Leading article

The importance of identifying novel biomarkers of microvascular damage in type 1 diabetes.

M. Loredana Marcovecchio

Department of Paediatrics, University of Cambridge, Cambridge Biomedical Campus, CB2 0QQ, Cambridge, UK

Email: mlm45@medschl.cam.ac.uk
ORCID: 0000-0002-4415-316X

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Address correspondence to:
Dr M. Loredana Marcovecchio; MD, PhD
University Department of Paediatrics, Level 8, Box 116, Addenbrooke's Hospital
Hills Road, Cambridge CB2 0QQ, UK
Tel.: +44 (0) 1223 768615, Fax: +44 (0) 1223 336996
Email: mlm45@medschl.cam.ac.uk

Abstract

Microvascular complications of type 1 diabetes, which primarily include diabetic kidney disease, retinopathy and neuropathy, are characterized by damage to the microvasculature of the kidney, retina and neurons. The pathogenesis of these complications is multifactorial, and several pathways are implicated. These complications are often silent during their early stages, and once symptoms develop, there might be little to be done to cure them. Thus, there is a strong need for novel biomarkers to identify individuals at risk of microvascular complications at an early stage and guide the implementation of new therapeutic options for preventing their development and progression.

Recent advancements in proteomics, metabolomics and other 'omics' have led to the identification of several potential biomarkers of microvascular complications. However, biomarker discovery has met several challenges and, up to now, there are no new biomarkers which have been implemented into clinical practice. This highlights the need of further work in this area to move towards better diagnostic and prognostic approaches.

Key points

- Early detection of microvascular complications is of paramount importance and new biomarkers are required to achieve that.
- New 'omics'-based biomarkers are promising, and they could be included in a multi-biomarker approach together with traditional markers of complications to support early diagnosis and management of microvascular complications.
- Many challenges remain in biomarkers discovery and translation into clinical practice, and future larger collaborative studies for a better characterisation of new biomarkers, methodological standardization and uniformity and cost evaluations are required.

1. Introduction

Mortality rates in individuals with type 1 diabetes (T1D) still exceed that of the background population by 3-4 fold [1–3], even though over the last decades there have been key advancements in the management of this condition. The burden associated with T1D is largely due to the associated micro- and macro-vascular complications, which develop in a high percentage of patients after a variable diabetes duration [4,5].

Prevention of vascular complications relies on the ability to identify high-risk individuals at an early stage when tissue damage may be more responsive to interventions and reversible [4–6]. Many of the biomarkers currently in use do not allow for early diagnosis of vascular damage, thus highlighting the need for novel biomarkers reflecting earlier stages of the development of vascular complications, to identify subjects at risk and implement additional preventive strategies.

Recent advancements in proteomics, metabolomics and other 'omics' and the integration of these different approaches continue to unveil new potential biomarkers in several fields, including T1D vascular complications [7,8].

This review provides an overview of the state-of-the-art on biomarkers of microvascular damage, specifically in the context of T1D. The focus of the review is on the value of biomarkers of vascular complications and the challenges related to their development and implementation into clinical practice. Some examples of biomarkers identified during most recent years, mainly arising from the application of proteomics and metabolomics, are also provided.

2. Diabetes microvascular complications

Vascular complications of T1D are generally classified in microvascular, affecting small vessels in the retina, kidney and nerves, and primarily including diabetic kidney disease (DKD), diabetic retinopathy (DR), and diabetic neuropathy (DNeu), and macrovascular complications, such as cardiovascular, cerebrovascular and peripheral vascular disease, where large vessels are predominantly affected [4–6,9]. However, this is a simplified classification and it is important to bear in mind that microvascular damage can also occur in other tissues, such as the heart, brain, myocardium, skin [5,9]. Furthermore, significant associations between microvascular and macrovascular complications have been reported,

whereby patients with one complication often present a second one, suggesting common risk factors and/or underlining mechanisms [10]. Some of the common risk factors for vascular complications are hyperglycemia, dyslipidemia, hypertension, diabetes duration, smoking, overweight, insulin resistance [11]. Endothelial dysfunction has been suggested as one of the potential links between different vascular complications [12]. Inflammation and oxidative stress are two key mechanisms implicated in the pathogenesis of all vascular complications as well as of T1D comorbidities, such as hypertension and dyslipidemia [13]. Cardiovascular disease, mainly in the form of coronary artery disease, is the main cause of mortality in people with T1D [14]. However, there is also strong evidence that microvascular complications substantially contribute to morbidity and mortality in individuals with T1D [15–17]. This was recently confirmed by data from the large cohort with T1D from the Steno Diabetes Center, showing a 2.2-fold increased mortality rate in the presence of DKD and 1.7-fold increase in individual with Dneu [18].

2.1 Epidemiology, natural history and diagnosis
2.1.1 Diabetes kidney disease

DKD is one of the main microvascular complications affecting up to 50% of all people with T1D over the course of their lifetime [19]. DKD represents the leading cause of End Stage Renal Disease (ESRD) worldwide and a main determinant of cardiovascular disease and mortality [18,19]. DKD reflects structural and functional changes occurring in the kidney, manifesting as renal hemodynamic changes, progressive increases in albuminuria and decline in renal function, and hypertension. Major renal structural changes include mesangial expansion, glomerular and tubular basement membrane thickening and glomerular sclerosis [20]. Renal hemodynamic changes, oxidative stress, inflammation, hypoxia and abnormalities in the renin-angiotensin-aldosterone system (RAAS) are involved in the pathogenesis of DKD [21].

