Clinical Chemistry 63:1
186–195 (2017)

Reviews

Use of Biomarkers in Predicting the Onset, Monitoring the Progression, and Risk Stratification for Patients with Type 2 Diabetes Mellitus

Benjamin M. Scirica[1*]

BACKGROUND: As the worldwide prevalence of type 2 diabetes mellitus (T2DM) increases, it is even more important to develop cost-effective methods to predict and diagnose the onset of diabetes, monitor progression, and risk stratify patients in terms of subsequent cardiovascular and diabetes complications.

CONTENT: Nonlaboratory clinical risk scores based on risk factors and anthropomorphic data can help identify patients at greatest risk of developing diabetes, but glycemic indices (hemoglobin A_{1c}, fasting plasma glucose, and oral glucose tolerance tests) are the cornerstones for diagnosis, and the basis for monitoring therapy. Although family history is a strong predictor of T2DM, only small populations of patients carry clearly identifiable genetic mutations. Better modalities for detection of insulin resistance would improve earlier identification of dysglycemia and guide effective therapy based on therapeutic mechanisms of action, but improved standardization of insulin assays will be required. Although clinical risk models can stratify patients for subsequent cardiovascular risk, the addition of cardiac biomarkers, in particular, high-sensitivity troponin and natriuretic peptide provide, significantly improves model performance and risk stratification.

CONCLUSIONS: Much more research, prospectively planned and with clear treatment implications, is needed to define novel biomarkers that better identify the underlying pathogenic etiologies of dysglycemia. When compared with traditional risk features, biomarkers provide greater discrimination of future risk, and the integration of cardiac biomarkers should be considered part of standard risk stratification in patients with T2DM.

In North America alone, type 2 diabetes mellitus (T2DM)[2] is currently estimated to affect more than 40 million patients. The global projections suggest that more than 600 million patients will have T2DM by 2040 (http://www.diabetesatlas.org). Moreover, almost twice as many patients are felt to have prediabetes or be at risk for developing diabetes *(1)*. As the obesity epidemic continues in the developed world and spreads with greater intensity in developing countries, the medical, economic, and social burden of diabetes will over the coming decades challenge health systems and societies. Patients with diabetes are at increased risk of micro- and macrovascular complications. Patients with T2DM are most likely to die from cardiovascular complications. The 2 most common initial cardiovascular diagnoses in a patient with diabetes are heart failure and peripheral arterial disease *(2)*.

T2DM is a chronic, progressive disease that traditionally is characterized by insulin resistance in the skeletal muscles, increased liver gluconeogenesis, and insufficient pancreatic β-cell function followed by eventual failure. The underlying pathologic processes that result in manifest diabetes begin years and even decades before the actual diagnosis. After the initial diagnosis, diabetes progresses as those same pathologic processes evolve and in most cases, worsen. Although all patients with T2DM and even those patients with "prediabetes" are at increased risk of both micro- and macrovascular complications, there are certain subgroups that are at particularly high risk and therefore should be identified, ideally as early as possible.

[1] TIMI Study Group, Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.
* Address correspondence to the author at: Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115. Fax 617-7347329; e-mail bscirica@partners.org.
Received July 21, 2016; accepted November 1, 2016.
Previously published online at DOI: 10.1373/clinchem.2016.255539

[2] Nonstandard abbreviations: T2DM, type 2 diabetes mellitus; A_{1c}, hemoglobin A_{1c}; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; BMI, body mass index; OGTT, oral glucose tolerance test; NGSP, National Glycohemoglobin Standardization Program; POC, point-of-care; ADA, American Diabetes Association; T1DM, type 1 diabetes mellitus; HOMA, homeostasis model assessment; tPA, tissue plasminogen activator; CRP, C-reactive protein; IL2-RA, interleukin-2 receptor A; CVD, cardiovascular disease; MODY, maturity-onset-diabetes of the young; NT-proBNP, amino-terminal pro-B-type natriuretic peptide; CKD, chronic kidney disease; UACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; hsTnT, high-sensitivity troponin T; hsTn, high-sensitivity cardiac troponin; LoB, limit of blank; SAVOR-TIMI, Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus–Thrombolysis in Myocardial Infarction.

Biomarkers in T2DM

The diagnosis of T2DM is based on the biochemical parameters of plasma glucose or hemoglobin A_{1c} (A_{1c}). Once the diagnosis is made, treatment goals focus on achieving optimal, patient-centered A_{1c} targets. Monitoring disease progression and evidence of complications, such as renal nephropathy, are similarly anchored by laboratory testing. Thus biomarkers have always played a central role in each stage of T2DM management. The relevant question is if novel biomarkers can provide incremental improvement in the diagnosis, progression monitoring, and risk stratification of patients with T2DM.

The most clinically useful biomarker, in addition to having adequate analytic performance and high-throughput platforms, improves the diagnosis of a disease, assesses progression, appropriately risk stratifies patients beyond standard risk metrics, and carries therapeutic implications, such that the biomarker results will directly lead to a change in treatment strategy. Ideally, a biomarker will provide insight into the underlying physiology, although that is not an absolute requirement to become a clinically useful test. To demonstrate that a biomarker provides an incremental improvement in risk stratification, it must be better than the standard metric in terms of discrimination (*c*-statistics, absolute and relative IDI (index of discrimination improvement), calibration, and NRI (net reclassification index) *(3, 4)*.

Predicting Onset

Because the conditions that eventually result in T2DM are present years before an actual diagnosis, it is reasonable to believe that identifying patients at the earliest stage of the dysglycemic continuum would be useful to begin interventions to delay or prevent progression. A biomarker may simply predict risk, in other words "discriminate future disease," or it can "reveal pathophysiology" *(5)*. Different biomarkers may or may not fulfill both criteria.

DIABETES PREDICTION RISK MODELS AND SCORES

Historically, the risk of diabetes was assessed via fasting plasma glucose concentrations or glucose tolerance tests. Impaired fasting glucose (IFG) is defined as 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) and impaired glucose tolerance (IGT) as a 2-h plasma glucose between 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) 2 h after a 75-g glucose load. Patients with either IGT or IFG are at increased risk of developing T2DM with the highest risk in those patients who meet both criteria *(6)* (see Table 1 in the Data Supplement that accompanies the online version of this review at http://www.clinchem.org/content/vol63/issue1).

Oral glucose tolerance tests (OGTTs) are difficult to arrange in clinical practice and infrequently performed in general practice. Even fasting glucose concentrations can be impractical as a population screening technique. Thus the strategy of identifying a nonlaboratory clinical risk score that could identify those patients at highest risk for subsequent focused testing or direct intervention is appealing.

