Uncontrolled hypertension

Cardiovascular risk, monitoring, and medication adherence

Eline Groenland

UNCONTROLLED HYPERTENSION

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Ongecontroleerde hypertensie: cardiovasculair risico, monitoring en therapietrouw

(met een samenvatting in het Nederlands)

Proefschrift

Ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. H. Kummeling, ingevolge het besluit van het college door promoties in het openbaar te verdedigen op dinsdag 1 november 2022 des middags te 2.15 uur

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CHAPTER 1

General introduction

General introduction

Systemic arterial hypertension (hereafter referred to as hypertension) is characterized by a persistently elevated blood pressure (BP) in the vascular system. BP is typically expressed as the ratio of the pressure that the blood exerts on the arterial walls during contraction of the heart, the systolic BP, and the pressure in the vascular system when the heart relaxes, the diastolic BP. In a clinical office setting a BP of 140/90 mmHg obtained by repeated BP measurements is most often used as the cut-off for the presence of hypertension (1). The global prevalence of hypertension is estimated at 31%, translating to approximately 1.4 billion adults worldwide (2).

Hypertension is one of the most important modifiable risk factors for cardiovascular disease (CVD; including cerebrovascular disease, coronary heart disease, atrial fibrillation, heart failure, and peripheral vascular disease), chronic kidney disease (CKD) and cognitive impairment, and is the leading risk factor to all-cause mortality worldwide (3). The likelihood of having a cardiovascular event increases as BP increases, starting as low as 115/75 mmHg (4). For every 20 mmHg higher systolic and 10 mmHg higher diastolic BP, the risk of death from CVD doubles (4,5). This relationship is independent of other CVD risk factors and thus BP level is a major component in all CVD risk prediction models (6).

Lowering BP to non-hypertensive levels through non-pharmacological and pharmacological treatment reduces the risk for CVD events by 20-40%, making control of BP an essential factor in reducing the global burden of morbidity and mortality (4,5). Although advances in the diagnosis and treatment of hypertension have led to a decrease in the overall prevalence of hypertension, rates of BP control have stagnated, with about 50% of hypertensive patients still having uncontrolled hypertension (2). Therefore, the search for further improvements in the management of patients with (uncontrolled) hypertension continues and is essential to reduce the (cardiovascular) disease burden and promote life expectancy in these patients.

I. Cardiovascular risk

An important step in the management of patients with (uncontrolled) hypertension is the estimation of CVD risk by using established risk calculators (7–9). A particularly relevant group of patients at very high risk for future CVD includes patients who have already experienced a vascular event (10). Since these patients have a high probability to benefit from antihypertensive drug therapy in addition to lifestyle changes, additional focus on this group is warranted (11).

To calculate CVD risk in patients with hypertension, risk estimation tools use well-established risk factors such as cholesterol, systolic BP, and smoking. However, despite

optimal management of these conventional risk factors, a significant residual risk of (recurrent) CVD remains. Therefore, there is an ongoing effort to identify more pathways beyond conventional risk factors for CVD. Characterizing these pathways may play a critical role in the development of novel strategies aimed at further reducing the residual CVD risk.

Understanding the role of genetics

One potential pathway currently studied is the genetic pathway. Blood pressure and hypertension are highly heritable traits, and their regulation depends on a typically polygenic contribution (12). Over 1000 single nucleotide polymorphisms (SNPs) at more than 900 genetic loci influencing BP have been identified through several genome-wide association studies (GWAS) (13,14). However, these genetic variants typically have small effects on the order of only 1 mmHg systolic BP and 0.5 mmHg diastolic BP per BP-raising allele. A polygenic risk score (PRS) is a common tool used to aggregate these small effects into a single score. Polygenic risk scores for BP have been shown to be of value in estimating risk of CVD in the general population (15,16). However, the value of such PRS in risk evaluation of patients with established vascular disease remains to be determined.

Lifestyle-related cardiovascular risk: the importance of salt consumption

Although the genetic predisposition to hypertension is non-modifiable and poses a lifelong CVD risk, the risk of hypertension is modifiable and largely preventable through a significant contribution of lifestyle factors. Among others, excessive dietary sodium and inadequate potassium intake are important lifestyle factors contributing to suboptimal BP control (17). Sodium is a crucial regulator of blood volume: high serum sodium concentration promotes fluid retention, thereby increasing blood volume and BP (18,19). At the same time, high potassium intake mitigates the effect that high sodium has on BP levels, thereby lowering BP and decreasing the risk of CVD (19,20).

In recent years, determining the optimal level of daily sodium and potassium intake has been an important topic of debate (21). The World Health Organization (WHO) has set the optimal level of sodium intake at less than 2 grams per day and that of potassium at more than 3.5 grams per day, as this is considered sufficient for metabolic balance and physiological function (22,23). Since these recommendations are largely based on evidence obtained by studies performed in the general population, it is unclear whether these recommendations can also be applied to patients with established vascular disease. More clarity is needed on the optimal dietary sodium and potassium intake for these patients, as these are the patients who are most likely to receive these recommendations in daily clinical practice.

High-risk subgroup of hypertension: apparent treatment-resistant hypertension

When BP is still not below target (office BP <140/90 mmHg), despite effective sodium restriction in combination with other lifestyle measures and BP-lowering drugs, one

should consider the presence of treatment-resistant hypertension (TRH). Treatment-resistant hypertension is defined as a BP above target despite the concurrent use of three antihypertensive drugs from different classes (commonly including a long-acting calcium channel blocker, an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, and a diuretic) in optimal doses, or a BP below target achieved by treatment with ≥4 antihypertensive drugs (1,24). Often, the term "apparent" TRH (aTRH) is used rather than TRH because comprehensive workups to rule out improper BP measurement, nonadherence, and secondary causes are not performed, and thus individuals with "pseudo-resistance" cannot be identified and excluded.

Albeit somewhat arbitrary with respect to the number of drugs required, aTRH is defined in this manner to identify patients at higher risk for CVD events and mortality. Apparent TRH has been associated with an up to 3-fold increased risk of CVD compared with treatment-responsive hypertensive patients (25–27). Although focus on this severe form of hypertension has increased during recent years, data regarding the impact of aTRH on adverse cardiovascular events in patients with established CVD is scarce. Understanding the relation between aTRH and recurrent CVD in this high-risk patient population could be of great value in motivating patients with aTRH to adhere to their risk factor management.

II. Monitoring

Monitoring of BP

Accurate measurement and recording of BP is essential to identify hypertension, ascertain BP-related CVD risk and guide management. Although office BP (OBP) measurement is still the most commonly used technique for screening and diagnosis of hypertension, it is inaccurate and importantly influenced by measurement errors and observers' bias (28). Inaccurate OBP measurements can lead to overdiagnosis ('white-coat hypertension') and unnecessary treatment, or underdiagnosis ('masked hypertension') and increased risk of CVD (29).

In an attempt to improve BP assessment in the office, unattended automated office BP (uAOBP) was developed (30). This approach involves multiple BP readings taken with a fully automated device in absence of health care providers after the patient has been resting quietly alone for a few minutes. Although preliminary studies suggest that the white-coat effect associated with conventional attended OBP can be virtually eliminated by using uAOBP (30), a number of issues related to uAOBP still need to be clarified, including its actual ability to predict outcomes better than other BP measurement methods.

These limitations, along with advances in technology and the availability of standardized validation protocols, stimulated the introduction of out-of-office BP monitoring methods

in routine clinical practice (31). Out-of office BP monitoring include home BP monitoring (HBPM) and 24-hour ambulatory BP monitoring (24-h ABPM). Home BP monitoring refers to the measurement of BP at regular intervals by an individual at their home or elsewhere outside the clinic setting. Twenty-four hour ABPM consists of measuring and recording the BP at regular intervals (usually every 20-30 minutes) during a period of 24 hours while individuals are performing their daily activities. Both methods were shown to be superior to OBP measurements for prediction of target organ damage and CVD (32,33). Therefore, current guidelines recommend out-of-office BP measurement for diagnosing hypertension (1,34). Of the two methods, the 24-hour ABPM is considered the reference standard because of the larger evidence base demonstrating its strong association with future CVD (35).

Although 24-hour ABPM has several unique advantages such as its capability of monitoring BP during sleep and daily activities, it is a burdensome and costly method that is not widely available, especially in primary care settings (28). HBPM is not only easier to use and less expensive than 24-hour ABPM, but also enables patients to take a greater role in self-management of their health, which may have a beneficial effect on medication adherence and BP control (36). However, the need for manual notation of self-measured BP by the patient, especially in the home setting, is prone to (unintentional) errors, which could compromise the reliability of HBPM (37,38). The introduction of smartphone application-assisted HBPM, in which BP measurements taken with a validated BP device can be automatically transferred to a smartphone application, might improve reliability and wide spread use of HBPM in clinical practice (39). How such app-assisted HBPM methods compare to uAOBP and the reference standard 24-hour ABPM in the measurement of BP and diagnosing hypertension is not yet well established. In addition, more clarity is needed on the HBPM protocol that provides a reliable and reproducible assessment of home BP.

Monitoring of salt intake

Reduction of sodium intake and an increase in potassium intake are considered to be among the most effective non-pharmacological interventions for lowering BP and reducing cardiovascular risk (1). A pooled analysis showed that an estimated 1 gram (43.5 mmol) reduction in daily sodium intake resulted in a 2.1 and 1.2 mmHg decrease in systolic BP among hypertensive and normotensive patients, respectively (40). Since the BP-lowering effect of sodium reduction depends on maintaining the intervention, effective monitoring of adherence to the recommended dietary salt intake is essential.

Monitoring of salt intake can accurately be done by the collection of multiple non-consecutive 24-hour urines (41). However, this method is costly and neither easy nor practical for patients (42). A more convenient method is the estimation of salt intake based on spot urine samples using formulas that estimate 24-hour salt excretion (43–45). Although these formulas can reliably be used to estimate the average salt intake level of

the population, overestimations in the low salt ranges and underestimations in the high salt ranges can occur (46). To overcome these issues, the sodium-to-potassium ratio (Na/K ratio) has been proposed as an easier and potentially more reliable alternative (47).

To further enhance effective monitoring of salt intake, self-monitoring devices that provide individuals with quick feedback on their salt excretion, are increasingly being developed and used (48,49). However, the validity of the estimated salt excretion by these self-monitoring devices has not yet been thoroughly investigated. Since the use of a self-monitoring device might motivate its users to continue sodium restriction, it is important to clarify whether changes in salt intake can be accurately estimated by such a device.

III. Medication adherence

Another important cause of poor BP control and treatment resistance is poor treatment adherence (50–52). Treatment adherence is defined by the WHO as the extent to which a patient's history of therapeutic medication-taking coincides with the prescribed treatment regimen, and the failure to do so is termed as nonadherence (53). The estimated prevalence of nonadherence in patients with hypertension varies between 10 and 86% and greatly depends on the population (uncontrolled versus treatment-resistant hypertension), the definition of nonadherence, and the method used to measure nonadherence (54,55).

Identification of nonadherence to antihypertensive drugs is of great importance since nonadherence is associated with an increased risk of CVD and mortality (56,57). According to a large meta-analysis of prospective studies, about 9% of cardiovascular events may be attributable to poor adherence to cardiovascular medications (58). In addition, early recognition of nonadherence might reduce the number of costly diagnostic tests and invasive device-based treatments (59).

Assessing adherence by chemical drug screening

Several methods are available to assess adherence, but most are indirect, subjective and poorly reliable as they have been shown to frequently overestimate adherence (60). As recommended by the current guidelines, chemical drug screening by liquid chromatography tandem mass spectrometry (LC-MS/MS) in plasma or urine is one of the most reliable methods for the assessment of medication adherence (1,61). Chemical adherence testing is mostly performed in a qualitative manner, evaluating the presence or absence of antihypertensive drugs or metabolites using the limit of detection (LOD), the lowest amount of a drug in a sample which can be detected. An important limitation of this qualitative method is that the LOD highly depends on the sensitivity of the analytical assay and not on the therapeutic range of the drug (62). Ongoing improvements of the

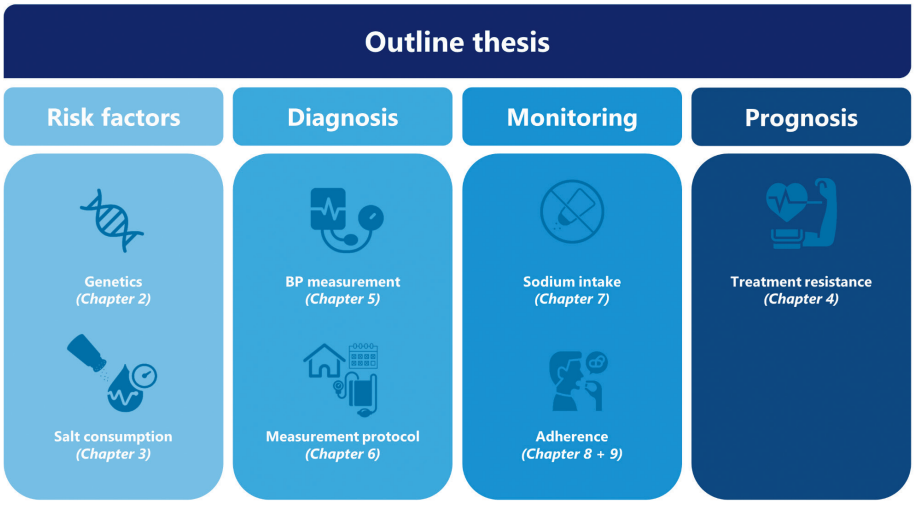
analytical assay, resulting in lower detection limits, will therefore increase the risk of misclassification of nonadherent patients.

Chemical drug screening may be improved by performing it in a quantitative manner, evaluating measured drug concentrations. One approach to quantitative chemical drug screening could be to compare the measured plasma concentration of the drug with the trough concentration, the minimum plasma concentration at steady state (63). With this approach, it is assumed that adherent patients will have at least a plasma drug concentration above this level. For this, a reliable trough level concentration should be established for each antihypertensive drug.

Identifying chemical nonadherence

The costs and infrastructure related to chemical drug screening limit wide application of this method in healthcare settings with limited (financial) resources where the prevalence of hypertension is higher, and the control of hypertension much lower (64). To reduce healthcare costs and make chemical drug screening more accessible in these settings, a clinical screening tool that creates the opportunity to carefully identify patients with a low probability of nonadherence, and therefore do not need to undergo further testing, would be desirable. Screening tools developed so far were either based on suboptimal methods (e.g. pharmacy refill data) (65–67), did not specify model coefficients (68), or were not externally validated (65–67), making them futile in clinical practice. Therefore, there is a need to develop a tool that overcomes these limitations by using reliable adherence data.

Figure 1. Outline thesis.



Thesis objectives and outline

This thesis explores several approaches to improve clinical care of patients with hypertension (**Figure 1**). The general objectives are to (1) explore cardiovascular risks of (causes of) uncontrolled BP in patients with established vascular disease, (2) to evaluate monitoring strategies in patients with uncontrolled hypertension, and (3) to improve identification of nonadherence in patients with uncontrolled hypertension.

Part I will focus on the cardiovascular risks of (causes of) uncontrolled BP in patients with established vascular disease. **Chapter 2** aims to evaluate the effect of polygenic risk scores (PRSs) for known genetic variants associated with LDL-C or SBP on the risk of recurrent cardiovascular events. In **Chapter 3**, the relation between estimated 24-hour sodium and potassium urinary excretion and the risk of recurrent vascular events and mortality is investigated. **Chapter 4** aims to quantify the relation between apparent treatment resistant hypertension (aTRH) and the risk of recurrent major adverse cardiovascular events and mortality in hypertensive patients with stable vascular disease.

Part II of this thesis focuses on the monitoring of patients with (uncontrolled) hypertension. **Chapter 5** investigates the (diagnostic) agreement between app-assisted home BP monitoring (HBPM), automated office BP, and the reference standard ambulatory BP monitoring (ABPM). In **Chapter 6**, the number of BP measurement days needed for a reliable estimation of true home BP and hypertension status is assessed. In **Chapter 7**, the validity of spot urine assay methods in estimating the 24-hour urinary sodium, potassium, and sodium-to-potassium ratio during three different sodium diets is evaluated.

Part III focuses on the problem of medication nonadherence in patients with uncontrolled hypertension. In **Chapter 8**, a literature review and meta-analysis of pharmacokinetic studies to determine plasma trough concentrations of amlodipine, hydrochlorothiazide and valsartan is performed. **Chapter 9** presents the development and validation of a clinical decision tool to carefully identify patients with a low probability of nonadherence, and therefore do not need to undergo further testing. The main findings of this thesis are discussed in **Chapter 10** and summarized in **Chapter 11**.

References

1. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
2. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet (London, England). 2021 Sep;398(10304):957–80.
3. Rapsomaniki E, Timmis A, George J, Pujades-Rodriguez M, Shah AD, Denaxas S, et al. Blood pressure and incidence of twelve cardiovascular diseases: lifetime risks, healthy life-years lost, and age-specific associations in 1·25 million people. Lancet (London, England). 2014 May;383(9932):1899–911.
4. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet (London, England). 2002 Dec;360(9349):1903–13.
5. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease across different levels of blood pressure: an individual participant-level data meta-analysis. Lancet (London, England). 2021 May;397(10285):1625–36.
6. Goff DCJ, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RBS, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014 Jul;63(25 Pt B):2935–59.
7. SCORE2-OP risk prediction algorithms: estimating incident cardiovascular event risk in older persons in four geographical risk regions. Eur Heart J. 2021 Jul;42(25):2455–67.
8. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021 Jul;42(25):2439–54.
9. Hageman SHJ, McKay AJ, Ueda P, Gunn LH, Jernberg T, Hagström E, et al. Estimation of recurrent atherosclerotic cardiovascular event risk in patients with established cardiovascular disease: the updated SMART2 algorithm. Eur Heart J. 2022 Feb;
10. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur J Prev Cardiol. 2022 Feb;29(1):5–115.
11. Muntner P, Whelton PK. Using Predicted Cardiovascular Disease Risk in Conjunction With Blood Pressure to Guide Antihypertensive Medication Treatment. J Am Coll Cardiol. 2017 May;69(19):2446–56.
12. Padmanabhan S, Caulfield M, Dominiczak AF. Genetic and molecular aspects of hypertension. Circ Res. 2015 Mar;116(6):937–59.
13. Giri A, Hellwege JN, Keaton JM, Park J, Qiu C, Warren HR, et al. Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. Nat Genet. 2019 Jan;51(1):51–62.
14. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet. 2018 Oct;50(10):1412–25.
15. Giontella A, Sjögren M, Lotta LA, Overton JD, Baras A, Minuz P, et al. Clinical Evaluation of the Polygenetic Background of Blood Pressure in the Population-Based Setting. Hypertens (Dallas, Tex 1979). 2021 Jan;77(1):169–77.
16. Ference BA, Bhatt DL, Catapano AL, Packard CJ, Graham I, Kaptoge S, et al. Association of Genetic Variants Related to Combined Exposure to Lower Low-Density Lipoproteins and Lower Systolic Blood Pressure With Lifetime Risk of Cardiovascular Disease. JAMA. 2019 Oct;322(14):1381–91.
17. Filippini T, Malavolti M, Whelton PK, Naska A, Orsini N, Vinceti M. Blood Pressure Effects of Sodium Reduction: Dose-Response Meta-Analysis of Experimental Studies. Circulation. 2021 Feb;

18. Aburto NJ, Ziolkovska A, Hooper L, Elliott P, Cappuccio FP, Meerpohl JJ. Effect of lower sodium intake on health: systematic review and meta-analyses. BMJ. 2013 Apr;346:f1326.

19. Gay HC, Rao SG, Vaccarino V, Ali MK. Effects of Different Dietary Interventions on Blood Pressure: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Hypertens (Dallas, Tex 1979). 2016 Apr;67(4):733–9.

20. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013 Apr;346:f1378.

21. Cook NR, He FJ, MacGregor GA, Graudal N. Sodium and health-concordance and controversy. BMJ. 2020 Jun;369:m2440.

22. World Health Organization (WHO). Guideline: sodium intake for adults and children. World Health Organization; 2012.

23. World Health Organization (WHO). Guideline: potassium intake for adults and children. World Health Organization; 2012.

24. Judd E, Calhoun DA. Apparent and true resistant hypertension: definition, prevalence and outcomes. J Hum Hypertens. 2014 Aug;28(8):463–8.

25. Kumbhani DJ, Steg PG, Cannon CP, Eagle KA, Smith SCJ, Crowley K, et al. Resistant hypertension: a frequent and ominous finding among hypertensive patients with atherothrombosis. Eur Heart J. 2013 Apr;34(16):1204–14.

26. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012 Apr;125(13):1635–42.

27. Pierdomenico SD, Lapenna D, Bucci A, Di Tommaso R, Di Mascio R, Manente BM, et al. Cardiovascular outcome in treated hypertensive patients with responder, masked, false resistant, and true resistant hypertension. Am J Hypertens. 2005 Nov;18(11):1422–8.

28. Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, et al. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. J Hypertens. 2021 Jul;39(7):1293–302.

29. Petruski-Ivleva N, Viera AJ, Shimbo D, Muntner P, Avery CL, Schneider ALC, et al. Longitudinal Patterns of Change in Systolic Blood Pressure and Incidence of Cardiovascular Disease: The Atherosclerosis Risk in Communities Study. Hypertens (Dallas, Tex 1979). 2016 Jun;67(6):1150–6.

30. Zhang Z-Y, Vanassche T, Verhamme P, Staessen JA. Implementing Automated Office Blood Pressure Measurement. Hypertens (Dallas, Tex 1979). 2019 Sep;74(3):441–9.

31. Stergiou GS, Alpert B, Mieke S, Asmar R, Atkins N, Eckert S, et al. A Universal Standard for the Validation of Blood Pressure Measuring Devices: Association for the Advancement of Medical Instrumentation/European Society of Hypertension/International Organization for Standardization (AAMI/ESH/ISO) Collaboration Statement. Hypertens (Dallas, Tex 1979). 2018 Mar;71(3):368–74.

32. Sega R, Facchetti R, Bombelli M, Cesana G, Corrao G, Grassi G, et al. Prognostic value of ambulatory and home blood pressures compared with office blood pressure in the general population: follow-up results from the Pressioni Arteriose Monitorate e Loro Associazioni (PAMELA) study. Circulation. 2005 Apr;111(14):1777–83.

33. Bliziotis IA, Destounis A, Stergiou GS. Home versus ambulatory and office blood pressure in predicting target organ damage in hypertension: a systematic review and meta-analysis. J Hypertens. 2012 Jul;30(7):1289–99.

34. Whelton PK, Carey RM, Aronow WS, Casey DEJ, Collins KJ, Dennison Himmelfarb C, et al. 2017 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 P. J Am Coll Cardiol. 2018 May;71(19):e127–248.

35. Krist AH, Davidson KW, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for Hypertension in Adults: US Preventive Services Task Force Reaffirmation Recommendation Statement. JAMA. 2021 Apr;325(16):1650–6.

36. McManus RJ, Mant J, Haque MS, Bray EP, Bryan S, Greenfield SM, 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. JAMA. 2014 Aug;312(8):799–808.

37. Nordmann A, Frach B, Walker T, Martina B, Battegay E. Comparison of self-reported home blood pressure measurements with automatically stored values and ambulatory blood pressure. Blood Press. 2000;9(4):200–5.

38. Nordmann A, Frach B, Walker T, Martina B, Battegay E. Reliability of patients measuring blood pressure at home: prospective observational study. BMJ. 1999 Oct;319(7218):1172.

39. Santo K, Redfern J. The Potential of mHealth Applications in Improving Resistant Hypertension Self-Assessment, Treatment and Control. Curr Hypertens Rep. 2019 Oct;21(10):81.

40. Mente A, O'Donnell M, Rangarajan S, Dagenais G, Lear S, McQueen M, et al. Associations of urinary sodium excretion with cardiovascular events in individuals with and without hypertension: a pooled analysis of data from four studies. Lancet (London, England). 2016 Jul;388(10043):465–75.

41. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich). 2019 Jun;21(6):700–9.

42. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of Urine Biomarkers to Assess Sodium Intake: Challenges and Opportunities. Annu Rev Nutr. 2015;35:349–87.

43. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol. 2013 Jun;177(11):1180–92.

44. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens. 2002 Feb;16(2):97–103.

45. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol. 1993 Jan;20(1):7–14.

46. Polonia J, Lobo MF, Martins L, Pinto F, Nazare J. Estimation of populational 24-h urinary sodium and potassium excretion from spot urine samples: evaluation of four formulas in a large national representative population. J Hypertens. 2017 Mar;35(3):477–86.

47. Iwahori T, Miura K, Ueshima H. Time to Consider Use of the Sodium-to-Potassium Ratio for Practical Sodium Reduction and Potassium Increase. Nutrients. 2017 Jul;9(7).

48. Yasutake K, Horita N, Umeki Y, Misumi Y, Murata Y, Kajiyama T, et al. Self-management of salt intake: clinical significance of urinary salt excretion estimated using a self-monitoring device. Hypertens Res. 2016 Mar;39(3):127–32.

49. Yamasue K, Tochikubo O, Kono E, Maeda H. Self-monitoring of home blood pressure with estimation of daily salt intake using a new electrical device. J Hum Hypertens. 2006 Aug;20(8):593–8.

50. Jung O, Gechter JL, Wunder C, Paulke A, Bartel C, Geiger H, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. J Hypertens. 2013 Apr;31(4):766–74.

51. Peacock E, Krousel-Wood M. Adherence to Antihypertensive Therapy. Med Clin North Am. 2017 Jan;101(1):229–45.

52. Redon J, Mourad J-J, Schmieder RE, Volpe M, Weiss TW. Why in 2016 are patients with hypertension not 100% controlled? A call to action. J Hypertens. 2016 Aug;34(8):1480–8.

53. World Health Organization (WHO). Adherence to long-term therapies: evidence for action. World Health Organization; 2003.

54. Abegaz TM, Shehab A, Gebreyohannes EA, Bhagavathula AS, Elnour AA. Nonadherence to antihypertensive drugs: A systematic review and meta-analysis. Medicine (Baltimore). 2017 Jan;96(4):e5641.

55. Durand H, Hayes P, Morrissey EC, Newell J, Casey M, Murphy AW, et al. Medication adherence among patients with apparent treatment-resistant hypertension: systematic review and meta-analysis. J Hypertens. 2017 Dec;35(12):2346–57.

56. Mazzaglia G, Ambrosioni E, Alacqua M, Filippi A, Sessa E, Immordino V, et al. Adherence to antihypertensive medications and cardiovascular morbidity among newly diagnosed hypertensive patients. Circulation. 2009 Oct;120(16):1598–605.

57. Lee H, Yano Y, Cho SMJ, Heo JE, Kim D-W, Park S, et al. Adherence to Antihypertensive Medication and Incident Cardiovascular Events in Young Adults With Hypertension. Hypertens (Dallas, Tex 1979). 2021 Apr;77(4):1341–9.

58. Corrao G, Parodi A, Nicotra F, Zambon A, Merlino L, Cesana G, et al. Better compliance to antihypertensive medications reduces cardiovascular risk. J Hypertens. 2011 Mar;29(3):610–8.

59. Berra E, Azizi M, Capron A, Høieggen A, Rabbia F, Kjeldsen SE, et al. Evaluation of Adherence Should Become an Integral Part of Assessment of Patients With Apparently Treatment-Resistant Hypertension. Hypertens (Dallas, Tex 1979). 2016 Aug;68(2):297–306.

60. Pandey A, Raza F, Velasco A, Brinker S, Ayers C, Das SR, et al. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension. J Am Soc Hypertens. 2015 Jun;9(6):420-426.e2.

61. Lam WY, Fresco P. Medication Adherence Measures: An Overview. Biomed Res Int. 2015;2015:217047.

62. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Drug adherence in hypertension: from methodological issues to cardiovascular outcomes. J Hypertens. 2017 Jun;35(6):1133–44.

63. Punt AM, Stienstra NA, van Kleef MEA, Lafeber M, Spiering W, Blankestijn PJ, et al. Screening of cardiovascular agents in plasma with LC-MS/MS: A valuable tool for objective drug adherence assessment. J Chromatogr B, Anal Technol Biomed life Sci. 2019 Jul;1121:103–10.

64. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016 Aug;134(6):441–50.

65. Krousel-Wood M, Joyce C, Holt EW, Levitan EB, Dornelles A, Webber LS, et al. Development and evaluation of a self-report tool to predict low pharmacy refill adherence in elderly patients with uncontrolled hypertension. Pharmacotherapy. 2013 Aug;33(8):798–811.

66. Steiner JF, Ho PM, Beaty BL, Dickinson LM, Hanratty R, Zeng C, et al. Sociodemographic and clinical characteristics are not clinically useful predictors of refill adherence in patients with hypertension. Circ Cardiovasc Qual Outcomes. 2009 Sep;2(5):451–7.

67. Gao W, Liu H, Ge C, Liu X, Jia H, Wu H, et al. A Clinical Prediction Model of Medication Adherence in Hypertensive Patients in a Chinese Community Hospital in Beijing. Am J Hypertens. 2020 Jul;

68. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Maresova V, et al. Risk Factors for Nonadherence to Antihypertensive Treatment. Hypertension. 2017;69(6):1113–20.

PART I

Cardiovascular risk in patients with uncontrolled hypertension

CHAPTER 2

Genetic variants associated with low-density lipoprotein cholesterol and systolic blood pressure and the risk of recurrent cardiovascular disease in patients with established vascular disease.

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Abstract

Background and aims: Polygenic risk scores (PRSs) can be used to quantify the effect of genetic contribution to LDL-cholesterol (LDL-C) and systolic blood pressure (SBP). Several PRSs for LDL-C and SBP have been shown to be associated with cardiovascular disease (CVD) in the general population. This study aimed to evaluate the effect of an LDL-C PRS and an SBP PRS on the risk of recurrent CVD in patients with CVD.

Methods: Genotyping was performed in 4,416 patients included in the UCC-SMART study. A weighted LDL-C PRS (279 LDL-C related SNPs) and SBP PRS (425 SBP related SNPs) were calculated. Linear regression models were used to evaluate the relation between both PRSs and LDL-C and SBP. The effects of the LDL-C PRS and SBP PRS, and its combination on the risk of recurrent CVD (stroke, myocardial infarction, and vascular death) were analyzed with Cox proportional-hazard models.

Results: Per SD increase in LDL-C PRS, LDL-C increased by 0.18 mmol/L (95%CI 0.15–0.21). Per SD increase in SBP PRS, SBP increased by 3.19 mmHg (95%CI; 2.60–3.78). During a follow-up of 11.7 years (IQR 9.2–15.0) 1,198 recurrent events occurred. Neither the LDL-C nor the SBP PRS were associated with recurrent CVD (HR 1.05 per SD increase in LDL-C PRS; 95%CI; 0.99–1.11 and HR 1.04 per SD increase in SBP PRS (95%CI 0.98–1.10). The combination of both scores was neither associated with recurrent CVD (HR 1.09; 95%CI 0.93–1.28).

Conclusion: In patients with vascular disease, an LDL-C PRS and SBP PRS, both separately and in combination, were not significantly associated with recurrent CVD

Introduction

Increased low-density lipoprotein cholesterol (LDL-C) and systolic blood pressure (SBP) are among the most important risk factors for the development and progression of cardiovascular disease (1). SBP and LDL-C are highly heritable traits, involving a large set of genes contributing to disease (2). Hundreds of single nucleotide polymorphisms (SNPs) associated with plasma LDL-C and SBP, have been identified through genome-wide association studies (GWAS) and this is still increasing (3-5). These genetic variants represent lifelong exposure to LDL-C or SBP in which the small individual effects of each SNP are assumed to be cumulative. Polygenic risk scores (PRSs) aggregate the modest effects of multiple SNPs into a single score as a proxy for lifelong exposure to a given trait (6). As demonstrated earlier, including genetic information in risk models could potentially contribute to the improvement of personalized cardiovascular risk prediction or to the identification of high-risk patients who might benefit from stricter treatment goals (7-9). Previous studies in the general population showed that a PRS for LDL-C and SBP is associated with an increased risk of incident cardiovascular events (8, 10-12). However, very few studies have reported on the association between such PRSs and recurrent cardiovascular events. So far, only one study evaluated the effect of an LDL-C PRS in a selected study population that underwent carotid endarterectomy (13). This study found no significant association between the LDL-C PRS and the occurrence of cardiovascular events including cardiovascular death, non-fatal stroke, non-fatal myocardial infarction, or vascular interventions. Treatment with lipid-lowering- and antihypertensive medications could modulate the effects of genetic variants on LDL-C and SBP in patients with stable vascular disease. In addition, the effects of these genetic variants on recurrent vascular events may be different compared to first events, because patients with few risk alleles may have other risk factors that caused the first event that also increase the risk of recurrent vascular events (14).

The aim of the present study is therefore twofold. First, to replicate the effect of PRSs for known genetic variants associated with LDL-C or SBP on these risk factors within a cohort of patients with established vascular disease. Second, to evaluate the effect of these PRSs for LDL-C and SBP on the risk of recurrent cardiovascular events in this high-risk patient population.

Methods

Study population

Data from patients enrolled in the Utrecht Cardiovascular Cohort – Second Manifestations of Arterial Disease (UCC-SMART) study were used. The UCC-SMART study is an ongoing, single-center, prospective cohort at the tertiary referral center University Medical Center Utrecht (UMCU) in the Netherlands. Patients aged 18-80 years referred to the UMCU with established cardiovascular disease (coronary artery disease (CAD), cerebrovascular disease (CeVD), peripheral arterial disease (PAD) or abdominal arterial aneurysm (AAA) underwent vascular screening. A description of the study rationale has been published previously (15). The UCC-SMART study was approved by the Medical Ethics Committee of the UMCU, and all patients provided written informed consent prior to inclusion. For the current study, data of patients that were included between September 1996 and August 2010 were used, as these patients were genotyped (n=6,971).

Baseline measurements

At baseline, all patients underwent a standardized vascular screening protocol including a health questionnaire, physical examination, laboratory testing, ankle-branchial index, and an abdominal, aortic and carotid ultrasound. Office blood pressure measurements were performed with automated blood pressure monitors (Iso-Stabil 5; Speidel & Keller, Jungingen, Germany) on the arm with the highest blood pressure. The mean of 3 measurements on that arm was recorded. Smoking, alcohol use, and medication use were self-reported. Lipid-lowering medication included use of statins, fibrates, bile acid sequestrants or nicotinic acid. Prescription of high intensity statins was defined as atorvastatin ≥40 mg or rosuvastatin ≥20 mg. Antihypertensive medications were grouped based on drug class (angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, beta-blockers, alpha-blockers, calcium antagonists, diuretics, aldosterone antagonists, central acting antihypertensives, direct vasodilators). Type 2 diabetes mellitus (T2DM) was defined as either a referral or self-reported diagnosis of T2DM, or a fasting plasma glucose ≥7 mmol/L at study inclusion with initiation of glucose-lowering treatment within 1 year, or baseline use of hypoglycemic agents or insulin.

Laboratory measurements

Laboratory blood testing was performed in the fasting state. Total cholesterol (TC) and triglycerides (TG) were measured with a commercial enzymatic dry chemistry kit (Johnson & Johnson, New Brunswick, USA). High-density lipoprotein-cholesterol (HDL-C) was measured with a commercial enzymatic kit (Boehringer, Mannheim, Germany) and LDL-C was calculated using the Friedewald formula up to triglyceride levels of 9 mmol/L to reduce missing values in this analysis (16, 17). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (18).

Genotyping and quality control

Genotyping of the cohort was performed using the Illumina GSA array. All SNPs went through a thorough quality control (QC) check using PLINK v. 1.9 (19). Genotype imputation has been performed using IMPUTE2 v2.3.0. After imputation 91.3 million SNPs were available. SNPs with an imputation quality (R2) <0.3 (n=36.8 million), a minor allele frequency below 0.1% (n=71.2 million) and SNPs with a Hardy-Weinberg equilibrium p-value <1 x 10-6 (n=90) were also excluded, resulting in 19.9 million imputed SNPs available. Patients of non-European ancestry (n=543), with low quality genotyping (n=212) or those who were related to each other (n=203) were excluded. In case of the latter, the patient with the most recent date of inclusion was excluded. Other reasons for exclusion during quality control were samples with likely sample contamination based on high degree of relatedness with other samples (n=37), or when samples were >5 standard deviations from median for inbreeding coefficient (n=32), with a sex mismatch between genotype and phenotype (n=18), and samples without phenotype data available (n=43). In total, 1,088 patients were excluded after quality control, resulting in 5,883 patients. Lastly, patients without established cardiovascular disease were excluded (n=1,467) resulting in 4,416 patients with vascular disease eligible for the analyses.

SNP selection and calculation of the polygenic risk scores

To identify SNPs for both PRSs we first retrieved the most recent (at the time of conducting the analysis) meta-analyses of GWAS describing genetic variants associated with either LDL-C (5) or SBP (3, 4, 20) at genome-wide level of significance ($p < 5x10-8$). From these meta-analyses, a total of 444 SNPs and 616 SNPs were identified as potentially relevant for the construction of each PRS. To remove highly correlated variants, we performed LD pruning on the summary data of these SNPs extracted from the Pan-ancestry genetic analysis of the UK biobank (21) using PLINK v.1.9 (22). To this end we used the '--indep-pairwise 1,000 10 0.2' flag in PLINK, which means that we used a window of 1,000 SNPs, calculated LD between each pair of SNPs in the window, removed one of a SNP pair if the LD was greater than r2 = 0.2, shifted the window 10 SNPs forward and then repeated the procedure. This resulted in a final selection of 279 and 425 SNPs associated with LDL-C and SBP, respectively.

For each patient, two weighted PRSs were calculated by summing the dosages of effect alleles (labeled as the alternate alleles; ranging from 0 to 2) of an individual patient at each SNP multiplied by the β-coefficient of the respective alternate allele. Because the UCC-SMART study population is from European descent, we used the β-coefficients from European ancestry sub-analysis of the Pan-UKB. These β-coefficients were adjusted for use of medication (row 4,491 for LDL-C and row 4,519 for SBP) (23). A list of genetic variants and their β-coefficients used to derive both PRSs is provided in Supplemental Table 1a and 1b.

Follow-up

Follow-up duration was defined as time from inclusion in the cohort until development of first cardiovascular event, death, loss to follow-up or the preselected date of July 1, 2019. From 1996 till July 1, 2019, 360 patients were lost to follow-up (8%). During follow-up patients received questionnaires on hospital admissions and outpatient clinic visits twice a year. If an event was reported, all relevant hospital documents, and laboratory and radiologic findings were collected. All events were audited independently by three physicians of the UCC-SMART endpoint committee. The primary outcome for this study was the combination of non-fatal and fatal vascular events, consisting of non-fatal myocardial infarction (MI), non-fatal stroke and vascular death. Secondary outcomes were the separate components of the composite outcome (non-fatal MI, non-fatal stroke and vascular death). For detailed description of the outcomes see Supplemental Table 2.

Data analysis

Baseline characteristics are presented in four groups, according to the median of both polygenic risk scores (the distributions of both PRSs are displayed in Supplementary Figure 1); one reference group with genetically lower LDL-C and SBP (LDL-C PRS < median and SBP PRS < median), one group with genetically higher SBP (LDL-C PRS < median, SBP PRS > median), one group with genetically higher LDL-C (LDL-C PRS > median, SBP PRS < median), and one group with both genetically higher SBP and LDL-C (LDL-C PRS > median, SBP PRS > median). The organization of patients according to both PRSs is provided in Supplemental Figure 2).

Baseline data are presented as number and percentage for categorical variables, mean ± standard deviation (SD) for normally distributed variables or median with interquartile range (IQR) in case of a skewed distribution. For the association between the LDL-C PRS and LDL-C and the SBP PRS and SBP values, respectively, linear regression models were fitted. Three models were built. The first model was adjusted for age, sex, and the first five principal components. The second model was additionally adjusted for BMI, T2DM, smoking, alcohol use, eGFR, and triglycerides. The third model was additionally adjusted for use of lipid-lowering- or antihypertensive medication. For these analyses the LDL-C - and SBP PRS were standardized. Hence, the beta coefficient corresponds to the change per SD increase in the PRS. In addition, the beta-coefficients derived from the linear regression models were plotted according to quartiles of the LDL-C and SBP PRS.

Cox proportional hazard models were used to determine the relationship between the (standardized) LDL-C PRS and SBP PRS and recurrent events. Linearity of the relationships between LDL-C PRS and SBP PRS with recurrent vascular events was assessed with restricted cubic splines. The Cox proportional hazard assumption was visually checked and confirmed by plotting Schoenfeld residuals against time. Two models were built. The first model was adjusted for age, sex, and the first five principal components. The second model was additionally adjusted for body mass index (BMI), T2DM, smoking, alcohol

use, eGFR, triglycerides, and systolic blood pressure and lipid-lowering medication (in model for LDL PRS), or LDL-C and antihypertensive medication (in model for SBP PRS). Additionally, to evaluate potential effect modification between the LDL-C and SBP PRS Cox models were fitted between the combined LDL-C and SBP PRS groups and recurrent cardiovascular events. To evaluate whether several key characteristics (T2DM, sex, age, type of vascular disease at baseline, and use of lipid-lowering- and antihypertensive medication) might modify the association between both PRSs and recurrent vascular events, we included interaction terms into the models.

Several sensitivity analyses were performed. To assess whether a different distribution of patient groups would influence the results, we classified patients according to the highest quintile and decile of both PRSs and compared the hazard of recurrent MACE in those with genetically higher LDL-C and SBP (top quintiles and top deciles of both PRSs) versus all others. Also, to evaluate whether the results were influenced by pleiotropy, we performed a sensitivity analysis by excluding SNPs that were significantly associated with either SBP or LDL-C PRS (p-value adjusted for multiple testing = 0.018 for LDL-C and p-value adjusted for multiple testing = 0.012 for SBP, Supplemental Tables 7 and 8).

To improve statistical accuracy, missing values of variables of interest [BMI (n=9; 0.2%), smoking status (n=17, 0.4%), eGFR (n=19, 0.4%), triglycerides (n=28, 0.6%), systolic blood pressure (n=9, 0.2%), LDL-C (n=38, 0.9%)] were completed by single regression imputation using predictive mean matching (24). There were no missing values for age, sex, T2DM, lipid-lowering- and antihypertensive medication. All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics

Baseline characteristics of the patients stratified according to the medians of both PRSs are shown in Table 1. The mean age was 61 ± 10 years and 75% of the patients were male, 61% had a history of CAD, 27% of CeVD, 21% of PAD, and 9% of AAA. Compared to the reference group (genetically lower LDL-C and SBP), the group with genetically higher LDL-C and SBP had a higher mean SBP (143 ± 21 mmHg versus 139 ± 20 mmHg) and a higher mean LDL-C (3.02 ± 1.07 mmol/L versus 2.87 ± 1.04 mmol/L). This group also had a higher prescription rate for lipid-lowering- (68% versus 59%) and antihypertensive medications (75% versus 70%) compared to the reference group. There were no clinically relevant differences with respect to the other variables at baseline between the four groups.

Relation between polygenic risk scores and traits

LDL-C polygenic risk score and LDL-C

Supplemental Table 3 shows that the LDL-C PRS was significantly associated with LDL-C (per SD increase in PRS, LDL-C increased by 0.11 mmol/L; 95% CI 0.08 – 0.14). Additional adjustment for the use of lipid-lowering medication further strengthened this relation (β-coefficient per SD 0.18 mmol/L; 95% CI 0.15 – 0.21). To evaluate whether the effect of the PRS was different in patients with or without lipid-lowering, we added use of lipid-lowering medication as an interaction term in the model. (p=0.08). Figure 1 shows mean LDL-C levels according to LDL-C PRS quartiles stratified for use of lipid-lowering medication after adjustment for age, sex, BMI, SBP, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components. Mean LDL-C levels were higher in patients without lipid-lowering medication in all quartiles.

SBP polygenic risk score and SBP

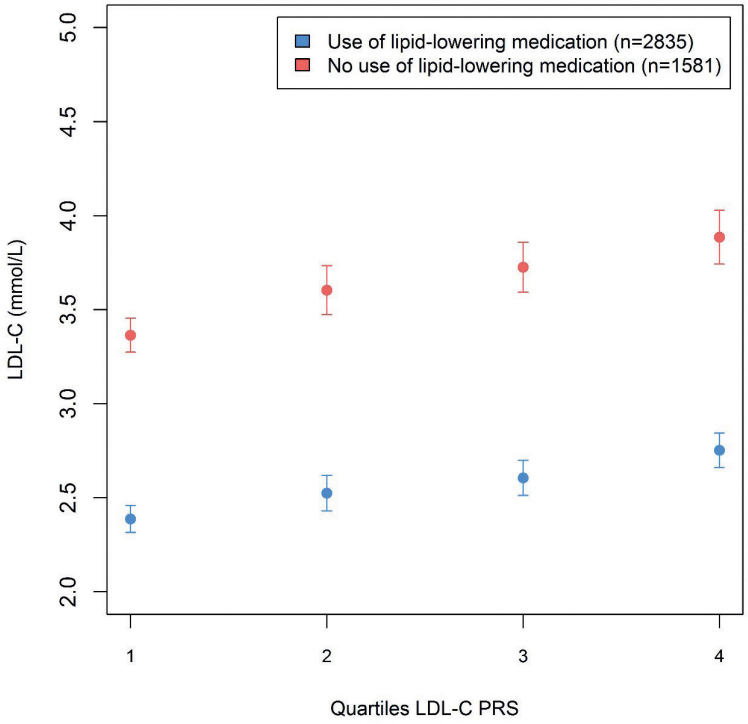
The SBP PRS was significantly associated with SBP, as shown in Supplemental Table 4. One SD increase in the SBP PRS corresponded to an increment of 3.15 mmHg (95% CI 2.56 – 3.74) in SBP. Additional adjustment for use of antihypertensive medication did not change the results meaningfully (β 3.19; 95% CI 2.60 – 3.78). Figure 2 shows mean SBP according to SBP PRS quartiles, stratified for use of antihypertensive medication after adjustment for age, sex, BMI, LDL-C, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components. SBP levels were similar in patients with and without antihypertensive medication indicating that the effect of the SBP does not depend on the use of antihypertensive drugs, which was confirmed by the non-significant interaction between SBP PRS and use of antihypertensive drugs (p = 0.17).

Table 1. Baseline characteristics according to combined LDL-C and SBP polygenic risk score.

	Reference group	LDL-C PRS ≤ median, SBP PRS > median	LDL-C PRS > median, SBP PRS ≤ median	LDL-C PRS and SBP PRS > median	Total
	n = 1,123	n = 1,085	n = 1,085	n = 1,123	n = 4,416
Male sex	840 (75%)	808 (74%)	815 (75%)	831 (74%)	3294 (75%)
Age (years)	61 ± 10	61 ± 10	60 ± 10	60 ± 10	61 ± 10
Current smoker	402 (36%)	348 (32%)	372 (34%)	354 (32%)	1476 (33%)
Current alcohol use	550 (49%)	536 (49%)	548 (51%)	577 (51%)	2211 (50%)
Body mass index (kg/m2)	26.8 ± 3.8	26.9 ± 3.9	26.7 ± 4.0	26.7 ± 3.9	26.7 ± 3.9
Systolic blood pressure (mmHg)	139 ± 20	144 ± 22	138 ± 21	143 ± 21	141 ± 21
Diastolic blood pressure (mmHg)	81± 11	82 ± 11	80 ± 12	83 ± 11	81 ± 11
History of vascular disease					
Diabetes mellitus type 2	173 (15%)	199 (18%)	156 (14%)	177 (16%)	705 (16%)
Coronary artery disease	651 (58%)	632 (58%)	702 (65%)	720 (64%)	2705 (61%)
Peripheral artery disease	231 (21%)	251 (23%)	217 (20%)	237 (21%)	936 (21%)
Cerebrovascular disease	338 (30%)	305 (28%)	260 (24%)	300 (27%)	1203 (27%)
Abdominal aortic aneurysm	107 (10%)	90 (8%)	95 (9%)	101 (9%)	393 (9%)
Laboratory values					
Total cholesterol (mmol/L)	4.82 ± 1.19	4.84 ± 1.21	5.03 ± 1.23	5.04 ± 1.31	4.93 ± 1.24
HDL-cholesterol (mmol/L)	1.23 ± 0.36	1.21 ± 0.38	1.20 ± 0.35	1.21 ± 0.37	1.21 ± 0.36
LDL-cholesterol (mmol/L)	2.87 ± 1.04	2.89 ± 1.08	3.08 ± 1.08	3.02 ± 1.07	2.97 ± 1.07
Triglycerides (mmol/L)	1.3 (0.9 - 1.9)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.5 (1.1 - 2.2)	1.4 (1.0 - 2.0)
Estimated GFR (ml/min/1.73m2)	75 ± 17	74 ± 18	76 ± 17	76 ± 18	75 ± 18
hsCRP (mg/L)	2.2 (1.0 - 4.6)	2.3 (1.0 - 4.9)	1.9 (0.9 - 4.3)	2.0 (1.0 - 4.4)	2.1 (1.0 - 4.5)
Medication use					
Lipid-lowering medication	660 (59%)	641 (59%)	770 (71%)	764 (68%)	2835 (64%)
High-intensity statins	54 (5%)	61 (6%)	85 (8%)	79 (7%)	279 (6%)
Antihypertensive medication	789 (70%)	819 (75%)	783 (72%)	845 (75%)	3236 (73%)
Number of antihypertensive drugs	1.2 (0 – 5)	1.4 (0 – 7)	1.3 (0 – 5)	1.4 (0 – 6)	1.3 (0 – 7)
Platelet inhibitors	819 (73%)	796 (73%)	813 (75%)	864 (77%)	3292 (75%)

All data in n (%), mean ± standard deviation, or mean (range). Abbreviations: HDL; high-density lipoprotein, LDL; low-density lipoprotein, SBP; systolic blood pressure, GFR; glomerular filtration rate, hsCRP; high sensitivity C-reactive protein

Figure 1. Relation LDL-C polygenic risk score and LDL-C values in quartiles in patients with and without use of lipid-lowering medication.



Linear regression analyses describing the association between mean LDL-C level and quartiles of LDL-C PRS, stratified for use of lipid-lowering medication. Models were adjusted for age, sex, BMI, SBP, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components.

Relation between polygenic risk scores and recurrent cardiovascular events

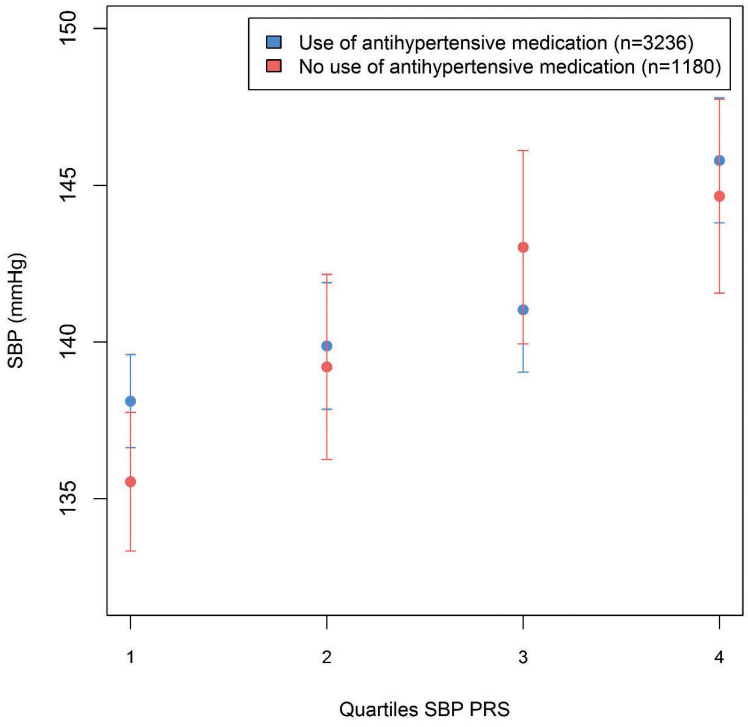
During a median follow-up of 11.7 years IQR: 9.2 – 15.0 years; 51,991 person-years), the composite outcome (consisting of non-fatal myocardial infarction, non-fatal stroke, and vascular death) occurred in 1,198 patients.

LDL-C polygenic risk score and recurrent cardiovascular events

After adjustment for traditional cardiovascular risk factors including age, sex, BMI, T2DM, smoking, alcohol use, eGFR, triglycerides, SBP, and lipid-lowering medication, the LDL-C PRS was not associated with the risk of recurrent cardiovascular events (hazard ratio (HR) per one SD increase in PRS; 1.05; 95% CI 0.99 – 1.11) (Table 2). There was no interaction with use of lipid-lowering medication (p for interaction=0.39). Also, there was no effect modification by age, sex, T2DM and type of vascular disease at baseline in the relation

between LDL-C PRS and recurrent cardiovascular events (p for all interactions >0.05). Exploratory analyses examining secondary outcomes showed similar results (non-fatal MI (HR 1.05; 95% CI 0.96 - 1.16), non-fatal stroke (HR 1.00; 95% CI 0.90 – 1.12), and vascular death (HR 1.05; 95% CI 0.98 – 1.13) (Supplemental Table 5).

Figure 2. Relation SBP polygenic risk score and SBP values in quartiles in patients with and without use of antihypertensive medication.



Linear regression analyses describing the association between mean SBP and quartiles of SBP PRS, stratified for use of antihypertensive medication. Models were adjusted for age, sex, BMI, LDL-C, smoking, alcohol use, T2DM, eGFR, triglycerides, and the first 5 principal components

SBP polygenic risk score and recurrent cardiovascular events

The SBP PRS was not associated with recurrent cardiovascular events (HR 1.04 per one SD increase in PRS; 95% CI; 0.98 – 1.10) (Table 2). The effects were similar in patients with or without antihypertensive mediation (p for interaction=0.79). No interaction was observed with age, sex, T2DM and type of vascular disease at baseline (p for all interactions >0.05). Analyses examining secondary outcomes also found no statistically significant association between the SBP PRS and non-fatal MI (HR 1.03; 95% CI 0.94 – 1.13)

and non-fatal stroke (HR 0.99; 95% CI 0.89 – 1.10), but did find a significant association with vascular death (HR 1.11; 95% CI 1.03 – 1.19) (Supplemental Table 5).

Table 2. LDL-C and SBP polygenic risk score and recurrent cardiovascular events (non-fatal MI, non-fatal stroke and vascular death).

			LDL-C PRS	**SBP PRS**
			N = 4416	**N = 4416**
		Model	HR per SD increase in PRS (95% CI)	HR per SD increase in PRS (95% CI)
	#events		1198	1198
Recurrent cardiovascular events		I	1.02 (0.96 - 1.08)	1.04 (0.99 - 1.10)
		II	1.05 (0.99 - 1.11)	1.04 (0.98 - 1.10)

Model I: adjusted for age and sex, and the first five principal components.
Model II: *LDL-C PRS:*
Model I + additional adjustment for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, SBP, and lipid-lowering medication
SBP PRS: Model I + additional adjustment for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, LDL-C, and antihypertensive medication

Combined polygenic risk scores and recurrent cardiovascular events

Patients with a genetically higher LDL-C and SBP experienced 303 recurrent cardiovascular events during follow-up (incidence rate 25.2 per 1,000 person-years). Patients with a genetically lower LDL-C and SBP experienced 295 recurrent cardiovascular events (incidence rate 24.8 per 1,000 person-years). Compared to patients with a genetically lower LDL-C and SBP, there was no statistically significant difference in the risk of recurrent cardiovascular events in patients with a genetically higher LDL-C and SBP (HR 1.09; 95% CI 0.93 – 1.28) (Table 3). Also, there was no significant difference in the risk of the separate cardiovascular outcomes (non-fatal MI (HR 1.10; 95% CI 0.84 – 1.44), non-fatal stroke (HR 1.02; 95% CI 0.75 – 1.39) and vascular death (HR 1.14; 95% CI 0.93 – 1.40)) when comparing both groups (Supplemental Table 6).

Sensitivity analyses

Repeating the analyses after classification of patients according to the highest quintile and decile of both PRSs showed comparable results (Supplemental Tables 9 - 10). Furthermore, to determine whether the results were influenced by pleiotropy, we performed a sensitivity analysis in which we excluded SNPs that were significantly associated with both LDL-C and SBP. For the LDL-C PRS, a total of 81 SNPs were excluded, and for the SBP PRS, a total of 77 SNPs. Exclusion of these SNPs from both PRSs did not change the estimates meaningfully (Supplemental Tables 11 - 14).

Discussion

In this prospective cohort study of patients with vascular disease, we replicated the association of a PRS for LDL-C and a PRS for SBP with these risk factors, constructed by SNPs identified through the latest large-scale genome-wide association studies. However, no statistically significant association was observed between these PRSs and recurrent cardiovascular events.

Results of the current study are in line with the results from a study that investigated an LDL-C PRS in patients that underwent carotid endarterectomy. This study also found no association between an LDL-C PRS and recurrent cardiovascular events within a follow-up of three years (HR per one SD increase 1.03 (95% CI 0.92 – 1.15)) (13).

To our knowledge, the combined effect of a PRS for LDL-C and a PRS for SBP on cardiovascular events only has been evaluated in apparently healthy individuals enrolled in the UK biobank (10). In contrast to our study, this study found that relatively small absolute differences in combined exposure to genetically lower LDL-C and SBP translated into a large difference in the risk for major coronary events (odds ratio (OR) 0.61 (95% CI 0.59 – 0.64)) (10). Although a direct comparison of PRS effect sizes may be challenging due to use of varying (number of) SNPs and outcomes it remains somewhat notable that the present study found no effect of either PRSs on the risk of recurrent cardiovascular events, also given the abundant evidence on LDL-C and SBP as causal contributors to cardiovascular risk. Several mechanisms may explain why no association was observed in this study.

First, the present study was conducted in a relatively small cohort compared to previous studies evaluating a PRS (10, 11). This may have resulted in limited power to demonstrate a genuine lack of associations, especially when the magnitude of the effect is small. This is supported by the ambivalent results we obtained: both PRSs were not associated with the primary outcome, but we did observe a nominally significant association between the PRS for SBP and the secondary outcome vascular death. Hence, before drawing any definitive conclusions, replication in larger cohorts of patients with vascular disease is needed. Second, index-event bias has been proposed as an explanation for differences in associations of PRS in patients with cardiovascular events compared to patients without prior cardiovascular disease (25). This can be understood by considering the onset of vascular events as the sum of the effect of multiple causal factors. If one important causal risk factor (such as a high genetically determined LDL-C or SBP (reflected in a high LDL-C or SBP PRS)) is already present, less effect of other factors is required for disease onset. Subsequently, comparing patients with a genetically unfavorable LDL-C and/or SBP profile to patients with a genetically favorable LDL-C and/or SBP profile who already have developed vascular disease, leads to a relatively healthy risk profile in the former compared to the latter and hence a bias of the results towards null.

Table 3. Combined LDL-C and SBP polygenic risk score and recurrent cardiovascular events (non-fatal MI, non-fatal stroke and vascular death).

			LDL-C PRS ≤ median, SBP PRS < median (Reference group)	LDL-C PRS ≤ median, SBP PRS > median	LDL-C PRS > median, SBP PRS ≤ median	LDL-C PRS > median, SBP PRS > median
			n=1123	**n= 1085**	**n= 1085**	**n= 1123**
	Model			HR (95% CI)	HR (95% CI)	HR (95% CI)
Recurrent cardiovascular events	# events		295	320	280	303
		I	Reference	1.08 (0.92 - 1.26)	0.98 (0.83 - 1.15)	1.06 (0.91 - 1.25)
		II	Reference	1.06 (0.90 - 1.24)	1.03 (0.87 - 1.22)	1.09 (0.93 - 1.28)

Model I: adjusted for age, sex, and the first 5 principal components
Model II: Model I + additionally adjusted for BMI, type 2 diabetes mellitus, smoking, alcohol use, eGFR, triglycerides, lipid-lowering medication, antihy

This type of bias is recently investigated in a study using data from the UK biobank (26). The authors demonstrated that associations of a CAD PRS with incident cardiovascular outcomes were greatly attenuated among those with established CAD compared to those without CAD. Nonetheless, the estimates did not change after adjustment for most known risk factors for vascular disease, making index event bias a less likely explanation.

Finally, use of lipid-lowering- or antihypertensive medication and healthy lifestyle may have contributed to the lack of an association between both PRSs and recurrent vascular events. As demonstrated in the baseline table, patients with both the LDL-C PRS and SBP PRS above the median had a higher prescription rate for lipid-lowering- and antihypertensive medication compared to patients with both PRS below median. Moreover, patients with a genetically higher LDL-C and SBP may be more likely to be treated more intensively with these type of medications and potentially adopt a more healthy lifestyle during follow-up, which eventually compensates for the higher genetically determined LDL-C and SBP levels. Moreover, these types of medication and the change to a healthy lifestyle may be more effective in patients with genetically higher LDL-C and SBP. This concept is supported by previous studies showing that both statins, Proprotein Convertase Subtilisin-Kexin type 9 (PCSK9) monoclonal antibodies, and also a healthy lifestyle are able to modify the risk of (recurrent) cardiovascular events associated with a high PRS (27-30).

This study shows that genetically determined LDL-C and SBP do not explain differences in residual cardiovascular risk in patients with established vascular disease. Although this is an etiologic study, these results support the recommendations in international guidelines not to routinely collect genetic information for CVD risk stratification. In general, the position of genetic risk scores in clinical practice is under debate. Currently, PRSs are considered of limited use for the prediction of CVD events (31). Moreover, in the scenario that PRSs will play an important role in clinical practice in the future, it is likely that its greatest value lies in the first decades of life, prior to clinical events and even prior to definable plaque burden by imaging.

Strengths of the present study include the prospective cohort study design reflecting clinical practice of patients with vascular disease being treated according to national guidelines, the substantial follow-up duration and the large number of validated clinically relevant outcomes. Also, genotyping and quality control were performed according to a highly standardized protocol by experts in the field. Lastly, elaborate sensitivity analyses were performed to further investigate the main findings of this study.

Some limitations need to be considered. In the present study two PRSs were used based on 704 different SNPs related to either LDL-C or SBP identified through GWAS in the general population. Some have argued that such PRSs are of limited value in populations with established vascular disease and advocate the design and use of dedicated GWAS

of disease progression (26, 32, 33). However, this study demonstrated a robust effect of the selected SNPs on plasma LDL-C and SBP levels in patients with vascular disease, independent of the use of lipid-lowering- or antihypertensive medication. Moreover, differences in LDL-C and SBP levels when stratified for LDL-C or SBP PRS, were comparable with the differences observed in the general population (7, 8). In addition, the allele frequencies of the selected SNPs in the current study population were comparable to the allele frequencies found in the general European population (Supplemental Table 1). Another important limitation is that use of medication such as lipid-lowering- and antihypertensive medication was only recorded at baseline. Although the use of these types of medication probably increased during follow-up, since treatment advice was part of the screening for this study, we were not able to account for these changes in the analyses. Lastly, the PRSs used in this study are only applicable to populations of European descent, which may limit the generalizability of the results and poses an ethical dilemma (34, 35).

In conclusion, in patients with established cardiovascular disease, we replicated the known association of PRSs for LDL-C and SBP with these risk factors. We found no statistically significant association between an LDL-C PRS and an SBP PRS, nor in combination, and recurrent cardiovascular events. These results suggests that genetically determined LDL-C and SBP do not explain the differences in residual cardiovascular risk in patients with established vascular disease.

References

1. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. European heart journal. 2021;42(34):3227-337.
2. Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, et al. The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. 2019;47(D1):D1005-d12.
3. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. Nat Genet. 2018;50(10):1412-25.
4. Giri A, Hellwege JN, Keaton JM, Park J, Qiu C, Warren HR, et al. Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. Nat Genet. 2019;51(1):51-62.
5. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, et al. Exome-wide association study of plasma lipids in >300,000 individuals. Nature genetics. 2017;49(12):1758-66.
6. Lambert SA, Abraham G, Inouye M. Towards clinical utility of polygenic risk scores. Hum Mol Genet. 2019;28(R2):R133-r42.
7. Abraham G, Malik R, Yonova-Doing E, Salim A, Wang T, Danesh J, et al. Genomic risk score offers predictive performance comparable to clinical risk factors for ischaemic stroke. Nature Communications. 2019;10(1):5819.
8. Khera AV, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018;50(9):1219-24.
9. Inouye M, Abraham G, Nelson Christopher P, Wood Angela M, Sweeting Michael J, Dudbridge F, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults. Journal of the American College of Cardiology. 2018;72(16):1883-93.
10. Ference BA, Bhatt DL, Catapano AL, Packard CJ, Graham I, Kaptoge S, et al. Association of Genetic Variants Related to Combined Exposure to Lower Low-Density Lipoproteins and Lower Systolic Blood Pressure With Lifetime Risk of Cardiovascular Disease. JAMA. 2019;322(14):1381-91.
11. Giontella A, Sjögren M, Lotta LA, Overton JD, Baras A, Minuz P, et al. Clinical Evaluation of the Polygenetic Background of Blood Pressure in the Population-Based Setting. Hypertension (Dallas, Tex : 1979). 2021;77(1):169-77.
12. Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults: Implications for Primary Prevention. Journal of the American College of Cardiology. 2018;72(16):1883-93.
13. Siemelink MA, van der Laan SW, van Setten J, de Vries JP, de Borst GJ, Moll FL, et al. Common variants associated with blood lipid levels do not affect carotid plaque composition. Atherosclerosis. 2015;242(1):351-6.
14. Reilly MP, Li M, He J, Ferguson JF, Stylianou IM, Mehta NN, et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet. 2011;377(9763):383-92.
15. Simons PC, Algra A, van de Laak MF, Grobbee DE, van der Graaf Y. Second manifestations of ARTerial disease (SMART) study: rationale and design. Eur J Epidemiol. 1999;15(9):773-81.
16. Tremblay AJ, Morrissette H, Gagne JM, Bergeron J, Gagne C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. Clin Biochem. 2004;37(9):785-90.

17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604-12.

19. PLINK [Internet]. Harvard [2022 Jan 15]. Available from: http://pngu.mgh.harvard.edu/purcell/plink/.

20. Liu C, Kraja AT, Smith JA, Brody JA, Franceschini N, Bis JC, et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. Nat Genet. 2016;48(10):1162-70.

21. Pan-ancestry genetic analysis of the UK Biobank [Internet]. Pan UKBB Team; 2021 [2022 Jan 15.]. Available from: https://pan.ukbb.broadinstitute.org.

22. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. 2015;4(1).

23. Pan-UK Biobank phenotype manifest [Internet]. [2022 Jan 15]. Available from: https://docs.google.com/spreadsheets/d/1AeeADtT0U1AukliiNyiVzVRdLYPkTbruQSk38DeutU8/edit#gid=511623409.

24. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol. 2006;59(10):1087-91.

25. Dahabreh IJ, Kent DM. Index event bias as an explanation for the paradoxes of recurrence risk research. JAMA. 2011;305(8):822-3.

26. Howe LJ, Dudbridge F, Schmidt AF, Finan C, Denaxas S, Asselbergs FW, et al. Polygenic risk scores for coronary artery disease and subsequent event risk amongst established cases. Hum Mol Genet. 2020;29(8):1388-95.

27. Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield M, Devlin JJ, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. Lancet. 2015;385(9984):2264-71.

28. Khera AV, Emdin CA, Drake I, Natarajan P, Bick AG, Cook NR, et al. Genetic Risk, Adherence to a Healthy Lifestyle, and Coronary Disease. N Engl J Med. 2016;375(24):2349-58.

29. Damask A, Steg PG, Schwartz GG, Szarek M, Hagström E, Badimon L, et al. Patients With High Genome-Wide Polygenic Risk Scores for Coronary Artery Disease May Receive Greater Clinical Benefit From Alirocumab Treatment in the ODYSSEY OUTCOMES Trial. Circulation. 2020;141(8):624-36.

30. Marston NA, Kamanu FK, Nordio F, Gurmu Y, Roselli C, Sever PS, et al. Predicting Benefit From Evolocumab Therapy in Patients With Atherosclerotic Disease Using a Genetic Risk Score: Results From the FOURIER Trial. Circulation. 2020;141(8):616-23.

31. Paquette M, Baass A. Polygenic risk scores for cardiovascular disease prediction in the clinical practice: Are we there? Atherosclerosis. 2022;340:46-7.

32. Patel RS, Tragante V, Schmidt AF, McCubrey RO, Holmes MV, Howe LJ, et al. Subsequent Event Risk in Individuals With Established Coronary Heart Disease. Circ Genom Precis Med. 2019;12(4):e002470.

33. Wünnemann F, Sin Lo K, Langford-Avelar A, Busseuil D, Dubé MP, Tardif JC, et al. Validation of Genome-Wide Polygenic Risk Scores for Coronary Artery Disease in French Canadians. Circ Genom Precis Med. 2019;12(6):e002481.

34. Martin AR, Gignoux CR, Walters RK, Wojcik GL, Neale BM, Gravel S, et al. Human Demographic History Impacts Genetic Risk Prediction across Diverse Populations. Am J Hum Genet. 2017;100(4):635-49.

35. Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. Nat Genet. 2019;51(4):584-91.

Supplemental Material

Supplemental Table 1 to 14 and Supplemental Figure 1 and 2 can be accessed by using the QR code below.

CHAPTER 3

The relation between urinary sodium and potassium excretion and risk of cardiovascular events and mortality in patients with cardiovascular disease

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Abstract

Background: Most evidence on the relationship between sodium and potassium intake and cardiovascular disease originated from general population studies. This study aimed to evaluate the relation between estimated 24-hour sodium and potassium urinary excretion and the risk of recurrent vascular events and mortality in patients with vascular disease.

Methods: 7561 patients with vascular disease enrolled in the UCC-SMART cohort (1996-2015) were included. Twenty-four hour sodium and potassium urinary excretion were estimated (Kawasaki formulae) from morning urine samples. Cox proportional hazard models with restricted cubic splines were used to evaluate the relation between estimated urinary salt excretion and major adverse cardiovascular events (MACE; including myocardial infarction, stroke, cardiovascular mortality) and all-cause mortality.

Results: After a median follow-up of 7.4 years (interquartile range: 4.1-11.0), the relations between estimated 24-hour sodium urinary excretion and outcomes were J-shaped with nadirs of 4.59 gram/day for recurrent MACE and 4.97 gram/day for all-cause mortality. The relation between sodium-to-potassium excretion ratio and outcomes were also J-shaped with nadirs of 2.71 for recurrent MACE and 2.60 for all-cause mortality. Higher potassium urinary excretion was related to an increased risk of both recurrent MACE (HR 1.25 per gram potassium excretion per day; 95%CI 1.13–1.39) and all cause-mortality (HR 1.13 per gram potassium excretion per day; 95%CI 1.03–1.25).

