Validity of Biomarkers Predicting Onset or Progression of Nephropathy in Patients with Type 2 Diabetes: A Systematic Review

Merel E. Hellemons, MD [1*], Julia Kerschbaum, MD [2*], Stephan J.L. Bakker, MD, PhD [1], Hannes Neuwirt, MD [2], Bernd Mayer, PhD [3], Gert Mayer, MD [2], Dick de Zeeuw, MD, PhD [1] Hiddo J. Lambers Heerspink, PhD [1], Michael Rudnicki, MD [2]

* Contributed equally to this paper

[1] Department of Clinical Pharmacology, University Medical Center of Groningen, University of Groningen, the Netherlands

[2] Department of Internal Medicine IV – Nephrology and Hypertension, Medical University Innsbruck, Austria

[3] Emergentec Biodevelopment GmbH, Vienna, Austria

Author of correspondence: Michael Rudnicki, MD, FASN

Medical University Innsbruck

Department of Internal Medicine IV – Nephrology and Hypertension

Anichstrasse 35, 6020 Innsbruck, Austria

Telephone: +43 512 504 81337

Fax: +43 512 504 25857

Email: michael.rudnicki@i-med.ac.at

Funding source: EU FP7/SysKid. **Conflicts of interest:** None to declare.

Word count: Abstract: 217 words/1536 char.; main text: 3960 words/27249 char.

Keywords: Biomarker, biological marker, nephropathy, diabetic nephropathy, non-insulin dependent diabetes mellitus, type 2 diabetes mellitus, prediction, progression, validity, methodology

ABSTRACT

Aims: Novel biomarkers predicting onset or progression of nephropathy in patients with type 2 diabetes have been recently identified. We performed a systematic review to assess the validity of biomarkers predicting onset or progression of nephropathy in patients with type 2 diabetes in longitudinal studies.

Methods: Methodological quality of the studies was scored using STARD criteria, and the independent predictive value of the biomarkers beyond conventional risk factors was scored according to the adjustment for these risk factors. Validity of the biomarkers was determined by summarizing the methodological quality and the adjustment score.

Results: We identified 15 studies describing 27 biomarkers. Six studies had sufficient methodological quality. These studies identified 13 valid and significant markers for nephropathy in diabetes: serum IL-18, plasma ADMA, and urinary ceruloplasmin, IgG and transferrin were considered valid markers predicting onset of nephropathy. Plasma ADMA, VCAM1, IL6, vWF and ICAM1 were considered valid biomarkers predicting progression of nephropathy. Plasma hsCRP, E-selectin, TPA, vWF and triglycerides were considered valid markers predicting onset and progression of nephropathy.

Conclusion: In conclusion, several novel biomarkers for prediction of nephropathy in diabetes have been published which can potentially be applied in clinical practice and research in future. Due to the heterogeneous quality of biomarker studies in this field a more rigorous evaluation of these biomarkers and validation in larger trials are advocated.

INTRODUCTION

Diabetic nephropathy is the leading cause of chronic renal failure in the United States and Western countries. As such it is not only associated with considerable morbidity and premature mortality but it also negatively affects patient's quality of life and their social environment, and it poses a significant burden on national health-care budgets [1].

Albuminuria is one of the first asymptomatic clinical manifestations of micro-vascular damage in diabetes [2]. It has been shown that the presence of micro- or macroalbuminuria is associated with progressive renal function loss and an increased risk of cardiovascular disease [3]. Therefore, screening for and quantification of albuminuria is recommended in all patients with diabetes to identify those who are at risk for long-term complications [4]. In recent years multiple urinary and serum/plasma biomarkers for the prediction of onset of microalbuminuria and for progression of nephropathy in patients with micro- and macroalbuminuria have been investigated in patients with type 2 diabetes. The term "biomarker" describes a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention without necessarily being causally related to the clinical endpoint (e.g. troponin T in myocardial infarction) [5]. Biomarkers can be used as diagnostic tools, staging tools, prognostic tools or tools for prediction/monitoring of clinical response to an intervention [6]. For the latter purpose, certain biomarkers are eligible to substitute clinical endpoints in intervention trials. Such a surrogate marker, also referred to as surrogate endpoint, is expected to predict clinical benefit as evidence exists that this marker is associated causally with a certain clinical outcome (e.g. elevated blood pressure as marker for cardiovascular disease). Therefore, only a subset of biomarkers will achieve surrogate marker status. In this review we focus on biomarkers for prognosis and risk prediction, which potentially can become surrogate markers in future studies. A more prominent role for biomarkers is proposed in early non-invasive screening and assessing overall renal risk in patients with type 2 diabetes [7]. In this perspective biomarkers may be

important tools in guiding early and more aggressive therapy in high risk patients in order to prevent long-term renal complications.

Before a putative biomarker can be applied in clinical practice as a prediction tool, rigorous evaluation is advocated and several criteria should be met [8-11]. First of all, biomarkers should be tested in longitudinal, methodologically well-designed studies with sufficient power in order to ascertain the generalisability of the results [10]. The association between the biomarker and disease should be independent of potential confounders and should add to risk prediction beyond conventional risk factors [8, 11, 12]. Last, results on biomarkers should be reproduced in other studies to validate the results [8-10]. So far, validity of biomarkers for the prediction onset or progression of nephropathy in patients with diabetes has not been critically appraised.

