Urinary proteomics combined with home blood pressure telemonitoring for health care reform trial: rational and protocol

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ABSTRACT

Background: Hypertension and diabetes cause chronic kidney disease (CKD) and diastolic left ventricular dysfunction (DVD) as forerunners of disability and death. Home blood pressure telemonitoring (HTM) and urinary peptidomic profiling (UPP) are technologies enabling prevention.

Methods: UPRIGHT-HTM (Urinary Proteomics Combined with Home Blood Pressure Telemonitoring for Health Care Reform [NCT04299529]) is an investigator-initiated 5-year clinical trial with patient-centred design, which will randomise 1148 patients to be recruited in Europe, sub-Saharan Africa and South America. During the whole study, HTM data will be collected and freely accessible for patients and caregivers. The UPP, measured at enrolment only, will be communicated early during follow-up to 50% of patients and their caregivers (intervention), but only at trial closure in 50% (control). The hypothesis is that early knowledge of the UPP risk profile will lead to more rigorous risk factor management and result in benefit. Eligible patients, aged 55–75 years old, are asymptomatic, but have ≥5 CKD- or DVD-related risk factors, preferably including hypertension, type-2 diabetes, or both. The primary endpoint is a composite of new-onset intermediate and hard cardiovascular and renal outcomes. Demonstrating that combining UPP with HTM is feasible in a multicultural context and defining the molecular signatures of early CKD and DVD are secondary endpoints.

KEYWORDS

Chronic kidney disease; diabetic nephropathy; home blood pressure telemonitoring; hypertension; left ventricular function; type-2 diabetes mellitus
Expected outcomes: The expected outcome is that application of UPP on top of HTM will be superior to HTM alone in the prevention of CKD and DVD and associated complications and that UPP allows shifting emphasis from treating to preventing disease, thereby empowering patients.

Background

The epidemiological transition is a global demographic change characterised by a longer life expectancy, but the number of years added to the human life comes at a cost of lower quality of life, i.e. a greater number of years lived with disability [1]. This demographic change represents a huge social and economic challenge. Health care will have to adjust to remain sustainable by moving emphasis from the resource-intensive and costly management of established disease to prevention. Given this context, UPRIGHT-HTM (Urinary Proteomics Combined with Home Blood Pressure Telemonitoring for Health Care Reform [NCT04299529]) focuses on chronic kidney disease (CKD) and diastolic left ventricular (LV) dysfunction (DVD), as archetypes of chronic age-related diseases, and as outlined below on two diagnostic modalities that might contribute to an improved prevention of CKD and DVD, as forerunners of premature mortality and morbidity.

Blood pressure telemonitoring

Guidelines unanimously recommend out-of-the-office monitoring as the technique of choice to assess blood pressure and to diagnose and manage hypertension [2–4]. According to expert committees, home blood pressure telemonitoring (HTM) complies with the state-of-the-art for the long-term follow-up of blood pressure [5–7]. HTM offers several of the well-recognized advantages of the more complex approach of ambulatory monitoring. The greater number of readings and the absence of the white-coat effect contribute to the higher diagnostic accuracy as compared with office blood pressure readings. If automated devices are used, the self-recorded blood pressure is free of observer bias [8]. Two randomised clinical trials, i.e. the Treatment of Hypertension Based on Home or Office Blood Pressure study (THOP) [9] and the Home Versus Office Measurement – Reduction of Unnecessary Treatment (HOMERUS) trial [10], demonstrated that adjustment of antihypertensive treatment based on the self-measured home blood pressure avoids needless treatment of white-coat hypertensive patients with no differences in general well-being or target organ damage. More recently, the HOMED-BP trial (Hypertension Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure) proved that the long-term HTM was well received by hypertensive patients and that adjusting treatment based on HTM is feasible [11].

Urinary proteomics

Urine is a stable biofluid [12] and contains over 20,000 low-molecular-weight peptides, which can be analysed on a 10-ml mid-morning urine sample without additional manipulation, such as proteolysis. Approximately 60% of the total mass of urinary peptides consist of collagen fragments [13]. CKD and DVD are characterised by a disturbed balance between collagen synthesis and breakdown, leading to fibrosis and remodelling of the extracellular matrix (ECM) [14]. The urinary peptidomic profile (UPP) does not undergo significant changes when urine is stored for 6 h at room temperature [15] or for 3 days at 4°C [16]. For studies running over a long time period, urine can be stored for years at –20°C without UPP alteration [12]. The UPP is well characterised and reference standards are available [17]. These characteristics facilitate the application of UPP in clinical practice or in clinical trials, as for instance evidenced by the PRIORITY study [18].

UPP based on capillary electrophoresis coupled to mass spectrometry (CE-MS) is a powerful technology to improve the management of chronic diseases (Figure S1). The CE-MS platform enables the separation of naturally occurring peptides in a single step, using a strong electrical field and subsequent detection by mass spectrometry [19,20]. It is a robust and operator-independent technology, allowing the high-resolution detection of several thousands of peptides (0.8 ≤ 18 kDa) in a single urine sample. A detailed description of urine sample preparation, proteome analysis by CE-MS, data processing and sequencing of the urinary peptides allowing the identification of parental proteins has been published [19,20] and is summarised in the online only Data Supplement.

Design

UPRIGHT-HTM is an investigator-initiated randomised clinical trial, comparing UPP combined with
HTM (experimental group) with HTM alone (control group) in risk profiling and as guide to starting or intensifying management of risk factors to prevent established disease. This multicentre trial will run in different countries and continents, is therefore open for patients of multiple ethnicities, who will be randomised in a 1:1 proportion to the experimental or control group. UPRIGHT-HTM complies with the Helsinki declaration, General Data Protection Regulations, and received or is seeking ethical approval in the countries where the study will be running (currently available ethics approvals listed at https://www.appremed.org/news).

Overview

In short, UPRIGHT-HTM consists of: (i) a run-in period of variable length (2–5 weeks or longer) during which the eligibility of patients will be checked; (ii) after stratification for centre and sex a randomised follow-up period informed by either HTM plus UPP (experimental group) or UPP alone (control group); and (iii) for patients leaving randomised follow-up prematurely a supervised follow-up period during which the incidence of the primary composite endpoint will be further monitored in consenting patients (Figure 1). The incidence of endpoints during supervised follow-up will contribute to the intention-to-treat analysis.

Inclusion and exclusion criteria

UPRIGHT-HTM aims to enrol 1148 asymptomatic patients with high-risk profile, based on their clinical history, biochemical data, and routine technical examinations as available in medical records or to be performed in compliance with current guidelines.

Inclusion criteria

Asymptomatic patients whose age ranges from 55 to 75 (inclusive) can be enrolled, after written informed consent has been obtained. In addition to age, patients must have at least five additional guideline-defined risk factors, preferably including hypertension, type-2 diabetes mellitus (T2DM), or both (Table 1). Furthermore, patients must have an email address and internet access via smartphone (only android will

Data to be collected on top of home BP

Figure 1. UPRIGHT-HTM design. eCRF: electronic report forms completed by investigators; EP: absence (-)/incidence (+) of the components of the primary endpoint during the run-in/follow-up periods, respectively; IC: written informed consent; pINF: patients received the UPRIGHT-HTM information sheet and were familiarised with operating the blood pressure monitoring devices; pQ: patient-administered questionnaires; R: randomisation after stratification for centre and sex; S: initial screening; UPP: urinary proteomic profiling (mandatory prior to randomisation – optional at the end of follow-up).
Table 1. Risk factors.

- type-2 diabetes mellitus
- fasting glycaemia ≥100 mg/dl
- random glycaemia ≥200 mg/dl
- positive oral glucose tolerance test
- HOMA-IR ≥2.8
- metabolic syndrome
- fasting serum insulin ≥21 μU/mL
- HbA1c ≥6.5%
- body mass index 30.0–39.9 kg/m²
- waist circumference ≥88/102 cm (♀/♂)
- current smoking
- total cholesterol >190 mg/dl
- high-density lipoprotein cholesterol <46/<40 mg/dl (♀/♂)
- low-density lipoprotein cholesterol >115 mg/dl
- non-HDL cholesterol ≥130 mg/dl
- serum triglycerides >150 mg/dl
- aortic pulse wave velocity >10 m/s
- Carotid intima-media thickness >900 μm

Risk factors were taken from the 2018 European guideline for the management of hypertension (reference 3). To conduct an oral glucose tolerance test, WHO recommends a 75-gm orally administered dose of glucose. The fasting plasma glucose should be less than 100 mg/dl. To be normal, the 1-hour and 2-hour plasma glucose levels should be <180 mg/dl and <140 mg/dl, respectively. HOMA-IR indicates Homeostatic Model Assessment of Insulin Resistance and is computed as (fasting glucose in mg per decilitre x insulin in μU)/405 (PMID 1895955). Metabolic syndrome is the combination of an increased waist circumference, hyperglycaemia (≥150 mg/dl), decreased high-density lipoprotein cholesterol (<50 mg/dl in women and <40 mg/dl in men), office hypertension (blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic), and a (fasting) plasma glucose of ≥100 mg/dl. Masked hypertension is a normal office blood pressure associated with an increased out-of-office blood pressure. Physical inactivity in older individuals is engaged in less than 150 min of moderate-intensity aerobic exercise (3–6 METS) throughout the week (https://www.who.int/dietphysicalactivity/physical-activity-recommendations-65years.pdf?ua=1). One metabolic equivalent (MET) is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 ml O₂ per kilogram body weight per minute (PMID 2204507).

Exclusion criteria

Type-1 diabetic patients do not qualify for UPRIGHT-HTM. Study-specific exclusion criteria include symptoms related to renal or LV dysfunction, stage-3B CKD (i.e. eGFR >45 ml/min/1.73 m²), a history of cardiovascular or renal disease within 1 year prior to enrolment, symptomatic patients, presence of microalbuminuria combined with systolic or diastolic LV dysfunction at enrolment, and hemodynamic significant valvular heart disease. Patients who during the run-in period have difficulties in completing the electronically administered questionnaires or are not adherent to HTM will not be randomised. Patients with impracticable echocardiographic window or with atrial fibrillation or flutter or frequent extrasystoles, will also be excluded, because these conditions do not allow a reliable assessment of diastolic LV function. Patients with stage-3A CKD or asymptomatic DVD at enrolment qualify for entry, but not those with DVD combined with CKD. Other exclusion criteria are common to all research in humans and include serious previous or concurrent cardiovascular or noncardiovascular disease, cancer within 5 years prior to enrolment, suspected substance abuse, psychiatric illness, or participation in other studies. However, patients who experienced a cardiovascular or noncardiovascular event or a renal complication one year or longer before being considered for randomisation qualify for entry, if they fully recovered and are symptomless.