Conclusions: In patients with established vascular disease, lower and higher sodium intake were associated with higher risk of recurrent MACE and all-cause mortality. Higher estimated 24-hour potassium urinary excretion was associated with a higher risk of recurrent MACE and all-cause mortality.

Introduction

Blood pressure (BP) control is an essential target for the prevention and management of recurrent cardiovascular disease (CVD) in patients with established vascular disease. In adults with and without hypertension, higher sodium intake is linearly associated with higher BP levels (1,2), and therefore most treatment guidelines advocate dietary sodium restriction to levels between 1.5 and 2.4 g per day to lower the risk of (recurrent) CVD (3–5).

However, previous cohort studies evaluating the association between sodium intake and CV events in primary prevention populations have shown conflicting results. While some studies report a neutral or positive linear association between sodium intake and CVD and total mortality (6–8), others demonstrate a J- or U-shaped relationship between estimated sodium intake and CVD risk with lower and higher sodium intake both being associated with higher risk of CVD, all-cause mortality, and longevity (9–12). Thus, guideline recommendations on dietary sodium intake conflict with findings from several observational studies regarding CVD risk.

In contrast to sodium, higher potassium intake has been inversely related to BP levels and may have a protective effect, thereby modifying the association between sodium intake, BP and CVD (10,13). Consequently, both the World Health Organization (WHO) and recent guidelines on the primary prevention of CVD recommend an intake of at least 3.5 grams per day (4,5,14). In addition, emerging evidence suggest that the sodium-to-potassium excretion ratio represents a more important risk factor for CVD than sodium and potassium separately (6,15). Since most of the evidence on the relationship between sodium and potassium intake and CVD originated from general population studies, the question is whether the above guideline recommendations can be applied to patients with established vascular disease. Clarifying the optimal dietary sodium and potassium intake is especially important in patients with clinical manifest arterial disease who are most likely to receive recommendations regarding dietary salt intake.

Hence, the aim of this study was to examine the relation between estimates of 24-hour sodium and potassium urinary excretion (as proxies for dietary intake), as well as their ratio, and the risk of recurrent major adverse cardiovascular events (MACE) and all-cause mortality in a high-risk population cohort with stable CVD.

Methods

Study design and participants

Patients originated from the Utrecht Cardiovascular Cohort-Second Manifestation of ARTerial disease (UCC-SMART) cohort. The UCC-SMART cohort is an ongoing, prospective cohort study starting from 1996 and comprised of 18 to 79-year-old patients referred to the University Medical Center Utrecht (UMCU), the Netherlands, for management of atherosclerotic disease or cardiovascular risk factors. A detailed description of the study rationale and design has been previously described (16). The study is in accordance with the 1964 Helsinki declaration, was approved by the institutional review board of the Utrecht University Medical Center, and all patients gave written informed consent.

For the current study, patients with established vascular disease (coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) at baseline between January 1996 and February 2015 were included (n = 7561).

Baseline assessment

At baseline, the patients underwent a standardized vascular screening protocol consisting of a health questionnaire including medical history and risk factors, physical examination and laboratory testing.

Office BP was measured with a nonrandom sphygmomanometer (Iso-Stabil 5; Speidel & Keller, Jungingen, Germany) three times simultaneously at the right and left upper arm in an upright position with an interval of 30 seconds. The mean of the last two BP measurements from the arm with the highest BP was used. Hypertension was defined as a prescription of antihypertensive medication and/or an office systolic BP of ≥140 or diastolic BP of ≥90 mmHg.

Laboratory blood testing was performed in fasting state for total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, creatinine, and high-sensitivity C-reactive protein (CRP). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (17) up to a plasma triglycerides level of 9 mmol/L (18). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (19).

Upon arrival at the study clinic, usually in the morning, a urine sample was collected in fasting state and stored at -20°C. Urinary sodium and potassium levels were measured using an ARCHITECT ci8200 analyzer (Abbott Laboratories, Lake Bluff, Illinois, USA). The coefficient of variation for both sodium and potassium was 3%, and 6% for creatinine. The Kawasaki formula was used to estimate 24-hour sodium and potassium urinary excretion from a fasting morning urine sample, and these estimates were used as proxies for sodium and potassium intake (20) (Supplemental Table 1). We chose to use the Kawasaki formula

to allow comparability between this and previous studies and because this formula is considered the least biased method for estimating 24-hour sodium excretion compared to other formula-based approaches (21).

Outcome assessment

Patients received a bi-annual health questionnaire concerning hospitalizations and outpatient clinic visits. Outcomes of interest for this study were first occurrence of myocardial infarction, stroke, vascular death, and a composite of these events (all vascular events). All-cause mortality was recorded as well. Definitions of events are shown in Supplemental Table 2. When a possible event was reported, hospital records including radiology examinations, laboratory reports, and hospital discharge letters, were collected. Death and cause of death were reported by relatives of the participant, the general practitioner, or the vascular specialist. The medical records and information from the questionnaire and/or the family were subsequently assessed by three separate physicians from the study end-point committee. Duration of follow-up was defined as the time between study enrollment and first cardiovascular event or death from any cause, date of loss to follow-up (n=407 (5.4%)), or the preselected date of March 1st, 2015.

Statistical analysis

Baseline characteristics are presented stratified in quintiles of estimated 24-hour sodium and potassium urinary excretion. Because complete case analysis would lead to loss of statistical power and possibly bias (22), missing data of determinants and possible confounders (urine sodium (n=510, 6.7%), potassium (n=440, 5.8%), urine creatinine (n=200, 2.6%), CRP (n=179, 2.4%) and ≤1% for other variables) was imputed using single regression imputation (aregImpute-algorithm in R, Hmisc package).

Linear regression models were fitted to examine the association between estimated 24-hour sodium and potassium urinary excretion and blood pressure. Restricted cubic-spline functions with four knots were used to explore the shape of the association between baseline salt measures (estimated 24-hour sodium urinary excretion, estimated 24-hour potassium urinary excretion, and the ratio between the two) and the outcomes (23). Based on visual inspection of the restricted-cubic spline plots, a quadratic relation between outcomes and estimated 24-hour sodium urinary excretion and the sodium-to-potassium excretion ratio seemed present. Hence, we fitted multivariable Cox proportional-hazards models, including linear and quadratic terms for estimated 24-hour sodium urinary excretion and the sodium-to-potassium excretion ratio. As the restricted cubic-spline plots of the relationship between the estimated 24-hour potassium urinary excretion and outcomes showed no sign of non-linearity, these Cox proportional-hazards model only included a linear term. Proportional hazards assumptions were tested by visual inspection of Schoenfeld residuals plots and no violation was observed.

Analyses were adjusted for age, sex, body mass index (BMI), smoking, presence of diabetes, eGFR, and non-HDL cholesterol. The p-values of the effects of baseline salt measures on the occurrence of vascular events and mortality were based on the $\chi 2$ statistic. Nadirs (value of salt measures associated with lowest hazard) were derived for the non-linear relations. Hazard ratios (HR's) with 95% confidence intervals (CIs) were reported for the linear associations. Nadirs were derived as the minimum of the quadratic function that models the relation between outcomes and baseline salt measures. For graphic representation of the relationship between estimated sodium urinary excretion and the sodium-to-potassium excretion ratio and cardiovascular events and mortality, hazard ratios and 95% CIs were plotted, taking the corresponding nadir as a reference.

We performed interaction analyses for key characteristics that might modify the association between salt measures and CV events (age (<65 years versus ≥ 65 years), sex, use of blood-pressure lowering medication, and hypertension). Moreover, we tested the interaction between estimated 24-hour sodium and potassium urinary excretion. When a significant interaction was found, the analyses were stratified according to the effect modifying characteristic.

Sensitivity analyses were performed to evaluate the likelihood of reverse causality. Because reverse causality, if present, affects short-term rather than long-term results, analyses were repeated excluding patients with events within 1, 2, and 5 year(s) after inclusion. Furthermore, we performed analyses excluding patients treated with loop diuretics at baseline since this is often prescribed in the treatment of heart failure and often also accompanied by sodium restriction. Lastly, to evaluate whether patients with low levels of salt excretion had lower survival rates in the first years of follow-up, Kaplan-Meier survival curves were plotted by quintile of each salt measure (estimated 24-hour sodium excretion, estimated potassium excretion, and stage-to-potassium ratio) for recurrent CVD and all-cause mortality.

All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed, with statistical significance set at 0.05.

Results

Baseline characteristics

Baseline characteristics for all subjects categorized by quintile of estimated 24-hour sodium urinary excretion and estimated 24-hour potassium urinary excretion are summarized in Table 1 and Supplemental Table 3, respectively. The mean estimated 24-hour sodium urinary excretion was 4.91 g/day (standard deviation (SD) 1.41), and the mean estimated 24-hour potassium urinary excretion was 2.18 g/day (SD 0.53). Patients with low estimated 24-hour sodium and potassium urinary excretion were younger, had lower BMI, were less likely to have a history of diabetes mellitus or coronary artery disease; and generally had a lower blood pressure. Furthermore, they were more likely to be current smokers, have a history of cerebrovascular disease, and use diuretics.

During a median follow-up of 7.4 years (interquartile range (IQR): 4.1-11.0 years; 58,386 person-years), the composite outcome of myocardial infarction, stroke, or vascular death occurred in 1332 patients. A total of 1502 deaths were reported.

Relation between estimated 24-hour sodium and potassium excretion and blood pressure

Adjusted linear regression models assessing the relationship between baseline estimated 24-hour sodium urinary excretion and baseline blood pressure showed that every 1 g/day increase of sodium urinary excretion was associated with a higher mean (95% CI) systolic blood pressure and diastolic blood pressure of 1.28 mmHg (0.95-1.62) and 0.46 mmHg (0.28-0.65), respectively. Every 1 g/day increase of potassium urinary excretion was also associated with a higher mean (95% CI) systolic blood pressure and diastolic blood pressure of 1.04 mmHg (0.15-1.93) and 0.61 mmHg (0.11-1.11), respectively.

Relation between 24-hour sodium excretion and recurrent cardiovascular events and all-cause mortality

The relationship between estimated 24-hour sodium urinary excretion and the incidence of vascular events followed a J-shaped curve, with increased hazard ratios at low and high sodium urinary excretions. This was initially explored by a Cox proportional-hazards model with restricted cubic splines (Supplemental Figure 1) and confirmed by a non-linear Cox proportional-hazards model including linear and quadratic sodium urinary excretion terms ($p=0.02$; non-linear term $p<0.01$) (Figure 1A). Similarly, the relationship between estimated 24-hour sodium urinary excretion and all-cause mortality followed a J-shaped curve (Figure 1B; $p<0.01$; non-linear term $p<0.01$). The nadir for vascular events was 4.59 g/day and 4.97 g/day for all-cause mortality. No association was found between estimated 24-hour sodium urinary excretion and the occurrence of stroke ($p=0.91$, non-linear term $p=0.61$) (Supplemental Figure 2) and the occurrence of myocardial infarction ($p=0.97$; non-linear term $p=0.76$) (Supplemental Figure 3). Still, the relationship between sodium urinary excretion and vascular mortality was J-shaped ($p<0.01$, non-linear term $p<0.01$, nadir 4.98) (Supplemental Figure 4).

Table 1. Baseline characteristics of all participants, according to estimated 24-hour sodium excretion.

		Estimated urinary sodium excretion, g/day; quintiles				
	Overall	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Range quintiles (g/day)		**[1.28 - 3.73]**	**[3.74-4.47]**	**[4.48-5.13]**	**[5.14-5.97]**	**[5.98-16]**
Mean Sodium (g/day)	4.9 ± 1.4	3.1 ± 0.5	4.1 ± 0.2	4.8 ± 0.2	5.5 ± 0.2	7.0 ± 1.0
	n = 7561	n = 1513	n = 1512	n = 1512	n = 1512	n = 1512
Male sex	5574 (74%)	864 (57%)	1036 (69%)	1153 (76%)	1227 (81%)	1294 (86%)
Age (years)	60 ± 10	58 ± 11	60 ± 10	60 ± 10	61 ± 10	61 ± 10
Current smoker	2396 (32%)	606 (40%)	487 (32%)	496 (33%)	414 (27%)	393 (26%)
Physical examination						
Body mass index (kg/m2)	26.8 ± 4.0	26.0 ± 4.1	26.3 ± 3.8	26.7 ± 3.7	27.2 ± 3.9	28.0 ± 4.3
Systolic blood pressure (mmHg)	140 ± 21	137 ± 20	139 ± 21	140 ± 20	141 ± 21	143 ± 21
Diastolic blood pressure (mmHg)	81 ± 11	80± 11	80 ± 11	81 ± 11	82 ± 11	82 ± 11
History of vascular disease						
Diabetes mellitus	1327 (18%)	218 (14%)	221 (15%)	225 (15%)	287 (19%)	376 (25%)
Coronary artery disease	4576 (61%)	784 (52%)	880 (58%)	930 (62%)	990 (65%)	992 (66%)
Peripheral artery disease	1408 (19%)	312 (21%)	290 (19%)	264 (17%)	273 (18%)	269 (18%)
Cerebrovascular disease	2247 (30%)	545 (36%)	468 (31%)	438 (29%)	397 (26%)	399 (26%)
Abdominal aortic aneurysm	650 (9%)	124 (8%)	126 (8%)	107 (7%)	132 (9%)	161 (11%)
Laboratory values						
Potassium excretion (g/day)	2.2 ± 0.5	1.9 ± 0.5	2.0 ± 0.4	2.1 ± 0.5	2.3 ± 0.5	2.6 ± 0.6
Total cholesterol (mmol/L)	4.9 ± 1.2	5.0 ± 1.2	4.9 ± 1.2	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.2
HDL-cholesterol (mmol/L)	1.2 ± 0.4	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.4
LDL-cholesterol (mmol/L)	2.9 ± 1.1	3.0 ± 1.1	2.9 ± 1.1	2.8 ± 1.1	2.8 ± 1.0	2.8 ± 1.1
Triglycerides (mmol/L)	1.4 (1.0-2.0)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.1)	1.4 (1.0 - 2.0)
Estimated GFR (ml/min/1.73m2)	76 ± 18	77 ± 19	76 ± 17	77 ± 17	76 ± 18	77 ± 19
CRP (mg/L)	2.1 (2.1- 4.4)	2.4 (1.1 - 4.9)	2.0 (1.0 - 4.2)	1.9 (0.9 - 4.0)	1.9 (0.9 - 4.5)	2.1 (1.0 - 4.5)
Medication use						
Lipid lowering	5091 (67%)	981 (65%)	994 (66%)	1033 (68%)	1039 (69%)	1044 (69%)
Platelet inhibitor	5762 (76%)	1109 (73%)	1165 (77%)	1141 (75%)	1184 (78%)	1163 (77%)
Antihypertensives	5599 (74%)	1105 (73%)	1061 (70%)	1093 (72%)	1164 (77%)	1176 (78%)
Diuretics	1574 (21%)	467 (31%)	305 (20%)	251 (17%)	262 (17%)	289 (19%)
Loop diuretics	617 (8%)	253 (17%)	109 (7%)	82 (5%)	89 (6%)	84 (6%)
Thiazide diuretics	874 (12%)	191 (13%)	178 (12%)	159 (11%)	156 (10%)	190 (13%)
ACE-inhibitors	2298 (30%)	523 (35%)	419 (28%)	475 (31%)	442 (29%)	439 (29%)
Beta-blockers	4023 (53%)	751 (50%)	738 (49%)	838 (55%)	863 (57%)	833 (55%)
Calcium antagonists	1568 (21%)	278 (18%)	268 (18%)	268 (18%)	323 (21%)	431 (29%)

All data in n (%) or mean ± standard deviation (except for triglycerides and CRP: median with IQR). HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index; eGFR, estimated glomerular filtration rate (calculated with Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula).

Relation between 24-hour potassium excretion and recurrent cardiovascular events and all-cause mortality

No evidence of non-linearity in the relations between estimated 24-hour potassium urinary excretion and any outcome was found in the fully adjusted models; all non-linear p-values were >0.05 (Supplemental Figure 1). Therefore, Cox proportional-hazards models that investigated the relation between potassium urinary excretion and recurrent MACE and all-cause mortality only included linear terms for potassium urinary excretion. In the fully adjusted models, potassium urinary excretion was observed to have a positive relation with the primary composite outcome (MI, stroke, and cardiovascular mortality) (HR 1.25; 95%CI 1.13 – 1.39) (Figure 1C) and the separate components myocardial infarction (HR 1.26; 95%CI 1.07-1.48) and cardiovascular mortality (HR 1.20; 95%CI 1.06-1.37) (Supplemental Figures 2-4). Also, potassium urinary excretion was positively associated with all-cause mortality (HR 1.13; 95%CI 1.03 – 1.25) (Figure 1D).

Relation between sodium-to-potassium excretion ratio and recurrent cardiovascular events and all-cause mortality

The relationship between sodium-to-potassium excretion ratio and the incidence of vascular events followed a J-shaped curve, with increased hazard rates at low and high ratios (Figure 1E; p<0.01; non-linear term p<0.01). Also, the relationship between sodium-to-potassium excretion ratio and all-cause mortality followed a J-shaped curve (Figure 1F; p<0.01; non-linear term p<0.01). The nadir for vascular events was 2.71 and 2.60 for all-cause mortality. No association was found between the sodium-to-potassium excretion ratio and the occurrence of stroke (p=0.72, non-linear term p=0.52) (Supplemental Figure 2) and the occurrence of myocardial infarction (p=0.14; non-linear term p=0.23) (Supplemental Figure 3). Still, the relationship between sodium-to-potassium excretion ratio and vascular mortality was J-shaped (p<0.01, non-linear term p<0.01, nadir 2.64) (Supplemental Figure 4).

Interactions

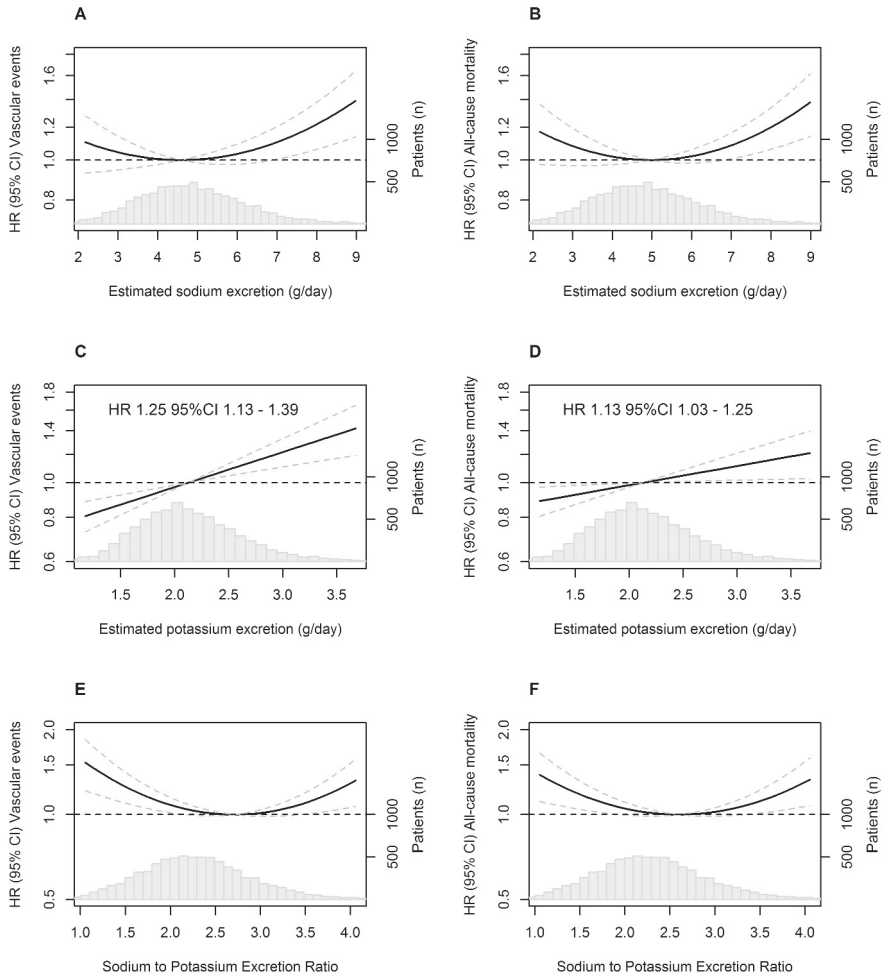
Results of the interaction tests are shown in Supplemental Table 4. The effect of sodium-to-potassium excretion ratio on all-cause mortality was modified by age (<65 versus ≥65 years). Hence, results were stratified according to age (Supplemental Figure 5). In patients aged ≥65 years, the sodium-to-potassium excretion ratio was not associated with all-cause mortality. There were no other significant interaction terms.

Sensitivity analysis

The shape of the relationship between sodium and potassium urinary excretion and vascular events and mortality did not materially change after exclusion of patients who experienced events or died within 1, 2, and 5 year(s) after inclusion and after exclusion of patients treated with loop diuretics (n=617) (a surrogate for heart failure patients) (Supplemental Figures 6 and 7). In the first years of follow-up, survival rates for patients in

the lower quintiles of salt excretion were similar to those of patients in the other quintiles of salt excretion (Supplemental Figure 8).

Figure 1. Relation between salt excretion and recurrent cardiovascular events and mortality.



Adjusted hazard ratios for vascular events and mortality by baseline estimated salt excretion (distribution shown by histogram) **A.** Relation between estimated 24-hour urinary sodium excretion and vascular events (linear term P=0.02; non-linear term P<0.01). Nadir: 4.59 g/day. **B.** Relation between estimated 24-hour urinary sodium excretion and mortality (linear term P<0.01; non-linear term <0.01). Nadir: 4.97 g/day. **C.** Relation between 1 gram/day higher estimated 24-hour urinary potassium excretion and vascular events. **D.** Relation between 1 gram/day higher estimated 24-hour urinary potassium excretion and mortality. **E.** Relation between sodium-to-potassium excretion ratio and vascular events (linear term P<0.01; non-linear term <0.01). Nadir: 2.71 g/day. **F.** Relation between sodium-to-potassium excretion ratio and mortality (linear term P<0.01; non-linear term <0.01). Nadir: 2.60 g/day. All hazard ratios were plotted between the 1st and 99th percentile of the corresponding salt measure. Dotted lines represent 95% confidence intervals. All models were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Discussion

In the current study we found a J-shaped relation between estimated 24-hour sodium urinary excretion and recurrent vascular events and mortality in patients with vascular disease. The optimum estimated sodium urinary excretion found was between 4.5 grams per day and 5.0 grams per day, which is generally viewed as an excess in sodium intake. This J-shaped relation was even more pronounced when accounting for potassium intake, using the sodium-to-potassium excretion ratio, with an optimum ratio between 2.5 and 3.0. Increasing values of estimated 24-hour potassium urinary excretion increased the risk of recurrent vascular events and mortality, and this relation was linear.

Several previous observational studies in populations at high cardiovascular risk have also found a J-shaped curve between sodium urinary excretion levels and the risk of CVD and mortality (24–26). In line with our findings, an observational post hoc analysis of 28,880 participants of the ONTARGET and TRANSCEND trials with established CVD or high-risk diabetes mellitus found a sodium excretion between 4 and 5.99 gram per day as the optimum level of sodium excretion using cardiovascular death, myocardial infarction, stroke, and hospitalization for congestive heart failure as outcome (24). Studies in patients with diabetes (type 1 and 2) also found lower 24-hour urinary sodium excretion to be associated with increased cardiovascular (25) and all-cause mortality (25,26). Results from the current study add to the limited amount of evidence on the relation between sodium and cardiovascular events and mortality in a population with vascular disease.

Reverse causality has been proposed as an explanation for the relation observed between low sodium excretion and vascular events and mortality (27). Observations suggestive of reverse causality include that a J-shaped association is seen during short, but not during prolonged follow-up (28) or that an initially present J-shaped relation becomes linear after exclusion of study participants having conditions that lead to reduced sodium intake and are simultaneously associated with an increased risk of adverse events . Sensitivity analyses of the present study showed that exclusion of patients with events within 1, 2, and 5 year(s) after start of the study and exclusion of patients treated with loop diuretics, considered as a proxy for a diagnosis of congestive heart failure, did not materially alter the shape of the relations. Still, we recognize that reverse causality cannot be completely ruled out and may partly account for the increased risk observed in patients with low sodium excretion.

Second, systematic error in sodium measurement has been proposed as an explanation for the paradoxical U- or J-shape relation (29). Similar to this study, previous cohort studies often used formulas to estimate an individual's usual sodium intake based on a single spot urine rather than multiple non-consecutive 24-hour urine collections (30,31). Although the latter is cumbersome and logistically more challenging, the formula-based approach may result in systematic errors with overestimation at lower levels

and underestimation at higher levels of sodium intake (32,33). This may even change the shape of the dose-response curve; placing subjects in poor health into groups with low sodium intake and falsely ascribe higher mortality to low sodium (33). Although, a J-shaped relationship was also described in studies that measured sodium intake by 24-hour urine collections (9,26), it cannot be ruled out that the formula-based approach may in part lead to these paradoxical findings.

Third, it is also possible that the J-shaped relation is due to selection on the index event (34). This can be understood by considering the onset of vascular events as the sum of the effect of multiple causal factors. If one important causal risk factor such as high sodium intake is already present, less effect of other factors is required for disease onset. Subsequently, comparing high sodium consumers with low sodium consumers who already have developed vascular disease, leads to the high sodium consumers having a relatively healthy risk profile compared to low sodium consumers in both measured and unmeasured factors. Nonetheless, the observed associations in this study remained after adjustment for most known risk factors for vascular disease, making index event bias a less likely explanation.

Besides methodological explanations, a causal mechanism explaining the relation observed between low sodium excretion and vascular events and mortality should also be considered. Sodium is an important electrolyte in the extracellular fluid and has an essential role in regulating the intra- and extracellular fluid. Previous neuroscience studies in animals have revealed neural networks that play a role in the regulation of sodium appetite to ensure a certain level of sodium intake (35). From these studies, it is hypothesized that sodium is under strict control, which is supported by the observation that sodium is often within a narrow range. For example, the mean estimated 24-hour sodium excretion level in our study is close to the mean range for sodium intake defined by previous analyses of worldwide 24-hour urinary sodium excretion data (36–38). Low sodium intake may therefore result in activation of a physiological mechanism to balance sodium concentration including an increase in plasma renin activity and aldosterone which consequently increase in sympathetic nerve activity (39), serum cholesterol and triglyceride levels, adrenalin secretion (40), and resistance to insulin (41,42), which may counteract the benefit of lowering blood pressure.

In the current study, a positive linear relationship between estimated 24-hour urinary potassium excretion and the risk of recurrent MACE and all-cause mortality was observed. Considering the separate components of MACE, the effect of potassium excretion on recurrent MACE was mainly driven by an increased risk of myocardial infarction. These findings differ compared to previous studies in primary and secondary prevention cohorts describing non-significant associations between potassium intake and coronary heart disease and significant inverse associations between potassium intake and MACE, respectively (13,43,44). The discrepancies between our study and previous studies may

be due to the difference in case-mix (patients with versus without vascular disease) and use of different statistical approaches. For example, previous studies were able to adjust for additional lifestyle factors (i.e. caloric, fruit, and vegetable intake), which reduced the risk of residual confounding (24). However, these studies often analyzed 24-hour urinary potassium excretion categorically rather than continuously (using non-linear terms), potentially leading to a loss of power and inaccurate estimations (45,46). Moreover, reverse causality and index events bias may also have played a role here. However, sensitivity analyses evaluating the likelihood of these biases showed similar results, making these explanations less likely.

As with all studies of observational nature, no definitive causal conclusions can be drawn. To guide clinical practice, these findings need to be replicated by large and long-term randomized controlled trials evaluating the effect of different targets for dietary salt intake on clinical (cardiovascular) outcomes in patients with clinically manifest vascular disease. In the recently published Salt Substitute and Stroke Study (SSaSS) (47), involving 20.995 persons with either a history of stroke or a high BP from 600 villages in rural China, the effect of regular salt (100% sodium chloride) was compared with a salt substitute (75% sodium chloride and 25% potassium chloride) with respect to stroke. The combined use of lower sodium and higher potassium, by means of this substitute, led to a lower rate of stroke than the use of regular salt (rate ratio 0.86; 95%CI 0.77-0.96). Although SSaSS provides some answers, it remains unclear whether the effect can be attributed to lower sodium intake, higher potassium intake or both.

Strengths of the present cohort study include the large number of patients with manifest vascular disease with extensive and standardized measurement of risk factors at baseline and a long follow-up with a low proportion of patients lost to follow-up. Furthermore, the generalizability of the results is high as the UCC-SMART cohort consists of a referred patient population with a broad spectrum of vascular disease. A limitation of the study includes the possibility of measurement error when using the Kawasaki formulas for the conversion of spot urine sodium and potassium measurements into estimated 24-hour urinary excretion. Since a lower proportion (~77%) of ingested potassium is excreted renally (48), the estimated 24-hour urinary potassium excretion in this study is likely a suboptimal reflection of actual potassium intake in this population. Lastly, patient characteristics were only measured at baseline which made it unable to address the time-varying nature of sodium and potassium excretion.

In conclusion, in this observational study, relations between both estimated 24-hour sodium urinary excretion and sodium-to-potassium excretion ratio and recurrent MACE and all-cause mortality were J-shaped, with sodium excretion above and below 4.5-5.0 gram per day both being associated with higher risk of recurrent MACE and all-cause mortality. Furthermore, higher estimated 24-hour potassium urinary excretion was associated with a higher risk of recurrent MACE, mainly driven by an increased risk of

myocardial infarction, and all-cause mortality. These results provide no evidence for dietary sodium restriction to levels between 1.5 and 2.4 g per day as a means of reducing the risk of recurrent CVD in patients with vascular disease and underline the need for further investigation into the relation between salt intake and cardiovascular disease in this population.

References

1. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. N Engl J Med. 2014 Aug;371(7):624–34.
2. Filippini T, Malavolti M, Whelton PK, Naska A, Orsini N, Vinceti M. Blood Pressure Effects of Sodium Reduction: Dose-Response Meta-Analysis of Experimental Studies. Circulation. 2021 Feb;
3. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
4. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Sep;140(11):e596–646.
5. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep;42(34):3227–337.
6. Cook NR, Obarzanek E, Cutler JA, Buring JE, Rexrode KM, Kumanyika SK, et al. Joint effects of sodium and potassium intake on subsequent cardiovascular disease: the Trials of Hypertension Prevention follow-up study. Arch Intern Med. 2009 Jan;169(1):32–40.
7. Tuomilehto J, Jousilahti P, Rastenyte D, Moltchanov V, Tanskanen A, Pietinen P, et al. Urinary sodium excretion and cardiovascular mortality in Finland: a prospective study. Lancet (London, England). 2001 Mar;357(9259):848–51.
8. Welsh CE, Welsh P, Jhund P, Delles C, Celis-Morales C, Lewsey JD, et al. Urinary Sodium Excretion, Blood Pressure, and Risk of Future Cardiovascular Disease and Mortality in Subjects Without Prior Cardiovascular Disease. Hypertens (Dallas, Tex 1979). 2019 Jun;73(6):1202–9.
9. Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerová J, Richart T, et al. Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. JAMA. 2011 May;305(17):1777–85.
10. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. N Engl J Med. 2014 Aug;371(7):612–23.
11. Graudal N, Jürgens G, Baslund B, Alderman MH. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: a meta-analysis. Am J Hypertens. 2014 Sep;27(9):1129–37.
12. McCarron DA, Geerling JC, Alderman MH. Urinary sodium excretion measures and health outcomes. Vol. 393, Lancet (London, England). England; 2019. p. 1294–5.
13. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013 Apr;346:f1378.
14. Organization WH. Guideline: potassium intake for adults and children. World Health Organization; 2012.
15. Yang Q, Liu T, Kuklina E V, Flanders WD, Hong Y, Gillespie C, et al. Sodium and potassium intake and mortality among US adults: prospective data from the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2011 Jul;171(13):1183–91.
16. Simons PC, Algra A, van de Laak MF, Grobbee DE, van der Graaf Y. Second manifestations of ARTerial disease (SMART) study: rationale and design. Eur J Epidemiol. 1999 Oct;15(9):773–81.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499–502.

18. Tremblay AJ, Morrissette H, Gagne J-M, Bergeron J, Gagne C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. Clin Biochem. 2004 Sep;37(9):785–90.

19. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009 May;150(9):604–12.

20. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol. 1993 Jan;20(1):7–14.

21. Mente A, O'Donnell MJ, Dagenais G, Wielgosz A, Lear SA, McQueen MJ, et al. Validation and comparison of three formulae to estimate sodium and potassium excretion from a single morning fasting urine compared to 24-h measures in 11 countries. J Hypertens. 2014 May;32(5):1005–14; discussion 1015.

22. Donders ART, van der Heijden GJMG, Stijnen T, Moons KGM. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol. 2006 Oct;59(10):1087–91.

23. Harrell Jr FE. Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis. Springer; 2015.

24. O'Donnell MJ, Yusuf S, Mente A, Gao P, Mann JF, Teo K, et al. Urinary sodium and potassium excretion and risk of cardiovascular events. JAMA. 2011 Nov;306(20):2229–38.

25. Ekinci EI, Clarke S, Thomas MC, Moran JL, Cheong K, MacIsaac RJ, et al. Dietary salt intake and mortality in patients with type 2 diabetes. Diabetes Care. 2011 Mar;34(3):703–9.

26. Thomas MC, Moran J, Forsblom C, Harjutsalo V, Thorn L, Ahola A, et al. The association between dietary sodium intake, ESRD, and all-cause mortality in patients with type 1 diabetes. Diabetes Care. 2011 Apr;34(4):861–6.

27. Cobb LK, Anderson CAM, Elliott P, Hu FB, Liu K, Neaton JD, et al. Methodological issues in cohort studies that relate sodium intake to cardiovascular disease outcomes: a science advisory from the American Heart Association. Circulation. 2014 Mar;129(10):1173–86.

28. Lawlor DA, Hart CL, Hole DJ, Davey Smith G. Reverse causality and confounding and the associations of overweight and obesity with mortality. Obesity (Silver Spring). 2006 Dec;14(12):2294–304.

29. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health. Int J Epidemiol. 2018 Dec;47(6):1784–95.

30. Sun Q, Bertrand KA, Franke AA, Rosner B, Curhan GC, Willett WC. Reproducibility of urinary biomarkers in multiple 24-h urine samples. Am J Clin Nutr. 2017 Jan;105(1):159–68.

31. Birukov A, Rakova N, Lerchl K, Olde Engberink RH, Johannes B, Wabel P, et al. Ultra-long-term human salt balance studies reveal interrelations between sodium, potassium, and chloride intake and excretion. Am J Clin Nutr. 2016 Jul;104(1):49–57.

32. Polonia J, Lobo MF, Martins L, Pinto F, Nazare J. Estimation of populational 24-h urinary sodium and potassium excretion from spot urine samples: evaluation of four formulas in a large national representative population. J Hypertens. 2017 Mar;35(3):477–86.

33. He FJ, Ma Y, Campbell NRC, MacGregor GA, Cogswell ME, Cook NR. Formulas to Estimate Dietary Sodium Intake From Spot Urine Alter Sodium-Mortality Relationship. Hypertens (Dallas, Tex 1979). 2019 Sep;74(3):572–80.

34. Dahabreh IJ, Kent DM. Index event bias as an explanation for the paradoxes of recurrence risk research. JAMA. 2011 Feb;305(8):822–3.

35. Lowell BB. New Neuroscience of Homeostasis and Drives for Food, Water, and Salt. N Engl J Med. 2019 Jan;380(5):459–71.

36. McCarron DA, Geerling JC, Kazaks AG, Stern JS. Can dietary sodium intake be modified by public policy? Clin J Am Soc Nephrol. 2009 Nov;4(11):1878–82.

37. McCarron DA, Kazaks AG, Geerling JC, Stern JS, Graudal NA. Normal range of human dietary sodium intake: a perspective based on 24-hour urinary sodium excretion worldwide. Am J Hypertens. 2013 Oct;26(10):1218–23.

38. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open. 2013 Dec;3(12):e003733.

39. Grassi G, Dell'Oro R, Seravalle G, Foglia G, Trevano FQ, Mancia G. Short- and long-term neuroadrenergic effects of moderate dietary sodium restriction in essential hypertension. Circulation. 2002 Oct;106(15):1957–61.

40. Graudal NA, Hubeck-Graudal T, Jurgens G. Effects of low sodium diet versus high sodium diet on blood pressure, renin, aldosterone, catecholamines, cholesterol, and triglyceride. Cochrane database Syst Rev. 2020 Dec;12:CD004022.

41. Petrie JR, Morris AD, Minamisawa K, Hilditch TE, Elliott HL, Small M, et al. Dietary sodium restriction impairs insulin sensitivity in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1998 May;83(5):1552–7.

42. Feldman RD, Schmidt ND. Moderate dietary salt restriction increases vascular and systemic insulin resistance. Am J Hypertens. 1999 Jun;12(6):643–7.

43. Kieneker LM, Gansevoort RT, de Boer RA, Brouwers FP, Feskens EJ, Geleijnse JM, et al. Urinary potassium excretion and risk of cardiovascular events. Am J Clin Nutr. 2016 May;103(5):1204–12.

44. Pickering RT, Bradlee ML, Singer MR, Moore LL. Higher Intakes of Potassium and Magnesium, but Not Lower Sodium, Reduce Cardiovascular Risk in the Framingham Offspring Study. Nutrients. 2021 Jan;13(1).

45. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. Lippincott Williams & Wilkins; 2020.

46. Bennette C, Vickers A. Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. BMC Med Res Methodol. 2012 Feb;12:21.

47. Neal B, Wu Y, Feng X, Zhang R, Zhang Y, Shi J, et al. Effect of Salt Substitution on Cardiovascular Events and Death. N Engl J Med. 2021 Aug;

48. Holbrook JT, Patterson KY, Bodner JE, Douglas LW, Veillon C, Kelsay JL, et al. Sodium and potassium intake and balance in adults consuming self-selected diets. Am J Clin Nutr. 1984 Oct;40(4):786–93.

Supplemental Material

Supplemental Table 1. Kawasaki formula used to predict 24-hour urinary sodium and potassium excretion from spot urine samples.

	Equation for estimating predicted 24-hour urine sodium or potassium excretion	Equation for estimating predicted 24-hour urine creatinine excretion (Pr24UCr mg/day)
Sodium (mg/day)	$23 \times (16.3 \times XNa^{0.5})$, where XNA = [spot Na (mmol/ l)/spot creatinine (mg/dL) × 10] × Pr24UCr (mg/day)	Pr24UCr (mg/day) for men = (12.63 × age (year)) + (15.12 × weight (kg)) + (7.39 × height (cm))− 79.9 Pr24UCr (mg/day) for women = (− 4.72 × age (year)) + (8.58 × weight (kg)) + (5.09 × height (cm))− 74.5
Potassium (mg/day)	$39 \times (7.2 \times XK^{0.5})$, where XK = [spot K (mmol/ L)/spot creatinine (mg/dL) × 10] × Pr24UCr (mg/day)	Pr24UCr (mg/day) for men = (12.63 × age (year)) + (15.12 × weight (kg)) + (7.39 × height (cm))− 79.9 Pr24UCr (mg/day) for women = (− 4.72 × age (year)) + (8.58 × weight (kg)) + (5.09 × height (cm))− 74.5

Supplemental Table 2. Definitions of vascular outcomes.

Outcome	Defined as
Myocardial infarction	(Non-)fatal myocardial infarction defined by ≥2 of the following: -Acute chest pain for at least 20 min -ST-elevation >1 mm in two adjacent leads or a left bundle branch block (LBBB) on ECG -Elevated troponin or elevated CK ≥2 times the normal value of CK and a MB-fraction >5% of the total CK; Or; - Coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI) -Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence).
Stroke	(Non-) fatal ischemic or hemorrhagic stroke: Relevant clinical features for at least 24 hours causing an increase in impairment of at least one grade of the modified Rankin scale, with/ without a new infarction or hemorrhage on CT or MRI.
Vascular mortality	Death from myocardial infarction, stroke, heart failure, or rupture of abdominal aortic aneurysm; vascular death from other causes; or sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence)).
Major Adverse Cardiovascular Events (MACE)	Composite of the above mentioned outcomes
All-cause mortality	All deaths during follow-up, irrespective of the cause of death.

Supplemental Table 3. Baseline characteristics of all participants, according to estimated 24 hour potassium excretion.

		Estimated urinary potassium excretion, g/d; quintiles				
	Overall	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**
Range quintiles (g/day)		**[0.72-1.76]**	**[1.77-2.01]**	**[2.02-2.25]**	**[2.26-2.57]**	**[2.58-7.09]**
Mean Potassium (g/day)	2.2 ± 0.5 n = 7561	1.5 ± 0.2 n = 1513	1.9 ± 0.1 n = 1512	2.1 ± 0.1 n = 1512	2.4 ± 0.1 n = 1512	3.0 ± 0.4 n = 1512
Male sex	5574 (74%)	784 (52%)	1058 (70%)	1162 (77%)	1257 (83%)	1313 (87%)
Age (years)	60 (10%)	58 ± 11	60 ± 10	61 ± 10	61 ± 10	61 ± 10
Current smoker	2396 (32%)	552 (36%)	502 (33%)	441 (29%)	472 (31%)	429 (28%)
Physical examination						
Body mass index (kg/m2)	26.8 ± 4.0	26.0 ± 3.9	26.5 ± 4.0	26.9 ± 3.9	27.2 ± 3.9	27.5 ± 4.2
Systolic blood pressure (mmHg)	140 ± 21	138 ± 21	140 ± 21	140 ± 20	141 ± 21	141 ± 21
Diastolic blood pressure (mmHg)	81 ± 11	80± 11	81 ± 11	81 ± 11	81 ± 11	82 ± 12
History of vascular disease						
Diabetes mellitus	1327 (18%)	224 (15%)	247 (16%)	261 (17%)	279 (18%)	316 (21%)
Coronary artery disease	4576 (61%)	813 (54%)	892 (59%)	933 (62%)	975 (64%)	963 (64%)
Peripheral artery disease	1408 (19%)	307 (20%)	296 (20%)	250 (17%)	293 (19%)	262 (17%)
Cerebrovascular disease	2247 (30%)	517 (34%)	473 (31%)	451 (30%)	388 (26%)	418 (28%)
Abdominal aortic aneurysm	650 (9%)	109 (7%)	125 (8%)	116 (8%)	144 (10%)	156 (10%)
Laboratory values						
Sodium excretion (g/day)	4.9 ± 1.4	4.0 ± 1.1	4.6 ± 1.1	4.9 ± 1.2	5.2 ± 1.3	5.9 ± 1.6
Total cholesterol (mmol/L)	4.9 ± 1.2	5.0 ± 1.3	4.8 ± 1.2	4.8 ± 1.2	4.8 ± 1.1	4.8 ± 1.2
HDL-cholesterol (mmol/L)	1.2 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.4
LDL-cholesterol (mmol/L)	2.9 ± 1.1	3.0 ± 1.1	2.8 ± 1.1	2.9 ± 1.0	2.9 ± 1.0	2.8 ± 1.1
Triglycerides (mmol/L)	1.4 (1.0 - 2.0)	1.4 (1.0 - 1.9)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.4 (1.0 - 2.0)	1.5 (1.0 - 2.1)
Estimated GFR (ml/min/1.73m2)	76 ± 18	78 ± 17	77 ± 17	75 ± 18	76 ± 18	76 ± 19
CRP (mg/L)	2.1 (1.0 - 4.4)	2.0 (0.9 - 4.4)	2.0 (1.0 - 4.4)	2.1 (1.0 - 4.5)	2.2 (1.0 - 4.4)	2.0 (0.9 - 4.4)
Medication use						
Lipid lowering	5091 (67%)	978 (65%)	1006 (67%)	1061 (70%)	1009 (67%)	1037 (69%)
Platelet inhibitor	5762 (76%)	1107 (73%)	1147 (76%)	1180 (78%)	1161 (77%)	1167 (77%)
Antihypertensives	5599 (74%)	1056 (70%)	1110 (73%)	1159 (77%)	1127 (75%)	1147 (76%)
Diuretics	1574 (21%)	324 (21%)	303 (20%)	341 (23%)	288 (19%)	318 (21%)
Loop diuretics	617 (8%)	115 (8%)	118 (8%)	130 (9%)	120 (8%)	134 (9%)
Thiazide diuretics	874 (12%)	189 (12%)	179 (12%)	191 (13%)	151 (10%)	164 (11%)
ACE-inhibitors	2298 (30%)	439 (29%)	477 (32%)	464 (31%)	435 (29%)	483 (32%)
Beta-blockers	4023 (53%)	776 (51%)	770 (51%)	851 (56%)	811 (54%)	815 (54%)
Calcium antagonists	1568 (21%)	260 (17%)	320 (21%)	315 (21%)	342 (23%)	331 (22%)

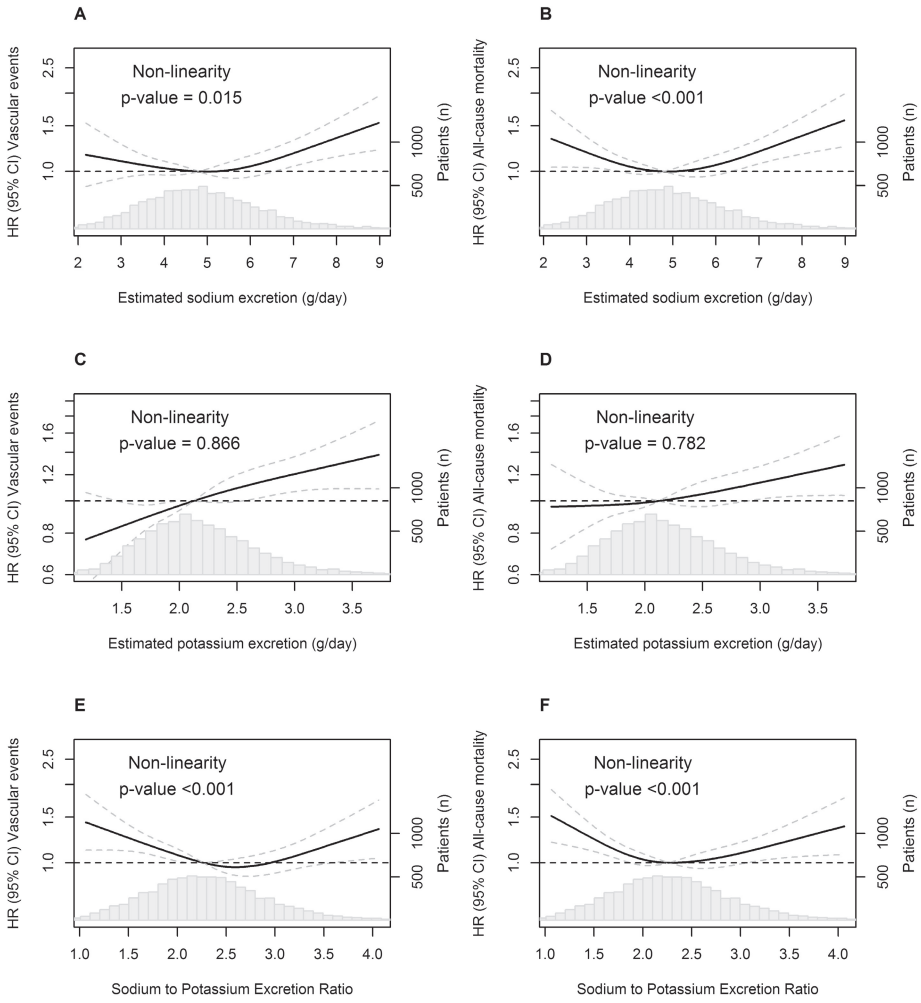
All data in n (%) or mean ± standard deviation (except for triglycerides and CRP: median with IQR). HDL, high-density lipoprotein; LDL, low-density lipoprotein; Hs-CRP, high-sensitivity C-reactive protein; BMI, body mass index; eGFR, estimated glomerular filtration rate (calculated with Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula).

Supplemental Table 4. P-values for interaction.

	Recurrent MACE			All-cause mortality		
Interaction variable:	*24h Na excretion*	*24h K excretion*	*Na-to-K ratio*	*24h Na excretion*	*24h K excretion*	*Na-to-K ratio*
Sex	0.93	0.35	0.54	0.52	0.89	0.95
quadratic term	0.86		0.59	0.51		0.88
Age	0.27	0.84	0.21	0.27	0.37	0.01*
quadratic term	0.15		0.11	0.27		0.01*
Hypertension	0.88	0.41	0.84	0.21	0.9	0.13
quadratic term	0.72		0.92	0.19		0.11
Use of antihypertensive drugs	0.11	0.53	0.11	0.35	0.41	0.18
quadratic term	0.06		0.11	0.19		0.12
24h K excretion	0.82			0.42		
quadratic term	0.27			0.11		
24h Na excretion		0.55			0.98	
quadratic term						

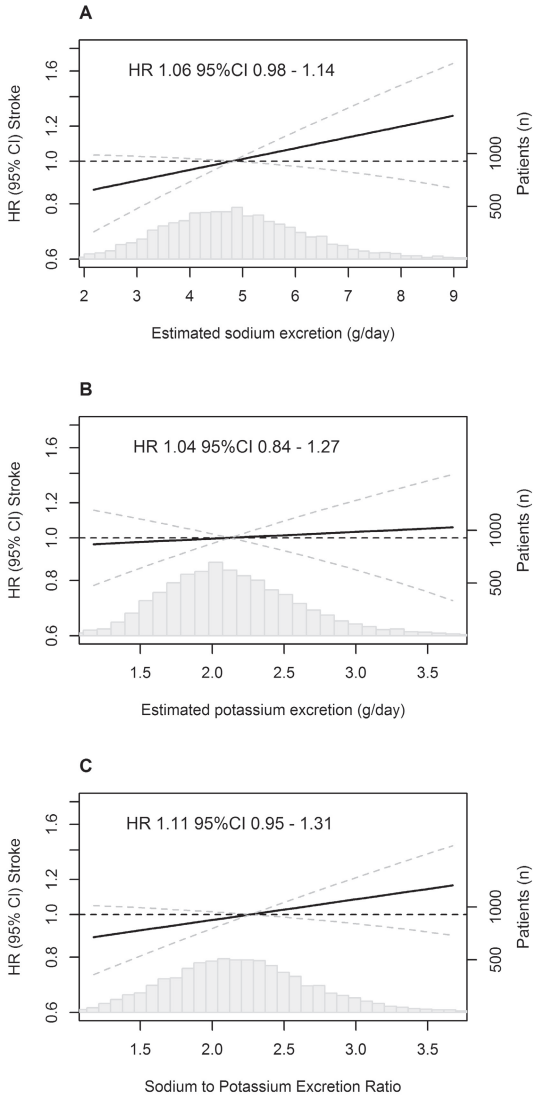
**significant interaction*

Supplemental Figure 1. Restricted-cubic-spline plots of the association between estimated salt excretion and recurrent major adverse cardiovascular events and all-cause mortality.



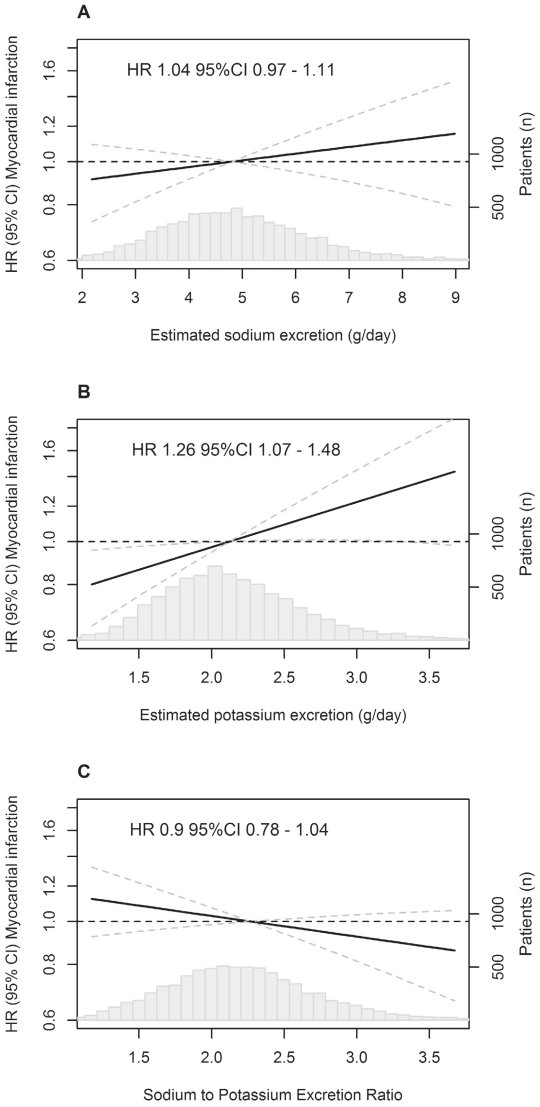
Restricted-cubic-spline plots of association between estimated 24-hour urinary excretion of sodium **(A-B),** potassium **(C-D)**, and their ratio **(E-F)** and recurrent MACE (left column) and all-cause mortality (right column). Histograms demonstrate distributions of different salt measures. The median of each salt measure (4.80 g/day, 2.12 g/day and 2.27 for sodium, potassium and their ratio, respectively) was taken as a reference (HR=1.0). Spline curves were plotted between the 1st and 99th percentile of the corresponding salt measure. Dotted lines represent 95% confidence intervals. All plots were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Supplemental Figure 2. Relationship between salt excretion and the occurrence of stroke.



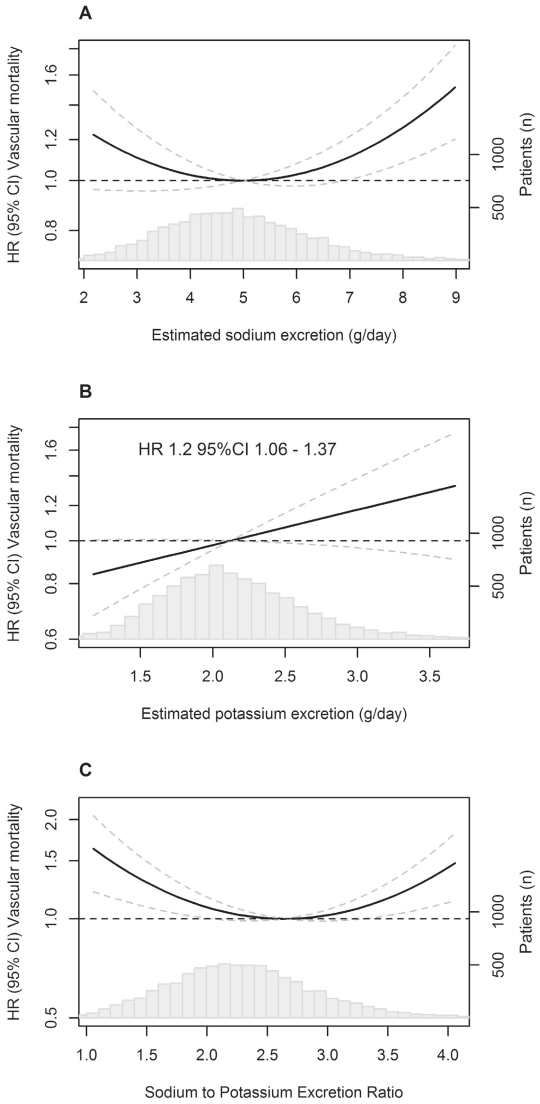
A. Relation between 1 gram/day higher estimated 24-hour urinary sodium excretion and the occurrence of stroke. **B.** Relation between 1 gram/day higher estimated 24-hour urinary potassium excretion and the occurrence of stroke. **C.** Relation between 1 unit higher sodium-to-potassium excretion ratio and the occurrence of stroke. Histograms demonstrate distributions of different salt measures. The median of each salt measure (4.80 g/day, 2.12 g/day and 2.27 for sodium, potassium and their ratio, respectively) was taken as a reference (HR=1.0). All hazard ratios were plotted between the 1st and 99th percentile of the corresponding salt measure. Dotted lines represent 95% confidence intervals. All plots were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Supplemental Figure 3. Relationship between salt excretion and the occurrence of myocardial infarction.



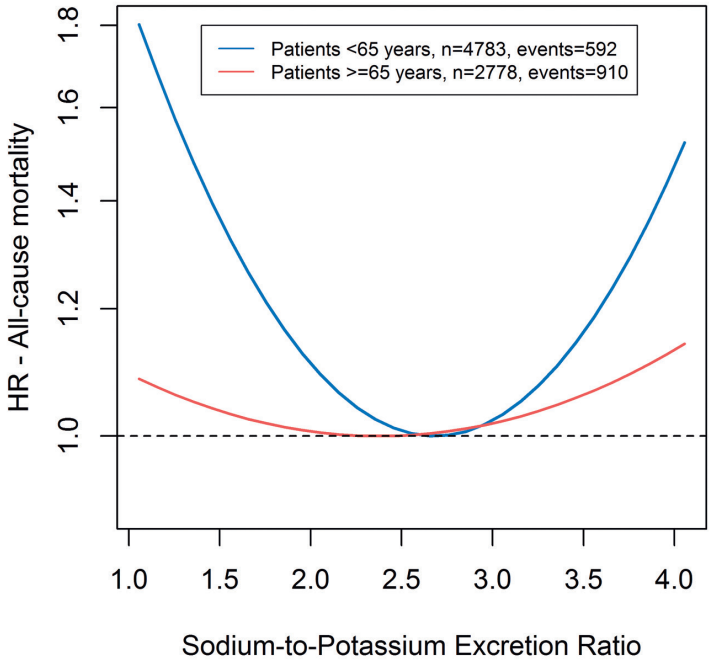
A. Relation between 1 gram/day higher estimated 24-hour urinary sodium excretion and the occurrence of myocardial infarction. **B.** Relation between 1 gram/day higher estimated 24-hour urinary potassium excretion and the occurrence of myocardial infarction. **C.** Relation between 1 unit higher sodium-to-potassium excretion ratio and the occurrence of myocardial infarction. Histograms demonstrate distributions of different salt measures. The median of each salt measure (4.80 g/day, 2.12 g/day and 2.27 for sodium, potassium and their ratio, respectively) was taken as a reference (HR=1.0). All hazard ratios were plotted between the 1st and 99th percentile of the corresponding salt measure. Dotted lines represent 95% confidence intervals. All plots were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Supplemental Figure 4. Relationship between salt excretion and vascular mortality.



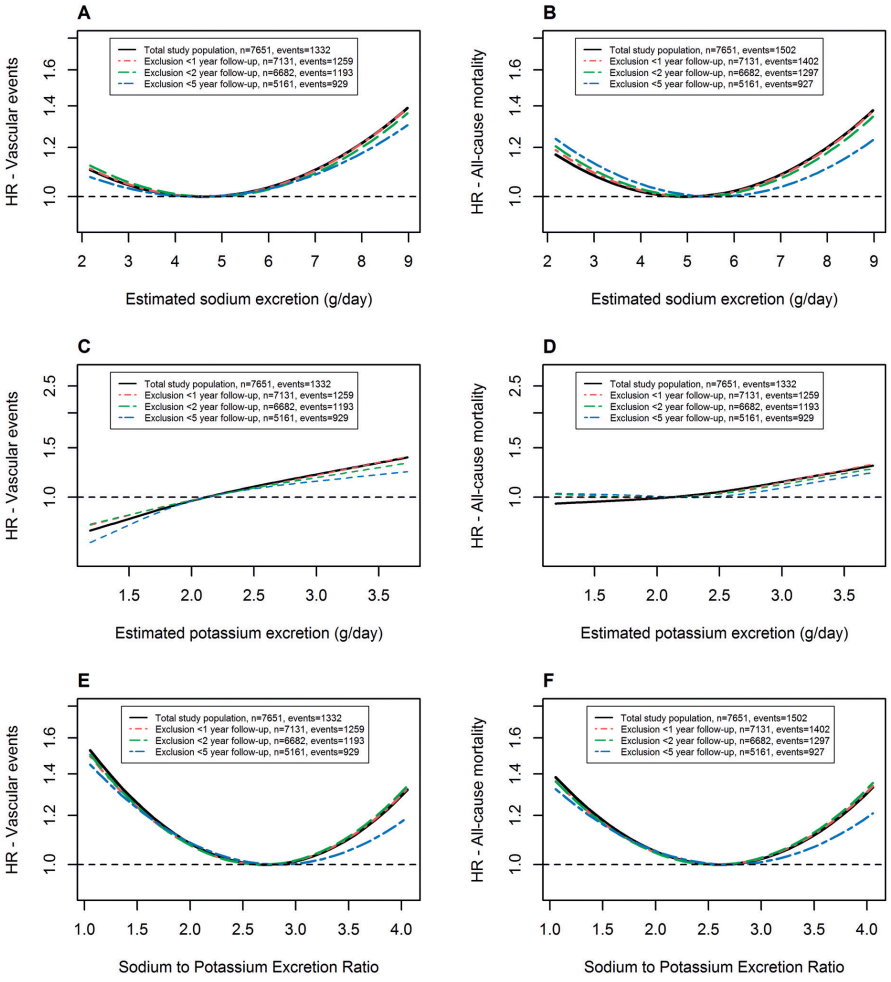
A. Relation between estimated 24-hour urinary sodium excretion and vascular mortality (linear term P<0.01; non-linear term P<0.01). Nadir: 4.98 g/day. **B.** Relation between 1 gram/day higher estimated 24-hour urinary potassium excretion and vascular mortality. **C.** Relation between sodium-to-potassium excretion ratio and vascular mortality (linear term P<0.01, non-linear term P<0.01). Nadir 2.64. Histograms demonstrate distributions of different salt measures. All hazard ratios were plotted between the 1st and 99th percentile of the corresponding salt measure. Dotted lines represent 95% confidence intervals. All plots were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Supplemental Figure 5. Stratified analyses for patients <65 years and ≥65 years of age.



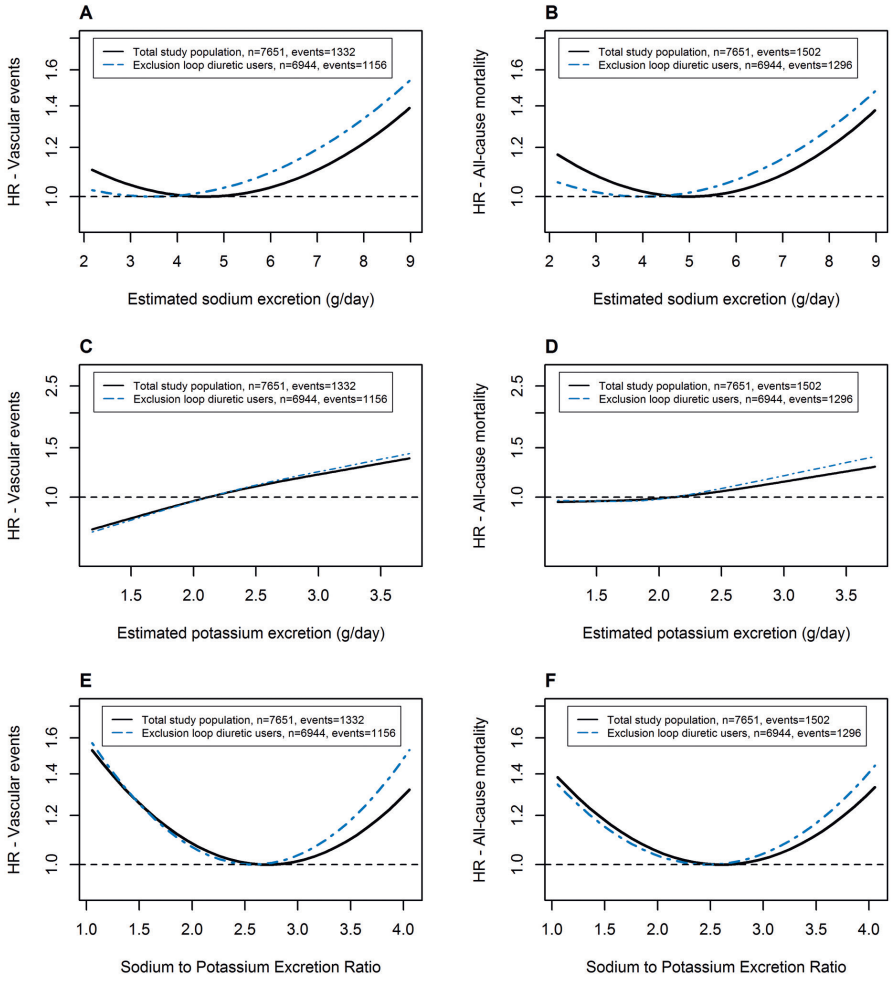
Adjusted hazard ratio for mortality by baseline sodium-to-potassium excretion ratio. Hazard ratios were plotted between the 1st and 99th percentile of the sodium-to-potassium excretion ratio. Plots were adjusted for age, sex, current smoking, BMI (kg/m^2), presence of diabetes, eGFR, and non-high-density lipoprotein cholesterol. HR = Hazard ratio.

Supplemental Figure 6. Sensitivity analysis excluding patients with short follow-up.



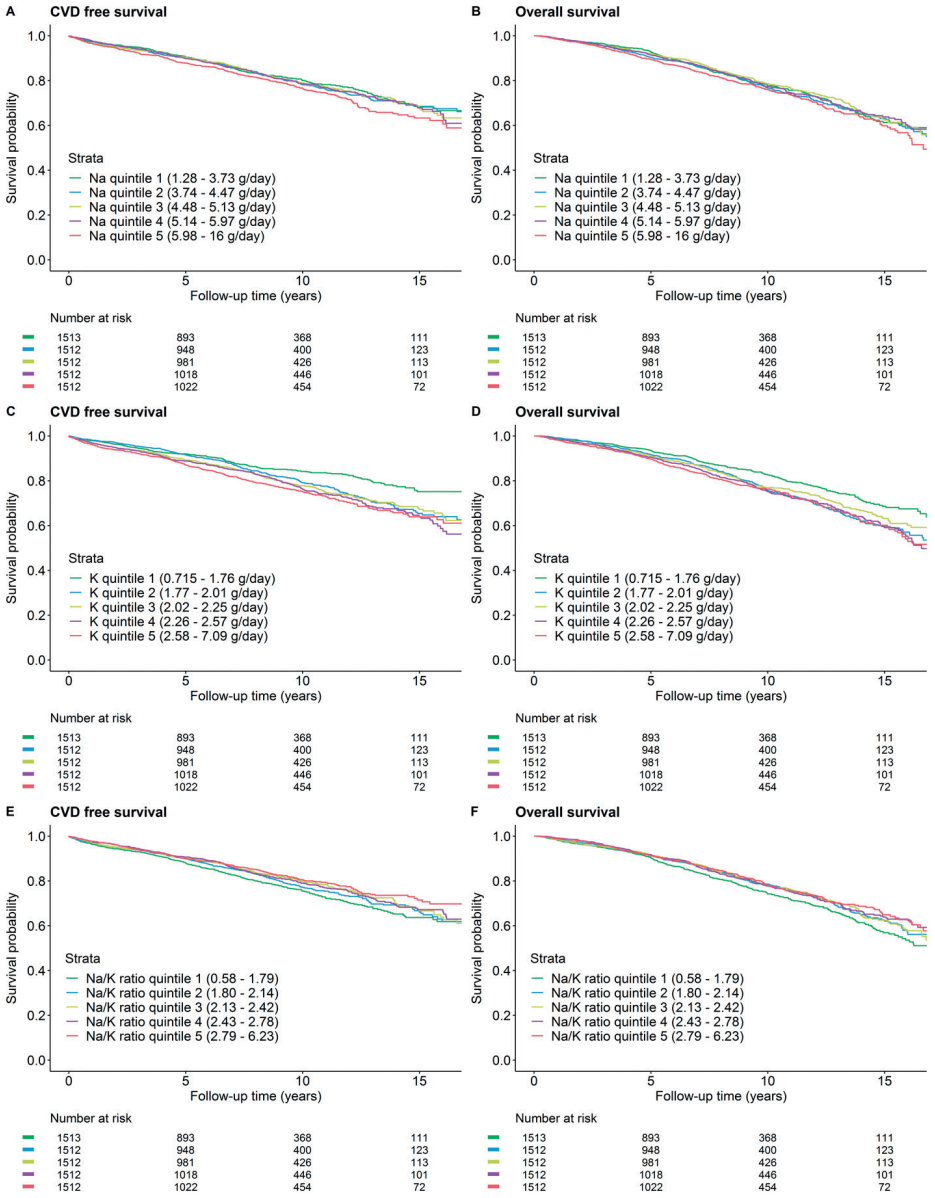
A-B. Change in estimated effect between estimated 24-hour sodium urinary excretion and vascular events (A) and mortality (B) after exclusion of patients who experienced events or died within 1 year (dashed red line), 2 years (dashed green line), and 5 years (dashed blue line) after inclusion. Black lines depict the main analysis. **C-D.** Change in estimated effect between 24-hour potassium urinary excretion and vascular events (C) and mortality (D) after exclusion of patients who experienced events or died within 1 year (dashed red line), 2 years (dashed green line), and 5 years (dashed blue line) after inclusion. **E-F.** Change in estimated effect between sodium-to-potassium excretion ratio and vascular events (E) and mortality (F) after exclusion of patients who experienced events or died within 1 year (dashed red line), 2 years (dashed green line), and 5 years (dashed blue line) after inclusion. HR = Hazard ratio.

Supplemental Figure 7. Sensitivity analysis excluding patients treated with loop diuretics.



A-B. Change in estimated effect between estimated 24-hour sodium urinary excretion and vascular events (A) and mortality (B) after exclusion of patients who were treated with loop diuretics (dashed blue line). Black lines depict the main analysis. **C-D.** Change in estimated effect between 24-hour potassium urinary excretion and vascular events (C) and mortality (D) after exclusion of patients who were treated with loop diuretics (dashed blue line). **E-F.** Change in estimated effect between sodium-to-potassium excretion ratio and vascular events (E) and mortality (F) after exclusion of patients who were treated with loop diuretics (dashed blue line). HR = Hazard ratio.

Supplemental Figure 8. Sensitivity analysis evaluating survival curves for quintiles of salt excretion.



A-B. Survival curves in quintiles of estimated 24-hour sodium excretion for (A) recurrent cardiovascular disease; (B) all-cause mortality. **C-D.** Survival curves in quintiles of estimated 24-hour potassium excretion for (C) recurrent cardiovascular disease; (D) all-cause mortality. **E-F.** Survival curves in quintiles of the sodium-to-potassium ratio for (E) recurrent cardiovascular disease; (F) all-cause mortality.

CHAPTER 4

Apparent resistant hypertension and the risk of recurrent cardiovascular events and mortality in patients with established vascular disease

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Abstract

Aim: To quantify the relation between apparent treatment resistant hypertension (aTRH) and the risk of recurrent major adverse cardiovascular events (MACE including stroke, myocardial infarction and vascular death) and mortality in patients with stable vascular disease.