The intent of this paper was to systematically assess the validity of biomarkers predicting onset or progression of nephropathy in patients with type 2 diabetes on the basis of methodological quality of the studies, and by determination of the independent predictive value over conventional risk factors.

MATERIALS AND METHODS

Identification of relevant studies

Relevant studies were identified by searches of Medline via Pubmed, Embase and the Cochrane Library database (Cochrane Central Register of Controlled Trials), with relevant text words and medical subject headings that consisted of the term “biomarker or biological marker” and one of the following terms: “(diabetic) nephropathy”, “(non insulin dependent) diabetes mellitus”, “microalbuminuria”, “albuminuria”, “proteinuria”, “chronic kidney disease”, “diabetes complications”. The search was limited to longitudinal studies on humans and adults, published between 1995 and 2010, without language restrictions. We extended our search by reviewing the references from the eligible papers and the papers that cited the eligible papers through Web of Science.

Study selection

Studies were considered eligible if they were longitudinal cohort studies or randomized controlled trials with at least 20 patients, reporting on biomarkers for the prediction of onset or progression of nephropathy in type 2 diabetic patients. We focused on biomarkers which can be measured in urine, plasma or serum. Studies on conventional risk markers/biomarkers (i.e. age, sex, race, hypertension, HbA1c, BMI, diabetes duration, hypercholesterolemia, retinopathy, smoking, albuminuria and use of RAAS-inhibitors) were excluded because we were mainly interested in novel biomarkers that could potentially improve risk prediction beyond conventional risk markers. Therefore, the conventional markers rather served as a basis for quality assessment of the studies (*vide infra*). The literature search, data extraction, and scoring were done by two reviewers independently by use of a standardized approach (JK, MH). Any disagreement was resolved by a third reviewer (MR).

Terminology

Diabetic nephropathy is histopathologically characterized by several changes in the kidney such as nodular glomerulosclerosis, mesangial expansion, basement membrane thickening and interstitial fibrosis. Clinically, diabetic nephropathy is usually a constellation of persistent albuminuria, elevated arterial blood pressure, and decline in kidney function [13]. Changes in albuminuria are considered a hallmark of onset or progression of nephropathy. Albuminuria levels have been categorized into normoalbuminuria (<30 mg/day or <20 µg/min), microalbuminuria (30-300 mg/day or 20-200 µg/min) and macroalbuminuria (>300 mg/day or >200 µg/min). Consequently, studies often report transition in albuminuria class (from normo- to microalbuminuria and from micro- to macroalbuminuria) or doubling of serum creatinine from baseline as indicators of nephropathy onset or progression. In our study onset of nephropathy was defined as the development of microalbuminuria in previously normoalbuminuric patients (early nephropathy). Progression of nephropathy was defined as either the transition from normo- or micro- to macroalbuminuria, a longitudinal change in the extent of albuminuria or doubling of serum creatinine in micro- and macroalbuminuric patients (late nephropathy).

Study analysis

Studies were divided into three groups: studies on biomarkers predicting onset of nephropathy in patients with normoalbuminuria (early nephropathy), studies predicting progression of nephropathy in patients with micro- or macroalbuminuria (late nephropathy), and studies predicting onset and progression of nephropathy in cohorts that included patients with normoalbuminuria and microalbuminuria.

Quality assessment

Studies were assessed for validity using a modified checklist of the STARD criteria (table 1). The STARD initiative developed a set of 25 criteria for reporting of studies of diagnostic accuracy in order to improve the quality [14]. For the purpose of this review, we limited the

quality assessment to 11 items, mainly focusing on methodological quality of the study. Items focusing on specific laboratory methods or tests were not included in this review. Study quality was considered as “good” if the score was ≥ 10, “average” if the score was 8-9, “fair” if the score was 6-7, and “poor” if the score was <6.

Adjustment score and biomarker validity score

In order to be clinically useful, biomarkers for onset and progression of nephropathy should have additional predictive value on top of conventional risk markers (age, sex, race, hypertension, HbA1c, BMI, diabetes duration, hypercholesterolemia, retinopathy, smoking, albuminuria and use of RAAS-inhibitors [15-24]). As a quality assessment we scored whether the studies took conventional risk markers into account. One point was attributed to each of the following conventional factors that was adjusted for: Age and sex, blood pressure/hypertension and/or use of antihypertensive agents, HbA1c or fasting plasma glucose, BMI, diabetes duration, total cholesterol and/or HDL cholesterol, retinopathy, smoking, use of RAAS-inhibitors, UAER or albumin-to-creatinine ratio (ACR). Race was not applicable because all of the studies were conducted in homogenous Asian, Caucasian or Native American populations. The maximum adjustment score was 10 points. Adjustment was considered “good” if the score was ≥ 9, “average” if the score was 7-8, “fair” if the score was 5-6, and “poor” if the score was <5.

The adjustment score when added to the methodological quality score resulted in a combined biomarker validity score. Biomarkers were considered as valid biomarker candidates if both the methodological quality score and the adjustment score were at least “good” or “average” (biomarker validity score ≥ 15).

