Universidade do Minho
Escola de Ciências da Saúde

Pedro Miguel Guimarães Marques da Cunha

Study to determine the cardiovascular risk of the population of Guimarães/Vizela, including the prevalence of arterial stiffness and early vascular aging syndrome

julho de 2015

Universidade do Minho
Escola de Ciências da Saúde

Pedro Miguel Guimarães Marques da Cunha

Study to determine the cardiovascular risk of the population of Guimarães/Vizela, including the prevalence of arterial stiffness and early vascular aging syndrome

Tese de Doutoramento
Medicina

Trabalho efectuado sob a orientação do
Professor Doutor Jorge Cotter
Escola de Ciências da Saúde
Universidade do Minho, Braga – Portugal

julho de 2015

DECLARAÇÃO DE INTEGRIDADE

Declaro ter atuado com integridade na elaboração da presente tese. Confirmo que em todo o trabalho conducente à sua elaboração não recorri à prática de plágio ou a qualquer forma de falsificação de resultados.

Mais declaro que tomei conhecimento integral do Código de Conduta Ética da Universidade do Minho.

Universidade do Minho, 14 de Julho de 2015

Nome completo: Pedro Miguel Guimarães Marques da Cunha

Assinatura: _____

Acknowledgments

- Ao Sr. Professor Jorge Cotter, que me incutiu o gosto pela prática da Medicina tal como hoje a exerço, e que me ensinou que a caminhada em direcção a um objectivo se faz todos os dias, com pequenos ou grandes passos, sempre com a certeza de que os atingiremos. Aqui chegamos.
- Ao Sr. Professor Pedro Oliveira, pela paciência infinda, pelo trabalho e dedicação incessantes, e acima de tudo pelo seu apoio, zelo, lealdade e amizade constantes.
- Ao Sr. Professor Nuno Sousa, por me ter acolhido na Escola de Ciências de Saúde, incentivado e apoiado todos os projectos que apresentei e impulsionado o meu crescimento académico, apresentando-me a novas dimensões de interação no domínio da investigação científica.
- Às Senhoras Doutoras Glória Alves e Helena Sarmento cujo apoio incansável, lealdade e amizade são parte integrante da minha vida profissional e pessoal.
- À Srª Enfermeira Isabel Vila, pelo seu profissionalismo e dedicação extremos; pelas suas qualidades humanas, de rigor científico e espírito de sacrifício.
- Ao Serviço de Medicina Interna do Hospital Senhora da Oliveira, Guimarães – que me fez crescer como médico e como homem e onde sempre encontrei colegas cujo entusiasmo e gosto pela procura da excelência me impulsionaram.
- Aos Senhores Doutores Rui Sampaio, Castro Freitas e Novais de Carvalho, bem como a todos os Directores e colegas das diferentes unidades de saúde de Guimarães e Vizela, por toda a colaboração e empenho.
- À Drª Paula Mota e a todo o Serviço de Patologia Clínica do Hospital Senhora da Oliveira, Guimarães.
- A toda a equipa de investigadores que se envolveu neste trabalho com zelo, desinteressadamente e com um entusiasmo enternecedor e moralizador.

- Aos meus pais, pelos valores e disciplina que me transmitiram. Pelo que sou.
- À minha esposa e às minhas filhas, por quem quero ser, todos os dias, um homem melhor.

Estudo para determinar o risco cardiovascular da população de Guimarães/Vizela, incluindo a prevalência de rigidez arterial e de Envelhecimento Vascular Precoce.

Resumo

A doença cardiovascular (DCV) é a principal causa de morte em Portugal, um dos 10 países do mundo em que as taxas de mortalidade por AVC são maiores do que as por doença coronária isquémica, e aquele que apresenta a mais alta incidência de acidente vascular cerebral (AVC) da Europa Ocidental. Neste contexto, pretendemos compreender as características existentes na comunidade respeitantes ao risco CV (RCV) e às manifestações de DCV, usando um rigoroso método de fenotipagem. O nosso objectivo nuclear é o de contribuir para uma melhor estratégia de identificação precoce dos sujeitos em risco e promover um início atempado de medidas. Os principais objectivos foram: i) estabelecer a prevalência de factores de RCV tradicionais e não tradicionais; ii) avaliar a distribuição dos valores médios de velocidade de onda de pulso (VOP) e de pressão arterial central (PAC) na população e reconhecer sinais existentes de envelhecimento vascular precoce (EVP) ou rigidez arterial; iii) estabelecer a distribuição da população pelas diferentes classes de RCV, seguindo diferentes estratégias de estimativa de risco e analisando a dimensão da reclassificação conseguida através de uma cuidadosa fenotipagem; iv) avaliar a prevalência de DCV e doença renal estabelecidas, bem como de lesão em órgão alvo (LOA).