Methods: 7455 hypertensive patients with symptomatic vascular disease were included from the ongoing UCC-SMART cohort between 1996 and 2019. Apparent TRH was defined as an office blood pressure ≥140/90 mmHg despite treatment with ≥3 antihypertensive drugs including a diuretic. Cox proportional hazard models were used to quantify the relation between aTRH and the risk of recurrent MACE and all-cause mortality. In addition, survival for patients with aTRH was assessed, taking competing risk of non-vascular mortality into account.

Results: A total of 1557 MACE and 1882 deaths occurred during a median follow-up of 9.0 years (interquartile range 4.8–13.1 years). Compared to patients with non-aTRH, the 614 patients (8%) with aTRH were at increased risk of cardiovascular mortality (HR 1.27;95%CI 1.03-1.56) and death from any cause (HR 1.25; 95%CI 1.07-1.45) but not recurrent MACE (HR 1.13;95%CI 0.95–1.34). At the age of 50 years, patients with aTRH after a first cardiovascular event on average had a 6.4 year shorter median life expectancy free of recurrent MACE than patients with non-aTRH.

Conclusion: In hypertensive patients with clinically manifest vascular disease, aTRH is related to a higher risk of vascular death and death from any cause. Moreover, patients with aTRH after a first cardiovascular event have a 6.4 year shorter median life expectancy free of recurrent cardiovascular disease.

Introduction

Globally, hypertension affects an estimated 31% (1.4 billion) of the adult population and is an important treatable risk factor for cardiovascular disease (CVD) and mortality (1,2). Although awareness and treatment have improved considerably, still about 50% of patients medically treated for hypertension do not reach the blood pressure (BP) targets recommended by guidelines (1).

Treatment resistant hypertension (TRH), a particularly severe form of hypertension, has been extensively studied during the last decades. The European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) define TRH as when patients treated with optimal or best-tolerated doses of three or more antihypertensive drugs, which should include a diuretic, fail to achieve office systolic BP and diastolic BP values of <140 mmHg and/or <90 mmHg, respectively (3). A more liberal definition has been adopted by the American College of Cardiology (ACC) and American Heart Association (AHA) who consider patients resistant when office BP is greater than or equal to 130/80 mmHg despite use of three antihypertensive drugs with complementary mechanisms of action (a diuretic should be 1 component) or when BP control is achieved but requires ≥4 medications (4). The diagnosis of TRH requires exclusion of pseudo-resistance, including medication nonadherence, improper BP measurement, white coat hypertension, and treatment inertia (5). After exclusion of pseudo-resistance, the true prevalence of TRH is likely to be <10% of treated patients (3). Population-based studies often use the term apparent TRH (aTRH) to clarify that pseudo-resistance was not excluded (6–10).

Previous studies among patients with hypertension have shown that patients with resistant hypertension are almost 50% more likely to experience outcomes such as death, myocardial infarction, heart failure, stroke, or chronic kidney disease (CKD) compared with treated hypertensive patients with controlled BP (7,8,10,11,12). Also, in hypertensive patients with coronary artery disease (CAD) the presence of aTRH was associated with a 27-77% higher risk of all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke compared with treated hypertensive patients with controlled BP (9,13,14).

Although considerable amount of data on the relative risk of (recurrent) major adverse cardiovascular events (MACE) is available, there remains a paucity of data regarding the impact of aTRH on life expectancy (LE) with and without CVD (15,16). Especially in patients with clinically manifest vascular disease, insight and quantification of the potential gain in life years could be of great value in motivating patients with aTRH to adhere to their risk factor management.

Therefore, the aim of the present study is twofold. First, to examine the risk of aTRH on recurrent MACE and all-cause mortality in patients with established CVD. Second, to evaluate the difference in life expectancy free of recurrent MACE in patients with and without aTRH in a large cohort of hypertensive patients with manifest vascular disease.

Methods

Study population

The population in this study originated from the Utrecht Cardiovascular Cohort – Second Manifestations of ARTerial disease (UCC-SMART), a single-center, ongoing prospective cohort study. Since September 1996, patients aged 18-80 referred to the University Medical Center Utrecht (UMCU), the Netherlands with a clinically stable manifestation of arterial disease (coronary artery disease (CAD), cerebrovascular disease (CeVD), peripheral arterial disease (PAD), or abdominal aortic aneurysm (AAA)) or known risk factors for atherosclerosis (dyslipidemia, hypertension or diabetes mellitus) were included. A detailed description of the study design has been published previously (17). The study was approved by the local Ethics Committee and all study participants gave written informed consent.

For the present study, data were used from 7455 hypertensive patients with a history or recent diagnosis of CAD, CVD, PAD or AAA included between 1996 and January 1st 2019. CAD was defined as either a diagnosis of myocardial infarction (MI), angina pectoris, coronary artery stenosis (>50% in ≥1 major coronary artery), or self-reported history of MI, cardiac arrest or cardiac surgery. CeVD was defined as either diagnosis of transient ischemic attack, ischemic or haemorrhagic stroke. PAD was defined as Fontaine stage IIa (i.e. intermittent claudication and rest ankle-brachial index (ABI) <0.9 in at least one leg) or worse, a self-reported history of amputation or vascular interventions of the lower extremities. AAA was notated when the patient had undergone vascular surgery because of an AAA or when an aneurysm of the abdominal aorta (distal aortic diameter ≥ 3 cm) was detected during screening.

Data collection

At baseline participants underwent a standardized vascular screening protocol consisting of a questionnaire regarding medical health and lifestyle, physical examination, fasting laboratory testing, ankle-brachial index, and an ultrasound of the abdominal aortic and carotid artery.

Between 1996 and 1999, office BP was measured with a semi-automatic oscillometric device (Omega 1400; Invivo Research Laboratories Inc., Broken Arrow, Oklahoma, USA) every 4 minutes at the right brachial artery in supine position during a total of 25 minutes. From April 1999 on, BP was measured with a nonrandom sphygmomanometer (Iso-Stabil 5; Speidel & Keller, Jungingen, Germany) three times simultaneously at the right and left upper arm in an upright position with an interval of 30 seconds. Before 1999 the mean BP of all measurements were taken, after April 1999 the mean of the last two BP measurements from the highest arm were taken as the BP.

Data from the self-reported use of medication have been recoded into drug classes. Smoking and the amount of pack-years were self-reported. Current smoking was defined as smoking within the last year. Diabetes mellitus (DM) at baseline was either self-reported DM type 1 or 2 or a fasting plasma glucose ≥7 mmol/L or non-fasting glucose ≥11.1 mmol/L at screening and receiving glucose-lowering therapy within 1 year from baseline measurements. Body mass index was calculated as weight (kg) divided by height (m) squared (kg/m2). Non-high density lipoprotein cholesterol (non-HDL-c) was defined as total cholesterol minus HDL-c and was measured from fasting venous blood samples. Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula (18) up to a plasma triglycerides level of 9 mmol/L (19). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (20). Albumin was determined in the first morning-void urine sample. Albuminuria was defined by an albumin/creatinine ratio of ≥3 mg/mmol. Carotid intima-media thickness was defined as the mean of the left and right common carotid artery measurements. Left ventricular (LV) hypertrophy on electrocardiography was defined according to the Sokolow-Lyon criterion when the voltage amplitude sum of either S wave in V1 (SV1) and R wave in V5 or SV1 and R wave in V6 was equal to or above 3.5 mV (21).

Definition of apparent treatment resistant hypertension

Patients with hypertension were defined as those who were prescribed antihypertensive medication and/or had an office systolic BP of ≥140 or diastolic BP of ≥90 mmHg. The definition of aTRH was based on the European guidelines and included an office BP above target (≥140/90 mmHg) at baseline with the concomitant use of at least three antihypertensive medications, including a diuretic. Since no information on medication adherence and out-of-office BP was available, we were not able to fully adopt the ESC/ESH definition of resistant hypertension.

Outcome assessment

The primary outcome for this study was MACE, a composite outcome consisting of non-fatal MI, non-fatal stroke and vascular death. The outcomes of secondary interest were the separate components of MACE; MI, stroke (ischemic or hemorrhagic) or vascular death, and all-cause mortality. For detailed description of the outcomes see Supplemental Table 1. During follow-up patients received questionnaires on a biannual basis to gather information on occurrence of the primary and secondary outcomes. When a potential event was reported, additional information was gained by collecting hospital or general practitioners' data. Three experienced physicians from the UCC-SMART endpoint committee independently evaluated the reported events and conflicting classifications were resolved through discussion. Duration of follow-up was defined as the period between inclusion and development of the primary outcome, death from any cause, date of loss to follow-up (n= 496, 6.7%), or the preselected date of January 1st 2019.

Data analyses

The baseline data are presented as counts (percentages) for categorical variables, means (standard deviation (SD)) for normally distributed variables or medians (interquartile range (IQR)) in case of a skewed distribution. Multivariable Cox-proportional hazard models were used to estimate the hazard ratios and corresponding 95% confidence intervals (95% CI) for the association between aTRH and recurrent MACE and all-cause mortality. When a patient experienced multiple vascular events, the first recorded event was used in the analyses. Three Cox models were fitted, a crude model, a model adjusted for age and sex (model II), and a model additionally adjusted for smoking, BMI, eGFR, non-HDL cholesterol and DM (model III). The Cox proportional hazards assumption, examined graphically by plotting scaled Schoenfield residuals against time, seemed appropriate. Formal testing of the proportional hazards assumption confirmed this (p-value 0.31 for recurrent MACE and 0.82 for all-cause mortality). To investigate whether the relation between aTRH and recurrent MACE was modified by sex, type of vascular disease at baseline or year of inclusion we included interaction terms into the models.

In addition, survival curves for patients with aTRH and patients without aTRH were created. These were based on the Cox proportional hazard models as described above and accounted for non-vascular mortality by applying the Fine and Gray competing risk method (22). Instead of follow-up time, age at enrolment and age at event were used. This was done in patients aged ≥50 years at time of inclusion to ensure a sufficient large sample size for visualization of the survival curve. Models were adjusted for previous mentioned confounders.

Several sensitivity analyses were performed. First, to investigate whether the increased cardiovascular risk seen in patients with aTRH is related solely to the persistent BP elevation we added office BP to the model (model IV). Second, to determine the influence of markers of hypertension mediated organ damage (HMOD) on the relation between aTRH and recurrent MACE and all-cause mortality we included LV hypertrophy, albuminuria and carotid intima-media thickness in the model (model V). Third, to evaluate whether the change of BP measurement protocol in 1999 might have influenced the results we repeated the analysis in only patients who were included after 1999. Fourth, as beta-blockers are often prescribed for reasons other than BP regulation, analyses were performed in which normotensive patients using a β blocker as the sole antihypertensive agent were excluded. Lastly, we repeated the primary analysis with a more liberal definition of aTRH based on the ACC/AHA guidelines (BP ≥130/80 mmHg on three antihypertensive medications (including a diuretic), or the use of ≥ four antihypertensive medications (including a diuretic), irrespective of BP).

Because complete case analysis may lead to loss of statistical power and possible bias (23), values of the determinant or potential confounders were imputed by single imputation using bootstrapping and predictive mean matching based on multivariable

regression including independent variables and outcome data ('AregImpute' function of the 'Hmisc' package in R). Missing data were <1.0%, except for albuminuria (n=389, 4.5%) and LV hypertrophy (n=599, 7.0%).

All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed, with statistical significance set at 0.05.

Results

Clinical characteristics

The study population consisted of 7455 patients, of whom 614 (8%) had aTRH. Compared to patients without aTRH, patients with aTRH had a higher mean age (63.7 ± 9.3 versus 60.7 ± 9.8 years), diabetes mellitus was more prevalent (34% versus 17%) and the average eGFR was lower (68 ± 21 versus 77 ± 18 mL/min/1.73m2) (Table 1). Baseline cholesterol levels were similar in both groups. Mean number of antihypertensive medications prescribed in patients with aTRH was 3.5 (SD 0.8) compared to 1.5 (SD 1.0) in patients without aTRH (Table 2). Prescription of lipid lowering and anti-platelet medication was comparable in both groups (Table 1).

Table 1. Baseline characteristics according to stage of hypertension.

	Non-aTRH	**aTRH**
	n = 6841	n = 614
Male sex	5149 (75%)	431 (70%)
Age (years)	60.7 ± 9.8	63.7 ± 9.3
Current smoker	1941 (28%)	149 (24%)
Former smoker	3380 (49%)	306 (50%)
Body mass index (kg/m2)	27.0 ± 4.0	28.2 ± 4.4
Systolic blood pressure (mmHg)	140 ± 20	159 ± 18
Diastolic blood pressure (mmHg)	81 ± 11	88 ± 13
Carotid intima–media thickness (mm)	0.92 ± 0.28	0.96 ± 0.26
Left ventricular hypertrophy	453 (7%)	69 (11%)
History of vascular disease		
Diabetes mellitus	1180 (17%)	206 (34%)
Coronary artery disease	4613 (67%)	405 (66%)
Abdominal aortic aneurysm	547 (8%)	77 (13%)
Cerebrovascular disease	1783 (26%)	191 (31%)
Peripheral artery disease	1069 (16%)	113 (18%)
Laboratory values		
Total cholesterol (mmol/L)	4.8 ± 1.2	4.8 ± 1.2
LDL-cholesterol (mmol/L)	2.8 ± 1.0	2.7 ± 1.0
HDL-cholesterol (mmol/L)	1.2 ± 0.4	1.2 ± 0.4
Triglycerides (mmol/L)	1.4 (1.0 – 2.0)	1.6 (1.1 - 2.3)
Estimated GFR (mL/min/1.73m2)	77 ± 18	68 ± 21
HbA1c (%)	5.9 (0.8)	6.2 (1.0)
Albuminuria	910 (13%)	162 (26%)

Table 1. *Continued.*

	Non-aTRH	**aTRH**
	n = 6841	n = 614
Prescribed medication		
Statin	4431 (65%)	422 (69%)
Platelet inhibitor	5492 (80%)	468 (76%)
Oral anticoagulants	751 (11%)	109 (18%)

Data are presented as number (%), mean±standard deviation or median (interquartile range).
Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate (calculated with Chronic Kidney Disease Epidemiology Collaboration [CKDEPI] formula); CKD-EPI, chronic kidney disease epidemiology collaboration

Table 2. Antihypertensive medication according to stage of hypertension.

	Non-aTRH	**aTRH**
	n = 6841	n = 614
Number of antihypertensive drugs	1.5 (0 – 6)	3.5 (3 – 7)
<3 antihypertensive drugs	5861 (86%)	0 (0%)
3 antihypertensive drugs	769 (11%)	375 (61%)
4 antihypertensive drugs	185 (3%)	182 (30%)
5 antihypertensive drugs	23 (0.3%)	39 (6%)
≥6 antihypertensive drugs	3 (0.1%)	18 (3%)
ACE-inhibitors	2389 (35%)	369 (60%)
Angiotensin II-receptor blockers	795 (12%)	214 (35%)
Aldosterone antagonists	180 (3%)	87 (14.2%)
Beta-blockers	4187 (61%)	478 (78%)
Calcium antagonist	1522 (22%)	313 (51%)
Thiazide diuretics	660 (10%)	380 (62%)
Loop diuretics	475 (7%)	214 (35%)
Potassium sparing diuretics	94 (1%)	66 (11%)
Alpha blockers	47 (1%)	37 (6%)
Central acting antihypertensives	11 (0.2%)	3 (0.5%)
Direct vasodilators	3 (0%)	3 (0.5%)

Data are presented as mean (range) or number (%).

Relation between aTRH and recurrent cardiovascular events and all-cause mortality

During a median follow-up of 9.0 years (IQR 4.8 – 13.1) and a total of 68,581 person-years of follow-up, 1557 patients experienced a recurrent cardiovascular event. Of these events 524 were myocardial infarctions, 371 strokes and 662 cardiovascular deaths. The crude event rates per 1000 person-years were higher for stroke (10 versus 6), vascular death (24 versus 13) and all-cause mortality (44 versus 26) in patients with aTRH compared to patients without aTRH (Table 3).

Table 3. Incidence rates according to stage of hypertension.

	Non-aTRH (n=6841)	**aTRH** (n=614)
	Events per 1000 py	Events per 1000 py
MACE	24	35
Myocardial infarction	9	8
Stroke	6	10
Cardiovascular mortality	13	24
All cause mortality	26	44

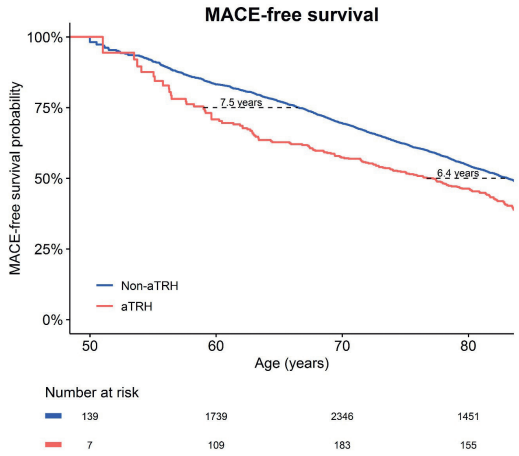
MACE, major adverse cardiovascular events; py, person-years

Patients with aTRH were at higher risk of the primary composite outcome (MI, stroke, and cardiovascular mortality) (HR 1.53; 95%CI 1.30-1.81) and the separate components stroke (HR 1.66; 95%CI 1.22-2.25) and cardiovascular mortality (HR 2.03; 95%CI 1.66-2.48) compared to patients without aTRH (Table 4, model I). However, after correction for confounding factors, this increased risk was not significant anymore (HR 1.13; 95%CI 0.95 – 1.34, HR 1.31; 95%CI 0.96-1.8, and HR 1.27; 95%CI 1.03-1.56, respectively) (Table 4, model III). After multivariate adjustment, presence of aTRH was related to a higher risk of the cardiovascular mortality (HR 1.27; 95%CI 1.03-1.56), and all-cause mortality (HR 1.25; 95%CI 1.07-1.45) but not to non-vascular mortality (HR 1.15; 95%CI 0.90 – 1.47) (Table 4, model III). Sex, type of vascular disease at baseline, and year of inclusion were no significant effect modifiers in the relation between aTRH and the risk of MACE and all-cause mortality (p for interaction >0.05).

aTRH and life expectancy free of recurrent cardiovascular events

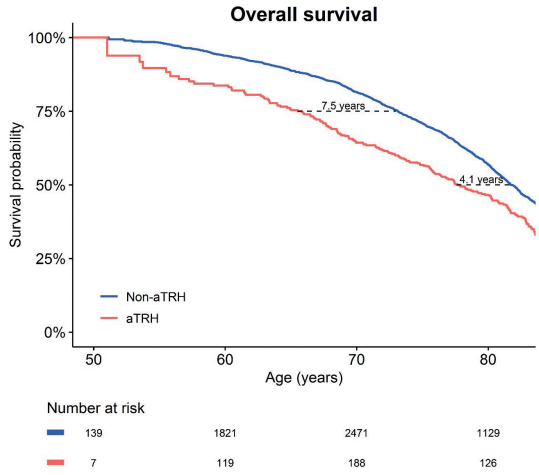
In Figure 1 and 2, MACE free survival and overall survival for patients with aTRH and patients without aTRH is presented for patients aged ≥50 years at onset of vascular disease. At the age of 50 years, compared to patients without aTRH, patients with aTRH, on average had a 4.1 year shorter median life expectancy (81.7 years 95%CI 81.2-82.4 versus 77.6 years 95%CI 73.3.-81.7) and a 6.4 year shorter median life expectancy free of recurrent cardiovascular disease (83.1 years 95%CI 81.3-84.9 versus 76.7 years 95%CI 67.6-83.1).

Figure 1. Competing risk adjusted MACE-free survival between patients with and without apparent treatment resistant hypertension (aTRH).



Risk of recurrent MACE and all-cause mortality in hypertensive patients with symptomatic vascular disease aged ≥50 years (n = 6479), according to the stage of hypertension. Competing risk-adjusted survival curve with 95% confidence intervals were obtained from Fine and Gray analyses with all-cause mortality as competing risk, adjusted for the same confounders as the main analysis (sex, smoking status, body mass index, non-HDL cholesterol, renal function and diabetes mellitus). MACE; major adverse cardiovascular disease. aTRH; apparent treatment resistant hypertension

Figure 2. Overall survival between patients with and without apparent treatment resistant hypertension (aTRH).



Risk of all-cause mortality in hypertensive patients with symptomatic vascular disease aged ≥50 years (n = 6479), according to the stage of hypertension. Survival curve with 95% confidence intervals, additionally adjusted for sex, smoking status, body mass index, renal function and diabetes mellitus. aTRH; apparent treatment resistant hypertension.

Sensitivity analyses

Sensitivity analyses were performed to determine whether the results were influenced by the inclusion of normotensive patients using a β blocker as the sole antihypertensive agent. Estimates and competing risk adjusted survival curves for patients with aTRH and without aTRH did not considerably differ after exclusion of these patients (n=924) (Supplemental Table 2). Adding office BP to the model did not have an important effect on the observed associations (Table 4, model IV). For all outcomes, except vascular death, additional adjustment for LV hypertrophy, albuminuria and carotid intima thickness did not change the hazard ratios substantially. For vascular death as outcome, the hazard ratio decreased and the relation became insignificant (Table 4, model V).

When the ACC/AHA-based definition for resistant hypertension (BP ≥130/80 mmHg on ≥3 agents, any BP on ≥4 agents) was used, the number of patients with aTRH increased to 994 patients (13%). This definition was associated with an increased risk of recurrent MACE (HR 1.25; 95%CI 1.08-1.44) and all-cause mortality (HR 1.33 95%CI 1.17-1.52) on multivariate adjusted analyses (Supplemental Table 3, model III). Use of this definition did not substantially alter the relation between aTRH and life expectancy (free of recurrent cardiovascular events) (Supplemental Figure 2). Lastly, performing the analysis in patients included after 1999 revealed similar results (Supplemental Table 4 and Supplemental Figure 3).

Table 4. Hazard ratios for recurrent vascular events associated with aTRH.

			Non-aTRH	**aTRH**
			n = 6841	n = 614
		Model	HR (95%CI)	HR (95%CI)
Major adverse cardiovascular events (MACE)	#events		1406	151
		I	1 (reference)	1.53 (1.30-1.81)*
		II	1 (reference)	1.34 (1.13-1.59)*
		III	1 (reference)	1.13 (0.95-1.34)
		IV	1 (reference)	1.10 (0.92-1.31)
		V	1 (reference)	1.09 (0.91-1.29)
Myocardial infarction (MI)	#events		537	35
		I	1 (reference)	0.90 (0.64-1.27)
		II	1 (reference)	0.90 (0.64-1.27)
		III	1 (reference)	0.79 (0.56-1.12)
		IV	1 (reference)	0.83 (0.58-1.18)
		V	1 (reference)	0.79 (0.55-1.11)
Stroke	#events		390	46
		I	1 (reference)	1.66 (1.22-2.25)*
		II	1 (reference)	1.43 (1.05-1.95)*
		III	1 (reference)	1.31 (0.96-1.80)
		IV	1 (reference)	1.25 (0.91-1.71)

Table 4. *Continued.*

			Non-aTRH	**aTRH**
			n = 6841	n = 614
		Model	HR (95%CI)	HR (95%CI)
		V	1 (reference)	1.25 (0.91-1.71)
Vascular mortality	#events		803	110
		I	1 (reference)	2.03 (1.66-2.48)*
		II	1 (reference)	1.57 (1.28-1.92)*
		III	1 (reference)	1.27 (1.03-1.56)*
		IV	1 (reference)	1.21 (0.98-1.49)
		V	1 (reference)	1.20 (0.97-1.47)
Non-vascular mortality	#events	742	74	
		I	1 (reference)	1.53 (1.21 – 1.95)*
		II	1 (reference)	1.21 (0.95 – 1.53)
		III	1 (reference)	1.15 (0.90 – 1.47)
		IV	1 (reference)	1.13 (0.88 – 1.45)
		V	1 (reference)	1.14 (0.89 – 1.46)
All-cause mortality	#events		1680	202
		I	1 (reference)	1.83 (1.58-2.12)*
		II	1 (reference)	1.43 (1.24-1.66)*
		III	1 (reference)	1.25 (1.07-1.45)*
		IV	1 (reference)	1.20 (1.03-1.40)*
		V	1 (reference)	1.21 (1.04–1.40)*

HR, hazard ratios; 95% CI, 95% confidence intervals. Model I: crude model; Model II: adjusted for age and sex; Model III: adjusted for age, sex, smoking, non-HDL-c, BMI, eGFR, DM. Model IV: adjusted for age, sex, smoking, non-HDL-c, BMI, eGFR, DM, Office systolic and diastolic BP. Model V: adjusted for age, sex, smoking, non-HDL-c, BMI, eGFR, DM, LV hypertrophy, albuminuria and carotid intima media thickness.* P<0.05

Discussion

The present study shows that in patients with a recent manifestation of vascular disease aTRH, based on the ESH/ESC definition, was associated with an increased risk of cardiovascular death and all-cause mortality. At the age of 50 years, compared to patients without aTRH, patients with aTRH, on average had a 4.1 year shorter median life expectancy and a 6.4 year shorter median life expectancy free of recurrent cardiovascular disease.

Results of the present study correspond to results of a previous analysis performed in the Reduction of Atherothrombosis for Continued Health (REACH) Registry (15). This study demonstrated a 10% (HR 1.10; 95%CI 1.01-1.20) higher risk of recurrent MACE for patients with aTRH (defined according to the ESH/ESC guidelines) compared to non TRH which is in line with our findings. Different from the current study, the increased risk of MACE remained significant after adjustment for multiple confounders and was mainly due to non-fatal stroke (HR 1.28; 95%CI 1.10-1.48). This difference could possibly be explained by the smaller sample size of our study (n=7455 versus n=53,530 in the REACH registry) resulting in broader confidence intervals such that existing associations could have stayed unnoticed. Moreover, in contrast to the UCC-SMART cohort that mainly consists of Caucasian subjects, the REACH registry contains an ethnically diverse population including more African-American subjects who are known to have a higher risk of recurrent stroke (24). However, in contrast to the REACH registry, our cohort is ongoing and has a substantial follow-up which made us able to extend their findings over a longer time period.

Use of the definition for resistant hypertension based on the ACC/AHA guidelines (BP ≥ 130/80 mmHg on ≥3 agents, any BP on ≥4 agents) not only increased the prevalence of aTRH to 13% but also resulted in a stronger association between aTRH and recurrent MACE. This suggests that in addition to the degree of BP control the number of antihypertensive medications is also relevant in the relation between aTRH and adverse clinical outcomes. The precise mechanism by which aTRH increases the risk of cardiovascular death and death from any cause is unknown. As was demonstrated in our analysis, adjustment for systolic and diastolic BP at baseline did not substantially alter the magnitude of the effect estimates, suggesting that the mechanism underlying the increased risk of recurrent MACE is not solely related to a difference in BP control at baseline. This finding corresponds to results of the post-hoc analysis of the INVEST (International Verapamil SR-Trandolapril Study) trial (13) and the REGARDS study (Reasons for Geographic and Racial Differences in Stroke) (8) showing that differences in BP control among patients with aTRH were not associated with differences in cardiovascular outcomes. As the difference in BP control cannot solely explain the increased risk of recurrent MACE and all-cause mortality, it seems reasonable to assume that requiring a greater number of antihypertensive drugs to achieve BP control reflects a larger

combination of adverse underlying disease processes. These processes may include, for example, increased sympathetic nervous system activation, renin–angiotensin system activation, excess aldosterone production, increased arterial stiffness, or subclinical atherosclerotic diseases that have been linked with increased cardiovascular risk (24,25). Alternatively, it may be that patients with aTRH have had a greater lifetime BP burden resulting in HMOD, relative to those without aTRH. This hypothesis is partly supported by the moderate decrease in the hazard ratio after additional adjustment for LV hypertrophy, albuminuria and carotid intima-media thickness, markers of HMOD. Lastly, since aTRH is associated with high rates of nonadherence to antihypertensive drugs (26), the increased risk of cardiovascular death in patients with aTRH could also result from poor adherence to other cardiovascular treatments such as glucose- or lipid- lowering drugs. Regardless of the exact cause, these findings suggest that for most fatal outcomes, the presence of aTRH better identifies a high-risk subpopulation of patients with hypertension than BP level or control alone.

In contrast to the unadjusted Kaplan-Meier survival curves published by previous studies in patients with aTRH (9,13,14,27) we were able to demonstrate (recurrent CVD free) life-expectancy in patients with aTRH by applying the Fine and Gray competing risk method and using age at enrolment and age at event instead of follow-up time. We showed that aTRH is associated with an important reduction in the median number of years lived without recurrent CVD and median life expectancy. Other studies have quantified the life expectancy of patients with hypertension compared to patients with normotension. According to these reports, normotensive adults from the age of 50 survived approximately 5 years longer and spent approximately 7.2 years fewer years of life without cardiovascular disease (28–30). Our study however, only includes patients with hypertension and demonstrates a similar reduction in median (CVD free) life expectancy for patients with aTRH. This is a novel finding and emphasizes the need to improve (adherence to) cardiovascular risk factor management, including the development of alternative treatments such as device-based antihypertensive treatments, in patients with aTRH.

Strengths of the present study include the large and relevant patient population and the prospective design with substantial follow-up, limited loss to follow-up (6.7%), large numbers of events, and the standardized measurement and completeness of baseline data which enabled us to correct for possible confounders. A limitation of this study is that we did not have data to rule out pseudo-resistant hypertension and therefore were not able to use the definition treatment resistant hypertension. The lack of an out-of-office BP measurement (i.e. home blood pressure monitoring (HBPM) and/or ambulatory blood pressure monitoring (ABPM)) did not allow us to detect patients with isolated clinic BP elevations (white coat hypertension). Moreover, data regarding medication adherence and treatment inertia were not collected. Therefore, some patients were possibly misclassified as apparently resistant which may have biased the results to

weaker associations between aTRH and the risk of MACE and mortality. Another limitation of this study is that baseline characteristics, including BP and medication use, were only recorded at the start of the study. Therefore, it was not possible to investigate changes in BP or medication use over time. Lastly, because the UCC-SMART study population almost completely consists of Caucasian patients, generalizability of our results to non-Caucasian populations is an issue.

In conclusion, in hypertensive patients with clinically manifest vascular disease, aTRH is related to a higher risk of vascular death and death from any cause. Moreover, patients with aTRH after a first cardiovascular event have a 6.4 year shorter median life expectancy free of recurrent cardiovascular disease. These findings, support the need for greater efforts toward improving BP control in patients with apparent treatment resistant hypertension and established vascular disease.

References

1. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016 Aug;134(6):441–50.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet (London, England). 2012 Dec;380(9859):2095–128.
3. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
4. Whelton PK, Carey RM, Aronow WS, Casey DE, Collins KJ, Himmelfarb CD, et al. Clinical Practice Guideline 2017 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 / . 2018. 13–115 p.
5. Carey RM, Calhoun DA, Bakris GL, Brook RD, Daugherty SL, Dennison-Himmelfarb CR, et al. Resistant Hypertension: Detection, Evaluation, and Management: A Scientific Statement From the American Heart Association. Hypertens (Dallas, Tex 1979). 2018 Nov;72(5):e53–90.
6. Judd E, Calhoun DA. Apparent and true resistant hypertension: definition, prevalence and outcomes. J Hum Hypertens. 2014 Aug;28(8):463–8.
7. Sim JJ, Bhandari SK, Shi J, Reynolds K, Calhoun DA, Kalantar-Zadeh K, et al. Comparative risk of renal, cardiovascular, and mortality outcomes in controlled, uncontrolled resistant, and nonresistant hypertension. Kidney Int. 2015 Sep;88(3):622–32.
8. Irvin MR, Booth JN 3rd, Shimbo D, Lackland DT, Oparil S, Howard G, et al. Apparent treatment-resistant hypertension and risk for stroke, coronary heart disease, and all-cause mortality. J Am Soc Hypertens. 2014 Jun;8(6):405–13.
9. Smith SM, Huo T, Delia Johnson B, Bittner V, Kelsey SF, Vido Thompson D, et al. Cardiovascular and mortality risk of apparent resistant hypertension in women with suspected myocardial ischemia: a report from the NHLBI-sponsored WISE Study. J Am Heart Assoc. 2014 Feb;3(1):e000660.
10. Pierdomenico SD, Lapenna D, Bucci A, Di Tommaso R, Di Mascio R, Manente BM, et al. Cardiovascular outcome in treated hypertensive patients with responder, masked, false resistant, and true resistant hypertension. Am J Hypertens. 2005 Nov;18(11):1422–8.
11. Daugherty SL, Powers JD, Magid DJ, Tavel HM, Masoudi FA, Margolis KL, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012 Apr;125(13):1635–42.
12. Hung C-Y, Wang K-Y, Wu T-J, Hsieh Y-C, Huang J-L, Loh E-W, et al. Resistant hypertension, patient characteristics, and risk of stroke. PLoS One. 2014;9(8):e104362.
13. Smith SM, Gong Y, Handberg E, Messerli FH, Bakris GL, Ahmed A, et al. Predictors and outcomes of resistant hypertension among patients with coronary artery disease and hypertension. J Hypertens. 2014 Mar;32(3):635–43.
14. Bangalore S, Fayyad R, Laskey R, Demicco DA, Deedwania P, Kostis JB, et al. Prevalence, predictors, and outcomes in treatment-resistant hypertension in patients with coronary disease. Am J Med. 2014 Jan;127(1):71-81.e1.
15. Kumbhani DJ, Steg PG, Cannon CP, Eagle KA, Smith SCJ, Crowley K, et al. Resistant hypertension: a frequent and ominous finding among hypertensive patients with atherothrombosis. Eur Heart J. 2013 Apr;34(16):1204–14.

16. van der Sande NGC, de Beus E, Bots ML, Voskuil M, Blankestijn PJ, Visseren F, et al. Apparent resistant hypertension and the risk of vascular events and mortality in patients with manifest vascular disease. J Hypertens. 2018 Jan;36(1):143–50.

17. Simons PC, Algra A, van de Laak MF, Grobbee DE, van der Graaf Y. Second manifestations of ARTerial disease (SMART) study: rationale and design. Eur J Epidemiol. 1999 Oct;15(9):773–81.

18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499–502.

19. Tremblay AJ, Morrissette H, Gagne J-M, Bergeron J, Gagne C, Couture P. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. Clin Biochem. 2004 Sep;37(9):785–90.

20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009 May;150(9):604–12.

21. SOKOLOW M, LYON TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J. 1949 Feb;37(2):161–86.

22. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94(446):496–509.

23. Zhang Z. Missing data imputation: focusing on single imputation. Ann Transl Med. 2016 Jan;4(1):9.

24. Albright KC, Huang L, Blackburn J, Howard G, Mullen M, Bittner V, et al. Racial differences in recurrent ischemic stroke risk and recurrent stroke case fatality. Neurology. 2018 Nov;91(19):e1741–50.

25. Smith SM. Epidemiology, prognosis, and treatment of resistant hypertension. Pharmacotherapy. 2013 Oct;33(10):1071–86.

26. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Drug adherence in hypertension: from methodological issues to cardiovascular outcomes. J Hypertens. 2017 Jun;35(6):1133–44.

27. Thomas G, Xie D, Chen H-Y, Anderson AH, Appel LJ, Bodana S, et al. Prevalence and Prognostic Significance of Apparent Treatment Resistant Hypertension in Chronic Kidney Disease: Report From the Chronic Renal Insufficiency Cohort Study. Hypertens (Dallas, Tex 1979). 2016 Feb;67(2):387–96.

28. Kiiskinen U, Vartiainen E, Puska P, Aromaa A. Long-term cost and life-expectancy consequences of hypertension. J Hypertens. 1998 Aug;16(8):1103–12.

29. Franco OH, Peeters A, Bonneux L, de Laet C. Blood pressure in adulthood and life expectancy with cardiovascular disease in men and women: life course analysis. Hypertens (Dallas, Tex 1979). 2005 Aug;46(2):280–6.

30. Clarke R, Emberson J, Fletcher A, Breeze E, Marmot M, Shipley MJ. Life expectancy in relation to cardiovascular risk factors: 38 year follow-up of 19,000 men in the Whitehall study. BMJ. 2009 Sep;339:b3513.

Supplemental Material

Supplemental Table 1. Definitions of vascular outcomes.

Outcome	Defined as
Myocardial infarction	(Non-)fatal myocardial infarction defined by ≥2 of the following: -Acute chest pain for at least 20 min -ST-elevation >1 mm in two adjacent leads or a left bundle branch block (LBBB) on ECG -Elevated troponin or elevated CK ≥2 times the normal value of CK and a MB-fraction >5% of the total CK; Or; - Coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI) -Sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence).
Stroke	(Non-) fatal ischemic or hemorrhagic stroke: Relevant clinical features for at least 24 hours causing an increase in impairment of at least one grade of the modified Rankin scale, with/ without a new infarction or hemorrhage on CT or MRI.
Vascular mortality	Death from myocardial infarction, stroke, heart failure, or rupture of abdominal aortic aneurysm; vascular death from other causes; or sudden death (unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence)).
Major Adverse Cardiovascular Events (MACE)	Composite of the above mentioned outcomes
All-cause mortality	All deaths during follow-up, irrespective of the cause of death.

Supplemental Table 2. Sensitivity analysis: effects of excluding normotensive patients using a β blocker as the sole antihypertensive agent.

			Non-aTRH	**aTRH**
			N= 5917	N = 614
		Model	HR (95%CI)	HR (95%CI)
Major adverse cardiovascular events (MACE)	#events		1261	151
		I	1 (reference)	1.45 (1.22-1.71)*
		II	1 (reference)	1.28 (1.08-1.52)*
		III	1 (reference)	1.11 (0.92-1.30)
Myocardial infarction (MI)	#events		447	35
		I	1 (reference)	0.92 (0.65-1.30)
		II	1 (reference)	0.89 (0.63-1.26)
		III	1 (reference)	0.79 (0.56-1.13)
Stroke	#events		366	46
		I	1 (reference)	1.50 (1.10-2.04)*
		II	1 (reference)	1.33 (0.98-1.81)
		III	1 (reference)	1.24 (0.90-1.70)
Vascular mortality	#events		756	110
		I	1 (reference)	1.83 (1.49-2.23)*
		II	1 (reference)	1.48 (1.21-1.81)*
		III	1 (reference)	1.23 (1.00-1.50)*
All-cause mortality	#events		1544	202
		I	1 (reference)	1.68 (1.45-1.95)*
		II	1 (reference)	1.37 (1.18-1.59)*
		III	1 (reference)	1.22 (1.05-1.42)*

HR, hazard ratios; 95% CI, 95% confidence intervals. Model I: crude model; Model II: adjusted for age and sex; Model III: adjusted for age, sex, smoking, BMI, non-HDL cholesterol, eGFR, DM. * P<0.05

Supplemental Table 3. Hazard ratios for recurrent vascular events associated with aTRH (according to definition ACC/AHA guidelines).

			Non-aTRH	**aTRH**
			n = 6461	n = 994
		Model	HR (95%CI)	HR (95%CI)
Major adverse cardiovascular events (MACE)	#events		1315	242
		I	1 (reference)	1.63 (1.44-1.87)*
		II	1 (reference)	1.46 (1.27-1.68)*
		III	1 (reference)	1.25 (1.08-1.44)*
Myocardial infarction (MI)	#events		509	63
		I	1 (reference)	1.06 (0.82-1.38)
		II	1 (reference)	1.06 (0.82-1.38)
		III	1 (reference)	0.94 (0.71-1.23)
Stroke	#events		371	65
		I	1 (reference)	1.49 (1.15-1.95)*
		II	1 (reference)	1.33 (1.02-1.73)*
		III	1 (reference)	1.25 (0.95-1.64)
Vascular mortality	#events		741	172
		I	1 (reference)	2.15 (1.82-2.54)*
		II	1 (reference)	1.75 (1.48-2.07)*
		III	1 (reference)	1.42 (1.19-1.70)*
All-cause mortality	#events		1579	303
		I	1 (reference)	1.85 (1.63-2.09)*
		II	1 (reference)	1.51 (1.34-1.71)*
		III	1 (reference)	1.33 (1.17-1.52)*

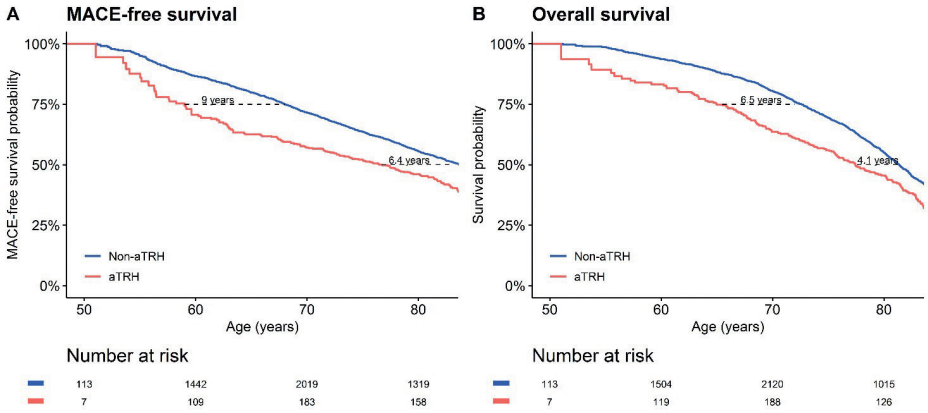
HR, hazard ratios; 95% CI, 95% confidence intervals. Model I: crude model; Model II: adjusted for age and sex; Model III: adjusted for age, sex, smoking, non-HDL cholesterol, BMI, eGFR, DM. * P<0.05

Supplemental Table 4. Hazard ratios for recurrent vascular events associated with apparent treatment resistant hypertension after exclusion of patients who were included in the cohort before 1999

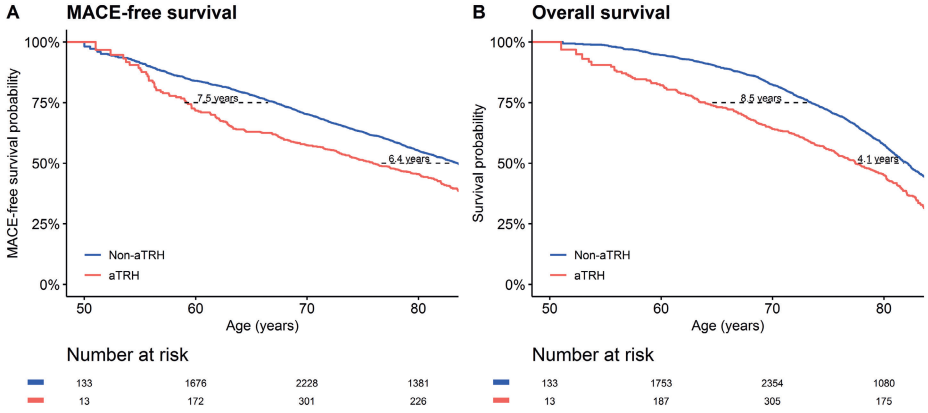
			Non-aTRH	**aTRH**
			N= 5935	N = 554
		Model	HR (95%CI)	HR (95%CI)
Major adverse cardiovascular events (MACE)	#events		1000	121
		I	1 (reference)	1.64 (1.35 - 1.98)*
		II	1 (reference)	1.46 (1.21-1.77)*
		III	1 (reference)	1.17 (0.97-1.43)
Myocardial infarction (MI)	#events		408	33
		I	1 (reference)	1.06 (0.74 - 1.52)
		II	1 (reference)	1.06 (0.74 -1.52)
		III	1 (reference)	0.90 (0.63-1.29)
Stroke	#events		291	34
		I	1 (reference)	1.56 (1.09 - 2.23)*
		II	1 (reference)	1.37 (0.96 - 1.96)
		III	1 (reference)	1.21 (0.84 - 1.74)
Vascular mortality	#events		513	82
		I	1 (reference)	2.22 (1.76 - 2.81)*
		II	1 (reference)	1.76 (1.39 - 2.23)*
		III	1 (reference)	1.32 (1.04 - 1.69)*
All-cause mortality	#events		1138	155
		I	1 (reference)	1.94 (1.64 - 2.30)*
		II	1 (reference)	1.54 (1.30 - 1.82)*
		III	1 (reference)	1.29 (1.08 - 1.53)*

HR, hazard ratios; 95% CI, 95% confidence intervals. Model I: crude model; Model II: adjusted for age and sex; Model III: adjusted for age, sex, smoking, non-HDL cholesterol, BMI, eGFR, DM. * P<0.05

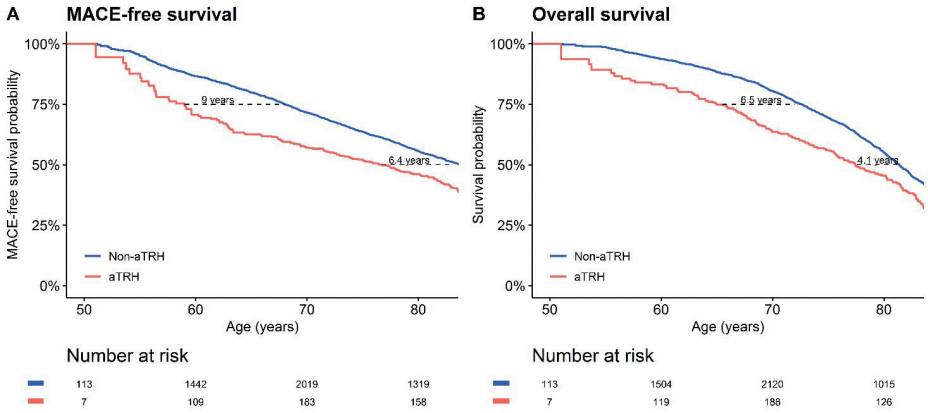
Supplemental Figure 1. Competing risk-adjusted MACE-free survival (A) and overall survival curves (B) for patients with and without aTRH, excluding normotensive patients using a β blocker as the sole antihypertensive agent aged ≥50 years (n= 5729).

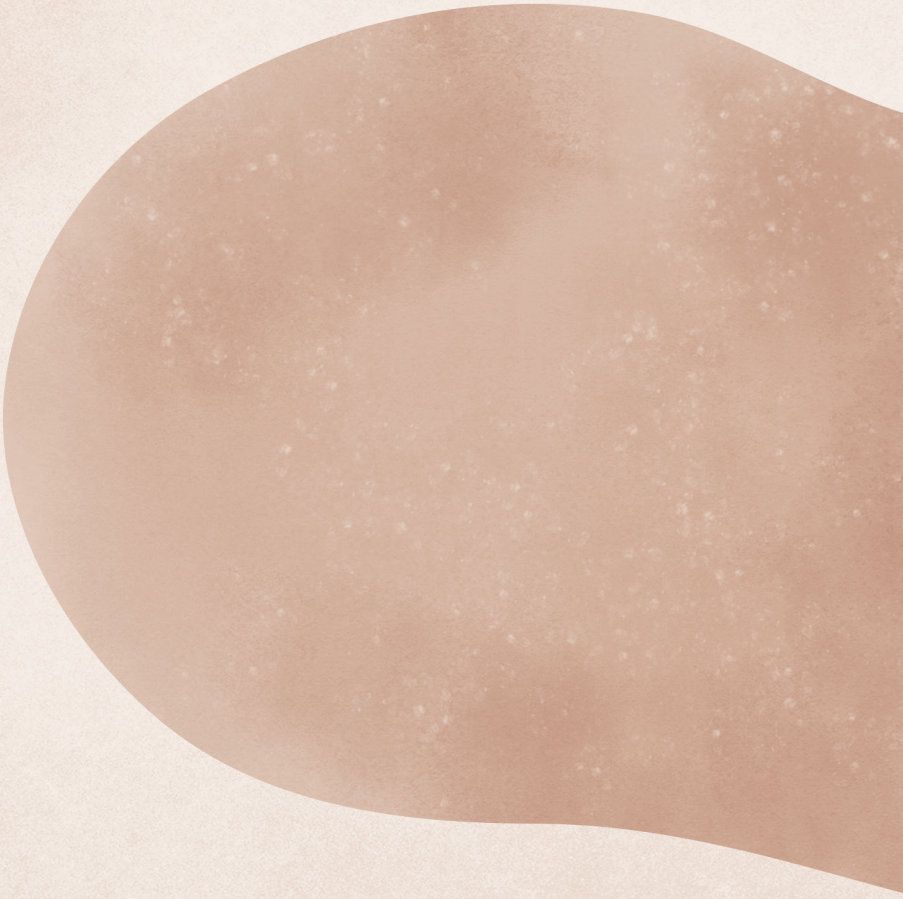


Supplemental Figure 2. Competing risk-adjusted MACE-free survival (A) and overall survival curves (B) between patients with and without aTRH defined based on the American College of Cardiology (ACC) and American Heart Association (AHA) guidelines.



Supplemental Figure 3. Competing risk-adjusted MACE-free survival (A) and overall survival curves (B) for patients with and without aTRH after exclusion of patients who were included in the cohort before 1999 and were aged ≥50 years (n=5633).





PART II

Monitoring in patients with uncontrolled hypertension

CHAPTER 5

Smartphone application-assisted home blood pressure monitoring compared with office and ambulatory blood pressure monitoring in patients with hypertension: the AMUSE-BP study

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Abstract

Background: The development of automated, smartphone application-assisted home blood pressure monitoring (HBPM) allows for standardized measurement of blood pressure (BP) at home. The aim of this study was to evaluate the (diagnostic) agreement between app-assisted HBPM, automated office BP (OBP), and the reference standard 24-hour ambulatory BP monitoring (ABPM).

Methods: In this open randomized five-way cross-over study, patients diagnosed with hypertension were randomized to one of ten clusters, each containing five BP measurement methods (ABPM, HBPM, attended OBP, unattended OBP, and unattended 30-minute BP) in different order.

Results: In total, 113 patients were included. The average 24-hour ABPM was 126±11/73±8 mmHg compared to 141±14/82±10 mm Hg with app-assisted HBPM, 134±13/80±9 mm Hg with unattended 30-minute BP, 137±16/81±11 mm Hg with attended OBP, and 135±15/81±10 mm Hg with unattended OBP monitoring. Diagnostic agreement between app-assisted HBPM and 24-hour ABPM for diagnosing sustained (OBP >140/90 mm Hg and ABPM ≥130/80 mm Hg or HBPM ≥135/85 mm Hg), white-coat (OBP ≥140/90 mm Hg and ABPM <130/80 mm Hg or HBPM <135/85 mm Hg), and masked hypertension (OBP <140/90 mm Hg and ABPM ≥130/80 mm Hg or HBPM ≥135/85 mm Hg) was fair to moderate (kappa statistics ranging from 0.34-0.40). App-assisted HBPM had high sensitivities (78-91%) and negative predictive values (90-97%) for diagnosing sustained and masked hypertension.

Conclusions: This study showed a considerable (diagnostic) disagreement between app-assisted HBPM and ABPM. App-assisted HBPM had high sensitivity in the diagnosis of sustained and masked hypertension, and may therefore be used as complementary to, but not a replacement of, ABPM.

Introduction

Proper diagnosis and monitoring of hypertension relies on accurate measurement of the blood pressure (BP) level (1,2). Traditionally, the diagnosis and monitoring of hypertension is based on conventional office BP measurements, either taken by a mercury sphygmomanometer or, nowadays more commonly, an automatic oscillometric device (3). Although office BP readings are obtained conveniently and rapidly, they are easily confounded, leading to incorrect diagnoses of normo- and hypertension called 'masked hypertension' and 'white-coat hypertension', respectively (4,5). Moreover, several meta-analyses have shown that compared to office BP measurements, out-of-office BP measurements have a stronger association with cardiovascular risk (6,7). Therefore, out-of-office BP monitoring, either performed by home blood pressure monitoring (HBPM) or 24-hour ambulatory blood pressure measurement (ABPM), is recommended for the diagnosis and monitoring of hypertension by several guidelines (1,2,8,9). Currently, ABPM is considered the reference standard because of the large evidence base demonstrating its strong association with future cardiovascular events (10).

Although ABPM has several unique advantages such as its capability of monitoring BP during sleep and daily activities, it is a burdensome and costly method that is not widely available, especially in primary care settings (11). Home BP monitoring is not only able to capture day-to-day variability and less expensive than ABPM, but also allows patients to take a greater role in self-management of hypertension, which may have a beneficial effect on medication adherence and BP control (12). However, the need for manual notation of self-measured BP by the patient, especially in the home setting, is prone to (unintentional) errors, which could compromise the reliability of HBPM (13,14). The introduction of smartphone application-assisted (app-assisted) HBPM, in which BP measurements taken with a validated BP device can be automatically transferred to a smartphone application, might improve reliability and widespread use of HBPM in clinical practice (15). How such app-assisted HBPM methods compare to the reference standard ABPM and automated office BP monitoring has not been clarified yet.

Therefore, the objectives of this study were to: (i) compare BP measured by automated office BP (attended, unattended, and unattended 30-minute), app-assisted HBPM, and the reference standard ABPM; (ii) to evaluate the agreement between app-assisted HBPM, automated office BP (attended, unattended, and unattended 30-minute), and ABPM in diagnosing hypertension; and (iii) to evaluate the agreement between app-assisted HBPM and ABPM in diagnosing sustained, white-coat, and masked hypertension, in patients with hypertension.

Methods

Study design and participants

The 'AMbulant versus Unattended & attended office versus SElf-home Blood Pressure measurement' (AMUSE-BP) study was an open randomized five-way cross-over study that included patients diagnosed with hypertension in three hypertension clinics (University Medical Center Utrecht, Ziekenhuis Gelderse Vallei in Ede, and Rijnstate in Arnhem) in The Netherlands between March 2020 and February 2022. Participants were ≥18 years of age, were stable on anti-hypertensive medication for at least 2 months, and were familiar with the use of a smartphone or tablet. Participants were ineligible if they had a systolic/diastolic BP (SBP/DBP) ≥180/110 mm Hg or ≤90/60 mm Hg during the screening visit, or suffered from conditions that may result in unstable blood pressure (e.g. pregnancy, endocrine disorders, arrhythmias, heart failure ≥ New York Heart Association (NYHA) class II). File S1 provides further details on the in- and exclusion criteria. The study protocol was approved by the institutional review board, and all participants provided written informed consent. The study is registered at www.trialregister.nl (ID NL8277).

Study procedures

At screening, BP was measured three times simultaneously on the right and left upper arm in an upright position with 1-minute intervals by a trained research nurse using the Microlife WatchBP Office AFIB (Microlife Corp, Widnau, Switzerland) device (16). Screening BP was recorded as the average of these three measurements. The arm with the highest average BP value was considered the reference. To collect demographic information a structured questionnaire was administered.

Eligible participants were randomly assigned to one of the 10 randomization arms, each of which prescribed to perform 5 BP measurements methods (attended office BP, unattended office BP, unattended 30-minute office BP, HBPM, and 24-hour ABPM) in different predefined orders over a 3-week period (Figure S1). The randomization arms were generated by the Latin Square method which allows to control for the carry-over effect (17). In addition, we applied a wash-out period of 2-4 days between all BP measurement methods, except when an out-of-office BP measurement followed an office BP measurement. If a measurement failed, the measurement was repeated until a valid measurement was obtained. If this was not possible, the measurement was noted as missing data.

Office blood pressure monitoring

Three types of office BP measurements were obtained: an attended office BP, an unattended office BP, and a unattended 30-minute office BP. All office BP measurements were taken by the research nurse who activated the BP monitor.

Attended office BP included triplicate measurements with 1-minute intervals using an automatic oscillometric device (Microlife WatchBP Office AFIB; Microlife Corp, Widnau, Switzerland) device (16) that was programmed to measure the BP after the patient had rested for at least 5 minutes in a sitting position. Measurement was performed with an appropriately sized cuff on the reference arm. The research nurse stayed in the office until all measurements were completed. Office BP was determined as the mean of all three measurements. Unattended office BP was obtained in exactly the same manner as attended office BP, but without the research nurse attending the programmed 5-minute resting period and the triplicate BP measurements.

Thirty-minute office BP was obtained from the reference arm, with the Microlife WatchBP Office AFIB 30 min (Microlife Corp, Widnau, Switzerland) device (18). This device was programmed to perform 6 consecutive measurements with 5-minute intervals after 5 minutes of seated rest (19). Thirty-minute office BP was performed without the research nurse in the office which also makes it an unattended BP measurement. However, an important difference compared to the unattended office BP described above is that in this measurement, the average value of 6 rather than 3 BP measurements was used for the analysis.

Home blood pressure monitoring

HBPM was carried out with the Microlife A6 BT (Microlife Corp, Widnau,Switzerland) (20) combined with the EmmaHBPM application (Medicine Men, Utrecht, the Netherlands) (21) with an appropriately sized cuff around the reference arm. The EmmaHBPM application is able to graphically display BP measurements, indicate whether the BP is within the normal range, and thus give patients more insight into their BP.

Prior to HBPM, participants were trained on the conditions of HBPM, the use of the device, and use of the EmmaHBPM application. Participants were instructed to measure their BP at home every morning and evening for 7 consecutive days, after 5 minutes of rest in a sitting position. Morning BP had to be measured between 6AM - 9AM, and evening BP had to be measured between 6PM - 9PM. BP was measured using the Microlife Average Mode (MAM) mode, which calculates a weighted average of a minimum of 3 consecutive BP readings with standardized 15-second intervals. In this mode, a specific algorithm takes into account the change in BP between sequential readings to determine the weight for the average of all readings. If the difference in consecutive measurements exceeds 40 mm Hg for SBP and 25 mm Hg for DBP, the highest measurement is rejected and an additional fourth measurement is taken. If the difference is between 18 and 40 mm Hg for SBP and 12 to 25 mm Hg for DBP, the higher measurement contributes only 50% to the average (22). After calculation of the weighted average, the device discards the three separate measurements obtained. Blood pressure measurements obtained with the Microlife A6 BT device had to be synchronized by the patient via Bluetooth with the EmmaHBPM application installed on the patient's smartphone or tablet. A valid HBPM was defined

as having at least 11 valid MAM readings within a 7-day time period. For calculation of the mean home BP, readings from the first measurement day were discarded, which is in line with the current practice guidelines (11).

Ambulatory blood pressure monitoring

Ambulatory BP was monitored using the Microlife WatchBP O3 BP AFIB device (Microlife Corp, Widnau,Switzerland) (23) with an appropriate-sized cuff on the non-dominant arm. If the interarm BP difference at screening was ≥20/10 mm Hg, ABPM had to be performed on the arm with the highest BP. BP measurements were taken at 20-min intervals over a 24-hour period. All individuals were instructed to follow their usual daily activity pattern and to report the performed activities. Reported activities were reviewed and discussed with the patient. If patients performed activities that did not fit into their usual daily pattern, including activities that potentially resulted in extreme blood pressure readings, a repeat ABPM could be considered. Mean awake and asleep BP were calculated using predefined nighttime (10PM – 6AM) and daytime (6AM - 10PM) periods. In line with the European guidelines, a valid ABPM was defined as having ≥20 daytime readings and ≥7 nighttime readings with at least 70% of all attempted BP readings being successful (2). The BP readings during the 24-hour, daytime, and nighttime period were averaged to obtain mean 24-hour, mean daytime, and mean nighttime BP, respectively.

Definition of hypertension categories

In line with the current hypertension guidelines (1,2,11), we defined office hypertension as a mean systolic BP ≥140 mm Hg and/or mean diastolic BP ≥90 mm Hg when based on the attended/unattended office measurements, and as a mean systolic BP ≥135 mm Hg and/or mean diastolic BP ≥85 mm Hg when based on 30-min BP readings. The corresponding thresholds were 135 mm Hg systolic and 85 mm Hg diastolic for home hypertension, 130 mm Hg systolic and 80 mm Hg diastolic for 24-hour ambulatory hypertension, and 135 mm Hg systolic and 85 mm Hg diastolic for daytime ambulatory hypertension. Sustained hypertension was defined as a consistently elevated office and out-of-office BP (home or 24-hour ambulatory). White-coat hypertension was defined as an elevated office and a normal out-of-office BP, and masked hypertension as the reverse (normal office and elevated out-of-office BP).

Data analysis

To obtain 90% power of detecting a clinical important mean difference in BP of >3 mm Hg between app-assisted HBPM and ABPM assuming a standard deviation (SD) of the difference of 8.9 mm Hg (24), a sample of at least 95 participants completing the study was required. Taking into account 25% drop-out, we aimed to include 120 patients. Patient characteristics were presented as categorical (n (%)), normal distributed continuous (mean ± SD) or non-normal distributed continuous (median [interquartile range (IQR)]). Means and proportions were compared by the Student's t-test and McNemar's test, respectively. To evaluate the differences between absolute values of the various BP

measurement methods, a linear mixed effects model was fitted. Random intercepts for patients accounted for the dependence of repeated measurements and the variability between patients. Models were adjusted for age, sex, body mass index (BMI), and smoking. Fundamental assumptions of the linear mixed model (e.g., normality of the residuals and homogeneity of variance) were tested to ensure the accuracy of results. The use of a mixed model allowed for appropriate handling of missing data in the outcome variable, assuming that the data were missing at random (MAR) (25). In addition, Bland-Altman plots were used to provide a visualized assessment of the agreement between the different BP measurement methods. For these plots, the average of measurements evaluated by two different methods (eg. HBPM and ABPM) is plotted against their difference for both systolic BP and diastolic BP. A priori, we defined a difference greater than 3 mm Hg as clinically relevant, based on the previously observed effect on cardiovascular morbidity and mortality associated with this difference (26). The diagnostic agreement between HBPM and ABPM in detecting sustained, white-coat, and masked hypertension was assessed using the kappa (κ) statistic. A κ statistic ≥0.80 was a priori considered to represent good agreement (27). In addition, we computed sensitivity, specificity, positive, and negative predictive values. For the primary analyses of this study, the average ABPM was based on all BP readings taken during the 24-hour measurement period. However, since office and home BP are only based on BP readings taken during daytime, we also performed a sensitivity analysis in which the average ABPM was based on BP readings taken between 6AM – 10PM. All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed, with statistical significance set at 0.05.

Results

Patient characteristics

Between February 2020 and March 2022, a total of 120 individuals were screened (Figure S2). Of these, 5 did not meet the eligibility criteria, one due to a recent change in prescribed medication and four due to extremely elevated BP (≥180/110 mm Hg). During the study, two patients dropped out due to comorbidities unrelated to the study, one of whom was later rescreened. The characteristics of the 113 study participants included in the analyses are shown in Table 1. Mean age of the study population was 61±10 years and 70 (62%) patients were male. Most patients were prescribed antihypertensive drugs (90%) with an average number of 2 antihypertensive drug classes.

Table 1. Characteristics of the study population.

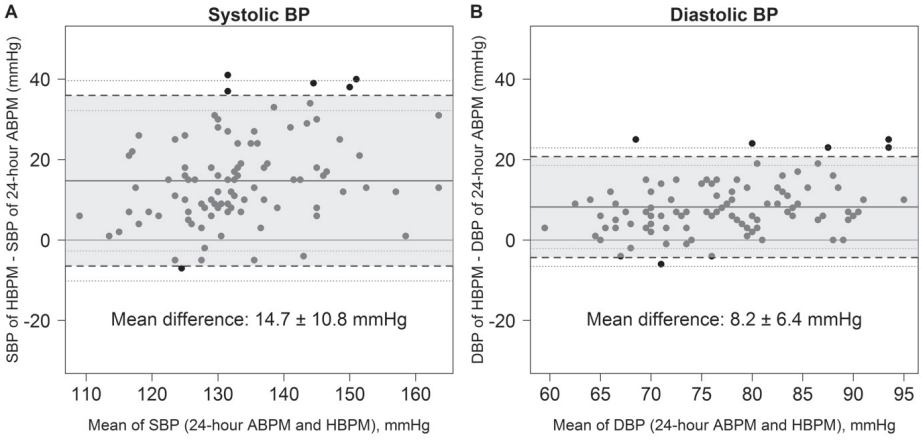
	n = 113
Male sex	70 (62%)
Age (years)	61 ± 10
Body mass index (kg/m^2)	29.0 ± 5.1
Smoking status	
Never	56 (50%)
Former smoking	51 (45%)
Current smoker	6 (5%)
Antihypertensive drug use	102 (90%)
Number of antihypertensive drugs	2 (1 - 3)
1	23 (20%)
2	31 (27%)
≥3	47 (42%)
Antihypertensive drug classes	
ACE inhibitors/ARB	79 (70%)
β-blocker	32 (28%)
Calcium channel blocker	55 (49%)
Diuretic	54 (48%)
Other*	3 (3%)
Blood pressure at screening	
Office systolic blood pressure (mm Hg)	141 ± 16
Office diastolic blood pressure (mm Hg)	84 ± 10
Interarm BP difference ≥ 20/10 mm Hg	0 (0%)

All data in n (%), mean ± standard deviation or median (interquartile range). ACE = angiotensin-converting enzyme, ARB = angiotensin II receptor blocker. *other antihypertensive drug classes including mineralocorticoid receptor antagonists, alpha-blockers, direct vasodilators and centrally acting drugs.

Agreement between automated office, app-assisted home, and 24-hour BP

Table 2 reports the mean systolic and diastolic BP for each measurement method and their difference compared to 24-hour ABPM as the reference method. Since not all patients completed all 5 BP measurements, the number of measurements varies per BP measurement method. Of the 113 patients, 101 (89%) had valid ABPM measurements, 109 (96%) had valid HBPM measurements, and 112 (99%) had valid office BP measurements. Systolic BP measured by HBPM and the office BP measurement methods were significantly different from 24-hour ABPM, with the latter being the lowest (Table 2). The mean difference between the HBPM and 24-hour ABPM systolic BP was 15 mm Hg (95% of the differences were between -6 and 36 mm Hg; 95% limits of agreement (95% LoA)) (Figure 1), whereas the mean differences between the unattended 30-minute, attended, and unattended office BP and 24-hour ABPM were 8 mm Hg (95% LoA: -14-31 mm Hg), 11 mm Hg (95% LoA: -16-38 mm Hg), and 10 mm Hg (95% LoA: -16-36 mm Hg), respectively (Figure 2). With respect to the diastolic BPs, differences showed a similar pattern (Figure 2). When daytime ABPM was used as the reference, the differences in BP with all BP methods were smaller, but still clinically relevant (>3 mm Hg) (Table S1 and Figure S3).

Figure 1. Bland-Altman plots of agreement between home and ambulatory systolic (a) and diastolic (b) BP.



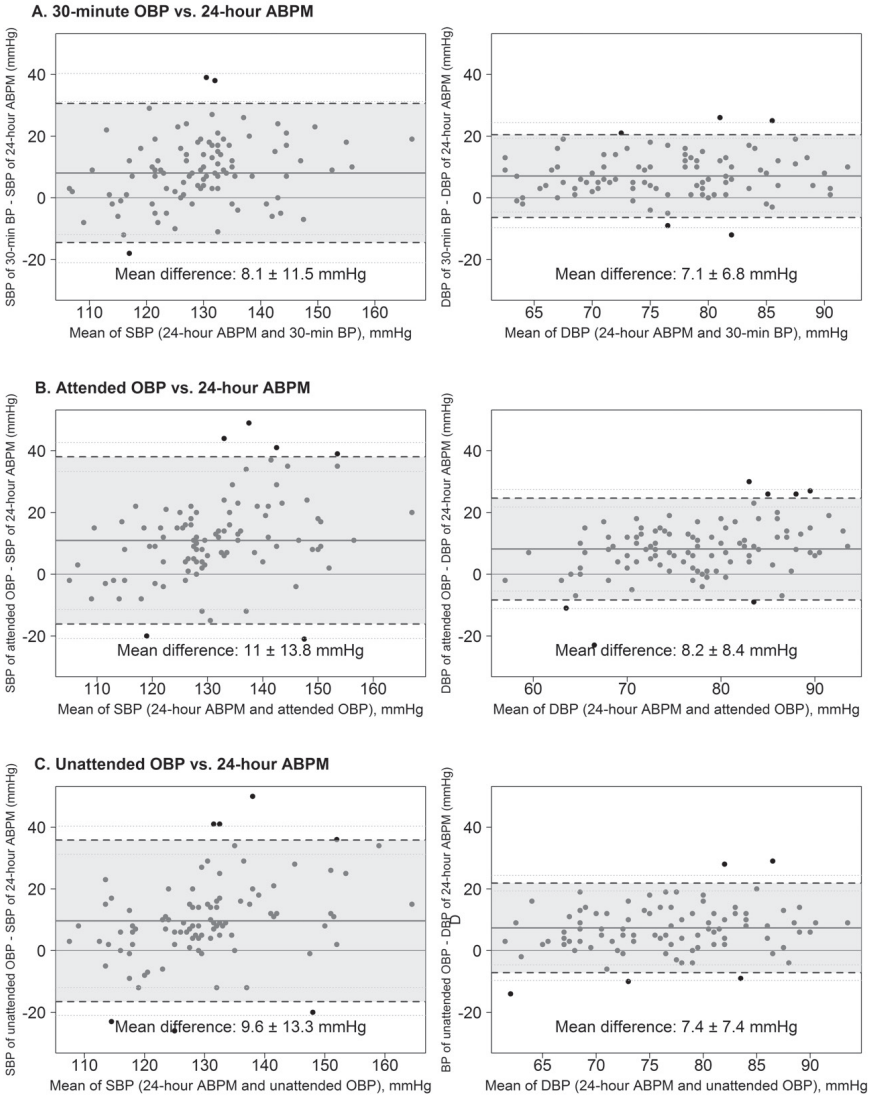
Plots comparing the difference between app-assisted HBPM and ABPM systolic (**A**) and diastolic (**B**) BP on the y-axis with the mean of the two methods on the x-axis.

Diagnostic agreement between automated office, app-assisted HBPM, and 24-hour ABPM in diagnosing hypertension

Figure S4 shows the prevalence of hypertension according to each BP measurement method using the method-specific hypertension thresholds. Prevalence of hypertension was 39% for 24-hour ABPM, 69% for app-assisted HBPM, 47% for attended office BP, 38% for unattended office BP, and 56% for unattended 30-minute BP. Compared to the office-based BP measurement methods, app-assisted HBPM showed a higher sensitivity (92% versus 62-77%) and negative predictive value (90% versus 76-83%) for the diagnosis of

hypertension (Table 3). Sensitivity analyses using daytime ABPM instead of 24-hour ABPM gave similar results with the main analysis (Table S2).

Figure 2. Bland-Altman plots of agreement between automated office and ambulatory systolic (left panes) and diastolic (right panes) BP.



Plots comparing the difference between 30-minute (**A**), attended office (**B**), and unattended office (**C**) and 24-hour ambulatory BP on the y-axis with the mean of the two methods on the x-axis.