The most characteristic biomarker, currently in use for the diagnosis of DKD, is albuminuria, which is associated with renal disease progression and cardiovascular events [15]. In addition, glomerular filtration rate (GFR) is another renal marker currently in use [22,23]. However, there is ongoing debate on the sensitivity and specificity of these biomarkers and a strong need for new biomarkers reflecting earlier subclinical manifestations of DKD.

2.1.2 Diabetic retinopathy

DR is the most common eye disease and the leading cause of blindness among patients with T1D and its overall prevalence is about 35% [24]. It occurs following damage in the retinal microvasculature, manifested as basement membrane thickening, increased capillary permeability, vascular tortuosity, retinal haemorrhage, microaneurysms, cotton-wool spots and lipid exudates [25]. These changes can be clinically silent for many years and then become evident as features of non-proliferative retinopathy. Further progression of retinal microvascular changes can lead to intravascular coagulation, resulting in retinal ischemia and consequent formation of new vessels within the retina. These new vessels are fragile, and their rupture leads to retinal bleeds and manifest as proliferative retinopathy. Fluid accumulation within the central neural retina, referred to as diabetic macular edema, manifests as abnormal retinal thickening and cystoid formation and is the most common cause of visual loss in individuals with DR [25].

Retinal fundus examination is the basis of current screening for DR; however, standard assessment can miss very early functional and structural abnormalities [23]. There are no circulating biomarkers of retinopathy currently in use. New non-invasive imaging techniques, such as retinal microvascular geometry assessment, are currently being explored to allow the identification of early damage within the retinal microcirculation [26]. However, at present, these new techniques are only for research use and not yet implemented into clinical practice.

2.1.3 Diabetic neuropathy

DNeu refers to a spectrum of various neurological disorders associated with diabetes. It is defined by a clinical or subclinical disorder, without any additional causes of peripheral neuropathy other than diabetes and can be either somatic or autonomic [5,27,28]. Chronic distal symmetric polyneuropathy is the most common form of DNeu and is characterized by symmetric damage of peripheral small sensory and large motor nerve fibers [27]. It occurs in about 20% of patients with T1D after a disease duration of 20 years and up to 50% at 10 years of disease [29]. DNeu is a significant contributor to overall morbidity and mortality. Of note, about 50%–70% of non-traumatic amputations are due to this complication [29].

The diagnostic approach is complicated and not well standardized and comprises clinical assessments based on signs, symptoms and questionnaires; instrumental tests, such as the 10g monofilament or the gold standard nerve conduction studies [23,27]. However, the latter tests are labour intensive, time consuming, costly and not easily implementable in

daily clinical practice. Therefore, peripheral DNeu is often diagnosed late when irreversible nerve injury has occurred, and its first presentation may be with a diabetic foot ulcer. Thus, an area of unmet need is the implementation of effective screening for early abnormalities preceding the appearance of overt clinical manifestations.

Some new techniques, such as corneal confocal microscopy and point-of-care devices for an early diagnosis of peripheral neuropathy are currently being explored [27]. No circulating biomarkers are currently available in clinical practice to support the diagnosis and management of DNeu.

2.2 Pathogenesis of microvascular complications

The pathogenesis of microvascular complications is incompletely understood, but it is likely the result of an interplay between several metabolic and hemodynamic factors, which occur as a consequence of hyperglycemia, dyslipidemia, hypertension, genetic predisposing factors and other environmental factors [5,30].

Hyperglycemia is a key determinant of vascular complications of T1D, and there is extensive evidence showing that both acute and chronic hyperglycemia have a deleterious effect [30–32]. Hyperglycemia contributes to the development of vascular complications through several mechanisms: activation of diacylglycerol-protein kinase C, polyol and hexosamine pathways; increased oxidative stress and advanced glycation end-products, subclinical inflammation, RAAS dysregulation. These factors can, in turn, induce diffuse altered blood flow, endothelial permeability, extravascular protein deposition and coagulation resulting in the progressive development of microvascular complications and organ dysfunction [30–32].

Of interest, recent animal studies have highlighted potential differences in the pathways activated by hyperglycemia in the context of each individual microvascular complication [33]. In addition to the main effect of hyperglycemia, altered lipid metabolism has also emerged as a key player in the pathogenesis of diabetic complications, with distinct contributing effects in the context of different vascular beds [33].

Microvascular complications are characterized by a long subclinical phase before becoming clinically manifest [4]. Early functional and structural changes in the eyes, kidney and nerves can occur soon after diagnosis, but be silent for many years [4]. This highlights the importance of early detection of subclinical signs of complications to prevent their

progression. However, it is currently impossible to reliably predict when and who will develop any of the microvascular complications. Their early detection relies on the availability of sensitive and specific biomarkers, which should be easily implemented in daily clinical practice.