Primary and secondary endpoints

The primary endpoint is a composite of intermediary and hard cardiovascular-renal endpoints.

Intermediary endpoints

The intermediate endpoints are: new-onset microalbuminuria, doubling of serum creatinine, a decrease in the estimated glomerular filtration rate (eGFR) by 30% or more or eGFR declining below 45 ml/min/1.73 m² new-onset hypertensive retinopathy (Keith-Wagener classification [21] or diabetic retinopathy [22], electrocardiographic or echocardiographic LV hypertrophy, cardiac arrhythmias (atrial fibrillation or flutter, frequent ventricular or supraventricular extrasystoles present in ≥20% of cardiac cycles [23], and DVD [24,25]. eGFR will be derived from serum creatinine, using the Chronic Kidney Disease Epidemiology Collaboration equation [26]. CKD will be staged according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative guideline [27].

In line with the PRIORITY study, the presence of microalbuminuria should be confirmed in two of three morning urine samples collected on consecutive days [18]. In low-resource or primary care settings a validated dipstick test is acceptable, but a positive dipstick test should preferably be confirmed by
measurement of the albumin-to-creatinine ratio [28–30]. In asymptomatic patients, atrial peptides do not provide reliable diagnostic information on the presence of silent LV dysfunction [31,32]. Diagnosis of DVD in the preclinical phase (heart failure stage B) requires echocardiography. DVD is an abnormally low age-specific transmitial E/A ratio, indicative of impaired relaxation, or a mildly-to-moderately elevated left ventricular filling pressure (E/e' >8.5) with normal or decreased age-specific E/A ratio [24,25]. The ejection fraction should be 50% or higher [24,25]. Higher e' and lower E/e' on transmitral/tissue Doppler echocardiographic imaging, respectively, reflect faster early diastolic LV relaxation and slower LV filling pressure (Figure S2).

**Hard cardiovascular-renal endpoints**

The hard cardiovascular endpoints include cardiovascular mortality (ICD10: I00-I99), and nonfatal myocardial infarction (I21, I22), nonfatal hospitalised heart failure (I50), nonfatal stroke, not including transient ischaemic attack (I60-I63), and coronary or peripheral revascularisation. The hard renal outcomes include macroalbuminuria, the need for renal-replacement therapy, and death due to renal causes (N17, N18), as defined in recent outcome trials [33].

**Sample size**

The population [8,34–37] and patient [38–42] studies and the randomised clinical trials [18,43–49] informing the sample size calculations are summarised in the online-only Data Supplement. Sample size calculations were based on the log-rank test, using SAS software, version 9.4. We assumed a study duration of 5 years with patients being recruited over two years, an annual dropout rate of 2.5%, a 4-year primary endpoint rate of 20%, and a risk reduction of 30% in the experimental group. Under these assumptions, with the α-level set at 0.05 and power at 0.80, 1148 patients would have to be randomised in a 1:1 proportion to the experimental and control group, respectively (574 per group). Sample sizes based on alternative assumptions appear in Table S1.

**Data collection**

UPRIGHT-HTM will run as a patient-centred study, mainly positioned at the patients' homes and the practices of their caregivers by implementation of modern information technology, under supervision of the patients’ own primary or specialist care providers.

**Data collected by patients**

Most data will be collected at the patients’ homes via web-based applications. For HTM, patients will use validated [50] OMRON HEM 9210-T (Europe) or OMRON HEM 9210 T-E (other regions) monitors (Omron Healthcare Co., Ltd., Kyoto, Japan) fitted with a cuff that accommodates the range of adults upper-arm circumferences. Each patient identification number will be uniquely linked with the serial number of the HTM device handed over to the patient. If a patient opts to drop out from the randomised trial or the supervised follow-up (Figure 1), the careprovider will recuperate the HTM device, check its functionality, and pass it on to another patient. Patients will be encouraged to measure their blood pressure after 5 min rest in the sitting position preferably within 1 h after awakening, before breakfast and before taking any medication, if possible, daily. Patients who fail to practice HTM at less than weekly intervals, will receive automatically generated email reminders but will not be excluded from the intention-to-treat analysis.

Other data to be collected from patients during the run-in period, at randomisation and at 6-monthly intervals during the study (Figure 1) via the web-based interface are the EQ-5D quality of life questionnaire (www.euroqol.org) and the World Health Organisation (G. Rose) questionnaires on chest pain, dyspnoea, claudication, cough and expectorations [51]. Both questionnaires were translated into all required local languages. Logistics will be set up in such a way that patients can collect a 10-ml mid-morning urine sample for UPP at their homes or at the practice of their caregiver. These urine samples, to be collected at baseline, will be stored locally at -20°C and dispatched in batches to Mosaiques-Diagnostics GmbH, Hannover, Germany, for UPP analysis. Investigators will be encouraged to collect an optional 10-ml urine sample from patients, when they leave the study allowing a longitudinal UPP assessment.

**Data collected by caregivers**

Caregivers will be responsible for recruiting patients, obtaining informed written consent, distributing the HTM devices (one device per patient) and the UPP test kits, and for collecting depersonalised clinical data via web-administered electronic case report
forms (eCRFs). Caregivers are free in organising the follow-up and nonpharmacological and pharmacological treatment of their patients according to current national or international guidelines. The online-only Data Supplement provides suggestions for the management of hypertension (Figure S3), antidiabetic therapy (Figure S4) and statin treatment (Table S2).

At screening, caregivers will explain the objectives of the UPRIGHT-HTM trial to patients verbally and by handing over the patient information leaflet together with the HTM monitor. They will complete an eCRF to document each patient’s anthropometrics, office blood pressure and heart rate. This information is mandatory, because it allows comparing screened patients not randomised with those randomised. Prior to randomisation, caregivers will collect informed written consent from each patient willing to be enrolled into the study. The caregivers will locally archive the consent forms, only available on paper to protect the patients’ privacy. Prior to randomisation, caregivers will complete the prerandomisation eCRF showing that enrolled patients meet all eligibility criteria. Other information to be collected via the prerandomisation eCRF includes: (i) anthropometrics; (ii) use of antihypertensive, lipid-lowering, antidiabetic and antiplatelet medications (only by drug class); (iii) the patient’s medical history; and (iv) the presence vs absence of components of the primary endpoint. During follow-up, the same eCRF will be used at annual follow-up visit, the end-of-the-randomised follow-up visit, and the end-of-trial close-out visit. Thus, the contents of all follow-up eCRFs are similar to that used prior to randomisation. These eCRFs allow reporting the incidence of the components of the primary endpoint, and if applicable, the reason why a patient wishes to discontinue randomised follow-up, or the justification why a caregiver withdrew a patient from randomised or supervised follow-up (Figure 1).

On clinical indication while observing guidelines, probably at baseline and annual intervals for most patients, caregivers will collect urine samples required for the diagnosis of incident microalbuminuria and refer patients for echocardiography (Table S3) at baseline and at least once during follow-up. Routine biochemical data collected within 6 to 9 months of the due date of the eCRF qualify, so that in most cases no blood sampling for the specific purpose of the study is necessary.

### Database management and statistical analysis

For data management and statistical analysis, SAS software, version 9.4, maintenance level 5 (SAS Institute, Cary, NC, USA) will be used. The HTM readings, the electronic questionnaire data collected directly from patients and the eCRFs completed by caregivers will reach the study coordinating team via the WiPam platform (www.wipam.net) as CSV (comma-separated values) or XML (extensible mark-up language) files. The UPP data will be emailed to study team as Excel (XLS or XLSX) email attachments. After quality control, the CSF, XML, XLS and XLSX files will be directly imported into a SAS database, using the SAS PC File Server, version 9.4.

### Intention-to-treat and per-protocol analysis

The main analysis will be implemented using the intention-to-treat dataset. This analysis will address the intermediate and hard events making up the primary composite endpoint, which occurred during randomised and supervised follow-up (Figure 1). This dataset will include all randomised patients, who were free of the endpoints of interest at enrolment and who had at least one endpoint assessed after randomisation, i.e. during randomised or supervised follow-up. The per-protocol dataset is a subset of the intention-to-treat dataset excluding patients randomised, but not complying with all inclusion and exclusion criteria, patients deviating from the study protocol to such extent that they might introduce bias in the analysis, and excluding the data that accrued during supervised follow-up (Figure 1).

### Stratification and randomisation

Eligible patients will be stratified by centre and sex and subsequently randomised in a 1:1 proportion within each stratum to the experimental group (HTM plus UPP) and control group (HTM alone), using a random function and permuted blocks. Stratification and randomisation will be automated via the WiPam platform.

### Masking

Caregivers will know the group to which their patients were randomly assigned. In the two groups, both patients and caregivers will have full access to the HTM data. In both treatment groups, patients and caregivers will be informed about the UPP risk profile. However, in the experimental group, patients will be informed about their UPP risk profile shortly after randomisation and in the control group, only when they leave randomised or supervised follow-up.
or at the completion of the trial. As implemented in other randomised controlled trials [52,53], the central study coordinating team will run interim analyses as part of the quality control programme, excluding any component of the primary endpoint, and have these analyses published in peer-reviewed journals to report on the progress of the trial. However, the study coordinating team will remain blinded to the primary endpoint and all of its components until completion of the trial and until the statistical analysis plan has been written and all datasets have been cleaned and frozen for the final analysis.

For endpoint validation, as implemented in other investigator-initiated trials [54], each centre will appoint two junior residents and one senior supervisor, who will remain blinded to randomisation. Using a special eCRF, at annul intervals, the study coordinating team will request validation of potential endpoints, as reported via the follow-up eCRFs.

**Statistical analysis**

The primary and secondary endpoints measured on a continuous scale will be analysed using mixed models. Between-group comparisons will be executed with adjustment for baseline. The fixed effects in the model include randomisation group and, if there is imbalance between the randomised groups at baseline, confounders. To account for the correlation between a patient’s repeated blood pressure and other measurements, models will also account for patient-level random effects. Centre-level random effects will be used to model the possible correlation of measurements between patients recruited at the same centre. Binary endpoints, such as the incidence of endpoints, will be analysed by means of McNemar’s test and hierarchical mixed-effects regression models. All statistical tests will be two-sided.

**Molecular analyses**

The primary analyses will focus on the urinary markers CKD273 and HF1, which have been widely validated and for which diagnostic thresholds have been established and published in the peer-reviewed literature [18,32,55,56]. However, in pursuit of the secondary objective to gain deeper insight in the molecular pathophysiology of early CKD and DVD, the urinary peptides with known amino-acid sequence allowing identification of the parent proteins will be carried through to further analyses as summarised in Table S4. Peptides linked to a shared pathophysiological process can then be combined in novel multidimensional classifiers, which after validation can be proposed for clinical use.