Diagnostic agreement between app-assisted HBPM and 24-hour ABPM in diagnosing sustained, white-coat, and masked hypertension

Compared with 24-hour ABPM, app-assisted HBPM showed a significant higher prevalence of sustained (39% versus 69%; $p<0.05$) and masked hypertension (10% versus 23%; $p<0.05$), but showed a significant lower prevalence of white-coat hypertension (23% versus 10%; $p<0.05$) (Figure S5).

If the 24-hour ABPM was considered as the standard for the diagnosis of white-coat, masked, and sustained hypertension, app-assisted HBPM showed fair to moderate diagnostic agreement (κ statistics 0.34-0.40; Table 4). Overall, the sensitivity, specificity, positive predictive value, and negative predictive value for each diagnosis indicated moderate diagnostic performance. However, for the diagnosis of sustained and masked hypertension, app-assisted HBPM had relatively high sensitivities (range 80-90%) and negative predictive values (range 90-97%) (Table 4). Sensitivity analyses using daytime ABPM instead of 24-hour ABPM gave similar results with the main analysis (Table S3 and Figure S6).

Discussion

The present study shows that in patients with hypertension, BP measured by the reference standard 24-hour ABPM was overestimated by 15/8 mm Hg using app-assisted HBPM, 8/7 mm Hg using 30-min BP, 11/8 mm Hg using attended office BP, and 10/7 mmHg using unattended office BP. In addition, app-assisted HBPM showed better performance in diagnosing hypertension than automated office BP measurements, using ABPM as the reference. App-assisted HBPM showed fair to moderate diagnostic agreement with ABPM for the diagnosis of sustained, white-coat, and masked hypertension.

Although current hypertension guidelines evenly recommend home and ambulatory measurements for the diagnosis and monitoring of BP, the different diagnostic thresholds for hypertension already suggest an essential difference between both methods (1,2). However, the magnitude of the difference between app-assisted home and ambulatory BP found in this study has rarely been described. The Home versus Office MEasurements, Reduction of Unnecessary treatment Study (HOMERUS), a randomized clinical trial in patients with hypertension that investigated whether one can safely base antihypertensive treatment decisions on HBPM, also described a considerable difference of +12/5 mm Hg between 7-day home and 24-hour ambulatory BP (28). Likewise, a study that evaluated HBPM usefulness in the management of patients with resistant hypertension showed a difference of +11/1 mm Hg between 4-day HBPM and 24-hour ABPM (29).

Several explanations for this clinically relevant difference may be considered. Although HBPM and ABPM both measure BP outside the office, the conditions in which BP is measured for both methods greatly differ. HBPM is performed in the morning and evening, whereas ABPM is performed over a period of 24 hours in ambulatory conditions (at home or work, during active and inactive phases, without a period of rest before measurements, and during sleeping hours). Both methods might therefore simply reflect different aspects of BP profile and behavior. Also, patients often ensure that they have a quiet schedule on the day of ABPM because of the burdensome aspect (BP recording at 20 minute intervals) of this measurement which might have resulted in a lower BP. In addition, the app-assisted HBPM method as applied in this study was a newly developed method that requires experience using a smartphone and a number of additional actions from the patient. Since this may be more difficult and stressful than standard HBPM methods, it may have led to higher home BP readings. Lastly, in contrast to many previous studies (24,30,31), home BP values obtained by this study were not self-reported. It has been shown that HBPM readings reported by patients frequently differ from the actually measured values automatically stored in the device memory (13). Therefore, previous studies might suffer from misreporting by the patient which potentially resulted in an underestimated difference between HBPM and ABPM.

Table 2. Mean BP values for each BP measurement method and the difference compared to 24-hour ABPM (reference).

	24-h ABPM	App-assisted HBPM	Unattended 30-min BP	Attended OBP	Unattended OBP
	n = 101	n = 109	n = 112	n = 112	n = 112
Systolic blood pressure (mm Hg)	125.8 ± 11.1	140.6 ± 13.6	133.5 ± 13.4	136.7 ± 16.0	135.3 ± 15.3
Mean difference (95%CI)	-	15.2 (12.9 – 17.5)	7.7 (5.5 – 10.0)	11.1 (8.8 – 13.3)	9.5 (7.2 – 11.8)
Diastolic blood pressure (mm Hg)	73.0 ± 7.7	81.5 ± 9.8	80.2 ± 9.1	81.3 ± 10.7	80.6 ± 9.7
Mean difference (95%CI)	-	8.5 (7.1 – 9.9)	6.9 (5.6 – 8.3)	8.3 (7.0 – 9.7)	7.3 (6.0 – 8.7)

All data in mean ± standard deviation or mean difference (95% confidence interval). Mean differences were obtained by fitting a linear mixed model. This model was adjusted for age, sex, body mass index (BMI), and smoking. ABPM = ambulatory blood pressure monitoring, HBPM = home blood pressure monitoring, OBP = office blood pressure.

Table 3. Diagnostic performance of app-assisted home and automated office blood pressure monitoring in detecting hypertension diagnosed by 24-hour ambulatory blood pressure monitoring (reference).

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa coefficient
Home blood pressure monitoring	92 (78 - 98)	46 (33 - 59)	51 (38 - 63)	90 (74 - 98)	0.33 (0.18 - 0.47)
Unattended 30-min blood pressure	77 (61 - 89)	56 (42 - 68)	53 (39 - 66)	79 (64 - 90)	0.30 (0.13 - 0.47)
Attended office blood pressure	77 (61 - 89)	73 (60 - 83)	64 (49 - 77)	83 (71 - 92)	0.48 (0.31 - 0.65)
Unattended office blood pressure	62 (45 - 77)	79 (66 - 88)	65 (47 - 80)	76 (64 - 86)	0.41 (0.22 - 0.59)

Values in the parentheses are 95% confidence interval. PPV = positive predictive value, NPV = negative predictive value. Cut-off values hypertension; HBPM: ≥135/85 mm Hg, 30-min BP: ≥135/85 mm Hg, attended OBP: ≥140/90 mm Hg, unattended OBP: ≥140/90 mm Hg.

Table 4. Diagnostic performance of app-assisted home against 24-hour ambulatory blood pressure monitoring (reference) in detecting different hypertension phenotypes.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa coefficient
Sustained hypertension	92 (78 - 98)	46 (33 - 59)	51 (38 - 63)	90 (74 - 98)	0.33 (0.18 - 0.47)
White-coat hypertension	32 (16 – 52)	99 (92 - 100)	90 (55 - 100)	81 (68 - 86)	0.38 (0.18 - 0.58)
Masked hypertension	80 (44 - 97)	84 (75 - 91)	36 (17 - 59)	97 (91 - 100)	0.42 (0.20 - 0.64)

Values in the parentheses are 95% confidence interval. PPV = positive predictive value, NPV = negative predictive value. Sustained hypertension = consistently elevated BP on office and home or 24-hour ambulatory measurements. White-coat hypertension = an elevated BP in the office and a normal home or 24-hour ambulatory BP. Masked hypertension = an elevated home or 24-hour ambulatory BP with normal office BP.

While understanding the mechanisms underlying the difference between app-assisted HBPM and 24-hour ABPM is important, the practical question is whether these BP differences actually result in different diagnoses and treatment decisions. The current study showed moderate diagnostic agreement between app-assisted HBPM and ABPM in diagnosing sustained, white-coat, and masked hypertension, which is consistent with findings from previous studies (32,33). Similar to our study, a recent meta-analysis of 4 studies that evaluated the effectiveness of HBPM compared with ABPM for the diagnosis of hypertension found a pooled sensitivity of 0.84 (95% CI, 0.76-0.90) and a pooled specificity of 0.60 (95% CI, 0.48-0.71) (34). Due to this limited diagnostic performance, (app-assisted) HBPM should not be relied on for making the final diagnosis of sustained, white-coat, or masked hypertension. However, the high sensitivity and negative predictive value for the diagnosis of sustained hypertension and masked hypertension indicate that app-assisted HBPM seems suitable to be used as a screening method for these diagnoses, which, if positive, requires confirmation with 24-hour ABPM. This is further supported by the fact that in the current study, automated office BP, still the most widely used method for hypertension detection and management today (11), showed worse diagnostic agreement with ABPM for the diagnosis of sustained hypertension than app-assisted HBPM. Since HBPM has also been shown to be a more reliable predictor of cardiovascular outcomes than office BP (35), app-assisted HBPM should therefore be the preferred method for screening on sustained hypertension.

Based on the above-mentioned findings, app-assisted HBPM and ABPM appear to have a complementary rather than a competitive role in the evaluation of hypertension and provide similar but also different information about the BP profile and behavior of a patient. This is supported by findings from an outcome study where patients with partial masked hypertension (elevated ambulatory but normal home BP values or the reverse, and normal office BP values) were at increased cardiovascular risk compared with patients with sustained normotension (normal BP on office BP, ABPM, and HBPM), but at lower risk compared to patients with sustained hypertension (elevated office and out-of-office BP), implying additive prognostic information provided by each method (36).

Although out-of-office BP monitoring by 24-hour ABPM or HBPM is increasingly used and endorsed by the recent hypertension guidelines (1,2,9,11), diagnosis and monitoring of hypertension is still frequently based on office BP measurements, especially in settings with limited financial resources and time. Since conventional auscultatory office BP is known to suffer from observer-related bias and unstandardized measurement conditions (37), validated automated BP devices are increasingly used for this purpose. Such devices can be used with (“attended”) or without (“unattended”) the presence of a physician or a nurse. Consistent with results of the Systolic Blood Pressure Intervention Trial (SPRINT) (38) and with findings of a previous meta-analysis (37), this study found an unattended-attended automated office BP difference of -1.5/-1.0 mm Hg (Table 2) which is considered a non-clinically relevant difference (<3 mm Hg). These findings suggest that

when automated office BP measurements are performed under standardized conditions (resting period, triplicate measurements, no talking), the "presence" of the observer itself has minimal or no effect on measured office BP. Although unattended automated office BP has the advantage of avoiding several sources of error, it requires additional resources within a routine office visit (office space and time) limiting its application in all healthcare settings. Therefore, in case out-of-office BP monitoring is not available, attended automated office BP should be considered the most feasible option.

A major strength of this study is the direct comparison of automated office, home, and ambulatory BP levels in a well-defined population within a short time frame. Moreover, BP was assessed with highly standardized BP monitoring protocols consistent with recommendations in the current guidelines. Also, assessment of home BP was based on readings exported by the device memory, thus avoiding potential issues related to inaccurate reporting of readings by patients. Finally, by application of a randomized balanced design and wash-out periods we minimized the risk of order-effect and carry-over bias.

Some limitations also need to be considered. Since the majority of the population was prescribed antihypertensive medication, the findings may not be generalizable to individuals (in primary care) who have not yet been prescribed antihypertensive medications, in whom BP variability is likely to be greater (39). Further, the cross-sectional design of this study did not allow the performance of HBPM to be assessed based on the occurrence of clinical outcomes, which is considered an important aspect in determining the best BP measurement method.

In conclusion, the present study showed that office and app-assisted HBPM substantially overestimate ABPM. Overall, app-assisted HBPM showed fair-to-moderate diagnostic agreement with ABPM for the diagnosis of sustained, white-coat, and masked hypertension. The high sensitivity and negative predictive value for diagnosing sustained and masked hypertension suggest that app-assisted HBPM may be suitable for screening on these hypertension phenotypes.

Perspectives

The emergence of mobile health applications offers an important new strategy to more actively involve patients in their own hypertension management. Smartphone application-assisted HBPM is an important example of such a strategy. Teletransmission of BP readings self-measured by patients at home, especially when combined with education and counselling, appears to be able to improve adherence, the doctor-patient relationship, as well as BP control. However, the present study showed considerable (diagnostic) disagreement between app-assisted HBPM and the reference standard

24-hour ABPM, suggesting that app-assisted HBPM and ABPM have a complementary rather than a competitive role in the evaluation of patients with hypertension. When app-assisted HBPM is considered, the results of this study also suggest that it is important to do so in an objective manner in order to avoid misreporting by the patient. Whether guiding antihypertensive therapy based on app-assisted HBPM also results in different effects on morbidity and mortality compared to ABPM-guided treatment remains to be studied.

References

1. Whelton PK, Carey RM, Aronow WS, Casey DEJ, Collins KJ, Dennison Himmelfarb C, et al. 2017 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 P. J Am Coll Cardiol. 2018 May;71(19):e127–248.
2. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
3. Muntner P, Shimbo D, Carey RM, Charleston JB, Gaillard T, Misra S, et al. Measurement of Blood Pressure in Humans: A Scientific Statement From the American Heart Association. Hypertens (Dallas, Tex 1979). 2019 May;73(5):e35–66.
4. Mancia G, Zanchetti A. White-coat hypertension: misnomers, misconceptions and misunderstandings. What should we do next? Vol. 14, Journal of hypertension. England; 1996. p. 1049–52.
5. Bobrie G, Clerson P, Ménard J, Postel-Vinay N, Chatellier G, Plouin P-F. Masked hypertension: a systematic review. J Hypertens. 2008 Sep;26(9):1715–25.
6. Roush GC, Fagard RH, Salles GF, Pierdomenico SD, Reboldi G, Verdecchia P, et al. Prognostic impact from clinic, daytime, and night-time systolic blood pressure in nine cohorts of 13,844 patients with hypertension. J Hypertens. 2014 Dec;32(12):2332–40; discussion 2340.
7. Bliziotis IA, Destounis A, Stergiou GS. Home versus ambulatory and office blood pressure in predicting target organ damage in hypertension: a systematic review and meta-analysis. J Hypertens. 2012 Jul;30(7):1289–99.
8. Nerenberg KA, Zarnke KB, Leung AA, Dasgupta K, Butalia S, McBrien K, et al. Hypertension Canada's 2018 Guidelines for Diagnosis, Risk Assessment, Prevention, and Treatment of Hypertension in Adults and Children. Can J Cardiol. 2018 May;34(5):506–25.
9. National Institute for Health and Care Excellence (NICE). Hypertension in Adults: Diagnosis and . 2019;
10. Krist AH, Davidson KW, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for Hypertension in Adults: US Preventive Services Task Force Reaffirmation Recommendation Statement. JAMA. 2021 Apr;325(16):1650–6.
11. Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, et al. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. J Hypertens. 2021 Jul;39(7):1293–302.
12. McManus RJ, Mant J, Haque MS, Bray EP, Bryan S, Greenfield SM, 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. JAMA. 2014 Aug;312(8):799–808.
13. Nordmann A, Frach B, Walker T, Martina B, Battegay E. Reliability of patients measuring blood pressure at home: prospective observational study. BMJ. 1999 Oct;319(7218):1172.
14. Nordmann A, Frach B, Walker T, Martina B, Battegay E. Comparison of self-reported home blood pressure measurements with automatically stored values and ambulatory blood pressure. Blood Press. 2000;9(4):200–5.
15. Santo K, Redfern J. The Potential of mHealth Applications in Improving Resistant Hypertension Self-Assessment, Treatment and Control. Curr Hypertens Rep. 2019 Oct;21(10):81.
16. Kollias A, Ntineri A, Kyriakoulis KG, Stambolliu E, Lagou S, Boubouchairopoulou N, et al. Validation of the professional device for blood pressure measurement Microlife WatchBP Office in adults and children according to the American National Standards Institute/Association for the Advancement of Medical Instrumentation/International Organizat. Blood Press Monit. 2018 Apr;23(2):112–4.

17. Cornu C, Kassai B, Fisch R, Chiron C, Alberti C, Guerrini R, et al. Experimental designs for small randomised clinical trials: an algorithm for choice. Orphanet J Rare Dis. 2013 Mar;8:48.

18. Cheng H-M, Sung S-H, Shih Y-T, Chuang S-Y, Yu W-C, Chen C-H. Measurement accuracy of a stand-alone oscillometric central blood pressure monitor: a validation report for Microlife WatchBP Office Central. Am J Hypertens. 2013 Jan;26(1):42–50.

19. Bos MJ, Buis S. Thirty-Minute Office Blood Pressure Monitoring in Primary Care. Ann Fam Med. 2017 Mar;15(2):120–3.

20. Stergiou GS, Giovas PP, Neofytou MS, Adamopoulos DN. Validation of the Microlife BPA100 Plus device for self-home blood pressure measurement according to the International Protocol. Blood Press Monit. 2006 Jun;11(3):157–60.

21. MedicineMen. EmmaHBPM [Internet]. [cited 2022 Apr 26]. Available from: https://medicinemen.eu/consument/producten/emmahbpm/

22. Wilton A, De Greef A, Shennan A. Rapid assessment of blood pressure in the obstetric day unit using Microlife MaM technology. Hypertens pregnancy. 2007;26(1):31–7.

23. Ragazzo F, Saladini F, Palatini P. Validation of the Microlife WatchBP O3 device for clinic, home, and ambulatory blood pressure measurement, according to the International Protocol. Blood Press Monit. 2010 Feb;15(1):59–62.

24. Park J-S, Rhee M-Y, Namgung J, Lee SY, Cho D-K, Choi T-Y, et al. Comparison of Optimal Diagnostic Thresholds of Hypertension With Home Blood Pressure Monitoring and 24-Hour Ambulatory Blood Pressure Monitoring. Am J Hypertens. 2017 Nov;30(12):1170–6.

25. Snijders TAB, Bosker RJ. Multilevel analysis: An introduction to basic and advanced multilevel modeling. sage; 2011.

26. Blood Pressure Lowering Treatment Trialists' Collaboration. Age-stratified and blood-pressure-stratified effects of blood-pressure-lowering pharmacotherapy for the prevention of cardiovascular disease and death: an individual participant-level data meta-analysis. Lancet (London, England). 2021 Sep;398(10305):1053–64.

27. McHugh ML. Interrater reliability: the kappa statistic. Biochem medica. 2012;22(3):276–82.

28. Verberk WJ, Kroon AA, Lenders JWM, Kessels AGH, van Montfrans GA, Smit AJ, et al. Self-measurement of blood pressure at home reduces the need for antihypertensive drugs: a randomized, controlled trial. Hypertens (Dallas, Tex 1979). 2007 Dec;50(6):1019–25.

29. Muxfeldt ES, Barros GS, Viegas BB, Carlos FO, Salles GF. Is home blood pressure monitoring useful in the management of patients with resistant hypertension? Am J Hypertens. 2015 Feb;28(2):190–9.

30. Di Monaco S, Rabbia F, Covella M, Fulcheri C, Berra E, Pappaccogli M, et al. Evaluation of a short home blood pressure measurement in an outpatient population of hypertensives. Clin Exp Hypertens. 2016;38(8):673–9.

31. Almeida AEM, Stein R, Gus M, Nascimento JA, Arévalo JRG, Fuchs FD, et al. Improved diagnostic accuracy of a 3-day protocol of home blood pressure monitoring for the diagnosis of arterial hypertension. Blood Press Monit. 2013 Apr;18(2):119–26.

32. Viera AJ, Yano Y, Lin F-C, Simel DL, Yun J, Dave G, et al. Does This Adult Patient Have Hypertension?: The Rational Clinical Examination Systematic Review. JAMA. 2021 Jul;326(4):339–47.

33. Hodgkinson J, Mant J, Martin U, Guo B, Hobbs FDR, Deeks JJ, et al. Relative effectiveness of clinic and home blood pressure monitoring compared with ambulatory blood pressure monitoring in diagnosis of hypertension: systematic review. BMJ. 2011 Jun;342:d3621.

34. Guirguis-Blake JM, Evans C V, Webber EM, Coppola EL, Perdue LA, Weyrich MS. Screening for Hypertension in Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2021 Apr;325(16):1657–69.

35. Ward AM, Takahashi O, Stevens R, Heneghan C. Home measurement of blood pressure and cardiovascular disease: systematic review and meta-analysis of prospective studies. J Hypertens. 2012 Mar;30(3):449–56.

36. Satoh M, Asayama K, Kikuya M, Inoue R, Metoki H, Hosaka M, et al. Long-Term Stroke Risk Due to Partial White-Coat or Masked Hypertension Based on Home and Ambulatory Blood Pressure Measurements: The Ohasama Study. Hypertens (Dallas, Tex 1979). 2016 Jan;67(1):48–55.

37. Kollias A, Stambolliu E, Kyriakoulis KG, Gravvani A, Stergiou GS. Unattended versus attended automated office blood pressure: Systematic review and meta-analysis of studies using the same methodology for both methods. J Clin Hypertens (Greenwich). 2019 Feb;21(2):148–55.

38. Johnson KC, Whelton PK, Cushman WC, Cutler JA, Evans GW, Snyder JK, et al. Blood Pressure Measurement in SPRINT (Systolic Blood Pressure Intervention Trial). Hypertens (Dallas, Tex 1979). 2018 May;71(5):848–57.

39. Eguchi K, Imaizumi Y, Kaihara T, Hoshide S, Kario K. Comparison of valsartan and amlodipine on ambulatory blood pressure variability in hypertensive patients. Clin Exp Hypertens. 2016;38(8):721–4.

Supplemental Material

File S1. Detailed description of study in- and exclusion criteria.
Inclusion criteria

1. Age of 18 years or older.
2. Documented medical history of hypertension in local hospital electronic patient record.
3. Stable dose of anti-hypertensive medication for at least 2 months, includes no current antihypertensive medication, diagnosis hypertension is sufficient.
4. Systolic blood pressure (SBP) >90 and <180 mm Hg and diastolic blood pressure (DBP) >60 and <110 mm Hg at inclusion screening attained by attended automated office blood pressure (AOBP).
5. Dutch and/or English language capable for reading PIF and in-app instructions.
6. Smartphone or tablet. Operating system (OS) requirements: iOS 8.0 or higher, Android version 4.1 or higher.

Exclusion criteria

1. SBP >180 mm Hg and/or DBP >110mm Hg at inclusion screening visit (measured by attended AOBP method).
2. Any BP that according to the treating physician is not adequately controlled and needs medication adjustment < 2 months or within the study time period.
3. Recent (<2 months) anti-hypertensive medication changes (including diuretics). Includes no current antihypertensive medication, diagnosis hypertension is sufficient.
4. Recent start or change in dosing of alpha-blockers prescribed for other purpose than blood pressure control (for example benign prostate hypertrophy).
5. Unstable or uncontrolled endocrine disease (e.g. thyroid disease, Cushing's or Addison's disease) with the exception of diabetes mellitus.
6. Arrhythmias that prevent any BP measurement device to correctly measure BP during inclusion screening visit; such as supraventricular arrhythmias or atrial ventricular block. Known arrhythmias, but not clinically present during inclusion screening is not an exclusion criterion.
7. Heart failure grade 2 or higher on the New York Heart Association (NYHA) Functional Classification.
8. Documented missed outpatient clinic appointments (2 or more the last 6 months).
9. Documented therapy nonadherence (e.g. biochemically proven medication nonadherence, known or highly suspected medication nonadherence by treating physician, proven direct observed therapy effect in BP).

10. Participants cannot plan a measurement schedule with a minimum of 15 and a maximum of 29-day period participation or a minimum of 4 and maximum of 5 hospital visits due to logistical issues or scheduling issues of any kind.
11. Physical inability to perform an home BP measurement, use the Microlife A6 BT BP device or the EmmaHBPM app.
12. Active pregnancy or planning trying to get pregnant during the study period.

Table S1. Mean BP values for each BP measurement method and the difference compared to daytime ABPM.

	Daytime ABPM	**App-assisted HBPM**	**30-min BP**	**Attended OBP**	**Unattended OBP**
	n = 101	n = 109	n = 112	n = 112	n = 112
Systolic blood pressure (mm Hg)	130.4 ± 11.1	140.6 ± 13.6	133.5 ± 13.4	136.7 ± 16.0	135.3 ± 15.3
Mean difference (95%CI)	-	10.4 (8.2 – 12.7)	2.9 (0.7 – 5.2)	6.3 (4.0 – 8.5)	4.7 (2.5 – 7.0)
Diastolic blood pressure (mm Hg)	77.0 ± 8.1	81.5 ± 9.8	80.2 ± 9.1	81.3 ± 10.7	80.6 ± 9.7
Mean difference (95%CI)	-	4.5 (3.2 – 5.9)	2.9 (1.6 – 4.3)	4.3 (3.0 – 5.7)	3.4 (2.0 – 4.7)

All data in mean ± standard deviation or mean difference (95% confidence interval). Mean differences were obtained by fitting a linear mixed model. This model was adjusted for age, sex, body mass index (BMI), and smoking. ABPM = ambulatory blood pressure monitoring, HBPM = home blood pressure monitoring, OBP = office blood pressure.

Table S2. Diagnostic performance of app-assisted home and automated office BP monitoring in detecting hypertension diagnosed by daytime ambulatory BP monitoring.

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Kappa coefficient**
Home blood pressure monitoring	86 (71 - 95)	42 (30 - 55)	46 (34 - 59)	84 (67 - 95)	0.24 (0.10 - 0.39)
30-min blood pressure	79 (64 - 91)	58 (45 - 70)	53 (40 - 67)	82 (68 - 92)	0.34 (0.17 - 0.51)
Attended office blood pressure	74 (58 - 87)	69 (57 - 80)	59 (44 - 73)	82 (69 - 91)	0.41 (0.24 - 0.59)
Unattended office blood pressure	64 (47 - 79)	80 (68 - 89)	66 (49 - 80)	78 (67 - 88)	0.44 (0.26 - 0.62)

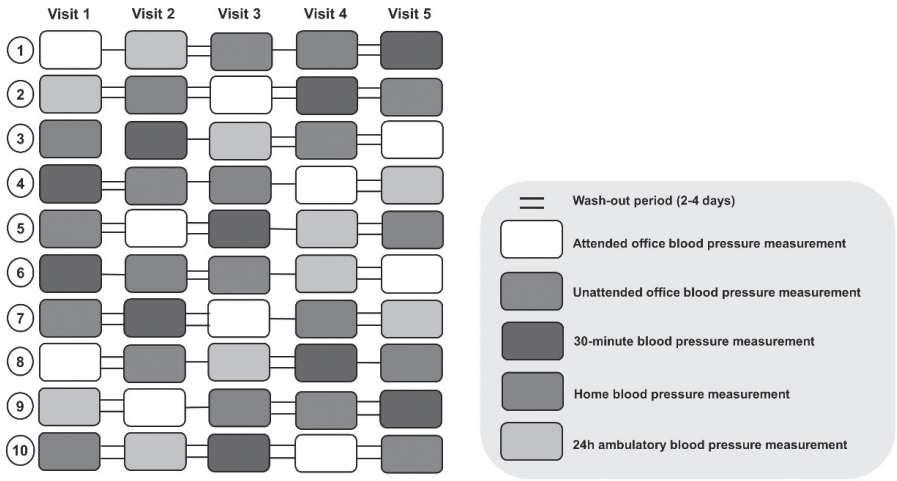
Values in the parentheses are 95% confidence interval. PPV = positive predictive value, NPV = negative predictive value. Cut-off values hypertension; HBPM: ≥135/85 mm Hg, 30-min BP: ≥135/85 mm Hg, attended OBP: ≥140/90 mm Hg, unattended OBP: ≥140/90 mm Hg.

Table S3. Diagnostic performance of app-assisted home against daytime ambulatory BP monitoring in detecting different hypertension phenotypes.

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Kappa coefficient**
Sustained hypertension	86 (71 - 95)	42 (30 - 55)	46 (34 - 59)	84 (67 - 95)	0.24 (0.10 - 0.39)
White-coat hypertension	27 (12 - 46)	97 (90 - 100)	80 (44 - 97)	76 (66 - 84)	0.30 (0.11 - 0.48)
Masked hypertension	70 (35 - 93)	84 (74 - 90)	32 (14 - 55)	96 (89 - 99)	0.35 (0.12 - 0.57)

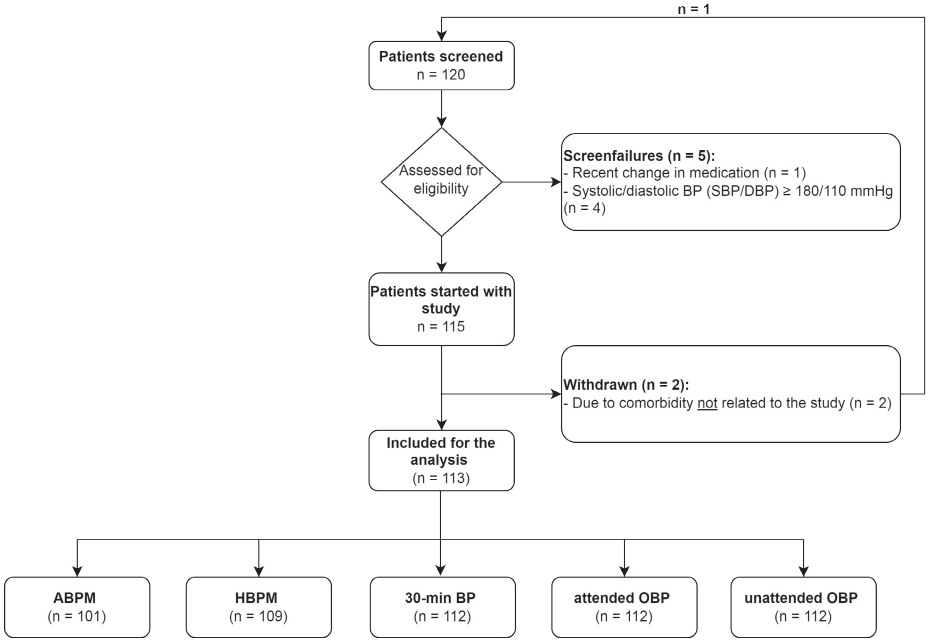
Values in the parentheses are 95% confidence interval. PPV = positive predictive value, NPV = negative predictive value. Sustained hypertension = consistently elevated BP on office and home or 24-hour ambulatory measurements. White-coat hypertension = an elevated BP in the office and a normal home or 24-hour ambulatory BP. Masked hypertension = an elevated home or 24-hour ambulatory BP with normal office BP.

Figure S1. Schematic overview of randomization clusters.



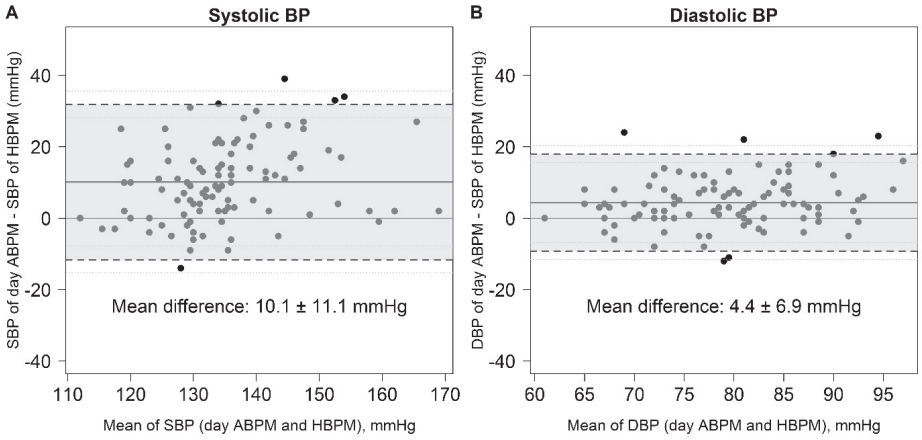
Overview of all 10 randomization clusters. Each patient underwent all five BP measurement methods. To minimize the carry-over effect, a wash-out period of 2 - 4 days was incorporated before and after each out-of-office BP measurement (ABPM and HBPM).

Figure S2. Flow diagram of patients enrolled in the study.



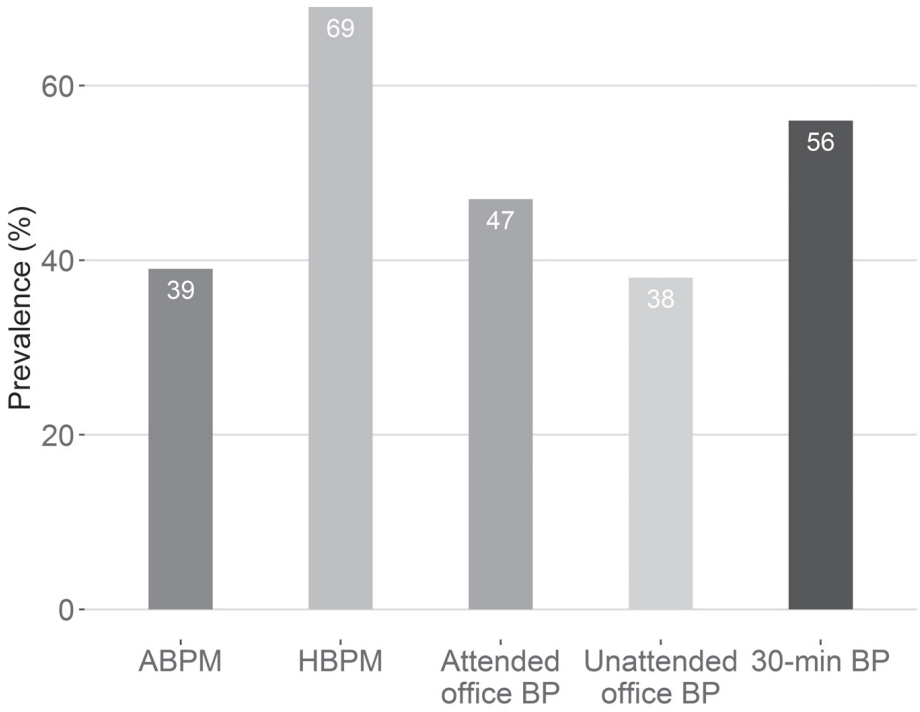
ABPM = Ambulatory Blood Pressure Monitoring, HBPM = Home Blood Pressure Monitoring, OBP = Office Blood Pressure

Figure S3. Bland-Altman plots of agreement between home and daytime ambulatory systolic (a) and diastolic (b) BP.



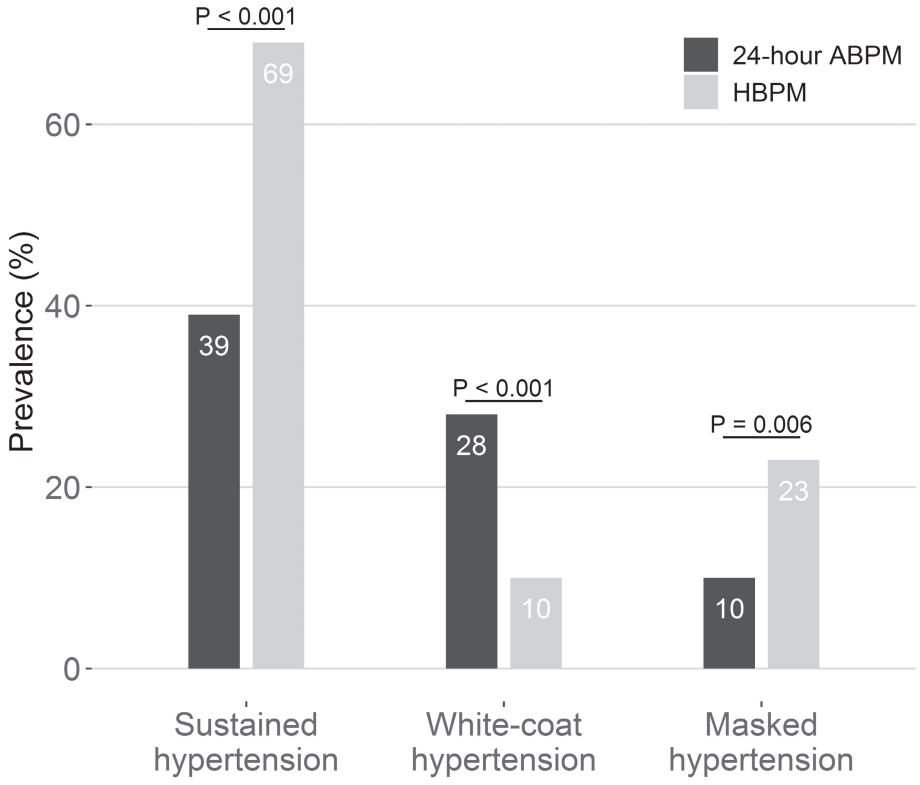
Plots comparing the difference between app-assisted HBPM and daytime ABPM systolic (**A**) and diastolic (**B**) BP on the y-axis with the mean of the two methods on the x-axis.

Figure S4. Prevalence of hypertension according to different BP measurement methods.



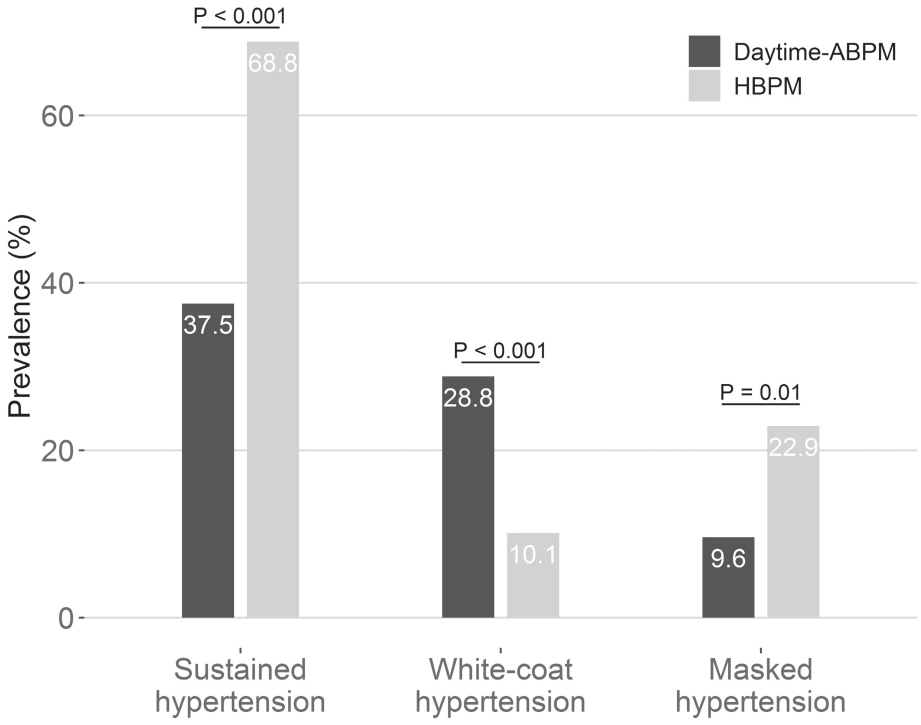
ABPM = Ambulatory Blood Pressure Monitoring, HBPM = Home Blood Pressure Monitoring. Cut-off values hypertension; HBPM: ≥135/85 mm Hg, 30-min BP: ≥135/85 mm Hg, attended OBP: ≥140/90 mm Hg, unattended OBP: ≥140/90 mm Hg.

Figure S5. Prevalence of sustained, white-coat, masked hypertension according to 24-hour ambulatory or home BP monitoring.



Sustained hypertension = consistently elevated BP on office and home or 24-hour ambulatory measurements. White-coat hypertension = an elevated BP in the office and a normal home or 24-hour ambulatory BP. Masked hypertension = an elevated home or 24-hour ambulatory BP with normal office BP. McNemar's test was used to test the difference in prevalence for each hypertension phenotype.

Figure S6. Prevalence of sustained, white-coat, masked hypertension according to daytime ambulatory or home BP monitoring.



Sustained hypertension = consistently elevated BP on office and home or 24-hour ambulatory measurements. White-coat hypertension = an elevated BP in the office and a normal home or 24-hour ambulatory BP. Masked hypertension = an elevated home or 24-hour ambulatory BP with normal office BP. McNemar's test was used to test the difference in prevalence for each hypertension phenotype.

CHAPTER 6

Number of measurement days needed for obtaining a reliable estimate of home blood pressure and hypertension status

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Abstract

Purpose: Out-of-office blood pressure (BP) measurements are essential for the diagnosis and monitoring of hypertension. Current guidelines vary in their recommendations on the protocol for home blood pressure monitoring (HBPM). We aimed to assess the number of blood pressure (BP) measurement days needed for a reliable estimation of true home BP (the expected BP level over time) and hypertension status, using the European guideline-based 7-day HBPM protocol as a reference.

Materials and Methods: Data from 567 adults who performed a 7-day HBPM were analyzed. Blood pressure was measured twice daily (morning and evening readings) using the Microlife Average Mode (MAM), which takes a weighted average of 3 consecutive BP readings. The variability of average BP for an increasing number of measurements was assessed using a linear mixed model including a random intercept per individual and correlated residuals. The reliability of home hypertension status was assessed by the κ statistic.

Results: Mean home BP of the population was 143±16/84±10 mm Hg. On average, the first BP measurements gave the highest values which then decreased over time. Systolic BP in the morning was systematically lower than systolic BP in the evening (142±17mm Hg versus 144±17 mm Hg, $p<0.05$). The average of 7 twice-daily MAM BP measurements was at most 5.2/3.3 mm Hg higher and 9.5/4.8 mmHg lower than the true home BP for 95% of the individuals. Reducing this protocol to 3 days increased this variability by 1.5/1.0 mm Hg and 4.8/2.3 mm Hg, respectively. For diagnosing home hypertension, there was good agreement with a minimum of 4.5 days of HBPM (κ-statistic 0.88; 95% Confidence Interval: 0.82-0.94).

Conclusion: Twice-daily MAM BP measurements for 3 consecutive days provide a reliable estimate of home BP. At least 4.5 consecutive days of HBPM are required for a reliable diagnosis of home hypertension.

Introduction

Guidelines for the management of hypertension recommend the use of out-of-office blood pressure (BP) measurements, either by home blood pressure monitoring (HBPM) or ambulatory blood pressure monitoring (ABPM), to diagnose and monitor hypertension(1,2). Out-of-office BP measurements are not only essential for the detection of white-coat and masked hypertension, but are also superior to conventional office BP measurements in predicting cardiovascular events (3). Advantages of HBPM over ABPM include its practicality (lower costs and greater patient tolerability) and the ability to take BP measurements over multiple days, allowing evaluation of BP trends (4). Furthermore, by enabling self-monitoring and feedback, HBPM has been shown to increase patient engagement, improve medication adherence, and lower BP (5,6).

To date, several studies evaluated the HBPM protocol that provides a reliable and reproducible assessment of home BP of an individual (7). However, protocols of these studies varied widely in terms of population (normotensive versus hypertensive subjects), number of participants, type of analysis (ranging from use of test–retest correlations and the standard deviation (SD) of differences to use of ANOVA models), and method of BP measurement (e.g. different number of measurements per occasion). This resulted in diverging conclusions regarding the optimal HBPM protocol (7). Consequently, recommendations on the HBPM protocol for diagnosing hypertension differ between guidelines as they are mainly based on expert-opinion. For example, the 2021 European Society of Hypertension (ESH) practice guidelines recommend twice-daily measurements for at least 3 but preferably 7 consecutive days, whereas the 2017 American College of Cardiology (ACC) / American Heart Association (AHA) guidelines recommend that home BP should only be based on an average of readings on ≥2 occasions, which is a less intensive and more patient-friendly protocol (2,8). More clarity is needed on the precision of the estimate of home BP obtained with different HBPM protocols to ultimately make a recommendation on the HBPM protocol that will provide a sufficiently reliable estimate of home BP while minimizing patient burden.

Therefore, the aim of this study was to evaluate the number of BP measurement days needed to obtain a reliable estimate of home BP using the current 7-day HBPM protocol as recommended by the European guidelines as a reference. In addition, we examined the required number of BP measurement days to reliably diagnose home hypertension.

Methods

Study design and population

This was an observational study in which data were gathered between October 2017 and July 2021 via the self-management platform EmmaHBPM developed by MedicineMen B.V. (Hilversum, The Netherlands) (9). This platform enables physicians (from both general practice and hypertension clinics) to support their patients in monitoring their blood pressure by obtaining BP data via the Emma smartphone application. Blood pressure measurements taken with the validated Microlife BP A6 BT AFIB device (Microlife Corp, Widnau, Switzerland) (10) can be transferred to the EmmaHBPM application using Bluetooth. The EmmaHBPM application is then able to graphically display the data and provide the patients and their physicians more insight into the patient's BP. Due to the observational nature of the study (anonymized data collected in routine clinical care) in which individuals were not subjected to procedures and were not required to follow rules of behaviour, no formal consent was needed (11). This was approved by the institutional ethics committee (Medisch Ethische Toetsingscommissie Utrecht, University Medical Center Utrecht, Utrecht, The Netherlands).

Home BP measurements

For this study, data were used from adults who completed their first HBPM with EmmaHBPM. Home BP monitoring was performed in accordance with the recommendations in the 2018 ESC/ESH guidelines for the management of hypertension (8). Blood pressure measurements were performed at the subject's home, twice a day, once in the morning (6:00-9:00) and once in the evening (18:00-21:00) for a 7-day period. Subjects were instructed to perform BP measurements before drug intake (if treated), after 5 minutes of rest, in a sitting position using Microlife BP A6 BT AFIB device that features a Microlife Average Mode (MAM) mode. The MAM mode calculates a weighted average of a minimum of 3 consecutive BP readings with standardized 15-second intervals. In this mode, a specific algorithm takes into account the change in BP between sequential readings to determine the weight for the average of all readings. If the difference in consecutive measurements exceeds 40 mm Hg for systolic BP (SBP) and 25 mmHg for DBP, the highest measurement is rejected and an additional fourth measurement is taken. If the difference is between 18 and 40 mm Hg for SBP and between 12 and 25 mm Hg for diastolic BP (DBP), the higher measurement contributes only 50% to the average (12). After calculation of the weighted average, the device discards the three separate measurements obtained.

Statistical analysis

Characteristics of subjects included in the current analysis were summarized as number and percentage for categorical variables and mean ± SD for continuous variables. Fourteen MAM measurements (based on 42 underlying BP readings) were averaged to give a single estimate of home BP per patient. Average BP was also calculated for each

day separately. Student's paired t-test was used for the exploratory comparison of home BP obtained at different measurement occasions.

To evaluate the variability of the average of an increasing number of home BP measurements linear mixed models (LMM) were fitted (see Supplemental File 1). Based on the pattern in average blood pressure over time, day (days 1-7) and part of the day (morning/evening) were included as fixed effects in the models (see Supplemental Tables 1-2). Random intercepts for subjects accounted for the dependence of repeated measurements and the variability between subjects. To account for remaining correlations between the BP measurements within each subject, various correlation structures for the model residuals were evaluated. Based on Akaike Information Criterion (AIC), the autoregressive-moving average correlation structure of order p=5 (13) was selected as the best fitting correlation structure. Fundamental assumptions of LMM (e.g., normality of the residuals and homogeneity of variance) were tested to ensure the accuracy of results.

Maximal deviation from the true home BP, defined as the expected level of BP over time and the BP that is ultimately responsible for the adverse effects of hypertension (14), for 95% of the individuals was calculated by two steps. First, the standard error of the average BP (derived from the covariance matrix of the fitted model (see Supplemental Tables 3-4) was multiplied by 1.96 (approximate value of the 97.5 percentile point of the standard normal distribution) (15). Second, to correct for the systematic difference related to day of measurement and moment of measurement (morning/evening), identified via the exploratory analyses before fitting the LMM, the confidence interval (CI) obtained at the first step was shifted by subtracting the estimates of the fixed effects from both CI limits. To quantify sampling variability of this maximal deviation, the estimation procedure described above was repeated on 1000 datasets simulated from the fitted multivariate normal distribution.

A maximum increase of 5 mm Hg in the maximal deviation of the current 7-day HBPM protocol for both SBP and DBP was considered acceptable based on the previously observed reduction in cardiovascular morbidity and mortality associated with this increase (16).

The reliability of home hypertension status between the current HBPM protocol and protocols with a reduced number of BP measurements was assessed using the kappa (κ) statistic. For this analysis, the average of all fourteen BP measurements was considered the true home BP per patient. Home hypertension was defined as mean home systolic BP ≥135 mm Hg and/or mean home diastolic BP ≥85 mm Hg, which is in line with the current European guidelines (1). We considered a 95% lower confidence limit (LCL) of the κ statistic ≥0.80 as the criterion for good agreement (17,18).

To evaluate whether variability of the average home BP and reliability of home hypertension status differed by sex, age (<65 years and ≥ 65 years), and healthcare domain (general practice or hospital), strata specific estimates were assessed graphically.

All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed, with statistical significance set at 0.05.

Results

Characteristics study population

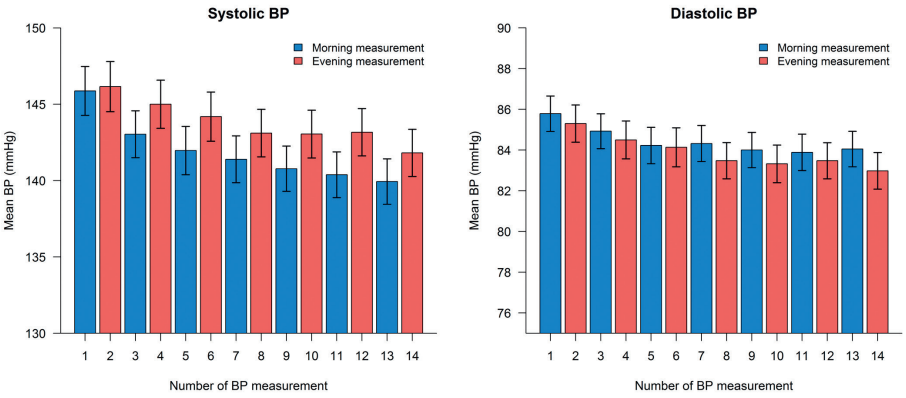
Table 1 shows the characteristics of the 567 subjects included in the analysis. The mean age was 62±14 years, 299 (53%) subjects were male, and most of the subjects were from general practice (82%). Mean home BP for the study population was 143±16/84±10 mm Hg (based on all fourteen BP measurements). Figure 1 displays the sample mean of each BP reading during a 7-day period. For both systolic and diastolic home BP, the first two measurements (first day) gave the highest value which then decreased over time (average SBP first day; 146±19 mm Hg versus average SBP day 2-7; 142±16 mm Hg, mean difference 3.8 mm Hg, 95%CI 2.9-4.7, p<0.05). For systolic home BP, morning BP was consistently lower compared to evening BP (average morning BP; 142±17 mm Hg versus average evening BP; 144±17 mm Hg, mean difference 2.0 mm Hg, 95%CI 1.8-2.3, p<0.05,). For diastolic home BP, morning BP was slightly higher compared to evening BP (average morning BP; 84.5±10 mm Hg versus average evening BP; 83.9±10 mm Hg, mean difference 0.6 mm Hg, 95%CI 0.1-1.0, p<0.05).

Table 1. Characteristics of the study population.

	n = 567
Age (years)	62 ± 14
Male sex	299 (53%)
Healthcare domain	
General practice	373 (82%)
Hospital	82 (18%)
Home Blood Pressure Measurement	
Systolic blood pressure (mmHg)	143 ± 16
Morning systolic blood pressure (mmHg)	142 ± 17
Evening systolic blood pressure (mmHg)	144 ± 17
Diastolic blood pressure (mmHg)	84 ± 10
Morning diastolic blood pressure (mmHg)	84 ± 10
Evening diastolic blood pressure (mmHg)	84 ± 10
Heart rate (beats/min)	71 ± 10
Morning heart rate (beats/min)	69 ± 10
Evening heart rate (beats/min)	73 ± 10

All data in n (%) or mean ± standard deviation

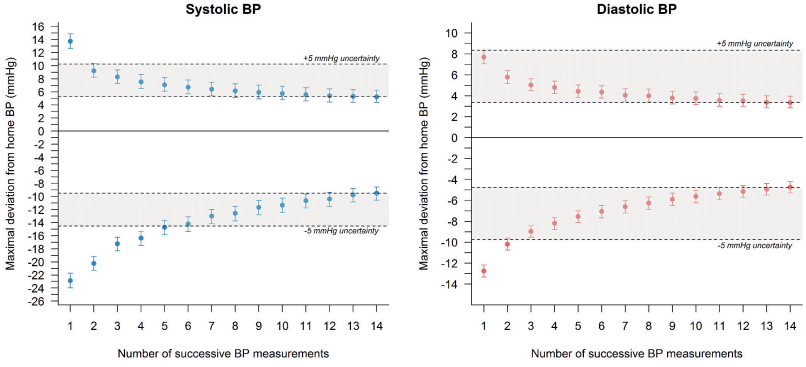
Figure 1. Mean home blood pressure (BP) during the current European guideline-based 7-day HBPM protocol (2 measurements per day).



Precision of home BP measurements

Figure 2 shows the variability of average home BP for an increasing number of BP measurements obtained on succeeding days. The 7-day HBPM protocol resulted in an average BP that is at most 5.2/3.3 mmHg higher and 9.5/4.8 mmHg lower than the true home BP for 95% of the individuals. Most decline in variability of the average home BP was achieved by averaging 6 successive readings (3 days) for systolic home BP and 3 successive readings (1.5 day) for diastolic home BP (increase in positive deviation of 1.5/1.0 mm Hg and negative deviation of 4.8/2.3 mm Hg) with little further decline (<5 mm Hg; shaded grey area) by averaging more readings. Results were similar for different subgroups (males and females, younger (≤ 65 years) and older (>65 years) persons, and healthcare domain (general practice or hospital)) (see Supplementary Figure 1).

Figure 2. Maximal deviation from home blood pressure of an increasing number of successive home BP readings for 95% of individuals.

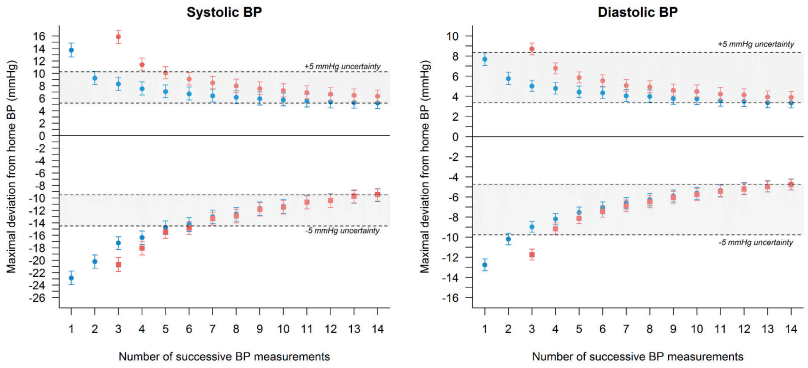


Positive deviation calculated as 1.96*standard error of average BP + systematic bias of average BP. Negative deviation calculated as -1.96*standard error of average BP - systematic bias of average BP.

First day versus consecutive days of home BP

Based on the observed higher average BP on the first day of HBPM, variability was also evaluated by omitting the first two measurements (first day). Figure 3 shows the variability of the average home BP by an increasing number of succeeding BP measurements with (blue circles) and without (red squares) BP measurements performed on the first day. Excluding first-day BP measurements from the analysis resulted in a slightly higher variability of the cumulative average home BP on the second and third measurement day, but did not affect the variability of the cumulative average BP after 7 measurement days.

Figure 3. Maximal deviation from home blood pressure of an increasing number of successive home BP readings for 95% of individuals with (blue circles), and without (red squares) BP readings taken on day 1.



Positive deviation calculated as 1.96*standard error of average BP + systematic bias of average BP. Negative deviation calculated as -1.96*standard error of average BP - systematic bias of average BP.

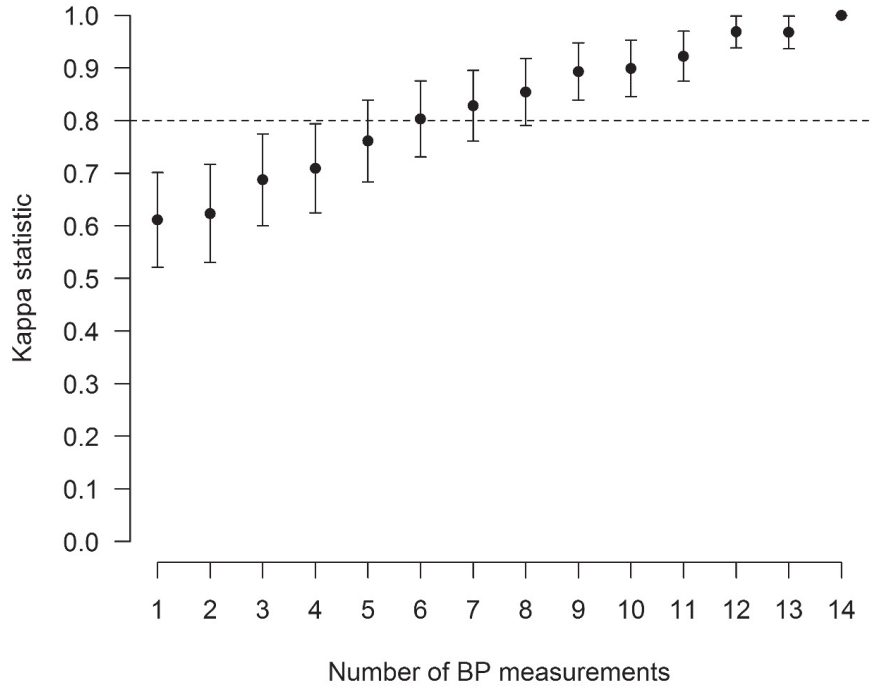
Reliability of home hypertension status

Using the BP thresholds recommended in the 2018 ESC/ESH hypertension guidelines (1), seventy-five percent of the study population had home hypertension. To obtain good agreement (LCL κ statistic ≥0.80) with the current 7-day HBPM protocol, a minimum of 9 consecutive BP readings (5 morning readings and 4 evening readings) was needed (κ statistic 0.88; 0.82-0.94) (see Table 2 and Figure 4; 95% LCL of the κ statistic ≥0.80 at 9 consecutive BP readings). This number of 9 consecutive BP measurements carried a sensitivity of 0.99 (95%CI 0.97-1.00) and a negative predictive value of 0.96 (95%CI 0.90-0.99) (see Table 2). Subgroup analyses showed similar results (see Supplemental Figure 2).

Table 2. Agreement between increasing number of successive home BP measurements and the current 7-day HBPM protocol for the diagnosis of home hypertension (mean home systolic BP ≥135 mm Hg and/or mean home diastolic BP ≥85 mm Hg).

No. of measurements	Kappa statistic (95%CI)	Sensitivity (95%CI)	Specificity (95%CI)	Positive predictive value (95%CI)	Negative predictive value (95%CI)
1	0.61 (0.52-0.70)	0.94 (0.91-0.96)	0.64 (0.54-0.73)	0.88 (0.85-0.92)	0.78 (0.68-0.86)
2	0.63 (0.54-0.72)	0.95 (0.92-0.97)	0.63 (0.53-0.73)	0.88 (0.84-0.92)	0.82 (0.71-0.90)
3	0.69 (0.60-0.77)	0.96 (0.93-0.98)	0.68 (0.58-0.77)	0.90 (0.86-0.93)	0.86 (0.76-0.93)
4	0.70 (0.62-0.79)	0.96 (0.93-0.98)	0.70 (0.60-0.79)	0.90 (0.86-0.93)	0.86 (0.76-0.93)
5	0.76 (0.69-0.84)	0.97 (0.94-0.99)	0.75 (0.65-0.83)	0.92 (0.88-0.95)	0.90 (0.81-0.96)
6	0.80 (0.72-0.87)	0.98 (0.95-0.99)	0.78 (0.68-0.86)	0.92 (0.89-0.95)	0.93 (0.84-0.97)
7	0.83 (0.76-0.90)	0.97 (0.94-0.99)	0.84 (0.75-0.91)	0.94 (0.91-0.97)	0.91 (0.83-0.96)
8	0.86 (0.79-0.92)	0.98 (0.96-1.00)	0.84 (0.75-0.91)	0.94 (0.91-0.97)	0.95 (0.88-0.99)
9	0.89 (0.84-0.95)	0.99 (0.97-1.00)	0.88 (0.80-0.94)	0.96 (0.93-0.98)	0.96 (0.90-0.99)
10	0.90 (0.85-0.95)	0.99 (0.96-1.00)	0.89 (0.81-0.95)	0.96 (0.93-0.98)	0.96 (0.90-0.99)
11	0.92 (0.88-0.97)	0.99 (0.97-1.00)	0.91 (0.83-0.96)	0.97 (0.94-0.99)	0.98 (0.92-1.00)
12	0.97 (0.94-1.00)	1.00 (0.98-1.00)	0.96 (0.89-0.99)	0.98 (0.96-1.00)	1.00 (0.96-1.00)
13	0.97 (0.94-1.00)	0.99 (0.97-1.00)	0.98 (0.92-1.00)	0.99 (0.97-1.00)	0.98 (0.92-1.00)
14	1.00 (1.00-1.00)	1.00 (0.98-1.00)	1.00 (0.96-1.00)	1.00 (0.98-1.00)	1.00 (0.96-1.00)

Figure 4. Agreement of home hypertension status determined with an increasing number of successive BP readings expressed by the kappa (κ) statistic.



Home hypertension: Mean home systolic blood pressure (BP) ≥135 mm Hg or home diastolic BP ≥85 mm Hg.
Good agreement: 95% lower confidence limit (LCL) of the κ statistic ≥0.80

Discussion

The present study shows that the 7-day HBPM protocol, based on the European guidelines, resulted in an average BP that is at most 5.2/3.3 mm Hg higher and 9.5/4.8 mm Hg lower than the true home BP for 95% of the individuals. Reducing this protocol to a minimum of 3 days will maintain this variability within acceptable limits (within 5 mm Hg) of the variability of the 7-day HBPM protocol. Moreover, to reliably diagnose home hypertension, a minimum of 4.5 consecutive measurement days is required.

In recent years, several studies investigated the optimal number of home BP measurements to obtain a reliable assessment of someone's true home BP. A systematic review published in 2019 retrieved 27 studies examining the reproducibility and/or accuracy of HBPM and 10 studies that related HBPM protocols to cardiovascular prognosis (7). This review concluded that the measurement of home BP should be measured for at least 3 days, which was primarily based on findings from studies evaluating its association with prognosis that showed little further increase in prognostic power after 3 days. However, the large heterogeneity and variable methodological quality of the included studies prevented drawing firm conclusions regarding the exact number and timing of BP measurements. A more recent study, not included in this systematic review, conducted in community-dwelling adults not taking antihypertensive medication supported this recommendation by showing that the average of 2 morning and 2 evening readings or 1 morning and 1 evening reading over 3 days of HBPM were needed to reliably estimate true home BP (19). In contrast to our study, most previous studies evaluated total variability of the home BP, which is composed of within- and between-subject variability. However, to make a statement about the maximal deviation of a measured average BP from an individual's true home BP, within-subject variability is needed. By using a mixed model (to obtain this within-subject variability) the current study demonstrated that a greater number of BP measurements, until the maximum available number of 14 BP measurements, resulted in a progressively lower variability of individual's true home BP. However, acceptable variability of the average BP (within 5 mm Hg of the variability of the 7-day BP average) was already achieved within the first 3 measurement days, which is consistent with the recommendations of the abovementioned studies. While a larger number of home BP measurements may improve precision, a longer HBPM protocol may also lower a patient's adherence to such a protocol. Therefore, a shorter, and thus probably less burdensome measurement protocol might increase adherence and is thus preferred in clinical practice and by patients (20). The relatively small benefit in precision obtained by more than 3 measurement days suggests that a prolonged HBPM protocol is likely to be useful only around diagnostic or treatment thresholds.

In line with previous studies (21–26), this study demonstrated a higher average BP on the first measurement day compared to subsequent days. This behavior of home BP is comparable to that of office BP, which is known to decline on repeated measurements

during the same visit (27). For calculation of the average home BP, previous studies suggest that the first day(s) of HBPM should therefore be discarded (21,24). However, as shown in this study, discarding first-day measurements did not alter the maximal deviation of the cumulative average BP on the last measurement day. Discarding first-day measurements even resulted in a somewhat higher variability of the cumulative average BP on the second and third measurement day. This suggests that despite the presence of systematic difference on the first days of measurement, this does not outweigh the additional variability due to reduction in the number of measurements. Based on this, first-day measurements should be included in the calculation of the average BP. This is consistent with recommendations from previous studies that evaluated the correlation of HBPM protocols in- and excluding first-day BP measurements with ABPM (28–30).

The consistently lower SBP in the morning compared to the evening, as observed in this study, is in accordance with several previous studies (22,24,26). Some studies, however, report higher home BP values in the morning (31,32). This difference can potentially be explained by the fact that these studies were conducted in Asian populations in which evening measurements were taken before bedtime and after bathing (33), whereas in the present study, evening measurements were taken during a more active part of the day (between 18:00-21:00), which is generally several hours before bedtime. Furthermore, a depressor effect of alcohol intake on evening BP, combined with a pressor effect in the morning, resulting in a net increase in morning BP, has been demonstrated by several studies (33,34).

A major strength of this study is the large set of routinely collected data, which enabled us to fit more complex models including correlation structures and to perform subgroup analyses. Moreover, the inclusion of correlation structures into our models reduced the risk of underestimation of the variability of the average BP. Also, application of the MAM algorithm by the Microlife BP A6 BT device ensured standardized measurements (3 consecutive BP readings with 15-second intervals) for the entire study population and thereby minimized the impact of measurement error on the variability.

Some limitations also need to be considered. First, data on several relevant patient characteristics such as comorbidities and use of antihypertensive medication were not available. Additional subgroup analyses, to make more specific statements about the heterogeneity of variability of average BP, were therefore not possible. Moreover, some classes of antihypertensive drugs (eg. β-blockers and calcium channel blockers) can affect BP variability (35,36), which could have resulted in an underestimation of the estimated variability of home BP. Second, due to use of the MAM algorithm, only a weighted average of 3 consecutive measurements was available for the analysis. Consequently, it was not possible to evaluate the optimal number of BP readings per measurement occasion. Moreover, use of the MAM algorithm may have led to an underestimation of the variability, since less weight is given to extreme measurements in the calculation of

the average. Therefore, application of current findings into clinical practice requires the use of a BP monitor equipped with a MAM mode (37). Lastly, an important limitation of this study is the lack of data regarding the reference standard ABPM and cardiovascular outcomes. For defining the most optimal HBPM protocol, the relation to both ABPM and cardiovascular outcomes should be taken into account. To investigate the relation between ABPM and the HBPM protocol as applied in this study, a randomized cross-over study called Ambulant versus Unattended & Attended office versus Self home Blood Pressure measurement (AMUSE-BP) is now being conducted (Netherlands Trial Register: NL8277).

In conclusion, this study showed that measurement of home BP twice daily (1 morning and 1 evening MAM reading) for 3 consecutive days provides a reliable estimate of home BP. At least 4.5 consecutive measurement days are required for a reliable diagnosis of home hypertension. These findings suggest that the 7-day HBPM protocol as recommended by the European guidelines can be reduced to 4.5 consecutive days when the goal is to confirm the diagnosis of hypertension. When the goal is monitoring, the HBPM protocol could even be tailored to 3 consecutive days without substantially affecting the variability of the average home BP as obtained by a 7-day HBPM protocol.

References

1. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
2. Whelton PK, Carey RM, Aronow WS, Casey DEJ, Collins KJ, Dennison Himmelfarb C, et al. 2017 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 P. J Am Coll Cardiol. 2018 May;71(19):e127–248.
3. Parati G, Stergiou GS, Asmar R, Bilo G, de Leeuw P, Imai Y, et al. European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. J Hypertens. 2008 Aug;26(8):1505–26.
4. Kikuya M, Ohkubo T, Metoki H, Asayama K, Hara A, Obara T, et al. Day-by-day variability of blood pressure and heart rate at home as a novel predictor of prognosis: the Ohasama study. Hypertens (Dallas, Tex 1979). 2008 Dec;52(6):1045–50.
5. Cramer JA. Microelectronic systems for monitoring and enhancing patient compliance with medication regimens. Drugs. 1995 Mar;49(3):321–7.
6. McManus RJ, Little P, Stuart B, Morton K, Raftery J, Kelly J, et al. Home and Online Management and Evaluation of Blood Pressure (HOME BP) using a digital intervention in poorly controlled hypertension: randomised controlled trial. BMJ. 2021 Jan;372:m4858.
7. Hodgkinson JA, Stevens R, Grant S, Mant J, Bray EP, Hobbs FDR, et al. Schedules for Self-monitoring Blood Pressure: A Systematic Review. Am J Hypertens. 2019 Mar;32(4):350–64.
8. Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, et al. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. J Hypertens. 2021 Jul;39(7):1293–302.
9. MedicineMen. EmmaHBPM [Internet]. [cited 2021 Sep 11]. Available from: https://medicinemen.eu/consument/producten/emmahbpm/
10. Stergiou GS, Giovas PP, Neofytou MS, Adamopoulos DN. Validation of the Microlife BPA100 Plus device for self-home blood pressure measurement according to the International Protocol. Blood Press Monit. 2006 Jun;11(3):157–60.
11. Central commity on research involving Human subjects C. Legal framework for medical scientific research: Your research: Is it subject to the WMO or not? [Internet]. [cited 2021 Sep 11]. Available from: https://english.ccmo.nl/investigators/legal-framework-for-medical-scientific-research/your-research-is-it-subject-to-the-wmo-or-not
12. Wilton A, De Greef A, Shennan A. Rapid assessment of blood pressure in the obstetric day unit using Microlife MaM technology. Hypertens pregnancy. 2007;26(1):31–7.
13. Pinheiro J, Bates D. Mixed-effects models in S and S-PLUS. Springer Science & Business Media; 2006.
14. Pickering TG, Pieper C, Schechter CB. Ambulatory monitoring and blood pressure variability. Science Press; 1991.
15. Tong YL. The multivariate normal distribution. Springer Science & Business Media; 2012.
16. Blood Pressure Lowering Treatment Trialists' Collaboration. Age-stratified and blood-pressure-stratified effects of blood-pressure-lowering pharmacotherapy for the prevention of cardiovascular disease and death: an individual participant-level data meta-analysis. Lancet (London, England). 2021 Sep;398(10305):1053–64.
17. Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. Fam Med. 2005 May;37(5):360–3.

18. McHugh ML. Interrater reliability: the kappa statistic. Biochem medica. 2012;22(3):276–82.

19. Bello NA, Schwartz JE, Kronish IM, Oparil S, Anstey DE, Wei Y, et al. Number of Measurements Needed to Obtain a Reliable Estimate of Home Blood Pressure: Results From the Improving the Detection of Hypertension Study. J Am Heart Assoc. 2018 Oct;7(20):e008658.

20. Grant S, Hodgkinson JA, Milner SL, Martin U, Tompson A, Hobbs FR, et al. Patients' and clinicians' views on the optimum schedules for self-monitoring of blood pressure: a qualitative focus group and interview study. Br J Gen Pract J R Coll Gen Pract. 2016 Nov;66(652):e819–30.

21. Stergiou GS, Skeva II, Zourbaki AS, Mountokalakis TD. Self-monitoring of blood pressure at home: how many measurements are needed? J Hypertens. 1998 Jun;16(6):725–31.

22. Stergiou GS, Nasothimiou EG, Kalogeropoulos PG, Pantazis N, Baibas NM. The optimal home blood pressure monitoring schedule based on the Didima outcome study. J Hum Hypertens. 2010 Mar;24(3):158–64.