The mechanisms implicated in the pathogenesis of microvascular damage can lead to an altered expression of local and circulating molecules, which could be used as biomarkers of disease development and progression, as well as potential targets for future interventions.

3. The role of Biomarkers in the field of diabetic microvascular complications

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [34,35]. Biomarkers can provide a robust approach to understand the spectrum of a disease from the earliest silent signs up to the most advanced stages. The ideal biomarker should be readily quantifiable in accessible biological samples, such as blood or urines, be sensitive and specific and show a good correlation with the progression of the outcome of interest. It also needs to be economical and feasible to be measured in most clinical laboratories. (Box 1)

In the context of microvascular complications, it is essential to discover new biomarkers which could allow the identification of vascular damage during its early subclinical phases, predict it progression and provide support for the development and implementation of tailored interventions. Sensitive and specific biomarkers could guide screening programmes, improve risk stratification, predict response to treatment as well as provide a way of monitoring response to treatment. (Figure 1)

In clinical practice, the value of new biomarkers of vascular complications is to replace or improve the predictive value of markers currently in use, such as clinical and biochemical parameters or imaging tests, for an early identification of microvascular damage and predict those patients at risk of developing complications as well as those most at risk of progressing to more advanced stages [36]. From a research perspective, new biomarkers could help to understand signalling pathways related to microvascular damage and discover novel therapies to prevent and treat complications. New biomarkers could also support selection of participants for future clinical trials exploring new interventions and predict and

monitor response to treatment [37,38]. This would improve the power of future studies and their efficiency.

Given the complexity and the multiple mechanisms and pathways implicated in the pathogenesis of microvascular complications, it is likely that there is not a single optimal diagnostic and prognostic biomarker, but more likely multiple biomarkers. The possibility to combine several different biomarkers in a multi-marker approach could improve the ability of detecting subjects most at risk and separate them from those at lower risk.

Biomarkers discovery for microvascular complications has been based on hypothesis-based as well as hypothesis-free approaches [39]. Hypothesis-based approaches rely on the knowledge of the pathogenetic mechanisms implicated in the development of complications. Hypothesis-based biomarkers are primarily those reflecting biochemical consequences of the diabetes milieu, such as hyperglycemia-related pathways including inflammation, oxidative stress, fibrosis, hypoxia, mythocondrial dysfunction, or lipid-related pathways [30]. In the context of each individual complication, tissues-specific biomarkers may also be of enormous relevance, and this can include glomerular or renal tubular proteins, endothelial cell markers and nerve components.

However, hypothesis-free technologies can provide additional support to identify novel biomarkers, and this is an emerging and active field for biomarkers discovery for vascular complications, which has been supported by recent advancements in proteomics, metabolomics, as well as genomics, transcriptomics, lipidomics and microRNA [40]. (Figure 1)

It is envisaged that a multi-omics approach could facilitate biomarkers discovery. Findings from different 'omics' and 'non-omics' approaches could be combined to generate scores and be included into predictive models together with clinical predictors to improve prediction of complications [41].

4. Challenges in biomarker discovery for diabetes microvascular complications

The development of clinically relevant biomarkers of microvascular damage has been met with numerous challenges, many of which are in common with other fields.

One key issue is biomarkers tissue specificity. It would be optimal to measure biomarkers directly in the tissues affected by the disease of interest. For vascular complications this implies using renal tissue obtained through kidney biopsies, retinal or nerves biopsies, which

are not easy to obtain and, for research purposes, pose relevant ethical considerations. Thus, so far biomarkers have been assessed mainly in biological fluids, such as blood or urine [42]. However, the obvious question is as to whether circulating biomarkers reliably reflect tissue mechanisms and local concentrations of any specific biomarker. To overcome this issue, alternative potential sources of biomarkers have been explored, such as tears in the context of DR [43].

Biomarkers discovery can be difficult due to different phenotypes associated with the individual diabetic vascular complications. For DKD, for example, studies have explored biomarkers in relation to different renal outcomes, such as GFR decline, ESRD, micro- or macroalbuminuria [42]. For DR, different studies have recruited populations with different phenotypes, including non-proliferative retinopathy, proliferative retinopathy, macular edema [44]. This can explain discordant findings between studies and make comparisons difficult.

In addition, although one can expect that some biomarkers, such as those reflecting inflammation, oxidative stress, endothelial dysfunction, could be in common to all microvascular complications, most of them are likely tissue specific [28,42,44]. Given the evidence that microvascular complications often tend to co-occur in the same individuals, it would be of interest to explore biomarkers in individual with one vs those with multiple complications, as well as the potential additional contribution of the effect of comorbidities, such as hypertension or dyslipidemia on biomarkers.

In addition, for any specific complication, there are differences in biomarkers in individuals with T1D vs type 2 diabetes (T2D) and so far there have been more studies in adults with T2D [42].

Another important aspect to consider is that most of the studies on biomarkers for diabetic complications have been performed in adult populations. In general, biomarkers discovery in pediatrics has been limited, although they will be invaluable in this age group for the early diagnosis and prevention of chronic conditions, such as diabetes and its complications [45]. Given that biomarkers can show age-specific differences, those identified in populations of adults with diabetes cannot necessarily be translated to youth with diabetes.

