

Systematic Survey

Systematic Survey of Creatinine-Based Versus Cystatin C-based Estimated GFR in People with Diabetes

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Abstract

Background

Diabetes is a common cause of kidney failure, and most anti-diabetic agents are excreted through the kidneys. Therefore, it is critical to adjust medication dosage and anti-diabetic agents based on kidney function. There are different methods to estimate kidney function, but the common practice is to use creatinine to estimate the glomerular filtration rate.

Objective

In this systematic review, we identify and review publications in order to assess differences between creatinine-based and cystatin C-based estimated glomerular filtration rates in diabetic patients.

Methods

The articles were identified using 3 databases and were assessed for eligibility. A total of 4 articles were included. Comparisons of the 2 patient groups as well as the patient characteristics were compiled into 2 tables.

Results

Two studies showed significant differences between creatinine-based and cystatin C-based estimated glomerular filtration rates in patients with type 1 diabetes. There were no significant differences in control or type 2 diabetes groups.

Conclusions

Although cystatin C-based estimation of kidney function looks promising, it fails to show superiority over creatinine-based estimation. Most studies included in this systematic review, however, had serious limitations to them. Further research with standardized ways of measuring creatinine and cystatin C is required.

Keywords

cystatins/blood; creatinine/blood; glomerular filtration rate; eGFR; diabetes mellitus type 1; diabetes mellitus type 2; cystatin C; diabetic nephropathies

Introduction

1.1 Diabetes and Hyperglycemia

Diabetes is defined by an impaired homeostasis in the glucose metabolism resulting in hyperglycemia. In 2014, the International Diabetes Federation estimated world-wide prevalence of diabetes to be at 387 million, and in the year 2035, to be beyond 592 million, of whom 75–85% are classified as type 2 diabetes (T2D).¹ The most prevalent types of diabetes are T2D and type 1 diabetes (T1D). Although rare, there

are several other types of diabetes, including maturity onset diabetes of the young (MODY) and late autoimmune diabetes in adults (LADA).

Prolonged hyperglycemia gives increased risk for dysfunction in multiple organs as well as premature death. This risk can be lowered with the help of anti-diabetic agents (ADA). The first line of ADA treatment in T2D is metformin. Metformin cannot be metabolized; it

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can only be excreted by glomerular filtration in the kidneys.² Other diabetes medications and antihypertensives are also excreted renally. The estimation of glomerular filtration rate (eGFR) is, therefore, pivotal in managing diabetes, as medications commonly taken for the condition need careful renal dosing.

1.2 Diabetic Nephropathy and eGFR

Diabetic nephropathy is a term used for kidney damage caused by diabetes. The progression of kidney disease is monitored and assessed by eGFR. A decreasing eGFR indicates declining kidney function.³ Clinicians routinely use eGFR to decide appropriate medication and dosage.

The gold standard for measuring GFR is through a clearance test with iohexol. Iohexol is a contrast agent that is excreted strictly by glomerular filtration. This exogenous, tracer-based, clearance test is the method of choice to accurately calculate GFR. Actual clearance tests are expensive, impractical, and demand extra resources, e.g., extra personnel.⁴ The most common and convenient way of measuring GFR is by estimating it. Serum creatinine (crea) and serum cystatin-C (cysc) are both used for estimation purposes.

Modification of diet in renal disease (MDRD) is the recommended formula to calculate eGFR since 2009. MDRD incorporates age and sex but not muscle mass or diet, 2 factors that directly affect crea concentrations. eGFR can also be calculated through the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which incorporates the same variables as MDRD. The CDK-EPI equation has less bias, and more appropriately, categorizes individuals in respect to their long-term clinical risk and reduces the risk for misclassification of kidney function.⁵ In summary, our current methodologies to estimate GFR have all been built upon equations that do not account for the important variables of dietary habits and body mass, which in most patients, can vary widely.

While creatinine levels are affected by diet and body-mass index (BMI), cystatin C is not.⁶ Cystatin C is an endogenous protein synthesized by all nucleated cells. It is exclusively excreted via the glomerulus, metabolized and reabsorbed in the proximal tubule. However,

there is an individual variation in cystatin C not related to the kidney function. This variation is based on how much cystatin C an individual produces, reabsorbs, and eliminates. These factors can be altered if there is an ongoing infection, inflammation and various other conditions, e.g., diabetes.^{7,8}

1.3 Clinical Relevance and Previous Studies

An estimation of GFR is of absolute importance when treating patients with pharmaceuticals eliminated via the kidneys. If eGFR overestimates the actual GFR, renally-dosed medications could potentially lead to intoxication. Whereas, an eGFR that underestimates the actual GFR might lead to an ineffective treatment regimen. Sometimes the difference between eGFR (crea) and eGFR (cysc) is significant without any reasonable explanation. It is not uncommon that these differences range over eGFR thresholds for clinical decisions.⁹ In a study on 3,418 patients, Stevens et al.¹⁰ found that factors like advanced age, high C-reactive protein (CRP), high white blood cells and diabetes gave a lower eGFR (cysc) while giving a higher eGFR (crea) compared to actual measured GFR. The included patients had no other comorbidities and were selected by previously known increases in serum creatinine.^{8,10} Another study found a significant difference between eGFR (crea) and eGFR (cysc) in neuro-intensive and oncological patients compared to cardiology and primary care patients.¹¹ These studies stress the impact that patient characteristics can have on eGFR, hence the importance of unveiling the key mediators affecting creatinine and cystatin C levels.