23. Stergiou GS, Parati G. The optimal schedule for self-monitoring of blood pressure by patients at home. J Hypertens. 2007 Oct;25(10):1992–7.

24. Verberk WJ, Kroon AA, Kessels AGH, Lenders JWM, Thien T, van Montfrans GA, et al. The optimal scheme of self blood pressure measurement as determined from ambulatory blood pressure recordings. J Hypertens. 2006 Aug;24(8):1541–8.

25. Imai Y, Obara T, Ohkubo T. How many times should we ask subjects to measure blood pressure at home on each occasion? J Hypertens. 2007 Oct;25(10):1987–91.

26. Celis H, De Cort P, Fagard R, Thijs L, Staessen JA. For how many days should blood pressure be measured at home in older patients before steady levels are obtained? J Hum Hypertens. 1997 Oct;11(10):673–7.

27. Stergiou GS, Skeva II, Baibas NM, Kalkana CB, Roussias LG, Mountokalakis TD. Diagnosis of hypertension using home or ambulatory blood pressure monitoring: comparison with the conventional strategy based on repeated clinic blood pressure measurements. J Hypertens. 2000 Dec;18(12):1745–51.

28. Niiranen TJ, Johansson JK, Reunanen A, Jula AM. Optimal schedule for home blood pressure measurement based on prognostic data: the Finn-Home Study. Hypertens (Dallas, Tex 1979). 2011 Jun;57(6):1081–6.

29. Di Monaco S, Rabbia F, Covella M, Fulcheri C, Berra E, Pappaccogli M, et al. Evaluation of a short home blood pressure measurement in an outpatient population of hypertensives. Clin Exp Hypertens. 2016;38(8):673–9.

30. Johansson JK, Niiranen TJ, Puukka PJ, Jula AM. Optimal schedule for home blood pressure monitoring based on a clinical approach. J Hypertens. 2010 Feb;28(2):259–64.

31. Asayama K, Ohkubo T, Kikuya M, Obara T, Metoki H, Inoue R, et al. Prediction of stroke by home "morning" versus "evening" blood pressure values: the Ohasama study. Hypertens (Dallas, Tex 1979). 2006 Oct;48(4):737–43.

32. Stergiou G, Parati G. Further insights into the 24-h blood pressure profile by home blood pressure monitoring: the issue of morning hypertension. Vol. 27, Journal of hypertension. England; 2009. p. 696–9.

33. Fujiwara T, Hoshide S, Nishizawa M, Matsuo T, Kario K. Difference in evening home blood pressure between before dinner and at bedtime in Japanese elderly hypertensive patients. J Clin Hypertens (Greenwich). 2017 Jul;19(7):731–9.

34. Kawano Y, Pontes CS, Abe H, Takishita S, Omae T. Effects of alcohol consumption and restriction on home blood pressure in hypertensive patients: serial changes in the morning and evening records. Clin Exp Hypertens. 2002;24(1–2):33–9.

35. Umemoto S, Ogihara T, Matsuzaki M, Rakugi H, Ohashi Y, Saruta T. Effects of calcium channel blocker-based combinations on intra-individual blood pressure variability: post hoc analysis of the COPE trial. Hypertens Res. 2016 Jan;39(1):46–53.

36. Eguchi K, Imaizumi Y, Kaihara T, Hoshide S, Kario K. Comparison of valsartan and amlodipine on ambulatory blood pressure variability in hypertensive patients. Clin Exp Hypertens. 2016;38(8):721–4.

37. Microlife. MAM (Microlife Average Mode) [Internet]. [cited 2021 Dec 8]. Available from: https://www.microlife.com/technologies/blood-pressure/mam-technology

Supplemental Material

Supplemental File 1. Linear mixed model for home blood pressure measurements.

$$y_{ij} = \beta_0 + u_{0i} + \boldsymbol{\beta} \boldsymbol{X}_{\boldsymbol{ij}}^{\boldsymbol{T}} + \varepsilon_{ij}$$

Where:

- y_{ij}: j^{th} blood pressure measurement for i^{th} individual
- β_0: average home BP in population on day 7 ($j = 7$)
- u_{0i}: random intercept for i^{th} individual with $u_{0i} \sim N(0, \sigma^2_{\text{between}})$
- $\boldsymbol{\beta}$: (row) vector with fixed effects
- $\boldsymbol{X}_{ij}$: (row) vector with explanatory covariates

  (day after start HBPM, moment of measurement (morning or evening))
- ε_{ij} : residual variance with $\boldsymbol{\varepsilon_i} \sim N(\mathbf{0}, \Sigma)$ for covariance matrix Σ

Such that $\beta_0 + u_{0i}$ equals the true home BP for individual i, and ε_{ij} equals the deviation of the measurement from the expected BP at the j^{th} measurement.

With this model the average of k BP measurements, $\bar{y}_{ik} = \frac{1}{k}\sum_{j=1}^{k} y_{ij}$, is normally distributed: $\bar{y}_{ik} \sim N(\beta_0 + u_{0i} + \frac{1}{k}\sum_{j=1}^{k} \boldsymbol{\beta} \boldsymbol{X}_{\boldsymbol{ij}}^{\boldsymbol{T}}, \boldsymbol{z}\Sigma_{\mathrm{k}}\mathbf{z}^{\mathrm{T}})$, where Σ_{k} is the sub-matrix consisting of the first k rows and columns of the covariance matrix Σ and $\boldsymbol{z} = \left(\frac{1}{k}, \frac{1}{k}, \ldots, \frac{1}{k}\right)$ is a row vector with k elements. Thus, $\boldsymbol{z}\Sigma_{\mathrm{k}}\mathbf{z}^{\mathrm{T}}$ equals the sum of all elements of Σ_{k} divided by k^2.

Supplemental Table 1. Parameter estimates from model for systolic BP measurements.

β_0	140.82
σ_{between}	15.89
$\beta_{morning}$	-0.94
$\beta_{evening}$	0.94
$\beta_{day\ 1}$	5.51
$\beta_{day\ 2}$	3.33
$\beta_{day\ 3}$	2.34
$\beta_{day\ 4}$	1.59
$\beta_{day\ 5}$	1.10
$\beta_{day\ 6}$	0.96

Supplemental Table 2. Parameter estimates from model for diastolic BP measurements.

β_0	83.57
σ_{between}	9.36
$\beta_{morning}$	0.32
$\beta_{evening}$	-0.32
$\beta_{day\ 1}$	2.22
$\beta_{day\ 2}$	1.18
$\beta_{day\ 3}$	0.67
$\beta_{day\ 4}$	0.43
$\beta_{day\ 5}$	0.15
$\beta_{day\ 6}$	0.20

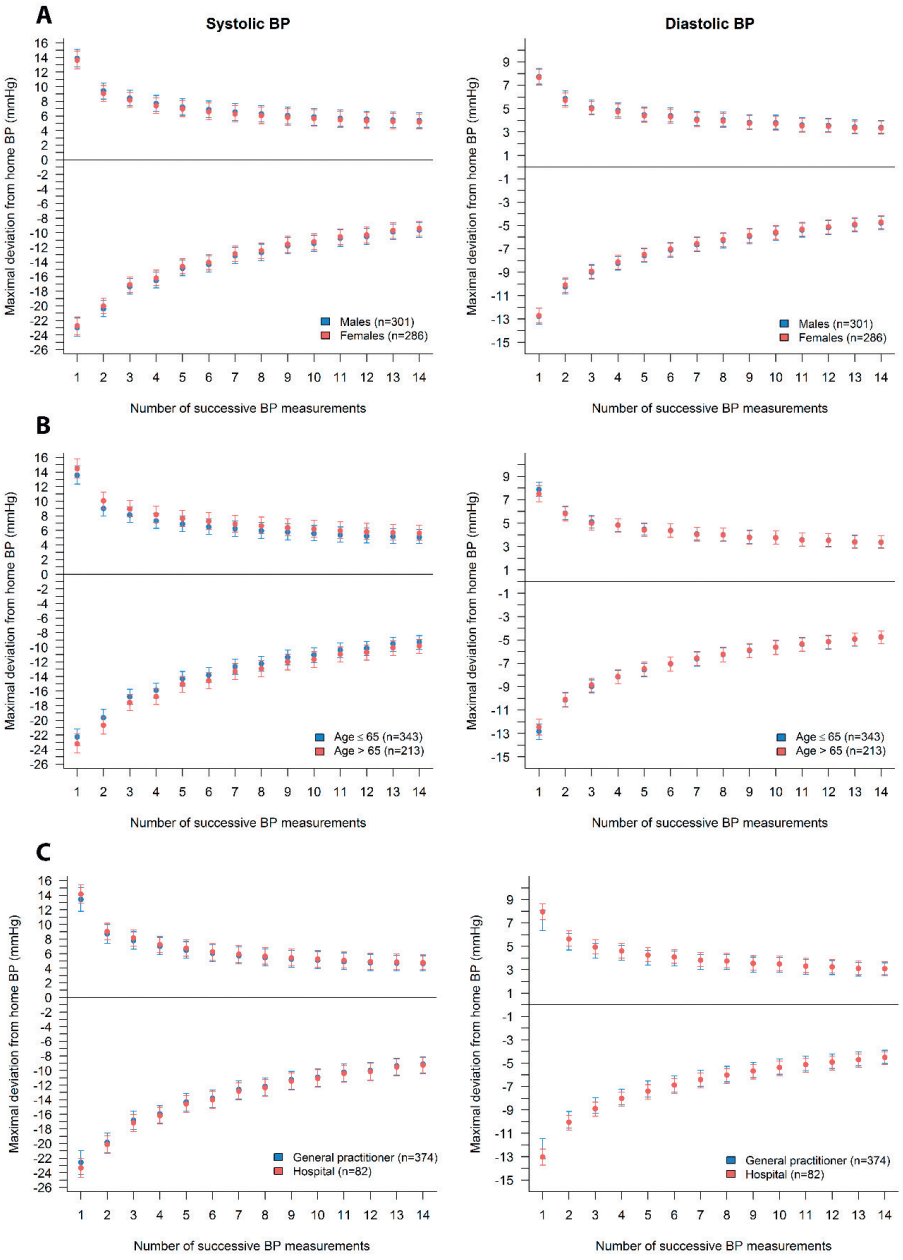
Supplemental Table 3. Covariance matrix (∑)estimate from model for systolic BP measurements.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	86.95	3.46	29.84	-8.72	18.92	-11.12	9.19	-8.28	5.99	-5.97	3.85	-3.94	2.59	-2.64
2	3.46	129.55	3.46	44.46	-8.72	28.20	-11.12	13.69	-8.28	8.92	-5.97	5.73	-3.94	3.86
3	29.84	3.46	86.95	3.46	29.84	-8.72	18.92	-11.12	9.19	-8.28	5.99	-5.97	3.85	-3.94
4	-8.72	44.46	3.46	129.55	3.46	44.46	-8.72	28.20	-11.12	13.69	-8.28	8.92	-5.97	5.73
5	18.92	-8.72	29.84	3.46	86.95	3.46	29.84	-8.72	18.92	-11.12	9.19	-8.28	5.99	-5.97
6	-11.12	28.20	-8.72	44.46	3.46	129.55	3.46	44.46	-8.72	28.20	-11.12	13.69	-8.28	8.92
7	9.19	-11.12	18.92	-8.72	29.84	3.46	86.95	3.46	29.84	-8.72	18.92	-11.12	9.19	-8.28
8	-8.28	13.69	-11.12	28.20	-8.72	44.46	3.46	129.55	3.46	44.46	-8.72	28.20	-11.12	13.69
9	5.99	-8.28	9.19	-11.12	18.92	-8.72	29.84	3.46	86.95	3.46	29.84	-8.72	18.92	-11.12
10	-5.97	8.92	-8.28	13.69	-11.12	28.20	-8.72	44.46	3.46	129.55	3.46	44.46	-8.72	28.20
11	3.85	-5.97	5.99	-8.28	9.19	-11.12	18.92	-8.72	29.84	3.46	86.95	3.46	29.84	-8.72
12	-3.94	5.73	-5.97	8.92	-8.28	13.69	-11.12	28.20	-8.72	44.46	3.46	129.55	3.46	44.46
13	2.59	-3.94	3.85	-5.97	5.99	-8.28	9.19	-11.12	18.92	-8.72	29.84	3.46	86.95	3.46
14	-2.64	3.86	-3.94	5.73	-5.97	8.92	-8.28	13.69	-11.12	28.20	-8.72	44.46	3.46	129.55

Supplemental Table 4. Covariance matrix (Σ) estimate from model for diastolic BP measurements.

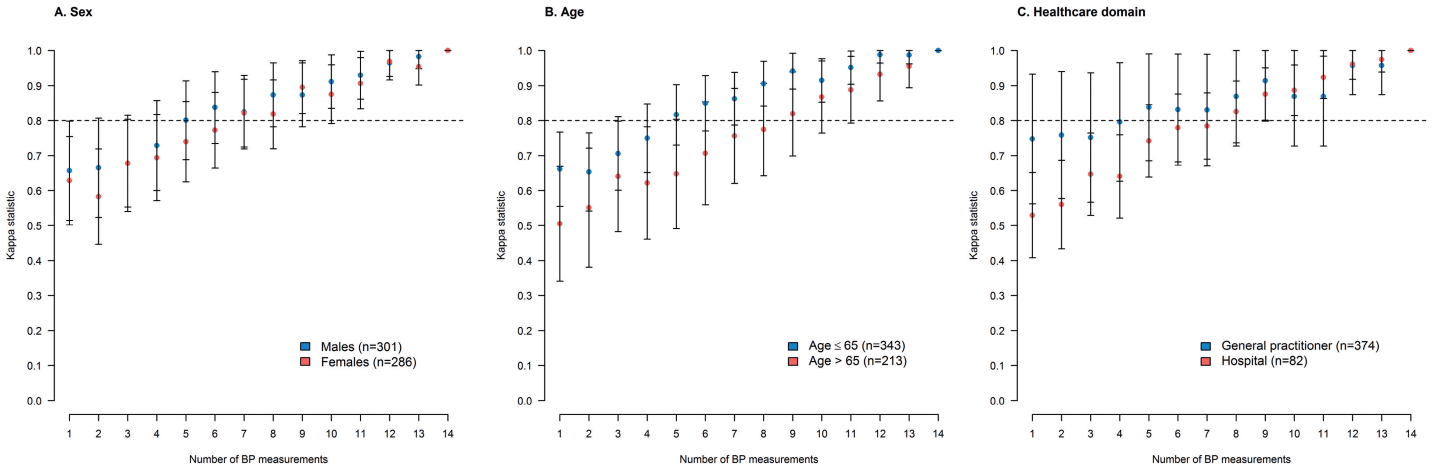
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	27.04	0.91	9.35	-2.21	6.47	-3.52	3.11	-2.61	2.10	-2.03	1.38	-1.36	0.96	-0.95
2	0.91	36.37	0.91	12.57	-2.21	8.70	-3.52	4.18	-2.61	2.82	-2.03	1.85	-1.36	1.29
3	9.35	0.91	27.04	0.91	9.35	-2.21	6.47	-3.52	3.11	-2.61	2.10	-2.03	1.38	-1.36
4	-2.21	12.57	0.91	36.37	0.91	12.57	-2.21	8.70	-3.52	4.18	-2.61	2.82	-2.03	1.85
5	6.47	-2.21	9.35	0.91	27.04	0.91	9.35	-2.21	6.47	-3.52	3.11	-2.61	2.10	-2.03
6	-3.52	8.70	-2.21	12.57	0.91	36.37	0.91	12.57	-2.21	8.70	-3.52	4.18	-2.61	2.82
7	3.11	-3.52	6.47	-2.21	9.35	0.91	27.04	0.91	9.35	-2.21	6.47	-3.52	3.11	-2.61
8	-2.61	4.18	-3.52	8.70	-2.21	12.57	0.91	36.37	0.91	12.57	-2.21	8.70	-3.52	4.18
9	2.10	-2.61	3.11	-3.52	6.47	-2.21	9.35	0.91	27.04	0.91	9.35	-2.21	6.47	-3.52
10	-2.03	2.82	-2.61	4.18	-3.52	8.70	-2.21	12.57	0.91	36.37	0.91	12.57	-2.21	8.70
11	1.38	-2.03	2.10	-2.61	3.11	-3.52	6.47	-2.21	9.35	0.91	27.04	0.91	9.35	-2.21
12	-1.36	1.85	-2.03	2.82	-2.61	4.18	-3.52	8.70	-2.21	12.57	0.91	36.37	0.91	12.57
13	0.96	-1.36	1.38	-2.03	2.10	-2.61	3.11	-3.52	6.47	-2.21	9.35	0.91	27.04	0.91
14	-0.95	1.29	-1.36	1.85	-2.03	2.82	-2.61	4.18	-3.52	8.70	-2.21	12.57	0.91	36.37

Supplemental Figure 1. Subgroup analyses for maximal deviation from home blood pressure for 95% of individuals.



Maximal deviation from home systolic (left column) and diastolic (right column) blood pressure (BP) stratified according to sex (**A**), age (≤65 and >65) (**B**), and healthcare domain (**C**).

Supplemental Figure 2. Subgroup analyses for agreement of home hypertension status determined with an increasing number of successive BP readings compared to the maximum of 14 BP readings.



Agreement (expressed by the κ statistic) of home hypertension status determined with an increasing number of successive BP readings stratified according to sex (**A**), age (≤65 and >65) (**B**), and healthcare domain (**C**).

CHAPTER 7

Validation of spot urine in estimating 24-hour urinary sodium, potassium, and sodium-to-potassium ratio during three different sodium diets in healthy adults

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Abstract

Purpose: To evaluate the validity of spot urine assay methods in estimating the 24-hour urinary sodium, potassium, and sodium-to-potassium ratio during three different sodium diets.

Methods: Twelve healthy volunteers were asked to adhere to 3 dietary sodium targets (3.3-5.0g/day, <3.3g/day, and >5.0g/day) for 3 consecutive weeks and to measure salt excretion daily in spot urine samples using a self-monitoring device. On day 7 of each week, 24-hour urine was collected to compare measured with estimated 24-hour salt excretion (by the Kawasaki, Tanaka, and INTERSALT equations) using correlation coefficients, Bland–Altman plots, and relative and absolute differences at the individual level.

Results: Correlation coefficients relating measured and estimated 24-hour salt excretion were low and not significant for Kawasaki and INTERSALT, and moderate for the Tanaka equation (τ 0.56-0.64, $p<0.05$). Bland-Altman plots showed considerable differences between estimated and measured salt excretion across all salt diets. Over 40% of the participants showed an absolute difference between measured and estimated 24-hour sodium of more than 1000 mg/day. The correlation coefficients between 24-hour and spot Na/K ratio were 0.67, 0.94, and 0.85 ($p<0.05$), and mean differences were 0.59, 0.06, and 0.48 for the intermediate, low, and high sodium diets, respectively. Using spot Na/K ratio, sixty-six percent of the participants were classified into the matching 24-hour Na/K ratio tertiles.

Conclusion: These findings do not support estimation of individual 24-hour urinary salt excretion from spot urine by the Kawasaki, Tanaka, or INTERSALT formula. Spot urine Na/K ratio may be an appropriate alternative for monitoring daily salt intake.

Introduction

High dietary salt intake is a major contributor to the onset and progression of hypertension (1), a leading modifiable risk factor for cardiovascular disease (CVD) and mortality worldwide (2). Of the global number of deaths due to CVD, approximately 1.65 million per year have been attributed to excessive salt intake (3). As a consequence, the reduction in sodium consumption to levels below 5 gram per day has been encouraged by the World Health Organization (WHO) and various international treatment guidelines (4–6). However, despite rigorous governmental campaigns to reduce salt intake, a large gap remains between the recommended and actual daily intake of salt (7). In addition to the fact that conventional population-based approaches have failed to close this gap (8), individuals are still poorly aware of their daily salt intake, with individuals who reported that they were on a low-salt diet actually showing salt intake levels similar to those who were not on a low-salt diet (9).

Self-monitoring devices that give individuals immediate feedback on their salt intake could provide support in achieving the goals as set by the WHO. At present, measurement of salt excretion in multiple non-consecutive 24-hour urinary collections is considered the gold standard for measuring dietary sodium intake (10). However, this method is burdensome, time-consuming, and error prone (11). Hence, several formulas for estimating 24-hour urinary salt excretion from casual spot urines have been developed (12–14). Although this formula-based approach offers a lower patient burden, the most commonly used formulas for estimating the 24-hour urine sodium excretions showed poor agreement with measured 24-hour sodium excretion (15). In addition, these formulas require information on parameters such as body mass index and creatinine levels, which complicates the purpose of self-monitoring. To overcome these issues, the sodium-to-potassium ratio (Na/K ratio) has been proposed as an easier and potentially more reliable alternative for self-monitoring (16–18). However, few studies evaluated the Na/K ratio and the formula-based approach in parallel. Therefore, the aim of the current study was to assess and compare the validity of both the estimated 24-hour urinary sodium and potassium excretion and the Na/K ratio from spot urine samples measured by a self-monitoring device under three different sodium diets using 24-hour urine collections as a reference standard.

Methods

Study participants

Between June 2018 and October 2018, twelve healthy volunteers from the general population who expressed interest during the initial research and development phase were approached and prospectively enrolled in the University Medical Center Utrecht (UMCU), the Netherlands. All participants were 18 years or older and motivated to adhere to the study protocol. Eligible participants were apparently healthy not having a known medical history of cardiovascular disease, chronic kidney disease, uncontrolled hypertension (blood pressure >140 and/or >90 mm Hg at screening), pregnancy, incontinence, or an impaired vision. Moreover, subjects that were prescribed diuretics were not eligible for inclusion in the study. The study was approved by Medical Ethics Committee Utrecht (approval number 18-002/D) and conforms to the 1964 Declaration of Helsinki ethical guidelines. Participants were given oral and written information regarding the purpose and outline of the study, and written informed consent was obtained from each individual.

Dietary protocol

All participants started with their intermediate salt diet (3.3-5.0 gram sodium per day), subsequently followed by a low salt diet (< 3.3 gram sodium per day) and finished with a high salt diet (> 5.0 gram sodium per day). Each dietary period lasted for 7 days. This particular order of the diets was chosen for two reasons. First, since the intermediate salt diet was expected to be easier to follow than the other two diets, it was planned first such that the participants could get used to the protocol for collecting and measuring urine samples. Second, because it takes several days for renal salt excretion to adjust to a new salt diet (19–21), the high salt diet was planned last to minimize its influence on salt excretion during the other two diets. To support participants in reaching the weekly sodium intake target, all participants received oral and written dietary recommendations compiled by an experienced dietician. Participants received no dietary advice regarding potassium intake.

Spot urine collection

The urine collection schedule is shown in Table 1. Participants were instructed to collect daily spot urine samples and measure sodium, potassium, and creatinine concentration by using the validated Medimate self-monitoring device (Fisic BV, Enschede, the Netherlands) (Supplemental Table 1) (22,23). Urine had to be collected in a pre-filled plastic container in which the urine sample was mixed with both an internal standard and buffer solution. By applying a drop of this mixed urine onto the disposable cartridge (using a pipette), sodium, potassium and creatinine concentrations were measured using microchip capillary electrophoresis (μCE) combined with conductivity detection. The device displayed the results within a few minutes and then stored them in the device's internal memory. On day 1, 3, 5, and 6, participants had to collect a morning spot urine

sample in fasting state. On day 2 and 4, this had to be a random urine sample collected in a non-fasting state. On day 7, four spot urine samples (single morning fasting urine, before lunch (09:00 -13:00), before diner (13:00 - 17:00), and after dinner (17:00 - 23:00)) had to be collected.

Table 1. Schedule of urine collections.

Sodium intake	Week 1 'Intermediate Salt' <3.3 gram/day	Week 2 'Low Salt' 3.3-5.5 gram/day	Week 3 'High Salt' >5.0 gram/day	Week 4
Day 1	MFU	MFU	MFU	MFU
Day 2	CSU	CSU	CSU	-
Day 3	MFU	MFU	MFU	-
Day 4	CSU	CSU	CSU	-
Day 5	MFU	MFU	MFU	-
Day 6	MFU	MFU	MFU	-
Day 7	24hUc, MFU, CSU2, CSU3, CSU4	24hUc, MFU, CSU2, CSU3, CSU4	24hUc, MFU, CSU2, CSU3, CSU4	-

24hUc = 24-hour urine collection, MFU = morning fasting urine sample, CSU = casual spot urine

Spot urine measurement by the Medimate self-monitoring device

The Medimate 2017 is a point-of-care self-test device for whole blood and urine. This device is able to measure lithium, sodium, potassium, magnesium, creatinine, chloride, phosphate and lactate in blood and urine using lab-on-a-chip technology, also referred to as "Micro Total Analysis Systems" (μTAS). Measurements are performed using microchip capillary electrophoresis (μCE) combined with conductivity detection. The device consists of two main components: a multireader and a disposable cartridge (Supplemental Figure 1A). The multireader contains all of the measurement electronics including a programmable black and white display on top and buttons to help the user perform a self-test, navigate through user-settings, and scroll through stored measurements. The power connector is 100-240V and at has a build in mini USB connector. The disposable cartridge called 'the Medimate Lab-Chip' (Supplemental Figure 1B) consists of three parts: a glass chip (LoC) for measurement analysis (Supplemental Figure 1C), a plastic housing, and a seal. Measurements are performed by applying a drop of urine onto the disposable cartridge and inserting it into the multireader. Measurement time is less than 8 minutes.

Estimation of 24-hour urine salt excretion

The methods for the estimation of 24-hour urinary sodium excretion by using spot urine samples were the Kawasaki method (12), the Tanaka method (13), and the INTERSALT method (14). The INTERSALT formula was not designed to estimate urinary potassium excretion, and therefore, only the Kawasaki and Tanaka method were used to estimate

24-hour urinary potassium excretion. The estimation formulas used are listed in Supplemental Table 2. In accordance with the original design, for the Kawasaki formula, the estimations of 24-hour sodium and potassium were based on the concentration of sodium, potassium, and creatinine of the morning spot urine sample taken on the day following the 24-hour urine collection, and for Tanaka and INTERSALT formulas, this was based on the average of spot urine samples 2, 3, and 4 taken on the same day as the 24-hour urine collection (Table 1).

Twenty four-hour urine collection

Twenty-four-hour urine was collected on day 7 of each week. To ensure a complete urine collection, written and oral instructions were provided to the participants. Participants were asked to discard their first voided urine after getting up and to collect all the urine they voided during the subsequent 24 hours, including the first urine of the following morning. In addition, participants were asked to record the start and finish times of the collections and report any wasting of urine to the study team. After completion of the 24-hour urine collection, a vacuum test tube was filled with urine from the collection container and sent to the UMCU chemical laboratory. Twenty-four-hour urine collection was considered to be successful if the participant reported collecting urine ≥ 24 hours, the total urine volume was greater than 1000 ml, and the difference between the expected 24-hour creatinine excretion (based on sex, age, and weight (24)) was within 25% of the actual measured values of 24-h urinary creatinine excretion. Urine collections were considered to be unsuccessful when the participant reported that portions of urine were missing from the collection. Unsuccessful samples were excluded from the analysis. Urinary creatinine, sodium and potassium values were determined by the ion-selective electrode method. All specimen measurements were conducted on a Beckman Coulter analyzer (DxAU 5811) (Brea, CA, USA).

Statistical analysis

The differences between urinary salt excretion measured by 24-hour urine collection and that estimated by each of the 3 formulas were calculated. Data were presented as means ± SD. Paired t-tests were used to evaluate the differences between the estimated and measured 24-hour values for significance. The correlation between estimated and measured 24-hour urinary salt excretion was evaluated by the Kendall rank correlation coefficient. Bland-Altman analysis was also applied to provide a visualized assessment of the agreement between the measured and estimated 24-hour salt excretion values. In addition, we analyzed the absolute and relative differences between the estimated and measured values, where the absolute differences were calculated as (estimated value – measured value) and the relative difference as [(estimated value – measured value)/measured value × 100%]. The proportional distribution of the relative and absolute differences provided a graphical representation of the accuracy at the individual level. The relative difference in this study was defined in five groups: within 10%, 10% to 19%, 20% to 29%, 30% to 39%, and over 40%. The absolute difference was also divided into

five groups that were within 500, 500 to 1000, 1000 to 1500, 1500 to 2000, and over 2000 mg/day in sodium or potassium amount. Furthermore, we categorized measured 24-hour salt excretion into tertiles and then compared for each estimation method the fraction of participants misclassified into the wrong group. Lastly, all of the above analyses were repeated to compare the average Na/K ratio measured in multiple casual spot urine samples on the 7th day with the Na/K ratio measured in the 24-hour urine collections. All analyses were performed with R statistical software (Version 3.5.1; R foundation for Statistical Computing, Vienna, Austria). All p-values were two-tailed, with statistical significance set at 0.05.

Results

Characteristics study population

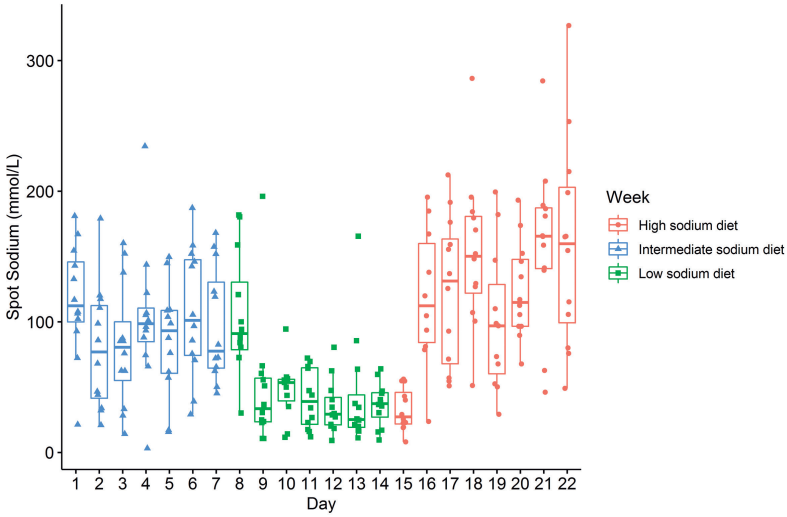
Characteristics of the 12 participants included in the study are presented in Table 2. The mean age of subjects was 47 (interquartile range (IQR) 35 - 69) years, 11 subjects (92%) were male. Median body mass index (BMI) was 24 kg/m^2 (IQR 23-27) and median blood pressure was 130/77 mmHg (IQR 125-133/ 74-81). Figure 1 and 2 demonstrate the overall trend in daily spot sodium and Na/K ratio levels, respectively. Spot sodium and Na/K ratio levels changed in accordance with the sodium diet applied; low values during the low sodium diet and high values during the high sodium diet. Spot potassium levels remained stable over time (Supplemental Figure 2).

Table 2. Characteristics of the study population.

	n = 12
Male sex	11 (92%)
Age (years)	47 [35 - 59]
Body mass index (kg/m^2)	24 [23 - 27]
Systolic blood pressure (mmHg)	130 [125 - 133]
Diastolic blood pressure (mmHg)	77 [73 - 81]

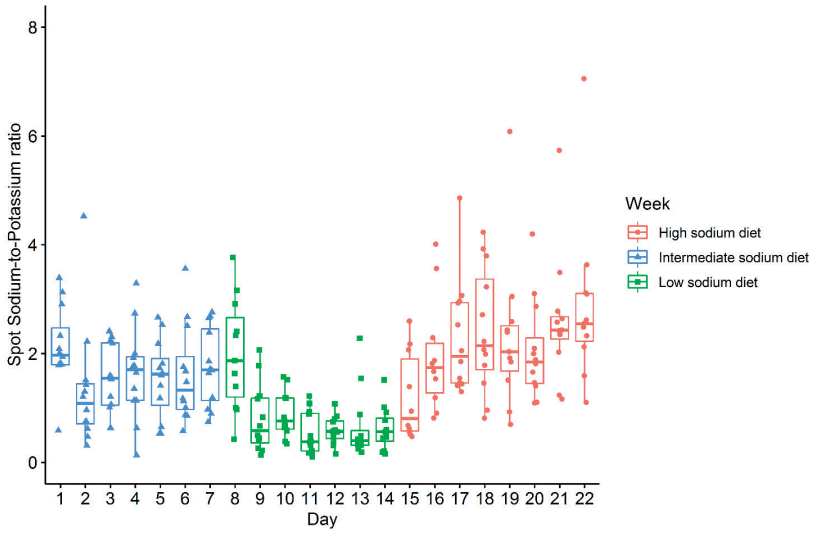
All data in n (%) or median [IQR]

Figure 1. Change in the spot urinary sodium levels over time during three different sodium diets as determined using a monitoring device.



Time-series boxplot of spot sodium levels (in mmol/L) during intermediate sodium diet (3.3-5.0 gram sodium/day), low sodium diet (<3.3 gram sodium/day), and high sodium diet (>5.0 gram sodium/day).

Figure 2. Change in the spot Na/K ratio over time during three different sodium diets as determined using a monitoring device.



Time-series boxplot of spot Na/K ratio during intermediate sodium diet (3.3-5.0 gram sodium/day), low sodium diet (<3.3 gram sodium/day), and high sodium diet (>5.0 gram sodium/day).

The number of successful 24-hour urine collections was 10, 11, and 12 for week 1, week 2, and week 3, respectively. The mean ± standard deviation (SD) of the measured 24-hour sodium excretion was 4011 ± 1369 mg in the intermediate sodium week, 1292 ± 562 mg in the low sodium week, and 5488 ± 1493 mg in the high sodium week (Table 3). Mean measured 24-hour potassium excretion was 3376 ± 987 mg in the intermediate sodium week, 3557 ± 719 mg in the low sodium week, and 3844 ± 1128 mg in the high sodium week (Supplemental Table 3).

Measured versus estimated 24-hour urine salt excretion

The differences between estimated and measured 24-hour urinary sodium excretion during each sodium diet are displayed in Table 3. During the intermediate sodium diet, the INTERSALT formula had the least mean difference between estimated and measured 24-hour sodium excretion (-61 mg/day, 95% confidence interval (95%CI) -1310 – 1188). During the low sodium diet, all formulas consistently overestimated 24-hour sodium excretion with the Tanaka formula having the lowest mean bias (754 mg/day, 95%CI 586-922) and during the high sodium diet, all formulas underestimated the 24-hour sodium excretion with the Kawasaki formula showing the least mean bias (-340 mg/day, 95%CI -1234 – 553). For potassium, both the Kawasaki and Tanaka formula consistently underestimated the 24-hour excretion (Supplemental Table 3).

Table 3. Measured 24-hour urinary sodium excretion compared to estimated 24-hour urinary sodium excretion (mg/day).

	Measured 24h sodium excretion (mg)	**Estimated 24h sodium excretion by Kawasaki[*] (mg)**	**Estimated 24h sodium excretion by Tanaka[$] (mg)**	**Estimated 24h sodium excretion by INTERSALT[$] (mg)**
Week 1 - Intermediate sodium diet (3.3 - 5.0 gram/day) (n=10)	4011 ± 1369	4155 ± 1088	3469 ± 656	3786 ± 1449
Mean difference (95%CI)	-	144 (-431 - 718)	-378 (-1016 - 260)	-61 (-1310 - 1188)
Week 2 - Low sodium diet (<3.3 gram/day) (n=11)	1292 ± 562	2152 ± 547	2144 ± 535	2955 ± 1548
Mean difference (95%CI)	-	860 (463 - 1258)	754 (586 - 922)	1565 (398 - 2731)
Week 3 - High sodium diet (>5.0 gram/day) (n=12)	5488 ± 1493	5148 ± 1247	3890 ± 690	4103 ± 1202
Mean difference (95%CI)	-	-340 (-1234 - 553)	-1415 (-2048 - -782)	-1202 (-2227 - -177)

All data in mean ± standard deviation. [*]Using the first morning urine sample collected on the day after the 24-hour urine collection. [$] Using the average of casual urine spot 2, 3, and 4 collected on the same day as 24-hour urine collection.

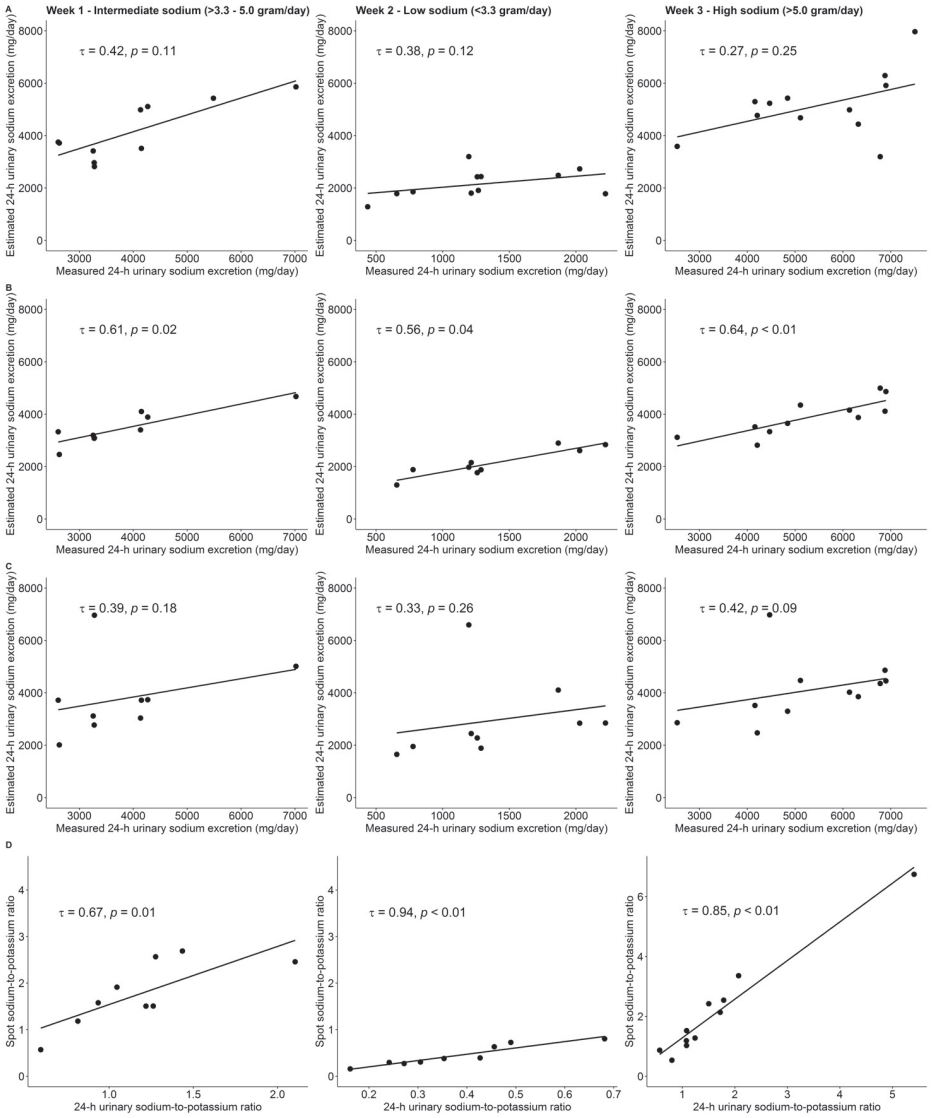
Relationships between the measured and estimated 24-hour urinary salt excretion
The Kendall rank correlation coefficients for the relationships between the measured values and the estimated 24-hour sodium excretion values using the Kawasaki and INTERSALT methods were weak and not significant (Figure 3A and C). The estimated 24-hour sodium excretion by the Tanaka method showed reasonable correlation with the measured 24-hour sodium (τ 0.56-0.64, $p<0.05$) (Figure 3B). For potassium, similar results were obtained for the Kawasaki method. However, the correlation coefficients between measured and estimated 24-hour potassium excretion by the Tanaka formula during the intermediate and high sodium diet were 1.0 ($p<0.05$) and 0.56 ($p<0.05$), respectively (Supplemental Figure 3).

Agreement between the measured and estimated 24-hour urinary salt excretion
The Bland-Altman plots analysis showed that the mean differences between estimated and measured 24-hour sodium excretion for the three formulas had great variations across the different salt diets (Figure 4A-C). For potassium, the Bland-Altman plots showed that both the Kawasaki and Tanaka formula underestimated the 24-hour potassium excretion and that variation in mean differences remained stable over the three diets (Supplemental Figure 4A-B).

Relative and absolute difference between measured and estimated 24-hour urinary salt excretion
The proportions of relative differences in 24-hour sodium excretion within ±10% were 18% for Kawasaki, 21% for Tanaka, and 3% for INTERSALT. The proportions beyond ±40% were 36%, 28%, and 38%, respectively (Figure 5A). The proportions of absolute differences within 500 mg sodium (1.25 gram salt) for the 3 equations were 21%, 21%, and 10%, respectively, and the proportions of absolute differences beyond 2000 mg (5 gram salt) were 6%, 14%, and 34%, respectively (Figure 5B).

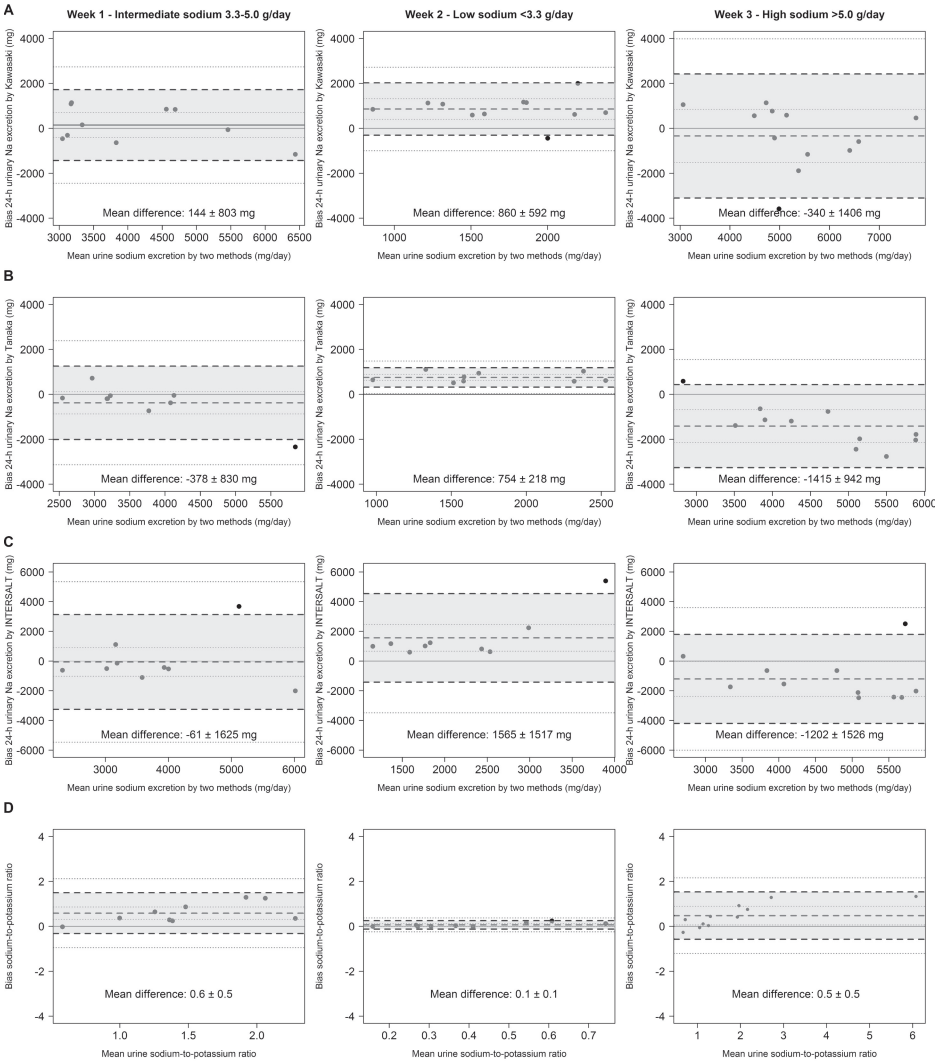
Misclassification analysis
The individual salt intake classification group was also compared according to their measured salt intake compared with their estimated salt intake by the three estimation equations. (Table 4). The results showed that the percentage of individuals that were misclassified when using the Kawasaki method was 27%, and those for the Tanaka and INTERSALT methods were 24% and 30%, respectively. The overall underestimation of potassium by the Kawasaki and Tanaka formula resulted in a misclassification rate of 67% and 64%, respectively (Supplemental Table 4).

Figure 3. Correlation between measured and estimated 24-hour sodium excretion.



Scatter plots with regression lines and Kendall rank correlation coefficients for measured versus estimated 24-hour urinary sodium excretion by the Kawasaki method (**A**), Tanaka method (**B**), and INTERSALT method (**C**), and for the spot Na/K ratio and 24-hour Na/K ratio (**D**).

Figure 4. Agreement between measured and estimated 24-hour urinary sodium excretion.



Bland-Altman plots presenting measured versus estimated 24-hour urinary sodium excretion using the Kawasaki method (**A**), the Tanaka method (**B**), the INTERSALT method (**B**), and spot Na/K ratio versus 24-hour Na/K ratio (**D**). The blue dashed line is the mean difference. The upper and lower limits of agreement (red dashed lines) are the mean difference ± 1.96 × standard deviation

Figure 5. Absolute (A) and relative (B) difference distributions of measured and estimated 24-hour urinary sodium excretions.

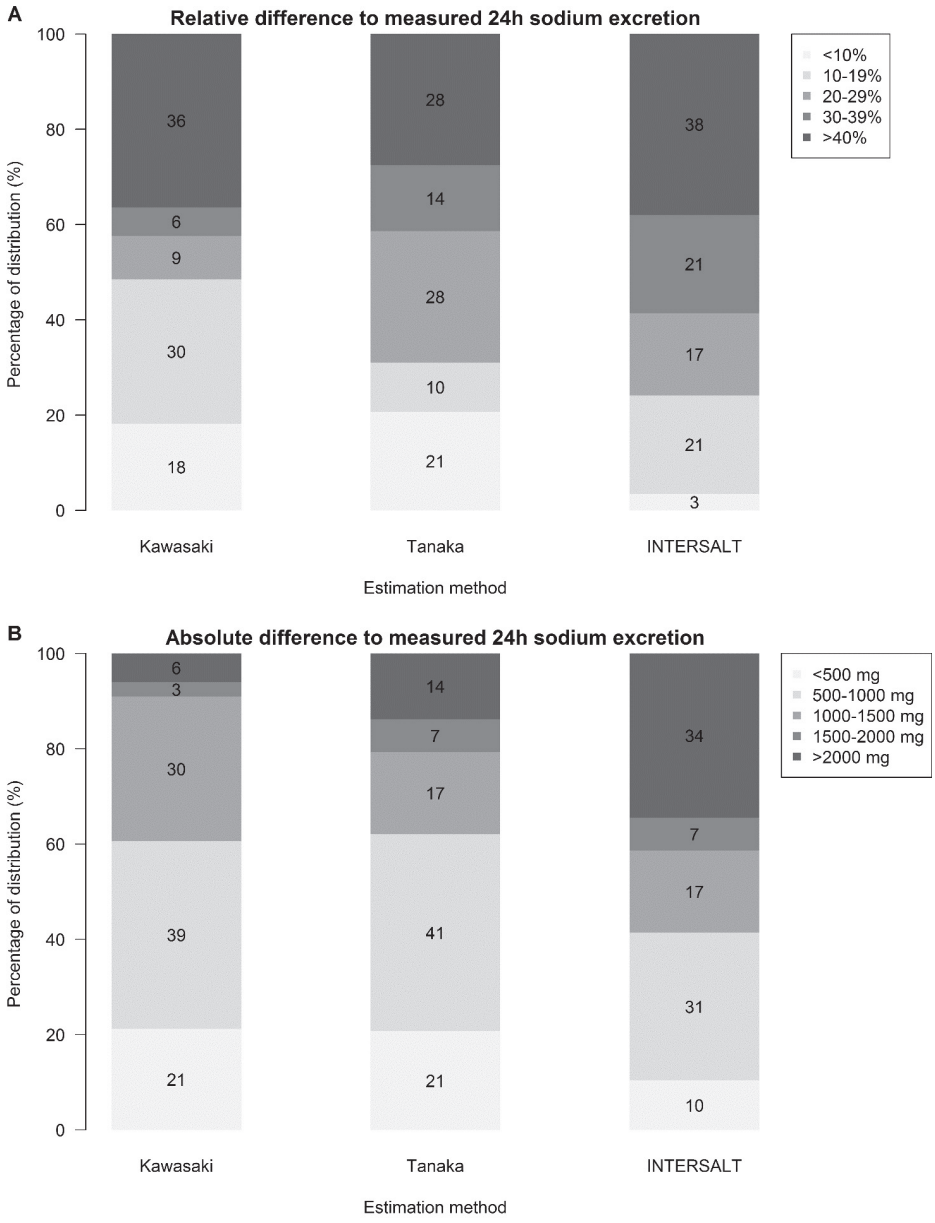


Table 4. Misclassification of the three estimation methods for individual sodium excretion.

	Tertiles measured 24h sodium excretion			
	< 2.3 gram	2.3 - 4.3 gram	> 4.3 gram	**Total**
	n = 11	n = 11	n = 11	n = 33
Kawasaki				
< 2.3 gram	7 (64%)	0 (0%)	0 (0%)	
2.3 - 4.3 gram	4 (36%)	7 (64%)	1 (9%)	
> 4.3 gram	0 (0%)	4 (36%)	10 (91%)	
Misclassification	4 (36%)	4 (36%)	1 (9%)	9 (27%)
Tanaka				
< 2.3 gram	6 (67%)	0 (0%)	0 (0%)	
2.3 - 4.3 gram	3 (33%)	11 (100%)	5 (56%)	
> 4.3 gram	0 (0%)	0 (0%)	4 (44%)	
Misclassification	3 (33%)	0 (0%)	5 (56%)	8 (24%)
INTERSALT				
< 2.3 gram	4 (44%)	1 (9%)	0 (0%)	
2.3 - 4.3 gram	4 (44%)	9 (82%)	3 (33%)	
> 4.3 gram	1 (11%)	1 (9%)	6 (67%)	
Misclassification	5 (89%)	2 (18%)	3 (33%)	10 (30%)

Values are expressed as n (%)

Spot sodium-to-potassium ratio versus 24-hour sodium-to-potassium ratio

The overall median value of Na/K ratio in 24-hour urine was 1.2 (IQR 1.0 – 1.4) during the intermediate sodium diet, 0.4 (0.3 - 0.4) during the low sodium diet, and 1.4 (1.1 – 1.9) during the high sodium diet (Table 5). The average Na/K ratio of 3 casual spot urines taken on the same day as the 24-hour urine collection was higher than 24-hour urine Na/K ratios during the intermediate and high sodium diet, and similar during the low sodium diet (Table 5).

Relationships between spot Na/K ratio and 24-hour Na/K ratio

Correlation analysis on Na/K ratios from spot urine indicated high positive correlations with 24-hour Na/K ratios (Figure 3D). Kendall rank correlation coefficients between the spot Na/K ratio and 24-hour Na/K ratio were 0.67, 0.94, and 0.85 during the intermediate, low, and high sodium diets, respectively (p-values all <0.05).

Table 5. Measured 24-hour sodium-to-potassium ratio compared to spot sodium-to-potassium ratio.

	24h sodium-to-potassium ratio	**Spot sodium-to-potassium ratio[$]**
Week 1 - Intermediate sodium diet (3.3 - 5.0 gram/day) (n=9)	1.2 [1.0 - 1.4]	1.6 [1.5 - 2.5]
Mean difference (95%CI)	-	0.6 (0.2 - 0.9)
Week 2 - Low sodium diet (<3.3 gram/day) (n=9)	0.4 [0.3 - 0.4]	0.4 [0.3 - 0.6]
Mean difference (95%CI)	-	0.1 (0.0 - 0.1)
Week 3 - High sodium diet (>5.0 gram/day) (n=11)	1.4 [1.1 - 1.9]	1.5 [1.1 - 2.5]
Mean difference (95%CI)	-	0.5 (0.1 - 0.8)

All data in median [Interquartile range] or mean (95% confidence interval). [$] Using the average of casual urine spot 2, 3, and 4 collected on the same day as 24-hour urine collection.

Agreement between spot Na/K ratio and 24-hour Na/K ratio

Using the Bland-Altman method, the bias between the 24-hour urine Na/K ratio and the spot urinary Na/K ratio was 0.59 in the intermediate sodium week, 0.06 in the low sodium week, and 0.48 in the high sodium week (Figure 4D).

Misclassification analysis Na/K ratio

Misclassification analysis showed that over all three diets, 66% of the participants were classified into the matching 24-hour Na/K ratio categories with the spot urine Na/K ratio (Table 6).

Table 6. Misclassification sodium-to-potassium ratio.

	Tertiles 24h sodium-to-potassium ratio			
Spot sodium-to-potassium ratio	< 0.6 n = 9	0.6 - 1.25 n = 11	> 1.25 n = 9	**Total** n = 29
< 0.6	6 (67%)	2 (18%)	0 (0%)	
0.6 - 1.25	3 (33%)	4 (36%)	0 (0%)	
> 1.25	0 (0%)	5 (45%)	9 (100%)	
Misclassification	3 (33%)	7 (64%)	0 (0%)	10 (34%)

Values are expressed as n (%)

Discussion

In the present study, in which apparently healthy people were subjected to three different sodium diets, we found that three commonly used equations (Kawasaki, Tanaka, and INTERSALT) performed poorly in estimating 24-hour urinary salt excretion from spot urine using a self-monitoring device. For all three sodium diets, estimated sodium and potassium excretion from each of the three equations showed low correlation with measured values. Moreover, all equations showed wide limits of agreement and relatively high rates of misclassification of salt intake groups. In contrast, the Na/K ratio based on multiple casual spot urines showed high correlation and acceptable agreement with 24-hour Na/K ratio, especially during the low sodium diet.

Collection of at least three non-consecutive 24-hour urine samples is considered the most accurate method for estimating salt intake (10). However, this method is cumbersome and imposes a large burden on the patient. Therefore, a simple and valid method for estimating 24-hour urinary salt excretion from casual spot urine samples would be desirable. However, this study, investigating three simple formulas to estimate salt excretion, found poor individual correlations of estimated and measured 24-hour urinary salt excretion, which is widely supported by previous studies in various populations (25–31). For example, results of previous studies showed correlation coefficients for the relationships between the values estimated using the Kawasaki, Tanaka, and INTERSALT formula of at most 0.54 in European populations (15,31), 0.43 in Chinese populations (27,29,32,33), and 0.18 in a South African population (30).

The Bland-Altman analysis in this study showed that the degree of under- and overestimation of all three formulas depended on the level of salt intake (= differential bias) which is in line with observations in previous studies (34–36). The INTERSALT equation appeared to overestimate at low levels, underestimate at high levels, and estimate correctly at intermediate levels of individual sodium intake. These findings are consistent with results from a study conducted in young American adults (34) but are inconsistent with findings from the PURE (Prospective Urban Rural Epidemiology) China Study which showed that the Kawasaki formula yielded the lowest bias (26). This may be explained by the fact that the Kawasaki formula was developed in a Japanese population, whereas the INTERSALT formula was developed in European and American populations, both with their own dietary patterns and behaviors.

To further analyze the bias of salt excretion estimates at the individual level we calculated relative and absolute differences between estimated and measured 24-hour urinary salt excretion. These analyses showed that the proportion of both relative and absolute differences above a certain amount was quite large for all three equations. More than 40% of the participants showed an absolute difference between measured and estimated 24-hour sodium excretion, regardless of the equation used, of more than 1000 mg/day (= 2.5

gram salt per day). When participants were further classified into tertiles of measured 24-hour sodium excretion, almost 30% of the participants were misclassified. These findings are in line with previous studies (27,32,37,38) and further illustrate the potential for large errors in the estimation of individual 24-hour urinary salt excretion.

Taken together, our findings suggest that the commonly used formula-based approach for estimating 24-hour salt excretion produces a high degree of variability that could lead to misclassification of salt intake at the individual level and thereby to erroneous associations between salt intake and cardiovascular risk (39,40). To support (self-) monitoring and evaluation of salt reduction, more accurate methods for evaluating individual 24-hour urinary salt excretion are needed. As shown in our study, the Na/K ratio based on multiple casual urine samples might be a plausible alternative. We found that the correlation coefficients for the 24-hour Na/K ratio and spot Na/K ratio were 0.67, 0.94, and 0.85 and mean biases were 0.59, 0.06, and 0.48 for the intermediate, low, and high sodium diets, respectively. This relatively good performance of the spot Na/K ratio in comparison with estimated salt excretion has been described previously (16–18,29) and can probably be explained by the ratio being independent of urine volume and creatinine excretion, of which the latter may degrade if samples are not kept at proper temperature (41). This makes estimation of the Na/K ratio from casual urine easier than estimating sodium or potassium excretion alone. Since the Na/K ratio has also been shown to be a superior measure than either sodium or potassium alone in relation to blood pressure, incident hypertension, and CVD (42,43), this appears to be a preferable method for self-monitoring of salt intake that may motivate individuals to reduce their sodium intake, increase their potassium intake, and thereby potentially improve blood pressure control and cardiovascular prognosis. At present, there is no generally accepted recommendation for the desired Na/K ratio. According to WHO reports, achieving the guidelines for both the sodium (<2 g/day) and potassium (>3.5 g/day) intake would result in a Na/K ratio of less than 1.00.

Strengths of this study include the collection of urine samples over a relatively long period covering different sodium diets enabling validation of the three equations and Na/K ratio over different levels of salt excretion. Also, use of repeated casual urine samples for the calculation of the Na/K ratio most likely increased precision of the individual measurement of Na/K ratio and is in line with previous recommendations regarding use of the Na/K ratio for estimating individual salt intake (17,18). Finally, to provide more insight into the accuracy of all methods at the individual level, we not only evaluated the validity of the three equations by analyzing the mean difference and correlation coefficients, but also assessed the distribution of relative and absolute differences, as well as the misclassification of salt intake groups.

A limitation of this study is the study population being limited to relatively young and apparently healthy men. Therefore, it is unknown whether our findings also apply to

individuals with other characteristics such as women, older individuals, and patients with various disease, e.g. chronic kidney disease, atherosclerotic diseases or diabetes. Lastly, we applied the Kawasaki equation, originally developed for use with second morning urine voids, to spot urine samples collected in the morning since the protocol did not require it to be second morning urine voids. Thus, by not specifically using second morning voids, we may have underestimated the performance of the Kawasaki equation.

In conclusion, three commonly used equations that estimate 24-hour urinary salt excretion (as a proxy for dietary intake) with the use of spot urine sodium and potassium measurements showed substantial bias, poor precision, and poor accuracy. Use of these formulas to monitor (changes in) salt intake is therefore not recommended. The Na/K ratio based on multiple casual urine samples may be a useful, low-burden, low-cost alternative method to 24-hour urine collection for monitoring daily salt intake.

References

1. Filippini T, Malavolti M, Whelton PK, Naska A, Orsini N, Vinceti M. Blood Pressure Effects of Sodium Reduction: Dose-Response Meta-Analysis of Experimental Studies. Circulation. 2021 Feb;
2. GBD 2016 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Stu. Lancet (London, England). 2018 Nov;392(10159):1923–94.
3. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. N Engl J Med. 2014 Aug;371(7):624–34.
4. World Health Organization (WHO). Guideline: potassium intake for adults and children. World Health Organization; 2012.
5. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019 Sep;140(11):e596–646.
6. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021 Sep;42(34):3227–337.
7. Drewnowski A, Rehm CD, Maillot M, Mendoza A, Monsivais P. The feasibility of meeting the WHO guidelines for sodium and potassium: a cross-national comparison study. BMJ Open. 2015 Mar;5(3):e006625.
8. Jacobson MF, Havas S, McCarter R. Changes in sodium levels in processed and restaurant foods, 2005 to 2011. JAMA Intern Med. 2013 Jul;173(14):1285–91.
9. Okuda N, Stamler J, Brown IJ, Ueshima H, Miura K, Okayama A, et al. Individual efforts to reduce salt intake in China, Japan, UK, USA: what did people achieve? The INTERMAP Population Study. J Hypertens. 2014 Dec;32(12):2385–92.
10. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich). 2019 Jun;21(6):700–9.
11. Cogswell ME, Maalouf J, Elliott P, Loria CM, Patel S, Bowman BA. Use of Urine Biomarkers to Assess Sodium Intake: Challenges and Opportunities. Annu Rev Nutr. 2015;35:349–87.
12. Kawasaki T, Itoh K, Uezono K, Sasaki H. A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. Clin Exp Pharmacol Physiol. 1993 Jan;20(1):7–14.
13. Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, et al. A simple method to estimate populational 24-h urinary sodium and potassium excretion using a casual urine specimen. J Hum Hypertens. 2002 Feb;16(2):97–103.
14. Brown IJ, Dyer AR, Chan Q, Cogswell ME, Ueshima H, Stamler J, et al. Estimating 24-hour urinary sodium excretion from casual urinary sodium concentrations in Western populations: the INTERSALT study. Am J Epidemiol. 2013 Jun;177(11):1180–92.
15. Polonia J, Lobo MF, Martins L, Pinto F, Nazare J. Estimation of populational 24-h urinary sodium and potassium excretion from spot urine samples: evaluation of four formulas in a large national representative population. J Hypertens. 2017 Mar;35(3):477–86.
16. Iwahori T, Miura K, Ueshima H, Chan Q, Dyer AR, Elliott P, et al. Estimating 24-h urinary sodium/potassium ratio from casual ('spot') urinary sodium/potassium ratio: the INTERSALT Study. Int J Epidemiol. 2017 Oct;46(5):1564–72.

17. Iwahori T, Ueshima H, Torii S, Saito Y, Fujiyoshi A, Ohkubo T, et al. Four to seven random casual urine specimens are sufficient to estimate 24-h urinary sodium/potassium ratio in individuals with high blood pressure. J Hum Hypertens. 2016 May;30(5):328–34.

18. Iwahori T, Ueshima H, Miyagawa N, Ohgami N, Yamashita H, Ohkubo T, et al. Six random specimens of daytime casual urine on different days are sufficient to estimate daily sodium/potassium ratio in comparison to 7-day 24-h urine collections. Hypertens Res. 2014 Aug;37(8):765–71.

19. Sagnella GA, Markandu ND, Singer DR, MacGregor GA. Kinetics of renal sodium excretion during changes in dietary sodium intake in man--an exponential process? Clin Exp Hypertens A. 1990;12(2):171–8.

20. Lucko AM, Doktorchik C, Woodward M, Cogswell M, Neal B, Rabi D, et al. Percentage of ingested sodium excreted in 24-hour urine collections: A systematic review and meta-analysis. J Clin Hypertens (Greenwich). 2018 Sep;20(9):1220–9.

21. Manitius J, Kliz J, Krupa-Wojciechowska B. The effect of dietary sodium loading on the kinetics of sodium excretion and blood pressure regulation in essential hypertensive men. Cor Vasa. 1985;27(1):29–35.

22. Fisic. Medimate Handleiding [Internet]. Fisic BV, Enschede, The Netherlands. 2017 [cited 2021 Nov 17]. p. 53. Available from: https://www.medimate.com/medimate/nl/downloads/Handleiding_Bubblegum_meting_Professional.pdf

23. Staal S, Ungerer M, Floris A, Ten Brinke H-W, Helmhout R, Tellegen M, et al. A versatile electrophoresis-based self-test platform. Electrophoresis. 2015 Mar;36(5):712–21.

24. Forni Ogna V, Ogna A, Vuistiner P, Pruijm M, Ponte B, Ackermann D, et al. New anthropometry-based age- and sex-specific reference values for urinary 24-hour creatinine excretion based on the adult Swiss population. BMC Med. 2015 Feb;13:40.

25. Rhee M-Y, Kim J-H, Shin S-J, Gu N, Nah D-Y, Hong K-S, et al. Estimation of 24-hour urinary sodium excretion using spot urine samples. Nutrients. 2014 Jun;6(6):2360–75.

26. Peng Y, Li W, Wang Y, Chen H, Bo J, Wang X, et al. Validation and Assessment of Three Methods to Estimate 24-h Urinary Sodium Excretion from Spot Urine Samples in Chinese Adults. PLoS One. 2016;11(2):e0149655.

27. Zhou L, Tian Y, Fu J-J, Jiang Y-Y, Bai Y-M, Zhang Z-H, et al. Validation of spot urine in predicting 24-h sodium excretion at the individual level. Am J Clin Nutr. 2017 Jun;105(6):1291–6.

28. Wan ER, Cross J, Sofat R, Walsh SB. 24-Hour vs. Spot Urinary Sodium and Potassium Measurements in Adult Hypertensive Patients: A Cohort Validation Study. Am J Hypertens. 2019 Sep;32(10):983–91.

29. Xu J, Du X, Bai Y, Fang L, Liu M, Ji N, et al. Assessment and validation of spot urine in estimating the 24-h urinary sodium, potassium, and sodium/potassium ratio in Chinese adults. J Hum Hypertens. 2020 Feb;34(2):184–92.

30. Charlton K, Ware LJ, Chidumwa G, Cockeran M, Schutte AE, Naidoo N, et al. Prediction of 24-hour sodium excretion from spot urine samples in South African adults: a comparison of four equations. J Hum Hypertens. 2020 Jan;34(1):24–33.

31. Vidal-Petiot E, Joseph A, Resche-Rigon M, Boutten A, Mullaert J, d'Ortho M-P, et al. External validation and comparison of formulae estimating 24-h sodium intake from a fasting morning urine sample. J Hypertens. 2018 Apr;36(4):785–92.

32. Zhang X, Liao H, Ye R, Li X, Gou Q, Zhang Z, et al. Assessment and validation of three spot urine assay methods for the estimation of 24-hour urinary sodium excretion in Chinese Tibetan adults living in the mountains. J Clin Hypertens (Greenwich). 2021 Aug;23(8):1588–98.

33. Ma W, Yin X, Zhang R, Liu F, Yang D, Fan Y, et al. Validation and Assessment of Three Methods to Estimate 24-h Urinary Sodium Excretion from Spot Urine Samples in High-Risk Elder Patients of Stroke from the Rural Areas of Shaanxi Province. Int J Environ Res Public Health. 2017 Oct;14(10).

34. Cogswell ME, Wang C-Y, Chen T-C, Pfeiffer CM, Elliott P, Gillespie CD, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr. 2013 Dec;98(6):1502–13.

35. Dong J, Yan Y, Fan H, Zhao X, Mi J. Accuracy Validation of 8 Equations to Estimate 24-Hour Sodium by Spot Urine in Young Adolescents. Am J Hypertens. 2019 Feb;32(3):257–64.

36. Mente A, O'Donnell MJ, Dagenais G, Wielgosz A, Lear SA, McQueen MJ, et al. Validation and comparison of three formulae to estimate sodium and potassium excretion from a single morning fasting urine compared to 24-h measures in 11 countries. J Hypertens. 2014 May;32(5):1005–14; discussion 1015.

37. Qian N, Jiang Y, Wang Y, Yan P, Yao F, Sun M, et al. Validity of five formulas in estimating 24-h urinary sodium via spot urine sampling in hypertensive patients living in Northeast China. J Hypertens. 2021 Jul;39(7):1326–32.

38. Dougher CE, Rifkin DE, Anderson CA, Smits G, Persky MS, Block GA, et al. Spot urine sodium measurements do not accurately estimate dietary sodium intake in chronic kidney disease. Am J Clin Nutr. 2016 Aug;104(2):298–305.

39. Cobb LK, Anderson CAM, Elliott P, Hu FB, Liu K, Neaton JD, et al. Methodological issues in cohort studies that relate sodium intake to cardiovascular disease outcomes: a science advisory from the American Heart Association. Circulation. 2014 Mar;129(10):1173–86.

40. He FJ, Campbell NRC, Ma Y, MacGregor GA, Cogswell ME, Cook NR. Errors in estimating usual sodium intake by the Kawasaki formula alter its relationship with mortality: implications for public health. Int J Epidemiol. 2018 Dec;47(6):1784–95.

41. Spierto FW, Hannon WH, Gunter EW, Smith SJ. Stability of urine creatinine. Clin Chim Acta. 1997 Aug;264(2):227–32.

42. Binia A, Jaeger J, Hu Y, Singh A, Zimmermann D. Daily potassium intake and sodium-to-potassium ratio in the reduction of blood pressure: a meta-analysis of randomized controlled trials. J Hypertens. 2015 Aug;33(8):1509–20.

43. Iwahori T, Miura K, Ueshima H. Time to Consider Use of the Sodium-to-Potassium Ratio for Practical Sodium Reduction and Potassium Increase. Nutrients. 2017 Jul;9(7).

Supplementary Table 1. Validation results Medimate self-monitoring device.

	Sodium	Potassium	Creatinine
Total error (mg/L)			
- Internal standard added with pipette	345 mg/L - 20%	780 mg/L	282.8 mg/L
- Internal standard added with cup	~ 414 mg/L - 23%	~ 897 mg/L	~ 339.3 mg/L
Correlation Medimate and Cobas 8000 Modular laboratory system			
- Regression line (mmol/L)	1.050x – 3.153	0.941x + 3.490	0.965x + 0.398
- R^2	0.96	0.87	0.97
Precision (2 x SD or 2 x CV) for levels:			
- Low	488 mg/L: 29.9 mg/L or 6%	690 mg/L: 72.5 mg/L or 11%	165 mg/L: 40 mg/L or 25%
- Intermediate	2284 mg/L: 202 mg/L or 4%	2621 mg/L: 210 mg/L or 8%	1003 mg/L: 78 mg/L or 8%
- High	4876 mg/L: 144 mg/L or 6%	5382 mg/L: 373 mg/L or 7%	2262 mg/L: 93 mg/L or 4%
Linearity (mg/L)	644 – 9108	858 - 7800	565.5 – 3393
Limit of detection (mg/L) / CV%	230 / 22%	234 / 50%	113.1 / 15%
Limit of quantification (mg/L) / CV%	230 / 22%	234 / 50%	113.1 / 15%

SD = Standard deviation; CV = Coefficient of Variation. To convert sodium from mg/L to mmol/L, divide by 23. To convert potassium from mg/L, divide by 39. To convert creatinine from mg/L to mmol/L divide by 113.1.
The reference data were measured on the AU5800 Clinical Chemistry System from Beckman-Coulter of the University Medical Center of Utrecht, the Netherlands and Cobas 8000/Modular type ISE unit/ISE900 of Medlon, Enschede, the Netherlands. Performance versus other laboratories or systems may differ.

Supplementary Table 2. Equations to estimate 24-hour salt excretion from spot urine samples.