Dozens of studies have evaluated a variety of clinical characteristics that are associated with developing T2DM *(7)*. Most include at least several of the following clinical features: age, body mass index (BMI), sex, ethnicity, parental history of diabetes, waist circumferences, smoking, systolic blood pressure or history of hypertension, and physical activity. Some models further incorporate laboratory testing of IFG, IGT, or lipoprotein concentrations, which may improve their performance but limit generalizability *(8)*. Not surprisingly, clinical components of the metabolic syndrome, regardless of the strict definition, will also identify patients at higher risk of developing diabetes.

Overall, these are "simple" risk scores that include on the order of 5–10 variables and provide very good discrimination of risk. Few have been validated in external data sets and therefore risk score performance is dependent on the populations studied and potentially in danger of "overfitting." Moreover, ascertainment of diabetes at baseline and definitions of incident diabetes have varied substantially based on available data. Based on the design of these analyses, none can demonstrate causal relationships between the risk factors and risk of diabetes *(9)*. One widely used score is the FINnish Diabetes RIsk SCore (www.diabetes.fi/english).

A much more exhaustive and nuanced model, named Archimedes, incorporates more than 50 continuously interacting and updated biological variables, including glycemic indices, other laboratory tests, treatments, and outcomes *(10)*. This model accurately predicted rates of matched incident T2DM when compared to actual data in a clinical trial *(11)*. This model is particularly useful for modeling different screening strategies based on the underlying risk of different populations. In 1 simulation applying this model in a representative US population, screening became cost-effective in patients aged 30–45 years, with screening repeated every 3–5 years *(12)*. Unfortunately, large, well-powered prospective studies assessing the clinical benefit of any screening strategy and their impact on clinical outcomes are still lacking.

HEMOGLOBIN A_{1c} AS RISK MARKER FOR T2DM

The widespread availability of standardized A_{1c} assays has changed the diagnostic and thereby screening strategy for T2DM. The A_{1c} assays should be certified by the National Glycohemoglobin Standardization Program

Table 1. 2016 American Diabetes Association criteria for the diagnosis of diabetes.

Fasting plasma glucose ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.[a]
OR
Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, by use of a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.[a]
OR
A_{1c} ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the Diabetes Control and Complications Trial assay.[a]
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose >200 mg/dL (11.1 mmol/L).

[a] In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

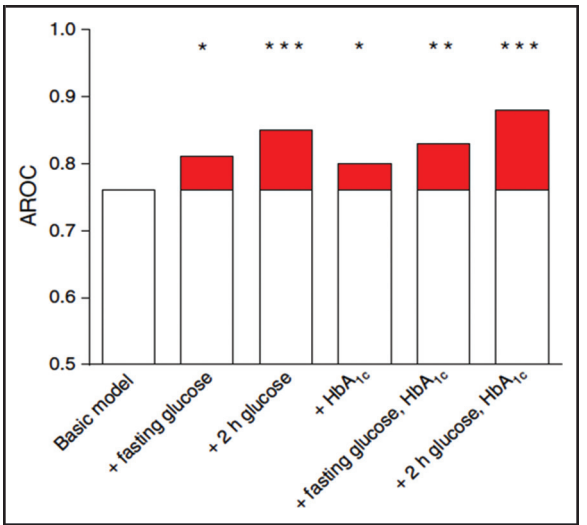

Fig. 1. Improvement in *c*-statistic by adding glycemic measures to basic clinical models.

Data from KORA S4/F4 Study [Rathmann et al. *(16)*]. Clinical model included age, sex, BMI, hypertension, parental diabetes, and former or present smoking. Diabetes was ascertained by validated self-report or OGTT. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs the basic model. AROC, area under the ROC curve; Hb A_{1c}, hemoglobin A_{1c}.

Image from Herder et al. *(67)*. Reproduced with permission.

(NGSP, www.ngsp.org), which directly links to the care goals and outcomes established in the Diabetes Control and Complication Trial (DCCT) or United Kingdom Prospective Diabetes Study (UKPDS) reference assays. The NGSP is traceable to the IFCC higher order reference method and ties the 2 standardization programs together. The IFCC reports values in mmol/mol while others such as the NGSP reports percentages (%). Point-of-care (POC) A_{1c} devices can be used for diagnosis of T2DM and for disease monitoring in patients with known T2DM. Because the diagnosis of T2DM is deemed a moderately complex indication, a POC device cannot be CLIA waived for this indication and requires proficiency testing. These POC devices are thus valid for diagnostic purposes, whereas any POC device can be used for the lower complexity indication of monitoring A_{1c} in patients with known T2DM. Compared with fasting plasma glucose and OGTT, A_{1c} has better preanalytic stability and is not affected by other physiologic processes such as infection, but it does tend to be less sensitive than OGTT and fasting plasma glucose, and does not correlate with average glucose in some cases.

The American Diabetes Association (ADA) criteria for the diagnosis of T2DM (Table 1) are based on plasma glucose concentrations (fasting, post-OGTT, or random with symptoms) and A_{1c} *(6)*. Why some patients will exhibit IGT vs IFG vs an increased A_{1c} pattern of dysglycemia is not well understood, but the different cutpoints will identify different patients. Moreover, there are racial differences in A_{1c} concentrations, which tend to be higher in blacks compared to whites, and differential correlations between A_{1c} and glucose concentrations among races. Supporting the notion for race-specific cutpoints for the diagnosis of T2DM is the observation that retinopathy is more prevalent at lower concentrations of A_{1c} in blacks compared to whites *(13)*.

A_{1c} reflects long-term glycemic exposure and is not affected by meals and is therefore easy to obtain as part of regular clinical practice. Current ADA guidelines suggest T2DM screening focus on obese and overweight patients with additional risk factors. A "negative" test should be repeated every 3 years. Patients are deemed to have "prediabetes" when the A_{1c} is between 5.7–6.4% (39–46 mmol/mol) *(6)*. The US Preventative Services Task Force recommends screening for abnormal blood glucose as part of cardiovascular risk assessment in adults aged 40–70 years who are overweight or obese, and providing patients with abnormal blood glucose intensive behavioral counseling interventions to promote a healthful diet and physical activity *(14)*.

Adding A_{1c} or glycemic measures to simple risk models will improve metrics of discrimination *(15, 16)* (Fig. 1). Thus 1 potential screening strategy could be to identify patients at higher risk based on noninvasive risk models in whom more frequent glycemic monitoring would be warranted and cost-effective.