RESULTS

The systematic search (performed July 1st 2010) for articles of longitudinal studies on biomarkers for prediction of the onset or progression of nephropathy in patients with type 2 diabetes resulted in 953 non-duplicate articles of which 841 were on conventional risk markers for nephropathy in diabetes type 2, and were therefore excluded. We excluded also studies on populations with chronic kidney diseases other than diabetic nephropathy, studies on nephropathy in patients with diabetes type 1, or studies on non-serum and non-urine biomarkers. The remainder of the articles (N=112) were reviewed in full text. Of these, only 14 studies met the inclusion criteria and were included in the present review. The main reason for exclusion in this phase was cross-sectional study design. Explosion search (the references of the selected papers) and forward citation search (all studies referring to the selected papers) resulted in identification of 1 additional paper. A flow-chart demonstrating the study selection process is shown in figure 1.

The 15 identified studies reported on 27 individual biomarkers, of which 19 were serum/plasma biomarkers, six were urinary biomarkers and IL-18 was measured in both serum and urine (and therefore counted as 2 individual biomarkers). Four studies reported on prediction of the onset of nephropathy in normoalbuminuric patients [25-28], two on progression of nephropathy in micro- or macroalbuminuric patients [29, 30] and seven reported biomarkers in combined normo- and microalbuminuric patient cohorts (i.e. onset and progression) and thus do not make a distinction between onset and progression [31-37]. Another two studies assessed biomarkers in combined normo- and microalbuminuric patient cohorts and report the results for onset and progression of nephropathy separately [38, 39].

Study characteristics

Study characteristics are shown in table 2. Individual study size ranged from 30 to 1103 patients with a total number of 3529 patients. Mean/median follow-up ranged from 0.5-9.0 years and mean age of the study populations ranged from 51.8-67.5 years. All but one study included both men and women in approximately equal proportion [37]. Eleven [25-28, 30, 33-

35, 37-39] out of 15 studies were conducted in Asian populations, one study in a Native American population [36], and 3 studies [29, 31, 32, 40] in Caucasian populations. In studies assessing biomarkers for prediction of onset of nephropathy the transition in albuminuria class (i.e. normo- to microalbuminuria) was the endpoint used in all studies. In studies assessing biomarkers for prediction of progression of nephropathy the endpoints used were transition in albuminuria class (i.e. micro- to macroalbuminuria) in microalbuminuric patients in 3 studies [29, 38, 39] and doubling of serum creatinine in macroalbuminuric patients in 1 study [30]. In studies of mixed normo-, micro- or macroalbuminuric patients the endpoints used were very heterogeneous and no distinction was made between onset and progression of nephropathy. Therefore, the identified biomarkers are summarized as the separate category “onset and progression”. Endpoints used by these studies were change of UAER over time (without indication of the baseline UAER) [37], the ratio of the UAER at baseline and UAER at end of follow-up [35], development of macroalbuminuria in combined normo- and microalbuminuric patients [32, 33, 36], transition from either normo- to microalbuminuria or micro- to macroalbuminuria [34]) and transition from normo- to either micro- or macroalbuminuria (18). Meta-analysis was not performed because of marked heterogeneity in biomarkers analysed, study endpoints, statistical methods used and biomarker cut-off levels.

Methodological quality score, adjustment score, and biomarker validity score

Overall, 7 of the 15 studies had good methodological quality, 6 studies were classified as average and 2 studies as fair. “Average” to “good” adjustment score corresponding to an adjustment for at least 7 of 10 of the conventional risk factors was only present in 6 studies (table 3). All of these 6 studies also had good or average methodological quality. The biomarkers from these 6 studies were considered as valid. These studies identified 17 valid biomarkers, of which 13 yielded significant results predicting nephropathy. Detailed results of all biomarkers are shown in tables 4, 5 and 6. Results were stratified for studies reporting on

biomarkers predicting the onset of nephropathy, the progression of nephropathy, and the onset and progression of nephropathy in combined patient cohorts, respectively.

Biomarkers predicting onset of nephropathy in diabetes (early nephropathy)

Five urinary biomarkers for prediction of the onset of nephropathy in diabetes were evaluated (table 4) [25, 26, 28]. Of these, urinary IgG, ceruloplasmin, and transferrin were predictive of nephropathy onset and had highest validity because of average study design and prediction beyond most conventional risk factors. Urinary transferrin was evaluated in two studies of good methodological quality, and it was significantly associated with onset of nephropathy in both.

Four serum/plasma biomarkers for the onset of nephropathy were evaluated. IL-18 (a marker of subclinical inflammation) and asymmetric dimethylarginine (ADMA; a marker of endothelial dysfunction) could be considered as most promising predictors of microalbuminuria, as these were identified in well-designed studies adjusting for (nearly) all conventional risk factors.

Although urinary N-acetylglucosaminidase (NAG) and serum C-reactive protein (CRP) were considered valid candidates, results were not significant. The remaining biomarkers (matrix metallopeptidase 9 [MMP9] in plasma and lipocalin-type prostaglandin D2 synthase [L-PGDS] in urine) were not considered valid due to lack of adjustment for conventional risk factors.