The Swedish Agency for Health Technology Assessment of Social Services (SBU) published a systematic review over the estimation of kidney function.¹² They found that eGFR (crea) and eGFR (cysc) were both reliable markers of measured GFR (mGFR) in most cases. Their conclusion was that both creatinine and cystatin C should be analyzed when estimating GFR. They also recommend that in patients with eGFR < 30 ml/min/1.73 m³, a mean of eGFR (crea) and eGFR (cysc) should be used. This recommendation was a consensus after long discussions between those with divided opinions. The recommendation does not suggest

that both methods are equivalent; it instead underlines the importance of further investigations.

An investigation of whether there is a discrepancy between the two major methods of estimating GFR would therefore have a great clinical relevance.

Methods

2.1 Objectives

The objective is to review publications that assessed differences between eGFR (crea) and eGFR (cysc) in patients with diabetes.

2.2 Search Strategy

The systematic literature search was conducted on three different databases. PubMed (last searched 2016-10-31), Cochrane (last searched 2016-10-31) and Web of Science Core Collection (last searched 2016-10-31) were all searched for relevant studies comparing eGFR (crea) to eGFR (cysc) in diabetics. The Medical Subject Headings (MeSH) terms used for this study were “diabetes,” “glomerular filtration rate,” “creatinine,” and “cystatin C”.

The detailed strategies for each electronic search can be found in the appendices: Appendix 1, PubMed; Appendix 2, Cochrane; and Appendix 3, and the Web of Science Core Collection.

2.3 Inclusion and Exclusion Criteria

Studies that were reviewed were: human studies—publication date from 2011-01-01 to 2016-10-31 with a sample size greater than 30 in the diabetic arm, English articles, and studies with available abstracts. Studies prior to 2011 were excluded since MDRD was implemented from that period of time. The sample size limitation in the diabetic arm was set to greater than 30 in order to increase the power of the included studies.

Inclusion criteria

- Participants: patients with type 1 or type 2 diabetes
- Intervention: eGFR (crea) and eGFR (cysc)
- Control: a group of non-diabetic people receiving the same interventions as the diabetic people
- Outcome: differences in eGFR (crea) and

eGFR (cysc) in the intervention group compared to the control group

- Study design: clinical and observational studies

Exclusion criteria

- Non peer-reviewed studies
- Reviews, systematic reviews, meta-analyses, case-control studies or editorial/comment articles
- Studies made on pediatric (<19 years) and octogenarian population (>79 years), certain indigenous populations (Pima Indians, etc.) and pregnant women
- Estimation of GFR calculated with methods other than with creatinine and cystatin C

2.4 Study Selection

The articles remaining after the initial search and screening were pooled in a reference manager program (Zotero). Further, the titles were screened, and any duplicates were removed. The abstracts were assessed for eligibility and excluded if not fitting the inclusion criteria. Full texts of the remaining articles were reviewed. Due to the nature of systematic reviews no ethical approvals were needed for the compilation of this study.

2.5 Data Extraction

The study identification, data extraction, data analyses and result reporting were all executed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹³ The process of extracting data and reviewing titles, abstracts and full texts were all independently handled by the author.

The extraction of data involved study characteristics (author, publication date, design, methods, number of participants in each arm, interventions and outcome), patient characteristics (sex-ratio, mean age, mean BMI, comorbidities and medications) and outcome (mean eGFR (crea) and mean eGFR (cysc)).

2.6 Quality and Risk of Bias Assessment

The risk of bias in the included studies was assessed according to the GRADE approach.¹⁴ Both Newcastle-Ottawa Scale for quality as-

assessment (NOS)¹⁵ and Cochrane Collaboration’s tool (CCT)¹⁶ are included in the GRADE method. Furthermore, the initial evidence grade for each study was derived using the same GRADE approach. For observational studies, the risk of bias was assessed using NOS. In this star-based scale, three domains (selection, comparability and outcome) were assessed for biases.¹⁵ CCT was used for randomized controlled studies. CCT works by evaluating 7 domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other biases) for their risks of biases.¹⁶

Both the initial evidence grades and the risk of bias assessment help to evaluate the final quality of evidence of the studies.

2.7 Data Synthesis

All of the extracted data were presented in either figure or table formats. The study design and assessment of study quality were derived using protocols¹³ and assessment scales¹⁴⁻¹⁶ and are demonstrated in the study characteristic section of this review.

Results

3.1 Search Results

The search revealed 698 papers from PubMed (MEDLINE), Web of Science Core Collection, and Cochrane databases. Most studies (n=295) were excluded due to their publication date. After the adaptation of limitations and removal of duplicates, there were a total of 213 papers left for the eligibility screening. Other limitations were also considered, and the articles further eliminated. At the end, there were 4 compatible studies that were analyzed in a systematic review. (Figure 1)

3.2 Included Studies and Outcome

A total of 1830 participants were included in the four studies. The participant composition was non-diabetic people (n=713), T1D or T2D (n=709), with the remaining having other types of diabetes. Despite differences in the objectives, all the studies included a comparison of eGFR (crea) and eGFR (cysc) in different groups of diabetics.