NONGLYCEMIC BIOMARKERS FOR PREDICTING T2DM

Nonglycemic biomarkers that predict the risk of diabetes in general attempt to define different underlying pathophysiologic perturbations of glucose metabolism. Initial research has focused on the traditional metabolic axis of β-cells, skeletal muscles, and the liver while more recent studies attempt to discern novel pathogenic pathways that lead to dysglycemia.

Classic physiologic axis: insulin resistance, β-cell function, and liver injury. In contrast to type 1 diabetes mellitus (T1DM), insulin concentrations in T2DM vary significantly between patients depending on their degree of insulin resistance and β-cell health. Early in the dysglycemic continuum, insulin concentrations may be quite high to combat greater peripheral insulin residence whereas they eventually fall with time. Therefore, insulin concentrations in isolation typically are not clinically useful in predicting diabetes. In addition, insulin resistance, while well recognized as central component of T2DM is similarly difficult to quantify. For research purposes in small cohorts, the euglycemic insulin clamp technique with either intravenous glucose or insulin tolerance testing can be used to define insulin resistance.

A more broadly applicable method, the homeostasis model assessment (HOMA) attempts to estimate insulin resistance (HOMA-IR) and β-cell function (HOMA-β). The original HOMA model uses fasting glucose and insulin concentrations, and has been extensively validated. The updated model (HOMA2) has been updated to provide nonlinear solutions to better reflect the variations in peripheral and liver resistance and insulin secretion. Moreover, it can use different insulin assays and c-peptide *(17)*. Even with this increased complexity, many limitations persist, most significantly the lack of standardized insulin and c-peptide assays, change in β-cell function over time, and cross-cultural/ethnic differences *(18)*. The eventual harmonization of these assays could offer a significant advance into potential screening strategies to detect insulin resistance before overt hyperglycemia *(19, 20)*.

The liver plays a central role in T2DM as the source of gluconeogenesis, and is also the target of end organ damage from hyperinsulinemia. Thus many biomarkers of liver injury [ALT (alanine aminotransferase), GTT (γ-glutamyl transferase), ferritin, PAI 1 (plasminogen activator inhibitor 1), tissue plasminogen activator (tPA) antigen, C-reactive proteins (CRPs), and triglycerides] are increased in patients with dysglycemia before the diagnosis of T2DM is met *(21)*. Liver injury through increased hepatic fat deposition can lead to nonalcoholic fatty liver disease, a common complication in T2DM and in those patients without T2DM who have a high clinical diabetes risk phenotype (i.e., obesity, central adiposity, IFG, and hypertension).

Novel biomarkers. Over the past decade, it has become apparent that the complexity of glucose dysregulation includes many organs and the hormonal axis extends beyond β-cell failure and insulin resistance. As DeFronzo has highlighted, the accelerated lipolysis of fat cells, deficiency in incretin axis, hyperglucagonemia from α-cell hyperactivity, and increased glucose reabsorption in the kidney are all key components in the pathogenesis and progression of T2DM *(22)*. Add markers of inflammation to these different metabolic axes and there are literally dozens of potential markers for the development of T2DM.

Advances in large scale "-omic" studies have expedited greater evaluation of potential biomarkers. Unfortunately, many studies test different sets of biomarkers and therefore lack external validation. One nested case control study of over 6000 patients tested 64 candidate proteins in over 6000 people. Only 6 biomarkers met rigorous inclusion criteria to remain in the ultimate model for predicting T2DM by 5 years—adiponectin, CRP, ferritin heavy chain 1, glucose, interleukin-2 receptor A (IL-2RA), and insulin *(23)*. Combining these markers into a risk score resulted in a *c*-statistic of 0.78, which was better than single glycemic measurements or HOMA-IR, but was not better than either an OGTT or 2-h insulin concentrations.

In another study examining 92 proteins associated with cardiovascular disease (CVD) or inflammation, only 6 were associated with insulin resistance (leptin, renin, IL-1RA, hepatocyte growth factor, fatty acid–binding protein 4, and tPA) and only 2 biomarkers (IL-1RA and t-PA) with incident T2DM, though this association was lost after adjusting for fasting glucose *(24)*. A systematic review performed in 2013 identified 24 studies and found that novel biomarkers failed to provide substantial improvement beyond clinical, behavioral, anthropometric, and in some cases, glycemic variables *(25)*.

Several studies have found certain microRNA "signatures" or "profiles" are associated with insulin resistance, incident T2D, or β-cell function *(26)*. A broader evaluation of exosomes and microvesicles (of which microRNA is 1 component) has also been highlighted as a potential avenue to identify and provide even more detailed evaluation of the different pathologic processes in the development and progression of T2D and other metabolic diseases *(27)*.

GENETICS

Despite obvious predisposition for T2DM among certain ethnic groups, within specific families, and between twins, genetic markers of incident T2D have failed to improve prediction above standard clinical risk parameters. In 1 case-cohort study, the inclusion of 20 diabetogenic SNPs did not improve discrimination beyond lifestyle risk factors *(15)*. A systematic review of several

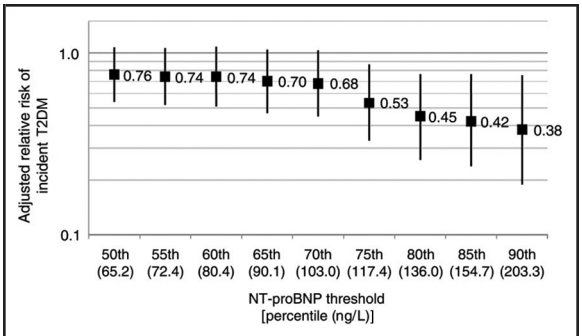

Fig. 2. Adjusted relative risk of incident T2DM according to NT-proBNP.

Image from Everett et al. *(32)*. Reproduced with permission.

studies that evaluated genetic variations found similar results *(25)*. In the Framingham Study, 18 loci associated with diabetes failed to improve a model for predicting incident diabetes that was adjusted for age, sex, family history, BMI, fasting glucose concentration, systolic blood pressure, HDL cholesterol concentration, and triglyceride concentration *(28)*. A European study examined 11 genes associated with the risk of T2D and found a similar lack in improvement beyond the clinical model *(29)*. A more recent study on 65 genetic variants did find that a weighted genetic risk score modestly improved discrimination, though validation is required and the generalizability of such strategy for a population remains questionable *(30)*.

The family of conditions termed maturity-onset-diabetes of the young (MODY) represents a rare situation where monogenetic mutations do lead to early onset of T2DM (typically before age 25) through early β-cell dysfunction. MODY subtypes are classified according to the underlying gene affected, such as hepatocyte nuclear factors, glucokinase, and insulin promoter factor 1, to name a few. MODY is autosomal dominant with variable penetration, but accounts for <5% of T2DM cases *(6)*.