**Discussion**

UPRIGHT-HTM is an investigator-steered, multi-centre, randomised trial set up in a multicultural context. The main objective is to assess the value of UPP administered on top of HTM compared with HTM alone in risk stratification and as guide to early intervention.

**Patient-centred design**

UPRIGHT-HTM is a patient-centred minimally intrusive study. Giving patients access to health-related tests is a double-edged asset, because test results can cause both anxiety or enhance well feeling [57–60]. However, a literature review revealed that, in general, web-based access to personal health-related information does not increase the patients’ anxiety levels, even in women with breast cancer given access to laboratory results and imaging studies [58]. What the studies revealed is that there is a great need to enhance the patients’ comprehension of results [58]. HTM is a powerful instrument in educating and empowering patients [61]. In a randomised clinical trial, involving 450 patients recruited at 59 primary care practices and followed up for 12 months, self-monitoring and self-titration of antihypertensive drugs lowered systolic blood pressure 8.8 mm Hg more than usual care based on office blood pressure measurement [62]. Moreover, self-measurement of blood pressure increases adherence to antihypertensive drugs [63], allows detecting symptoms that occur between clinic visits and reduces the number of clinic visits required for optimising drug treatment [64]. However, self-titration of medication was not considered as a practicable option in UPRIGHT-HTM, because of the multi-ethnic and multicultural settings of the study sites.

**Feasibility**

Both CKD and DVD are associated with specific UPP profiles. The multidimensional marker CKD273 predicts progression of CKD earlier than microalbuminuria does [56]. HF1 is a marker of subclinical DVD [65]. Both CKD273 [55,56] and HF1 [65] have been extensively validated in longitudinal patient and population studies, and CKD273 also in the
framework of the PRIORITY study [18]. Pharmacological treatment, including antihypertensive, lipid-lowering, antidiabetic and antiplatelet drugs and even immunosuppressive drugs in transplant patients [66], have no noticeable influence on the UPP or its association with study endpoints. As a working example, Figure 2 proposes how HF1 might be applied in clinical practice in asymptomatic high-risk individuals [32]. In the presence of clinical risk factors for DVD, in particular older age combined with overweight or abdominal obesity and hypertension (25.1% of the adult population [32]), HF1 might be used as a screening tool [32]. If its value is less than $-0.350$, managing risk factors over a 5-year time span is to be recommended. In contrast, if HF1 is $-0.350$ or higher, patients should not only have their risk factors addressed, but might be referred for echocardiography to confirm the presence of DVD [32]. An added benefit of HF1 is that the marker predicts worsening of renal function [55] and the 5-year incidence of cardiovascular and cardiac events [31]. In our studies [32,65], in line with other publications [67], N-terminal pro-B-type natriuretic peptide (NT-proBNP) did not add to the prediction of DVD over and beyond classical risk factors. Moreover, HF1 in the presence of NT-proBNP fully retained its prognostic value [32,65].

Two other considerations relate to the feasibility of URIGHT-HTM. First, drug-adherence is an important determinant of how well risk factors will be managed in the intervention and control group. Because of practicability, the clinical centres will not engage in a formal and standardised monitoring programme of drug adherence, other than the information to be collected by the caregivers and to be provided via the annual eCRFs. However, the home blood pressure recorded by the patients and the biochemical data, e.g. blood glucose and eGFR being mandatory fields in the eCRFs, will generate objective information on how well risk factors are being controlled. Previous experience in sub-Saharan Africa confirmed that adherence to treatment does not grossly differ compared with clinical trials in developed nations [68]. Second, URIGHT-HTM will be conducted in affluent communities, where drug expenses are largely covered by health insurance. However, cheap generic drugs are widely available in sub-Saharan Africa. Diuretics, the cheapest drug class, and generic long-acting calcium-channel blockers are widely available in sub-Saharan Africa and are recommended to initiate antihypertensive treatment in Blacks born and living in Africa [2–4]. This also applies to metformin, the first line agent to treat T2DM (Figure S4) [69].

**Early intervention**

All current guidelines state that risk factor management is the foundation for stopping progression of early towards established disease and in preventing the target organ damage and the cardiovascular-renal complications that make up the primary composite endpoint of URIGHT-HTM. Furthermore, guidelines concur on using angiotensin converting-enzyme inhibitors (ACEIs) in patients at risk of – or already having – CKD or heart failure with preserved ejection fraction, while angiotensin receptor blockers are indicated in ACEI-intolerant patients [5–7]. Addressing insulin resistance and glycemic control in T2DM are of primordial importance in patients at risk of DVD [24,34] and diabetic nephropathy [18]. Spironolactone did not stop progression of diabetic nephropathy in PRIORITY [18] or prevent the incidence of cardiovascular complications in patients with diastolic heart failure enrolled in the Spironolactone for Heart Failure with Preserved Ejection Fraction (TOPCAT) trial [70]. However, in the Diastolic Heart Failure (DHF) trial, a 12-month course with spironolactone improved diastolic LV function [71]. In patients with a high risk profile, such as those recruited into URIGHT-HTM, statins will be indicated in most (Table S2). In patients at risk of CKD, DVD or both, SGLT2-Is might be particularly indicated, if covered by health insurance. Indeed, in placebo-controlled trials of very high-risk T2DM patients, SGLT2-Is improved glycaemic control, lowered office and ambulatory blood pressure, protected against major cardiovascular and renal complications, reduced the incidence of heart failure, and lowered body weight with no risk of hypoglycaemia [33].

![Figure 2. Proposal for the clinical application of HF1 over a 5-year horizon. In a random population sample, 25% of participants combined three major risk factors for diastolic left ventricular dysfunction. Modified and reproduced with permission from reference [32].](image-url)
Molecular leads from previous studies

In stage-3 CKD [56,72], there is upregulation of mucin-1 subunit-α, a protein shed by the renal tubular epithelium [72]. Mucin-1 is a high-molecular weight (400 kDa) membrane-tethered glycoprotein [73], which normal kidneys express in the thick segment of Henle’s loop and in the distal tubules and collecting ducts. The main function of mucin-1 is to shield cell surfaces by maintenance of a luminal epithelial mucobARRIER [74]. Further evidence supporting mucin-1 as a marker of renal dysfunction originated from genetic studies. A frameshift mutation in the MUC1 gene, located on chromosome 1 (1q21) [75] creates a new peptide that accumulates inside the MUC1 expressing renal tubular cells and causes autosomal dominant medullary cystic kidney disease type-1.

With respect to DVD, the urinary proteome revealed a downregulation of WW domain-binding protein 11 [65]. The WBP-11 gene encodes a nuclear protein, which in cell nuclei co-localises with mRNA splicing factors [76]. In cardiomyocytes, the gene product, WBP-11, interacts with the 52-amino acid integral membrane protein phospholamban (PP-1) and thereby contributes to the regulation of the transmembrane Ca2⁺ flux via the Ca2⁺ pump (SERCA), which transports Ca2⁺ from the cytosol to the sarcoplasmic reticulum. Phosphorylation of PP-1 by protein kinase A and dephosphorylation by WBP-11, respectively, stimulates and inhibits SERCA [77]. Downregulation of WBP-11, as observed in DVD patients, might enhance SERCA activity and impair electromechanical coupling in the myocardium [78].

Perspectives

Governments typically invest over 95% of healthcare budgets in treatment of established disease, even though prevention generates a lower cost per additional quality-adjusted life-year [79]. If UPRIGHT-HTM fulfils the expectations underpinning its design, it will make a strong point for combining HTM with UPP as model for rigorous risk factor management to prevent target organ damage and age-related cardiovascular-renal complications.

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Urinary proteomics

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**Acknowledgments**

The authors gratefully acknowledge the expert clerical assistance of Vera De Leebeeck and Renilde Wolfs at the Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Belgium.

**Disclosure statement**

Professor Harald Mischak is the co-founder and co-owner of Mosaiques Diagnostics GmbH, Hannover, Germany.

**Funding**

The Non-Profit Research Institute Alliance for the Promotion of Preventive Medicine, Mechelen, Belgium (URL: www.appremed.org) received a nonbinding grant from OMRON Healthcare Co., Ltd, Kyoto, Japan. Mosaiques-Diagnostics GmbH will provide an in-kind contribution covering the UPP costs.

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**References**

[1] Murray CJ, Lopez AD. Measuring the global burden of disease. N Engl J Med. 2013;369(5):448–457.
[2] Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2018;71:e127–e248.
[3] Williams B, Mancia G, Spiering W, et al.; ESC Scientific Document Group. 2018 ESC/ESH guidelines for the management of arterial hypertension. Eur Heart J. 2018;39(33):3021–3104.
[4] Unger T, Borghi C, Charchar F, et al. 2020 International society of hypertension global hypertension practice guidelines. Hypertension. 2020;75(6):1334–1357.
[5] O’Brien E, Asmar R, Beilin L, et al. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. J Hypertens. 2003;21:821–848.
[6] Pickering TG, Miller NH, Ogedegbe G, et al. A joint scientific statement from the American Heart Association, American Society of Hypertension, and Preventive Cardiovascular Nurses Association. Hypertension. 2008;52(1):10–29.
[7] Pickering TG. Home blood pressure monitoring: a new standard method for monitoring hypertension control in treated patients. Nat Clin Pract Cardiovasc Med. 2008;5(12):762–763.
[8] Niiranen TJ, Hanninen MR, Johansson J, et al. Home-measured blood pressure is a stronger predictor of cardiovascular risk than office blood pressure: the Finn-Home study. Hypertension. 2010;55(6):1346–1351.
[9] Staessen JA, Den HE, Celis H, et al. Antihypertensive treatment based on blood pressure measurement at home or in the physician’s office. A randomized controlled trial. J Am Med Assoc. 2004;291(8):955–964.
[10] Verberk WJ, Kroon AA, Lenders JWM, et al. Self-measurement of blood pressure at home reduces the need for antihypertensive drugs. A randomized, controlled trial. Hypertension. 2007;50(6):1019–1025.
[11] Asayama K, Ohkubo T, Metoki H, et al.; on behalf of Hypertension Objective Treatment Based on Measurement by Electrical Devices of Blood Pressure (HOMED-BP) investigators. Cardiovascular outcomes in the first trial of antihypertensive
therapy guided by self-measured home blood pressure. Hypertens Res. 2012;35(11):1102–1110.

[12] Fliser D, Novak J, Thongboonkerd V, et al. Advances in urine proteome analysis and biomarker discovery. J Am Soc Nephrol. 2007;18(4):1057–1071.

[13] Coon JJ, Żuribig P, Dakna M, et al. CE-MS analysis of the human urinary proteome for biomarker discovery and disease diagnostics. Proteomics Clin Appl. 2008;2(7-8):964–973.