Biomarker discovery requires large sample sizes to have enough power, mainly when exploring multiple biomarkers through 'omics' approaches. In addition, biomarker utility

needs to be confirmed in two or more independent populations, including discovery and validation cohorts [46].

Differences in assays and mass spectrometry approaches across different laboratories can introduce a source of bias in biomarker measurements. Therefore, once a biomarker has been identified, harmonization of techniques for measurement and quality control measures, as well as defining reference values are essential to reduce measurement variability [47]. A wider availability of techniques, such as mass spectrometry and magnetic resonance spectroscopy, and expertise with them in clinical laboratories, are other essential steps to move towards the translation of biomarkers into clinical practice. Standardized procedures are also required in terms of sample collection, handling and storage. Furthermore, the added value of a novel biomarker in clinical practice compared with existing markers should also be carefully evaluated. New biomarkers can be more expensive than traditional markers used for screening or diagnostic purposes. Although there have been enormous progresses with 'omics' technologies, they remain costly and not yet of unlimited access. Therefore, for any new biomarker there is a need to assess its performance versus existing clinical predictors and biomarkers. It is essential to analyze the cost-effectiveness of their introduction into clinical practice to reduce risk of complications and improve patients' outlook.

5. Where are we in biomarkers discovery for microvascular complications?

5.1 Diabetic kidney disease

Over the last decades, several studies have assessed potential new biomarkers which could replace GFR and urinary albumin excretion or improve their predictive value for the identification of DKD and prediction of progression towards ESRD. Biomarkers of DKD have been extensively reviewed elsewhere [42,48]. Here, some key aspects related to biomarkers discovery will be reviewed along with some examples of relevant biomarkers identified so far.

Development and validation studies for DKD biomarkers in patients with T1D have been based on biological samples (urines, blood), with only few studies being based on kidney biopsy tissues. Most studies have been performed in cohorts of adults with T2D, and fewer in adults with T1D. In addition, limited studies have been performed in youth with diabetes [42,48].

The approaches used for DKD biomarkers discovery have been different, going from investigations of single biomarkers in biological samples to multiple biomarkers assessed in the same samples, individually or as part of panels, and more recently to proteomic- and metabolomics-based approaches [42,48].

When interpreting the available results on biomarkers for DKD it is important to take into account significant differences between studies in terms of characteristics of the study populations, i.e. stages of DKD, study design and selected renal outcomes, and the adjustments made in the predictive models, which not always have allowed for known predictors of DKD [42,48]. The search for new biomarkers has focused mainly on circulating molecules in the blood or urine able to improve prediction of clinically significant outcomes such as ESRD, marked decline in GFR or death. There remains a strong need for biomarkers reflecting early structural and functional changes occurring in the kidney [49].

Different approaches have been used in different studies and this is another factor complicating comparisons and explaining heterogeneous findings when comparing studies. These have included: hypothesis-based approaches, where single or multiple biomarkers have been assessed using ELISA or multiplexed platforms, and proteomics and metabolomics studies, mainly based on based on mass spectrometry and nuclear magnetic spectroscopy [42,48].

The DKD biomarkers identified so far belong to different categories: glomerular and renal tubular biomarkers, inflammation-, oxidative stress-, fibrosis-related biomarkers as well as cardiovascular biomarkers [42,48,50].

Several inflammatory biomarkers have been investigated in relation to DKD [51]. A recent systematic review and meta-analysis [52] provided an updated overview on the association between circulating tumor necrosis factor receptors (TNFRs), which have been widely studied in relation to DKD, and the risk of DKD progression as well as cardiovascular disease events and mortality in patients with diabetes. Overall the analysis showed a 2-fold increased risk of DKD progression per doubling increase in TNFR1 and TNFR2. In a recent study, Niewczas et al [53] quantified circulating concentrations of 194 inflammatory proteins in 3 cohorts with diabetes totalling 525 participants. Using a global proteomic profiling approach, 17 inflammatory proteins, defined as 'kidney risk inflammatory signature (KRIS)', were associated with a 10-year risk of developing ESRD. Of note, the protein signature was enriched in TNFR superfamily members [53]. All identified proteins had a

systemic, non-kidney source, providing strong evidence for a role of inflammation in the pathogenesis of DKD and a target for future intervention strategies.

Proximal tubular proteins, such as urinary Kidney Injury Molecule-1, Neuthrophil gelatinase-associated lipocalin, Liver-type fatty acid-binding protein have been associated with a faster decline in GFR in adults with T1D [54,55]. Studies have also highlighted the role of cardiovascular disease biomarkers as being associated with declining renal function, such as high-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide [56,57].

Urinary proteomics is a promising tool to identify biomarkers of DKD [48,58]. Recent urinary proteomics studies in patients with diabetes have highlighted some promising proteins/peptides associated with renal outcomes and reflecting potential pathogenetic mechanisms implicated in DKD [48,58].

