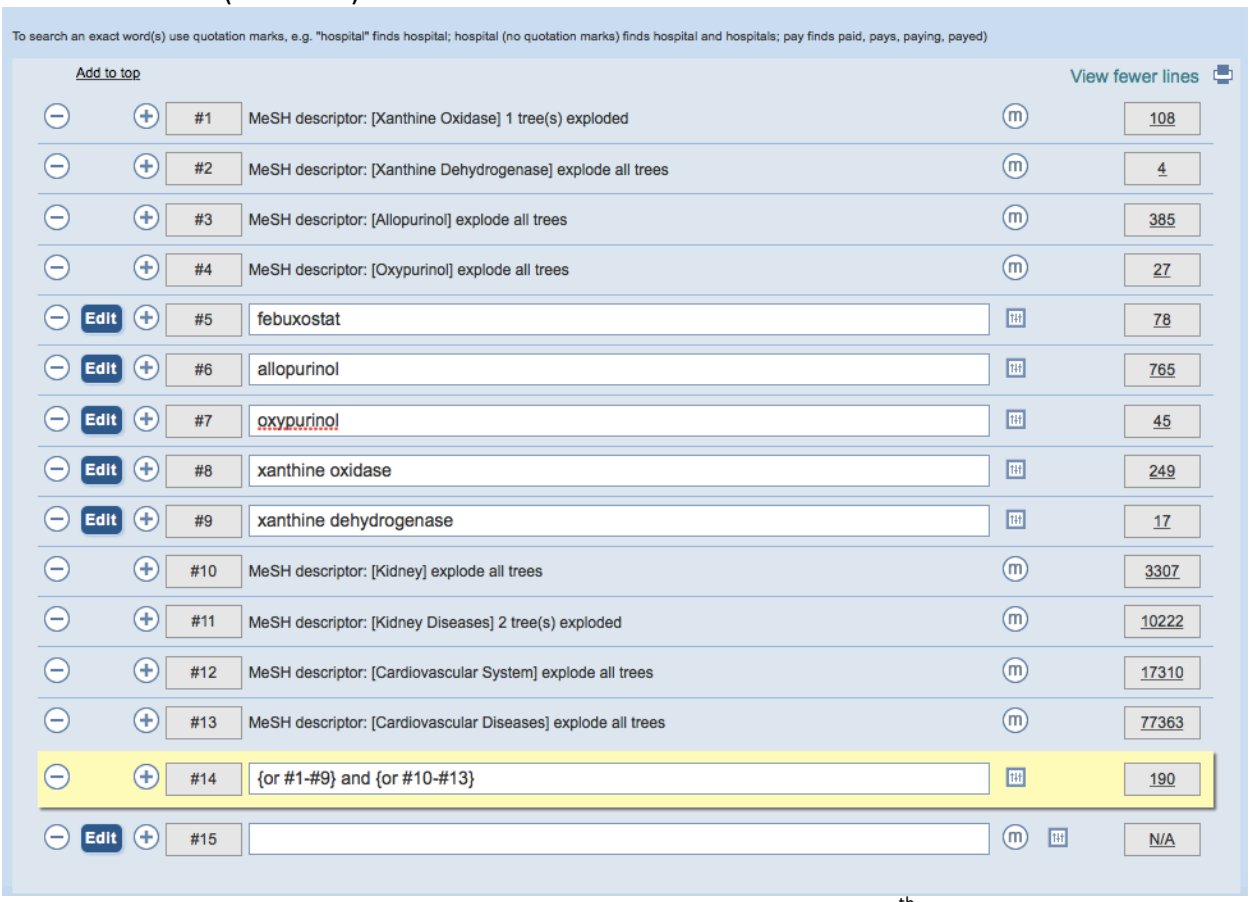
Table S2. Describtion of Ovid Embase search used in this thesis