The study by Natalia Nowak et al. (Study 1)¹⁷ evaluated cystatin C as a candidate biomarker for HNF1A-MODY (a specific genetic subtype

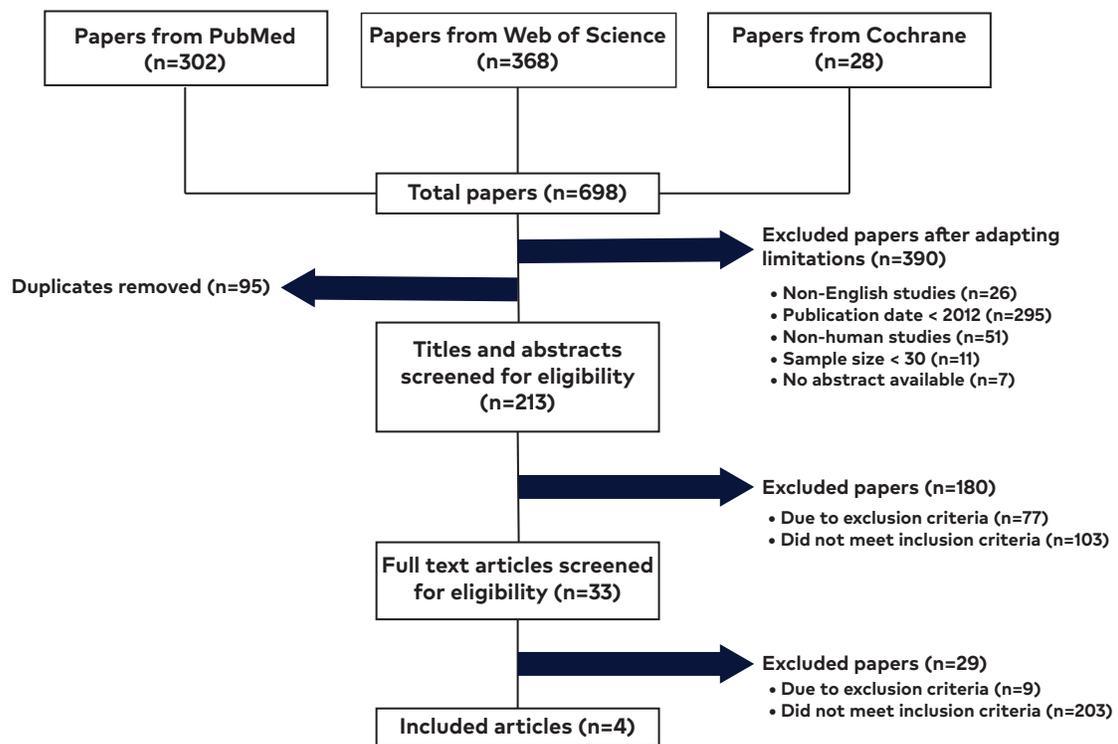


Figure 1. Flow chart with a full description of the different steps of study selection process.

of MODY). This retrospective study included 891 participants with HNF1A-MODY (n=268), T1D (n=114), T2D (n=244), GCK-MODY (n=170), HNF4A-MODY (n=39), HNF1B-MODY (n=17), and non-diabetic people (n=43). For the non-diabetic group and T2D, the difference between eGFR (crea) and eGFR (cysc) was insignificant. The same difference for T1D was significant with a value of 12.5 ml/min/1.73m³.

Magdalena Szopa et al. (**Study 2**)¹⁸ also compared eGFR measured with creatinine and cystatin C in HNF1A-MODY amongst other types of diabetes. The same intervention comparing eGFR (crea) and eGFR (cysc) was assessed in 332 participants, made up of HNF1A-MODY (n=72), GCK-MODY (n=72), T1D (n=53), T2D (n=70) and non-diabetic people (n=65). The non-diabetic control group showed a significant difference of -16.9 ml/min/1.73m³ in mean eGFR. The T1D group also showed a significant difference of 11.6 ml/min/1.73m³ whereas for T2D there was no significant difference.

Peter Bjornstad et al. (**Study 3**)¹⁹ measured GFR through the CKD-EPI formula in a prospective observational study. 1014 people were included and had either T1D (n=449) or were assigned to the non-diabetic control group (n=565). The participants were followed over a 6-year period in order to investigate whether insulin sensitivity could predict diabetic nephropathy. Baseline data (V1) suggests that the difference between eGFR (crea) and eGFR (cysc) for the non-diabetic control group was -6 ml/min/1.73m³. Whereas, in the T1D group, the same difference was -3 ml/min/1.73m³. Follow-up data (V3) suggest the difference in eGFR for the non-diabetic group increased -3.5 ml/min/1.73m³ to -9.5 ml/min/1.73m³. The difference for T1D increased to -4.1 ml/min/1.73m³. No statistical testing was done in this study.