[14] Zhang ZY, Ravassa S, Yang WY, et al. Diastolic left ventricular function in relation to urinary and serum collagen biomarkers in a general population. PLoS One. 2016;11(12):e0167582.

[15] Theodorescu D, Wittke S, Ross MM, et al. Discovery and validation of new protein biomarkers for urothelial cancer: a prospective analysis. Lancet Oncol. 2006;7(3):230–240.

[16] Schaub S, Wilkins J, Weiler T, et al. Urine protein profiling with surface-enhanced laser-desorption/ionization time-of-flight mass spectrometry. Kidney Intern. 2004;2004;65(1):323–332.

[17] Mischak H, Kolch W, Aivaliotis M, et al. Comprehensive human urine standards for comparability and standardization in clinical proteome analysis. Proteomics Clin Appl. 2010;4(4):464–478.

[18] Tofte N, Lindhardt M, Adamova K, 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 Diabet Endocrinol. 2020;8(4):301–312.

[19] Mischak H, Allmaier G, Apweiler R, et al. Recommendations for biomarker identification and qualification in clinical proteomics. Sci Transl Med. 2010;2(46):46ps42.

[20] Mischak H, Vlahou A, Ioannidis JPA. Technical aspects and inter-laboratory variability in native peptide profiling: the CE-MS experience. Clin Biochem. 2013;46(6):432–443.

[21] Breslin DJ, Gifford RW, Jr., Fairbairn JFII, et al. Prognostic importance of ophthalmoscopic findings in essential hypertension. JAMA. 1966;195(5):335–338.

[22] Klein R, Klein BE, Magli YL, et al. An alternative method of grading diabetic nephropathy. Ophthalmology. 1986;93(9):1183–1187.

[23] Eugenio PL. Frequent premature ventricular contractions: an electrical link to cardiomyopathy. Cardiol Rev. 2015;23(4):168–172.

[24] Kuznetsova T, Herbots L, López B, et al. Prevalence of left ventricular diastolic dysfunction in a general population. Circ Heart Fail. 2009;2(2):105–112.

[25] Kloch-Badelek M, Kuznetsova T, Sakiewicz W, et al.; European Project On Genes in Hypertension (EPOGH) Investigators. Prevalence of left ventricular diastolic dysfunction in European populations based on cross-validated diagnostic thresholds. Cardiovasc Ultrasound. 2012;10:10.

[26] Levey AS, Stevens LA, Schmid CH, et al.; for the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–612.

[27] National Kidney Foundation. KDOQI clinical practice guidelines for diabetes and CKD: 2012 update. Am J Kidney Dis. 2012;60:850–886.

[28] Zeller A, Sigle JP, Battegay E, et al. Value of a standard urinary dipstick test for detecting microalbuminuria in patients with newly diagnosed hypertension. Swiss Med Wkly. 2005;135(3-4):57–61.

[29] White SL, Yu R, Craig JC, Polkinghorne KR, et al. Diagnostic accuracy of urine dipsticks for detection of albuminuria in the general community. Am J Kidney Dis. 2011;58(1):19–28.

[30] Wen CP, Yang YC, Tsai MK, et al. Urine dipstick to detect trace proteinuria: an underused tool for an underappreciated risk marker. Am J Kidney Dis. 2011;58(1):1–3.

[31] Zhang ZY, Thijs L, Petit T, et al. Urinary proteome and systolic blood pressure as predictors of 5-year cardiovascular and cardiac outcomes in a general population. Hypertension. 2015;66(1):52–60.

[32] Zhang ZY, Nkuipou-Kenfack E, Yang WY, et al. Epidemiologic observations guiding clinical application of a urinary peptidomic marker of diastolic left ventricular dysfunction. J Am Soc Hypertens. 2018;12(6):438–447.

[33] Yan C, Thijs L, Cao Y, et al. Opportunities of anti-diabetic drug in cardiovascular medicine: a meta-analysis and perspectives for trial design. Hypertension. 2020;76:420–431.

[34] Kuznetsova T, Thijs L, Knez J, et al. Longitudinal changes in left ventricular diastolic function in a general population. Circ Cardiovasc Imaging. 2015;8(4):e002882.

[35] Kuznetsova T, Thijs L, Knez J, et al. Prognostic value of left ventricular diastolic dysfunction in a general population. J Am Heart Assoc. 2014;3:e000789.

[36] Yang WY, Melgarejo JD, Thijs L, et al.; for The International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcomes (IDACO) Investigators. Association of office and ambulatory blood pressure with mortality and cardiovascular outcomes. J Am Med Assoc. 2019;322(5):409–420.

[37] Li Y, Thijs L, Zhang ZY, et al.; On behalf of the International Database on Ambulatory and Home Blood Pressure in Relation to Cardiovascular Outcome Investigators. Opposing age-related trends in absolute and relative risk of adverse health outcomes associated with out-of-office blood pressure. Hypertension. 2019;74(6):1333–1342.

[38] Lacombe VA, Viatchenko-Karpinski S, Terentyev D, et al. Mechanisms of impaired calcium handling underlying subclinical diastolic dysfunction in diabetes. Am J Physiol Regul Integr Comp Physiol. 2007;293(5):R1787–R1797.

[39] Stahrenberg R, Edelmann F, Mende M, et al. Association of glucose metabolism with diastolic function along the diabetic continuum. Diabetologia. 2010;53(7):1331–1340.
[40] Jørgensen PG, Jensen MT, Mogelvang R, et al. Abnormal echocardiography in patients with type 2 diabetes and relation to symptoms and clinical characteristics. Diabet Vasc Dis Res. 2016;13(5):321–330.

[41] von Bibra H, Paulus W, St.John Sutton M. Cardiometabolic syndrome and increased risk of heart failure. Curr Heart Fail Rep. 2016;13(5):219–229.

[42] Jørgensen PG, Jensen MT, Biering-Sørensen T, et al. Burden of uncontrolled metabolic risk factors and left ventricular structure and function in patients with type 2 diabetes mellitus. J Am Heart Assoc. 2018;7:e008856.

[43] McManus RJ, Mant J, Bray EP, et al. Telemonitoring and self-management in the control of hypertension (TASMINH2): a randomised controlled trial. Lancet. 2010;376(9736):163–172.

[44] McManus RJ, Mant J, Franssen M, TASMINH4 investigators, et al. Efficacy of self-monitored blood pressure, with or without telemonitoring, for titration of antihypertensive medication (TASMINH4): an unmasked randomised controlled trial. Lancet. 2018;391(10124):949–959.

[45] Margolis KL, Asche SE, Bergdall AR, et al. Effect of home blood pressure telemonitoring and pharmacist management on blood pressure control: a cluster randomized clinical trial. J Am Med Assoc. 2013;310(1):46–56.

[46] Margolis KL, Asche SE, Dehmer SP, et al. Long-term outcomes of the effects of home blood pressure telemonitoring and pharmacist management on blood pressure among adults with uncontrolled hypertension: follow-up of a cluster randomized clinical trial. JAMA Netw Open. 2018;1(5):e181617.

[47] Staessen JA, Wang JG, Thijs L. Cardiovascular protection and blood pressure reduction: a meta-analysis. Lancet. 2001;358(9290):1305–1315.

[48] Haller H, Itô S, Izzo JL, Jr, et al. Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. N Engl J Med. 2011;364(10):907–917.

[49] The ACCORD Study Group. Effects of intensive blood-pressure control in type 2 diabetes mellitus. N Engl J Med. 2010;362:1575–1585.

[50] Alpert BS. Validation of the Omlron HEM-9210T by the ANSI/AAMI/ISO 81060-2: 2013 with two novel cuffs: wide-range and extra-large. Blood Press Monit. 2017;22(3):166–168.

[51] Rose GA, Blackburn H. Cardiovascular surgery methods. Monogr Ser World Health Organ. 1968;56:1–188.

[52] Staessen J, Amery A, Birkenhäger W, et al. Syst-Eur multicenter trial on the treatment of isolated systolic hypertension in the elderly: first interim report. J Cardiovasc Pharmacol. 1992;19(1):120–125.

[53] Staessen JA, Celis H, Den Hond E, et al.; THOP investigators. Comparison of conventional and automated blood pressure measurements: interim analysis of the THOP trial. Treatment of Hypertension According to Home or Office Blood PressureBlood Press Monit. 2002;7(1):61–62.

[54] Cleland JGF, Ferreira JP, Mariottoni B, et al. The effect of spironolactone on cardiovascular function and markers of fibrosis in people at increased risk of developing heart failure: the heart 'OMics' in AGEing (HOMAGE) randomized clinical trial. Eur Heart J. 2021;41:1711–1723.

[55] Gu YM, Thijs L, Liu YP, et al. The urinary proteome as correlate and predictor of renal function in a population study. Nephrol Dial Transplant. 2014;29(12):2260–2268.

[56] Pontillo C, Zhang ZY, Schanstra JP, et al. Prediction of chronic kidney disease stage 3 by CKD27, a urinary proteomic biomarker. KI Reports. 2017;2(6):1066–1075.

[57] Wiljer D, Leonard KJ, Urowitz S, et al. The anxious wait: assessing the impact of patient accessible EHRs for breast cancer patients. BMC Med Inform Decis Mak. 2010;10:46.

[58] Mäk G, Smith Fowler H, Leaver C, et al. The effects of web-based patient access to laboratory results in British Columbia: a patient survey on comprehension and anxiety. J Med Internet Res. 2015;17(8):e191.

[59] Robinson JO, Wynn J, Biesecker B, et al. Psychological outcomes related to exome and genome sequencing result disclosure: a meta-analysis of seven Clinical Sequencing Exploratory Research (CSER) Consortium studies. Genet Med. 2019;21(12):2781–2790.

[60] Hirst JA, Farmer AJ, Williams V. How point-of-care HbA1c testing changes the behaviour of people with diabetes and clinicians – a qualitative study. Diabet Med. 2020;37(6):1008–1015.

[61] Evangelista LS, Lee JA, Moore AA, et al. Examining the effects of remote monitoring systems on activation, self-care, and quality of life in older patients with chronic heart failure. J Cardiovasc Nurs. 2015;30(1):51–57.

[62] McManus RJ, Mant J, Haque MS, et al. Effect of self-monitoring and medication self-titration on systolic blood pressure in hypertensive patients at high risk of cardiovascular disease: the TASMIN-SR randomized clinical trial. J Am Med Assoc. 2014;312(8):799–808.

[63] Márquez-Contreras E, Martell-Claros N, Gil-Guillén V, et al.; Compliance Group of the Spanish Society of Hypertension (SEE). Efficacy of a home blood pressure monitoring programme on therapeutic compliance in hypertension: the EAPACUM-HTA study. J Hypertens. 2006;24(1):169–175.

[64] Staessen JA, Thijs L, Ohkubo T, et al. Thirty years of research on diagnostic and therapeutic thresholds for the self-measured blood pressure at home. Blood Press Monit. 2008;13(6):352–365.