Of particular interest is CDK273, a panel of 273 urinary biomarkers, discovered in an original comparison of the urinary proteome of 379 healthy participants and 230 participants with chronic kidney disease related to different renal conditions, which represents a good example on how to combine multiple biomarkers into a 'classifier' [59,60]. The CDK273 classifier has been associated with progression of albuminuria and loss of renal function in retrospective cohorts, mainly with T2D [42]. More recently, in a recent large prospective multicentre study (PRIORITY), the CKD273 classifier was used for risk stratification in individuals with normoalbuminuria and T2D [38]. A high-risk score based on CDK273 was able to predict progression of microalbuminuria, independently of clinical characteristics, and a 30% decline in GFR in those participants at risk of microalbuminuria [38]. However, the mineralocorticoid receptor antagonist spironolactone was not shown to delay or prevent development of microalbuminuria in those identified to be at high risk of progression by the CKD273 classifier [38]. Further studies are required to confirm the value of this classifier in T1D and explore ways of integrating it with other markers into clinical practice.

Metabolomics has also been applied in the field of DKD and unveiled several metabolites mainly represented by products of lipid metabolism, branched chain and aromatic amino acids, citric acid cycle metabolites related to mitochondrial dysfunction [60,61].

5.2 Diabetic retinopathy

Biomarkers of DR have been explored in blood samples, and in more specific fluids/tissues, such as vitreous humour, aqueous humour and retina tissue [43,44,62]. The retina, vitreous

and aqueous humours represent great sources to understand the pathogenesis of DR and identify biomarkers. However, their collection requires invasive procedures, which could be acceptable if performed for clinical indications, such as surgery for managing retinal diseases, but not for diagnostic or screening purposes. Most studies using these tissues have been performed in animal models or post-mortem in humans [43,44,62].

Of interest, over recent years, there has been a focus on tears as a potential source of new biomarkers of DR [43,44]. The main advantage of tear samples is that they can be collected noninvasively, they contain a relatively high concentration of proteins and tear proteome correlates with disease progression [43,44]. Starting from studies performed in 2012, a relevant number of proteins in the tears have been associated with different stages of DR. These include, among others, lipocalin A, lysozyme C, lipophilin A, immunoglobulin lambda chain, lactotransferrin, β-2-microglobulin [44].

Many systemic biomarkers have been associated with different stages of DR. Most of them are inflammatory molecules and endothelial dysfunction markers. This underscores a key role of inflammation in the pathogenesis of DR. Among novel biomarkers, it is important to mention VEGF, because it is implicated in retina neovascularization. Its key role into the pathogenies of proliferative retinopathy has led to a new intervention for this complication, with anti-VEGF drugs [63]. Additional biomarkers linked to DR are advanced glycation end products and angiogenic proteins, including fibroblast growth factor-21, adiponectin, cystatin C [44].

Metabolomics studies have also been performed for DR and have mainly highlighted abnormalities in metabolites from different pathways, including amino acids, such as tryptophan metabolites, indoleamine 2,3-dioxygenase, fatty acids, glucose metabolism [61]. As for DKD, different studies have led to discordant results due to various factors, such as differences in study populations and stages of DR under investigations. Different approaches have been used in different studies, including single or panel of biomarkers as well as wider approaches, such as proteomic and metabolomics, and this represents another source of variability between studies [43,44,61,64].

5.3 Diabetic neuropathy

A comprehensive review of the main biomarker discovery studies for DNeu has been recently published [28]. One key aspect is that, compared to DKD and DR, there are fewer data on biomarker discovery for DNeu based on hypothesis-free approaches using

proteomics or metabolomics. However, there are growing data on new potential imaging techniques, which could support and improve diagnosis and management of this complication in the future [28].

Systemic biomarkers of oxidative stress, inflammation, and vascular activation have been associated to peripheral DN. Pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, IL-8, monocyte chemoattractant protein-1 and C-reactive protein, vascular cell adhesion molecule-1, E-selectin and chemokines show an increased expression in patients with diabetic peripheral neuropathy [65]. Biomakers such as ICAM-1 and IL-1 receptor have been associated with progression of peripheral DNeu [66].

A promising novel biomarker of DNeu is Nrf2, a molecule which acts as a link in various inflammatory and apoptotic pathways impacting progression of DR [67]. There is evidence suggesting that while acute hyperglycemia increases the expression of Nrf2, chronic hyperglycemia decreases its expression. This downregulation of Nrf2 causes various microvascular changes, which result in diabetic neuropathy. Nrf2 activators have been suggested as a therapeutic potential for the treatment of diabetic neuropathy [67]. Of interest, preliminary data suggest that levels of substance P, which is implicated in maintaining corneal nerve health, in the tear film, are reduced in individuals with T1D and this is associated with both corneal changes and peripheral DNeu [68].

Further studies are required to confirm these new promising biomarkers along with a wider application of omics approaches to identify additional early biomarkers of DN.

6. Summary and Conclusions

Diabetic microvascular complications are often asymptomatic during their early stages, and once symptoms develop, there might little to be done to cure them. Therefore, there is clearly a need for novel biomarkers with high sensitivity and specificity for predicting the development and progression of vascular complications. The identification of reliable biomarkers, in addition to providing useful tools for early detection of complications and risk stratification, could also bring new insights into pathogenetic mechanisms, and lead to new therapeutic options.