An interesting series of observations suggest an inverse (protective) association between natriuretic peptides and the risk of T2DM with higher concentrations of amino-terminal pro–B-type natriuretic peptide (NT-proBNP) correlating with a lower incidence of T2DM *(31, 32)* (Fig. 2). A Mendelian randomization analyses further supported a causal relationship between natriuretic peptides and a lower rate of incident diabetes *(31)*. While an exact mechanism has yet to be defined, transgenic mice that overexpress BNP were resistant to the metabolic effects of a high-fat diet, possibly via increased mitochondrial biogenesis *(33)*. Intriguingly, there are also natriuretic peptide receptors on pancreatic β-cells *(34)*.

CLINICAL IMPLICATIONS

The potential for including novel biomarkers into T2DM screening strategies is thus limited given the strong association between readily obtainable clinical and anthropometric variables, and the ease of obtaining standardization glucose and A_{1c} measurements. Moreover, the primary interventions recommended in those patients at risk for T2DM are diet and lifestyle modifications, which are low-risk interventions with few to no side effects. There is thus little downside of a population screening strategy that counsels patients to try to live a healthier lifestyle. The benefit–risk balance would shift when considering more intensive therapies such as pharmacotherapy or bariatric surgery. In these cases, the risk of T2DM would likely require the presence of other risk factors for micro- and macrovascular disease to justify more intense intervention. The 1 area where novel biomarkers could play a role would be to identify those patients considered low risk based on noninvasive screening techniques who would otherwise be missed.

Monitoring Progression

Once the diagnosis T2DM is made, the principal tenets of T2DM care are glucose control, detection of microvascular disease complications, and control of cardiovascular risk factors. Currently, there is little emphasis on further assessment on the actual progression of diabetes beyond glucose control. All the pathophysiologic processes discussed above regarding the etiology T2DM remain in effect with diabetes progression and therefore could be used for monitoring if there were some clinical implications.

GLYCEMIC CONTROL

The natural progression of T2DM is that glucose will continue to rise over time *(35)*. Although the worsening dysglycemia is due in part to the underlying pathologic process that led to T2DM in the first place, much of the deterioration is felt to be due to the loss of β-cell function *(36)*. The ADA recommends measuring A_{1c} at a minimum of twice yearly for patients meeting treatment goals and at least quarterly in patients not meeting goals or with changes in therapy. Mean glucose concentrations, calculated from specific A_{1c}, are often reported to aid in the clinical interpretation of A_{1c} testing. A1c concentrations are affected by hemoglobinopathies, transfusions, and abnormal blood cell destruction or production *(6)*.

Estimating the degree of insulin resistance or the status of β-cell function for example, is not recommended in current practice recommendations, in part because of the lack of harmonization between assays *(6)*. Fasting glucose and A_{1c} are thus the integrators of diabetes progression. If glycemic indices are not at goal, then the glucose-lowering regimen is increased. Unfortu-

nately, the decision regarding the next therapeutic agent is rarely made on physiologic grounds, but rather based on algorithms that are based on historical practice patterns, cost, and side effects. In fact, some agents such as sulfonylureas may provide short-term glucose control but at the cost of more rapid β-cell destruction and subsequent hyperglycemia. A more physiologic approach would be to target the particular pathologic processes that are most affected *(22)*. For example, early in the course of T2DM, using insulin-sensitizing agents such as biguanides (metformin) or thiazolidinediones may help delay further insulin resistance. Patients with obesity may benefit from agents such as GLP-1 (glucagon-like peptide-1) receptor agonists or SGLT2 (sodium-glucose cotransporter 2) inhibitors that lower weight. Conversely, insulin should be started earlier in patients with premature β-cell destruction and low insulin concentrations.

CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a common complication of T2DM, affecting 30%–40% of such patients *(37)* To detect CKD, the ADA recommends screening at least once a year by measuring urinary albumin, e.g., spot urinary albumin-to-creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR) in patients with T1DM with a duration of 5 years, in all patients with T2DM, and in all patients with comorbid hypertension. eGFR is categorized into 6 stages with an eGFR <60 mL/min/1.73 m^2 *(2)* marking the threshold for the presence of mild to moderate kidney impairment *(38, 39)*.

Increased concentrations of urinary albumin indicate damage to the glomerular basement membrane and capillary endothelium, and when present, indicate the presence of CKD, even within different eGFR categories *(38, 40)*. In people without CKD, UACR is typically <10 mg/g. History, UACR concentrations ≥ 30 mg/g were deemed "microalbuminuria." Current nomenclature now labels this as mogderately increased concentrations of albuminuria [Kidney Disease: Improving Global Outcomes (KDIGO) category A2] and ≥ 300 mg/g as severe albuminuria *(38–40)* However, even smaller increases in urinary albumin between 10 mg/g and 30 mg/g have been associated with progression of renal disease and increased mortality *(41, 42)*.

One study has reported that increased concentrations of natriuretic peptides and high-sensitivity troponin T (hsTnT) were associated with increased risk of microvascular complications, in particular, nephropathy *(43)*. It is unknown whether there is any causal relationship between these markers and nephropathy. More likely, they identify patients at higher risk due to other comorbidities.

Cardiovascular Risk Stratification

Biomarkers provide insight into different pathophysiologic processes and improve risk stratification in many domains of CVD. T2DM promotes atherogenesis and worsens myocardial function such that T2DM is a well-recognized risk factor across the cardiovascular continuum *(44–47)*. Assessing cardiovascular risk in patients with T2DM is challenging because of the heterogeneity of T2DM patients. Moreover, the overall higher risk of patients with T2DM makes further risk discrimination challenging. This is particularly relevant when assessing risk in T2DM patients without manifest CVD, in whom the risk of ischemic complications varies widely based on age, duration of diabetes, and comorbidities.

GLYCEMIC METRICS

Many studies have demonstrated that the risk of cardiovascular events increases in patients with an increased A_{1c} *(48–51)*, though the relationship may be linear. Interesting, recent studies have not found a similar relationship between A_{1c} and heart failure *(51)*.

RENAL BIOMARKERS

The relationship between eGFR and cardiovascular death and overall mortality is "U-shaped" with the lowest risk in those patients with eGFR approximately 90–100 mL/min/m^2. The risk increases substantially with a lower eGFR, but also increases with eGFR >105 mL/min/m^2, likely representing the hyperfiltration seen with early diabetic nephropathy. In terms of urinary albumin, there is a linear relationship between UACR and outcomes. The increased risk in mortality and cardiovascular death is present even in those patients with concentrations between 10 mg/g and 30 mg/g who are typically not labeled as having CKD (Fig. 3).