Num estudo de cohort com uma amostra aleatoriamente seleccionada da população de duas cidades, observamos os sujeitos em duas ocasiões, registando história clínica, características biológicas, medidas hemodinâmicas centrais e periféricas e recolhendo ainda amostras de sangue e urina. Cada característica fenotípica foi atribuída a um indivíduo se verificada concordantemente em ambas as visitas.

4000 sujeitos foram randomizados, 3038 incluídos e 2542 completaram as duas visitas, constituindo uma amostra equilibrada com idade média de 45.5 anos e 55.1% de mulheres. Os nossos resultados principais foram:

1) A confirmação de uma sobre e/ou subestimação da prevalência de características de RCV, quando utilizada apenas uma avaliação desses parâmetros;

2) A prevalência de várias características de RCV é elevada, particularmente em faixas etárias mais jovens, e os riscos mínimos de exposição para o desenvolvimento de DCV são amplamente ultrapassados (glicose, pressão arterial (PA), índice de massa corporal);
3) Apesar da evolução positiva no número de sujeitos hipertensos tratados e controlados na última década, os actuais valores médios de PA posicionam-se entre os mais elevados da Europa; as tendências nacionais de declínio na prevalência de hipertensão e dos níveis de PA, não foram verificadas;
4) Diferentes estratégias de estratificação de RCV, permitiram reclassificar 18.2% da população para risco mais elevado; 38.5% dos sujeitos com risco Moderado foi reclassificada para classes de risco Alto e Muito Alto; a classe de Muito Alto risco aumentou cerca de 32 vezes. 62.7% dos hipertensos foram classificados como de Alto/Muito Alto risco; apenas 43% recebiam tratamento anti-lipídico: só 14.3% dos hipertensos de Muito Alto Risco atingiam alvos terapêuticos.
5) A prevalência de EVP foi de 12.5%; 26.1% dos sujeitos com menos de 30 anos apresentavam EVP. 18.7% da população apresentava valores de VOP > 10m/s, predominantemente no sexo masculino. Modelos de regressão logística indicam que as mulheres apresentam a mesma probabilidade de atingir valores de VOP> 10m/s, 10 anos mais tarde que os homens;
6) A PAC Sistólica (PACS) e a Pressão de Pulso Central (PPC) excederam em 10 a 20 mmHg o valor médio esperado, em várias faixas etárias. O ratio médio da Amplificação da PPC (PPCA) foi 1.32. 37.5% da população/72.4% dos hipertensos apresentaram valores de PACS > percentil 90 (P90); PPC> P90 foi documentada em 23.7/51.5% da população/dos hipertensos. Em hipertensos tratados e controlados, 33.9/20.5% dos sujeitos apresentavam valores de PACS/PPC> P90. LOA foi significativamente mais prevalente em sujeitos com PACS/PCC>P90 (2 a 4 vezes). O Sal apresentou-se como uma variável independente explicativa da PACS e da PPCA em análise de regressão multivariada, e como factor de risco para valores de PACS > P90.

Achados congruentes permitem estabelecer a imagem de uma população com risco mais elevado que o esperado para o desenvolvimento de DCV, começando por alterações fenotípicas que favorecem a doença, desde idades jovens.

Study to determine the cardiovascular risk of the population of Guimarães/Vizela, including the prevalence of Arterial Stiffness and Early Vascular Aging Syndrome.

Summary

Cardiovascular (CV) disease (CVD) is the leading cause of death in Portugal. The northern region registers the highest incidence of stroke in Western Europe; paradoxically, this country is one of the ten nations in the world where standardized mortality rates by stroke are higher than for coronary heart disease. In this setting, we wanted to better understand the community dwelling characteristics concerning CV risk (CVR) and CVD manifestations by using a more strict method to phenotype subjects. Our goal was to contribute to a better strategy of pinpointing earlier subjects at risk, and promote subsequent earlier initiation of measures to decrease the development of CVD. Our main aims were to: i) establish the prevalence of traditional and non-traditional CVR factors; ii) evaluate the distribution of pulse wave velocity (PWV) and central blood pressure values in the population and recognize the existing signs of early vascular aging (EVA) or pathologic arterial stiffness; iii) establish the distribution of the population by the different CV risk classes, following different risk estimation strategies, and analyse the reclassification of risk achieved through thorough phenotyping; iv) evaluate the prevalence of established CV and renal disease and target organ damage (TOD).