During recent years, several new biomarkers have been identified, although often with discordant findings across studies and different populations with diabetes. Disappointingly, none of the discovered biomarkers have been implemented into clinical practice. In my

opinion there are still some key steps to be made before translation from bench to bedside can occur. First, there is a clear need of further larger collaborative studies for biomarkers discovery and validation based on standardized protocols for sample collection and processing, and data analysis. Well characterized study populations with a wider age range should be included in future studies.

The most promising approach to improve early detection and management of microvascular complication will likely rely on the integration of multiple biomarkers, reflecting different pathways and mechanisms implicated in microvascular damage, and emerging from different approaches, such as proteomics, metabolomics, genomics, by generating a biomarker score or classifier. However, omics technologies remain costly and of limited accessibility, and there will be a need of health economics assessments before recommending novel omics-based biomarkers for routine use.

This highlights the need of further work in this area, which is essential to move towards a personalized medicine approach.

References

1. Livingstone SJ, Levin D, Looker HC, Lindsay RS, Wild SH, Joss N, et al. Estimated life expectancy in a Scottish cohort with type 1 diabetes, 2008-2010. JAMA. 2015;313:37–44.

2. Rawshani A, Sattar N, Franzén S, Rawshani A, Hattersley AT, Svensson A-M, et al. Excess mortality and cardiovascular disease in young adults with type 1 diabetes in relation to age at onset: a nationwide, register-based cohort study. Lancet. 2018;392:477–86.

3. Svane J, Lynge TH, Pedersen-Bjergaard U, Jespersen T, Gislason GH, Risgaard B, et al. Cause-specific mortality in children and young adults with diabetes mellitus: A Danish nationwide cohort study. Eur J Prev Cardiol. 2019;2047487319836550.

4. Forbes JM, Fotheringham AK. Vascular complications in diabetes: old messages, new thoughts. Diabetologia. 2017;60:2129–38.

5. Barrett EJ, Liu Z, Khamaisi M, King GL, Klein R, Klein BEK, et al. Diabetic microvascular disease: An endocrine society scientific statement. J Clin Endocrinol Metab. 2017;102:4343–410.

6. Donaghue KC, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes. 2018;19:262–74.

7. Olivier M, Asmis R, Hawkins GA, Howard TD, Cox LA. The need for multi-omics biomarker signatures in precision medicine. Int. J. Mol. Sci 2019;20:4781.

8. Gan WZ, Ramachandran V, Lim CSY, Koh RY. Omics-based biomarkers in the diagnosis of diabetes. J Basic Clin Physiol Pharmacol. J Basic Clin Physiol Pharmacol; 2020;31.

9. Mauricio D, Alonso N, Gratacòs M. Chronic Diabetes Complications: The Need to Move beyond Classical Concepts. Trends Endocrinol. Metab. 2020;31:287-95.

10. Girach A, Vignati L. Diabetic microvascular complications--can the presence of one predict the development of another? J Diabetes Complications. J Diabetes Complications; 2006;20:228–37.

11. Girach A, Manner D, Porta M. Diabetic microvascular complications: can patients at risk be identified? A review. Int J Clin Pract. 2006;60:1471–83.

12. Schalkwijk CG, Stehouwer CDA. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond). 2005;109:143–59.

13. Jha JC, Jandeleit-Dahm KAM, Cooper ME. New Insights Into the Use of Biomarkers of Diabetic Nephropathy. Adv Chronic Kidney Dis. 2014;21:318–26.

14. Sharma H, Lencioni M, Narendran P. Cardiovascular disease in type 1 diabetes. Cardiovasc. Endocrinol. Metab. 2019:8:28–34.

15. Groop P-H, Thomas MC, Moran JL, Wadèn J, Thorn LM, Mäkinen V-P, et al. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes. American Diabetes Association; 2009;58:1651–8.

16. Soedamah-Muthu SS, Fuller JH, Mulnier HE, Raleigh VS, Lawrenson RA, Colhoun HM.

High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. Diabetes Care. 2006;29:798–804.

17. Garofolo M, Gualdani E, Giannarelli R, Aragona M, Campi F, Lucchesi D, et al. Microvascular complications burden (nephropathy, retinopathy and peripheral polyneuropathy) affects risk of major vascular events and all-cause mortality in type 1 diabetes: A 10-year follow-up study. Cardiovasc Diabetol. 2019;18:159.

18. Bjerg L, Hulman A, Carstensen B, Charles M, Witte DR, Jørgensen ME. Effect of duration and burden of microvascular complications on mortality rate in type 1 diabetes: an observational clinical cohort study. Diabetologia. 2019;62:633–43.

19. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017;12:2032–45.

20. Fu H, Liu S, Bastacky SI, Wang X, Tian XJ, Zhou D. Diabetic kidney diseases revisited: A new perspective for a new era. Mol. Metab. 2019; 30:250–63.

21. Warren AM, Knudsen ST, Cooper ME. Diabetic nephropathy: an insight into molecular mechanisms and emerging therapies. Expert Opin Ther Targets. 2019;23:579–91.