[65] Zhang ZY, Nkuipou-Kenfack E, Staessen JA. Urinary peptidomic biomarker for personalized prevention and treatment of diastolic left ventricular dysfunction. Proteomics Clin Appl. 2019;13(2):e1800174.

[66] Huang QF, Trenson S, Zhang ZY, et al. Biomarkers to assess right heart pressures in recipients of a heart transplant: a proof-of-concept study. Transplant Direct. 2018;4(5):e346.
Redfield MM, Rodeheffer RJ, Jacobsen SJ, et al. Plasma brain natriuretic peptide to detect preclinical ventricular systolic or diastolic dysfunction: a community-based study. Circulation. 2004;109(25):3176–3181.

M’Buyamba-Kabangu JR, Anisiuba BC, Ndiaye MB, et al.; on behalf of the Newer versus Older Antihypertensive Agents in African Hypertensive Patients Trial (NOAAH) Investigators. Efficacy of newer versus older antihypertensive drugs in black patients living in sub-Saharan Africa. J Hum Hypertens. 2013;27(12):729–735.

Buse JB, Wexler DJ, Tsapas A, et al. 2019 update to: management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2020;63(2):221–228.

Pitt B, Pfeffer MA, Assmann SF, et al.; TOPCAT Investigators. Spironolactone for heart failure with preserved ejection fraction. N Engl J Med. 2014;370(15):1383–1392.

Edelmann F, Wachter R, Schmidt AG, et al. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction. The Aldo-HF randomized controlled clinical trial. J Am Med Assoc. 2013;309(8):781–791.

Zhang ZY, Ravassa S, Pejchinovski M, et al. A urinary fragment of mucin-1 subunit a is a novel biomarker associated with renal dysfunction in the general population. KI Reports. 2017;2(5):811–820.

Apostolopoulos V, Stojanovska L, Gargosky SE. MUC1 (CD227): a multi-tasked molecule. Cell Mol Life Sci. 2015;72(23):4475–4500.

Eckardt KU, Alper SL, Antignac C, et al.; Kidney Disease: Improving Global Outcomes. Autosomal dominant tubulointerstitial kidney disease: diagnosis, classification, and management-A KDIGO consensus report. Kidney Int. 2015;88(4):676–683.

Kirby A, Gniirke A, Jaffe DB, et al. Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in MUC1 missed by massively parallel sequencing. Nat Genet. 2013;45(3):299–303.

Llorian M, Beullens M, Andrés I, et al. SIPP1, a novel pre-mRNA splicing factor and interactor of protein phosphatase-1. Biochem J. 2004;378(Pt 1):229–238.

Neumann J. Altered phosphatase activity in heart failure, influence on Ca2+ movement. Basic Res Cardiol. 2002;97(7):I1–I95.

Pfeiffer ER, Tangney JR, Omens JH, et al. Biomechanics of cardiac electromechanical coupling and mechanoelectric feedback. J Biomech Eng. 2014;136(2):021007.

Voigt J, Sasha John M, Taylor A, et al. A reevaluation of the costs of heart failure and its implications for allocation of health resources in the United States. Clin Cardiol. 2014;37(5):312–321.
DATA SUPPLEMENT

Urinary Proteomics Combined with Home Blood Pressure Telemonitoring for Health Care Reform Trial: Rational and Protocol

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Urinary proteomics

Sample preparation and CE-MS analysis

For proteomic analysis, a 0.7 mL aliquot of stored urine was thawed immediately before use and diluted with 0.7 ml of 2 M urea, 10 mM NH₄OH containing 0.02% sodium dodecyl sulphate. To remove higher molecular mass proteins, such as albumin and immunoglobulins, the sample was ultra-filtered, using Centrisart ultracentrifugation filter devices (20 kDa MWCO; Sartorius, Göttingen, Germany) at 3,000 relative centrifugal force units until 1.1 ml of filtrate was obtained. This filtrate was then applied onto a PD-10 desalting column (GE Healthcare, Uppsala, Sweden) equilibrated in 0.01% NH₄OH in HPLC-grade in H₂O (Carl Roth GmbH, Karlsruhe, Germany) to decrease matrix effects by removing urea, electrolytes, salts, and to enrich polypeptides. Finally, all samples were lyophilized, stored at 4°C, and suspended in HPLC-grade H₂O shortly before CE-MS analyses.

CE-MS analyses were performed using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) on-line coupled to a micrOTOF MS (Bruker Daltonics, Bremen, Germany).¹² The electrospray ionization device (Agilent Technologies, Palo Alto, CA, USA) was grounded, and the ion spray interface potential was set between -4 and -4.5 kV. Data acquisition and MS acquisition methods were automatically controlled by the CE via contact-close-relays. Spectra were accumulated every 3 seconds, over a mass-to-charge ratio (m/z) ranging from 350 to 3000.

Quality control

Accuracy, precision, selectivity, sensitivity, reproducibility (Figure S1) and stability of the CE-MS have been previously published.¹³ Quality control involves daily CE-MS analysis of a human urine standard.³ To prevent variability due to carry-over effects from one to the next analysis, capillaries are reconditioned between runs with 1 M NaOH. Figure S1 shows
the data from 6 CE-MS analyses of the human urine standard. The coefficient of variance estimated from over 600 human urine standard analyses for over 3 years was 5.8%.4

**Mass spectrometric data processing**

Mass spectral peaks representing identical molecules at different charge states were deconvoluted into single masses, using MosaiquesVisu software.5 Only signals with a charge >1 observed in a minimum of three consecutive spectra with a signal-to-noise ratio of at least 4 were considered. Reference signals of 1770 urinary polypeptides were used for CE-time calibration by locally weighted regression. For normalization of analytical and urine dilution variances, signal intensities were normalized relative to 29 “housekeeping” peptides.6,7 The obtained peak lists characterize each polypeptide by its molecular mass, normalized CE migration time and normalized signal intensity. All detected peptides were deposited, matched, and annotated in a Microsoft SQL database, allowing further statistical analysis.8 For clustering, peptides in different samples were considered identical, if the mass deviation was less than 50 ppm. CE migration time was controlled to be below 0.35 minutes after calibration.

**Sequencing of polypeptides**

CE-MS signals were in silico assigned to the previously sequenced peptides from Human Urinary Proteome Database, version 2.0 9. Peptides from this database were sequenced, as described elsewhere.10,11 Briefly, urinary peptides were fragmented, using different tandem mass spectrometric techniques with a prior separation step with CE or high-pressure liquid chromatography (HPLC). Fragmentation spectra were matched to the protein sequences from up-to-date public databases (IPI, NCBI Reference Sequence Database and Uniprot), using MS/MS search engines MASCOT (Matrix Sciences Ltd., London, UK) and OMSSA (National Center for Biotechnology Information, Bethesda, MD, USA). In matching, we
accounted for urinary proteins post-translational modifications, such as hydroxylation of lysine and proline, and specific MS characteristics. Peptide sequences from liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses were verified by the comparison of experimental and theoretical CE migration time, which is dependent on the number of basic and neutral polar amino acids.

Identified specific urinary peptides were combined into multidimensional classifiers, using the support vector machine-based MosaCluster software, version 1.7.0 12. MosaCluster calculates classification scores based on the amplitudes of the selected biomarkers. Classification is performed by determining the Euclidian distance (defined as the support-vector machine classification score) of the vector to a maximal margin hyperplane.

**Regulatory issues**

The CE-MS technology as developed by Mosaiques-Diagnostiques GmbH, Hannover, Germany (technology readiness level, 9) is commercialized by DiaPat®, a subsidiary of Mosaiques-Diagnostics GmbH. From a regulatory point of view, the clinical application of UPP, commercialized via DiaPat®, is authorized as an in-vitro diagnostic (IVD) tool, according to EU Directive 98/79/EG for IVD diagnostics, the Act on Medical Devices, and the DIN EN ISO 13485:2003 quality system regulations for medical devices. Certification in Germany (valid across the EU) has been obtained for kidney disease (DE/CA09/0829/IVD/001), ureteropelvic junction obstruction (DE/CA09/0829/IVD/004), prostate/bladder cancer (DE/CA09/0829/IVD/003), cholangiocellular carcinoma (DE/CA09/0829/IVD/006), coronary artery disease (DE/CA09/0829/IVD/005), and graft-versus-host disease (DE/CA09/0829/IVD/002). In 2016, FDA accepted UPP as valid diagnostic instrument in the context of CKD and issued a letter of support (https://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/UCM518268.pdf).
Sample size

Population research and a PubMed search informed the sample size calculations.

- In the Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO), a family-based study of the general population in Flanders, Belgium, the incidence of asymptomatic new-onset or worsening DVD was 9.1% over a median follow-up of 4.7 years,\textsuperscript{13} which would translate into 7.8% over 4 years. Of these incident cases, 30% also had CKD stage-3 or higher. Avoiding the overlap between DVD and CKD, the 4-year incidence of DVD without CKD in this population sample was 5.5%.

- In FLEMENGHO, DVD was a forerunner of a composite cardiovascular endpoint, occurring at a rate of 7.8% over 4.7 years,\textsuperscript{14} which would translate into a 4-year incidence of 6.7%.

- In the T2DM patients enrolled in PRIORITY (Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention of Early Diabetic Nephropathy in Type-2 Diabetic Patients with Normal Albumin Excretion),\textsuperscript{15-17} the incidence of microalbuminuria and new-onset CKD stage-3 or worse over a median follow-up of 2.7 years were 11.2% and 9.7%, respectively, corresponding with 4-year rates of 16.1% and 14.0%, respectively.

- The main driver of new-onset or worsening DVD and its progression to heart failure is insulin resistance.\textsuperscript{18-22} Unfortunately, an extensive PubMed search did not reveal any prospective cohort study in T2DM with serial echocardiographic imaging, from which the DVD incidence in diabetic patients could be deduced.