CARDIAC BIOMARKERS

Natriuretic peptides. Similar to almost every other population studied, increased concentrations of natriuretic peptides in patients with T2DM are associated with increased cardiovascular risk, in both acute and chronic scenarios, and in particular for cardiovascular death and hospitalization for heart failure *(52–57)* (Fig. 4).

While natriuretic peptides are excellent discriminators of risk, they have not been shown to definitively change treatment decisions in cardiovascular care in patients with diabetes *(58)* The proof of concept NT-proBNP Selected Prevention of Cardiac Events in a Population of Diabetic Patients without a History of Cardiac Disease (PONTIAC) study, identified patients with T2DM at high risk based on increased concentrations of natriuretic peptides and randomized them to a strategy of aggressive risk management vs standard of care. Though

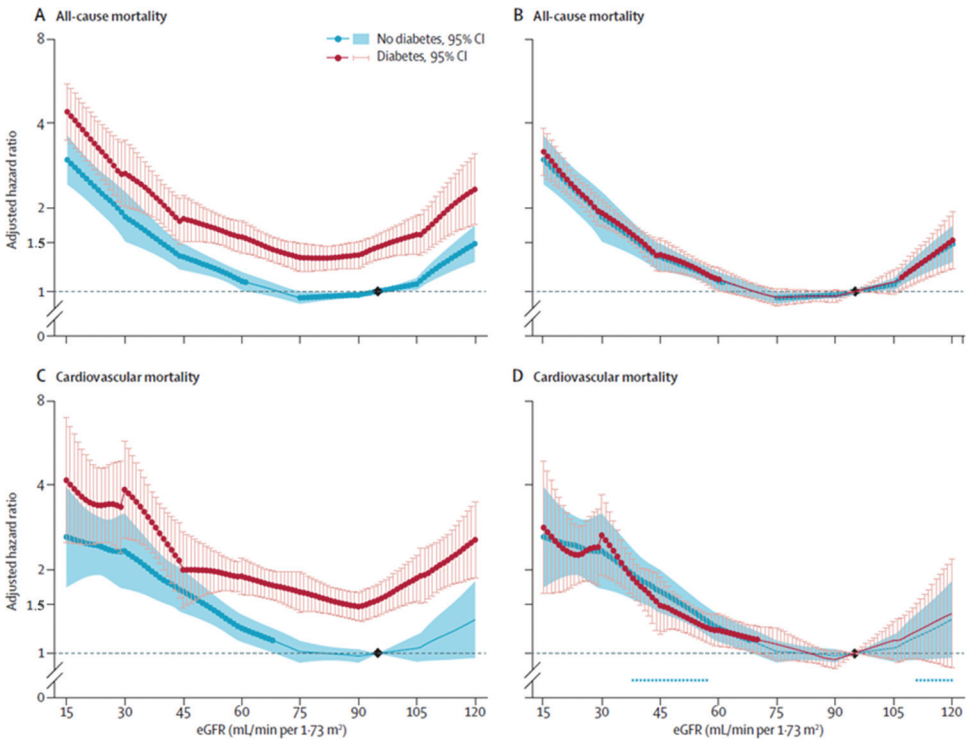

Fig. 3. Hazard ratios for all-cause and cardiovascular mortality in the combined general and high-risk populations according to eGFR in individuals with and without diabetes.
Image from Fox et al. *(41)*. Reproduced with permission.

underpowered, they found a significant reduction in the rates of cardiovascular death and hospitalization in patients randomized to more aggressive uptitration of inhibitors of the renin–angiotensin–aldosterone system and β-blockers *(59)*. Additional studies on larger populations with more extended observation will be required to validate these findings.

High-sensitivity cardiac troponin. The advent of high-sensitivity cardiac troponin (hsTn) assays has permitted the identification of low concentrations of circulating troponin in a large proportion of stable patients with diabetes *(56, 60–62)*. For example, among 512 women with T2DM but without any established cardiovascular disease, Everett et al. found hsTnT concentrations above the limit of blank (LoB, 3 ng/L) in 45.5% of their population and above the 99th percentile (14 ng/L) in 4%, compared to 30.3% and 2% in those without diabetes *(60)* (see Fig. 1 in the online Data Supplement). In T2DM patients with more advanced CVD, almost all had concentrations of hsTnT greater than the LoB (3 ng/L) and a large number of patients actually had hsTnT concentrations above the 99th percentile. In 2285 patients with stable ischemic heart disease in the Bypass Angioplasty Revascularization Investigation in Type 2 Diabetes (BARI 2D), 39.3% had concentrations above the 99th percentile (14 ng/L). In the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR-TIMI 53), almost all 12 310 patients with diabetes had a baseline concentration of hsTnT ≥3 ng/L (LoB). Overall 43.7% had a level above the 99th percentile (>15 ng/L for men and >10 ng/L for women). The proportion was higher in patients with established CVD (46.2%) compared to patients with cardiovascular risk factors only (31.4%) ($P < 0.001$) *(57)*. Increased concentrations of cTnT, even below the 99th percentile, were strongly and independently associated with increased cardiovascular risk, including cardiovascular death, myocardial infarction, and hospitalization for heart failure *(57, 60–63)*. In the SAVOR-TIMI 53 population, patients without manifest CVD but an increased hsTnT were at similar or higher risk of subsequent cardiovascular events than patients with established CVD but low hsTnT concentrations *(57)* (see Fig. 2 in the online Data Supplement).

The detection of concentrations of hsTn above the LoB, and in particular above the 99th percentile, has