Factors associated with overestimating GFR were assessed in the study by Akihiro Tsuda et al. (**Study 4**).²⁰ The study measured eGFR (crea), eGFR (cysc) and inulin-clearance in 80 participants (40 non-diabetic controls and 40 diabetic people). The difference in eGFR in the control group was -6.8 ml/min/1.73m³, while the difference in the diabetic group was -5.6 ml/min/1.73m³. No statistical testing regarding differences in eGFR (crea) compared to eGFR (cysc) exists for this study.

3.3 Risk of Bias Assessment

The first study is an observational study¹⁷ that has several crucial selection limitations. The representativeness of the different cohorts included in the study cannot, therefore, be determined. Hence, the selection bias may affect the outcome of the study.

The second observational study¹⁸ has an initial evidence type of 3. It has a few limitations in both the selection and outcome processes, but these limitations are interpreted as unlikely to affect the outcome of interest. Therefore, the limitations of this study are not as serious as the first study.

The third study is a prospective observational study¹⁹ with an initial evidence type of 2. It has serious limitations in the selection process since there are no descriptions for the ascertainment of exposure. This study possesses a high risk for selection bias because of the lack of explicit criteria for the selection of participants in the diabetic arm.

The final study is a randomized controlled study²⁰ with an initial evidence grade of 1. It has serious limitations due to insufficient generation of random sequence, selective reporting and other types of biases (e.g., strictly Japanese participants).

Details about the risk of bias assessment for each study are listed under study characteristics.

Discussion

Previous studies regarding the predictive values of eGFR, when calculated with creatinine compared to cystatin C in patients with diabetes, have not been conclusive.^{21, 22} There are several factors that affect creatinine concentration in serum, including age, muscle mass, and diet, while smoking, BMI, hyperglycemia, and inflammation can affect cystatin C levels.⁸ Finding a more reliable method of estimating GFR is therefore of importance as multiple factors clearly affects the level of creatinine and cystatin C. A more reliable eGFR value would mean better medicated patients, lowered risk for side-effects from medication, earlier detection of patients with risk for chronic kidney failure and more accurate detection of dialysis candidates.^{23, 24}

Previous studies by Tsuda et al. and Eisner et al. suggest that creatinine and cystatin C easily overestimate GFR in patients with diabetes.^{20,25} In order to accurately estimate GFR, we need to address the main factors affecting the secretion, excretion, metabolism and reabsorption of creatinine and cystatin C.

In the study by Tsuda et al. (**Study 4**),²⁰ we observe higher eGFR (crea) and eGFR (cysc) in the diabetic group compared to the control group. The author attributes this overestimation to poor glycemic control. As Tsuda et al. and Eisner et al. mention in their studies, it is hypothesized that sustained elevated glucose levels might lead to increased eGFR in people with diabetes.^{20,25} A suggestion like this would mean that eGFR would have to be adjusted for this patient group. For creatinine, this overestimation is explained and attributed to an increased secretion from renal tubule via hyperglycemia-induced upregulation of organic cation transporters. For cystatin C, it is explained as reduced metabolism and reabsorption of the substance in the proximal tubule.²⁰ However, the study has several limitations. It is a study performed on 80 Japanese participants. Besides the small sample size, the study also has an ethnic homogeneity that is hard to generalize on a larger and more diverse cohort. Also, as seen in the results for patient characteristics, the cohort is composed exclusively of CKD patients. All of these factors, combined with an insignificant difference between eGFR (crea) and eGFR (cysc) in both the diabetic and control group, make it hard to draw any reliable conclusions regarding the superiority of one eGFR-equation over the other.

A very simple and fundamental bias is not addressed by the author. The study does not bring up the fact that eGFR-equations are very dependent on the measured GFR. This means that eGFR is overestimated when mGFR is low and underestimated when mGFR is high.^{26,27} There are also laboratory differences in eGFR and mGFR, which are very difficult to assess. Since inulin and glucose are relatively similar in structure, the colorimetric detection of inulin for measurement of GFR is at risk for interference.²⁸ Therefore, poor glycemic control would automatically lead to falsely elevated inulin levels and also a false low clearance.

Other parameters associated with glycemic control are the level of hemoglobin A1c (HbA1c) as well as insulin sensitivity. In the study of Bjornstad et al. (**Study 3**),¹⁹ both parameters show an association with declining eGFR. The association is strongest when it comes to rapid declines of eGFR in T1D patients. Interestingly, cystatin C detects rapid eGFR decline more accurately than creatinine in T1D, which may be of clinical importance when assessing conditions like acute kidney failure. eGFR (cysc) also shows a greater association with insulin sensitivity index (ISI) than eGFR (crea).¹⁹ Hence, the study author insinuates that cystatin C-based equations might be superior to those of creatinine when it comes to monitoring diabetic nephropathy. However, further research is required in order to establish the superiority of cystatin C in patients with diabetes. The concentration of cystatin C varies and is affected by the degree of inflammation, cardiovascular disease and insulin sensitivity. The advantage of cystatin C is that it is an entirely glomerular-filtered endogenous substance that reacts fast to early signs of diabetes like microalbuminuria. (**Table 1**)