- In two cluster-randomized trials of HTM, both running over 12 months, the patient eligibility criteria included an age range of 18-85 years, receiving antihypertensive drugs, and having an office blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic. Compared with usual care, based on office blood pressure, the between-group
differences in systolic blood pressure (usual care minus HTM), were 3.7 mm Hg (95% confidence interval [CI], 2.4-8.5 mm Hg; p=0.013)\textsuperscript{23} and 5.5 mm Hg (CI, 2.4-7.0 mm Hg; p<0.001) at 6 and 12 months of follow-up, respectively.\textsuperscript{24} However, in another cluster randomized trial, in which HTM and a pharmacist-managed intervention was compared with usual care, 57.2% vs 30.0% of patients achieved blood pressure control after 6 and 12 months, in the intervention and control group, respectively,\textsuperscript{25} but at 54 months the differential reduction in systolic blood pressure in favour of the intervention group was only 2.5 mm Hg (CI, -6.3 to 1.2 mm Hg; p=0.18).\textsuperscript{26}

\begin{itemize}
  \item In a meta-regression analysis of 62,605 hypertensive patients enrolled in nine placebo-controlled trials, the relative risk reduction associated with a 3-mm Hg reduction in systolic blood pressure was approximately 15%.\textsuperscript{27}
  \item The incidence of a composite cardiovascular endpoint and the associated hazard ratio in relation to the out-of-the-office blood pressure was estimated by analysing the International Databases on Ambulatory (IDACO)\textsuperscript{28} and Home (IDHOCO)\textsuperscript{29} Blood Pressure in Relation to Cardiovascular Outcome. The database included 17,624 participants enrolled in 13 population studies.\textsuperscript{30} The composite cardiovascular outcome consisted of cardiovascular mortality combined with non-fatal coronary events, non-fatal heart failure and non-fatal stroke, but not including transient ischemic attack. The age band 55-75 years included 3224 participants at risk. Over 39,503 person-years of follow-up, 693 people experienced a composite cardiovascular endpoint at a rate of 21.3 events per 1000 person-years (CI, 19.6-23.0 events per 1000 person-years). The number of person-years expected to accrue during 4 years in UPRIGHT-HTM amounts to 3450 (assuming an “effective” median follow-up of approximately 3.5 years). Based on the IDACO combined with IDHOCO data,\textsuperscript{30} 43 “hard” cardiovascular events are expected to occur in the UPRIGHT-HTM control group and 30 in the intervention group.
\end{itemize}
Furthermore, in the age band 55-75 years, the hazard ratio of a composite cardiovascular endpoint associated with a 20 mm Hg higher home systolic blood pressure was 1.46 (CI, 1.30-1.64). Assuming in UPRIGHT-HTM a 3-mm Hg lower systolic blood pressure in the experimental compared with the control group, the risk of a “hard” cardiovascular outcome would be 5.5% lower in the experimental group (CI, 3.8-7.1%). However, the aforementioned risk and risk reduction estimates will be substantially higher in UPRIGHT-HTM, because this trial – by design – only includes high-risk patients instead of people randomly recruited from the population.

- The incidence of a composite cardiovascular endpoint in ROADMAP (Randomized Olmesartan and Diabetes Microalbuminuria Prevention), over a median follow-up of 3.2 years was 4.3%, translatable into 5.3% in UPRIGHT-HTM (108 events over 2000 patient-years in the UPRIGHT-HTM control group and 76 in the intervention group). Microalbuminuria developed in 8.2% of the patients in the olmesartan group (178 of 2160 patients who could be evaluated) and in 9.8% in the placebo group (210 of 2139); the time to the onset of microalbuminuria increased by 23% on olmesartan (hazard ratio for onset of microalbuminuria, 0.77; CI, 0.63-0.94; p=0.01). Extrapolation from these ROADMAP data, suggest an incidence of 172 events over 3.5 years of follow-up of the UPRIGHT-HTM control group). However, mean age in ROADMAP was 57.8 years (range, 18-75 years), and only 25% of the ROADMAP patients were 65 years or older.

- In ACCORD (Action to Control Cardiovascular Risk in Diabetes), over a median follow-up of 4.7 years, a composite cardiovascular endpoint, also including coronary revascularization, occurred at a rate of 21.7%, which would be equivalent to 18.8% over 4 years (368 events in the UPRIGHT-HTM control group over an effective median follow-up of 3.5 years). In ACCORD, mean age was 62.2 years.
Treatment guidelines

Caregivers will follow present-day national or international guidelines for the management of risk factors. The national/regional coordinators will supervise how caregivers inform eligible patients on the nonpharmacological and pharmacological management of their risk factors. In particular, at the screening visit, the patients will be counselled about the lifestyle measures to be implemented.

Lifestyle

Lifestyle changes will be recommended and reinforced during the whole trial, including stopping smoking, moderation of excessive alcohol intake, regular physical activity, and weight reduction in overweight and obese patients, dietary measures to control dyslipidaemia or improve glycaemic control, and a moderation of salt intake. Although the evidence is limited, observational studies suggest that a daily sodium intake of less than 4.6 grams per day (260 millimoles or 11.5 grams of salt), but not lower than 2.3 grams per day (100 millimoles or 5.75 grams of salt) might be optimal.33,34

Antihypertensive treatment

The blood pressure lowering treatment will be optimized, for instance as outlined below.

□ By using combinations of antihypertensive drugs with different mode of action in line with the AB/CD algorithm (figure 3);35

□ By using antihypertensive agents with a long duration of action, so-called forgiving drugs, based on their catabolic pathway, but not based on their galenic formulation (e.g., amlodipine vs nifedipine slow release);36

□ By enhancing adherence to antihypertensive drug treatment via a reduction of the pill load and by prescription of single-pill combination tablets, including two or three antihypertensive agents in varying doses;37
According to the 2018 ESC/ESH guideline, single pill combinations containing agents of two different antihypertensive drug classes can be prescribed to initiate antihypertensive treatment;37

Antihypertensive drug combinations, certainly at the stage of triple therapy, should include a diuretic;38

If not contra-indicated, aldosterone receptor antagonists and β-blockers should at least be attempted in treatment-resistant patients.39

**Lipid-lowering treatment**

Because UPRIGHT-HTM will enrol high-risk, albeit asymptomatic patients, in principle, most if not all, qualify for statin therapy on top of lifestyle intervention, unless there are contraindications or unless statins are not well tolerated.40,41 If a patient does not tolerate a high-intensity statin, the aim should be to treat the patient with the maximum tolerated dose.42 The following strategies apply if side-effects occur on statin therapy: (i) tell the patient that any statin at any dose reduces cardiovascular risk; (ii) discontinue the statin, but rechallenge the patients when the symptoms have resolved to check if they are truly related to the statin; (iii) reduce the dose within the same intensity group (table 5); and (iv) start a statin of a lower intensity group (table 5).42,43 An alternative is to combine a low-dose low-intensity statin with ezetimibe. For severe hypertriglyceridaemia, fibrates are indicated.44

**Antidiabetic treatment**

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) published an algorithm for blood glucose lowering in T2DM patients (Figure S5).45 What follows highlights key principles in the organization of drug treatment for controlling the blood glucose levels.
The target HbA1c must be personalized according to an individual patient’s needs and circumstances, including the patient’s preferences, risks from polypharmacy and from too tight glycaemic control. Clinical inertia should be avoided. HbA1c should be measured at 3/6-monthly intervals, as appropriate.

First-line therapy is lifestyle optimization, including dietary and weight management and physical activity. The 2020 WHO guidelines on physical activity propose 150-300 minutes of moderate-intensity aerobic physical activity, or at least 75-150 minutes of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate-intensity and vigorous-intensity activity throughout the week for substantial health benefits. Adults should also do muscle-strengthening activities at moderate or greater intensity that involve all major muscle groups on 2 or more days a week, as these provide additional health benefits. Adults should limit the amount of time spent being sedentary. Replacing sedentary time with physical activity of any intensity (including light intensity) provides health benefits.

If lifestyle management is not sufficient to reduce HbA1c below the personalized target, metformin is the first-choice medical therapy in combination with the ongoing lifestyle adaptations. Metformin should be slowly titrated towards the maximal tolerated dose (usually <2000 mg per day).

When lifestyle management and metformin in optimal dose are not sufficient, other medication classes should be considered. The treatment of choice is influenced by co-morbidities (atherosclerotic cardiovascular disease, heart failure), need to minimize hypoglycaemia or need for weight loss. Cost and local regulations on reimbursement can influence the choice. The 2019 update to the 2018 consensus report on glucose-

lowering medication in T2DM established by ADA/EASDEASD, updated in 2020, should be the guide for the most appropriate drug choices (Figure S4).45

- Sodium-glucose cotransporter 2 inhibitors (SGLT2-Is) are recommended in patients with T2DM and heart failure, particularly in those with reduced ejection fraction with the objective to reduce hospitalization for heart failure, major adverse cardiovascular events and cardiovascular death.45,47

- SGLT2-Is are also recommended to prevent the progression of CKD, hospitalized heart failure, major adverse cardiovascular events and cardiovascular death in T2DM patients with CKD.45,47

**Echocardiography**

Studies are to be performed with the state-of-the-art ultrasound device equipped with 2.5- to 3.5-MHz transducer with M-mode, two-dimensional (2D), and Doppler (pulsed, continuous-wave, colour-flow, and tissue) capabilities according to current guidelines.48-53 The ECG must be simultaneously recorded and blood pressure must be measured three times consecutively, using a validated blood pressure monitor. All echocardiographic examinations will be stored in a digital format for off-line analysis.

**Echocardiographic scanning sequence**

Parasternal long-axis view:

a. 2D of LV during quiet respiration: store 5 beats;

b. M-mode cursor perpendicular through left ventricle just below the level of the mitral leaflet tips: record M-mode over 5 beats;
c. Optional: colour Doppler myocardial imaging: posterior wall; 2 sets of 5 beats with a colour scale adjustment (for instance, 16 and 20, if feasible); >150 fps;
d. 2D of LVOT and aortic annulus over 5 beats;
e. M-mode cursor perpendicular through aorta and left atrium; record M-mode over 5 beats;
f. Colour-flow Doppler recordings to search for mitral and aortic (5 beats) regurgitation;
g. Turn 90 degrees into parasternal short axis view.

Parasternal short-axis view:

a. 2D at level of aorta and left atrium during quite respiration: 5 beats;
b. PW Doppler recording in pulmonary artery: 5 beats;
c. 2D of LV at level of mitral valve (basal): 5 beats (>150 fps);
d. Optional: colour Doppler myocardial imaging (basal level) – sets of 5 beats with a colour scale adjustment (preferable 16);
e. 2D of LV at level of papillary muscles (middle): 5 beats;
f. 2D of LV at apical level: 5 beats (>50 fps; no papillary muscles interference);
g. Optional: colour Doppler myocardial imaging (apex) – sets of 5 beats with a colour scale adjustment (preferable 16).