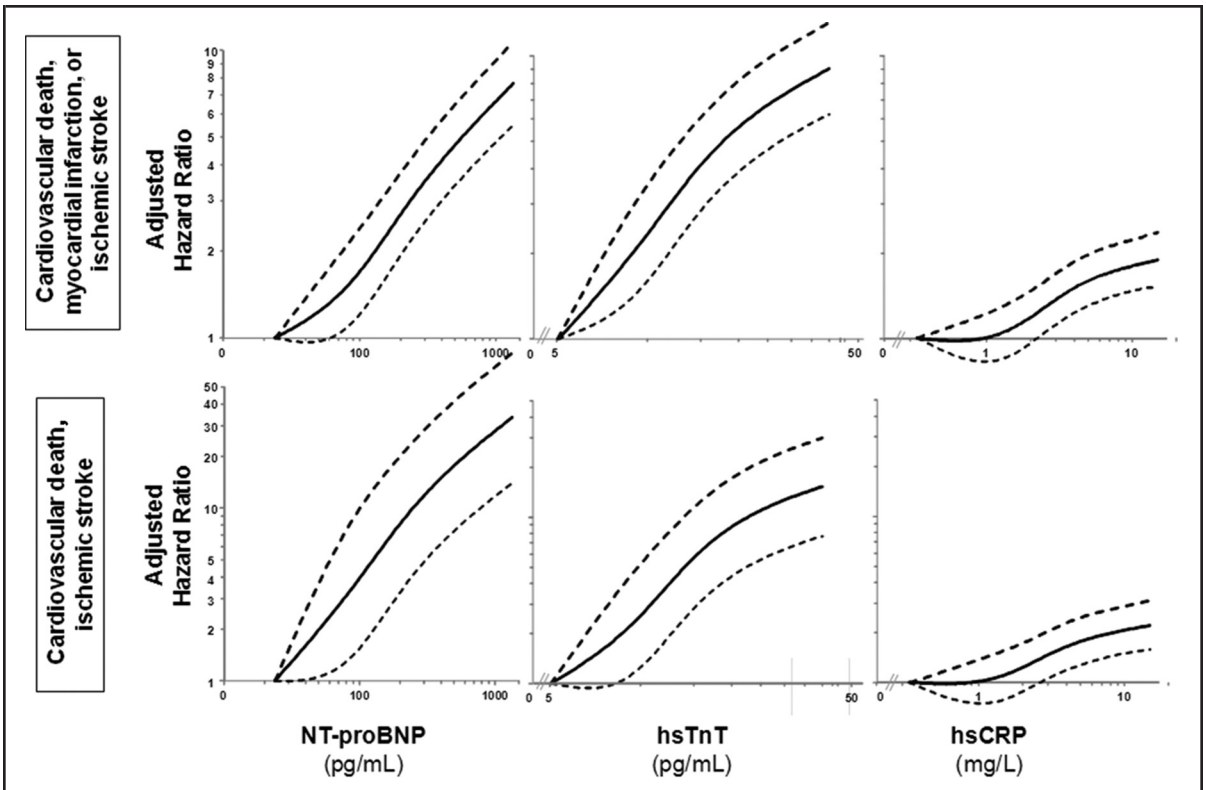

Fig. 4. Risk of cardiovascular death, myocardial infarction, or stroke (top) and cardiovascular death (bottom) by NT-proBNP, hsTnT, and hsCRP.

Hazard ratios were adjusted for treatment arms, age (continuous), systolic blood pressure (continuous), sex, history of heart failure, duration of diabetes, prior myocardial infarction, history of hypertension, history of hyperlipidemia, smoking, eGFR (continuous) and stratified by established CVD vs multiple risk factors.

Image from Scirica et al. *(57)*. Reproduced with permission.

several clinical implications. The evidence of ongoing myocardial injury in asymptomatic T2DM patients is likely due to persistent myocardial injury from glycemic dysregulation, endothelial and microvascular dysfunction, myocardial cell death, and subsequent fibrosis *(64)*. The fact that patients without established CVD, but with increased concentrations of hsTnT, are at equal or higher subsequent risk than patients with manifest CVD and lower concentrations of hsTnT thus challenges the traditional risk stratification of patients based on clinical diagnosis alone. Biomarkers may provide a better screening tool than clinical history. This may be particularly helpful for predicting the risk of heart failure. Current treatment recommendations do not incorporate these biomarkers *(65)*. Because a large proportion of patients with T2DM have increased circulating concentrations of hsTnT above the MI threshold of the 99th percentile, the triage of patients with T2DM presenting with an acute chest pain must integrate the rise and fall of hsTn rather than the absolute concentration into diagnostic algorithms *(66)*. The controversy surrounding sex-specific cutpoints is also relevant when considering population screening as lower concentrations of hsTn may still indicate higher risk in women compared to men. This is particularly important in T1DM, where a higher proportion of patients are female.

CRP. CRP as measured with a high sensitivity assay (hsCRP) is a well-established nonspecific marker of inflammation. Increased concentrations of hsCRP have been associated with increased cardiovascular risk in multiple patient populations. In T2DM patients, the association between increased concentrations of hsCRP and risk is present, but not as strongly associated as for other markers such as hsTn and natriuretic peptides. For example, in the SAVOR-TIMI 53 cohort, the risk of cardiovascular death increased by just 9% for each standard deviation higher change in concentration compared to 41% for hsTnT and more than 2-fold higher for NT-proBNP *(57)* (Fig. 4). The poor performance of hsCRP

in T2DM is likely due to the generalized higher level of chronic inflammation in this population that precludes better discrimination of risk.

Other biomarkers. May other biomarkers have been evaluated in T2DM. A broad evaluation of established and novel biomarkers in the ORIGIN (Outcome Reduction with an Initial Glargine Intervention) Trial found that after adjustment for confounders, NT-proBNP, growth differentiation factor 15, trefoil factor 3, α_2-macroglobulin, glutathione *S*-transferase α, peroxiredoxin-4, angiopoietin-2, macrophage derived chemokine, YKL-40, and insulin-like growth factor–binding protein 2 were all independently associated with mortality *(55)*. htTn was not measured in this study.

Future Directions

T2DM is a heterogeneous disease that manifests with increased glucose concentrations and increased risk of micro- and macrovascular complications. Currently, the diagnosis and progression of diabetes rely predominately on glycemic indices. Novel biomarkers that define better the underlying pathogenic etiologies of dysglycemia should offer the possibility to target those specific pathways that would benefit most from directed therapy. However, much research, prospectively planned and with clear treatment implications, is needed before we arrive at truly "personalized" diabetes care. Similarly, cardiac biomarkers provide greater discrimination of future risk when compared with traditional risk features, and thus integration of biomarkers such as natriuretic peptides and hsTn (where available) should be considered part of standard risk stratification in patients with T2DM.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: B.M. Scirica, AstraZeneca, Biogen Idec, Covance, Boehringer Ingelheim, Elsevier Practice Update Cardiology, Lexicon, Sanofi, Merck, GlaxoSmithKline, Dr. Reddy's Laboratory, NovoNordisk, and St. Jude's Medical.
Stock Ownership: B.M. Scirica, Health[at]Scale.
Honoraria: None declared.
Research Funding: B.M. Scirica, Research grants via the TIMI Study Group and Brigham and Women's Hospital from AstraZeneca, Eisai, and Poxel.
Expert Testimony: None declared.
Patents: None declared.