However, there are some limitations to the study done by Bjornstad et al.¹⁹ as well. The first limitation is that there are no direct measurements of GFR with either inulin or iohexol. Another limitation is the fact that there are no real measurements of insulin sensitivity. Instead the author chooses to estimate the sensitivity and calculate ISI through an equation based on waist circumference, body weight, daily insulin dose, triglyceride levels and diastolic blood pressure. There are many ways of overcoming this limitation, including acknowledged indices that estimate insulin sensitivity as well as the insulin glucose clamp, which is the gold standard for quantifying insulin sensitivity.²⁹ A third limitation regards the selection bias in this study. Some participants with T1D were excluded because of incomplete data at baseline. These participants reportedly also had worse renal function and lipid panel, meaning that the study group was not reflecting the initial T1D group or T1D patients in general.¹⁹ There seems, however, to be a clear linkage between glycemic control, insulin sensitivity, and eGFR as shown in this study as well as previous studies.^{19,20,30} Due to the serious limitations of

Table 1. Patient Demographics by Group Including Age, Sex and Charlson Comorbidity Index

| | Groups | Mean age | Sex ratio (F N (%)) | Mean BMI | Comorbidities | Medication |
|------------------------------------|-----------------------|---------------|------------------------|--------------|---|--|
| Natalia Nowak et al. 2013 | Control (n=43) | 32.3 ± 3.95 | 26 (60.5) | 23.6 ± 1.35 | No known. | No known. |
| | T1D (n=58) | 36.1 ± 3.05 | 37 (66.1) | 23.6 ± 1.05 | CKD (n=5) | No known. |
| | T2D (n=41) | 57.9 ± 2.8 | 25 (61.0) | 33.9 ± 2.4 | CKD (n=5) | No known. |
| Magdalena Szopa et al. 2015 | Control (n=65) | 38.02 ± 11.70 | 23 (35.4) | 23.91 ± 2.93 | Exclusion of participants with thyroid problems, infections, neoplasms, chronic respiratory disease, kidney and liver disease | Exclusion of participants on steroid therapy. |
| | T1D (n=53) | 31.72 ± 11.71 | 21 (55.7) | 24.43 ± 3.20 | | |
| | T2D (n=70) | 58.96 ± 10.25 | 39 (55.7) | 30.48 ± 4.76 | | |
| Peter Bjornstad et al. 2013 | Control V1 (n=565) | 40.2 ± 8.8 | 277 (49) | 26.0 ± 4.8 | Hypertension (n=16) | ACEi/ARB (n=3) Statins (n=7) |
| | T1D V1 (n=449) | 36.7 ± 8.7 | 238 (53) | 26.1 ± 4.3 | Hypertension (n=39) | ACEi/ARB (n=32) Statins (n=16) |
| | Control V3 (n=565) | 46.8 ± 8.8 | 283 (50) | 26.5 ± 4.9 | Hypertension (n=24) | ACEi/ARB (n=8) Statins (n=19) |
| | T1D V3 (n=449) | 43.3 ± 8.7 | 243 (54) | 26.7 ± 4.6 | Hypertension (n=54) | ACEi/ARB (n=46) Statins (n=47) |
| Akihiro Tsuda et al. 2014 | Control (n=40) | 48.3 ± 15.8 | 21 (52.5) | 24.3 ± 4.7 | Restricted to participants with chronic kidney disease (CKD). | Exclusion of participants with medication that may alter tubular creatinine secretion. |
| | Diabetic group (n=40) | 64.8 ± 9.5 | 24 (60.0) | 25.4 ± 3.4 | Exclusion of participants with thyroid problems. | |

this study (e.g., no mGFR), it is hard to agree that eGFR (cysc) is superior to eGFR (crea). (**Table 2**)

In order to answer the question of whether eGFR (crea) or eGFR (cysc) is the superior estimate in people with diabetes, we also need to discuss the results of the studies by Nowak et al. (**Study 1**)¹⁷ and Szopa et al. (**Study 2**).¹⁸ In both studies, eGFR (crea) was significantly higher than eGFR (cysc) in patients with T1D. No significant differences were measured for T2D. Furthermore, adjustments for confounders like gender, age, BMI, glucose level, CRP concentration, HDL level and total cholesterol level did not alter the observed differences in the studies, which implies that the observed difference in eGFR is independent from the aforementioned factors. The difference between eGFR (crea) and eGFR (cysc) can, therefore, not be attributed to muscle mass, degree of inflammation or glycemic control. Interestingly, previous studies have shown that cystatin

C is more effective at detecting mild diabetic nephropathy and that this superiority over creatinine-based equations is evened out at GFR values below 60 mL/min.^{31,32} The significant discrepancy observed between eGFR (crea) and eGFR (cysc) in T1D patients in the studies by Nowak et al.¹⁷ and Szopa et al.¹⁸ could then be a result of the superior cystatin C estimation. On the other hand, there was no significant difference between eGFR values in patients with T2D, which would indicate that cystatin C superiority in mild diabetic nephropathy probably is not the explanation for the significant difference seen in the T1D group.