Apical four-chamber view:

a. 2D of LV and left atrium: 5 beats to be recorded with maximum chamber dimensions and sharp LV endocardial LV endothelial delineation for measurement of the global longitudinal LV strain; >130 fps; left atrial dimensions (5 beats);
b. If possible, PW Doppler transmitral flow recordings with sample volume at leaflet tips during diastole (5 beats);
c. If possible, PW Doppler pulmonary vein recordings (5 beats) guided by colour Doppler;
d. PW Doppler recording positioned between LV inflow and LV outflow tract for IVRT measurement (5 beats);
e. PW Doppler LV outflow recording (5 beats);
f. CW Doppler aortic valve recordings (5 beats);
g. Colour-flow Doppler recordings of mitral valve (5 beats) and CW Doppler recordings of mitral valve in case of detection of moderate or severe mitral regurgitation (5 beats);
h. Colour-flow Doppler recordings of aortic valve (5 beats);
i. Colour-flow Doppler recordings over the tricuspid valve (5 beats) and CW Doppler recordings for detection of tricuspid regurgitation, if any (5 beats); estimation pf pulmonary arterial systolic blood pressure;
j. Optional: colour Doppler myocardial imaging: septum (2 sets of images with differently adjusted colour scale; >150 fps; 5 beats each) and lateral wall (2 sets; >150 fps; 5 beats each);
k. Mitral Annular Doppler Tissue Imaging: the DTI PW Doppler sample volume is placed at the medial (septal) corner (5 beats) and, subsequently, at the lateral corner of the mitral annulus (5 beats);
l. Turn 60 degrees counter clockwise.

Apical two-chamber view:

a. 2D of LV during quiet respiration: 5 beats; FPS >150; for global LV strain measurement by speckle tracking;
b. Optional: colour Doppler myocardial imaging: inferior wall (2 sets; FPS >150; 5 beats);

c. Optional: mitral annular Doppler Tissue Imaging: the DTI PW Doppler sample volume is placed at the inferior (left) wall corner of the mitral annulus (5 beats);

d. Turn another 60 degrees counter clockwise.

Apical three-chamber view:

a. 2D of LV during quiet respiration: 5 beats; >150 fps; for global LV strain measurement by speckle tracking;

b. Optional: colour Doppler myocardial imaging: posterior (inferolateral) wall (2 sets; >150 fps; 5 beats);

c. Optional: mitral annular Doppler Tissue Imaging: the DTI PW Doppler sample volume is placed at the posterior (inferolateral) (left) wall corner of the mitral annulus (5 beats).

Post-processing

Guidelines describe the methods to limit variability in the echocardiographic measurements within and between sessions and among readers. The quantitative echocardiographic measurements are summarized in Table S3.

1. Minimize the number of readers and monitor reader variability;

2. Monitoring of sonographers for technical quality; encode study quality in database;

3. Reading strategies:

   a. Batch read, when possible, to minimise systematic temporal drifts; or as an alternative to batch reading, periodically reread sample aliquots to measure trends in systematic temporal bias;
b. Average multiple beats (minimum of 3 in regular rhythm, 5 in atrial fibrillation);

4. Establish acquisition and reader variability
   a. Test-retest a small sample of participants, i.e., the same participant repeated over small interval, same machine, same sonographer, and same reader.
   b. Blind duplicates for inter-reader and intra-reader variability assessment.

5. Directly export individual patients' echocardiographic measurements from the echocardiographic software to an Excel format to avoid keying errors.
References

1. Theodorescu D, Wittke S, Ross MM, et al. Discovery and validation of new protein biomarkers for urothelial cancer: a prospective analysis. *Lancet Oncol.* 2006;7:230-240.

2. Wittke S, Mischak H, Walden M, Kolch W, Rädler T, Wiedemann K. Discovery of biomarkers in human urine and cerebrospinal fluid by capillary electrophoresis coupled to mass spectrometry: towards new diagnostic and therapeutic approaches. *Electrophoresis.* 2005;26:1476-1487.

3. Mischak H, Kolch W, Aivaliotis M, et al. Comprehensive human urine standards for comparability and standardization in clinical proteome analysis. *Proteomics Clin Appl.* 2010;4:464-478.

4. Mischak H, Vlahou A, Ioannidis JPA. Technical aspects and inter-laboratory variability in native peptide profiling: the CE-MS experience. *Clin Biochem.* 2013;46:432-443.

5. Neuhoff NV, Kaiser T, Wittke S, et al. Mass spectrometry for the detection of differentially expressed proteins: a comparison of surface-enhanced laser desorption/ionization and capillary electrophoresis/mass spectrometry. *Rapid Commun Mass Spectrom.* 2004;18:149-156.

6. Haubitz M, Good DM, Woywodt A, et al. Identification and validation of urinary biomarkers for differential diagnosis and evaluation of therapeutic intervention in anti-neutrophil cytoplasmic antibody-associated vasculitis. *Moll Cell Proteomics.* 2009;8:2296-2307.

7. Jantos-Siwy J, Schiffer E, Brand K, et al. Quantitative urinary proteome analysis for biomarker evaluation in chronic kidney disease. *J Proteome Res.* 2009;8:268-281.
8. Dakna M, He Z, Yu WC, Mischak H, Kolch W. Technical, bioinformatical and statistical aspects of liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) based clinical proteomics: a critical assessment. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2009;877:1250-1258.

9. Stalmach A, Albalat A, Mullen W, Mischak H. Recent advances in capillary electrophoresis coupled to mass spectrometry for clinical proteomic applications. *Electrophoresis.* 2013;34:1452-1464.

10. Coon JJ, Zürbig P, Dakna M, et al. CE-MS analysis of the human urinary proteome for biomarker discovery and disease diagnostics. *Proteomics Clin Appl.* 2008;2:964-973.

11. Rossing K, Mischak H, Dakna M, et al. Urinary proteomics in diabetes and CKD. *J Am Soc Nephrol.* 2008;19:1283-1290.

12. Delles C, Schiffer E, von Zur Muhlen C, et al. Urinary proteomic diagnosis of coronary artery disease: identification and clinical validation in 623 individuals. *J Hypertens.* 2010;28:2316-2322.

13. Kuznetsova T, Thijs L, Knez J, et al. Longitudinal changes in left ventricular diastolic function in a general population. *Circ Cardiovasc Imaging.* 2015;8:e002882.

14. Kuznetsova T, Thijs L, Knez J, Herbots L, Zhang Z, Staessen JA. Prognostic value of left ventricular diastolic dysfunction in a general population. *J Am Heart Assoc.* 2014;3:e000789.
15. Lindhardt M, Persson F, Zürbig P, et al. Urinary proteomics predict onset of microalbuminuria in normoalbuminuric type 2 diabetic patients, a sub-study of the DIRECT-Protect 2 study. *Nephrol Dial Transplant*. 2017;32:1866-1873.

16. Tofte N, Lindhardt M, Adamova K, et al. Characteristics of high- and low-risk individuals in the PRIORITY study: urinary proteomics and mineralocorticoid receptor antagonism for prevention of diabetic nephropathy in type 2 diabetes. *Diabet Med.* 2018;35:1375-1382.

17. Tofte N, Lindhardt M, Adamova K, 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 Diabet Endocrinol.* 2020;8:301-312.

18. Lacombe VA, Viatchenko-karpinski S, Terentyev D, et al. Mechanisms of impaired calcium handling underlying subclinical diastolic dysfunction in diabetes. *Am J Physiol Regul Integr Comp Physiol.* 2007;293:R1787-R1797.

19. Stahrenberg R, Edelmann F, Mende M, et al. Association of glucose metabolism with diastolic function along the diabetic continuum. *Diabetologia*. 2010;53:1331-1340.

20. Jørgensen PG, Jensen MT, Mogelv ang R, et al. Abnormal echocardiography in patients with type 2 diabetes and relation to symptoms and clinical characteristics. *Diabet Vasc Dis Res.* 2016;13:321-330.

21. von Bibra H, Paulus W, St.John Sutton M. Cardiometabolic syndrome and increased risk of heart failure. *Curr Heart Fail Rep.* 2016;13:219-229.
22. Jørgensen PG, Jensen MT, Biering-Sørensen T, et al. Burden of uncontrolled metabolic risk factors and left ventricular structure and function in patients with type 2 diabetes mellitus. *J Am Heart Assoc.* 2018;7:e008856.

23. McManus RJ, Mant J, Bray EP, et al. Telemonitoring and self-management in the control of hypertension (TASMINH2): a randomised controlled trial. *Lancet.* 2010;376:163-172.

24. McManus RJ, Mant J, Franssen M, et al. Efficacy of self-monitored blood pressure, with or without telemonitoring, for titration of antihypertensive medication (TASMINH4): an unmasked randomised controlled trial. *Lancet.* 2018;391:949-959.

25. Margolis KL, Asche SE, Bergdall AR, et al. Effect of home blood pressure telemonitoring and pharmacist management on blood pressure control: a cluster randomized clinical trial. *J Am Med Assoc.* 2013;310:46-56.

26. Margolis KL, Asche SE, Dehmer SP, et al. Long-term outcomes of the effects of home blood pressure telemonitoring and pharmacist management on blood pressure among adults with uncontrolled hypertension: follow-up of a cluster randomized clinical trial. *JAMA Network Open.* 2018;1:e181617.

27. Staessen JA, Wang JG, Thijs L. Cardiovascular protection and blood pressure reduction: a meta-analysis. *Lancet.* 2001;358:1305-1315.

28. Yang WY, Melgarejo JD, Thijs L, et al. Association of office and ambulatory blood pressure with mortality and cardiovascular outcomes. *J Am Med Assoc.* 2019;409-420.
29. Niiranen TJ, Hänninen MR, Johansson J, Reunanen A, Jula AM. Home-measured blood pressure is a stronger predictor of cardiovascular risk than office blood pressure: the Finn-Home study. *Hypertension.* 2010;55:1346-1351.

30. Li Y, Thijs L, Zhang ZY, *et al.* Opposing age-related trends in absolute and relative risk of adverse health outcomes associated with out-of-office blood pressure. *Hypertension.* 2019;74:1333-1342.

31. Haller H, Ito S, Izzo JL, Jr., *et al.* Olmesartan for the delay or prevention of microalbuminurinuria in type 2 diabetes. *N Engl J Med.* 2011;364:907-917.

32. The ACCORD Study Group. Effects of intensive blood-pressure control in type 2 diabetes mellitus. *N Engl J Med.* 2010;362:1575-1585.

33. O'Donnell M, Mente A, Rangarajan S, *et al.* Urinary sodium and potassium excretion, mortality, and cardiovascular events. *N Engl J Med.* 2014;371:612-623.

34. O'Donnell M, Mente A, Alderman MH, *et al.* Salt and cardiovascular disease: insufficient evidence to recommend low sodium intake. *Eur Heart J.* 2020;41:3363-3373.

35. National Institute for Health and Care Excellence. Hypertension in adults: diagnosis and treatment. NICE guideline [NG136]. Publication date: August 2019.

36. Hernández-Hernández R, Armas de Hernández MJ, Armas-Padilla MC, Carvajal AR, Guerrero-Pajuelo J. The effects of missing dose of enalapril versus amlodipine on ambulatory blood pressure. *Blood Press Monit.* 1996;1:121-126.