	Method	Equation for estimating predicted 24-hour urine sodium or potassium excretion	Equation for estimating predicted 24-hour urine creatinine excretion (Pr24UCr mg/day)
Sodium (mg/day)	Kawasaki	23 × (16.3 × $XNa^{0.5}$), where XNA = [spot Na (mmol/ L)/spot creatinine (mg/dL) × 10] × Pr24UCr (mg/day)	Pr24UCr (mg/day) for men = (12.63 × age (year)) + (15.12 × weight (kg)) + (7.39 × height (cm))− 79.9 Pr24UCr (mg/day) for women = (− 4.72 × age (year)) + (8.58 × weight (kg)) + (5.09 × height (cm))− 74.5
	Tanaka	23 × 21.98 × (spot Na (mmol/ L)/ spot creatinine (mg/dL) × 1/10 × $Pr24UCr)^{0.392}$	Pr24UCr = 16.14 × height (cm) + 14.89 × weight (kg) − 2.04 × age (year) − 2244.45
	INTERSALT	Male = 23 × 4.10 × BMI (kg/m^2) + (0.46 × spot Na (mmol/L)+ 25.46) − 2.75 × spot creatinine (mg/dL) − 0.13 × spot K (mmol/L) + 0.26 × age (year) Female = 23 × 2.39 × BMI (kg/m^2) + (0.34 × spot Na (mmol/L) + 5.07) − 2.16 × spot creatinine (mg/dL) − 0.09 × spot K (mmol/L) + 2.35 × age (year) − 0.03 × $age (year)^2$	NA
Potassium (mg/day)	Kawasaki	39 × (7.2 × $XK^{0.5}$), where XK = [spot K (mmol/L)/spot creatinine (mg/dL) × 10] × Pr24UCr (mg/day)	Pr24UCr (mg/day) for men = (12.63 × age (year)) + (15.12 × weight (kg)) + (7.39 × height (cm))− 79.9 Pr24UCr (mg/day) for women = (− 4.72 × age (year)) + (8.58 × weight (kg)) + (5.09 × height (cm))− 74.5
	Tanaka	39 × 7.59 × (K (mmol/L)/spot creatinine (mg/dL) × 1/10 × $Pr24UCr)^{0.431}$	Pr24UCr (mg/day) = 16.14 × height (cm) + 14.89 × weight (kg) − 2.04 × age (year) − 2244.45

NA = not applicable

Supplementary Table 3. Comparison between the measured 24-hour urinary potassium excretion and the estimated 24-hour urinary potassium excretion (mg/day).

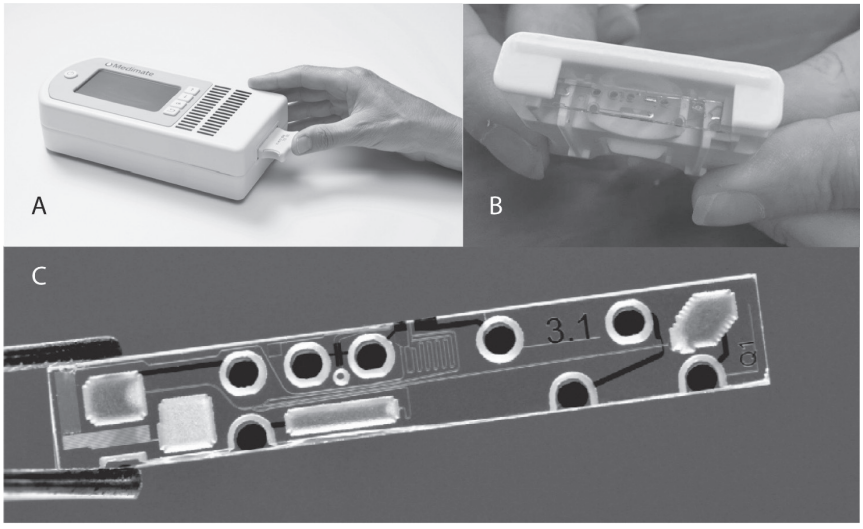
	Measured 24h potassium excretion (mg)	**Estimated 24h potassium excretion by Kawasaki (mg)***	**Estimated 24h potassium excretion by Tanaka (mg)$**
Week 1 - Intermediate sodium diet (3.3 - 5.0 gram/day) (n=10)	3376 ± 987	2154 ± 292	1976 ± 228
Mean difference (95%CI)	-	-1221 (-1923 - -520)	-1464 (-2084 - -843)
Week 2 - Low sodium diet (<3.3 gram/day) (n=11)	3557 ± 719	2181 ± 305	2112 ± 167
Mean difference (95%CI)	-	-1376 (-1831 - -920)	-1650 (-2126 - -1175)
Week 3 - High sodium diet (>5.0 gram/day) (n=12)	3844 ± 1128	2191 ± 426	2224 ± 369
Mean difference (95%CI)	-	-1654 (-2213 - -1095)	-1671 (-2251 - -1091)

All data in mean ± standard deviation. *Using the first morning urine sample collected on the day after the 24-hour urine collection. $ Using the average of casual urine spot 2, 3, and 4 collected on the same day as 24-hour urine collection.

Supplementary Table 4. Misclassification of the three estimation methods for individual potassium excretion.

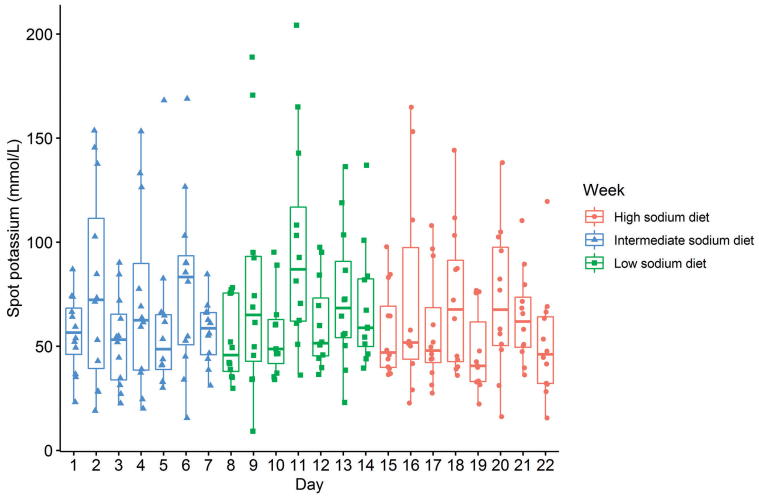
	Tertiles measured 24h potassium excretion			
	< 3.3 gram	3.3 - 4.1 gram	> 4.1 gram	**Total**
	n = 11	n = 11	n = 11	n = 33
Kawasaki				
< 3.3 gram	11 (100%)	11 (100%)	11 (100%)	
3.3 - 4.1 gram	0 (0%)	0 (0%)	0 (0%)	
> 4.1 gram	0 (0%)	0 (0%)	0 (0%)	
Misclassification	0 (0%)	11 (100%)	11 (100%)	22 (67%)
Tanaka				
< 3.3 gram	8 (100%)	10 (100%)	11 (100%)	
3.3 - 4.1 gram	0 (0%)	0 (0%)	0 (0%)	
> 4.1 gram	0 (0%)	0 (0%)	0 (0%)	
Misclassification	0 (0%)	10 (100%)	11 (100%)	21 (64%)

Supplementary Figure 1. Medimate device.



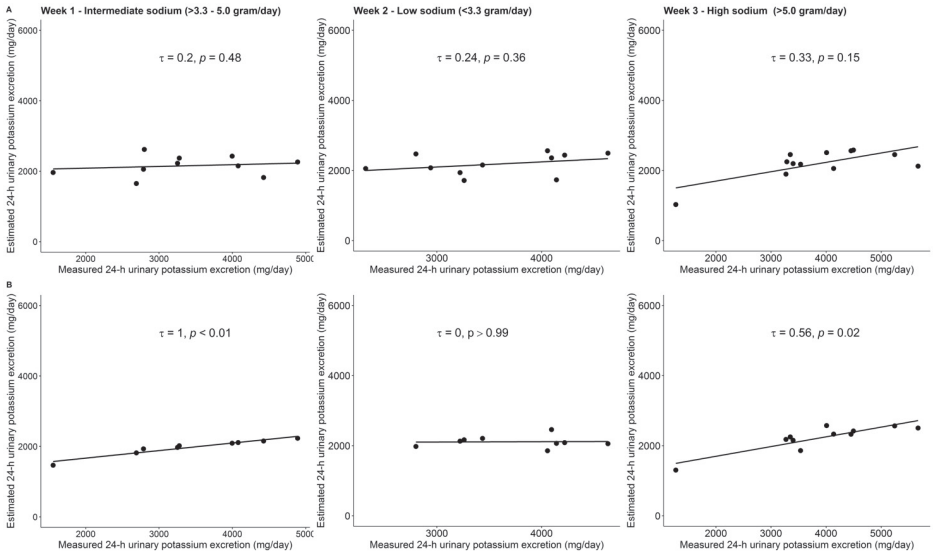
A.The Medimate Multireader device with adapter, the Medimate Lab-Chip cartridge (which include the Lab-on-a-Chip (not visible) and two beakers, with lit the beaker with Internal Standard and without lit the Urine collection beaker. **B.** 'The Medimate Lab-Chip', a smaller disposable cartridge which contains the glass Lab-on-a-Chip. **C.** Glass Lab-on-a-Chip (LoC). The LoC facilitates microchip capillary electrophoresis in combination with conductivity detection

Supplementary Figure 2. Change in the spot urinary potassium levels (mmol/L) over time during three different sodium diets as determined using a monitoring device.



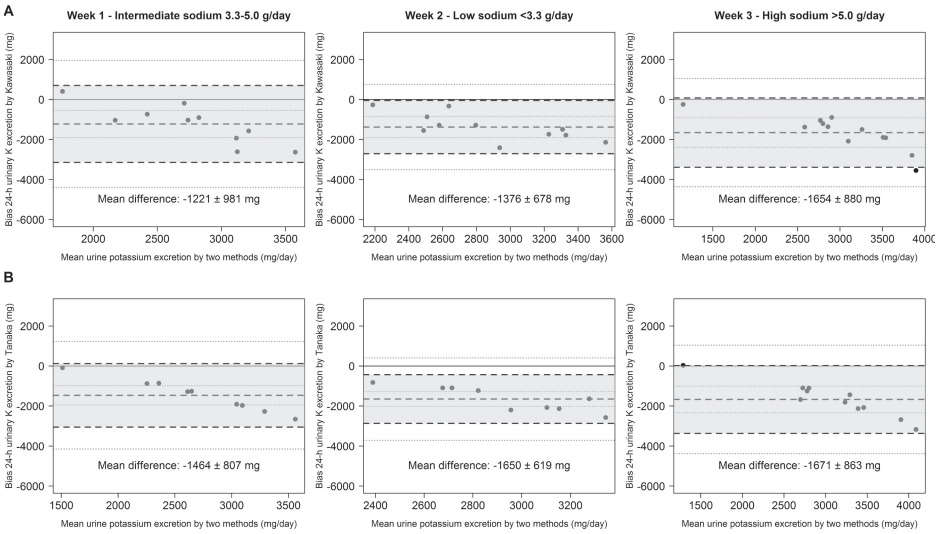
Time-series boxplot of spot potassium levels (in mmol/L) during intermediate sodium diet (3.3-5.0 gram sodium/day), low sodium diet (<3.3 gram sodium/day), and high sodium diet (>5.0 gram sodium/day).

Supplementary Figure 3. Correlation between measured and estimated 24-hour potassium excretion.



Scatter plots with regression lines and Kendall rank correlation coefficients for measured versus estimated 24-hour urinary potassium excretion by the Kawasaki method (**A**) and Tanaka method (**B**)

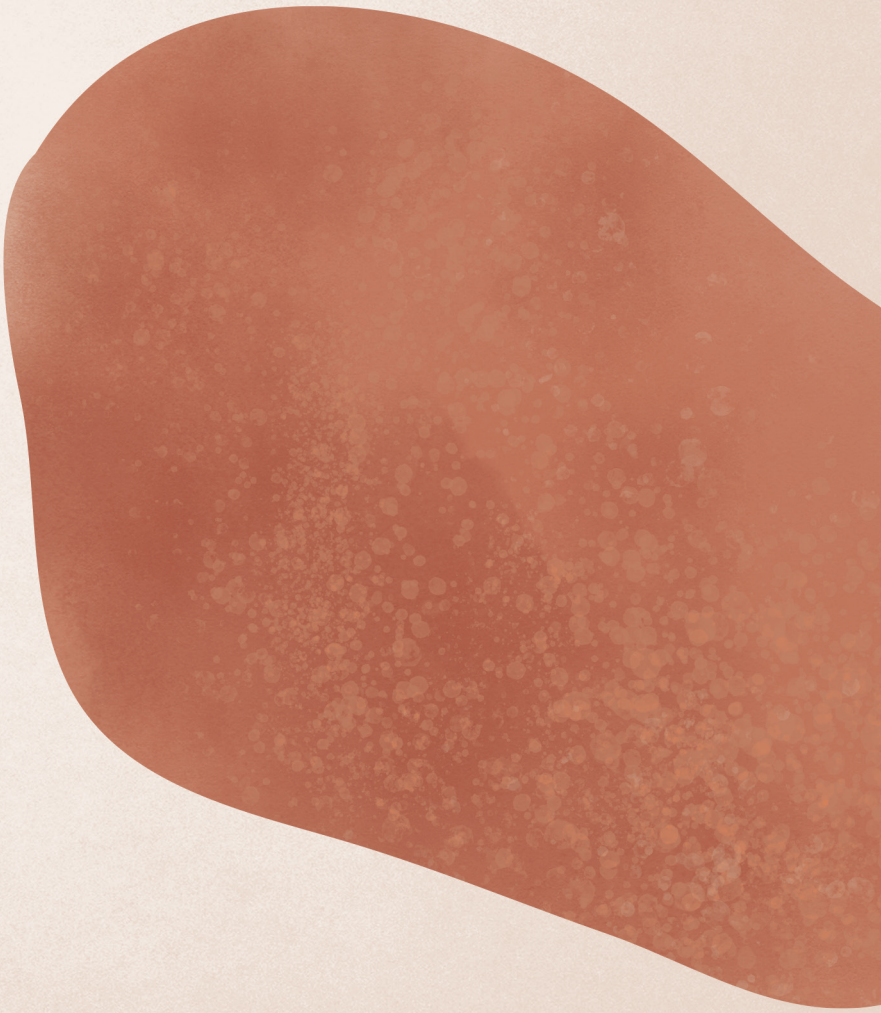
Supplementary Figure 4. Agreement between measured and estimated 24-hour urinary potassium excretion.



Bland-Altman plots presenting measured versus estimated 24-hour urinary potassium excretion using the Kawasaki method (**A**) and the Tanaka method (**B**). The blue dashed line is the mean difference. The upper and lower limits of agreement (red dashed lines) are the mean difference ± 1.96 × standard deviation

PART III

Medication adherence in patients with uncontrolled hypertension



CHAPTER 8

Plasma trough concentrations of antihypertensive drugs for the assessment of treatment adherence: a meta-analysis

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Abstract

Background: Biochemical drug screening by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in plasma is an accurate method for the quantification of plasma concentrations of antihypertensive medications in patients with hypertension. Trough concentrations could possibly be used as drug-specific cut-off values in the biochemical assessment of (non-)adherence.

Methods: We performed a literature review and meta-analysis of pharmacokinetic studies to determine plasma trough concentrations of amlodipine, hydrochlorothiazide and valsartan. PubMed was searched for pharmacokinetic studies up to September 2020. Eligible studies reported steady-state mean trough concentrations and their variance. Pooled trough concentrations were estimated using a three-level random effects meta-analytic model. Moderator analyses were performed to explore sources of heterogeneity.

Results: One thousand three hundred eighteen potentially relevant articles were identified of which 45 were eligible for inclusion. The pooled mean trough concentration was 9.2 ng/mL (95%CI: 7.5–10.80) for amlodipine, 41.0 ng/mL (95%CI 17.4–64.7) for hydrochlorothiazide, and 352.9 ng/mL (95%CI 243.5–462.3) for valsartan. Substantial heterogeneity was present for all three pooled estimates. Moderator analyses identified dosage as a significant moderator for the pooled trough concentration of amlodipine (β_1=0.9, p<0.05), mean age and mean body weight for the mean trough concentration of hydrochlorothiazide (β_1=2.2, p<0.001 respectively β_1=-4.0, p=0.04) and no significant moderators for valsartan.

Conclusion: Plasma trough concentrations of amlodipine, hydrochlorothiazide, and valsartan, measured with LC-MS/MS, are highly heterogeneous over the different studies. Use of the pooled trough concentration as a cut-off in the biochemical assessment of adherence can result in inaccurate diagnosis of (non-)adherence, which may seriously harm the patient-physician relationship, and is therefore not recommended.

Introduction

Blood pressure (BP) control is generally low in hypertensive populations with prevalence rates ranging from 10 to 44% (1,2). Medication nonadherence is a known behavioral contributor to poor BP control and is associated with an increased risk of cardiovascular disease (CVD), hospitalization, and increased health care costs (3,4). Moreover, nonadherent uncontrolled patients are at greater risk of being exposed to unnecessary and costly diagnostic tests for assessment of secondary causes of hypertension and invasive device-based therapies (5). Identification of nonadherence to antihypertensive drug treatment is therefore of major importance. Biochemical drug screening in plasma or urine by liquid chromatography tandem mass spectrometry (LC-MS/MS) is an objective method for medication adherence assessment (6). This method allows simultaneous and sensitive detection of different antihypertensive drugs and their metabolites and creates the opportunity to link medication exposure to BP when blood sampling is accompanied by BP measurement (7).

Biochemical drug screening is most often performed qualitatively with the goal of detecting the presence or absence of antihypertensive drugs or metabolites based on the limit of detection (LOD), the lowest amount of a drug in a sample that can be detected. Also used is lower limit of quantitation (LLOQ), the lowest amount of a drug in a sample which can be quantitatively determined with a certain accuracy, precision, and reproducibility (8). Patients will be classified as adherent to treatment when the drug or a metabolite is present at a concentration of at least its LLOQ or LOD and conversely is classified as nonadherent to treatment when the concentration of the drug or metabolite is less than its LLOQ or LOD. In general, approaches based on the LLOQ or the LOD are qualitative screening methods that can only detect complete nonadherence at one point in time. More erratic or irregular adherence behavior may not be detected. Moreover, qualitative LC-MS/MS is not able to identify white-coat adherence, defined as an increase in adherence to treatment regimens before a clinical appointment. Finally, the LLOQ and LOD highly depend on the sensitivity of the analytical assay and not on the therapeutic range of the drug (9). Ongoing improvements of the analytical assay, resulting in lower detection limits, will therefore increase the risk of misclassification of partially nonadherent patients.

The biochemical assessment of adherence may be improved by quantitative analysis, evaluating measured drug concentrations. A possible way to perform quantitative biochemical drug screening is to compare the measured plasma drug concentration (C_x) with the trough concentration (C_{min}), the minimum plasma concentration at steady state, assuming that adherent patients will at least have a plasma drug concentration above this limit. To implement this method in clinical practice, a reliable trough concentration per antihypertensive drug should be identified.

Therefore, the aim of this study was to perform a literature review and meta-analysis of pharmacokinetic studies to determine plasma population trough concentrations of three frequently prescribed antihypertensive drugs with different pharmacokinetic properties and from different antihypertensive drug classes; amlodipine, hydrochlorothiazide and valsartan.

Methods

Literature search

We conducted a literature search via PubMed (including articles up to September 1st, 2020) for studies describing the pharmacokinetics of amlodipine, hydrochlorothiazide, and valsartan. These antihypertensive drugs were selected because they belong to the most widely used classes of antihypertensive drugs, are the preferred three-drug class combination if BP is not controlled by a two-drug single-pill combination according to the 2018 ESC/ESH Guidelines (10), and possess different pharmacokinetic properties (e.g. bioavailability, $T_{max,}$ volume of distribution, and elimination). PubMed was searched for each drug separately with terms for the generic drug name and pharmacokinetics (Supplemental Table 1). All articles were screened for relevant title/abstracts using predefined in- and exclusion criteria. After title and abstract screening, full texts of the remaining articles were independently screened by two authors (E.H. Groenland and M.E.A.M van Kleef). In addition, reference lists of all eligible articles were hand-searched for additional eligible studies.

Inclusion and exclusion criteria

We included prospective cohort and pharmacokinetic intervention studies that reported the steady state plasma trough concentration and their variance (standard deviation (SD) or standard error (SE)) in healthy subjects or patients with hypertension. We excluded single-dose studies because the trough concentrations in these studies are unlikely to match with the trough concentrations in patients chronically treated with antihypertensive drugs, since steady-state concentration is not reached after a single dose. Moreover, we excluded studies that did not provide a measure of variability (or data to calculate the variability) for the mean trough concentration. Because of limited sensitivity of analytical methods other than liquid or gas chromatography, we excluded studies that applied such methods. Lastly, case reports, case series, narrative reviews, and articles in languages other than English, German or Dutch were also excluded.

Data extraction

Data extraction was carried out by one researcher (E.H. Groenland) and verified by another (M.E.A.M van Kleef). Extracted information included general study information such as journal, author, year of publication, study region, study design, and number of patients enrolled. Furthermore, we extracted information about drug dose, dosing frequency, treatment period, and analytical method. When available, age, sex, and body weight were extracted. Specifically, we extracted data on the plasma trough concentration of each drug (mean, SD, or SE). In case measures were only available in graphical format, the software Digitizelt version 2.3.2 (Digitizelt, Braunschweig, Germany) (11) was used to extract the data. Discrepancies observed between the extracted data were resolved through discussion, and when discrepancies could not be resolved, a third reviewer K.C.M. van der Elst was consulted.

Data analyses

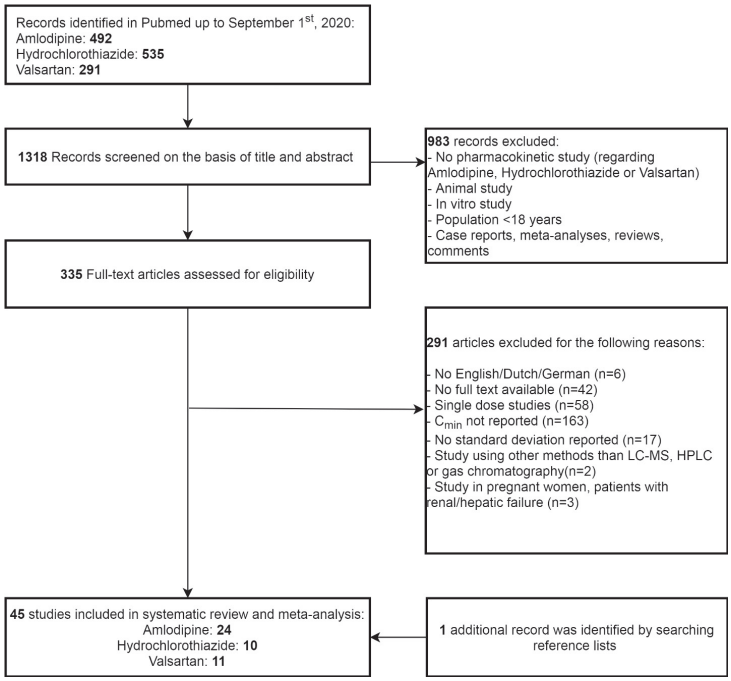
To provide an overall estimate of the mean trough concentration per antihypertensive drug, mean trough concentrations from individual studies were pooled. Since most of the studies provided multiple mean trough concentrations (ie, with and without co-medication, multiple dosages) we applied a three-level random effects meta-analytic model, taking dependency between the mean trough concentrations into account (12). Moreover, moderator analyses were performed to explore sources of heterogeneity (13). A detailed description of the used methodology is provided in the Supplemental File 1. Results were graphically presented in forest plots. All analyses were performed using the statistical software package R version 3.5.1. and the metafor package (14). For all analyses, a p-value of <0.05 was considered statistically significant.

Results

Literature search and review process

Figure 1 shows the flow diagram for the study inclusion. The search generated a total of 1318 potentially relevant studies; 492 for amlodipine, 535 for hydrochlorothiazide and 291 for valsartan. On the basis of the title and abstracts, we identified 335 possibly relevant articles. After full text screening, 44 studies met the eligibility criteria. Additionally, one study was identified from the reference lists (15). This additional study was not indexed with a term related to pharmacokinetics and therefore not included in our search results.

Figure 1. Flowchart showing the process of study selection.



LC-MS = Liquid Chromatography Mass Spectrometry, HPLC = High-Performance Liquid Chromatography

Description of the included studies

Supplemental Table 2 reports the characteristics and key findings from the included studies. Most studies were open-label trials set up to evaluate the pharmacokinetics of the antihypertensive drug alone or in relation to other drugs. Males were overrepresented in most study populations; twenty-two studies consisted of at least 80% males, with 13 studies containing exclusively men. Of the 45 included studies, 37 studies evaluated the interaction of the antihypertensive drug with other, mostly cardiovascular, medication.

Thirty-eight studies reported multiple mean trough concentration obtained from measurements in different populations, measurements after different drug dosages, or measurements after combination with other drugs. Therefore, ninety-three mean trough concentrations were included in the meta-analysis.

Amlodipine

Data on the trough concentration and variance of amlodipine was available in 24 studies (15–38). These 24 studies reported a total of 49 trough concentrations. The pooled mean trough concentration for amlodipine was 9.2 ng/mL (95% CI 7.5 – 10.80) (Figure 2A). We found significant variability of trough concentrations within studies (at level 2) (Likelihood Ratio Test (LRT) 476.4, $p < 0.05$) as well as between studies (at level 3) (LRT 29.3, $p < 0.05$). Of the total variance, 14% and 85% were distributed at levels 2 and 3, respectively, and 0.8% was the percentage of sampling variance that was calculated using the formula of Cheung (12). Moderator analyses showed a significant moderating effect for dose. The mean trough concentration increased as studies applied a higher dose (β_1=0.9; per mg increase in dose the mean trough concentration increased by 0.9 ng/mL, $p<0.05$) (Table 1).

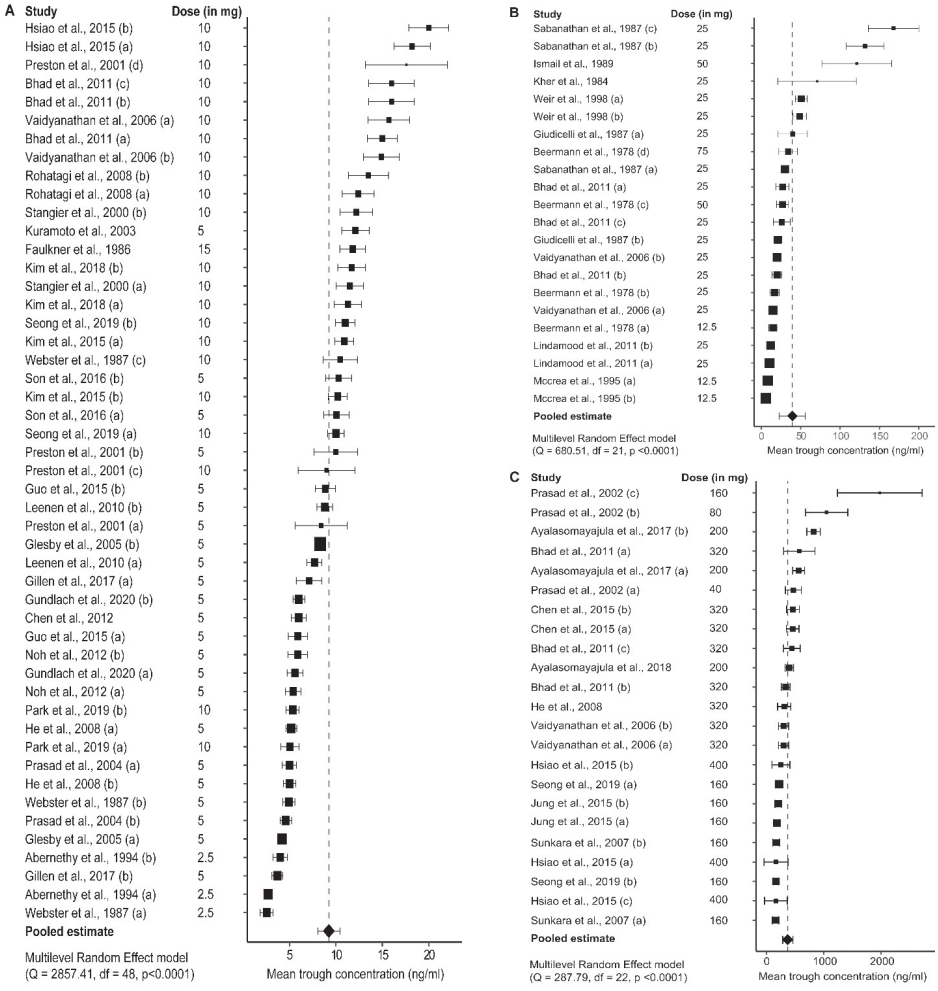
Hydrochlorothiazide

Data on the trough concentration and variance of hydrochlorothiazide was available in 10 studies (27,31,39–46). These 10 studies reported a total of 22 trough concentrations. The pooled mean trough concentration for hydrochlorothiazide was 41.0 ng/mL (95% CI 17.4 – 64.7) (Figure 2B). We found significant variability of trough concentrations within studies (at level 2) (LRT 98.7, $p < 0.0001$) as well as between studies (at level 3) (LRT 4.43, $p=0.04$). Of the total variance, 45% and 55% were distributed at levels 2 and 3, respectively, and 0.2% was the percentage of sampling variance that was calculated using the formula of Cheung (47). Moderator analyses showed a significant moderating effect for mean age and mean body weight. The pooled mean trough concentration increased as the mean age increased (β_1=2.2, $p<0.05$) and decreased as the mean body weight increased (β_1=-4.0, $p=0.04$). A multiple-moderators model, including these two covariates, showed that only mean age had a unique moderating effect on the mean trough concentration (β_1=2.1, $p<0.05$) (Table 1).

Valsartan

Data on the trough concentration of valsartan was available in 11 studies (17,27,28,31,37,38,48–52). These 11 studies reported a total of 23 trough concentrations. The pooled mean trough concentration for valsartan was 352.9 ng/mL (95% CI 243.5 – 462.3) (Figure 2C). The variability within studies (at level 2) was not significant (LRT 2.7, $p=0.1$). However, we did find significant variability of trough concentrations between studies (at level 3; LRT 12.8, $p=0.05$). Of the total variance, 7% and 88% were distributed at levels 2 and 3, respectively, and 5% was the percentage of sampling variance that was calculated using the formula of Cheung (47). Moderator analyses showed no significant moderating effect for the preselected study characteristics (Table 1).

Figure 2. Forest plots trough concentrations.



Forest plots of the trough concentration of amlodipine (**A**), hydrochlorothiazide (**B**), and valsartan (**C**) ordered by the height of the mean trough concentration. The diamonds indicate the pooled estimate for the mean trough concentration from the meta-analysis, based on the multilevel random-effect model. a-d indicate different trough concentrations derived from the same study, see Supplemental Table 1 for further specification.

Table 1. Results of moderator analyses.

	Moderator variables	N studies	NES	β_0, Mean [95% CI]	β_1 [95% CI]	F(df1, df2)[a]	p[b]
Amlodipine	Overall	24	49	9.15 [7.47 - 10.83]			
	Mean age (years)	20	40	8.06 [4.48 - 11.64]	0.05 [-0.04-0.14]	1.49 (1,38)	0.14
	Mean body weight (kg)	19	35	9.17 [-7.25 - 25.60]	0.003 [-0.23-0.23]	0.001 (1,33)	0.89
	Sex (% males)	21	42	10.62 [7.58-13.65]	-0.019 [-0.051-0.013]	1.48 (1,40)	0.23
	Dose (mg)	24	49	2.40 [-0.08 – 4.88]	0.92 [0.63 - 1.22]	39.07 (1,45)	<0.001
Hydrochlorothiazide	Overall	10	22	41.04 [17.37 - 64.71]			
	Mean age (years)	7	18	-64.29 [-101.18 - 27.39]	2.23 [1.73-2.74]	87.51 (1,16)	<0.001
	Mean body weight (kg)	6	14	334.39 [72.97 - 595.82]	-4.04 [-7.76 - -0.32]	5.61 (1,12)	0.036
	Sex (% males)	7	17	23.98 [-3.98-51.94]	-0.03 [-0.39-0.33]	0.03 (1,15)	0.86
	Dose (mg)	10	22	25.86 [-11.65-63.37]	0.56 [-0.53-1.64]	1.13 (1,20)	0.3
Valsartan	Overall	11	23	352.87 [243.46 - 462.29]			
	Mean age (years)	9	20	141.05 [-314.74-596.83]	6.68 [-5.36 - 18.72]	1.36 (1,18)	0.26
	Mean body weight (kg)	7	15	-914.35 [-2279.67 - 450.97]	16.16 [-1.63-33.95]	3.85 (1,13)	0.071
	Sex (% males)	10	22	519.36 [81.93 - 956.80]	-2.01 [-7.21-3.19]	0.65 (1,20)	0.43
	Dose (mg)	11	23	317.20 [15.03-619.37]	0.15 [-1.02-1.33]	0.07 (1,21)	0.79

NStudies = number of studies; NES = number of effect sizes(trough concentrations); β_0 (intercept) Mean = Mean trough concentration (ng/mL); β_1 = Regression coefficient; Per unit increase in the variable, the mean trough concentration increases by β_1; F(df1, df2) = omnibus test; [a]Omnibus test of all regression coefficients in the model. p[b] value of omnibus test.

Discussion

The present study was designed to formulate a pooled trough concentration for amlodipine, hydrochlorothiazide and valsartan; three frequently prescribed antihypertensive drugs from different classes with different pharmacokinetic properties, with the aim to use these values in the quantitative biochemical assessment of medication adherence in patients with uncontrolled hypertension. Our meta-analysis resulted in a pooled trough concentration of 9.3 ng/mL (95%CI 7.6 – 11.0) for amlodipine, 41.0 ng/mL (95% CI 17.4 – 64.7) for hydrochlorothiazide and 352.9 ng/mL (95% CI 243.5 – 462.3) for valsartan. However, substantial heterogeneity within- and between studies was present, which could only partly be explained by differences in dose in case of amlodipine and differences in mean age for hydrochlorothiazide.

The substantial heterogeneity within- and between studies in the present meta-analysis indicates large between-individual variability in trough concentrations. This substantial variability in pharmacokinetic parameters of antihypertensive drugs corresponds to results from previous studies investigating the variability in plasma concentrations of BP-lowering drugs (53,54). The large variability in trough concentrations is most likely explained by differences in drug-, dose-, and patient characteristics, including adherence behavior. In this meta-analysis, univariate moderator analysis revealed drug dosage as a significant moderator for the pooled mean plasma trough concentration of amlodipine with a value of 0.92 ng/mL per mg increase in dosage. This observation is in line with a previous study investigating the influence of dosage on the plasma concentration of amlodipine (54). Although not expected, dosage was not a significant moderator on the pooled mean trough concentrations of hydrochlorothiazide and valsartan. One of the reasons for this could be the limited amount of studies investigating different dosages for these antihypertensive drugs. The pooled mean trough concentration of hydrochlorothiazide significantly decreased with increasing mean body weight (β_1=-4.0, p=0.04) which is probably because of a higher volume of distribution in patients with increased body weight. Furthermore, the pooled mean trough concentration of hydrochlorothiazide increased with increase in mean age (β_1=2.2, p<0.05). This was in accordance with earlier findings that showed a reduced renal clearance of hydrochlorothiazide with increasing age, resulting in higher plasma concentrations (55). The lack of an effect of age on the trough concentration of amlodipine and valsartan in the current study is probably because of a limited number of studies including older people. Certainly, there are many other factors that can influence the mean plasma concentrations of these three antihypertensive drugs (eg. renal- and hepatic function, interacting co-medications and the degree of adherence). However, because of the limited availability of these data in the included studies, we were not able to evaluate the influence of these factors.

The large variation in plasma trough concentrations, as demonstrated in the present study, discourages the use of the pooled trough concentration as a reliable cut-off in the biochemical assessment of adherence. Few alternatives for quantitative drug screening have previously been proposed (56,57). In 2018, the concept of indexed plasma drug concentrations for drug adherence screening in hypertensive patients was proposed (56). This concept involves comparison of the measured plasma drug concentrations (C_x) of antihypertensive drugs with the expected C_{max} (C_x/C_{max}) for each drug and dose using published reference values. When these indexed plasma concentrations are used, different drugs and doses can be compared on the same relative scale. Moreover, plasma half-lives of the tested drugs, timing of the drug intake, and timing of blood sampling may be used to define a particular C_x/C_{max} value as a common threshold for same-day drug use. However, the choice of an appropriate C_{max} from published data is nevertheless a crucial prerequisite for the application of this method. Just like the retrieved trough concentrations in our meta-analysis, the C_{max} is also highly variable and should therefore not be used as a reliable threshold.

Use of published therapeutic reference ranges as a cut-off for adherence is further discouraged by findings from a recent German study that reported serum concentrations of antihypertensive drugs below the literature-based reference ranges despite supervised intake of these drugs (57). To overcome this limitation, a novel method which is based on the dose-related concentration (DRC) was introduced. This method compares the measured concentration of an antihypertensive drug with trough drug concentrations calculated individually for each patient. Although the cut-off values in that study were also based on parameters from pharmacokinetic studies conducted in selected study populations which do not entirely reflect the variability in the population, it was shown that all patients attending the nephrology ward had measured drug concentrations above the lower limit of the dose-related concentration, after supervised antihypertensive drug intake. Therefore, their approach might be a promising method for future quantitative drug screening.

Strengths of our meta-analysis include the application of a three-level random effects model for the meta-analysis of the trough concentration from the individual studies. By using this model, we were able to pool multiple trough concentrations derived from the same study since this model takes dependency between mean trough concentrations into account. An important advantage of the three-level approach is that all the relevant information produced in primary studies can be preserved and maximum statistical power can be achieved. In addition, we performed a moderator analysis that allowed us to explore within-study and between-study variance.

Several limitations of the present meta-analysis need to be taken into account. First, by limiting our search to the PubMed database, we could have missed some relevant studies. However, this limited search already resulted in highly variable trough concentrations.

Therefore, extension of the search to other databases will probably not change our main finding of substantial within- and between- study heterogeneity. Moreover, reference check of the included studies yielded only one additional reference, indicating that the amount of missed studies is limited. Second, because data on clinical characteristics of the individual studies were sparse, we were limited in our ability to perform moderator analyses. Consequently, there may be a true moderating effect of several study and/or patient characteristics, which we were unable to detect in the present study. Also, the limited amount of studies did not allow us to construct a multiple-moderator model to explore the presence of multicollinearity (20). Third, as most of the included studies were performed in young to middle aged, healthy, and mainly male individuals it is questionable whether these results could be translated to patients with hypertension. Patients with hypertension are characterized by a higher age, and generally suffer from multiple comorbidities (eg, renal insufficiency) which is often an indication for additional, possibly interacting, drug treatment. Use of co-medication may in theory further increase the between-individual pharmacokinetic variability (58). The pooled trough concentrations and the amount of heterogeneity reported in this study are therefore likely to be underestimated.

Perspectives

Then, how are we supposed to apply our findings into clinical practice? The goal of biochemical adherence assessment is to accurately distinguish adherent from nonadherent patients and to use this information in a shared decision-making approach to ultimately improve drug adherence and BP control. To implement quantitative screening in daily clinical practice, reliable cut-off values are required. As illustrated in our meta-analysis, trough concentrations of the three different antihypertensive drugs are highly variable, which means that a drug concentration below the trough concentration could also be the result of a deviation from typical pharmacokinetics. Therefore, trough concentrations are not suitable as cut-off values for the quantitative biochemical assessment of drug adherence as this increases the risk of misclassification of adherent patients. Performing biochemical assessment in urine instead of plasma will even further increase the risk of misclassification as some antihypertensive drugs are extensively metabolized or have a low urinary excretion. Classifying patients as nonadherent while they are actually adherent could seriously harm the patient-physician relationship and should therefore be avoided. Hence, as long as a reliable cut-off value for quantitative drug screening is lacking, a conservative approach is preferred and biochemical assessment of adherence should be performed qualitatively.

In conclusion, the plasma trough concentrations of amlodipine, hydrochlorothiazide, and valsartan are highly heterogeneous. Use of the pooled trough concentrations, retrieved by our meta-analysis, as a cut-off for the biochemical assessment of adherence in clinical

practice is therefore not recommended. Before implementation of a quantitative drug screening into clinical practice, drug-, dose-, and patient-specific lower limits based on individual patient data from pharmacokinetic studies are needed to take into account factors that influence drug exposure.

References

1. Fang J, Alderman MH, Keenan NL, Ayala C, Croft JB. Hypertension control at physicians' offices in the United States. Am J Hypertens. 2008 Feb;21(2):136–42.
2. Pereira M, Lunet N, Azevedo A, Barros H. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. J Hypertens. 2009 May;27(5):963–75.
3. Mazzaglia G, Ambrosioni E, Alacqua M, Filippi A, Sessa E, Immordino V, et al. Adherence to antihypertensive medications and cardiovascular morbidity among newly diagnosed hypertensive patients. Circulation. 2009 Oct;120(16):1598–605.
4. Will JC, Zhang Z, Ritchey MD, Loustalot F. Medication Adherence and Incident Preventable Hospitalizations for Hypertension. Am J Prev Med. 2016 Apr;50(4):489–99.
5. Rimoldi SF, Scherrer U, Messerli FH. Secondary arterial hypertension: when, who, and how to screen? Eur Heart J. 2014 May;35(19):1245–54.
6. Lam WY, Fresco P. Medication Adherence Measures: An Overview. Biomed Res Int. 2015;2015:217047.
7. Punt AM, Stienstra NA, van Kleef MEA, Lafeber M, Spiering W, Blankestijn PJ, et al. Screening of cardiovascular agents in plasma with LC-MS/MS: A valuable tool for objective drug adherence assessment. J Chromatogr B, Anal Technol Biomed life Sci. 2019 Jul;1121:103–10.
8. Burnier M, Wuerzner G, Struijker-Boudier H, Urquhart J. Measuring, analyzing, and managing drug adherence in resistant hypertension. Hypertens (Dallas, Tex 1979). 2013 Aug;62(2):218–25.
9. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Drug adherence in hypertension: from methodological issues to cardiovascular outcomes. J Hypertens. 2017 Jun;35(6):1133–44.
10. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
11. Bormann I. Digitizer Software—Digitize a Scanned Graph or Chart Into (x, y) Data. 2016. DigitizeIt;
12. Cheung MW-L. A Guide to Conducting a Meta-Analysis with Non-Independent Effect Sizes. Neuropsychol Rev. 2019 Dec;29(4):387–96.
13. Assink M, Wibbelink CJM. Fitting three-level meta-analytic models in R: A step-by-step tutorial. Quant Methods Psychol. 2016;12(3):154–74.
14. Viechtbauer W. Conducting meta-analyses in R with the metafor package. J Stat Softw. 2010;36(3):1–48.
15. Webster J, Robb OJ, Jeffers TA, Scott AK, Petrie JC, Towler HM. Once daily amlodipine in the treatment of mild to moderate hypertension. Br J Clin Pharmacol. 1987 Dec;24(6):713–9.
16. Abernethy DR. An overview of the pharmacokinetics and pharmacodynamics of amlodipine in elderly persons with systemic hypertension. Am J Cardiol. 1994 Jan;73(3):10A-17A.
17. Jung JA, Lee S-Y, Kim J-R, Ko J-W, Jang SB, Nam SY, et al. A pharmacokinetic and pharmacodynamic drug interaction between rosuvastatin and valsartan in healthy subjects. Drug Des Devel Ther. 2015;9:745–52.
18. Kim CO, Sil Oh E, Kim C, Park MS. Pharmacokinetic Interaction Between Amlodipine and Lobeglitazone, a Novel Peroxisome Proliferator-activated Receptor-gamma Agonist, in Healthy Subjects. Clin Ther. 2015 Sep;37(9):1999-2006.e1.
19. Kim J-R, Kim S, Huh W, Ko J-W. No pharmacokinetic interactions between candesartan and amlodipine following multiple oral administrations in healthy subjects. Drug Des Devel Ther. 2018;12:2475–83.
20. Kuramoto K, Ichikawa S, Hirai A, Kanada S, Nakachi T, Ogihara T. Azelnidipine and amlodipine: a comparison of their pharmacokinetics and effects on ambulatory blood pressure. Hypertens Res. 2003 Mar;26(3):201–8.

21. Leenen FHH, Coletta E. Pharmacokinetic and antihypertensive profile of amlodipine and felodipine-ER in younger versus older patients with hypertension. J Cardiovasc Pharmacol. 2010 Dec;56(6):669–75.

22. Noh Y-H, Lim H-S, Kim MJ, Kim YH, Choi HY, Sung HR, et al. Pharmacokinetic interaction of telmisartan with s-amlodipine: an open-label, two-period crossover study in healthy Korean male volunteers. Clin Ther. 2012 Jul;34(7):1625–35.

23. Park J-W, Kim K-A, Il Kim Y, Park J-Y. Pharmacokinetic and haemodynamic interactions between amlodipine and losartan in human beings. Basic Clin Pharmacol Toxicol. 2019 May;

24. Prasad PP, Stypinski D, Vyas KH, Gonasun L. Lack of Interaction between Modified-Release Fluvastatin and Amlodipine in Healthy Subjects. Clin Drug Investig. 2004;24(6):323–31.

25. Preston RA, Chung M, Gaffney M, Alonso A, Baltodano NM, Epstein M. Comparative pharmacokinetics and pharmacodynamics of amlodipine in hypertensive patients with and without type II diabetes mellitus. J Clin Pharmacol. 2001 Nov;41(11):1215–24.

26. Rohatagi S, Lee J, Shenouda M, Haworth S, Bathala MS, Allison M, et al. Pharmacokinetics of amlodipine and olmesartan after administration of amlodipine besylate and olmesartan medoxomil in separate dosage forms and as a fixed-dose combination. J Clin Pharmacol. 2008 Nov;48(11):1309–22.

27. Bhad P, Ayalasomayajula S, Karan R, Leon S, Riviere G-J, Sunkara G, et al. Evaluation of pharmacokinetic interactions between amlodipine, valsartan, and hydrochlorothiazide in patients with hypertension. J Clin Pharmacol. 2011 Jun;51(6):933–42.

28. Seong SJ, Ohk B, Kang WY, Gwon M-R, Kim BK, Cho S, et al. Pharmacokinetic Drug Interactions Between Amlodipine, Valsartan, and Rosuvastatin in Healthy Volunteers. Adv Ther. 2019 Jul;36(7):1642–56.

29. Son M, Guk J, Kim Y, Woo Chae D, Heo Y-A, Soh D, et al. Pharmacokinetic Interaction Between Rosuvastatin, Telmisartan, and Amlodipine in Healthy Male Korean Subjects: A Randomized, Open-label, Multiple-dose, 2-period Crossover Study. Clin Ther. 2016 Aug;38(8):1845–57.

30. Stangier J, Su CA, Roth W. Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. J Int Med Res. 2000;28(4):149–67.

31. Vaidyanathan S, Valencia J, Kemp C, Zhao C, Yeh C-M, Bizot M-N, et al. Lack of pharmacokinetic interactions of aliskiren, a novel direct renin inhibitor for the treatment of hypertension, with the antihypertensives amlodipine, valsartan, hydrochlorothiazide (HCTZ) and ramipril in healthy volunteers. Int J Clin Pract. 2006 Nov;60(11):1343–56.

32. Chen X, Hu P, Jiang J, Liu T, Zhong W, Liu H, et al. Pharmacokinetic and pharmacodynamic profiles of a fixed-dose combination of olmesartan medoxomil and amlodipine in healthy Chinese males and females. Clin Drug Investig. 2012 Dec;32(12):783–90.

33. Faulkner JK, McGibney D, Chasseaud LF, Perry JL, Taylor IW. The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily. Br J Clin Pharmacol. 1986 Jul;22(1):21–5.

34. Gillen M, Yang C, Wilson D, Valdez S, Lee C, Kerr B, et al. Evaluation of Pharmacokinetic Interactions Between Lesinurad, a New Selective Urate Reabsorption Inhibitor, and CYP Enzyme Substrates Sildenafil, Amlodipine, Tolbutamide, and Repaglinide. Clin Pharmacol drug Dev. 2017 Jul;6(4):363–76.

35. Glesby MJ, Aberg JA, Kendall MA, Fichtenbaum CJ, Hafner R, Hall S, et al. Pharmacokinetic interactions between indinavir plus ritonavir and calcium channel blockers. Clin Pharmacol Ther. 2005 Aug;78(2):143–53.

36. Guo C, Pei QI, Tan H, Huang Z, Yuan H, Yang G. Effects of genetic factors on the pharmacokinetics and pharmacodynamics of amlodipine in primary hypertensive patients. Biomed Reports. 2015 Mar;3(2):195–200.

37. He Y-L, Ligueros-Saylan M, Sunkara G, Sabo R, Zhao C, Wang Y, et al. Vildagliptin, a novel dipeptidyl peptidase IV inhibitor, has no pharmacokinetic interactions with the antihypertensive agents amlodipine, valsartan, and ramipril in healthy subjects. J Clin Pharmacol. 2008 Jan;48(1):85–95.

38. Hsiao H-L, Langenickel TH, Greeley M, Roberts J, Zhou W, Pal P, et al. Pharmacokinetic drug-drug interaction assessment between LCZ696, an angiotensin receptor neprilysin inhibitor, and hydrochlorothiazide, amlodipine, or carvedilol. Clin Pharmacol drug Dev. 2015 Nov;4(6):407–17.

39. Beermann B, Groschinsky-grind M. Antihypertensive effect of various doses of hydrochlorothiazide and its relation to the plasma level of the drug. Eur J Clin Pharmacol. 1978 May;13(3):195–201.

40. Giudicelli JF, Richer C, Mattei A. Pharmacokinetics and biological effects of captopril and hydrochlorothiazide after acute and chronic administration either alone or in combination in hypertensive patients. Br J Clin Pharmacol. 1987;23 Suppl 1:51S-63S.

41. Ismail Z, Triggs EJ, Smithurst BA, Parke W. The pharmacokinetics of amiloride-hydrochlorothiazide combination in the young and elderly. Eur J Clin Pharmacol. 1989;37(2):167–71.

42. Kher A, Fillastre JP, Fourtillan JB, Lefebvre MA, Ingrand I. Pharmacokinetics of a fixed combination of sotalol and hydrochlorothiazide in hypertensive patients with moderate renal insufficiency. Eur J Clin Pharmacol. 1984;27(3):361–2.

43. Lindamood C, Ortiz S, Shaw A, Rackley R, Gorski JC. Effects of commonly administered agents and genetics on nebivolol pharmacokinetics: drug-drug interaction studies. J Clin Pharmacol. 2011 Apr;51(4):575–85.

44. Sabanathan K, Castleden CM, Adam HK, Ryan J, Fitzsimons TJ. A comparative study of the pharmacokinetics and pharmacodynamics of atenolol, hydrochlorothiazide and amiloride in normal young and elderly subjects and elderly hypertensive patients. Eur J Clin Pharmacol. 1987;32(1):53–60.

45. McCrea JB, Lo MW, Tomasko L, Lin CC, Hsieh JY, Capra NL, et al. Absence of a pharmacokinetic interaction between losartan and hydrochlorothiazide. J Clin Pharmacol. 1995 Dec;35(12):1200–6.

46. Weir SJ, Dimmitt DC, Lanman RC, Morrill MB, Geising DH. Steady-state pharmacokinetics of diltiazem and hydrochlorothiazide administered alone and in combination. Biopharm Drug Dispos. 1998 Sep;19(6):365–71.

47. Cheung MW-L. Modeling dependent effect sizes with three-level meta-analyses: a structural equation modeling approach. Psychol Methods. 2014 Jun;19(2):211–29.

48. Sunkara G, Reynolds C V, Pommier F, Humbert H, Yeh C, Prasad P. Evaluation of a pharmacokinetic interaction between valsartan and simvastatin in healthy subjects. Curr Med Res Opin. 2007 Mar;23(3):631–40.

49. Ayalasomayajula S, Langenickel T, Pal P, Boggarapu S, Sunkara G. Clinical Pharmacokinetics of Sacubitril/Valsartan (LCZ696): A Novel Angiotensin Receptor-Neprilysin Inhibitor. Clin Pharmacokinet. 2017 Dec;56(12):1461–78.

50. Ayalasomayajula S, Schuehly U, Pal P, Chen F, Zhou W, Sunkara G, et al. Effect of the angiotensin receptor-neprilysin inhibitor sacubitril/valsartan on the pharmacokinetics and pharmacodynamics of a single dose of furosemide. Br J Clin Pharmacol. 2018 May;84(5):926–36.

51. Chen CL, Desai-Krieger D, Ortiz S, Kerolous M, Wright HM, Ghahramani P. A Single-Center, Open-Label, 3-Way Crossover Trial to Determine the Pharmacokinetic and Pharmacodynamic Interaction Between Nebivolol and Valsartan in Healthy Volunteers at Steady State. Am J Ther. 2015;22(5):e130-40.

52. Prasad PP, Yeh C-M, Gurrieri P, Glazer R, McLeod J. Pharmacokinetics of multiple doses of valsartan in patients with heart failure. J Cardiovasc Pharmacol. 2002 Nov;40(5):801–7.

53. Agesen FN, Weeke PE, Tfelt-Hansen P, Tfelt-Hansen J. Pharmacokinetic variability of beta-adrenergic blocking agents used in cardiology. Pharmacol Res Perspect. 2019 Aug;7(4):e00496.

54. Peeters LEJ, Feyz L, Boersma E, Daemen J, van Gelder T, Koch BCP, et al. Clinical Applicability of Monitoring Antihypertensive Drug Levels in Blood. Hypertens (Dallas, Tex 1979). 2020 Jul;76(1):80–6.

55. Williams RL, Mordenti J, Upton RA, Lin ET, Gee WL, Blume CD, et al. Effects of formulation and food on the absorption of hydrochlorothiazide and triamterene or amiloride from combination diuretic products. Pharm Res. 1987 Aug;4(4):348–52.

56. Sandbaumhuter FA, Haschke M, Vogt B, Bohlender JM. Indexed plasma drug concentrations for drug adherence screening in hypertensive patients. Ann Cardiol Angeiol (Paris). 2018 Jun;67(3):119–26.

57. Ritscher S, Hoyer M, Wunder C, Obermuller N, Toennes SW. Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and beta-blockers - drug classes with low adherence in antihypertensive therapy. Sci Rep. 2019 Oct;9(1):15652.

58. Peeters LEJ, Kester MP, Feyz L, Van Den Bemt P, Koch BCP, Van Gelder T, et al. Pharmacokinetic and pharmacodynamic considerations in the treatment of the elderly patient with hypertension. Expert Opin Drug Metab Toxicol. 2019;15(4):287–97.

Supplemental Material

Supplemental Methods

Since most of the studies included in our meta-analysis provided multiple mean trough concentrations (i.e. with and without co-medication, multiple dosages) we applied a three-level random effects meta-analytic model, taking dependency between the mean trough concentrations into account. This three-level meta-analytic model allows for three levels of variance, including the sampling variance of each trough concentration (level 1), the variance between trough concentrations retrieved from the same study (level 2), and variance between studies (level 3). First, an overall mean trough concentration was estimated using a model without moderators (i.e., an intercept-only model). To determine whether the within-study variance (level 2) and between-study variance (level 3) was significant, we conducted a separate log-likelihood test for each of these two levels. In both tests, the null hypothesis states that one of the variance components equals zero, whereas the alternative hypothesis states that the variance component is greater than zero. Moreover, we determined the distribution of the total variance over the three levels of the meta-analytic model by using the formula of Cheung (1). According to the 75% rule as described by Hunter and Schmidt heterogeneity was regarded as substantial, if less than 75% of the total amount of variance can be attributed to sampling variance (at level 1). When this was the case, the three-level intercept-only models were extended by including (possible) moderators as covariates, so that moderating effects could be examined. Based on availability of the data and their known influence on the pharmacokinetics of a drug, mean age, mean body weight, percentage males and dose were selected as moderators of interest. An omnibus test was performed to determine the significance of the included moderators. The null hypothesis in this omnibus test states that all regression coefficients are equal to zero, and the alternative hypothesis states that at least one of these regression coefficients is not equal to zero. To obtain reliable results, we conducted moderator analyses only if each category contained at least five studies (parameter estimates are poor when the number of studies is very small. Results were graphically presented in forest plots. The three-level analyses were conducted according to the three-level random-effects model guidelines formulated by Assink and Wibbelink (2).

Supplemental Table 1. Search strategy, performed on September 1st, 2020.

Antihypertensive medication	Search string	Results
Amlodipine	(((((((Amlodipine[Title/Abstract]) OR (amlodipine[MeSH Terms])) OR (amlodis[Title/Abstract])) OR (astudal[Title/Abstract])) OR (norvasc[Title/Abstract])) OR (Istin[Title/Abstract])) OR (amlor[Title/Abstract])) AND ((((((pharmacokinetics[MeSH Terms]) OR (pharmacokinetic*[Title/Abstract])) OR (drug monitoring[MeSH Terms])) OR (drug level[Title/Abstract])) OR (therapeutic drug monitoring[Title/Abstract])) OR (drug concentration[Title/Abstract]))	492
Hydrochlorothiazide	((((((((((((hydrochlorothiazide[Title/Abstract]) OR (hydrochloorthiazide[Title/Abstract])) OR (hydrochlorothiazide[MeSH Terms])) OR (HCTZ[Title/Abstract])) OR (Dichlothiazide[Title/Abstract])) OR (Dihydrochlorothiazide[Title/Abstract])) OR (HydroDIURIL[Title/Abstract])) OR (Oretic[Title/Abstract])) OR (Sectrazide[Title/Abstract])) OR (Esidrix[Title/Abstract])) OR (Esidrex[Title/Abstract])) OR (Hypothiazide[Title/Abstract])) AND ((((((pharmacokinetics[MeSH Terms]) OR (pharmacokinetic*[Title/Abstract])) OR (drug monitoring[MeSH Terms])) OR (drug level[Title/Abstract])) OR (therapeutic drug monitoring[Title/Abstract])) OR (drug concentration[Title/Abstract]))	535
Valsartan	(((((((valsartan[Title/Abstract]) OR (valsartan[MeSH Terms])) OR (diovan[Title/Abstract])) OR (kalpress[Title/Abstract])) OR (tareg[Title/Abstract])) OR (Nisis[Title/Abstract])) OR (provas[Title/Abstract])) OR (miten[Title/Abstract])) AND (((pharmacokinetic*[Title/Abstract]) OR (pharmacokinetics[MeSH Terms])) OR ((((drug monitoring[MeSH Terms]) OR (Therapeutic Drug Monitoring[Title/Abstract])) OR (drug level[Title/Abstract])) OR (drug concentration[Title/Abstract])))	291

Supplemental Table 2a. Characteristics of included studies – Amlodipine.

Author, year (Country)	Study design	Patient population	N	Sex (% male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Bhad 2011 (India)(3)	multicenter open-label, 4-cohort§, parallel-group	Hypertensive patients	23	79	42.8 ± 10.7	NS	10	18	Valsartan	15±4 (a)
			23	81	44.2 ± 9.6		10	18	Hydrochlorothiazide	16±6 (b)
			23	48	45.7 ± 7.1		10	18	Valsartan and Hydrochlorothiazide	16±6 (c)
Abernethy 1990 (USA)	open-label trial	Young hypertensive patients	13	80	28-45	NS	2.5	10		2.7±0.7* (a)
		Elderly hypertensive patients	15	77	65-73		2.5	10		4±1.5* (b)
Chen 2012 (China)(4)	single-centre, open-label, sequential single- study	Healthy adult volunteers	10	50	27.1 ± 6	58.8±6.1	5	10	Olmesartan	6±1.3*
Faulkner 1986 (Germany)(5)	double-blind parallel group study	Healthy volunteers	56	100	26.1 ± 36	68.2±7	15	14		11.8±5.3
Gillen 2017 (USA)(6)	open-label trial	Healthy volunteers	14	100	30 ± 0.4	77±8.25	5	14		7.1±2.6* (a)
			14	100	30 ± 0.4	77±8.25	5	14	Lesinurad	3.7±1.1* (b)
Glesby 2005 (USA)(7)	randomized, phase 1, open-label, 2-arm pharmacokinetic drug interaction study	Healthy volunteers	18	71	18-49	NS	5	7		4.2±0.8* (a)

Supplemental Table 2a. *Continued.*

Author, year (Country)	Study design	Patient population	N	Sex (% male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
			18	71	18-49		5	7	Indinavir and Ritonavir	8.3±0.3* (b)
Gundlach 2020 (Germany) (8)	open-label multiple-dose, fixed-sequence study	Healthy volunteers	18	100	31± 9.1	78±14.6	5	7		5.6±1.84 (a)
			18	100	31± 9.2	78±14.6	5	7	Candesartan and atorvastatine	6±1.4 (b)
Guo 2015 (China)(9)	open-label trial	Hypertensive patients	31	100	57.7 ± 7.9	66.6±11	5	28		5.91±2.93 (a)
			29	0	57.7 ± 7.9	66.6±11	5	28		8.86±2.98 (b)
He 2008 (USA)(10)	open-label, 3-period, randomized, crossover study	Healthy volunteers	19	61.9	28.3 ± 7.8	73.6±9.6	5	10		5.2±1.3* (a)
			19	61.9	28.3 ± 7.8	73.6±9.6	5	10	Vildagliptin	5±1.5* (b)
Hsiao 2015 (USA)(11)	open-label, single sequence and 3-period study	Healthy volunteers	26	79	42.6 ± 9.6	76.8±10.4	10	10		18.2±5.1* (a)
			26	79	42.6 ± 9.6	76.8±10.4	10	15	LCZ696	20±5.6* (b)
Kim 2015 (Korea)(12)	randomized, open-label, multiple-dose, 3- treatment, 3- period, 6- sequence cross-over study	Healthy volunteers	23	NS	26.6 ± 3.9	68.7±5.7	10	10		10.9±2.5* (a)
			23		26.6 ± 3.9	68.7±5.7	10	10	Lobeglitazone	10.2±2.5* (b)

Supplemental Table 2a. *Continued.*

Author, year (Country)	Study design	Patient population	N	Sex (% male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Kim 2018 (Korea)(13)	randomized, open-label, 2-sequence, 2-period, 2-intervention crossover study	Healthy volunteers	20	100	31 ± 8	69.6±8.1	10	10		11.3±3.35 (a)
			20	100	31 ± 8	69.6±8.1	10	10	Candesartan	11.7±3.42 (b)
Kuramoto 2003 (Japan) (14)	randomized double-blind trial	Hypertensive patients	23	43.5	54 ± 6.5	62.1±9.4	5	42		12.1±3.6*
Leenen 2010 (Canada)(15)	double-blind, placebo-controlled, parallel, comparative single-center study	Young hypertensive patients	28	35.7	41 ± 2	86±4	5	56		7.7±2.2* (a)
		Elderly hypertensive patients	35	42.9	67 ± 1	79±2	5	56		8.8±2.5* (b)
Noh 2012 (Korea) (16)	multiple-dose, open-label, 2-sequence, 2-period, crossover study	Healthy volunteers	12	100	28.1 ± 5.5	70.4±7.2	5	9		5.4±1.5* (a)
			11	100	28.1 ± 5.5	70.4±7.2	5	9	Telmisartan	5.9±1.8* (b)
Park 2019 (Korea)(17)	open-label, 3- period, fixed-sequence study	Healthy volunteers	20	100	19-38	59-86	10	9		5.06±2.28 (a)
			20	100	19-38	59-86	10	9	Losartan	5.34±1.63 (b)

Supplemental Table 2a. *Continued.*

Author, year (Country)	Study design	Patient population	N	Sex (% male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Prasad 2004 (USA)(18)	single-centre, 6-sequence,3- period, randomised, cross-over study	Healthy volunteers	19	33.3	25 ± 6	66±10	5	14		5±1.7 (a)
			19	33.3	25 ± 6	66±10	5	14	Fluvastatin	4.6±1.4 (b)
Preston 2001 (USA)(19)	open-label trial	Hypertensive patients	10	40	56.3 ± 10.2	NS	5	14		8.4±4.5 (a)
		Diabetic hypertensive patients	18	55.6	53.3 ± 7.6		5	14		10±5.1 (b)
		Hypertensive patients	9	40	56.3 ± 10.2		10	28		9±4.7 (c)
		Diabetic hypertensive patients	12	55.6	53.3 ± 7.6		10	28		17.6±7.8 (d)
Rohatagi 2008 (USA) (20)	multiple-dose, interaction study	Healthy volunteers	23	66.7	38.3 ± 11.5	81.7±11.4	10	10		12.4±4.2 (a)
			23	66.7	38.3 ± 11.5	81.7±11.4	10	10	Olmesartan	13.5±5.3 (b)
Seong 2019 (Korea)(21)	open-label, multiple-dose, two-period, fixed-sequence study	Healthy volunteers	30	100	24.7 ± 3.4	71.5±10.5	10	10	Valsartan	10±2.5* (a)
			30	100	24.7 ± 3.4	71.5±10.5	10	10	Valsartan and Rosuvastatine	11±3* (b)

Supplemental Table 2a. *Continued.*

Author, year (Country)	Study design	Patient population	N	Sex (% male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Son 2016 (Korea)(22)	randomized, open-label, multiple-dose, 2-part, 2-period crossover study	Healthy volunteers	22	100	28.5 ± 6.5	68.1±7.4	5	9	Telmisartan	10.06±3.3* (a)
			22	100	28.5 ± 6.5	68.1±7.4	5	9	Telmisartan and Rosuvastatin	10.3±3.3* (b)
Stangier 2000 (Germany) (23)	open-label 2-way cross-over, randomized trial	Healthy volunteers	12	100	34.7 ± 9.8	82.7±10.8	10	9		11.5±2.6* (a)
			12	100	34.7 ± 9.8	82.7±10.8	10	9	Telmisartan	12.2±3.1* (b)
Vaidyanathan 2006 (USA) (24)	open-label study	Healthy volunteers	20	66.70	33.7 ± 8.2	69.6±9.2	10	14		15.7±5.1 (a)
			18	66.7	33.7 ± 8.2	69.6±9.2	10	14	Aliskiren	14.9±4.2 (b)
Webster 1987 (Scotland) (25)	double-blind placebo-controlled parallel group study	Hypertensive patients	15	NS	31-60	NS	2.5	14		2.54±1.36 (a)
			15	NS	31-60		5	14		4.93±1.28 (b)
			13	NS	31-60		10	14		10.47±3.39 (c)

NS = not specified.* derived from concentration curve using software Digitizelt (Digitizelt, Braunschweig, Germany). †The 4 treatment cohorts were cohorts 1, valsartan + hydrochlorothiazide; cohort 2, amlodipine + valsartan; cohort 3, amlodipine + hydrochlorothiazide; and cohort 4, amlodipine + valsartan + hydrochlorothiazide. (a-d) indicates the different trough concentrations derived from the same study.

Supplemental Table 2b. Characteristics of included studies – *Hydrochlorothiazide.*

Author, year (Country)	Study design	Study population	N	Sex (%male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Beermann 1978 (Sweden) (26)	open-label study	Hypertensive patients	6	77.8	50-75	64-85	12.5	14		15±7 (a)
			8	77.8	50-75	64-85	25	14		17±8 (b)
			8	77.8	50-75	64-85	50	14		27±11 (c)
			8	77.8	50-75	64-85	75	14		34±17 (d)
Bhad 2011 (India)(3)	multicenter open-label, 4-cohort[§], parallel-group study	Hypertensive patients	25	76.7	44.5±10	NS	25	16	Valsartan	27±21 (a)
			23	80.8	44.2±9.6	NS	25	16	Amlodipine	20±15 (b)
			23	48.1	45.7±7.1	NS	25	16	Amlodipine and Valsartan	26±26 (c)
Giudicelli 1987 (France) (27)	open-label randomized study	Hypertensive patients	10	30	51.7±3.6	67.9±3.3	25	45		40±30* (a)
			8	37.5	54.9±2.4	67.1±5	25	45	Captopril	21±7* (b)
Ismail 1989 (Australia)(28)	open-label trial	Elderly hypertensive patients	11	NS	61-85	52.4±8.7	50	14	Amiloride	121.3±75.1
Kher 1984 (France) (29)	open-label trial	Hypertensive patients	4	NS	NS	NS	25	45	Sotalol	50.7±25.4
Lindamood 2011 (USA)(30)	open-label drug-drug interaction study	Healthy volunteers	13	84.6	19-45	NS	25	10		10,6±3.4 (a)
			13	84.6	19-45	NS	25	10	Nebivolol	11,8±5.6 (b)
Mccrea 1995 (USA) (31)	open, randomized, 3-period crossover study	Hypertensive patients	12	83	35-55	NS	12.5	7		8±2.8 (a)

Supplemental Table 2b. *Continued.*

Author, year (Country)	Study design	Study population	N	Sex (%male)	Age (years, (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
			12	83	35-55	NS	12.5	7	Losartan	5.8±2.5 (b)
Sabanathan 1987 (UK)(32)	open-label study	Healthy young volunteers	6	NS	22-29	53-81	25	7	Atenolol and Amiloride	30±5* (a)
		Healthy elderly volunteers	6	NS	69-77	55-91	25	7	Atenolol and Amiloride	132±30* (b)
		Elderly hypertensive volunteers	6	NS	67-76	48-70	25	7	Atenolol and Amiloride	168±40* (c)
Vaidyanathan 2006 (USA) (24)	open-label study	Healthy volunteers	22	40.9	28,1±8.8	NS	25	4		15±6.1 (a)
			22	40.9	28,1±8.8	NS	25	4	Aliskiren	20±7.1 (b)
Weir 1998 (USA)(33)	randomized, 3-way complete crossover, open-label study	Healthy volunteers	15	100	27.8±5.7	NS	25‡	5.5		50.7±14.7 (a)
			15	100	27.8±5.7	NS	25‡	5.5	Diltiazem	48.8±17.1 (b)

NS = not specified. * derived from concentration curve using software Digitizelt (Digitizelt, Braunschweig, Germany). †The 4 treatment cohorts were cohorts 1, valsartan + hydrochlorothiazide; cohort 2, amlodipine + valsartan; cohort 3, amlodipine + hydrochlorothiazide; and cohort 4, amlodipine + valsartan + hydrochlorothiazide. ‡ Dosed twice a day. (a-d) indicates the different trough concentrations derived from the same study.

Supplemental Table 2c. Characteristics of included studies – Valsartan.