Both of the studies fall short because of the fact that there were no actual mGFR assessments. Some other limitations to these studies are the relatively small sample sizes and the fact that T1D and T2D groups differed in some clinical features. Since both studies are done on different cohorts and show the same results, it may be tempting to jump to con-

Table 2. Primary outcome measures comparing eGFR (crea) with eGFR (cysc) in healthy and diabetic populations. All values are given in the unit of ml/min/1.73m³ as a measure of eGFR.

| | Control (n=43) | T1D (n=56) | T2D (n=41) | |
|------------------------------------|----------------------------------|-----------------------------------|----------------------------------|--------------------------------|
| Natalia Nowak et al. 2013 | Mean eGFR (crea) 111.9 ± 4.75 | Mean eGFR (crea) 101.2 ± 6.00 | Mean eGFR (crea) 82.4 ± 7.10 | |
| | Mean eGFR (cysc) 113.3 ± 5.80 | Mean eGFR (cysc) 88.7 ± 1.30 | Mean eGFR (cysc) 79.5 ± 7.85 | |
| | Difference in eGFR -1.4 | Difference in eGFR 12.5 | Difference in eGFR 2.9 | |
| | Control (n=65) | T1D (n=53) | T2D (n=70) | |
| Magdalena Szopa et al. 2015 | Mean eGFR (crea) 99.5 ± 14.35 | Mean eGFR (crea) 111.5 ± 15.8 | Mean eGFR (crea) 88.7 ± 16.99 | |
| | Mean eGFR (cysc) 116.2 ± 15.5 | Mean eGFR (cysc) 100.0 ± 17.55 | Mean eGFR (cysc) 89.6 ± 21.64 | |
| | Difference in eGFR -16.7 | Difference in eGFR 11.5 | Difference in eGFR -0.9 | |
| | Control V1 (n=565) | T1D V1 (n=449) | Control V3 (n=565) | T1D V3 (n=449) |
| Peter Bjornstad et al. 2013 | Mean eGFR (crea) 102 ± 20 | Mean eGFR (crea) 105 ± 24 | Mean eGFR (crea) 96.5 ± 14.5 | Mean eGFR (crea) 106 ± 21.4 |
| | Mean eGFR (cysc) 108 ± 12 | Mean eGFR (cysc) 108 ± 18 | Mean eGFR (cysc) 106 ± 14.0 | Mean eGFR (cysc) 102 ± 22 |
| | Difference in eGFR -6 | Difference in eGFR -3 | Difference in eGFR -9.5 | Difference in eGFR 4 |
| | Control (n=40) | Diabetic group (n=40) | | |
| Akihiro Tsuda et al. 2014 | Mean eGFR (crea) 53.2 ± 22.0 | Mean eGFR (crea) 75 ± 20.2 | | |
| | Mean eGFR (cysc) 60 ± 29.6 | Mean eGFR (cysc) 80.6 ± 22.4 | | |
| | Difference in eGFR -6.8 | Difference in eGFR -5.6 | | |

clusions. The studies should instead be taken cautiously due to their serious limitations.

4.1 Strengths and Limitations

Our study is a systematic review covering a field of medicine that is somewhat divided in research and opinions. We believe we have a high level of evidence and a potential recommendation for use of eGFR (cysc) over eGFR (Crea), specifically in T1D.

This systematic review should, however, be interpreted within its limitations. First and foremost, the quality assessments of the studies were only based on their risk of bias and initial evidence type. To determine the final evidence, a more complete quality assessment is required. Moreover, no statistical tests were performed in this study. When comparing two

interventions, statistical analysis, hence a meta-analysis, is of great value.

Mapping and investigating non-renal factors affecting cystatin C is essential for the clinical applicability of eGFR (cysc). The analysis method for cystatin C needs to be standardized between clinical settings and new reference intervals for patients with and without diabetes needs to be established. Furthermore, there is a need to show that eGFR (cysc) compared to eGFR (crea) improves the risk stratification for nephropathy. Until then, creatinine-based eGFR will remain the most clinically applicable method for the estimation of GFR.

4.2 Future Research

There are still some ambiguities and uncertainty regarding non-renal factors that regulate

creatinine and cystatin C levels. Therefore, the production, secretion, metabolism and reabsorption of these compounds need further investigation. Larger cohort sizes, adequate mGFR methods, standardized ways of measuring serum creatinine, and cystatin C, as well as urinary creatinine and cystatin C, needs to be adapted when conducting future studies.

Conclusion

Although cystatin C-based GFR equations look promising, they fail to show superiority over creatinine-based equations in this study. However, due to the serious limitations of the included articles, there is a need for further research in order to be certain of such a conclusion.

List of Abbreviations

| | |
|---------|--|
| ADA | Anti-diabetic agent |
| BMI | Body-mass index |
| CCT | Cochrane Collaboration's tool |
| CKD | Chronic Kidney Disease |
| CKD-EPI | Chronic Kidney Disease Epidemiology Collaboration equation |
| Crea | Creatinine |
| Cysc | Cystatin C |
| eGFR | Estimated glomerular filtration rate |
| HbA1c | Hemoglobin A1c |
| ISI | Insulin sensitivity index |
| MeSH | Medical subject headings |
| mGFR | Measured glomerular filtration rate |
| MDRD | Modification of Diet in Renal Disease |
| MODY | Maturity onset diabetes of the young |
| NOS | Newcastle-Ottawa Scale |

| | |
|--------|--|
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta-Analyses |
| SBU | Swedish Agency for Health Technology Assessment of Social Services |
| T1D | Type 1 diabetes |
| T2D | Type 2 diabetes |

Conflicts of Interest

The authors declare they have no conflicts of interest.