References

1. Menke A, Casagrande S, Geiss L, Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988–2012. JAMA 2015;314:1021–9.
2. Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1.9 million people. Lancet Diabetes Endocrinol 2015;3:105–13.
3. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation 2009;119:2408–16.
4. Pencina MJ, D'Agostino RB, Pencina KM, Janssens AC, Greenland P. Interpreting incremental value of markers added to risk prediction models. Am J Epidemiol 2012; 176:473–81.
5. Meigs JB. Multiple biomarker prediction of type 2 diabetes. Diabetes Care 2009;32:1346–8.
6. Professional Practice Committee for the Standards of Medical Care in Diabetes–2016. Diabetes Care 2016; 39(Suppl 1):S107–8.
7. Kengne AP, Beulens JW, Peelen LM, Moons KG, van der Schouw YT, Schulze MB, et al. Non-invasive risk scores for prediction of type 2 diabetes (EPIC-InterAct): a validation of existing models. Lancet Diabetes Endocrinol 2014;2:19–29.
8. Kahn HS, Cheng YJ, Thompson TJ, Imperatore G, Gregg EW. Two risk-scoring systems for predicting incident diabetes mellitus in U.S. adults age 45 to 64 years. Ann Intern Med 2009;150:741–51.
9. Noble D, Mathur R, Dent T, Meads C, Greenhalgh T. Risk models and scores for type 2 diabetes: systematic review. BMJ 2011;343:d7163.
10. Eddy DM, Schlessinger L. Archimedes: a trial-validated model of diabetes. Diabetes Care 2003;26:3093–101.
11. Eddy DM, Schlessinger L. Validation of the Archimedes diabetes model. Diabetes Care 2003;26:3102–10.
12. Kahn R, Alperin P, Eddy D, Borch-Johnsen K, Buse J, Feigelman J, et al. Age at initiation and frequency of screening to detect type 2 diabetes: a cost-effectiveness analysis. Lancet 2010;375:1365–74.
13. Tsugawa Y, Mukamal KJ, Davis RB, Taylor WC, Wee CC. Should the hemoglobin A_{1c} diagnostic cutoff differ between blacks and whites? A cross-sectional study. Ann Intern Med 2012;157:153–9.
14. Siu AL, US Preventive Services Task Force. Screening for abnormal blood glucose and type 2 diabetes mellitus: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med 2015;163:861–8.
15. Schulze MB, Weikert C, Pischon T, Bergmann MM, Al-Hasani H, Schleicher E, et al. Use of multiple metabolic and genetic markers to improve the prediction of type 2 diabetes: the EPIC-Potsdam Study. Diabetes Care 2009;32:2116–9.
16. Rathmann W, Kowall B, Heier M, Herder C, Holle R, Thorand B, et al. Prediction models for incident type 2 diabetes mellitus in the older population: KORA S4/F4 cohort study. Diabet Med 2010;27:1116–23.
17. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487–95.
18. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. Diabetes Care 2003;26:3320–5.
19. Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Diabetes Care 2011;34: e61–99.
20. Staten MA, Stern MP, Miller WG, Steffes MW, Campbell SE, Insulin Standardization Workgroup. Insulin assay standardization: leading to measures of insulin sensitivity and secretion for practical clinical care. Diabetes Care 2010;33:205–6.
21. Sattar N, McConnachie A, Ford I, Gaw A, Cleland SJ, Forouhi NG, et al. Serial metabolic measurements and conversion to type 2 diabetes in the west of Scotland coronary prevention study: specific elevations in alanine aminotransferase and triglycerides suggest hepatic fat accumulation as a potential contributing factor. Diabetes 2007;56:984–91.
22. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 2009;58:773–5.
23. Kolberg JA, Jorgensen T, Gerwien RW, Hamren S, McKenna MP, Moler E, et al. Development of a type 2 diabetes risk model from a panel of serum biomarkers from the Inter99 cohort. Diabetes Care 2009;32:1207–12.