#	Searches	Results	Search Type
1	exp cardiovascular system/	1678863	Advanced
2	exp cardiovascular disease/	3453261	Advanced
3	exp cardiovascular function/	1484194	Advanced
4	exp kidney disease/	780519	Advanced
5	exp kidney function/	192842	Advanced
6	exp kidney/	439450	Advanced
7	CKD.ti,ot,hw,ab,dv,kw.	24302	Advanced
8	"Chronic kidney disease*".ti,ot,hw,ab,dv,kw.	51532	Advanced
9	Heart failure.ti,ot,hw,ab,dv,kw.	286108	Advanced
10	CHF.ti,ot,hw,ab,dv,kw.	19458	Advanced
11	LVEF.ti,ot,hw,ab,dv,kw.	22608	Advanced
12	"Endothel*".ti,ot,hw,ab,dv,kw.	422211	Advanced
13	FMD.ti,ot,hw,ab,dv,kw.	8373	Advanced
14	Flow mediated vasodilation.ti,ot,hw,ab,dv,kw.	1237	Advanced
15	Vasodilation.ti,ot,hw,ab,dv,kw.	26460	Advanced
16	clearance.ti,ot,hw,ab,dv,kw.	219849	Advanced
17	renal function.ti,ot,hw,ab,dv,kw.	97769	Advanced
18	renal clearance.ti,ot,hw,ab,dv,kw.	8388	Advanced
19	Glomerular filtration.ti,ot,hw,ab,dv,kw.	48357	Advanced
20	GFR.ti,ot,hw,ab,dv,kw.	26977	Advanced
21	kidney function.ti,ot,hw,ab,dv,kw.	123077	Advanced
22	mortality.ti,ot,hw,ab,dv,kw.	1037384	Advanced
23	death.ti,ot,hw,ab,dv,kw.	901214	Advanced
24	morbidity.ti,ot,hw,ab,dv,kw.	466717	Advanced
25	cardiac failure.ti,ot,hw,ab,dv,kw.	17039	Advanced
26	myocardial infarction.ti,ot,hw,ab,dv,kw.	218887	Advanced
27	MI.ti,ot,hw,ab,dv,kw.	58413	Advanced
28	blood pressure.ti,ot,hw,ab,dv,kw.	509489	Advanced
29	"hypertens*".ti,ot,hw,ab,dv,kw.	731635	Advanced
30	exp mortality/	782118	Advanced
31	exp death/	588182	Advanced
32	exp morbidity/	266847	Advanced
33	xanthine oxidase.ti,ot,hw,ab,dv,kw.	14705	Advanced
34	xanthine dehydrogenase.ti,ot,hw,ab,dv,kw.	1671	Advanced

35	allopurinol.ti,ot,hw,ab,dv,kw.	20170	Advanced
36	"ox?purinol.".ti,ot,hw,ab,dv,kw.	1206	Advanced
37	febuxostat.ti,ot,hw,ab,dv,kw.	1041	Advanced
38	"xanthine oxidase inhibitor* ".ti,ot,hw,ab,dv,kw.	1867	Advanced
39	exp febuxostat/	995	Advanced
40	exp oxipurinol/	1079	Advanced
41	exp allopurinol/	19223	Advanced
42	exp xanthine oxidase/	10929	Advanced
43	exp xanthine dehydrogenase/	1296	Advanced
44	exp xanthine oxidase inhibitor/	20664	Advanced
45	human/	16258236	Advanced
46	exp controlled clinical trial/ or exp clinical trial/ or exp controlled study/ or exp randomized controlled trial/	5386520	Advanced
47	or/1-32	7017120	Advanced
48	or/33-44	33330	Advanced
49	45 and 46 and 47 and 48	2716	Advanced
50	limit 49 to english language	2616	Advanced
51	limit 50 to (clinical trial or randomized controlled trial or controlled clinical trial or multicenter study or phase 1 clinical trial or phase 2 clinical trial or phase 3 clinical trial or phase 4 clinical trial)	1369	Advanced
52	limit 51 to article	542	Advanced

Table describing the search conducted 20.08.15 in Embase Classic+Embase 1947 to August 19th 2015 used to identify records used in this thesis.

Figure S1. Description of search conducted in Cochrane Central Register of Controlled Trials (CENTRAL)



A search in Cochrane Central Register of Controlled Trials (CENTRAL) August 20th 2015– gave 190 results. Of these, 164 were clinical trials.

Table S3. Showing characteristics of excluded studies and reason for exclusion

Author	Year	Category of exclusion	Reason for exclusion
Hosoya, T(65)	2014	**Study protocol**	Study protocol on future study
Givertz, M(121)	2013	**Study protocol**	Study protocol of included study
White, W(68)	2012	**Study protocol**	Study protocol on future study
Freudenberg, R.S(67)	2004	**Study protocol**	Study protocol of included study
Tausche, A-K(69)	2014	**Population**	Tophaceous gout patients
Whelton, A(71)	2013	**Population**	Gout patients
Whelton, A(70)	2011	**Population**	Gout patients
Gibson,T(72)	1982	**Population**	Population with gout
Gibson, T(142)	1980	**Population**	Population with gout
Ogino, K(75)	2010	**Intervention**	Use of Benzobromarone as intervention
Pop-Busui(74)	2013	**Intervention**	Three drugs vs placebo
Kostka-Jeziorny, K(76)	2011	**Design**	Not an RCT-design
Terawaki, H(77)	2013	**Design**	Non-randomised kohort study
Wei, L(78)	2009	**Design**	Cohort study
Miranda, ME(79)	1994	**Design**	Case-study
Klinenberg, J(80)	1975	**Design**	No intervention
Ghosh, S(81)	2013	**Animal study**	Study on rats
Badkoobeh, R.S(82)	2011	**Language**	Not available in english
Shelmadine, B(64)	2009	**Not available**	No full text available

An overview of the records that were excluded after the initial screening of titles and abstract. The records are categorized after six reasons for exclusion and a further comment is given on the specific reason the record was excluded.

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