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Study Characteristics

Study 1: Natalia Nowak et al. 2012

Title: Cystatin C is not a good candidate biomarker for HNF1A-MODY

Study Design

| | |
|---------------|---|
| Type | Retrospective comparative study Cross-sectional study Observational study |
| Participants | 891 patients from three different centers HNFA1-MODY (n=268), T1DM (n=114), T2DM (n=244), GCK (n=170), HNF4A (n=39), HNF1B (n=17) and non-diabetic control (n=43) |
| Interventions | Comparison of eGFR CDK-EPI (crea) vs. MDRD eGFR (cysc) |
| Outcomes | In patients with HNF1A-MODY, cystatin C-based eGFR is higher than creatinine-based one. The study suggests that cystatin C may not be a good biomarker for HNF1A-MODY since the low CRP levels associated with HNF1A-MODY can affect cystatin C levels, thus overestimating GFR compared to creatinine-based equations. |
| Limitations | The intergroup difference seen in samples from the Polish center is not found in samples from the remaining centers in the UK. Different cystatin C assays or differences in recruitment procedures may be the cause. The study also uses different equations for calculation of eGFR (crea) and eGFR (cysc). |

Assessment of the Quality of Evidence

| | |
|---------------|---|
| Evidence type | 3 (retrospective comparative study) |
| Risk of bias | Very serious limitations (-2) Selection: -The representativeness of the exposed cohort cannot be determined since there is no description of the derivation of the cohort. -There is no description of the derivation of the non-exposed cohort. -There is no description regarding the ascertainment of exposure. -There are no demonstrations that the outcome of interest was not present at start of the study. Comparability: -The study controls for differences in eGFR (crea) and eGFR (cysc). * -The study controls for additional factors including sex-ratio, mean age and mean BMI. * Outcome: -There is a record linkage for the assessment of outcome (measurements, e.g., eGFR, BMI). * -The follow up was long enough for outcomes to occur. There was no follow-up but for the outcome of interest there is none needed. * -There is no statement of the adequacy of follow up of cohorts. Selection (0/4 stars). Comparability (2/2 stars). Outcome (2/3 stars). |

Study 2: Magdalena Spoza et al. 2015

Title: Comparison of Glomerular Filtration Rate Estimation from Serum Creatinine and Cystatin C in HNF1A-MODY and Other Types of Diabetes

Study Design

| | |
|---------------|---|
| Type | Retrospective comparative study Cross-sectional study Observational study |
| Participants | 332 participants from one center HNF1A-MODY (n=72), GCK-MODY (n=72), T1DM (n=53), T2DM (n=70) and non-diabetic control (n=65) |
| Interventions | Comparison of eGFR CDK-EPI (crea) vs eGFR CDK-EPI (cysc) |
| Outcomes | eGFR (cysc) has shown to be less biased in diabetes with poor glycemic control. Interestingly, for T1DM-patients in this study, eGFR (crea) is significantly higher than eGFR (cysc). The authors suggest that creatinine-based equations under euglycemic conditions can overestimate the inulin-based assessed GFR; whereas, under hyperglycemic conditions it can underestimate GFR. |
| Limitations | There is no reliable gold standard for measuring GFR (inulin or iothalamate). The study has a small sample size. The study group differs in terms of some clinical characteristics. The participants included have an eGFR of 60 ml/min/1.73 m or above, which means participants with advanced stage of renal disease were excluded. |

Assessment of the Quality of Evidence

| | |
|---------------|--|
| Evidence type | 3 (retrospective comparative study) |
| Risk of bias | Serious limitations (-1) Selection: -The exposed cohort is somewhat representative of the average T1D and T2D people in the community. * -The non-exposed cohort is drawn from the same community as the exposed cohort (white Caucasians residing in southeastern Poland).* -There is a clear ascertainment of exposure for both exposed cohort and control cohort (clinical symptoms and fasting blood glucose levels). * -There are no demonstrations that outcome of interest was not present at start of the study. Comparability: -The study controls for differences in eGFR (crea) and eGFR (cysc). * -The study controls for additional factors including sex-ratio, mean age, mean BMI, comorbidities and medications. * Outcome: -There is a record linkage for the assessment of outcome (measurements e.g. eGFR, BMI). * -The follow up was long enough for outcomes to occur. There was no follow-up but for the outcome of interest there is none needed. * -There is no statement of the adequacy of follow up of cohorts. Selection (3/4 stars). Comparability (2/2 stars). Outcome (2/3 stars). |

Study 3: Peter Bjornstad et al. 2013

Title: Early Diabetic Nephropathy: A complication of reduced insulin sensitivity in type 1 diabetes

Study Design

| | |
|---------------|---|
| Type | Prospective comparative study Cross-sectional study Observational study |
| Participants | 1014 participants T1DM (n=449) and non-diabetic controls (n= 565) |
| Interventions | Comparison of eGFR CDK-EPI (crea) vs. eGFR CDK-EPI (cysc) vs CDK-EPI (crea-cysc) Relate eGFR to insulin sensitivity index (ISI) |
| Outcomes | Rapid GFR decline is associated with worsened ISI. eGFR CDK-EPI (cysc) is less biased and better at detecting a rapid decline of GFR. |
| Limitations | There is no direct measurement of GFR or insulin-sensitivity. Some T1DM patients without baseline data have worse renal function and lipid profile, which may bias the results since less healthy patients were not included. |