Biomarkers predicting progression of nephropathy in diabetes (late nephropathy)

Twelve biomarkers for progression of nephropathy in diabetes were evaluated (table 5). Of these, 9 plasma biomarkers were evaluated by Persson et al. [29] in patients from the well-designed randomized control trial 'Irbesartan MicroAlbuminuria Type 2 Diabetes Mellitus in Hypertensive Patients' (IRMA-2) [41]. Vascular cell adhesion molecule 1 in serum (sVCAM1), interleukin 6 (IL-6), von Willebrand factor (vWf), and intercellular cell adhesion molecule 1 in serum (sICAM1) were significantly associated with albuminuria progression, whereas high sensitive CRP (hs-CRP), transforming growth factor β (TGF-β), fibrinogen, E-selectin, and

advanced glycation end-product (AGE) peptides were not significant. Interestingly, this study also tested the combined z-scores of multiple biomarkers (biomarker panel). The panel of markers of endothelial dysfunction (i.e. sVCAM1, vWF, sICAM1 and E-selectin) was found as an independent predictor of progression, whilst the panel of inflammation markers (i.e. hs-CRP, IL-6 and fibrinogen) was not. The valid biomarker ADMA that was earlier shown to predict onset of nephropathy also predicted progression of nephropathy [39]. In contrast, IL-18 predicted onset but not progression of nephropathy and hs-CRP neither predicted onset nor progression of nephropathy in the study by Araki et al. [38]. Whereas the methodological quality of the study on Lipoprotein(a) was good, the study did not address adequate correction for conventional risk factors resulting in low overall biomarker validity.

Biomarkers predicting onset and progression of nephropathy in diabetes

Fourteen biomarkers were evaluated in 7 studies in combined cohorts of normo-, micro- and macroalbuminuric patients with variable endpoints (table 6). Of these studies all but one had average to good methodological quality. However, only the study by Stehouwer et al. (assessing 6 biomarkers) performed adequate adjustment for conventional risk factors, and identified hs-CRP, E-selectin (E-SEL), tissue-type plasminogen activator (TPA), von Willebrand factor (vWf) and triglycerides, but not sVCAM1, as valid and promising biomarkers [31].

DISCUSSION

In this systematic review we identified 15 publications on longitudinal studies reporting on 27 candidate biomarkers for the prediction of nephropathy in type 2 diabetes. We scored the methodological quality of the identified studies and we evaluated whether these biomarkers add information on risk prediction. Using this approach we demonstrated that the overall study quality of these studies is in general modest. This not only limits proper assessment of the potential clinical value of the identified biomarkers, it also limits the generalisability and comparability of the results.

Most of the marker molecules identified in this systematic review represent subclinical systemic inflammation (CRP, IL-6, IL-18, fibrinogen), endothelial dysfunction (ADMA, vWF, VCAM, ICAM1, TPA), extracellular matrix synthesis (TGFb1, laminin, collagen type 4) or glomerular and tubular dysfunction (urinary IgG, ceruloplasmin, and transferrin). Due to the clear pathophysiological connection between these molecules and nephropathy in diabetes it is tempting to utilize these biomarkers in clinical practice. However, before these markers can be applied in practice, the clinical applicability of these biomarkers needs to be confirmed in high-quality validation studies.

We would like to highlight several important methodological issues that are relevant for the quality of biomarker research. First, we found that studies of predictive markers frequently calculated odds ratios or relative risks to demonstrate the strength of association between the biomarker and the outcome. However, odds ratios and hazard ratios inaccurately predict the risk for individual subjects. The ratios are only a measure of association between biomarker and outcome, but do not characterize the ability to discriminate between future health or disease [42]. Another limitation of odds ratios and relative risks is that the size of the ratio depends on the units of measurement [8]. Some form of standardization is necessary, for example by division of a continuous measure by its standard deviation. Moreover, even if very large odds ratios are reported, one cannot conclude that the marker has good predictive power, since each odds ratio could correspond to largely variable true-positive / false-positive fractions. Hence, instead of using odds ratios, the additive value of a

marker in risk prediction should be specified by prediction analyses such as false-positive / true-positive fractions, area under the receiver-operator characteristic (ROC)-curve, the net reclassification improvement (NRI), integrated discrimination improvement (IDI) [43] or the discriminative likelihood ratio (dLR) [44, 45]. Only one study included in this review [28] reported an area under the ROC-curve, and 4 other studies [25], [30, 34], [26] provided sufficient detail for the calculation of sensitivity and specificity.

A second important issue in biomarker research concerns the validation of results of a study in other patient populations. Biomarkers only have clinical value if the results are reproducible (external validation). Of the 27 identified biomarkers, the majority was evaluated in only 1 (longitudinal) study, and only 10 markers (plasma VCAM1, hs-CRP, vWF, fibrinogen, E-selectin, triglycerides, transferrin, serum IL18, homocysteine and urinary transferrin) were analyzed in at least two studies. In nearly all cases results could not be replicated (potentially due to limited power).

A last important methodological issue is the heterogeneity of endpoints in some of the studies. Moreover, the methods of albumin assessment and the number of albuminuria measurements were either not stated or highly heterogeneous between the studies. Lastly, the frequently used endpoint “transition in albuminuria class” has crucial limitations, e.g. a patient with a rise in albuminuria from 29 to 31 mg/day is defined as progressor, while a patient who increases from 31 to 299 mg/day is not. Thus, avoiding albuminuria classification itself or introduction of combined relative and absolute changes in albuminuria (e.g. class transition and at least 30% increase) may represent a valid alternative. This also highlights the importance to reach consensus on definitions of endpoints in biomarker research.