24. Nowak C, Sundstrom J, Gustafsson S, Giedraitis V, Lind L, Ingelsson E, Fall T. Protein biomarkers for insulin resistance and type 2 diabetes risk in two large community cohorts. Diabetes 2016;65:276–84.
25. Echouffo-Tcheugui JB, Dieffenbach SD, Kengne AP. Added value of novel circulating and genetic biomarkers in type 2 diabetes prediction: a systematic review. Diabetes Res Clin Pract 2013;101:255–69.
26. Guay C, Regazzi R. Circulating microRNAs as novel biomarkers for diabetes mellitus. Nat Rev Endocrinol 2013;9:513–21.
27. Muller G. Microvesicles/exosomes as potential novel biomarkers of metabolic diseases. Diabetes Metab Syndr Obes 2012;5:247–82.
28. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208–19.
29. Lyssenko V, Jonsson A, Almgren P, Pulizzi N, Isomaa B, Tuomi T, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. N Engl J Med 2008; 359:2220–32.
30. Talmud PJ, Cooper JA, Morris RW, Dudbridge F, Shah T, Engmann J, et al. Sixty-five common genetic variants and prediction of type 2 diabetes. Diabetes 2015;64: 1830–40.
31. Pfister R, Sharp S, Luben R, Welsh P, Barroso I, Salomaa V, et al. Mendelian randomization study of B-type natriuretic peptide and type 2 diabetes: evidence of causal association from population studies. PLoS Med 2011; 8:e1001112.
32. Everett BM, Cook NR, Chasman DI, Magnone MC, Bobadilla M, Rifai N, et al. Prospective evaluation of B-type natriuretic peptide concentrations and the risk of type 2 diabetes in women. Clin Chem 2013;59:557–65.
33. Miyashita K, Itoh H, Tsujimoto H, Tamura N, Fukunaga Y, Sone M, et al. Natriuretic peptides/cGMP/cGMP-dependent protein kinase cascades promote muscle mitochondrial biogenesis and prevent obesity. Diabetes 2009;58:2880–92.
34. Ropero AB, Soriano S, Tuduri E, Marroquí L, Téllez N, Gassner B, et al. The atrial natriuretic peptide and guanylyl cyclase-A system modulates pancreatic beta-cell function. Endocrinology 2010;151:3665–74.
35. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837–53.
36. UK Prospective Diabetes Study Group. UK prospective diabetes study 16: overview of 6 years' therapy of type II diabetes: a progressive disease. Diabetes 1995;44: 1249–58.
37. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 2011; 305:2532–9.
38. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease, Chapter 1, Definition and classification of CKD. Kidney Int Suppl 2013;3:19–62.
39. ADA.–[Section] 9. Microvascular complications and foot care. In: Standards of medical care in diabetes–2016. Diabetes Care 2016;39(Suppl 1):S72–80.
40. Levey AS, Becker C, Inker LA. Glomerular filtration rate and albuminuria for detection and staging of acute and chronic kidney disease in adults: a systematic review. JAMA 2015;313:837–46.
41. Fox CS, Matsushita K, Woodward M, Bilo HJ, Chalmers J, Heerspink HJ, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 2012;380:1662–73.
42. Tonelli M, Muntner P, Lloyd A, Manns BJ, Klarenbach S, Pannu N, et al. Risk of coronary events in people with chronic kidney disease compared with those with diabetes: a population-level cohort study. Lancet 2012; 380:807–14.
43. Welsh P, Woodward M, Hillis GS, Li Q, Marre M, Williams B, et al. Do cardiac biomarkers NT-proBNP and hsTnT predict microvascular events in patients with type 2 diabetes? Results from the ADVANCE trial. Diabetes Care 2014;37:2202–10.
44. Donahoe SM, Stewart GC, McCabe CH, Mohanavelu S, Murphy SA, Cannon CP, Antman EM. Diabetes and mortality following acute coronary syndromes. JAMA 2007;298:765–75.
45. Emerging Risk Factors Collaboration, Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet 2010;375:2215–22.
46. Bhatt DL, Eagle KA, Ohman EM, Hirsch AT, Goto S, Mahoney EM, et al. Comparative determinants of 4-year cardiovascular event rates in stable outpatients at risk of or with atherothrombosis. JAMA 2010;304:1350–7.
47. Cavender MA, Steg PG, Smith SC, Eagle K, Ohman EM, Goto S, et al. Impact of diabetes mellitus on hospitalization for heart failure, cardiovascular events, and death: outcomes at 4 years from the Reduction of Atherothrombosis for Continued Health (REACH) registry. Circulation 2015;132:923–31.
48. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010;362:800–11.
49. Schillinger M, Exner M, Amighi J, Mlekusch W, Sabeti S, Rumpold H, et al. Joint effects of C-reactive protein and glycated hemoglobin in predicting future cardiovascular events of patients with advanced atherosclerosis. Circulation 2003;108:2323–8.
50. Iribarren C, Karter AJ, Go AS, Ferrara A, Liu JY, Sidney S, Selby JV. Glycemic control and heart failure among adult patients with diabetes. Circulation 2001;103: 2668–73.
51. Cavender MA, Scirica BM, Raz I, Steg PG, McGuire DK, Leiter LA, et al. Cardiovascular outcomes of patients in SAVOR-TIMI 53 by baseline hemoglobin A_{1c}. Am J Med 2016;129:340.e1–8.
52. Omland T, Sabatine MS, Jablonski KA, Rice MM, Hsia J, Wergeland R, et al. Prognostic value of B-type natriuretic peptides in patients with stable coronary artery disease: the PEACE trial. J Am Coll Cardiol 2007;50: 205–14.
53. Bruno G, Landi A, Barutta F, Ghezzo G, Baldin C, Spadafora L, et al. N-terminal probrain natriuretic peptide is a stronger predictor of cardiovascular mortality than C-reactive protein and albumin excretion rate in elderly patients with type 2 diabetes: the Casale Monferrato population-based study. Diabetes Care 2013;36: 2677–82.
54. Tarnow L, Gall MA, Hansen BV, Hovind P, Parving HH. Plasma N-terminal pro-B-type natriuretic peptide and mortality in type 2 diabetes. Diabetologia 2006;49: 2256–62.
55. Gerstein HC, Pare G, McQueen MJ, Haenel H, Lee SF, Pogue J, et al. Identifying novel biomarkers for cardiovascular events or death in people with dysglycemia. Circulation 2015;132:2297–304.
56. Hillis GS, Welsh P, Chalmers J, Perkovic V, Chow CK, Li Q, et al. The relative and combined ability of high-sensitivity cardiac troponin T and N-terminal pro-B-type natriuretic peptide to predict cardiovascular events and death in patients with type 2 diabetes. Diabetes Care 2014;37:295–303.
57. Scirica BM, Bhatt DL, Braunwald E, Raz I, Cavender MA, Im K, et al. Prognostic implications of biomarker assessments in patients with type 2 diabetes mellitus at high cardiovascular risk: a secondary analysis of a randomized clinical trial. JAMA Cardiol [Epub ahead of print 2016 Sep 28].
58. Troughton RW, Frampton CM, Brunner-La Rocca HP, Pfisterer M, Eurlings LW, Erntell H, et al. Effect of B-type natriuretic peptide-guided treatment of chronic heart failure on total mortality and hospitalization: an individual patient meta-analysis. Eur Heart J 2014;35: 1559–67.
59. Huelsmann M, Neuhold S, Resl M, Strunk G, Brath H, Francesconi C, et al. PONTIAC (NT-proBNP Selected PreventiOn of cardiac eveNts in a populaTion of dIabetic patients without A history of Cardiac disease): a prospective randomized controlled trial. J Am Coll Cardiol 2013;62:1365–72.
60. Everett BM, Cook NR, Magnone MC, Bobadilla M, Kim E, Rifai N, et al. Sensitive cardiac troponin T assay and the risk of incident cardiovascular disease in women with and without diabetes mellitus: the Women's Health Study. Circulation 2011;123:2811–8.
61. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, et al. A sensitive cardiac troponin T assay in stable coronary artery disease. N Engl J Med 2009;361:2538–47.
62. Yiu KH, Lau KK, Zhao CT, Chan YH, Chen Y, Zhen Z, et al. Predictive value of high-sensitivity troponin-I for future adverse cardiovascular outcome in stable patients with type 2 diabetes mellitus. Cardiovasc Diabetol 2014;13: 63.
63. Everett BM, Brooks MM, Vlachos HE, Chaitman BR, Frye RL, Bhatt DL; BARI 2D Study Group. Troponin and cardiac events in stable ischemic heart disease and diabetes. N Engl J Med 2015;373:610–20.
64. Selvin E, Lazo M, Chen Y, Shen L, Rubin J, McEvoy JW, et al. Diabetes mellitus, prediabetes, and incidence of subclinical myocardial damage. Circulation 2014;130: 1374–82.
65. Fox CS, Golden SH, Anderson C, Bray GA, Burke LE, de Boer IH, et al. Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation 2015;132:691–718.
66. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD; Joint ESC/ACCF/AHA/WHF Task Force for Universal Definition of Myocardial Infarction Third universal definition of myocardial infarction. J Am Coll Cardiol 2012;60:1581–98.
67. Herder C, Kowall B, Tabak AG, Rathmann W. The potential of novel biomarkers to improve risk prediction of type 2 diabetes. Diabetologia 2014;57:16–29.