Author, year (Country)	Study design	Study population	N	Sex (%male)	Age (years (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
Ayalasomayajula 2017 (China)(34)	open-label, 3-period, single-sequence study.	Healthy volunteers	28	100	25.2±2.7	NS	200‡	4		822±318* (a)
			28	100	25.2±2.7	NS	200‡	4	Atorvastatin	562±281* (b)
Ayalasomayajula 2018 (USA)(35)	open-label, 2-period, single-sequence, single-center study.	Healthy volunteers	28	85.7	34.5	75.9	200‡	5	Sacubutril	400±200*
Bhad 2011 (India)(3)	multicenter open-label, 4-cohort[§], parallel-group study	Hypertensive patients	23	77	44.5±10	NS	320	18	Hydrochlorothiazide	573±670 (a)
			23	79	42.8±10.7	NS	320	18	Amlodipine	336±188 (b)
			23	48	45.7±7.1	NS	320	18	Hydrochlorothiazide and Amlodipine	444±356 (c)
Chen 2015 (USA) (36)	single-center, randomized, open-label, multiple-dose, 3-way crossover trial	Healthy volunteers	26	50	34.4±8.3	74±13.8	320	7		457.3±288.5 (a)

Supplemental Table 2c. *Continued.*

Author, year (Country)	Study design	Study population	N	Sex (%male)	Age (years (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
			26	50	34.4±8.3	74±13.8	320	7	Nebivolol	463.2±284.2 (b)
He 2008 (USA) (10)	open-label, multiple-dose, 3-period, randomized, crossover design	Healthy volunteers	28	59	NS	NS	320	7	Vidagliptine	309±310*
Hsiao 2015 (USA) (11)	open-label, single sequence, 3-period design	Healthy volunteers	28	82	42.7±9.7	79.2±10.2	400	4	Hydrochloorthiazide	168±566.2* (a)
			28	71	40.8±7.37	75.5±9.78	400	5	Carvedilol	253±423.3* (b)
			27	79	42.6±9.6	76.8±10.4	400	5	Amlodipine	165±529.2* (c)
Jung 2015 (Korea)(37)	randomized, open-label, multiple-dose,3-treatment, 3-period crossover study	Healthy volunteers	30	100	NS		160	4		183±114 (a)
			30	100	NS		160	4	Rosuvastatine	207±166 (b)
Prasad 2002 (USA)(38)	open-label, multiple-dose study	Patients with chronic stable heart failure	18	78	63.1±10.1	90.7±20.5	40ǂ	7	ACE-inhibitors, beta-blockers	470±300 (a)

Supplemental Table 2c. *Continued.*

Author, year (Country)	Study design	Study population	N	Sex (%male)	Age (years (mean±SD)/ (range))	Weight (kg, (mean±SD)/ (range))	Dose (mg)	Treatment period (days)	Comedication	Mean trough concentration ± SD (ng/ml)
			18	78	63.1±10.1	90.7±20.5	80‡	7	ACE-inhibitors, beta-blockers	1050±800 (b)
			18	78	63.1±10.1	90.7±20.5	160‡	7	ACE-inhibitors, beta-blockers	1980±1600 (c)
Seong 2019 (Korea)(21)	open-label, multiple-dose, 2-period, fixed-sequence study	Healthy volunteers	30	100	24.7±3.4	71.5±10.5	160	10	Amlodipine	220±78.4 (a)
			30	100	24.7±3.4	71.5±10.5	160	10	Amlodipine and Rosuvastatine	165±121 (b)
Sunkara 2007 (USA)(39)	open-label, randomized, multiple-dose, 3-period crossover design	Healthy volunteers	18	72	29±8	75±11	160	7		160±120 (a)
			18	72	29±8	75±11	160	7	Simvastatine	170±130 (b)
Vaidyanathan 2006 (USA)(24)	open-label study	Healthy volunteers	19	74	31.7±9.3	74.9±9.2	320	4		300±200 (a)
			19	74	31.7±9.3	74.9±9.2	320	4	Aliskiren	300±200 (b)

NS = not specified. * derived from concentration curve using software Digitizelt (Digitizelt,Braunschweig, Germany). †The 4 treatment cohorts were cohorts 1, valsartan + hydrochlorothiazide; cohort 2, amlodipine + valsartan; cohort 3, amlodipine + hydrochlorothiazide; and cohort 4, amlodipine + valsartan + hydrochlorothiazide.‡ Dosed twice a day. (a-d) indicates the different trough concentrations derived from the same study.

References supplemental material

1. Cheung MW-L. Modeling dependent effect sizes with three-level meta-analyses: a structural equation modeling approach. Psychol Methods. 2014 Jun;19(2):211–29.
2. Assink M, Wibbelink CJM. Fitting three-level meta-analytic models in R: A step-by-step tutorial. Quant Methods Psychol. 2016;12(3):154–74.
3. Bhad P, Ayalasomayajula S, Karan R, Leon S, Riviere G-J, Sunkara G, et al. Evaluation of pharmacokinetic interactions between amlodipine, valsartan, and hydrochlorothiazide in patients with hypertension. J Clin Pharmacol. 2011 Jun;51(6):933–42.
4. Chen X, Hu P, Jiang J, Liu T, Zhong W, Liu H, et al. Pharmacokinetic and pharmacodynamic profiles of a fixed-dose combination of olmesartan medoxomil and amlodipine in healthy Chinese males and females. Clin Drug Investig. 2012 Dec;32(12):783–90.
5. Faulkner JK, McGibney D, Chasseaud LF, Perry JL, Taylor IW. The pharmacokinetics of amlodipine in healthy volunteers after single intravenous and oral doses and after 14 repeated oral doses given once daily. Br J Clin Pharmacol. 1986 Jul;22(1):21–5.
6. Gillen M, Yang C, Wilson D, Valdez S, Lee C, Kerr B, et al. Evaluation of Pharmacokinetic Interactions Between Lesinurad, a New Selective Urate Reabsorption Inhibitor, and CYP Enzyme Substrates Sildenafil, Amlodipine, Tolbutamide, and Repaglinide. Clin Pharmacol drug Dev. 2017 Jul;6(4):363–76.
7. Glesby MJ, Aberg JA, Kendall MA, Fichtenbaum CJ, Hafner R, Hall S, et al. Pharmacokinetic interactions between indinavir plus ritonavir and calcium channel blockers. Clin Pharmacol Ther. 2005 Aug;78(2):143–53.
8. Gundlach K, Wolf K, Salem I, Randerath O, Seiler D. Safety of Candesartan, Amlodipine, and Atorvastatin in Combination: Interaction Study in Healthy Subjects. Clin Pharmacol drug Dev. 2020 May;
9. Guo C, Pei QI, Tan H, Huang Z, Yuan H, Yang G. Effects of genetic factors on the pharmacokinetics and pharmacodynamics of amlodipine in primary hypertensive patients. Biomed Reports. 2015 Mar;3(2):195–200.
10. He Y-L, Ligueros-Saylan M, Sunkara G, Sabo R, Zhao C, Wang Y, et al. Vildagliptin, a novel dipeptidyl peptidase IV inhibitor, has no pharmacokinetic interactions with the antihypertensive agents amlodipine, valsartan, and ramipril in healthy subjects. J Clin Pharmacol. 2008 Jan;48(1):85–95.
11. Hsiao H-L, Langenickel TH, Greeley M, Roberts J, Zhou W, Pal P, et al. Pharmacokinetic drug-drug interaction assessment between LCZ696, an angiotensin receptor neprilysin inhibitor, and hydrochlorothiazide, amlodipine, or carvedilol. Clin Pharmacol drug Dev. 2015 Nov;4(6):407–17.
12. Kim CO, Sil Oh E, Kim C, Park MS. Pharmacokinetic Interaction Between Amlodipine and Lobeglitazone, a Novel Peroxisome Proliferator-activated Receptor-gamma Agonist, in Healthy Subjects. Clin Ther. 2015 Sep;37(9):1999-2006.e1.
13. Kim J-R, Kim S, Huh W, Ko J-W. No pharmacokinetic interactions between candesartan and amlodipine following multiple oral administrations in healthy subjects. Drug Des Devel Ther. 2018;12:2475–83.
14. Kuramoto K, Ichikawa S, Hirai A, Kanada S, Nakachi T, Ogihara T. Azelnidipine and amlodipine: a comparison of their pharmacokinetics and effects on ambulatory blood pressure. Hypertens Res. 2003 Mar;26(3):201–8.
15. Leenen FHH, Coletta E. Pharmacokinetic and antihypertensive profile of amlodipine and felodipine-ER in younger versus older patients with hypertension. J Cardiovasc Pharmacol. 2010 Dec;56(6):669–75.
16. Noh Y-H, Lim H-S, Kim MJ, Kim YH, Choi HY, Sung HR, et al. Pharmacokinetic interaction of telmisartan with s-amlodipine: an open-label, two-period crossover study in healthy Korean male volunteers. Clin Ther. 2012 Jul;34(7):1625–35.

17. Park J-W, Kim K-A, Il Kim Y, Park J-Y. Pharmacokinetic and haemodynamic interactions between amlodipine and losartan in human beings. Basic Clin Pharmacol Toxicol. 2019 May;

18. Prasad PP, Stypinski D, Vyas KH, Gonasun L. Lack of Interaction between Modified-Release Fluvastatin and Amlodipine in Healthy Subjects. Clin Drug Investig. 2004;24(6):323–31.

19. Preston RA, Chung M, Gaffney M, Alonso A, Baltodano NM, Epstein M. Comparative pharmacokinetics and pharmacodynamics of amlodipine in hypertensive patients with and without type II diabetes mellitus. J Clin Pharmacol. 2001 Nov;41(11):1215–24.

20. Rohatagi S, Lee J, Shenouda M, Haworth S, Bathala MS, Allison M, et al. Pharmacokinetics of amlodipine and olmesartan after administration of amlodipine besylate and olmesartan medoxomil in separate dosage forms and as a fixed-dose combination. J Clin Pharmacol. 2008 Nov;48(11):1309–22.

21. Seong SJ, Ohk B, Kang WY, Gwon M-R, Kim BK, Cho S, et al. Pharmacokinetic Drug Interactions Between Amlodipine, Valsartan, and Rosuvastatin in Healthy Volunteers. Adv Ther. 2019 Jul;36(7):1642–56.

22. Son M, Guk J, Kim Y, Woo Chae D, Heo Y-A, Soh D, et al. Pharmacokinetic Interaction Between Rosuvastatin, Telmisartan, and Amlodipine in Healthy Male Korean Subjects: A Randomized, Open-label, Multiple-dose, 2-period Crossover Study. Clin Ther. 2016 Aug;38(8):1845–57.

23. Stangier J, Su CA. Pharmacokinetics of repeated oral doses of amlodipine and amlodipine plus telmisartan in healthy volunteers. J Clin Pharmacol. 2000 Dec;40(12 Pt 1):1347–54.

24. Vaidyanathan S, Valencia J, Kemp C, Zhao C, Yeh CM, Bizot MN, et al. Lack of pharmacokinetic interactions of aliskiren, a novel direct renin inhibitor for the treatment of hypertension, with the antihypertensives amlodipine, valsartan, hydrochlorothiazide (HCTZ) and ramipril in healthy volunteers. Int J Clin Pract. 2006;60(11):1343–56.

25. Webster J, Robb OJ, Jeffers TA, Scott AK, Petrie JC, Towler HM. Once daily amlodipine in the treatment of mild to moderate hypertension. Br J Clin Pharmacol. 1987 Dec;24(6):713–9.

26. Beermann B, Groschinsky-grind M. Antihypertensive effect of various doses of hydrochlorothiazide and its relation to the plasma level of the drug. Eur J Clin Pharmacol. 1978 May;13(3):195–201.

27. Giudicelli JF, Richer C, Mattei A. Pharmacokinetics and biological effects of captopril and hydrochlorothiazide after acute and chronic administration either alone or in combination in hypertensive patients. Br J Clin Pharmacol. 1987;23 Suppl 1:51S-63S.

28. Ismail Z, Triggs EJ, Smithurst BA, Parke W. The pharmacokinetics of amiloride-hydrochlorothiazide combination in the young and elderly. Eur J Clin Pharmacol. 1989;37(2):167–71.

29. Kher A, Fillastre JP, Fourtillan JB, Lefebvre MA, Ingrand I. Pharmacokinetics of a fixed combination of sotalol and hydrochlorothiazide in hypertensive patients with moderate renal insufficiency. Eur J Clin Pharmacol. 1984;27(3):361–2.

30. Lindamood C, Ortiz S, Shaw A, Rackley R, Gorski JC. Effects of commonly administered agents and genetics on nebivolol pharmacokinetics: drug-drug interaction studies. J Clin Pharmacol. 2011 Apr;51(4):575–85.

31. McCrea JB, Lo MW, Tomasko L, Lin CC, Hsieh JY, Capra NL, et al. Absence of a pharmacokinetic interaction between losartan and hydrochlorothiazide. J Clin Pharmacol. 1995 Dec;35(12):1200–6.

32. Sabanathan K, Castleden CM, Adam HK, Ryan J, Fitzsimons TJ. A comparative study of the pharmacokinetics and pharmacodynamics of atenolol, hydrochlorothiazide and amiloride in normal young and elderly subjects and elderly hypertensive patients. Eur J Clin Pharmacol. 1987;32(1):53–60.

33. Weir SJ, Dimmitt DC, Lanman RC, Morrill MB, Geising DH. Steady-state pharmacokinetics of diltiazem and hydrochlorothiazide administered alone and in combination. Biopharm Drug Dispos. 1998 Sep;19(6):365–71.

34. Ayalasomayajula S, Langenickel T, Pal P, Boggarapu S, Sunkara G. Clinical Pharmacokinetics of Sacubitril/Valsartan (LCZ696): A Novel Angiotensin Receptor-Neprilysin Inhibitor. Clin Pharmacokinet. 2017 Dec;56(12):1461–78.

35. Ayalasomayajula S, Schuehly U, Pal P, Chen F, Zhou W, Sunkara G, et al. Effect of the angiotensin receptor-neprilysin inhibitor sacubitril/valsartan on the pharmacokinetics and pharmacodynamics of a single dose of furosemide. Br J Clin Pharmacol. 2018 May;84(5):926–36.

36. Chen CL, Desai-Krieger D, Ortiz S, Kerolous M, Wright HM, Ghahramani P. A Single-Center, Open-Label, 3-Way Crossover Trial to Determine the Pharmacokinetic and Pharmacodynamic Interaction Between Nebivolol and Valsartan in Healthy Volunteers at Steady State. Am J Ther. 2015;22(5):e130-40.

37. Jung JA, Lee S-Y, Kim J-R, Ko J-W, Jang SB, Nam SY, et al. A pharmacokinetic and pharmacodynamic drug interaction between rosuvastatin and valsartan in healthy subjects. Drug Des Devel Ther. 2015;9:745–52.

38. Prasad PP, Yeh C-M, Gurrieri P, Glazer R, McLeod J. Pharmacokinetics of multiple doses of valsartan in patients with heart failure. J Cardiovasc Pharmacol. 2002 Nov;40(5):801–7.

39. Sunkara G, Reynolds C V, Pommier F, Humbert H, Yeh C, Prasad P. Evaluation of a pharmacokinetic interaction between valsartan and simvastatin in healthy subjects. Curr Med Res Opin. 2007 Mar;23(3):631–40.

CHAPTER 9

Clinical characteristics do not reliably identify nonadherence in patients with uncontrolled hypertension

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Abstract

Purpose: Chemical adherence testing is a reliable method to assess adherence to antihypertensive drugs. However, it is expensive and has limited availability in clinical practice. To reduce the number and costs of chemical adherence tests, we aimed to develop and validate a clinical screening tool to identify patients with a low probability of nonadherence in patients with uncontrolled hypertension.

Materials and Methods: In 495 patients with uncontrolled hypertension referred to the University Medical Center Utrecht (UMCU), the Netherlands, a penalized logistic regression model including seven pre-specified easy-to-measure clinical variables was derived to estimate the probability of nonadherence. Nonadherence was defined as not detecting at least one of the prescribed antihypertensive drugs in plasma or urine. Model performance and test characteristics were evaluated in 240 patients with uncontrolled hypertension referred to the Heartlands Hospital, United Kingdom.

Results: Prevalence of nonadherence to antihypertensive drugs was 19% in the UMCU and 44% in the Heartlands Hospital population. After recalibration of the model's intercept, predicted probabilities agreed well with observed frequencies. The c-statistic of the model was 0.63 (95%CI 0.53–0.72). Predicted probability cut-off values of 15%-22.5% prevented testing in 5%-15% of the patients, carrying sensitivities between 97% (64-100) and 90% (80-95), and negative predictive values between 74% (10-99) and 70% (50-85).

Conclusion: The combination of seven clinical variables is not sufficient to reliably discriminate adherent from non-adherent individuals to safely reduce the number of chemical adherence tests. This emphasizes the complex nature of nonadherence behavior and thus the need for objective chemical adherence tests in patients with uncontrolled hypertension.

Introduction

Hypertension globally affects 30-45% of the adult population and is an important treatable risk factor for cardiovascular disease (CVD) and mortality (1,2). Although awareness and treatment have improved considerably, still approximately 50% of those receiving treatment for hypertension do not reach the blood pressure (BP) targets recommended by guidelines (1). Nonadherence to antihypertensive treatment is a major contributor to suboptimal BP control at the population level (3,4). The estimated prevalence of nonadherence ranges from 16-53% in patients with uncontrolled BP to 10-86% in patients with resistant hypertension (5). Diagnosis of nonadherence is important as nonadherence is associated with a higher risk of acute cardiovascular events in the general hypertensive population (3,6,7). Moreover, early recognition of nonadherence might reduce the number of costly diagnostic tests and invasive device-based therapies (8).

Several methods are available to assess adherence, but most are indirect, subjective and poorly reliable since they are shown to often overestimate adherence (9). As recommended by the 2018 European Society of Cardiology (ESC)/ European Society of Hypertension (ESH) Guidelines, chemical drug screening by liquid chromatography tandem mass spectrometry (LC-MS/MS) in plasma or urine is one of the most reliable methods for medication adherence assessment (10,11). However, due to the related costs and infrastructure, LC-MS/MS–based analysis is unlikely to become available in healthcare settings with limited (financial) resources where the prevalence of hypertension is higher, and the control of hypertension much lower (1). To reduce healthcare costs and make LC-MS/MS more accessible in these settings a clinical screening tool that creates the opportunity to carefully identify patients with a low probability of nonadherence, and therefore do not need to undergo further testing, would be desirable. However, the limited clinical screening tools developed so far were either based on pharmacy refill data (12–14), did not specify model coefficients (15), or were not externally validated (12–14) making them futile in clinical practice. Therefore, the aim of this study was to develop and externally validate a screening tool, based on easy to collect clinical variables, to estimate the probability of nonadherence in patients with uncontrolled hypertension.

Methods

Study populations

For the development of the clinical screening tool we used data from 495 consecutive patients with uncontrolled hypertension referred to the outpatient clinic of the Vascular Medicine department of the University Medical Center Utrecht (UMCU) between November 2017 and November 2020. Patients were referred for diagnostic evaluation and/or treatment advice if BP targets were not met despite BP lowering treatment and/or suffered from target-organ damage. All patients underwent diagnostic evaluation according to a standardized protocol to identify underlying causes of hypertension. The details of this protocol have been described elsewhere (16). Patients who were prescribed at least one antihypertensive drug were included in this study. Patients in whom no biochemical drug screening was performed were excluded (n=14): nine patients were evaluated in early November before the biochemical drug screening was fully implemented, two patients used candesartan which could not be analyzed by the LC-MS/MS assay, and the results from three patients were missing for unknown reasons.

For external validation of the clinical screening tool, data from 240 patients who attended the hypertension clinic at Birmingham Heartlands Hospital in the United Kingdom (UK) between January 2015 and December 2018 were used. These patients were referred by their general practitioner or other medical specialists for the investigation and management of uncontrolled hypertension. Patients underwent biochemical drug screening in urine when either medication nonadherence was suspected by the treating hypertension specialist or when patients fulfilled the criteria for apparent resistant hypertension (in spite of concurrent use of three antihypertensive agents of different classes).

Since participants in this study were not subject to procedures and were not required to follow rules of behavior outside the scope of routine clinical practice, no formal consent was required which was approved by the institutional ethics committees.

Definition of outcome

The outcome of interest was nonadherence to antihypertensive drugs. According to the guidelines for reporting on medication adherence, the EMERGE taxonomy, nonadherence can occur in three different phases of medication adherence: (1) initiation, (2) implementation, and (3) persistence (17). Since the study population concerns patients with uncontrolled hypertension who have been referred to a specialist center in which the initiation phase has long passed, this study is mainly focused on the implementation and persistence phase of adherence.

Nonadherence to antihypertensive drugs was assessed by chemical adherence testing which was performed in accordance with the recommendations in a recently

published position paper on this method (18). For the UMCU population chemical adherence testing was performed using an LC-MS/MS method which is able to detect 39 antihypertensive drugs (covering >95% of the European antihypertensive drug prescriptions) simultaneously (18,19) (see Supplemental Table 1). In the UK population, the LC-MS/MS method was able to measure urine concentrations of 24 commonly prescribed antihypertensive drugs (covering over 90% of the UK antihypertensive drug prescriptions) (18,20) (see Supplemental Table 1). For both study populations, all patients provided verbal consent for chemical adherence testing in blood or urine on the day of their clinical appointment. Patients were not informed in advance that drug testing would be performed at their clinical visit.

We considered the lower limit of detection (LOD), the lowest quantity of a drug that can be distinguished from the absence of that drug, as a cut-off for adherence. Based on this cut-off, patients were divided into two main categories: adherent (all of the prescribed medications detected) or non-adherent (at least one of the prescribed medications not detected). Nonadherence was further categorized into full nonadherence (complete absence of any prescribed antihypertensive medications in the blood or urine sample) and partial nonadherence (presence of fewer medications than prescribed in blood or urine sample). In case of fixed-dose combinations, we considered all separate drug components.

Clinical model parameters

The screening tool was built with the following pre-specified clinical variables: age, sex, body mass index (BMI, kg/m^2), history of CVD (yes/no, defined according to the atherosclerotic cardiovascular disease (ASCVD) definition as proposed by the European Guidelines on Cardiovascular Disease Prevention (21)), office systolic BP (SBP, mmHg), office heart rate (beats/min) and total number of antihypertensive drug tablets. Selection of these variables was based on previous studies that reported clinical screening tools for nonadherence and etiologic studies that investigated factors that were independently associated with nonadherence measured by LC-MS/MS. These studies were identified through a systematic literature search. See Supplementary Table 2-4 and Supplemental Figure 1 for further details on this search and the selection process. After identification of potentially suitable variables, a final selection of the variables was made based on availability in the dataset and clinical availability in the hospital setting as well as in general practice, which will facilitate future widespread use of the screening tool.

Missing data

For both the derivation and validation dataset there was a considerable amount of missing data for the variables of interest, including systematically missing data for office heart rate in the validation set (Table 1). A complete case analysis excluding these patients would yield loss of efficiency and would provide biased results, since missing data rarely occur completely at random and are usually dependent on the outcome (22).

Therefore, missing data were handled using 10-fold multilevel multiple imputation with fully conditional specification to take the uncertainty of imputed values into account (jomoImpute-algorithm in R, mitml package). The choice of 10 imputations was based on simulation studies that showed that there tends to be little or no practical benefit to using more than 10 imputations (23). The resulting 10 completed datasets were analyzed separately and the results were combined using Rubin's rules (24,25).

Table 1. Baseline characteristics of the derivation set and validation set.

	University Medical Center Utrecht		**University Hospital Birmingham**	
	n = 495	Missings	n = 240	Missings
Clinical characteristics				
Male sex	250 (51%)	0 (0%)	123 (51%)	0 (0%)
Age (years)	57 ± 14	0 (0%)	57 ± 14	0 (0%)
Current smoker	53 (11%)	0 (0%)	39 (24%)	75 (31%)
Body mass index (kg/m2)	28.2 ± 5.3	5 (1%)	32.2 ± 5.9	65 (27%)
History of cardiovascular disease	133 (27%)	0 (0%)	58 (24%)	0 (0%)
Laboratory values				
Estimated GFR (ml/min/1.73m2)	81 ± 27	22 (4%)	75 ± 30	17 (7%)
HbA1c (mmol/mol)	39 ± 9	22 (4%)	46 ± 16	115 (48%)
Total cholesterol (mmol/L)	5.2 ± 1.2	22 (4%)	5.0 ± 1.3	58 (24%)
LDL-cholesterol (mmol/L)	3.1 ± 1.0	13 (3%)	3.0 ± 1.1	66 (28%)
HDL-cholesterol (mmol/L)	1.4 ± 0.4	22 (4%)	1.2 ± 0.4	60 (25%)
Triglycerides (mmol/L)	1.7 ± 1.2	22 (4%)	1.9 ± 2.0	58 (24%)
Blood pressure				
Office systolic blood pressure (mmHg)	165 ± 28	1 (0%)	165 ± 27	1 (0%)
Office diastolic blood pressure (mmHg)	94 ± 14	1 (0%)	93 ± 18	3 (1%)
Office heart rate (bpm)	73 ± 13	3 (1%)	NA	240 (100%)
Ambulatory systolic blood pressure (mmHg)	138 ± 19	18 (4%)	153 ± 20	199 (83%)
Ambulatory diastolic blood pressure (mmHg)	82 ± 11	18 (4%)	88 ± 13	199 (83%)
Medication use				
Antihypertensive medication tablets (n/day)	3 (1-12)	0 (0%)	3 (1-10)	0 (0%)
class A	336 (68%)	0 (0%)	210 (88%)	0 (0%)
class B	204 (41%)	0 (0%)	104 (43%)	0 (0%)
class C	314 (63%)	0 (0%)	194 (81%)	0 (0%)
class D	253 (51%)	0 (0%)	147 (61%)	0 (0%)
class E	122 (25%)	0 (0%)	139 (58%)	0 (0%)

Table 1. Continued.

	University Medical Center Utrecht		University Hospital Birmingham	
	n = 495	Missings	n = 240	Missings
Nonadherence	93 (19%)	0 (0%)	105 (44%)	0 (0%)
Partial nonadherence	73 (15%)	0 (0%)	37 (15%)	0 (0%)
Full nonadherence	20 (4%)	0 (0%)	67 (28%)	0 (0%)

All data in n (%) or mean ± standard deviation. GFR, glomerular filtration rate (calculated with Chronic Kidney Disease Epidemiology Collaboration [CKDEPI] formula), LDL, low-density lipoprotein, HDL, high-density lipoprotein, NA, Not Available, class A, ACE-inhibitors, angiotensin receptor blockers and direct renin inhibitors, class B, beta-blockers, class C, calcium channel blockers, class D, diuretics, class E, others

Statistical analyses

The clinical screening tool was developed and validated using the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) criteria (26).

Model derivation

Model derivation was performed by multivariable logistic regression, including the seven pre-specified clinical variables as described above. No stepwise variable selection was performed as this would increase the risk of selecting spurious variables (overfitting) and an increased risk for failing to include important variables (underfitting) (26). Continuous variables were truncated to the 1st and 99th percentile to limit influence of outliers. Next, by comparing Akaike's Information Criterion (AIC), we tested whether logarithmic or quadratic transformations of continuous variables improved model fit (27). The final model coefficients were estimated using penalized estimation methods using an L2 quadratic (i.e., "ridge") penalty to further prevent overfitting (28,29).

Model validation

Internal validity of the model was assessed with a calibration plot showing the agreement between the observed frequencies of nonadherence and the pooled probabilities of nonadherence of the 10 imputed datasets. Discrimination of the model was assessed by the ROC-curve and c-statistic that was obtained using bootstrapping with 1000 bootstrap samples. External validity of the model was tested in the Heartlands Hospital population. To adjust for variation in the underlying prevalence of nonadherence, the intercept of the derived model was recalibrated such that the mean predicted probability equals the observed prevalence in the validation set (30). In addition to discrimination and calibration, test characteristics (sensitivity, specificity, positive and negative predictive value) for different cut-off values of the predicted probability were determined. These estimates and their standard errors were logit transformed, pooled by using Rubin's rules, and then back transformed (25). The final model was presented after pooling the recalibrated intercepts and shrunken beta coefficients. All analyses were conducted with R statistical software version 4.0.3 (R Development Core Team, Vienna, Austria).

Results

Baseline characteristics and prevalence of nonadherence

In Table 1, the baseline characteristics based on the observed, non-imputed data of patients in the derivation and validation population are provided. In the derivation set, patients smoked less often and on average had a lower BMI and more often a history of CVD, compared to patients in the validation set. In the 495 UMCU patients with uncontrolled hypertension whose blood sample underwent LC-MS/MS analysis, the prevalence of nonadherence was 19% (fully non-adherent 4%, partially non-adherent 15%). The prevalence of nonadherence, determined based on LC-MS/MS in urine samples, among 240 patients recruited in the Heartlands Hospital was 44% (fully non-adherent 28%, partially non-adherent 15%). As Supplemental Table 5 illustrates, the percentage of fully adherent patients decreases as the number of prescribed antihypertensive drug classes increases.

Development and internal validation of the diagnostic model

Table 2 shows the pooled model coefficients and corresponding odds ratios. Logarithmic or quadratic transformations of continuous predictors did not improve the model fit. The model formula that was used to estimate probabilities of nonadherence is shown in Supplemental Table 6. Internal validation showed good agreement between the predicted probabilities and observed frequencies of nonadherence (Supplemental Figure 2A). The discriminative ability of the diagnostic tool in the development dataset was fair with a c-statistic of 0.73 (95% confidence interval (CI) 0.66 – 0.79) (Supplemental Figure 2B).

Table 2. Model coefficients and odds ratios.

	Coefficient	Odds ratio (95% CI)
Intercept	-6.5909	
Age (per year)	-0.0269	0.97 (0.95–0.99)
Sex (female)	-0.0359	0.96 (0.59-1.57)
Office systolic blood pressure (mmHg)	0.0215	1.02 (1.01-1.03)
Office heart rate (beats/min)	0.0053	1.01 (0.99-1.02)
Number of tablets (n/day)	0.3028	1.35 (1.16-1.58)
History of CVD (yes)	-0.1977	0.82 (0.46-1.47)
BMI (kg/m^2)	0.0614	1.06 (1.01-1.12)

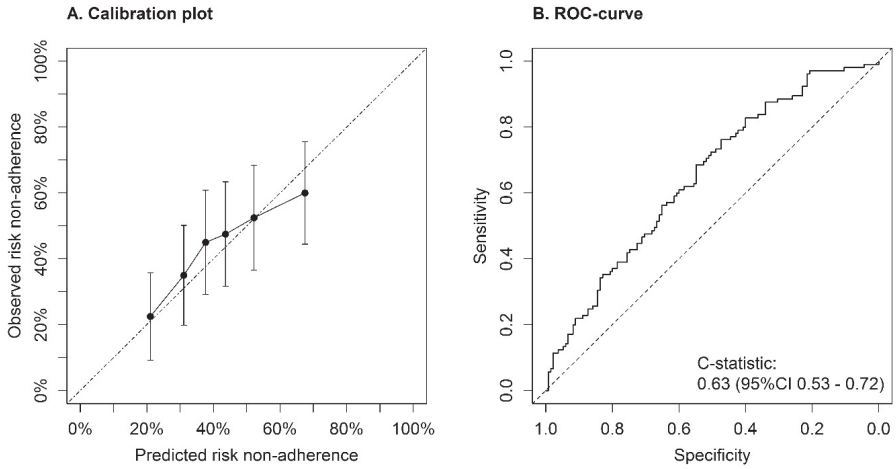
CVD, cardiovascular disease; BMI, body mass index

External validation of the diagnostic model

Figure 1A shows good agreement between predicted probabilities and observed frequencies of nonadherence after adjustment of the intercept (mean correction factor 0.394). This indicates that, after adjusting the mean predicted risk to the observed

risk in the validation set, the clinical screening tool could be applied to populations with uncontrolled hypertension with different overall prevalence of nonadherence. Discriminative performance of the diagnostic model in the Heartlands Hospital population was poor with a c-statistic of 0.63 (95% CI 0.53 – 0.72) (Figure 1B).

Figure 1. External validation of the clinical screening tool for nonadherence.



A. Plot of external calibration of clinical screening tool in Heartlands Hospital population showing the agreement between predicted and observed probabilities of nonadherence after recalibration. **B.** Receiver operating characteristics (ROC) curve showing the discriminative performance of the diagnostic tool.

Table 3 shows the test characteristics and proportion of patients spared testing for cut-off values of the predicted probability of nonadherence between 10 – 25%. This range was chosen because it showed the highest sensitivities and negative predictive values; characteristics that are desirable when the purpose of the diagnostic tool is to rule out nonadherence. The proportion of patients spared LC-MS/MS testing reflects the proportion of patients with a predicted probability equal or below the cut-off value in which (according to this screening tool) no further testing is needed. Predicted probability cut-off values of 15%-22.5% prevented testing in 5% (95% CI 2 - 8%) to 15% (95% CI 10 – 20%) of the patients with uncontrolled hypertension, carrying sensitivities between 97% (64 - 100) and 90% (80 - 95), and negative predictive values between 74% (10 - 99) and 70% (50 - 85) (Table 3).

Table 3. Test characteristics and proportion of patients spared chemical adherence testing.

	Cut-off value of predicted probability						
	10.0%	12.5%	15.0%	17.5%	20.0%	22.5%	25.0%
Sensitivity	0.99 (0.53 - 1.00)	0.98 (0.53 - 1.00)	0.97 (0.64 - 1.00)	0.95 (0.73 - 0.99)	0.93 (0.82 - 0.97)	0.90 (0.80 - 0.95)	0.86 (0.77 - 0.92)
Specificity	0.00 (0.00 - 1.00)	0.03 (0.00 - 0.74)	0.06 (0.02 - 0.18)	0.10 (0.05 - 0.18)	0.15 (0.09 - 0.23)	0.19 (0.12 - 0.27)	0.24 (0.17 - 0.34)
Positive predictive value	0.44 (0.37 - 0.50)	0.44 (0.37 - 0.50)	0.44 (0.38 - 0.51)	0.45 (0.38 - 0.51)	0.46 (0.39 - 0.52)	0.46 (0.39 - 0.53)	0.47 (0.40 - 0.54)
Negative predictive value	0.18 (0.00 - 1.00)	0.65 (0.00 - 1.00)	0.74 (0.10 - 0.99)	0.72 (0.23 - 0.96)	0.72 (0.45 - 0.89)	0.70 (0.50 - 0.85)	0.70 (0.53 - 0.83)
Proportion of patients spared testing (%)	1 (0 - 3)	3 (0 - 5)	5 (2 - 8)	8 (4 - 12)	12 (7 - 17)	15 (10 - 20)	20 (14 - 25)

The proportion of patients spared intensive testing is the proportion of patients with a predicted probability equal to or below the cut-off value. Estimates and corresponding Bootstrap-based 95% confidence intervals are presented for different cut-off values of the predicted probability by the diagnostic tool.

Discussion

In this cross-sectional, diagnostic study, we report the development and external validation of a screening tool, based on seven objective and easy-to-collect clinical variables, for estimating nonadherence to antihypertensive drugs in patients with uncontrolled hypertension. Validation showed good agreement between model predictions and observed frequencies of nonadherence. However, the discriminative ability of the screening tool was insufficient to reliably distinguish between adherence and nonadherence in patients with uncontrolled hypertension.

This is one of the first studies describing the development and validation of a clinical screening tool for biochemically confirmed adherence to antihypertensive drugs. In 2017, Gupta et al. also developed and validated two diagnostic models for biochemically confirmed nonadherence in patients with suboptimal BP control (15). These models, based on a smaller set of model parameters compared to this study, showed somewhat higher c-statistics upon external validation (0.710 and 0.708). However, Gupta et al. used selected study populations, including patients referred for chemical adherence testing, for both the development and validation of the models. Therefore, their results cannot be generalized to all patients referred with uncontrolled hypertension. Moreover, the lack of reporting of their model coefficients makes it impossible to validate their model externally, let alone use it in clinical practice.

The poor discriminative power of our model in external validation can probably be explained by differences in patient selection between the derivation and validation population. Whereas for the development of the model, all consecutive patients visiting the outpatient clinic of the UMCU were systematically screened by LC-MS/MS, patients in the Heartlands Hospital population were only subjected to drug screening when either the clinician suspected therapy nonadherence or when patients fulfilled the criteria for apparent resistant hypertension. As the clinician's suspicion of nonadherence is likely based (in part) on clinical characteristics included in the diagnostic model, only patients with a high probability of nonadherence were referred for drug screening. This probably resulted in partial verification bias (31) and underestimation of discriminative ability. For the screening tool to be clinically relevant and reliable, the use of an unselected population of patients with uncontrolled hypertension is essential for the development of the model, as this is the population in which the screening tool will ultimately be applied. Thus, further evaluation of the screening tool developed in the current study in unselected populations of patients with uncontrolled hypertension would be appropriate. Although this may result in a more reliable estimate of discriminative power of the tool, this measure will be at most 0.73 (c-statistic internal validation), indicating moderate discrimination (32).

Another explanation for the inadequate discrimination of the diagnostic model described in this study could be the homogeneous clinical characteristics of the patients in the Heartlands Hospital population compared to the UMCU population. Consequently, these patients had fewer distinguishing factors for predicting higher or lower probabilities of nonadherence. Also, adherence in the derivation population was assessed by LC-MS/MS in plasma compared to urine in the validation population. There is evidence that LC-MS/MS in urine may be less accurate than in serum for a number of compounds (33,34), especially for the evaluation of substances with low bioavailability, low renal excretion or high metabolism rate, which probably led to misclassification of nonadherence and eventually the discriminative ability of the model.

To establish an easily applicable screening tool that can reliably select patients with a very low probability of nonadherence, who would consequently not need to be exposed to a costly chemical adherence test, the tool requires a high negative predictive value to prevent non-adherent patients from not being identified. However, the validated screening tool presented here had poor discrimination resulting in a negative predictive value of 74% at best. Such a negative predictive value means that in case of a negative test result of the screening tool there is still a 26% chance of nonadherence. These results are in line with findings from previous studies reporting on tools to predict nonadherence in patients with uncontrolled hypertension (12,13). In summary, these results indicate that it is not possible to sufficiently accurately predict whether a patient will be adherent with antihypertensive treatment based on a combination of either clinical characteristics or self-reported barriers to medication adherence. This emphasizes the need for direct and objective chemical adherence testing in routine clinical practice.

The current study had several strengths, including the identification of clinical model parameters through a systematic literature search and the use of penalized estimation methods, both reducing the risk of overfitting. Another strength of this study is that clinical variables were routinely collected, resembling daily clinical practice, which is essential in a diagnostic study. Also, in contrast to previous studies on diagnostic models for nonadherence in patients with uncontrolled hypertension, we were able to externally validate the developed screening instrument. External validation is required to guarantee generalizability and should be done before a diagnostic model can be applied in clinical practice (26). In our case, external validation revealed an insufficient discriminatory power of the model which would otherwise have stayed unnoticed and therefore emphasizes the importance of external validation.

Limitations of the study should also be considered. The validation set had a relatively small sample size, which may have increased the risk of biased and imprecise estimates of model performance. Previous simulation studies indicated that validation studies need at least 100 events to provide reliable results (35,36). Although our study meets this condition (105 events), external validation in larger datasets would be preferable.

Another limitation is that some of the clinical variables in the validation set contained a considerable amount of missing values, including systematically missing data on the clinical variable heart rate. However, we applied multilevel multiple imputation to handle these missing data and thereby reduced the risk of bias and improved efficiency for the analysis (37). Furthermore, data on number of comorbidities, socio-economic status, and experience of side effects from antihypertensive drugs, which have previously been described as being associated with nonadherence (38) and thus could improve model performance, were not available in both datasets. Lastly, we acknowledge that, despite being an objective and direct method, there are limitations to the use of LC-MS/MS to diagnose adherence to antihypertensive drugs. For example, due to the long half-life of several antihypertensive drugs (e.g. amlodipine; half-life 30-50 hours (39)) these drugs would remain detectable in the patient's plasma or urine long after the last ingestion. This means that intermittent nonadherence could have been missed. Moreover, patients that only take their medications before visiting the doctor's office ('white-coat adherence') (37) are likely to be classified as biochemically adherent despite being non-persistent. Also, the LC-MS/MS methods used for this study, particularly the one used for the Heartlands Hospital population, could only measure a limited number of antihypertensive drugs. Although this assay still covers the majority of routinely prescribed antihypertensive drugs (40), this may have resulted in an underestimation of nonadherence to antihypertensive drugs.

In conclusion, the combination of seven easy-to-measure clinical variables is not sufficient to discriminate adherent from non-adherent individuals with uncontrolled hypertension to safely reduce the number of biochemical adherence tests. This emphasizes the complex nature of nonadherence behavior, which cannot simply be captured by a few clinical characteristics, and thus the need for objective chemical drug tests in patients with uncontrolled hypertension.

References

1. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016 Aug;134(6):441–50.
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet (London, England). 2012 Dec;380(9859):2095–128.
3. Mazzaglia G, Ambrosioni E, Alacqua M, Filippi A, Sessa E, Immordino V, et al. Adherence to antihypertensive medications and cardiovascular morbidity among newly diagnosed hypertensive patients. Circulation. 2009 Oct;120(16):1598–605.
4. Sokol MC, McGuigan KA, Verbrugge RR, Epstein RS. Impact of medication adherence on hospitalization risk and healthcare cost. Med Care. 2005 Jun;43(6):521–30.
5. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Drug adherence in hypertension: from methodological issues to cardiovascular outcomes. J Hypertens. 2017 Jun;35(6):1133–44.
6. Corrao G, Parodi A, Nicotra F, Zambon A, Merlino L, Cesana G, et al. Better compliance to antihypertensive medications reduces cardiovascular risk. J Hypertens. 2011 Mar;29(3):610–8.
7. Lee H, Yano Y, Cho SMJ, Heo JE, Kim D-W, Park S, et al. Adherence to Antihypertensive Medication and Incident Cardiovascular Events in Young Adults With Hypertension. Hypertens (Dallas, Tex 1979). 2021 Apr;77(4):1341–9.
8. Berra E, Azizi M, Capron A, Høieggen A, Rabbia F, Kjeldsen SE, et al. Evaluation of Adherence Should Become an Integral Part of Assessment of Patients With Apparently Treatment-Resistant Hypertension. Hypertens (Dallas, Tex 1979). 2016 Aug;68(2):297–306.
9. Pandey A, Raza F, Velasco A, Brinker S, Ayers C, Das SR, et al. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension. J Am Soc Hypertens. 2015 Jun;9(6):420-426.e2.
10. Lam WY, Fresco P. Medication Adherence Measures: An Overview. Biomed Res Int. 2015;2015:217047.
11. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
12. Krousel-Wood M, Joyce C, Holt EW, Levitan EB, Dornelles A, Webber LS, et al. Development and evaluation of a self-report tool to predict low pharmacy refill adherence in elderly patients with uncontrolled hypertension. Pharmacotherapy. 2013 Aug;33(8):798–811.
13. Steiner JF, Ho PM, Beaty BL, Dickinson LM, Hanratty R, Zeng C, et al. Sociodemographic and clinical characteristics are not clinically useful predictors of refill adherence in patients with hypertension. Circ Cardiovasc Qual Outcomes. 2009 Sep;2(5):451–7.
14. Gao W, Liu H, Ge C, Liu X, Jia H, Wu H, et al. A Clinical Prediction Model of Medication Adherence in Hypertensive Patients in a Chinese Community Hospital in Beijing. Am J Hypertens. 2020 Jul;
15. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Maresova V, et al. Risk Factors for Nonadherence to Antihypertensive Treatment. Hypertens (Dallas, Tex 1979). 2017 Jun;69(6):1113–20.
16. Van Der Sande NGC, Blankestijn PJ, Visseren FLJ, Beeftink MM, Voskuil M, Westerink J, et al. Prevalence of potential modifiable factors of hypertension in patients with difficult-to-control hypertension. J Hypertens. 2019 Feb;37(2):398–405.
17. De Geest S, Zullig LL, Dunbar-Jacob J, Helmy R, Hughes DA, Wilson IB, et al. ESPACOMP Medication Adherence Reporting Guideline (EMERGE). Ann Intern Med. 2018 Jul;169(1):30–5.

18. Lane D, Lawson A, Burns A, Azizi M, Burnier M, Jones DJL, et al. Nonadherence in Hypertension: How to Develop and Implement Chemical Adherence Testing. Hypertens (Dallas, Tex 1979). 2021 Nov;HYPERTENSIONAHA12117596.

19. Punt AM, Stienstra NA, van Kleef MEA, Lafeber M, Spiering W, Blankestijn PJ, et al. Screening of cardiovascular agents in plasma with LC-MS/MS: A valuable tool for objective drug adherence assessment. J Chromatogr B, Anal Technol Biomed life Sci. 2019 Jul;1121:103–10.

20. Lawson AJ, Shipman KE, George S, Dasgupta I. A Novel "Dilute-and-Shoot" Liquid Chromatography-Tandem Mass Spectrometry Method for the Screening of Antihypertensive Drugs in Urine. J Anal Toxicol. 2016;40(1):17–27.

21. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representat. Eur Heart J. 2016 Aug;37(29):2315–81.

22. Donders ART, van der Heijden GJMG, Stijnen T, Moons KGM. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol. 2006 Oct;59(10):1087–91.

23. Schafer JL. Multiple imputation: a primer. Stat Methods Med Res. 1999 Mar;8(1):3–15.

24. Rubin DB. Multiple imputation for survey nonresponse. New York: Wiley; 1987.

25. Wood AM, Royston P, White IR. The estimation and use of predictions for the assessment of model performance using large samples with multiply imputed data. Biom J. 2015 Jul;57(4):614–32.

26. Moons KGM, Altman DG, Reitsma JB, Ioannidis JPA, Macaskill P, Steyerberg EW, et al. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. Ann Intern Med. 2015 Jan;162(1):W1-73.

27. Steyerberg EW. Clinical prediction models. Springer; 2019.

28. Steyerberg EW, Eijkemans MJ, Harrell FEJ, Habbema JD. Prognostic modelling with logistic regression analysis: a comparison of selection and estimation methods in small data sets. Stat Med. 2000 Apr;19(8):1059–79.

29. Pavlou M, Ambler G, Seaman SR, Guttmann O, Elliott P, King M, et al. How to develop a more accurate risk prediction model when there are few events. BMJ. 2015 Aug;351:h3868.

30. Janssen KJM, Moons KGM, Kalkman CJ, Grobbee DE, Vergouwe Y. Updating methods improved the performance of a clinical prediction model in new patients. J Clin Epidemiol. 2008 Jan;61(1):76–86.

31. Whiting PF, Rutjes AWS, Westwood ME, Mallett S. A systematic review classifies sources of bias and variation in diagnostic test accuracy studies. J Clin Epidemiol. 2013 Oct;66(10):1093–104.

32. Alba AC, Agoritsas T, Walsh M, Hanna S, Iorio A, Devereaux PJ, et al. Discrimination and Calibration of Clinical Prediction Models: Users' Guides to the Medical Literature. JAMA. 2017 Oct;318(14):1377–84.

33. Ritscher S, Georges C, Wunder C, Wallemacq P, Persu A, Toennes SW. Assessment of adherence to diuretics and β-blockers by serum drug monitoring in comparison to urine analysis. Blood Press. 2020 May;1–8.

34. Ritscher S, Hoyer M, Georges C, Wunder C, Wallemacq P, Persu A, et al. Benefit of serum drug monitoring complementing urine analysis to assess adherence to antihypertensive drugs in first-line therapy. PLoS One. 2020;15(8):e0237383.

35. Vergouwe Y, Steyerberg EW, Eijkemans MJC, Habbema JDF. Substantial effective sample sizes were required for external validation studies of predictive logistic regression models. J Clin Epidemiol. 2005 May;58(5):475–83.

36. Collins GS, Ogundimu EO, Altman DG. Sample size considerations for the external validation of a multivariable prognostic model: a resampling study. Stat Med. 2016 Jan;35(2):214–26.

37. White IR, Carlin JB. Bias and efficiency of multiple imputation compared with complete-case analysis for missing covariate values. Stat Med. 2010 Dec;29(28):2920–31.

38. van der Laan DM, Elders PJM, Boons CCLM, Beckeringh JJ, Nijpels G, Hugtenburg JG. Factors associated with antihypertensive medication nonadherence: a systematic review. J Hum Hypertens. 2017 Nov;31(11):687–94.

39. Meredith PA, Elliott HL. Clinical pharmacokinetics of amlodipine. Clin Pharmacokinet. 1992 Jan;22(1):22–31.

40. NHS digital. Prescription Cost Analysis - England, 2018 [PAS] [Internet]. NHS. 2018 [cited 2021 Aug 31]. Available from: https://digital.nhs.uk/data-and-information/publications/statistical/prescription-cost-analysis/2018

Supplemental Material

Supplemental Table 1. Antihypertensive medications and/or their metabolites examined by the liquid chromatography-tandem mass spectrometry technique according to hypertension clinic.

Antihypertensive medication (metabolites)	UMC Utrecht	Heartlands Hospital
Aliskiren	X	-
Amlodipine	X	X
Atenolol	X	X
Barnidipine	X	-
Bisoprolol	X	X
Bumetanide	X	-
Spironolacton (Canrenone)	X	X
Captopril	X	-
Carvedilol	X	-
Chlortalidone	X	-
Diltiazem	X	X
Doxazosin	X	X
Enalapril (Enalaprilat)	X	X
Eplerenone	X	-
Felodipine	X	X
Fosinopril (Fosinoprilat)	X	-
Furosemide	X	X
Hydrochlorothiazide	X	X
Indapamide	X	X
Irbesartan	X	X
Labetalol	X	X
Lercanidipine	X	-
Lisinopril	X	X
Losartan (Losartan COOH)	X	X
Methyldopa	X	-
Metoprolol	X	X
Nebivolol	X	X
Nicardipine	X	-
Nifedipine	X	X
Olmesartan	X	-
Perindopril (Perindoprilat)	X	X
Propranolol	X	-
Quinalapril (Quinaprilat)	X	-
Ramipril	X	X
Sotalol	X	-
Telmisartan	X	-
Triamterene	X	-
Valsartan	X	-
Verapamil	X	X
Candesartan	-	X
Moxinidine	X	X
Bendroflumethiazide	-	X

Supplemental Table 2. Systematic search strategy for selection of predictors.

PubMed	((((hypertens*[Title/Abstract]) OR (hypertension[MeSH Terms])) OR ((high[Title/Abstract]) AND (blood pressure[Title/Abstract])))) AND (((diagnos*[Title/Abstract]) OR (predict*[Title/Abstract])) AND (((model[Title/Abstract]) OR (clinical prediction rule[MeSH Terms])) OR (clinical decision tool[Title/Abstract])))) AND (((((adherence[Title/Abstract]) OR (compliance[Title/Abstract])) OR (nonadherence[Title/Abstract])) OR (patient compliance[MeSH Terms])) OR (medication adherence[MeSH Terms]))
Embase	(('hypertension'/exp OR hypertens*:ti,ab,kw OR (high:ti,ab,kw AND 'blood pressure':ti,ab,kw)) AND (('clinical decision rule'/exp OR 'prediction model'/exp) OR ((diagnos*:ti,ab,kw OR predict*:ti,ab,kw) AND model:ti,ab,kw)) AND ('adherence'/exp OR 'medication compliance'/exp OR adherence:ti,ab,kw OR compliance:ti,ab,kw OR nonadherence:ti,ab,kw)) AND 'article'/it
Cochrane Library	(((high):ti,ab,kw AND ("blood pressure"):ti,ab,kw) OR "hypertension":ti,ab,kw OR "hypertension":ti,ab,kw) AND (("clinical decision rule"):ti,ab,kw OR ("prediction model"):ti,ab,kw OR MeSH descriptor: [Clinical Decision Rules] explode all trees)) AND (("adherence"):ti,ab,kw OR ("compliance"):ti,ab,kw OR ("nonadherence"):ti,ab,kw OR MeSH descriptor: [Treatment Adherence and Compliance] explode all trees)

Supplemental Table 3. Eligible studies reporting clinical screening tools for nonadherence to antihypertensive drugs.

Study	Year	Country	N	Population	Determinants	Outcome measurement	Internally validated	Externally validated	Model	Model performance	Sensitivity, specificity
Steiner et al. [14]	2009	USA	17 177	Patients with hypertension	1. Age >55 2. White race 3. Marital status 4. Acculturation USA 5. Diabetes mellitus 6. CVD 7. Substance abuse 8. Alcohol abuse 9. Number of antihypertensive medication	Nonadherence: medication possession ratio (MPR)<0.8	No	No	Multivariable logistic regression model	C-statistic: 0.606 Hosmer-lemeshow X^2: 14.7, p=0.07	Sensitivity of 57% Specificity of 57% *(cut-off: 9 points)*
Krousel-Wood et al. [13]	2013	USA	394	Community-dwelling adults ≥65 years with uncontrolled hypertension	1. Missing taking medications when feeling better 2. Forget taking medication 3. Unsure about taking medications all of the time when worried about taking them for the rest of their life 4. Health limiting moderate activities a lot	Nonadherence: medication possession ratio (MPR)<0.8	Yes (bootstrapping + shrinkage)	No	Multivariable logistic regression model	C-statistic: 0.704 (95% CI 0.683–0.714) Hosmer-Lemeshow X^2: 1.238, p=0.743	Sensitivity: 67.4% Specificity: 67.8% *(cut-off: ≥1 point)*

Supplemental Table 3. *Continued.*

Study	Year	Country	N	Population	Determinants	Outcome measurement	Internally validated	Externally validated	Model	Model performance	Sensitivity, specificity
Gupta et al. [16]	2017	UK	676	Patients suspected of nonadherence	1. Age 2. Sex 3. Number of antihypertensive drugs 4. Use of diuretics	Nonadherence: LC-MS/MS antihypertensive drug < LOD	No	Yes, in Czech Republic dataset	Multivariable logistic regression model	AUC after external validation: 0.708	NR
Gao et al. [15]	2020	China	7638	Hypertensive patients	1.Types of antihypertensive drugs used in the year before the first prescription 2. Body weight 3. Smoking 4. Hospital visits in previous year 5. Days of medication use in previous year 6. Age 7. Diabetes 8. Follow-up rate	Nonadherence: proportion of days covered (PDC) by the antihypertensive medications ≤ 0.8	Yes by splitting their dataset into a training and test dataset	No	Decision-tree model	AUC: 0.810	Sensitivity: 0.78 Specificity: 0.69

Supplemental Table 4. Etiologic studies reporting independent factors associated with nonadherence to antihypertensive drugs.

	Sex	Age	Office systolic blood pressure	Office diastolic blood pressure	Elevated heart rate	History of chronic kidney disease	Number of antihypertensive drugs	Use of diuretics	Use of calcium channel blockers	Number of total pills prescribed
Ceral et al. [39]		X	X	X	X					
Strauch et al. [40]		X								
Brinker et al. [41]		X		X	X					
Lawson et al. [42]	X	X		X			X		X	X
Jung et al. [43]			X	X	X					
Hamdidouche et al. [44]			X	X			X			X
Candace et al. [45]			X					X		
Jager et al. [46]			X	X						
Avataneo et al. [47]			X	X	X					
Pandey et al. [10]					X	X				

Supplemental Table 5A. Adherence according to the number of prescribed antihypertensive drug classes for UMC Utrecht population.

	Number of prescribed antihypertensive drug classes				
Adherence	1 (n = 125)	2 (n = 142)	3 (n = 120)	4 (n = 80)	5 (n = 28)
Fully adherent	116 (93%)	116 (82%)	95 (79%)	60 (75%)	15 (54%)
Non-adherent	9 (7%)	26 (28%)	25 (21%)	20 (25%)	13 (46%)

All data in n (%). Non-adherent: at least one of the prescribed medications not detected

Supplemental Table 5B. Adherence according to the number of prescribed antihypertensive drug classes for Heartlands hospital population.

	Number of prescribed antihypertensive drug classes				
Adherence	1 (n = 18)	2 (n = 38)	3 (n = 65)	4 (n = 88)	5 (n = 31)
Fully adherent	12 (67%)	25 (66%)	36 (55%)	51 (58%)	11 (35%)
Non-adherent	6 (33%)	13 (34%)	29 (45%)	37 (42%)	20 (65%)

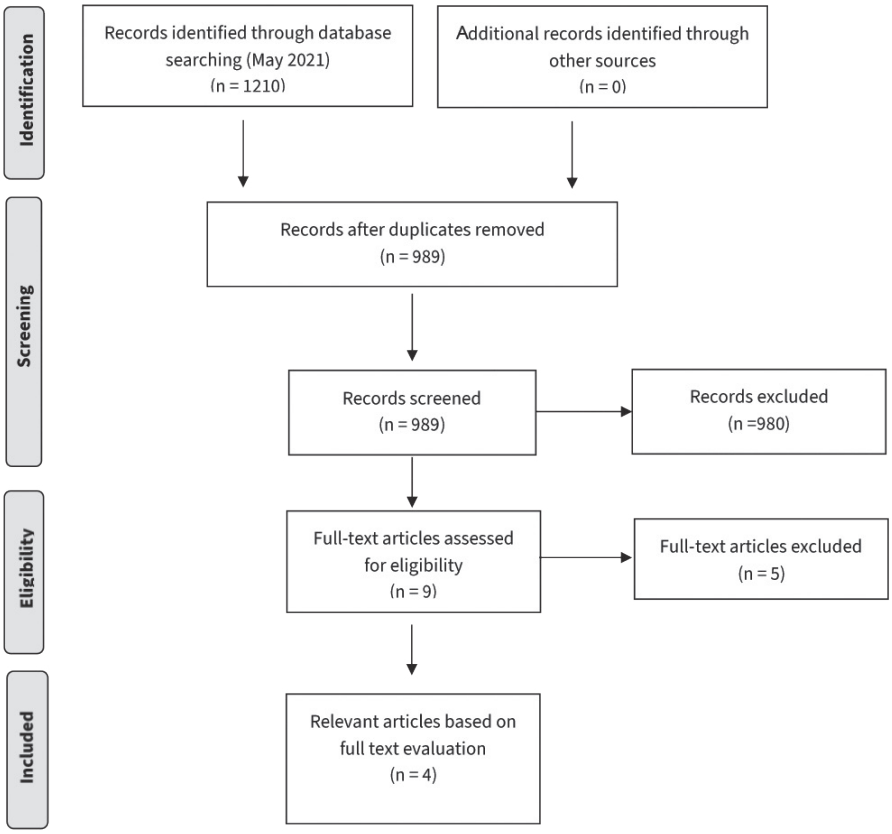
All data in n (%). Non-adherent: at least one of the prescribed medications not detected

Supplemental Table 6. Estimation of nonadherence to antihypertensive drugs for individual patients.

	Risk of nonadherence to antihypertensive drugs
Formula for risk estimation	(1/ 1 + e^-(-6.0001+ **A**)) * 100%
Linear predictor	**A =** 0.0195 * age (in years) - 0.0299 * male sex + 0.0173 * office systolic BP (in mmHg) – 0.0059 * office heart rate (beats/min) + 0.2551 * number of tablets (n/day) - 0.1780 * if history of CVD + 0.0549 * BMI(kg/m2) Coefficients after penalized estimation using an L2 quadratic (i.e., “ridge”) penalty are presented

BP = blood pressure; CVD = cardiovascular disease; BMI = body mass index

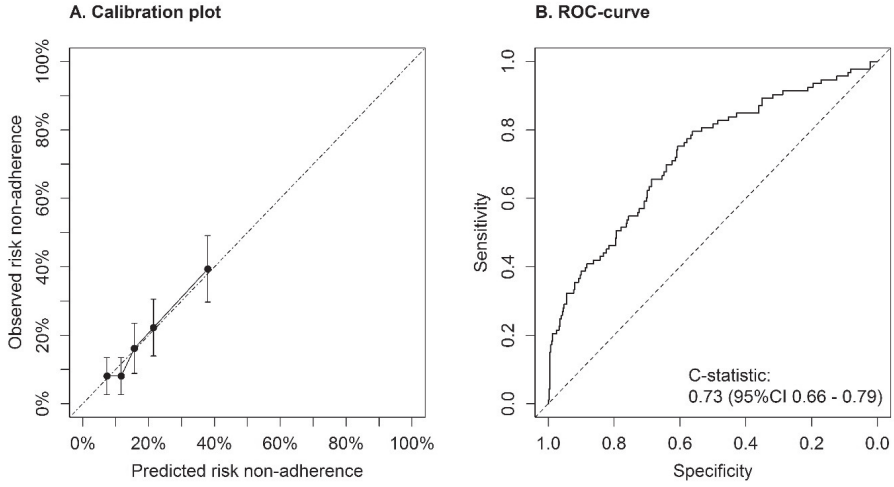
Supplemental Figure 1. Study selection.



Eligibility criteria

(a) Study population: participants with hypertension
(b) Cross-sectional or Longitudinal study design
(c) Outcome: adherence to antihypertensive drugs
(d) Model performance measures (c-statistic/AUC, calibration plot, Hosmer-Lemeshow test) with 95%CI should be reported
(e) Language: English or Dutch
(f) No geographical restrictions

Supplementary Figure 2. Internal validation of the clinical screening tool for nonadherence.



A. Plot of internal calibration of clinical screening tool in UMCU population showing the agreement between predicted and observed probabilities of nonadherence after recalibration. **B.** Receiver operating characteristics (ROC) curve showing the discriminative performance of the diagnostic tool.

References supplemental material

1. Steiner JF, Ho PM, Beaty BL, Dickinson LM, Hanratty R, Zeng C, et al. Sociodemographic and clinical characteristics are not clinically useful predictors of refill adherence in patients with hypertension. Circ Cardiovasc Qual Outcomes. 2009 Sep;2(5):451–7.
2. Krousel-Wood M, Joyce C, Holt EW, Levitan EB, Dornelles A, Webber LS, et al. Development and evaluation of a self-report tool to predict low pharmacy refill adherence in elderly patients with uncontrolled hypertension. Pharmacotherapy. 2013 Aug;33(8):798–811.
3. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Maresova V, et al. Risk Factors for Nonadherence to Antihypertensive Treatment. Hypertens (Dallas, Tex 1979). 2017 Jun;69(6):1113–20.
4. Gao W, Liu H, Ge C, Liu X, Jia H, Wu H, et al. A Clinical Prediction Model of Medication Adherence in Hypertensive Patients in a Chinese Community Hospital in Beijing. Am J Hypertens. 2020 Jul;
5. Ceral J, Habrdova V, Vorisek V, Bima M, Pelouch R, Solar M. Difficult-to-control arterial hypertension or uncooperative patients ? The assessment of serum antihypertensive drug levels to differentiate non-responsiveness from nonadherence to recommended therapy. Hypertens Res [Internet]. 2010;34(1):87–90. Available from: http://dx.doi.org/10.1038/hr.2010.183
6. Strauch B, Petrák O, Zelinka T, Rosa J, Somlóová Z, Indra T, et al. Precise assessment of noncompliance with the antihypertensive therapy in patients with resistant hypertension using toxicological serum analysis. J Hypertens. 2013 Dec;31(12):2455–61.
7. Brinker S, Mbbs AP, Ms CA, Price A, Raheja P, Arbique D, et al. Therapeutic Drug Monitoring Facilitates Blood Pressure Control in Resistant Hypertension. JAC [Internet]. 2014;63(8):834–5. Available from: http://dx.doi.org/10.1016/j.jacc.2013.10.067
8. Lawson AJ, Hameed MA, Brown R, Cappuccio FP, George S, Hinton T, et al. Nonadherence to antihypertensive medications is related to pill burden in apparent treatment-resistant hypertensive individuals. J Hypertens. 2020 Jun;38(6):1165–73.
9. Jung O, Gechter JL, Wunder C, Paulke A, Bartel C, Geiger H, et al. Resistant hypertension? Assessment of adherence by toxicological urine analysis. J Hypertens. 2013 Apr;31(4):766–74.
10. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Routine urinary detection of antihypertensive drugs for systematic evaluation of adherence to treatment in hypertensive patients. J Hypertens. 2017 Sep;35(9):1891–8.
11. McNaughton CD, Brown NJ, Rothman RL, Liu D, Kabagambe EK, Levy PD, et al. Systolic Blood Pressure and Biochemical Assessment of Adherence: A Cross-Sectional Analysis in the Emergency Department. Hypertens (Dallas, Tex 1979). 2017 Aug;70(2):307–14.
12. de Jager RL, van Maarseveen EM, Bots ML, Blankestijn PJ. Medication adherence in patients with apparent resistant hypertension: findings from the SYMPATHY trial. Br J Clin Pharmacol. 2018 Jan;84(1):18–24.
13. Avataneo V, De Nicolò A, Rabbia F, Perlo E, Burrello J, Berra E, et al. Therapeutic drug monitoring-guided definition of adherence profiles in resistant hypertension and identification of predictors of poor adherence. Br J Clin Pharmacol. 2018 Nov;84(11):2535–43.
14. Pandey A, Raza F, Velasco A, Brinker S, Ayers C, Das SR, et al. Comparison of Morisky Medication Adherence Scale with therapeutic drug monitoring in apparent treatment-resistant hypertension. J Am Soc Hypertens. 2015 Jun;9(6):420-426.e2.

CHAPTER 10

General discussion

General discussion

Despite being a largely controllable condition, the rates of awareness, treatment, and control of hypertension, defined as an office blood pressure (BP) above 140/90 mmHg, are disappointingly low (1,2). A recent comprehensive global analysis showed that the number of people aged 30–79 years with hypertension doubled from 1990 to 2019, from 331 (95% credible interval 306–359) million women and 317 (292–344) million men in 1990 to 626 (584–668) million women and 652 (604–698) million men in 2019 (3). Due to a growing and ageing population, this number is expected to rise further in the coming years. Since hypertension is one of the largest contributors to morbidity and mortality worldwide (4), this emphasizes the need for improvement throughout the entire process of screening, diagnosis, treatment, and follow-up of patients with (uncontrolled) hypertension. This thesis explored several approaches to improve this process.

Part I: Cardiovascular risk

Is there a role for genetics?
A crucial step in the management of patients with hypertension is the assessment of an individual's cardiovascular risk (5,6). This cardiovascular risk, combined with the patient's BP level and preferences, will influence the decision to initiate or intensify treatment. According to current hypertension guidelines, estimation of cardiovascular risk in patients with hypertension is preferably done on the basis of traditional risk factors including age, sex, smoking, cholesterol, and systolic BP, using the recently updated Systematic COronary Risk Evaluation (SCORE2) system (7). Hypertensive patients with documented cardiovascular disease (CVD) are automatically considered to be at very high or high 10-year cardiovascular risk (5–7). Although these high-risk patients show the greatest absolute reduction in cardiovascular outcomes with BP-lowering treatment, some of them continue to experience (recurrent) CVD despite lower BP levels (8). This is commonly referred to as residual risk (9).

There is ongoing interest to find other pathways explaining this residual CVD risk. One of these pathways is the one through genes. Over the past 15 years, substantial progress has been made in identifying genetic variants, also called single nucleotide polymorphisms (SNPs), associated with common polygenetic disorders such as hypertension. Since the first meta-analysis of genome-wide significant association studies (GWAS) for BP (10), hundreds of novel SNPs have been discovered. Subsequently, the use of a polygenic risk score (PRS), which aggregates the small effects of multiple SNPs into one weighted score, has been proposed to help personalize preventive measures. Because genetic risk is accumulated continuously over the entire lifespan, genetic risk scores may offer a potential advantage over traditional CVD risk factors. Yet, the clinical utility of a PRS to further improve CVD risk estimation beyond traditional risk factors, and to define treatment thresholds based on the PRS results is subject to substantial debate (11,12).

Polygenic risk scores have shown some potential to improve the prediction of CVD risk for primary prevention, but the incremental prediction accuracy is relatively modest and needs further evaluation (12–14). The extent to which genetic variants are associated with recurrent cardiovascular events in individuals with established CVD is less clear and was studied in **chapter 2** of this thesis. A PRS for systolic BP composed of 425 SNPs was not statistically significantly associated with recurrent cardiovascular events (HR 1.04 per SD increase in SBP PRS; 95%CI 0.98–1.10). Although the effect of including such a PRS in existing risk prediction scores for patients with established CVD remains to be determined, these findings do not support routine collection of genetic information in clinical care for high-risk hypertensive patients, which is in line with current guideline recommendations (5,6,15).

Sodium excretion in patients with vascular disease: the mechanism behind the J-shape
Sodium is essential in the regulation of important physiological functions such as nutrient absorption and maintaining fluid balance. However, in the vast majority of populations around the world, sodium intake is high (estimated global average: 3.66-4.00 g/day), and greatly exceeds the minimal physiological need (<2.00 g/day) (16–18). This is concerning because several cross-sectional observational studies have shown that in both patients with and without vascular disease, sodium excretion (as a proxy for sodium intake) was positively related to BP (19,20). In addition, sodium reduction trials found a significant dose-response relation between the size of sodium reduction and BP response (21). Because of the known relation between BP and CVD, this has led to the notion that "the lower is better". However, this predicate was questioned after publication of several studies, primarily in high-risk cohorts, suggesting that sodium excretion below and above an optimal range of 4.00–4.99 g/day is associated with increased risk for CVD, including heart failure, and mortality (22–25). In **chapter 3**, we also reach this conclusion. There has been much discussion about why some studies find a higher risk of CVD at lower sodium levels. J-shaped curves have been observed in many other areas of research, including BP interventions and obesity (26,27). All studies describing a J-shaped relation, including the one described in **chapter 3** of this thesis, are based on observational data. Causal inferences from observational data are prone to reverse causality and index event bias, two types of unmeasured confounding bias (28). Approaches to account for these types of biases, such as exclusion of patients with events within the first years of follow-up and exclusion of patients treated with loop diuretics (a proxy for a diagnosis of congestive heart failure), did not materially alter the shape of the relations in these studies. Nevertheless, it must be recognized that these approaches are not absolute and therefore bias cannot be completely ruled out. Another possible explanation for the increased risk of CVD and mortality in the lower ranges of sodium intake includes measurement error. Most studies examining the relation between sodium excretion and CVD used spot urine in combination with a formula to estimate 24-hour sodium excretion, rather than the potentially laborious and expensive but more accurate reference standard of multiple non-consecutive 24-hour urine collections. Such formula-based approaches

for estimating 24-hour salt excretion have been found to produce a high degree of variability that could lead to misclassification of salt intake at the individual level (as also shown in **chapter 7**) (29–31). A recent study found that this error could even change the shape of the dose-response curve (32).

A definitive answer to the question of which sodium target is most beneficial for patients with vascular disease requires a large-scale and long-term randomized controlled trial examining different targets in this patient population. An approach to such a trial is the recently published Salt Substitute and Stroke Study (SSaSS), a large cluster randomized controlled trial conducted in China. This study showed that using a salt substitute that was low in sodium and high in potassium resulted in a lower rate of stroke than the use of regular salt (rate ratio 0.86; 95%CI 0.77-0.96) (28). Although this study provides some answers, it remains unclear whether the effect can be attributed to lower sodium intake, higher potassium intake or both. Moreover, it is questionable whether the effect of using a salt substitute can simply be generalized to other countries. Unlike other developed countries, where about 80% of the salt consumed is added by the food industry, in China about 80% of the salt is added by the consumers during cooking and thus easier to adjust (33). Therefore, the effect of using a salt substitute is likely to be greater in the Chinese population.