Assessment of the Quality of Evidence

| | |
|---------------|---|
| Evidence type | 2 (prospective comparative study) |
| Risk of bias | Serious limitations (-1) Selection: -The exposed cohort is somewhat representative of the average T1D people in the community. * -The non-exposed cohort is drawn from the same community as the exposed cohort (19–56 year olds that were asymptomatic for cardiovascular disease).* -There is no description regarding the ascertainment of exposure. -There are no demonstrations that outcome of interest was not present at start of study. Comparability: -The study controls for differences in eGFR (crea) and eGFR (cysc). * -The study controls for additional factors including sex-ratio, mean age, mean BMI, comorbidities and medications. * Outcome: -There was a record linkage for the assessment of outcome (measurements, e.g., eGFR, BMI). * -The follow-up was long enough for outcomes to occur (6 years). * -There were a small number of participants lost at follow up. The 1–2% loss is unlikely to introduce bias. * Selection (2/4 stars). Comparability (2/2 stars). Outcome (3/3 stars). |

Study 4: Akihiro Tsuda et al. 2014

Title: Poor Glycemic Control Is a Major Factor in the Overestimation of Glomerular Filtration Rate in Diabetic Patients

Study Design

| | |
|---------------|--|
| Type | Randomized controlled study |
| Participants | 80 participants DM (n=40) and non-diabetic controls (n=40) |
| Interventions | Comparison between inulin clearance vs. eGFR (crea) and eGFR(cysc) |
| Outcomes | As glycemic control worsens, eGFR overestimates inulin clearance. Comparing inulin clearance agreement with eGFR (crea) and eGFR (cysc) shows eGFR overestimates the glomerular filtration rate, especially when calculated through serum-creatinine. Factors associated with overestimation includes HbA1c and glycolated albumin. It's suggested that the organic cation transporters responsible for creatinine secretion is modulated by high glucose. A new formula is suggested where creatinine is corrected for hba1c. |
| Limitations | The study has a small sample size. The study has only Japanese participants, which makes the results and the new formula harder to apply to other ethnic groups. The authors cannot offer an explanation why eGFR (cysc) also overestimates the glomerular filtration rate. |

Assessment of the Quality of Evidence

| | |
|---------------|--|
| Evidence type | 1 (randomized controlled study) |
| Risk of bias | Serious limitations (-1) Random Sequence Generation: -Allocation is based on clinical history or the results of laboratory tests, which has a high risk of bias. Allocation Concealment: -There is insufficient information regarding allocation concealment, creating an unclear risk of bias. Blinding of Participants and Personnel: -No blinding is used, but the outcome is not likely to be influenced by the absence of blinding, thus a low risk of bias. Blinding of Outcome Assessment: -No blinding is used, but the outcome is not likely to be influenced by the absence of blinding, thus a low risk of bias. Incomplete Outcome Data: -No missing outcome data is reported, thus a low risk of bias. Selective Reporting: -One or more primary outcomes are reported using measurements, analysis methods or subsets of the data that were not pre-specified, creating a high risk of bias. Other Bias: -The study has a potential source of bias (n=80 and all Japanese participants) related to the specific study design used, creating a high risk of bias. |

Appendices

Appendix 1

Search strategy for database: PubMed
Last search: 2016-10-31

Search strategy

1. Diabetes AND glomerular filtration rate AND creatinine AND cystatin c (302 hits)
2. English (287)
3. Date 2012–2016 (160)
4. Human (114)
5. Sample size > 30 (108)
6. No abstract available (105)

Query translation for the initial search: (*"diabetes mellitus"*[MeSH Terms] OR (*"diabetes"*[All Fields] AND *"mellitus"*[All Fields])) OR *"diabetes mellitus"*[All Fields] OR *"diabetes"*[All Fields] OR *"diabetes insipidus"*[MeSH Terms] OR (*"diabetes"*[All Fields] AND *"insipidus"*[All Fields]) OR *"diabetes insipidus"*[All Fields] AND (*"glomerular filtration rate"*[MeSH Terms] OR (*"glomerular"*[All Fields] AND *"filtration"*[All Fields] AND *"rate"*[All Fields]) OR *"glomerular filtration rate"*[All Fields]) AND (*"creatinine"*[MeSH Terms] OR *"creatinine"*[All Fields] AND (*"cystatin c"*[MeSH Terms] OR *"cystatin c"*[All Fields])).

Appendix 2

Search strategy for database: Cochrane
Last search: 2016-10-31

Search strategy

1. *Diabetes AND glomerular filtration rate AND creatinine AND cystatin c* (28 hits)
2. English (28)
3. Date 2012–2016 (21)
4. Human (21)
5. Sample size > 30 (20)
6. No abstract available (20)

Appendix 3

Search strategy for database: Web of Science Core Collection
Last search: 2016-10-31

Search strategy

1. *Diabetes AND glomerular filtration rate AND creatinine AND cystatin c* (368 hits)
2. English (357)
3. Date 2012–2016 (196)
4. Human (191)
5. Sample size > 30 (187)
6. No abstract available (183)

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