Aside from these limitations in study design, several biomarkers showed promising results. For the prediction of onset of nephropathy in diabetes, urinary IgG, ceruloplasmin and transferrin, serum IL-18 and plasma ADMA were most promising. The results on biomarkers for progression of diabetic nephropathy are not conclusive probably due to differences in adjustment for conventional risk factors.

This review has certain limitations due to its focus on longitudinal studies for prediction of nephropathy and its focus on methodological quality. We were unable to compare the measures of association of the described biomarkers due to marked heterogeneity in study endpoints, statistical methods and different cut-offs. Head to head comparison of biomarkers in a well-designed and sufficiently large longitudinal study is most likely the best way to compare biomarkers. Secondly, we did not take into consideration the individual time of follow-up in the individual studies. This is of particular importance in a slow progressive disease such as nephropathy in diabetes.

Based on the status of current biomarker research in this field, we recommend that future research should be directed at both further biomarker discovery and validation of published biomarkers in large well-designed longitudinal studies. Specific prediction analyses should be applied to assess the additive predictive value of novel and published biomarker candidates beyond conventional risk factors.

In the end, all effort of biomarker research should be directed towards the development of a reliable, accurate, reproducible and robust “Diabetic Nephropathy Biomarker Panel” that would compare to the biomarker panel consisting of creatine kinase (CK), CK-MB, Troponin I and Troponin T currently used in cardiology. Both clinical practice and clinical trials on the efficacy of various treatments on renal disease in type 2 diabetic patients would benefit of such a biomarker panel, thus developing such a biomarker panel would be a major step forward in nephrology.

The fact that many well-designed studies were not able to confirm the results on certain biomarkers emphasizes the remaining uncertainty of the clinical utility of many of the studied markers despite promising findings. Future research will have to elucidate the true value of the current biomarker candidates for prediction of onset and progression of nephropathy in diabetes. However, current results prevent us from making clear recommendations for clinical practice at this moment.

DISCLOSURE STATEMENT

The authors state that they have nothing to disclose. The authors have no relations to companies that might have a financial interest in the information contained in this manuscript.

TITLES AND LEGENDS

Figure 1 Flowchart of the identification process of eligible studies. [a] In at least one study

Table 1 Methodological quality assessment according to 11 relevant items of the STARD criteria. RCT; randomized controlled trial.

Table 2 Characteristics of selected studies, stratified for prediction of onset, progression, and onset and progression of nephropathy. [a]completed follow-up; [b]mean±standard deviation; [c]median and range; [d]median; [e]mean, [f]in the studies (25) and (26) normo- and microalbuminuric patients were analysed seperately; ACR; albumin-to-creatinine ratio. AM; albuminuria measurement. Long; longitudinal. Micro; microalbuminuric patients. Macro; macroalbuminuric patients. nat. Am.; native American. Normo; normoalbuminuric patients. sCr; serum creatinine. UAER; urinary albumin excretion rate.

Table 3 Adjustment score. ● criterion is met, ○ criterion is not met. Adj. score; Adjustment score. Dur. Diab.; duration of diabetes. UAER; urinary albumin excretion. BMI; body mass index. RAAS; Renin-Angiotensin-Aldosterone Systeme.

Table 4 Overview of biomarkers for onset of nephropathy in diabetes.

Tables 4-6: [a]median; [b]mean±2 standard deviations; n.s. not significant; 8-oxodG; 8-oxo-7, 8-dihydro-2'-deoxyguanosine. ADMA; asymmetric dimethylarginine. AGE; advanced glycation end-products. APO-B; Apolipoprotein B; AUC; area under the curve. CI; confidence interval. Cr; COL-IV; collagen type IV. Creatinine. HR; hazard ratio. hs-CRP. high sensitive C-reactive protein. IgG; Immunoglobulin G. IL-6; interleukin 6. IL-18, interleukin 18. L-PGDS; Lipocalin-type prostaglandin D2 synthase. MMP9; matrix metallopeptidase 9. NAG; N-acetylglucosaminidase. OR; odds ratio. P; plasma. R; Reference group. S; serum. Sens/Spec; Sensitivity/Specifity. SD; standard deviation. sICAM1; Intercellular cell adhesion molecule 1 in serum. sVCAM1; Vascular cell adhesion molecule 1 in serum. TGF-β;

transforming growth factor β. TPA; Tissue-type plasminogen activator. VCAM; U; urine. vascular cell adhesion molecule. vWf; von Willebrand factor.

Table 5 Overview of biomarkers for progression of nephropathy in diabetes.

Table 6 Overview of biomarkers for onset and progression of nephropathy in diabetes.

TABLES AND FIGURES

Table 1.

Section		Criteria	Scoring	Comments
Introduction	**1**	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	stated=1 not stated=0	Stated in all.
Methods	**2**	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	stated=1 not stated=0	Stated in all, but two (15, 24).
	3	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria/a randomized controlled trial?	consecutive/RCT=1 non-consecutive/not stated=0	Non-consecutive/RCT or not stated in eight (12, 14, 15, 19, 21, 22, 24, 25).
	4	Data collection: Was data collection planned before the index test and reference standard were performed (prospective) or after (retrospective)?	prospective=1 retrospective=0	Prospective in all, but one (23).
	5	The reference standard and its rationale.	stated=1 not stated=0	Stated in all, but two (23, 24).
	6	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	stated=1 not stated=0	Stated in all, but two (12, 17).
	7	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	stated=1 not stated=0	Stated in all, but one (21).
	8	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% conf. intervals).	stated=1 not stated=0	Not stated in four (14, 20, 22, 24).
Results	**9**	When study was performed, including beginning and end dates of recruitment.	stated=1 not stated=0	Not stated in five (13-16, 22).
	10	Clinical and demographic characteristics of the study population (at least	stated=1	Stated in all, but one (15).

	information on age, gender, spectrum of presenting symptoms).	not stated=0	
11	Distribution of severity of diasease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	stated=1 not stated=0	Stated in all, but two (20, 24).