Part II: Monitoring

Although the most beneficial sodium target is still under debate, a substantial proportion of the world's population exceeds even the optimal range suggested by the observational studies that demonstrated a J-shaped relationship between sodium intake and CVD (34). Therefore, reducing sodium intake currently remains one of the most important non-pharmacological interventions for lowering BP and reducing cardiovascular risk. However, changing dietary salt intake is difficult because most individuals are still poorly aware of their daily salt intake, with individuals who reported that they were on a low-salt diet actually showing salt intake levels similar to those who were not on a low-salt diet (35). To create awareness and encourage salt restriction, monitoring of salt intake in clinical practice or by the patient at home is essential.

How to monitor dietary salt intake?

Accurate monitoring of salt intake is challenging because in most circumstances an individuals' diets varies widely from meal to meal, day to day, and has other temporal sources of variation related to factors such as seasonal availability of foods, holidays, cultural practices, and climate change (causing altered food availability). Often, daily salt intake is estimated by food frequency questionnaires, dietary recalls, or 24-hour recall. However, these methods are prone to recall bias as they rely on the honesty and memory of the patient as well as the skill of the physician (36,37). In addition, it can be difficult to estimate the sodium content of the foods consumed, particularly the amount of salt

used during cooking or added as table salt. A more reliable method for assessing salt intake is 24-hour urine collection. This method is based on the assumption that 24-hour urinary salt excretion always reflects 90-95% (38) of ingested sodium and 70% of ingested potassium (39), given that the urine is collected on a representative day and that urine collection is complete. Since salt intake varies from day to day, it was determined that the collection of at least three non-consecutive, high-quality 24-hour urine collections is considered the gold standard for accurate assessment of an individual's salt intake (38). However, the costs and significant patient burden associated with this method limit easy application in clinical practice and in the patient's home. As a result, a variety of equations were developed to estimate 24-hour salt excretion from spot urine samples. Although collecting a spot urine sample is much easier than collecting a 24-hour urine sample, the most commonly used formulas for estimating the 24-hour urine salt excretions have a problematic bias and should therefore not be used for monitoring of salt intake at the individual level (**chapter 7**) (29,40).

A more reliable spot-based method to monitor salt intake is quantification of the urinary sodium-to-potassium (Na/K) ratio (**chapter 7**). Renal handling of potassium and sodium are highly interconnected; when potassium intake is high, it increases natriuresis by modulating the sodium-chloride cotransporter in the distal convoluted tubule (41). High sodium intake may thus differently affect BP and long-term outcome when combined with high or low potassium intake (42). Advantages of the urinary Na/K ratio include that the protective effects of both low sodium and high potassium intake are incorporated into one parameter and that conversion to 24-hour values is not required, which is likely to improve the accuracy and facilitate use in daily clinical practice. Data on how the dietary Na/K ratio can be best estimated are scarce. A previous study suggested that the mean urine Na/K ratio of six randomly collected spot urine samples was strongly related to the gold standard of seven consecutive 24-hour urine collections, indicating that repeated collection of spot urine samples might be a valid approach (43).

Self-monitoring of the Na/K ratio can be performed using urine containers, which are sent to the hospital laboratory where the Na/K ratio is measured. However, due to the time required for the collection, delivery, and measurement, this method may not provide prompt enough feedback to patients to further adjust their diet. The emergence of digital health, combining digital technologies and health care, offers a potential solution to this limitation through the development of portable self-monitoring devices. A recent study that examined such a device for self-monitoring of the urinary Na/K ratio found a trend toward greater reductions in the urinary Na/K ratio for the self-monitoring group compared to the control group (44). Further improvement in self-monitoring of salt intake lies in combining the use of such devices with educational programs and adequate feedback.

App-assisted HBPM: how can it be implemented in clinical practice?
Another challenge in the management of hypertension is monitoring of BP in a large number of hypertensive patients. Also here, digital health could provide promising support. Self-monitoring of BP by using mobile health applications actively engages the patient in their own hypertension management which could improve the detection and control rates of hypertension. An example of such a mobile health application is the EmmaHBPM application (Medicine Men, Utrecht, the Netherlands) that facilitates the storage and teletransmission of BP readings obtained outside of the clinic to the treating physician (**chapter 5**).

Together with 24-hour ambulatory BP monitoring (ABPM), HBPM is a well-accepted approach for measuring BP outside of the clinic (6,45). However, the utility of these methods, both in a conventional and app-based approach, for guiding patient care has been widely debated (46,47). There is controversy about which method is better for determining out-of-office BP. Both methods typically obtain more measurements than in the clinic setting, allow for identification of white coat hypertension and masked hypertension, and are superior to office BP for predicting end-organ damage and CVD (48,49). Yet, the prognostic value of 24-hour ABPM is more strongly supported compared to HBPM (50). Moreover, an important difference between ABPM and HBPM is that ABPM measures BP for 24 hours during daily routine activities, including sleep, whereas HBPM measures BP at specific times in the morning and evening for several days under standardized conditions (in resting sitting position and at home). These different measurement conditions lead to systematic differences in out-of-office BP measured by each method (**chapter 5**), which has also led to different thresholds for the diagnosis of hypertension in current guidelines (5,6). Despite considerable differences in absolute BP values, HBPM has been shown to be of diagnostic value in screening for uncontrolled and masked hypertension (**chapter 5**) (51). In clinical practice, therefore, ABPM and HBPM should be considered complementary techniques; with HBPM being used preferentially for screening and follow-up (also because of its better tolerability and lower cost) and ABPM being used to confirm the diagnosis suggested by screening.

When HBPM is used, training of patients, use of standardized procedures, and use of a validated oscillometric device are essential to ensure accurate and reliable BP measurements. Current European guidelines recommend to measure home BP twice in the morning and twice in the evening for a duration of preferably seven consecutive days (6,45). Although a 7-day protocol will provide adequate precision in home BP, a longer HBPM protocol may also lower patient's adherence to this protocol. Therefore, aiming for a shorter, less intensive and more patient-friendly protocol, might enhance adherence and is thus preferred in clinical practice and by patients (52). Shortening the HBPM protocol to three days will only result in a minor increase in variability of home BP compared to the 7-day protocol (**chapter 6**) (53,54). Thus, to improve usefulness and patient adherence, the HBPM protocol as recommended by the current European

guidelines should be reduced to three consecutive days. The relatively small benefit in precision from extending this protocol is likely to be useful only when average home BP is around diagnostic or treatment thresholds (**chapter 6**).

The usefulness of HBPM could further be improved by addition of telemonitoring, whereby patients receive feedback from healthcare professionals based on BP measurements sent electronically (55) (also possible with the EmmaHBPM application described in **chapter 5 and 6**). A telemedicine-based approach has been shown to significantly improve BP control compared to usual care (56,57). In addition, telemedicine allows effective health care to be provided in situations where close interaction is not possible, such as the recent COVID-19 pandemic or natural disasters.

Traditionally, BP telemonitoring relies on the use of automated upper-arm BP monitors which frequently lead to discomfort and sleep disturbance. Therefore, cuffless BP telemonitoring tools are becoming increasingly popular among patients (58). Examples of such cuffless tools are wearable devices including wrist-worn fitness bands and smartwatches. The most common types of smartwatches measure BP through the determination of pulse wave transit time, which is the time required for the arterial pressure wave to travel from the left ventricle to the wrist. Blood pressure can be estimated from pulse wave transit time because pulse wave transit time shortens when BP increases. Recently, several wearable device have been shown to perform well against current out-of-office BP measurement approaches and have received approval from the Food and Drug Administration as medical devices (59–61). Albeit wearables can bring several practical and clinical benefits in hypertension management, cultural (e.g. lack of knowledge on telemonitoring), structural (e.g. lack of integration of telehealth services into existing national health systems), and financial (e.g. high costs of wearables without reimbursement) barriers currently prevent widespread use of such technology among doctors and patients. Therefore, future research should focus on how to best incorporate these new technologies into clinical practice for both physicians and patients.

Part III: Medication adherence

Can we recognize non-adherent patients based on clinical characteristics and should we do so in clinical practice?

Medication nonadherence is a major barrier to achieving treatment goals for individuals with hypertension. About half of patients prescribed antihypertensive medications become non-adherent within the first year (62). Because nonadherence to antihypertensive drugs worsens health outcomes, clinicians have long sought to identify patients who do not take their medications as prescribed. Early studies showed that physician judgement on nonadherence is “no better than a coin toss” with less than 50% of the patients being correctly classified as non-adherent (63). In an effort to make the assessment of adherence more objective, many studies have attempted to identify

easy-to-collect clinical predictors of adherence (64). Among the characteristics most often proposed to predict adherence are age, sex, race or ethnicity, and substance abuse (**chapter 9**). Although these characteristics are statistically significant associated with nonadherence, the associations are often weak in epidemiologic terms, with odds ratios around 1.5. As also shown in **chapter 9** of this thesis, combining such easy-to-collect clinical characteristics into a multivariate regression model often result in poorly discriminating models (65,66). Besides missing the diagnosis nonadherence in a considerable number of patients, the use of such models, especially when they include characteristics such as race or substance abuse, may even perpetuate health disparities. For example, several studies have shown that clinicians are significantly more likely to view African-Americans, patients of lower socio-economic status, and substance abusers as less adherent to treatment than individuals without those characteristics (67–69). If these characteristics are converted into therapeutic decisions, patients may be denied effective treatments on the basis of anticipated nonadherence.

Adherence behavior should be viewed as a complex construct of beliefs and behaviors that interact with each other and are influenced by social and environmental considerations as well as clinical care. Patient-reported attitudes and beliefs could therefore be better predictors of adherence, but structured assessment of these attitudes is time-consuming, requires experienced personnel, and is therefore difficult to implement in daily clinical practice. Hence, rather than attempting to infer a behavior such as adherence from a set of weakly associated predictors, a far more useful clinical strategy is to measure adherence directly. According to the current hypertension guidelines, chemical drug screening by liquid chromatography tandem mass spectrometry (LC-MS/MS) in plasma or urine is one of the most reliable methods for direct measurement of medication adherence (6,70).

How to implement chemical drug screening of antihypertensive drugs in clinical practice?
Chemical drug screening is often done in a qualitative manner by using the limit of detection (LOD), the lowest amount of a drug in a sample which can be detected as a cut-off. This qualitative assay will report a positive result if the antihypertensive drug concentration is above the LOD, classifying the patient as adherent, and a negative result if it is below the LOD, classifying the patient as non-adherent. Since the LOD depends on the sensitivity of the LC-MS/MS assay and not on the exposure to the drug, the definition of adherence can be misleading and wrongly classify a patient as adherent or not (71). For example, if patients take their drugs very irregularly, the concentration in urine or plasma will remain above the LOD, especially when drugs with long half-lives are prescribed.

An alternative and possible improvement to the qualitative method could be quantitative analysis of antihypertensive drugs, taking into account the actual plasma concentration of the drug. However, there is currently no reference standard for quantitative drug screening. Several approaches have been proposed so far. One includes comparing

the measured drug concentration to the established peak (C_{max}) or trough (C_{min}) concentration of a given drug, as these may provide a putative range indicative of regular intake (72). However, as also shown in **chapter 8** of this thesis, such metrics are highly variable, indicating that a drug concentration above the C_{max} or below the C_{min} could also be the result of a deviation from typical pharmacokinetics. Since this increases the risk of classifying patients who are adherent as non-adherent, the C_{max} or C_{min} are not suitable as cut-off values for the quantitative assessment of chemical adherence. Other approaches to quantitative assessment include the use of cut-offs on an individual (rather than population) basis, using the prescribed dose alongside scoped pharmacokinetics to estimate a dose-dependent concentration (73). Such an approach might be a promising method for future quantitative drug screening. However, until this method is fully established, a conservative approach, to prevent false accusations, is preferred and chemical assessment of adherence should be performed qualitatively.

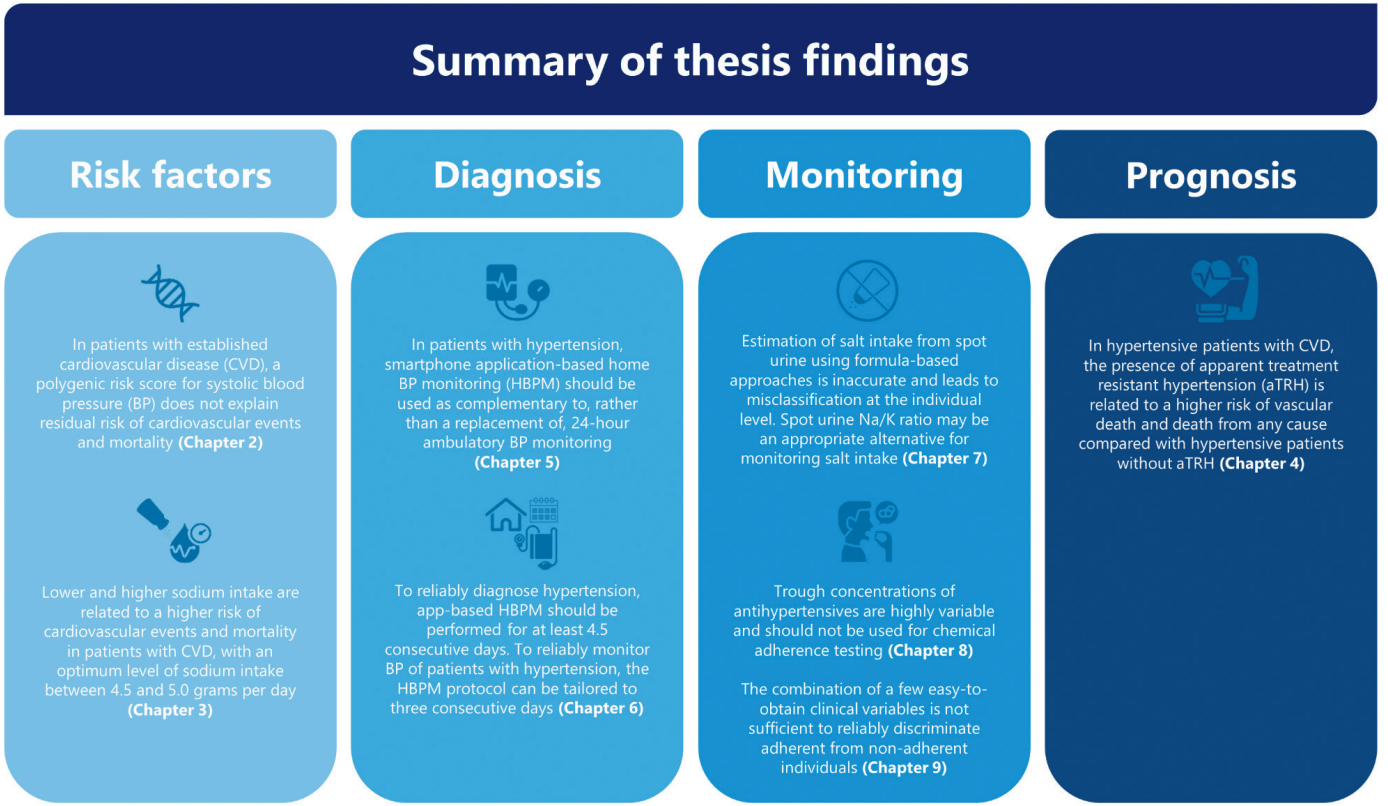
To date, chemical drug screening is used in a limited number of secondary and tertiary healthcare settings. For example, in the Netherlands, this method is only available in two centers. This limited availability may be due to lack of awareness, lack of qualified personnel, and the relatively high cost of the LC-MS/MS instrumentation (≈€450.000) (74). Ideally, objective chemical drug screening should become a part of the standard work-up of all patients with uncontrolled hypertension. However, since current availability and relatively high cost currently limit this goal, the European Society of Cardiology/ European Society of Hypertension recommend measuring at least patients who meet the definition of apparent resistant hypertension, which is considered a high-risk subgroup of hypertensive patients (**chapter 4**) (6,74). Assessment of adherence in these patients should be performed early in the diagnostic work-up, before expensive investigations, invasive device-based treatments (i.e., renal denervation or baroreflex amplification) as well as treatment escalations/dose alterations (75). This is considered to be an effective and cost-saving strategy (76).

Although identification of nonadherence is an important step in the management of patients with uncontrolled hypertension, follow-up steps are needed to ultimately improve BP control (77). The second step includes communicating the results of the biochemical drug screening to the patient. To this end, a no-blame approach is recommended, whereby patients are encouraged to discuss the barriers that lead to nonadherence in their situation (78). Barriers to medication adherence can either be practical, such as side effects and costs, or perceptual, such as doubts about safety or efficacy of antihypertensive drugs or the consequences of the disease (79). After such barriers have become clear, the third and final step is to find a personalized solution to resolve the issues that lead to suboptimal adherence. The effect of this three-step approach on adherence and BP is currently being investigated in a single-blinded randomized controlled trial in patients with resistant hypertension; RHYME-RCT (Resistant Hypertension: Measure to ReaCh Targets, Dutch trial register NL6736) (80).

Concluding remarks

With hypertension remaining a leading risk factor for cardiovascular disease and morbidity and mortality worldwide, there exists a persistent and urgent need to more effectively manage this chronic disease and reduce its burden on the healthcare system. The results of this thesis provide several new insights into cardiovascular risk, monitoring, and medication adherence of patients with uncontrolled hypertension (**Figure 1**). Further improvement seems to lie in better measurements, not only of blood pressure but also the effect of lifestyle interventions, such as sodium reduction, and medication adherence. The emergence of digital health, including smartphone applications and wearable devices, contributes to improving these measurements and offers an important new strategy to more actively involve patients in their own hypertension management. With these and other technological advances aimed at involving the patient, the management of hypertension will become more accessible and efficient, likely leading to improvement in BP control.

Figure 1. Summary of thesis findings.



References

1. Beaney T, Schutte AE, Stergiou GS, Borghi C, Burger D, Charchar F, et al. May Measurement Month 2019: The Global Blood Pressure Screening Campaign of the International Society of Hypertension. Hypertens (Dallas, Tex 1979). 2020 Aug;76(2):333–41.
2. Lu J, Lu Y, Wang X, Li X, Linderman GC, Wu C, et al. Prevalence, awareness, treatment, and control of hypertension in China: data from 1·7 million adults in a population-based screening study (China PEACE Million Persons Project). Lancet (London, England). 2017 Dec;390(10112):2549–58.
3. Worldwide trends in hypertension prevalence and progress in treatment and control from 1990 to 2019: a pooled analysis of 1201 population-representative studies with 104 million participants. Lancet (London, England). 2021 Sep;398(10304):957–80.
4. Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation. 2016 Aug;134(6):441–50.
5. Whelton PK, Carey RM, Aronow WS, Casey DEJ, Collins KJ, Dennison Himmelfarb C, et al. 2017 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 P. J Am Coll Cardiol. 2018 May;71(19):e127–248.
6. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J. 2018 Sep;39(33):3021–104.
7. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021 Jul;42(25):2439–54.
8. Thomopoulos C, Parati G, Zanchetti A. Effects of blood pressure lowering on outcome incidence in hypertension. 1. Overview, meta-analyses, and meta-regression analyses of randomized trials. J Hypertens. 2014 Dec;32(12):2285–95.
9. Matsuura Y, Kanter JE, Bornfeldt KE. Highlighting Residual Atherosclerotic Cardiovascular Disease Risk. Arterioscler Thromb Vasc Biol. 2019 Jan;39(1):e1–9.
10. Levy D, Larson MG, Benjamin EJ, Newton-Cheh C, Wang TJ, Hwang S-J, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. BMC Med Genet. 2007 Sep;8 Suppl 1(Suppl 1):S3.
11. Elliott J, Bodinier B, Bond TA, Chadeau-Hyam M, Evangelou E, Moons KGM, et al. Predictive Accuracy of a Polygenic Risk Score-Enhanced Prediction Model vs a Clinical Risk Score for Coronary Artery Disease. JAMA. 2020 Feb;323(7):636–45.
12. Khera A V, Chaffin M, Aragam KG, Haas ME, Roselli C, Choi SH, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018 Sep;50(9):1219–24.
13. Sun L, Pennells L, Kaptoge S, Nelson CP, Ritchie SC, Abraham G, et al. Polygenic risk scores in cardiovascular risk prediction: A cohort study and modelling analyses. PLoS Med. 2021 Jan;18(1):e1003498.
14. Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults: Implications for Primary Prevention. J Am Coll Cardiol. 2018 Oct;72(16):1883–93.
15. Visseren FLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur J Prev Cardiol. 2022 Feb;29(1):5–115.
16. Thout SR, Santos JA, McKenzie B, Trieu K, Johnson C, McLean R, et al. The Science of Salt: Updating the evidence on global estimates of salt intake. J Clin Hypertens (Greenwich). 2019 Jun;21(6):710–21.

17. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open. 2013 Dec;3(12):e003733.

18. World Health Organization (WHO). Guideline: sodium intake for adults and children. World Health Organization; 2012.

19. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. BMJ. 1988 Jul;297(6644):319–28.

20. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, et al. Association of urinary sodium and potassium excretion with blood pressure. N Engl J Med. 2014 Aug;371(7):601–11.

21. Filippini T, Malavolti M, Whelton PK, Naska A, Orsini N, Vinceti M. Blood Pressure Effects of Sodium Reduction: Dose-Response Meta-Analysis of Experimental Studies. Circulation. 2021 Feb;

22. Graudal N, Jürgens G, Baslund B, Alderman MH. Compared with usual sodium intake, low- and excessive-sodium diets are associated with increased mortality: a meta-analysis. Am J Hypertens. 2014 Sep;27(9):1129–37.

23. O'Donnell M, Mente A, Rangarajan S, McQueen MJ, Wang X, Liu L, et al. Urinary sodium and potassium excretion, mortality, and cardiovascular events. N Engl J Med. 2014 Aug;371(7):612–23.

24. Stolarz-Skrzypek K, Kuznetsova T, Thijs L, Tikhonoff V, Seidlerová J, Richart T, et al. Fatal and nonfatal outcomes, incidence of hypertension, and blood pressure changes in relation to urinary sodium excretion. JAMA. 2011 May;305(17):1777–85.

25. McCarron DA, Geerling JC, Alderman MH. Urinary sodium excretion measures and health outcomes. Vol. 393, Lancet (London, England). England; 2019. p. 1294–5.

26. Bhaskaran K, Dos-Santos-Silva I, Leon DA, Douglas IJ, Smeeth L. Association of BMI with overall and cause-specific mortality: a population-based cohort study of 3·6 million adults in the UK. lancet Diabetes Endocrinol. 2018 Dec;6(12):944–53.

27. Dorresteijn JAN, van der Graaf Y, Spiering W, Grobbee DE, Bots ML, Visseren FLJ. Relation between blood pressure and vascular events and mortality in patients with manifest vascular disease: J-curve revisited. Hypertens (Dallas, Tex 1979). 2012 Jan;59(1):14–21.

28. Dahabreh IJ, Kent DM. Index event bias as an explanation for the paradoxes of recurrence risk research. JAMA. 2011 Feb;305(8):822–3.

29. Zhou L, Tian Y, Fu J-J, Jiang Y-Y, Bai Y-M, Zhang Z-H, et al. Validation of spot urine in predicting 24-h sodium excretion at the individual level. Am J Clin Nutr. 2017 Jun;105(6):1291–6.

30. Vidal-Petiot E, Joseph A, Resche-Rigon M, Boutten A, Mullaert J, d'Ortho M-P, et al. External validation and comparison of formulae estimating 24-h sodium intake from a fasting morning urine sample. J Hypertens. 2018 Apr;36(4):785–92.

31. Cogswell ME, Wang C-Y, Chen T-C, Pfeiffer CM, Elliott P, Gillespie CD, et al. Validity of predictive equations for 24-h urinary sodium excretion in adults aged 18-39 y. Am J Clin Nutr. 2013 Dec;98(6):1502–13.

32. He FJ, Ma Y, Campbell NRC, MacGregor GA, Cogswell ME, Cook NR. Formulas to Estimate Dietary Sodium Intake From Spot Urine Alter Sodium-Mortality Relationship. Hypertens (Dallas, Tex 1979). 2019 Sep;74(3):572–80.

33. Anderson CAM, Appel LJ, Okuda N, Brown IJ, Chan Q, Zhao L, et al. Dietary sources of sodium in China, Japan, the United Kingdom, and the United States, women and men aged 40 to 59 years: the INTERMAP study. J Am Diet Assoc. 2010 May;110(5):736–45.

34. Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibzadeh S, Engell RE, et al. Global sodium consumption and death from cardiovascular causes. N Engl J Med. 2014 Aug;371(7):624–34.

35. Okuda N, Stamler J, Brown IJ, Ueshima H, Miura K, Okayama A, et al. Individual efforts to reduce salt intake in China, Japan, UK, USA: what did people achieve? The INTERMAP Population Study. J Hypertens. 2014 Dec;32(12):2385–92.

36. McLean RM, Farmer VL, Nettleton A, Cameron CM, Cook NR, Woodward M, et al. Twenty-Four-Hour Diet recall and Diet records compared with 24-hour urinary excretion to predict an individual's sodium consumption: A Systematic Review. J Clin Hypertens (Greenwich). 2018 Oct;20(10):1360–76.

37. McLean RM, Farmer VL, Nettleton A, Cameron CM, Cook NR, Campbell NRC. Assessment of dietary sodium intake using a food frequency questionnaire and 24-hour urinary sodium excretion: a systematic literature review. J Clin Hypertens (Greenwich). 2017 Dec;19(12):1214–30.

38. Campbell NRC, He FJ, Tan M, Cappuccio FP, Neal B, Woodward M, et al. The International Consortium for Quality Research on Dietary Sodium/Salt (TRUE) position statement on the use of 24-hour, spot, and short duration (<24 hours) timed urine collections to assess dietary sodium intake. J Clin Hypertens (Greenwich). 2019 Jun;21(6):700–9.

39. World Health Organization (WHO). Potassium intake for adults and children. World Heal Organ Geneva, Switz. 2012;1–52.

40. Mercado CI, Cogswell ME, Loria CM, Liu K, Allen N, Gillespie C, et al. Validity of predictive equations for 24-h urinary potassium excretion based on timing of spot urine collection among adults: the MESA and CARDIA Urinary Sodium Study and NHANES Urinary Sodium Calibration Study. Am J Clin Nutr. 2018 Sep;108(3):532–47.

41. Wang M-X, Cuevas CA, Su X-T, Wu P, Gao Z-X, Lin D-H, et al. Potassium intake modulates the thiazide-sensitive sodium-chloride cotransporter (NCC) activity via the Kir4.1 potassium channel. Kidney Int. 2018 Apr;93(4):893–902.

42. Aburto NJ, Hanson S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. BMJ. 2013 Apr;346:f1378.

43. Iwahori T, Ueshima H, Miyagawa N, Ohgami N, Yamashita H, Ohkubo T, et al. Six random specimens of daytime casual urine on different days are sufficient to estimate daily sodium/potassium ratio in comparison to 7-day 24-h urine collections. Hypertens Res. 2014 Aug;37(8):765–71.

44. Iwahori T, Ueshima H, Ohgami N, Yamashita H, Miyagawa N, Kondo K, et al. Effectiveness of a Self-monitoring Device for Urinary Sodium-to-Potassium Ratio on Dietary Improvement in Free-Living Adults: a Randomized Controlled Trial. J Epidemiol. 2018 Jan;28(1):41–7.

45. Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, et al. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. J Hypertens. 2021 Jul;39(7):1293–302.

46. Verdecchia P, Angeli F, Mazzotta G, Gentile G, Reboldi G. Home Blood Pressure Measurements Will Not Replace 24-Hour Ambulatory Blood Pressure Monitoring. Hypertens (Dallas, Tex 1979). 2009 Aug;54(2):188–95.

47. Parati G, Omboni S, Bilo G. Why Is Out-of-Office Blood Pressure Measurement Needed? Hypertens (Dallas, Tex 1979). 2009 Aug;54(2):181–7.

48. Peacock J, Diaz KM, Viera AJ, Schwartz JE, Shimbo D. Unmasking masked hypertension: prevalence, clinical implications, diagnosis, correlates and future directions. J Hum Hypertens. 2014 Sep;28(9):521–8.

49. Piper MA, Evans C V, Burda BU, Margolis KL, O'Connor E, Whitlock EP. Diagnostic and predictive accuracy of blood pressure screening methods with consideration of rescreening intervals: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2015 Feb;162(3):192–204.

50. Krist AH, Davidson KW, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for Hypertension in Adults: US Preventive Services Task Force Reaffirmation Recommendation Statement. JAMA. 2021 Apr;325(16):1650–6.

51. Guirguis-Blake JM, Evans C V, Webber EM, Coppola EL, Perdue LA, Weyrich MS. Screening for Hypertension in Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2021 Apr;325(16):1657–69.

52. Grant S, Hodgkinson JA, Milner SL, Martin U, Tompson A, Hobbs FR, et al. Patients' and clinicians' views on the optimum schedules for self-monitoring of blood pressure: a qualitative focus group and interview study. Br J Gen Pract J R Coll Gen Pract. 2016 Nov;66(652):e819–30.

53. Bello NA, Schwartz JE, Kronish IM, Oparil S, Anstey DE, Wei Y, et al. Number of Measurements Needed to Obtain a Reliable Estimate of Home Blood Pressure: Results From the Improving the Detection of Hypertension Study. J Am Heart Assoc. 2018 Oct;7(20):e008658.

54. Hodgkinson JA, Stevens R, Grant S, Mant J, Bray EP, Hobbs FDR, et al. Schedules for Self-monitoring Blood Pressure: A Systematic Review. Am J Hypertens. 2019 Mar;32(4):350–64.

55. Omboni S, McManus RJ, Bosworth HB, Chappell LC, Green BB, Kario K, et al. Evidence and Recommendations on the Use of Telemedicine for the Management of Arterial Hypertension: An International Expert Position Paper. Hypertens (Dallas, Tex 1979). 2020 Nov;76(5):1368–83.

56. Milani R V, Lavie CJ, Bober RM, Milani AR, Ventura HO. Improving Hypertension Control and Patient Engagement Using Digital Tools. Am J Med. 2017 Jan;130(1):14–20.

57. McLean G, Band R, Saunderson K, Hanlon P, Murray E, Little P, et al. Digital interventions to promote self-management in adults with hypertension systematic review and meta-analysis. J Hypertens. 2016 Apr;34(4):600–12.

58. Sim I. Mobile Devices and Health. N Engl J Med. 2019 Sep;381(10):956–68.

59. Kario K, Shimbo D, Tomitani N, Kanegae H, Schwartz JE, Williams B. The first study comparing a wearable watch-type blood pressure monitor with a conventional ambulatory blood pressure monitor on in-office and out-of-office settings. J Clin Hypertens (Greenwich). 2020 Feb;22(2):135–41.

60. Kuwabara M, Harada K, Hishiki Y, Kario K. Validation of two watch-type wearable blood pressure monitors according to the ANSI/AAMI/ISO81060-2:2013 guidelines: Omron HEM-6410T-ZM and HEM-6410T-ZL. J Clin Hypertens (Greenwich). 2019 Jun;21(6):853–8.

61. Islam SMS, Cartledge S, Karmakar C, Rawstorn JC, Fraser SF, Chow C, et al. Validation and Acceptability of a Cuffless Wrist-Worn Wearable Blood Pressure Monitoring Device Among Users and Health Care Professionals: Mixed Methods Study. JMIR mHealth uHealth. 2019 Sep;7(10):e14706.

62. Vrijens B, Vincze G, Kristanto P, Urquhart J, Burnier M. Adherence to prescribed antihypertensive drug treatments: longitudinal study of electronically compiled dosing histories. BMJ. 2008 May;336(7653):1114–7.

63. Meddings J, Kerr EA, Heisler M, Hofer TP. Physician assessments of medication adherence and decisions to intensify medications for patients with uncontrolled blood pressure: still no better than a coin toss. BMC Health Serv Res. 2012 Aug;12:270.

64. van der Laan DM, Elders PJM, Boons CCLM, Beckeringh JJ, Nijpels G, Hugtenburg JG. Factors associated with antihypertensive medication nonadherence: a systematic review. J Hum Hypertens. 2017 Nov;31(11):687–94.

65. Gupta P, Patel P, Strauch B, Lai FY, Akbarov A, Maresova V, et al. Risk Factors for Nonadherence to Antihypertensive Treatment. Hypertension [Internet]. 2017 Jun;69(6):1113–20. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L616372900

66. Steiner JF, Ho PM, Beaty BL, Dickinson LM, Hanratty R, Zeng C, et al. Sociodemographic and clinical characteristics are not clinically useful predictors of refill adherence in patients with hypertension. Circ Cardiovasc Qual Outcomes. 2009 Sep;2(5):451–7.

67. van Ryn M, Burke J. The effect of patient race and socio-economic status on physicians' perceptions of patients. Soc Sci Med. 2000 Mar;50(6):813–28.

68. Bogart LM, Catz SL, Kelly JA, Benotsch EG. Factors influencing physicians' judgments of adherence and treatment decisions for patients with HIV disease. Med Decis Mak an Int J Soc Med Decis Mak. 2001;21(1):28–36.

69. Lutfey KE, Ketcham JD. Patient and provider assessments of adherence and the sources of disparities: evidence from diabetes care. Health Serv Res. 2005 Dec;40(6 Pt 1):1803–17.

70. Unger T, Borghi C, Charchar F, Khan NA, Poulter NR, Prabhakaran D, et al. 2020 International Society of Hypertension Global Hypertension Practice Guidelines. Hypertens (Dallas, Tex 1979). 2020 Jun;75(6):1334–57.

71. Hamdidouche I, Jullien V, Boutouyrie P, Billaud E, Azizi M, Laurent S. Drug adherence in hypertension: from methodological issues to cardiovascular outcomes. J Hypertens. 2017 Jun;35(6):1133–44.

72. Sandbaumhüter FA, Haschke M, Vogt B, Bohlender JM. Indexed plasma drug concentrations for drug adherence screening in hypertensive patients. Ann Cardiol Angeiol (Paris). 2018 Jun;67(3):119–26.

73. Ritscher S, Hoyer M, Wunder C, Obermuller N, Toennes SW. Evaluation of the dose-related concentration approach in therapeutic drug monitoring of diuretics and beta-blockers - drug classes with low adherence in antihypertensive therapy. Sci Rep. 2019 Oct;9(1):15652.

74. Lane D, Lawson A, Burns A, Azizi M, Burnier M, Jones DJL, et al. Nonadherence in Hypertension: How to Develop and Implement Chemical Adherence Testing. Hypertens (Dallas, Tex 1979). 2022;79(1):12–23.

75. Patel P, Gupta PKC, White CMJ, Stanley AG, Williams B, Tomaszewski M. Screening for nonadherence to antihypertensive treatment as a part of the diagnostic pathway to renal denervation. J Hum Hypertens. 2016 Jun;30(6):368–73.

76. van Schoonhoven A V, van Asselt ADI, Tomaszewski M, Patel P, Khunti K, Gupta P, et al. Cost-Utility of an Objective Biochemical Measure to Improve Adherence to Antihypertensive Treatment. Hypertens (Dallas, Tex 1979). 2018 Nov;72(5):1117–24.

77. Peeters LEJ, van der Net JB, Schoenmakers-Buis K, van der Meer IM, Massey EK, van Dijk L, et al. Introducing the importance and difficulties of a three-step approach to improve nonadherence to antihypertensive drugs: a case series. J Hypertens. 2022 Jan;40(1):189–93.

78. NICE: National Institute for Health and Clinical Excellence. Medicines adherence: involving patients in decisions about prescribed medicines and supporting adherence [Internet]. Clinical guideline [CG76]. 2009. Available from: https://www.nice.org.uk/guidance/cg76

79. Linn AJ, van Weert JC, Schouten BC, Smit EG, van Bodegraven AA, van Dijk L. Words that make pills easier to swallow: a communication typology to address practical and perceptual barriers to medication intake behavior. Patient Prefer Adherence. 2012;6:871–85.

80. Netherlands Trial Register. RHYME-RCT: Resistente HYpertensie: MEten voor Resultaat op Controle Tensie [Internet]. 2018. Available from: https://www.trialregister.nl/trial/6736

APPENDICES

Summary

Hypertension, characterized by a persistently elevated blood pressure (BP) in the vascular system, is a major risk factor for the development and progression of cardiovascular disease (CVD) and the leading risk factor for all-cause mortality. Currently, its global prevalence is estimated at 31%. Although awareness and control of hypertension have improved in the last decade, the proportion of patients meeting BP targets has stagnated, with about 50% of hypertensive patients still having uncontrolled hypertension. This thesis explores potential strategies in different aspects of hypertension management with the aim to further improve these control rates. Part I will focus on the cardiovascular risks of (causes of) uncontrolled BP in patients with established vascular disease, part II focuses on the monitoring of patients with (uncontrolled) hypertension, and part III will focus on the problem of medication non-adherence in these patients.

An important step in the management of patients with hypertension is the assessment of the individual's absolute cardiovascular risk. On the basis of this risk, together with the severity of hypertension and the patient's preference, treatment is started or intensified. A particularly relevant group of patients at very high risk for future CVD includes patients who have already experienced a vascular event. Despite optimal treatment of the established risk factors such as smoking and hypercholesterolemia, some of these patients still experience recurrent CVD, a concept called "residual risk". In recent years, there has been increasing interest in clarifying the contribution of genes to this residual risk. Therefore, in chapter 2, we have studied the relation between multiple genetic variants associated with systolic BP, by means of a polygenic risk score (PRS), and the risk of recurrent cardiovascular events in 4416 patients with established vascular disease. During a follow-up of 11.7 years (IQR 9.2–15.0) this PRS for systolic BP was not significantly associated with recurrent CVD (HR 1.04 per SD increase in SBP PRS; 95%CI 0.98–1.10). These results suggests that genetically determined SBP does not explain the differences in residual cardiovascular risk in patients with established vascular disease.

Before initiating antihypertensive drug treatment in patients with hypertension, several non-pharmacological interventions need to be considered. Since higher sodium intake promotes fluid retention which results in higher BP levels and potassium mitigates this effect, important non-pharmacological interventions for the prevention and treatment of hypertension are the reduction of sodium intake and promotion of potassium intake. Most treatment guidelines advocate dietary sodium restriction to levels below 2 grams per day. However, these recommendations conflict with findings from several observational studies that demonstrate a J- or U-shaped relationship between estimated sodium intake and CVD risk, with lower and higher sodium intake both being associated with higher risk of CVD and all-cause mortality. In chapter 3, we assessed this relation between estimates of 24-hour sodium and potassium urinary excretion (as proxies for dietary intake) and the risk of recurrent major adverse cardiovascular events (MACE) and all-cause mortality in

patients with vascular disease who are most likely to receive recommendations regarding their dietary salt intake. We found that the relations between estimated 24-hour sodium urinary excretion and outcomes were J-shaped with nadirs of 4.59 gram/day for recurrent MACE and 4.97 gram/day for all-cause mortality. Interestingly, higher potassium urinary excretion was positively linearly related to the risk of both recurrent MACE (HR 1.25 per gram potassium excretion per day; 95%CI 1.13–1.39) and all cause-mortality (HR 1.13 per gram potassium excretion per day; 95%CI 1.03–1.25). Although these results do not support dietary sodium restriction to levels below 2 grams per day as a means of reducing the risk of recurrent CVD in patients with vascular disease, uncertainty remains whether these findings are due to a causal effect, measurement error, or statistical bias (e.g. reverse causality, residual confounding). This uncertainty provides a strong rationale for trials evaluating different salt intake targets.

A particularly severe form of hypertension is treatment resistant hypertension (TRH) defined as BP ≥140/90 mm Hg despite the use of antihypertensive drugs from ≥3 drug classes including a diuretic, or the use of ≥4 antihypertensive drugs irrespective of BP. The diagnosis of TRH requires exclusion of pseudo-resistance, including medication non-adherence, improper BP measurement, white coat hypertension, and treatment inertia. If pseudo-resistance is not excluded, the term apparent TRH (aTRH) is often used. In chapter 4 we evaluated the risk of subsequent vascular events and mortality in patients with aTRH. During a median follow-up of 9.0 years (interquartile range 4.8–13.1 years) patients with aTRH were at increased risk of cardiovascular mortality (HR 1.27;95%CI 1.03-1.56) and death from any cause (HR 1.25; 95%CI 1.07-1.45) but not recurrent MACE (HR 1.13;95%CI 0.95–1.34) compared with patients without aTRH. Moreover, patients with aTRH after a first cardiovascular event on average have a 6.4 year shorter median life expectancy free of recurrent cardiovascular disease. These findings, support the need for greater efforts toward improving BP control in patients with aTRH and established vascular disease.

Proper monitoring of BP control relies on accurate measurement of the BP level. Since office BP readings are associated with important limitations and can result in the misdiagnosis of hypertension, consequently leading to under- or overtreatment, out-of-office BP monitoring, either performed by home blood pressure monitoring (HBPM) or 24-hour ambulatory blood pressure measurement (ABPM), has become an important step in the management of hypertension. Although 24-hour ABPM has several unique advantages such as its capability of monitoring BP during sleep and daily activities, it is a burdensome and costly method that is not widely available, especially in primary care settings. HBPM is not only easier to use and less expensive than 24-hour ABPM, but also enables patients to take a greater role in self-management of their health, which may have a beneficial effect on medication adherence and BP control. However, the need for manual notation of self-measured BP by the patient, especially in the home setting, is prone to (intentional and unintentional) errors, which could compromise the reliability of HBPM. The introduction of smartphone application-assisted HBPM, in which

BP measurements taken with a validated BP device can be automatically transferred to a smartphone application, might reduce measurement error and stimulate widespread use of HBPM in clinical practice. Chapter 5 evaluated how such app-assisted HBPM compare to 24-hour ABPM and automated office BP in the measurement of BP and diagnosing sustained, white-coat, and masked hypertension in 113 patients with hypertension. The average 24-hour ABPM was 125.8±11.1/73.0±7.7 mm Hg compared to 140.6±13.6/81.5±9.8 mm Hg with app-assisted HBPM, 133.5±13.4/80.2±9.1 mm Hg with 30-minute BP, 136.7±16.0/81.3±10.7 mm Hg with attended office BP, and 135.3±15.3/80.6±9.7 mm Hg with unattended office BP monitoring. Diagnostic agreement between app-assisted HBPM and 24-hour ABPM for diagnosing sustained, white-coat, and masked hypertension was fair to moderate (kappa statistic ranging from 0.34 to 0.40). App-assisted HBPM had high sensitivities (78-91%) and negative predictive values (90-97%) for diagnosing sustained and masked hypertension. These findings suggest that app-assisted HBPM should be used as complementary to, but not as a replacement of, ABPM.

Chapter 6 aimed to assess the number of BP measurement days needed for a reliable estimation of true home BP, defined as the expected BP level over time, and hypertension status, using the European guideline-based 7-day HBPM protocol as a reference. For this, data from 567 adults who performed a 7-day HBPM were analyzed. The average of 7 twice-daily BP measurements was at most 5.2/3.3 mmHg higher and 9.5/4.8 mmHg lower than the true home BP for 95% of the individuals. Reducing this protocol to 3 days increased these variabilities by 1.5/1.0 mmHg and 4.8/2.3 mmHg, respectively. For diagnosing home hypertension, there was good agreement with a minimum of 4.5 days of HBPM (κ-statistic 0.88; 95% CI: 0.82-0.94). Therefore, we conclude that twice-daily BP measurements for 3 consecutive days provide a reliable estimate of home BP. At least 4.5 consecutive days of HBPM are required for a reliable diagnosis of home hypertension.

Besides monitoring of BP, monitoring of lifestyle changes, such as sodium intake reduction, is also essential to further improve BP control. However, accurate monitoring of salt intake is challenging because salt intake varies widely from day to day and from meal to meal. At present, measurement of salt excretion in multiple non-consecutive 24-hour urinary collections is considered the gold standard for measuring dietary sodium intake. However, this method is burdensome, time-consuming, and error prone. Therefore, methods based on easy-to-collect spot urine samples are increasingly being studied. In chapter 7 we assessed and compared the validity of several formula-based approaches to estimate 24-hour urinary sodium and potassium excretion and the Na/K ratio from spot urine samples measured by a self-monitoring device under three different sodium diets using 24-hour urine collections as the reference. Twelve healthy volunteers were asked to adhere to 3 dietary sodium targets (3.3-5.0g/day, <3.3g/day, and >5.0g/day) for 3 consecutive weeks and to measure salt excretion daily in spot urine samples using a self-monitoring device. On day 7 of each week, 24-hour urine was collected to compare measured with estimated 24-hour salt excretion (by the Kawasaki, Tanaka, and

INTERSALT equations). Correlation coefficients relating measured and estimated 24-hour salt excretion were low and not significant for Kawasaki and INTERSALT, and moderate for the Tanaka equation (τ 0.56-0.64, $p<0.05$). Bland-Altman plots showed considerable differences between estimated and measured salt excretion across all salt diets. Over 40% of the participants showed an absolute difference between measured and estimated 24-hour sodium of more than 1000 mg/day. The correlation coefficients between 24-hour and spot Na/K ratio were 0.67, 0.94, and 0.85 ($p<0.05$), and mean differences were 0.59, 0.06, and 0.48 for the intermediate, low, and high sodium diets, respectively. Using spot Na/K ratio, 66% of the participants were classified into the matching 24-hour Na/K ratio tertiles. We, therefore, conclude that use of three commonly used equations that estimate 24-hour urinary salt excretion result in substantial bias, poor precision, and poor accuracy and are therefore not recommended. The Na/K ratio based on multiple casual urine samples may be a useful, low-burden, low-cost alternative method to 24-hour urine collection for monitoring daily salt intake.

Non-adherence to antihypertensive drugs is a major reason for not reaching BP targets, which needs to be recognized before patients are subjected to extensive diagnostic work-ups and invasive device-based therapies. Biochemical drug screening in plasma or urine by liquid chromatography tandem mass spectrometry (LC-MS/MS) is an objective method for medication adherence assessment. This method is often performed qualitatively with the purpose to detect the presence or absence of antihypertensive drugs or metabolites using the limit of detection (LOD), the lowest amount of a drug in a sample which can be detected. However, the LOD highly depends on the sensitivity of the analytical assay and not on the therapeutic range of the drug. Ongoing improvements of the analytical assay will therefore likely result in lower detection limits and might increase the risk of misclassification of partially non-adherent patients. Biochemical assessment of adherence may be improved by quantitative analysis, by comparing the measured plasma drug concentration with the trough concentration, the minimum plasma concentration at steady state. In chapter 8 we describe the results of a literature review and meta-analysis of pharmacokinetic studies to determine plasma population trough concentrations of amlodipine, hydrochlorothiazide and valsartan which could be used for this purpose. The pooled mean trough concentration was 9.2 ng/ml (95% confidence interval: 7.5–10.80) for amlodipine, 41.0 ng/ml (95%CI 17.4–64.7) for hydrochlorothiazide and 352.9 ng/ml (95%CI 243.5–462.3) for valsartan. Substantial heterogeneity was present for all three pooled estimates. These findings imply that use of the pooled trough concentration as a cut-off in the quantitative biochemical assessment of adherence can result in inaccurate diagnosis of (non-) adherence, which may seriously harm the patient-physician relationship, and is therefore not recommended.

Although chemical drug screening is one of the most reliable methods of assessing adherence, it is only used in a limited number of secondary and tertiary health care settings. This limited availability can be partially attributed to a lack of qualified personnel

and the relatively high cost of the LC-MS/MS instrumentation (≈ €450.000). To reduce healthcare costs and make chemical drug screening more accessible, a clinical screening tool that creates the opportunity to carefully identify patients with a low probability of non-adherence, and therefore do not need to undergo further testing, would be desirable. Therefore, in chapter 9, we developed a clinical screening tool for non-adherence in 495 patients with uncontrolled hypertension referred to the University Medical Center Utrecht. Non-adherence to antihypertensive drugs was diagnosed in 93 (19%) patients. The screening tool was developed by penalized logistic regression analyses including seven pre-specified easy-to-measure clinical variables: age, sex, body mass index (BMI, kg/m2), history of CVD (yes/no), office systolic BP (SBP, mmHg), office heart rate (beats/min) and total number of antihypertensive drug tablets. The screening tool was validated in 240 patients with uncontrolled hypertension referred to the Heartlands Hospital, United Kingdom. After recalibration of the model's intercept, predicted probabilities agreed well with observed frequencies. The c-statistic of the model was 0.63 (95%CI 0.53–0.72). Predicted probability cut-off values of 15%-22.5% prevented testing in 5%-15% of the patients, carrying sensitivities between 97% (64-100) and 90% (80-95), and negative predictive values between 74% (10-99) and 70% (50-85). Therefore, we conclude that a combination of seven clinical variables is not sufficient to reliably discriminate adherent from non-adherent individuals to safely reduce the number of chemical adherence tests. This emphasizes the complex nature of non-adherence behavior and thus the need for objective chemical adherence tests in patients with uncontrolled hypertension.

In conclusion, with hypertension being one of the largest contributors to morbidity and mortality worldwide, there is a persistent and urgent need to manage this chronic condition more effectively and reduce its burden on the healthcare system. The results of this thesis provide insight in cardiovascular risk, monitoring, and treatment adherence of patients with uncontrolled hypertension. Further improvement seems to lie in better measurements, not only of BP but also the effect of lifestyle interventions, such as sodium reduction, and treatment adherence.

Samenvatting (voor niet ingewijden)

Hypertensie is een aandoening die wordt gekarakteriseerd door een continue verhoogde druk in de slagaders. Hypertensie is een belangrijke risicofactor voor de ontwikkeling en progressie van hart- en vaatziekten en is wereldwijd de belangrijkste risicofactor voor sterfte. Momenteel komt hypertensie voor bij 31% van de wereldbevolking wat neerkomt op ongeveer 1.4 miljard volwassenen. Hoewel het bewustzijn en de behandeling van hypertensie de laatste jaren zijn verbeterd, is het percentage patiënten dat de bloeddrukstreefwaarden behaalt gestagneerd rond de 50%. In dit proefschrift onderzoeken wij mogelijke strategieën in verscheidene aspecten van het behandelproces van hypertensie met als doel het percentage patiënten met een bloeddruk onder de streefwaarde te verhogen. Deel 1 van dit proefschrift richt zich op de kans op hart- en vaatziekten die gepaard gaat met (oorzaken van) ongecontroleerde hypertensie in patiënten met reeds bestaand vaatlijden. Deel 2 van dit proefschrift richt zich op het monitoren van patiënten met (ongecontroleerde) hypertensie en deel 3 gaat over het identificeren van therapie-ontrouw bij deze patiënten.

Een eerste belangrijke stap in de behandeling van patiënten met hypertensie is het bepalen van de kans op het krijgen van een hartvaatziekte binnen 10 jaar. Op basis van dit risico, samen met de hoogte van de bloeddruk en de voorkeuren van de patiënt, kan worden besloten om een behandeling te starten of te intensiveren. Een bijzonder relevante groep van patiënten met een zeer hoog risico op toekomstige hart- en vaatziekten omvat patiënten die reeds een hart- of vaatziekte hebben doorgemaakt. Ondanks optimale behandeling van bewezen risicofactoren zoals roken en een hoog cholesterol, hebben sommige patiënten nog steeds een verhoogd risico om opnieuw een hart- of vaatziekte door te maken, dit wordt ook wel het ‘residuele risico’ genoemd. In de laatste jaren is er toenemende belangstelling voor de bijdrage van genen aan dit residuele risico. Om die reden hebben wij in hoofdstuk 2 het gecombineerde effect van meerdere genetische varianten, die verband houden met systolische bloeddruk (bovendruk), op het risico op een nieuwe hart- of vaatziekte in 4416 patiënten die eerder een hart- en/of vaatziekten hebben doorgemaakt onderzocht. Er werd geen verband gevonden tussen de combinatie van dergelijke genetische varianten en het risico op het ontstaan van een nieuwe hart- of vaatziekte. Verschillen in genetische varianten lijken dus geen verklaring te zijn voor de verschillen in het residuele risico op hart- en vaatziekten bij de onderzochte patiëntengroep.

Voordat een behandeling met bloeddrukverlagende geneesmiddelen bij patiënten met hypertensie wordt gestart, moeten verschillende niet-medicamenteuze behandelingen worden overwogen. Een hogere natriuminname leidt tot het vasthouden van meer vocht, hetgeen de bloeddruk kan verhogen, terwijl een hogere kaliuminname dit effect afzwakt. Daarom zijn belangrijke niet-medicamenteuze strategieën voor het voorkomen en behandelen van hypertensie de vermindering van de natriuminname en

het bevorderen van de kaliuminname. De meeste behandelrichtlijnen adviseren een beperking van de natriuminname tot minder dan 2 gram per dag. Deze aanbevelingen passen echter niet bij de bevindingen van verschillende observationele studies waarin een lager en hoger dan gemiddeld natriuminname beide samenhangen met een hoger risico op hart- en vaatziekten en sterfte. Een dergelijk verband noemen we ook wel J- of U-vormig. In hoofdstuk 3 onderzochten wij deze relatie tussen de geschatte 24-uurs uitscheiding van natrium en kalium in de urine (als benadering voor de zoutinname via de voeding) en het risico op een nieuwe hart- of vaatziekte en sterfte in patiënten met vaatziekten. Laatstgenoemde patiënten hebben een grote kans om aanbevelingen te krijgen om hun zoutconsumptie aan te passen. Wij vonden dat er in deze patiëntengroep eveneens sprake was van een J-vormig verband. Verder concludeerden wij dat een 24-uurs natriumuitscheiding die afwijkt van 4.59-4.97 gram/dag een verhoogd risico geeft op hart- en vaatziekten en sterfte. Echter, het is opvallend dat een hogere kaliumuitscheiding ook samenhing met een hoger risico op hart- en vaatziekten en sterfte. Deze bevindingen ondersteunen de aanbevelingen in de huidige behandelrichtlijnen niet, maar het blijft onzeker of deze bevindingen te wijten zijn aan een direct oorzakelijk verband, een meetfout of statistische vertekening.

Een bijzonder ernstige vorm van hypertensie is therapieresistente hypertensie, gedefinieerd als een bloeddruk hoger of gelijk aan 140/90 mm Hg ondanks het gebruik van 3 of meer bloeddrukverlagende geneesmiddelen, waaronder een plasmedicijn, of het gebruik van 4 of meer bloeddrukverlagende geneesmiddelen, ongeacht de hoogte van de bloeddruk. Om de diagnose therapieresistente hypertensie te kunnen stellen is het belangrijk om uit te sluiten dat er sprake is van therapie-ontrouw, een foutieve bloeddrukmeting of witte jassen hypertensie (hoge bloeddruk door aanwezigheid van medisch personeel tijdens de meting). Indien therapieontrouw niet kan worden uitgesloten, wordt er gesproken van schijnbaar therapieresistente hypertensie. In hoofdstuk 4 hebben we gekeken naar het risico op nieuwe hart- of vaatziekten en sterfte in patiënten met schijnbaar therapieresistente hypertensie en reeds bestaand vaatlijden. Ten opzichte van patiënten zonder schijnbaar therapieresistente hypertensie hadden patiënten met schijnbaar therapieresistente hypertensie een sterk verhoogd risico op overlijden (door een nieuwe hart- of vaatziekte). Tevens bleken patiënten met schijnbaar therapieresistente hypertensie gemiddeld 6.4 jaar korter te leven dan patiënten zonder schijnbaar therapieresistente hypertensie. Deze bevindingen benadrukken de noodzaak tot verbetering van de bloeddrukcontrole bij patiënten met schijnbaar therapieresistente hypertensie en reeds bestaand vaatlijden.

Goede monitoring van hypertensie vereist een nauwkeurige meting van de bloeddruk. Bloeddrukmetingen die zijn verricht in de spreekkamer kunnen vaak minder betrouwbaar zijn en kunnen leiden tot een onjuiste diagnose van hypertensie en daarmee een verhoogd risico op hart- en vaatziekten. Daarom heeft het meten van de bloeddruk buiten de spreekkamer, door middel van geprotocolleerde thuismetingen (tweemaal daags

meten voor een duur van 7 dagen) of een 24-uurs meting, een belangrijke plaats gekregen in de behandeling van hypertensie. Hoewel een 24-uurs meting verschillende voordelen heeft, zoals de mogelijkheid om de bloeddruk tijdens de slaap en dagelijkse activiteiten te meten, is het een belastende en kostbare methode die niet overal beschikbaar is. Geprotocolleerde thuismetingen zijn gemakkelijker te gebruiken en minder kostbaar dan een 24-uurs meting en stellen een patiënt ook in staat om grotere rol te spelen in het management van hun eigen gezondheid. Laatstgenoemde kan een gunstig effect hebben op de therapietrouw en de bloeddrukcontrole. Echter, het meten van de bloeddruk door de patiënt zelf, vooral in de thuissituatie waarbij de gemeten waarden opgeschreven dienen te worden, is vatbaar voor (onbedoelde) fouten, wat de betrouwbaarheid van geprotocolleerde thuismetingen in gevaar kan brengen. Thuismetingen met behulp van een smartphone applicatie zouden de betrouwbaarheid en de toepasbaarheid in de zorg voor patiënten met hypertensie mogelijk kunnen verbeteren. In hoofdstuk 5 evalueren we hoe een dergelijke app-ondersteunde thuismeting zich verhoudt tot 24-uurs meting en geautomatiseerde bloeddrukmetingen in de spreekkamer bij 113 patiënten met hypertensie. De gemiddelde bloeddruk was 126/73 mm Hg bij de 24-uurs meting en 141/81 mm Hg met de geprotocolleerde thuismeting. De diagnostische overeenstemming tussen de app-geassisteerde thuismeting en de 24-uurs meting voor de diagnose van witte-jassen en gemaskeerde hypertensie was redelijk tot matig. Wij concluderen dat app-geassisteerde thuismetingen kunnen worden gebruikt als een aanvulling op, maar niet als een vervanging van, de 24-uurs meting

In hoofdstuk 6 onderzochten wij het aantal meetdagen dat nodig is om zowel een betrouwbare schatting van de thuisbloeddruk te verkrijgen als de diagnose hypertensie betrouwbaar te kunnen stellen. Hiervoor gebruikten wij data van 567 volwassenen die een 7-daagse thuismeting volgens de Europese richtlijn uitvoerden (referentie). Het gemiddelde van 7 tweemaal daagse bloeddrukmetingen was maximaal 5.2/3.3 mm Hg hoger en 9.5/4.8 mm Hg lager dan de werkelijke thuisbloeddruk voor 95% van de personen. Verkorting van dit protocol tot 3 dagen verhoogde deze afwijking met respectievelijk 1.5/1.0 mm Hg en 4.8/2.3 mm Hg, een verschil wat wordt beschouwd als klinisch niet relevant (<5 mm Hg). Voor de diagnose van hypertensie thuis was er een goede overeenkomst met een minimum van 4.5 dagen. Daarom concluderen wij dat tweemaal daagse bloeddrukmetingen gedurende 3 opeenvolgende dagen een betrouwbare schatting van de thuisbloeddruk geven. Ten minste 4.5 opeenvolgende meetdagen zijn nodig om de diagnose hypertensie betrouwbaar te kunnen stellen in de thuissituatie.

Naast het monitoren van de bloeddruk is het monitoren van veranderingen in levensstijl, zoals het verminderen van de zoutinname, ook essentieel om de bloeddruk beter onder controle te krijgen. Nauwkeurige monitoring van de zoutinname via voeding is echter een uitdaging omdat de zoutinname sterk varieert van dag tot dag en van maaltijd tot maaltijd. Op dit moment wordt het meten van de zoutuitscheiding in meerdere niet-

opeenvolgende 24-uurs urineverzamelingen beschouwd als de gouden standaard. Echter, deze methode is omslachtig, tijdrovend en foutgevoelig. Daarom wordt steeds meer onderzoek gedaan naar methoden die gebaseerd zijn op spot-urinemonsters (bijvoorbeeld alleen de ochtendurine die wordt verzameld) die gemakkelijk te verzamelen zijn. In hoofdstuk 7 vergeleken we de validiteit van verschillende, op formules gebaseerde, benaderingen om de 24-uurs natrium- en kaliumuitscheiding en de natrium-kalium ratio te schatten op basis van spot-urinemonsters. Hiervoor werden 12 gezonde vrijwilligers gevraagd om metingen in spot-urinemonsters met een zelf-monitoring apparaat te verrichten gedurende een normaal dieet, een zoutarm dieet en een zout verrijkt dieet. Wij observeerden grote verschillen tussen de geschatte en gemeten 24-uurs uitscheiding van natrium en kalium gedurende alle diëten. Meer dan 40% van de deelnemers toonde een absoluut verschil tussen gemeten en geschatte 24-uurs natrium van meer dan 1000 mg/dag. De spot-gebaseerde natrium-kalium ratio toonde daarentegen betere overeenkomsten met de 24-uurs natrium-kalium ratio met classificatie van 66% van de deelnemers in de juiste categorie. Wij concluderen daarom dat het gebruik van formule-gebaseerde methodes die de 24-uurs zoutuitscheiding in de urine schatten resulteert in een aanzienlijke systematische fout, een geringe precisie en een geringe nauwkeurigheid. Daarentegen kan de natrium-kalium ratio gebaseerd op meerdere spot-urinemonsters een bruikbaar, goedkoop en weinig belastend alternatief zijn voor 24-uurs urineverzameling voor het monitoren van de dagelijkse zoutinname.

Therapie-ontrouw is een belangrijke oorzaak voor het niet behalen van bloeddruk streefwaarden. Het is belangrijk om therapie-ontrouw tijdig te herkennen voordat patiënten worden onderworpen aan uitgebreide diagnostische trajecten en invasieve-, niet-medicamenteuze behandelingen. Het meten van medicatie in bloed of urine met behulp van vloeistofchromatografie-tandem massaspectrometrie is een objectieve methode om therapietrouw te beoordelen. Deze methode wordt vaak kwalitatief uitgevoerd, waarbij uitsluitend kan worden gezien of een geneesmiddel aanwezig is of niet. Deze kwalitatieve methode is sterk afhankelijk van de gevoeligheid van de analyse apparatuur (detectiegrens) en niet de daadwerkelijke concentratie van het bloeddrukverlagende medicijn. Het meten van medicatie in bloed of urine zou preciezer kunnen door dit kwantitatief te doen. Bijvoorbeeld door de gemeten medicatieconcentratie te vergelijken met de populatie dalconcentratie (de minimale plasmaconcentratie bij trouwe inname van de medicatie) van het medicament. In hoofdstuk 8 beschrijven wij daarom de resultaten van een literatuuronderzoek en een meta-analyse van farmacokinetische studies ter bepaling van de dalconcentraties van amlodipine, hydrochloorthiazide en valsartan. De dalconcentraties in de gevonden studies liepen zeer uiteen. Daarom concluderen wij dat gebruik van één enkele dalconcentratie voor de kwantitatieve beoordeling van gemeten medicatieconcentraties in bloed of urine kan leiden tot een onnauwkeurige beoordeling van therapietrouw. Omdat dit de arts-patiënt relatie potentieel ernstig zou kunnen schaden, raden wij gebruik van de populatie dalconcentratie niet aan.

Hoewel het meten van medicatie in bloed of urine een van de meest betrouwbare methodes voor het beoordelen van therapietrouw is, wordt het slechts in een beperkt aantal ziekenhuizen toegepast. Deze beperkte beschikbaarheid kan gedeeltelijk worden toegeschreven aan een gebrek aan gekwalificeerd personeel en de relatief hoge kosten van de apparatuur die nodig is voor vloeistofchromatografie-tandem massaspectrometrie (≈ €450.000). Een klinisch screeningsinstrument waarmee patiënten met een lage waarschijnlijkheid van therapie-ontrouw zorgvuldig kunnen worden geïdentificeerd zou wenselijk zijn. Deze patiënten hoeven dan geen verdere tests te ondergaan wat de kosten voor de gezondheidszorg zou kunnen verlagen. In hoofdstuk 9 ontwikkelden we daarom een klinisch screeningsinstrument bij 495 patiënten met ongecontroleerde hypertensie die verwezen werden naar het Universitair Medisch Centrum Utrecht. Therapie-ontrouw werd gediagnosticeerd bij 93 (19%) patiënten. Met behulp van zeven gemakkelijk te meten klinische karakteristieken (leeftijd, geslacht, body mass index (kg/m2), voorgeschiedenis van hart- of vaatziekten (ja/nee), systolische spreekkamerbloeddruk (mm Hg), hartslag (slagen/min) en het totaal aantal bloeddrukverlagende tabletten per dag) werd de kans op therapie-ontrouw geschat. Het screeningsinstrument werd gevalideerd bij 240 patiënten met ongecontroleerde hypertensie die naar het Heartlands Hospital in het Verenigd Koninkrijk werden verwezen. De voorspelde kans op therapie-ontrouw kwam goed overeen met de geobserveerde frequentie van therapie-ontrouw. Echter, het model toonde onvoldoende vermogen om therapietrouwe van therapie-ontrouwe patiënten te kunnen onderscheiden. Dit benadrukt de complexe aard van therapie-ontrouw en dus de behoefte aan objectieve testen om therapietrouw te beoordelen bij patiënten met ongecontroleerde hypertensie.

Concluderend, hypertensie is wereldwijd een van de belangrijkste oorzaken van morbiditeit en mortaliteit. Om die reden bestaat er een dringende noodzaak om deze chronische aandoening effectiever te behandelen, daarmee hart- en vaatziekten te voorkomen en de druk op het gezondheidszorgsysteem te verminderen. De resultaten van dit proefschrift geven inzicht in het risico op hart- en vaatziekten, monitoring en therapietrouw van patiënten met (ongecontroleerde) hypertensie. Verdere verbetering lijkt te liggen in betere metingen, zowel van de bloeddruk zelf, van therapietrouw als van het effect van leefstijlinterventies, zoals zoutbeperking.

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List of publications

Publications included in this thesis:

Groenland EH, Vendeville ACJ, Bemelmans RHH, Monajemi H, Bots ML, Visseren FLJ, Spiering W, Smartphone application-assisted home blood pressure monitoring compared with office and ambulatory blood pressure monitoring in patients with hypertension: the AMUSE-BP study. Hypertension. 2022 August; Online ahead of print

Groenland EH, Dasgupta I, Visseren FLJ, vn der Elst KCM, Lorde N, Lawson AJ, Bots ML, Spiering W. Clinical characteristics do not reliably identify non-adherence in patients with uncontrolled hypertension. Blood Pressure.2022.Dec;31(1):178-186.

Groenland EH, Bots ML, Visseren FLJ, McManus RJ, Spiering W. Number of measurement days needed for obtaining a reliable estimate of home blood pressure and hypertension status. Blood Pressure. 2022.Dec;31(1),100–108.

Groenland EH, Heidemann BE, van der Laan SW, van Setten J, Koopal C, Bots ML, et al. Genetic variants associated with low-density lipoprotein cholesterol and systolic blood pressure and the risk of recurrent cardiovascular disease in patients with established vascular disease. Atherosclerosis. 2022 Jun;350:102-108.

Groenland EH, Vendeville ACJ, Bots ML, de Borst GJ, Nathoe HM, Ruigrok YM, et al. The relation between urinary sodium and potassium excretion and risk of cardiovascular events and mortality in patients with cardiovascular disease. PLoS One. 2022;17(3):e0265429.

Groenland EH, Bots ML, Asselbergs FW, de Borst GJ, Kappelle LJ, Visseren FLJ, Spiering W; UCC-SMART Study Group. Apparent treatment resistant hypertension and the risk of recurrent cardiovascular events and mortality in patients with established vascular disease. Int J Cardiol. 2021 Apr 29:S0167-5273(21)00736-1.

Groenland EH, van Kleef MEAM, Bots ML, Visseren FLJ, van der Elst KCM, Spiering W. Plasma Trough Concentrations of Antihypertensive Drugs for the Assessment of Treatment Adherence: A Meta-Analysis. Hypertension. 2021 Jan;77(1):85-93.

Publications not included in this thesis:

Groenland EH, van Kleef MEAM, Hendrikse J, Spiering W and Siero JCW. The effect of endovascular baroreflex amplification on central sympathetic nerve circuits and cerebral blood flow in patients with resistant hypertension: A functional MRI study. Front. Neuroimaging, 2022 Jul

Groenland EH, van der Elst KCM, Spiering W. Biochemische screening van antihypertensiva. FocusVasculair. Jaargang 7 – nummer 2. Juli 2022.

Groenland EH, Spiering W. Baroreflex Amplification and Carotid Body Modulation for the Treatment of Resistant Hypertension. Curr Hypertens Rep. 2020 Mar 12;22(4):27.

Groenland EH, Bemelmans R, Van Vugt S, Westerink J. Onvoldoende bewijs dat vitamine D bij moeheid helpt. Huisarts Wet 2019;62.

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had ik toen niet verwacht. Achteraf kan ik zeggen dat ik die weg met niemand anders had willen bewandelen dan met jou, we mogen trots zijn op het resultaat! Je bent een hele fijne collega en vriendin, veel dank ook voor al je hulp bij de logistiek van het uitvoeren van een klinische studie, het uitzoeken van (bruiloft)kleding(accessoires) (zeer geslaagd!) en de zoektocht naar een ruggengraat (minder geslaagd..).

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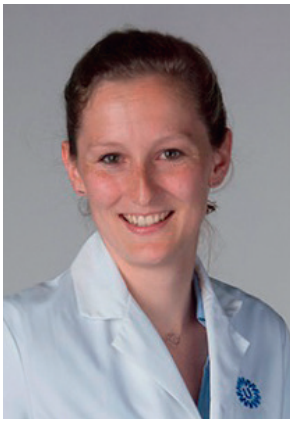
Curriculum vitae



Eline Groenland was born on January 9th, 1993 in Woerden, the Netherlands. After she graduated from secondary school at “College de Heemlanden” in Houten in 2011, she studied Medicine at the University of Utrecht. During her Masters programme she was a member of the Education and Research Council in which she represented students and advised the Dean of Medicine of the Faculty of Medicine at Utrecht University.

After a senior internship in internal medicine at hospital ‘Gelderse Vallei’ (Ede, the Netherlands), she obtained her medical degree in 2018, upon which she was offered the opportunity to continue working at this hospital. In addition to the educational and enjoyable experiences she had in the clinic, her enthusiasm for medical research was fueled by participation in a clinically oriented research project under the supervision of dr. R.H.H. Bemelmans. Her work experience in internal medicine and scientific interest motivated her to start her PhD research project at the department of Vascular Medicine of the University Medical Center Utrecht under the supervision of prof. dr. F.L.J. Visseren, prof. dr. M.L. Bots, and dr. W. Spiering in 2019. She combined her PhD research with a Masters in Clinical Epidemiology at Utrecht University from which she graduated in 2021. In June 2022, she resumed her work as a medical doctor in internal medicine at hospital ‘Gelderse Vallei’.