We established a cohort study, with a randomly selected sample of the population of two adjacent cities. Subjects were observed in a two visit plan where clinical history, biologic characteristics, peripheral and central hemodynamic variables were recorded and blood and urine samples collected. A phenotypic characteristic would be attributable to an individual if it was concordantly verified in the two visits.

Four thousand subjects were randomized, 3038 were included and 2542 completed the two visit plan, constituting a balanced sample with 45.5 years of mean age and 55.1% of women. Our main results were:

1) The confirmation of an over and/or underestimation of the prevalence of CVR characteristics usually obtained with a single evaluation of those parameters;

2) The prevalence of several CVR features is elevated, particularly in younger age groups, and theoretical minimum risk exposure for the development of CVD are largely surpassed (glucose, blood pressure (BP), body mass index);
3) In spite of the positive evolution on the number of treated and controlled hypertensive subjects over the last decade, current mean BP levels rank amongst the highest in European countries; the reported national trends of BP levels and hypertension prevalence decline have not been confirmed;
4) Using different CVR stratification strategies, 18.2% of the population was reclassified into higher risk classes; 38.5% of Moderate risk subjects were reclassified into High and Very/High risk categories, increasing the number of Very High risk subjects by 32 times. 62.7% of hypertensives were classified with High or Very High risk; only 43% of them were receiving anti-lipidic treatment and only 14.3% of the Very High Risk group achieved treatment targets.
5) The overall prevalence of EVA was 12.5%; 26.1% of individuals under 30 years presented this feature. 18.7% of the population exhibited PWV values above 10 m/s, with male predominance. Logistic regression models indicated gender differences concerning the risk of developing large artery damage, with women having the same odds, of reaching PWV>10m/s, 10 years later than men.
6) Central Systolic Blood Pressure (CSBP) and Central Pulse Pressure (CPP) exceeded 10 - 20 mmHg the expected mean values for healthy subjects, in several age groups. CPP Amplification (CPPA) mean ratio was 1.32. 37.5% of the population and 72.4% of hypertensives presented CSBP>90^{th} percentile (90^{th}p); CPP>90^{th}p was registered in 23.7 of the population and in 51.5% of hypertensives. In treated and controlled hypertensives, 33.9/20.5% of the subjects persist with CSBP/CPP values >90^{th}p. TOD was significantly more prevalent in subjects above CSBP/CPP 90^{th}p (2 to 4 times). Salt was an independent explanatory variable of CSBP and CPPA in multivariate regression analysis, and it increased the risk of having CSBP >90^{th}p.

Congruent findings construct a snapshot of a population with higher than expected risk for the development of CVD, starting with phenotypic changes favouring disease at young ages.

Table of Contents

Abbreviation List

BP – blood pressure

BMI – body mass index

CBP – central blood pressure

CHD – Coronary Heart Disease

CI – Confidence Interval

CKD – chronic kidney disease

CPP – central pulse pressure

CPPA – central pulse pressure amplification

CSBP – central systolic blood pressure

CDBP – central diastolic blood pressure

CV – cardiovascular

CVD – cardiovascular disease

CVE – cardiovascular events

CVR – cardiovascular risk

DBP – diastolic blood pressure

ERVC – European reference values collaboration

ESC/ESH – European societies of Cardiology / Hypertension

EVA – early vascular aging

GFR – glomerular filtration rate

HTN - hypertension

HR – Heart Rate

ISH – International Society of Hypertension

LVH – left ventricular hypertrophy

MARE – "metabolic syndrome and arteries research in Europe"