Table 2.

Study	Year	Ethnicity	Patients[a] (n)	Men [n (%)]	Normo (n)	Micro (n)	Macro (n)	Age[b]	Type of AM	Follow-up	Endpoint
Onset of nephropathy											
Ebihara (14)	1998	Asian	30	17 (57)	30	0	0	55.5 ±15.5	UAER	4	Normo to micro
Kazumi (13)	1999	Asian	77	50 (52)	77	0	0	56.3 ± 10.6	ACR	2	Normo to micro
Narita (12)	2006	Asian	117	60 (51)	117	0	0	60.4 ± 8.9	ACR	5	Normo to micro
Uehara (15)	2008	Asian	121	-	121	0	0	-	ACR	2	Normo to micro
Araki (25)	2007	Asian	249	130 (52)	173	76[f]	0	61 ± 9	UAER	7 [3-8][c]	Normo to micro
Hanai (26)	2009	Asian	225	144 (64)	183	42[f]	0	64 ± 10	ACR	5.2[d]	Normo to micro
Progression of nephropathy											
Persson (16)	2008	Caucasian	269	184 (68)	0	269	0	57.8 ± 8.5	UAER	2	Micro to macro
Song 817)	2005	Asian	81	36 (44)	0	0	81	59 ± 9.2	UAER	2	Doubling of baseline sCr
Araki (25)	2007	Asian	249	130 (52)	173[f]	76	0	61 ± 9	UAER	7 [3-8][c]	Micro to macro
Hanai (26)	2009	Asian	225	144 (64)	183[f]	42	0	64 ± 10	ACR	5.2[d]	Micro to macro
Onset and progression of nephropathy											
Fukui (24)	2009	Asian	162	162 (100)	-	-	-	63.9 ± 11.1	ACR	2	Longitudinal change in UAER
Bruno (19)	2003	Caucasian	1103	477 (43)	677	426	0	67.5 ± 10.2	UAER	5.3 [0.1-7.9][c]	Normo/micro to macro
Hinokio (21)	2002	Asian	396	190 (48)	115	281	0	51.8 ± 10.3	ACR	5	Normo to micro/micro to macro
Looker (23)	2003	Nat.American	229	-	152	77	0	-	ACR	8.6[e]	Normo/micro to macro
Okazaki (20)	1995	Asian	66	57 (66)	-	-	-	-	dipstick	3.5 [3-4][c]	Normo/micro to macro
Nakamura (22)	2005	Asian	76	34 (41)	41	31	10	62.5 ± 7.5	UAER	0.5	UAER-post/ UAER-pre ratio
Stehouwer (18)	2002	Caucasian	328	191 (58)	191	92	45	54.3 ± 8.2	UAER	9±2.9[b]	Normo to micro/macro

Table 3.

Study	*UAER*	*Duration of Diabetes*	*Age&Sex*	*Hypertension*	*HbA1c*	*Smoking*	*Retinopathy*	*Lipids*	*BMI*	*RAAS-Inhibition*	*Adjustment score*
Araki (25)	●	●	●	●	●	●	●	●	●	●	**10**
Bruno (19)	○	●	○	●	●	○	○	○	○	○	**3**
Ebihara (14)	○	○	○	○	○	○	○	○	○	○	**0**
Fukui (24)	○	●	○	●	●	●	○	●	●	○	**6**
Hanai (26)	●	●	●	●	●	○	●	●	●	●	**9**
Hinokio (21)	○	●	○	●	●	○	○	○	○	○	**3**
Kazumi (13)	●	○	●	●	●	●	○	●	●	○	**7**
Looker(23)	●	●	●	○	●	○	○	○	○	○	**4**
Nakamura (22)	●	○	○	○	○	○	○	○	○	○	**1**
Narita (12)	●	●	●	●	●	●	●	●	●	●	**10**
Okazaki (20)	○	●	●	●	●	○	○	○	○	○	**4**
Persson (16)	●	○	●	●	●	●	●	●	○	●	**8**
Song (17)	●	○	○	●	●	○	○	○	○	○	**3**
Stehouwer (18)	●	●	●	●	●	●	○	●	●	○	**8**
Uehara (15)	○	○	○	○	○	○	○	○	○	○	**0**

Table 4.