37. Williams B, Mancia G, Spiering W, *et al.* 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur Heart J.* 2018;39:3021-3104.
38. Whelton PK, Carey RM, Aronow WS, et al. ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol.* 2018;71:e127-e248.

39. Wei FF, Zhang ZY, Huang QF, Staessen JA. Diagnosis and management of resistant hypertension: state of the art. *Nat Rev Nephrol.* 2018;14:428-441.

40. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J.* 2016;37:2999-3058.

41. National Institute for Health and Care Excellence. Lipid modification therapy for preventing cardiovascular disease. NICE pathway last updated: 19 November 2019.

42. National Institute for Health and Care Excellence. Lipid-modifying drugs (ktt3). 2015.

43. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *Br Med J.* 2003;326:1423.

44. National Institute for Health and Care Excellence. Hypertriglyceridaemia management according to the 2018 AHA/ACC guideline (accessed 13 February 2020).

45. Buse JB, Wexler DJ, Tsapas A, et al. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia.* 2020;63:221-228.
46. Bull FC, Al-Ansari SS, Biddle S, et al. World Health Organization 2020 guideline on physical activity and sedentary behaviour. Br J Sports Med. 2020;54:1451-1462.

47. Yan C, Thijs L, Cao Y, et al. Opportunities of antidiabetic drugs in cardiovascular medicine: a meta-analysis and perspectives in trial design. Hypertension. 2020;76:420-431.

48. Gottdiener JS, Bednarz J, Devereux R, et al. A report from the American Society of Echocardiography's Guidelines and Standard Committee and the Task Force on Echocardiography in Clinical Trials. J Am Soc Echocardiogr. 2004;17:1086-1119.

49. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr. 2009;22:107-133.

50. Galderisi M, Henein MY, D'Hooge J, et al. Recommendations of the European Association of Echocardiography. Eur J Echocardiogr. 2011;12:339-353.

51. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015;16:233-270.

52. Manwick TH, Gillebert TC, Aurigemma G, et al. Recommendations on the use of echocardiography in adult hypertension: a report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography. J Am Soc Echocardiogr. 2015;28:727-754.
53. Nagueh SF. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr.* 2016;29:277-314.
Table S1. Sample size

| Rate in the control group (%) | Rate in the experimental group (%) | Rate decrease in the experimental group (%) | N per group |
|-------------------------------|-----------------------------------|------------------------------------------|------------|
| 0.20                          | 0.14                              | 30                                       | 574        |
| 0.20                          | 0.12                              | 40                                       | 310        |
| 0.25                          | 0.18                              | 30                                       | 504        |
| 0.25                          | 0.15                              | 40                                       | 234        |
| 0.30                          | 0.21                              | 30                                       | 343        |
| 0.30                          | 0.18                              | 40                                       | 186        |

All sample size calculation assumed a two-sided $\alpha$-level of 0.05 and 80% power. Rates refer to the incidence of the primary endpoint (%) over 4 years.
Table S2. Grouping of statins by potency to reduce low-density lipoprotein cholesterol

| Daily dose (mg/day) | 5   | 10  | 20  | 40  | 80  |
|---------------------|-----|-----|-----|-----|-----|
| Fluvastatin         | …   | …   | -21%| -27%| -33%|
| Pravastatin         | …   | -20%| -24%| -29%| …   |
| Simvastatin         | …   | -27%| -32%| -37%| -42%|
| Atorvastatin        | …   | -37%| -43%| -49%| -55%|
| Rosuvastatin        | -38%| -43%| -48%| -53%| …   |

Reproduced with permission from references 42,43.
| Echocardiographic window and view | Measurements | Calculated variables |
|---------------------------------|--------------|----------------------|
| Parasternal long-axis; M-mode (short axis might also be used) | LV internal dimension (LVID), interventricular septal (IVS) and posterior wall (PW) thicknesses measured at end-diastole (d) and end-systole (s) | LV structure - LV mass, relative wall thickness |
|                                 |              | LV systolic function - systolic endocardial fractional shortening, ejection fraction; end systolic stress (ESS) |
| Parasternal long-axis; M-mode   | Left atrial dimension (end-systole), aortic root dimension | |
| Parasternal long-axis; 2D       | Aortic annular diameter (systole) | LV systolic function - Doppler stroke volume |
| 4-chamber apical; pulsed Doppler of LVOT | Velocity-time integral (VTI) | |
| 4-chamber apical; 2D            | LV cavity volume measurements at the end diastole and end systole (methods of discs). | LV systolic function – ejection fraction |
| 4-chamber apical; pulsed Doppler of transmitral flow | Peak early diastolic mitral flow (E peak) and late diastolic mitral flow (A peak, atrial filling fraction) wave velocities, deceleration times of E peak and duration of A peak, isovolumetric relaxation time (IVRT) | LV diastolic function - E/A ratio |
| 4-chamber apical; pulsed Doppler of pulmonary vein | Pulmonary vein systolic (PVs), diastolic (PVD), and atrial reversal (PVA) wave velocities, and duration of flow (a-dur) | |
| 4-chamber and 2-chamber apical, mitral annulus velocities and strain rate of medial, anterior, inferior and lateral walls | Peak systolic myocardial (Sm), early diastolic myocardial (Em), and late diastolic myocardial (Am) velocities, deceleration time of Em and duration of Am. | LV diastolic function - Em/Am ratio, E/Em ratio, Se/Sa ratio |
|                                 | Peak systolic (S), time to peak systolic S, peak SR E (Se) and peak SR A (Sa), time to onset of Se. | |
### Table S4. Schematic representation of the statistical workflow for the molecular analyses

| Analysis step                      | Statistical method                                                                                                                                 |
|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Preparing for analysis             | Checking distributions, logarithmic transformation, rank normalization, removal of outliers                                                   |
| Basic statistical approaches       | Large-sample z test, t-test or ANOVA (means); $\chi^2$ statistic or Fisher exact test (proportion); log-rank test (survival functions); analyses across quantiles of biomarkers; scatterplots; standardization of rates |
| Identification of covariables      | Stepwise linear or stepwise logistic regression                                                                                                   |
| Analyses with **continuous** outcome |                                                                                                                                                  |
| Single urinary peptides, one at a time |                                                                                                                                                  |
| Cross-sectional analyses           | Multivariable-adjusted linear regression, correction for multiple testing                                                                         |
| Longitudinal analyses              | Multivariable-adjusted linear regression (including adjustment for the baseline value of the outcome, if available) with correction for multiple testing |
| All markers                        |                                                                                                                                                  |
| Cross-sectional analyses           | Partial least squares analysis                                                                                                                    |
| Longitudinal analyses              | Partial least squares analysis                                                                                                                    |
| Analyses with **categorical** outcome |                                                                                                                                                  |
| Single markers, one at a time      |                                                                                                                                                  |
| Cross-sectional analyses           | Multivariable-adjusted logistic regression with correction for multiple testing                                                               |
| Longitudinal analyses              | Multivariable-adjusted Cox regression with correction for multiple testing                                                                      |
| All markers                        |                                                                                                                                                  |
| Cross-sectional analyses           | Partial least squares discriminant analysis                                                                                                      |
| Longitudinal analyses              | Partial least squares discriminant analysis                                                                                                      |
| Prediction of adverse outcomes     | Integrated discrimination improvement, net reclassification improvement, optimized thresholds, 2-by-2 classification tables, log-rank test, receiver operating characteristic curve, c-statistic |
| Molecular pathways                 | PANTHER, DAVID, IPA, Cytoscape, Proteasix, ...                                                                                                    |
Figure S1.
Proteome coverage of 6 CE-MS runs (A–F) of human urine standards. The molecular mass on a logarithmic scale (0.8-20 kDa on the y-axis) was plotted against the normalised CE migration time (15-45 min on the x-axis). Peak height and colour represent average signal intensity. The human urine standard was a urine sample from a randomly selected healthy person, used for quality control. Reproduced with permission from reference 3.
Table showing clinical interpretation of echocardiographic measurements related to diastolic left ventricular function:

| Measurement | Description | Interpretation |
|-------------|-------------|----------------|
| E, A, E/A  | Peak velocities of the transmitral blood flow, as measured by standard Doppler techniques during rapid LV filling in early diastole (E) and during atrial contraction in late diastole (A). | E (cm/s) decreases with impaired relaxation during the early stage of diastolic LV dysfunction, but increases in the later stages with onset of LV stiffening. During progression from mild to severe diastolic LV dysfunction, the A wave (cm/s) changes in directions opposite to E. The E/A ratio is decreased in early diastolic LV dysfunction. Because mitral inflow patterns are highly sensitive to preload (left atrial pressure) and change as diastolic dysfunction progresses, the use of mitral valve inflow patterns to assess diastolic function remains limited. |
| a', a, a'W | Peak velocities of the mitral annular movement, as measured by tissue Doppler imaging (TDI) during rapid LV filling in early diastole (a') and during atrial contraction in late diastole (a). | a' (cm/s) progressively decreases during the progression of diastolic LV dysfunction. a' (cm/s) increases in the early stage of diastolic LV dysfunction, but thereafter decreases with advancing disease. TDI assessment of diastolic LV function is less load dependent than that provided by standard Doppler techniques. a' is resistant to changes in LV filling pressure. The a'/a ratio decreases in early diastolic LV dysfunction, but increases with further progression. |
| E/Em | Transmural E / mitral annular a' | This ratio reflects LV filling pressure and increases during progression of diastolic LV dysfunction. |
| Ad, ARd, LAVI | Duration of the transmitral A wave and of the reversal of flow in the pulmonary veins; left atrial volume normalised to body surface area. | Ad = ARd + 10 (ms) and LAVI ≤ 28 mL/m² confirm that LV filling pressure is high. |
| DT, IVRT | Deceleration time and isovolumetric relaxation time | DT (ms) and IVRT (ms) initially lengthen as LV relaxation is impaired, but shorten with more severe LV dysfunction. |

Figure S2.
Clinical interpretation of echocardiographic measurements related to diastolic left ventricular function.
Figure S3.
Recommendation for combining blood pressure lowering drugs according to the AB/CD rule. Modified and reproduced with permission from the 2019 National Institute for Health and Care Excellence (reference 35).
The first-line therapy to reach individualized HbA1c target is metformin and lifestyle changes (weight ↓ - physical activity ↑)

Figure S4.
ADA/EASD algorithm for blood-glucose lowering therapy in adults with type-2 diabetes mellitus
Abbreviations: ADA/EASD, American Diabetes Association/European Association for the study of Diabetes; DPP-4i, dipeptityl peptidase-4 inhibitors; GLP-1Ra, glucagon-like peptide-1 receptor agonists; SGLT2i, sodium-glucose cotransporter 2 inhibitors; TZD, thiazolidinediones; SU, sulfonylurea; INS, insulin. Modified and reproduced with permission from reference 45.