22. Stephen R, Jolly SE, Nally J V., Navaneethan SD. Albuminuria: When urine predicts kidney and cardiovascular disease. Cleve. Clin. J. Med. 2014;81:41–50.

23. American Diabetes Association. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020; 43:S135–51.

24. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. N. Engl. J. Med. 2012;366:1227–39.

25. Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. Lancet. 2010; 376:124–36.

26. Rasmussen ML, Broe R, Frydkjaer-Olsen U, Olsen BS, Mortensen HB, Peto T, et al. Retinal vascular geometry and its association to microvascular complications in patients with type 1 diabetes: the Danish Cohort of Pediatric Diabetes 1987 (DCPD1987). Graefe's Arch Clin Exp Ophthalmol. 2017;255:293–9.

27. Selvarajah D, Kar D, Khunti K, Davies MJ, Scott AR, Walker J, et al. Diabetic peripheral neuropathy: advances in diagnosis and strategies for screening and early intervention. Lancet Diabetes Endocrinol. 2019;7:938–48.

28. Bönhof GJ, Herder C, Strom A, Papanas N, Roden M, Ziegler D. Emerging Biomarkers, Tools, and Treatments for Diabetic Polyneuropathy. Endocr Rev. 2019;40:153–92.

29. Pop-Busui R, Braffett BH, Zinman B, Martin C, White NH, Herman WH, et al. Cardiovascular Autonomic Neuropathy and Cardiovascular Outcomes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study. Diabetes Care. 2017;40:94–100.

30. Forbes JM, Cooper ME. Mechanisms of Diabetic Complications. Physiol Rev. 2013;93:137–88.

31. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54:1615–25.

32. Marcovecchio ML, Lucantoni M, Chiarelli F. Role of chronic and acute hyperglycemia in the development of diabetes complications. Diabetes Technol Ther. 2011;13:389–94.

33. Eid S, Sas KM, Abcouwer SF, Feldman EL, Gardner TW, Pennathur S, et al. New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. Diabetologia. 2019;62:1539–49.

34. Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. John Wiley & Sons, Ltd; 2001;69:89–95.

35. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5:463–6.

36. Radcliffe NJ, Seah J-M, Clarke M, MacIsaac RJ, Jerums G, Ekinci EI. Clinical predictive factors in diabetic kidney disease progression. J Diabetes Investig. 2017;8:6–18.

37. Colombo M, Valo E, McGurnaghan SJ, Sandholm N, Blackbourn LAK, Dalton RN, et al. Biomarker panels associated with progression of renal disease in type 1 diabetes. Diabetologia. 2019;62:1616–27.

38. Tofte N, Lindhardt M, Adamova K, Bakker SJL, Beige J, Beulens JWJ, et al. Early detection of diabetic kidney disease by urinary proteomics and subsequent intervention with spironolactone to delay progression (PRIORITY): a prospective observational study and embedded randomised placebo-controlled trial. Lancet Diabetes Endocrinol. 2020;8:301-12.

39. Frangogiannis NG. Biomarkers: Hopes and challenges in the path from discovery to clinical practice. Transl. Res. 2012;159:197–204.

40. Karahalil B. Overview of Systems Biology and Omics Technologies. Curr Med Chem. Curr Med Chem; 2016;23:4221–30.

41. Eddy S, Mariani LH, Kretzler M. Integrated multi-omics approaches to improve classification of chronic kidney disease. Nat Rev Nephrol. 2020; May 18. doi: 10.1038/s41581-020-0286-5.

42. Colhoun HM, Marcovecchio ML. Biomarkers of diabetic kidney disease. Diabetologia. 2018;61:996–1011.

43. Ting DSW, Tan K-A, Phua V, Tan GSW, Wong CW, Wong TY. Biomarkers of Diabetic Retinopathy. Curr Diab Rep. 2016;16:125.

44. Youngblood H, Robinson R, Sharma A, Sharma S. Proteomic biomarkers of retinal inflammation in diabetic retinopathy. Int. J. Mol. Sci. 2019;20:4755.

45. Shores DR, Everett AD. Children as Biomarker Orphans: Progress in the Field of Pediatric Biomarkers. J Pediatr. 2018;193:14-20.e31.

46. Lyons TJ, Basu A. Biomarkers in diabetes: Hemoglobin A1c, vascular and tissue markers. Transl. Res. 2012;159:303–12.

47. Kraus VB. Biomarkers as drug development tools: Discovery, validation, qualification and use. Nat. Rev. Rheumatol. 2018;14:354–62.

48. Pena MJ, Mischak H, Heerspink HJL. Proteomics for prediction of disease progression

and response to therapy in diabetic kidney disease. Diabetologia. 2016;59:1819–31.

49. Looker HC, Mauer M, Nelson RG. Role of Kidney Biopsies for Biomarker Discovery in Diabetic Kidney Disease. Adv. Chronic Kidney Dis. 2018;25:192–201.

50. Dubin RF, Rhee EP. Proteomics and Metabolomics in Kidney Disease, including Insights into Etiology, Treatment, and Prevention. Clin J Am Soc Nephrol. 2020;15:404-11.