Biomarker	U/P/S	Reference	Meth. Quality Score	Adj. Score	Biomarker Validity Score	Cut-off	Unit	Results	Sensitivity/ specificity
IL-18	Serum	(25)	10	10	20	134.6[a]	ng/L	OR [95%CI]: 3.6 [1.2-10.4]	-
ADMA	Plasma	(11)	11	9	20	0.46 µmol/l[a]	µmol/L	HR [95%CI]: 2.61 [1.06-6.43]	-
IgG	Urine	(12)	9	10	19	-	mg/gCr	OR [95%CI]: 8.99 [3.16-25.6]	0.47/0.91
Ceruloplasmin	Urine	(12)	9	10	19	-	mg/gCr	OR [95%CI]: 4.67 [1.67-13.1]	0.47/0.84
Transferrin	Urine	(12)	9	10	19	-	µg/gCr	OR [95%CI]: 5.52 [1.81-16.8]	0.35/0.91
Transferrin	Urine	(13)	10	7	17	>107 µg/mmolCr[b]	µg/mmolCr	OR [95%CI]: 7.04 [1.02-48.5]	0.56/0.84
MMP9	Plasma	(14)	8	0	8	-	µg/L	P<0.001 (48 months)	-
L-PGDS	Urine	(15)	7	0	7	4.2 mg/gCr	mg/gCr	AUC [95%CI]: 0.759 [0.725-0.791]	-
hs-CRP	Serum	(25)	10	10	20	-	mg/L	Not reported (n.s.)	-
NAG	Urine	(12)	9	10	19	-	U/gCr	Not reported (n.s.)	-

Table 5.

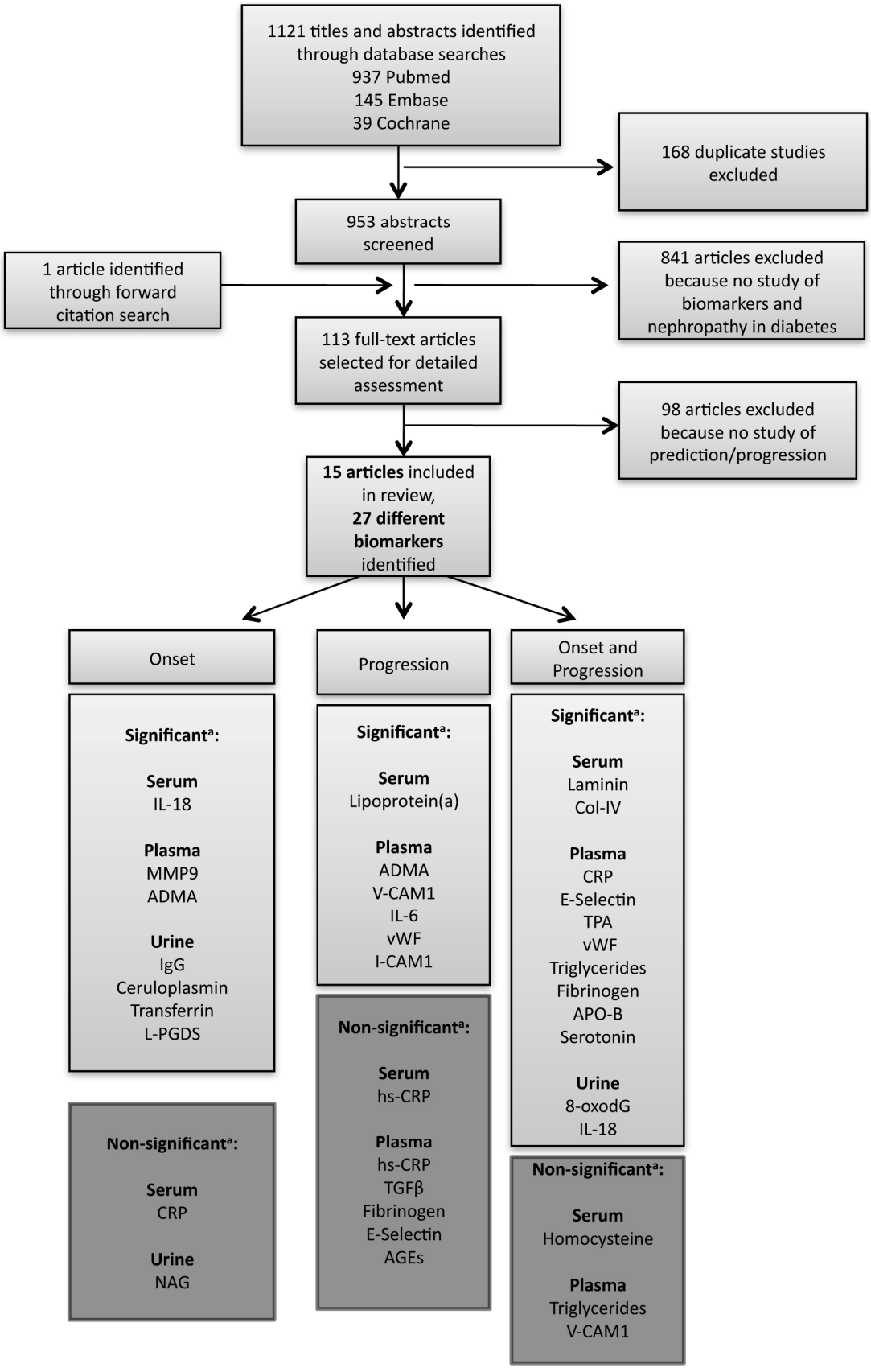
Biomarker	*U/P/S*	*Reference*	*Meth. Quality Score*	*Adj. Score*	*Biomarker Validity Score*	*Cut-off*	*Unit*	*Results*	*Sensitivity/ specificity*
ADMA	Plasma	(26)	11	9	20	0.51 µmol/l[a]	µmol/L	HR [95%CI]: 7.57 [1.42-40.38], P=0.018	-
sVCAM-1	Plasma	(16)	10	8	18	1 SD change	ng/mL	HR [95%CI]: 2.06 [1.26-3.36], P=0.004	-
IL-6	Plasma	(16)	10	8	18	1 SD change	pg/mL	HR [95%CI]: 1.72 [1.12-2.66], P=0.014	-
vWf	Plasma	(16)	10	8	18	1 SD change	%	HR [95%CI]: 1.69 [1.10-2.59], P=0.016	-
sICAM-1	Plasma	(16)	10	8	18	1 SD change	ng/mL	HR [95%CI]: 1.99 [1.04-3.82], P=0.038	-
Lipoprotein(a)	Serum	(17)	10	3	13	per 10 mg/dL	mg/dL	OR [95%CI]: 1.418 [1.040-1.934], P=0.027	0.93/0.55
hs-CRP	Serum	(25)	10	10	20	Not reported	mg/L	n.s.	-
IL-18	Serum	(25)	10	8	18	1 SD change	mg/L	n.s.	-
hs-CRP	Plasma	(16)	10	10	20	Not reported	ng/L	n.s.	-
TGF-β	Plasma	(16)	10	8	18	1 SD change	ng/mL	n.s.	-
Fibrinogen	Plasma	(16)	10	8	18	1 SD change	g/L	n.s.	-
E-Selectin	Plasma	(16)	10	8	18	1 SD change	ng/mL	n.s.	-
AGEs	Plasma	(16)	10	8	18	1 SD change	%	n.s.	-