51. Matoba K, Takeda Y, Nagai Y, Kawanami D, Utsunomiya K, Nishimura R. Unraveling the role of inflammation in the pathogenesis of diabetic kidney disease. Int J Mol Sci. 2019;20:3393.

52. Cheng D, Fei Y, Saulnier PJ, Wang N. Circulating TNF receptors and risk of renal disease progression, cardiovascular disease events and mortality in patients with diabetes: a systematic review and meta-analysis. Endocrine. 2019;68:32-43.

53. Niewczas MA, Pavkov ME, Skupien J, Smiles A, Md Dom ZI, Wilson JM, et al. A signature of circulating inflammatory proteins and development of end-stage renal disease in diabetes. Nat Med. 2019;25:805–13.

54. Abbasi F, Moosaie F, Khaloo P, Dehghani Firouzabadi F, Fatemi Abhari SM, Atainia B, et al. Neutrophil Gelatinase-Associated Lipocalin and Retinol-Binding Protein-4 as Biomarkers for Diabetic Kidney Disease. Kidney Blood Press Res. S. 2020;45:222–32.

55. Colombo M, Valo E, McGurnaghan SJ, Sandholm N, Blackbourn LAK, Dalton RN, et al. Biomarker panels associated with progression of renal disease in type 1 diabetes. Diabetologia. 2019;62:1616–27.

56. Jenks SJ, Conway BR, McLachlan S, Teoh WL, Williamson RM, Webb DJ, et al. Cardiovascular disease biomarkers are associated with declining renal function in type 2 diabetes. Diabetologia. 2017;60:1400-8.

57. Bidadkosh A, Lambooy SPH, Heerspink HJ, Pena MJ, Henning RH, Buikema H, et al. Predictive Properties of Biomarkers GDF-15, NTproBNP, and hs-TnT for Morbidity and Mortality in Patients With Type 2 Diabetes With Nephropathy. Diabetes Care. 2017;40:784–92.

58. Mischak H, Delles C, Vlahou A, Vanholder R. Proteomic biomarkers in kidney disease: issues in development and implementation. Nat Rev Nephrol. 2015;11:221–32.

59. Good DM, Zürbig P, Argilés À, Bauer HW, Behrens G, Coon JJ, et al. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol Cell Proteomics. 2010;9:2424–37.

60. Tofte N, Persson F, Rossing P. Omics research in diabetic kidney disease: new biomarker dimensions and new understandings? J. Nephrol. 2020; May 30. doi: 10.1007/s40620-020-00759-4.

61. Wu T, Qiao S, Shi C, Wang S, Ji G. Metabolomics window into diabetic complications. J. Diabetes Investig. 2018;9:244–55.

62. Kwan CC, Fawzi AA. Imaging and Biomarkers in Diabetic Macular Edema and Diabetic Retinopathy. Curr. Diab. Rep. 2019;19:95.

63. Mansour SE, Browning DJ, Wong K, Flynn HW, Bhavsar AR. The evolving treatment of diabetic retinopathy. Clin. Ophthalmol. 2020;14:653–78.

64. Liew G, Lei Z, Tan G, Joachim N, Ho I-V, Wong TY, et al. Metabolomics of Diabetic Retinopathy. Curr Diab Rep. 2017;17:102.

65. Jin HY, Park TS. Role of inflammatory biomarkers in diabetic peripheral neuropathy. J. Diabetes Investig. 2018;9:1016–8.

66. Herder C, Kannenberg JM, Huth C, Carstensen-Kirberg M, Rathmann W, Koenig W, et al. Proinflammatory cytokines predict the incidence and progression of distal sensorimotor polyneuropathy: KORA F4/FF4 study. Diabetes Care. 2017;40:569–76.

67. Kumar A, Mittal R. Nrf2: a potential therapeutic target for diabetic neuropathy. Inflammopharmacology. 2017; 25:393–402.

68. Tummanapalli SS, Willcox MDP, Issar T, Yan A, Pisarcikova J, Kwai N, et al. Tear film substance P: A potential biomarker for diabetic peripheral neuropathy. Ocul Surf. 2019;17:690–8.

Box 1. Characteristics of an ideal biomarker for microvascular complications:

1. Highly sensitive and specific
2. Correlated with the severity of microvascular damage
3. Non-invasive
4. Easy to measure and cost-effective
5. Applicable across different populations
6. Provide risk stratification and prognostic information
7. Identify possible pathogenetic mechanisms and targets for new interventions
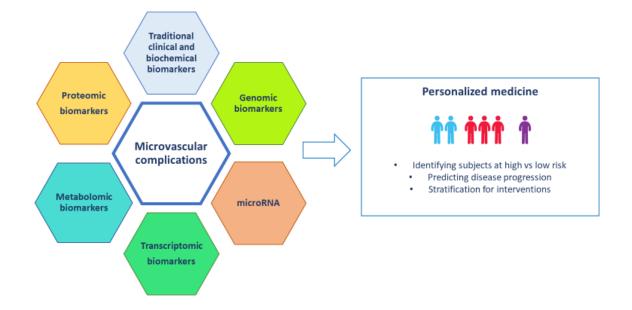8. Support stratification for interventions
9. monitor

Figure 1. Model of multi-biomarkers development for microvascular complications