Table 6.

Biomarker	U/P/S	Reference	Meth. Quality Score	Adj. Score	Biomarker Validity Score	Cut-off	Unit	Results	Sensitivity/ specificity
CRP	Plasma	(18)	11	8	19	per 1.0 mg/L	mg/L	OR [95%CI]: 1.06 [1.02-1.11]	-
E-SEL	Plasma	(18)	11	8	19	per 10 µg/L	µg/L	OR [95%CI]: 1.08 [1.03-1.13]	-
TPA	Plasma	(18)	11	8	19	per 10 µg/L	µg/L	OR [95%CI]: 1.02 [1.00-1.04]	-
vWf	Plasma	(18)	11	8	19	per 10%	%	OR [95%CI]: 1.05 [1.00-1.10]	-
Triglycerides	Plasma	(18)	11	8	19	per 1.0 mmol/L	mmol/L	OR [95%CI]: 1.10 [1.00-1.20]	-
Fibrinogen	Plasma	(19)	11	3	14	3.49-4.12 (R:<3.00)	g/L	OR [95%CI]: 1.93 [1.18-3.16]	-
APO-B	Plasma	(19)	11	3	14	95-112 (R:<74)	mg/dL	OR [95%CI]: 1.73 [1.05-2.87]	-
Laminin	Serum	(20)	9	4	13	-	U/ml	P<0.01	-
COL-IV	Serum	(20)	9	4	13	-	ng/mL	P<0.05	-
8-oxodG	Urine	(21)	9	3	12	>400 (R:<200)	pmol/kg/day	OR [95%CI]: 2.71 [1.78-3.88]	0.45/0.87
Serotonin	Plasma	(24)	6	6	12	per log unit	ng/mL	β=0.284, P=0.0013	-
IL-18	Urine	(22)	8	1	9	-	pg/ml	r = 0.234, P=0.042	-
IL-18	Serum	(22)	8	1	9	-	pg/ml	r = 0.268, P=0.018	-
VCAM	Plasma	(18)	11	8	19	per 100 µg/L	µg/L	n.s.	-

Triglycerides	Plasma	(19)	11	3	14	-	mmol/L	Not reported (n.s.)	-
Homocysteine	Serum	(23)	9	4	13	per 5 µmol/L (±1SD)	µmol/L	n.s.	-

Figure 1.


1121 titles and abstracts identified through database searches
937 Pubmed
145 Embase
39 Cochrane
168 duplicate studies excluded
953 abstracts screened
1 article identified through forward citation search
841 articles excluded because no study of biomarkers and nephropathy in diabetes
113 full-text articles selected for detailed assessment
98 articles excluded because no study of prediction/progression
15 articles included in review, 27 different biomarkers identified
Onset
Progression
Onset and Progression
Significant[a]:
Serum
IL-18
Plasma
MMP9
ADMA
Urine
IgG
Ceruloplasmin
Transferrin
L-PGDS
Non-significant[a]:
Serum
CRP
Urine
NAG
Significant[a]:
Serum
Lipoprotein(a)
Plasma
ADMA
V-CAM1
IL-6
vWF
I-CAM1
Non-significant[a]:
Serum
hs-CRP
Plasma
hs-CRP
TGFβ
Fibrinogen
E-Selectin
AGEs
Significant[a]:
Serum
Laminin
Col-IV
Plasma
CRP
E-Selectin
TPA
vWF
Triglycerides
Fibrinogen
APO-B
Serotonin
Urine
8-oxodG
IL-18
Non-significant[a]:
Serum
Homocysteine
Plasma
Triglycerides
V-CAM1

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ACKNOWLEDGEMENT

“The work leading to this paper has received funding from the European Community's Seventh Framework Programme under grant agreement n° HEALTH–F2–2009–241544.”