OR – odds ratio

PBP – peripheral blood pressure

PWA – pulse wave analysis

PWV – pulse wave velocity

REF - Reference

SBP – systolic blood pressure

SD – standard deviation

SE – standard error

SMR – standardized mortality rates

TOD – target organ damage

Tx - treatment

YLL – years of life lost

WHO – world health organization

Chapter 1

Introduction

Introduction

In many manuscripts, related to cardiovascular (CV) risk or cardiovascular disease (CVD), throughout the literature (and throughout the years) we can find an initial reference to the amount of morbidity and mortality that these pathologies input to different populations. The wording is many times different, but the sense is the same: CVD ranks amongst the first (or, is the first) causes of morbidity and mortality around the world. In fact, between 1990 and 2010, we observed a decrease in communicable diseases and an increase in non-communicable ones; during this period, not only did coronary heart disease (CHD) and ischemic stroke consolidated their leadership as the first two causes of death in the world, as they have also become one of the top three diseases promoting more years of life lost (1). Europe (in the entire length of its territory) was no exception to the rule, but it has evolved in a different sense in what corresponds to the consolidation of the relative weight of these diseases: the standardized death rates by CHD or stroke have been declining progressively (2). In the latest available update in Europe, CVD accounts for 42%/51% of the mortality causes in females and males, respectively(3).

Portugal has accompanied the same trends in these European ranks and trends, with two particularities: a) it is the only country in Western Europe (and one of the 2 countries in the entire European continent, alongside with Macedonia) where stroke standardized mortality rates (SMR) surpass CHD's SMR (4, 5); b) it has the highest incidence rate of stroke of Western Europe (6, 7) .

Aging. The demographic pyramid of several European countries, and especially our own, has been growing in the sense of progressively increasing the number of older and elderly people, with the parallel decrease of younger generations. This has been also a consequence of medical progress, socioeconomic, education and sanitary improvement (amongst others), promoting an increase in life expectancy that has been recorded in the last 40 years (8). This has also led to an increased awareness towards the progression and expression of diseases in older individuals, like CVD, cognitive impairment and frailty (9, 10).

Aging has become an integrative part of the cardiovascular disease continuum, and a well established non-modifiable cardiovascular risk factor (11). The increased interest recorded concerning the effects of age on CV disease, has been translated into researching, on one hand, the structural changes in the CV system that occur as a consequence of age advancement and, on the other, the changes that are imprinted into that same system as a consequence of the interaction between age and known or unknown insults (from prenatal *in utero* influences, to increased blood pressure, oxidative stress, genetics, inflammation, and of course, the well known CVR factors) (12-17). Arterial stiffness has become the "gold-standard" measurement of the deterioration of the arterial wall with age, with or without the influence of other factors. Its independent value as predictor of CVD and CV events (CVE) has been well established (18-26).

Brain damage and cognitive deterioration have been also linked to the aging process, and related, at least in part, to vascular disease. Accumulating evidence has surfaced, linking the parallel progression of both ailments (27-32), a worrisome fact from the public health point of view, in an ageing population, when considering the extended life expectancy.

Riding the perfect storm. A particularly wise and contemporary observation by Niccolo Machiavelli, in the year of 1513, seems more appropriate than ever: "At the beginning, a disease is easy to cure, but difficult to diagnose; as time passes, not having been treated or recognized at the outset, it becomes easy to diagnose, but difficult to cure".

The apparent and deceiving enthusiasm with the progressive lowering of SMR related to CVD in our country must be countered with concern with two other rising problems. One of them has already been addressed here, and pertains to the increase in the older population and age-related diseases, from cognitive impairment to full blown CVD. The other problem pertains with the cardiovascular health and risk of younger generations, and its evolution for the upcoming years.

In Portugal, 32% of children with 11 years of age are overweight, the second highest prevalence (in that age range) in the European Union (33). According to the same report, almost 60% of the subjects ≥ 15 years of age have insufficient physical

activity. Portuguese primary schools are ranked amongst the highest, concerning body mass index levels measured in students (34). Several reports have also found high levels of hypertension prevalence in convenience samples, ranging from 9.8% in children with an average age of 13 years (35) to 34% in adolescents (16-19 years) (36). Finally, a recent evaluation of children aged 10 to 12 years, has recorded a daily uptake of salt of approximately 8g, significantly above the recommended daily dose for that age range (37). These data must be put into context with what has been shown concerning the early effect of cardiovascular risk factors on the future development of CVD in adulthood. Firstly, the Bogalusa Heart study has shown how elevated blood pressure during childhood (especially in the highest quintile of distribution) can significantly increase the risk of hypertension in adult life (38). The Amsterdam Growth and Health Longitudinal Study has documented the relationship of adolescent higher levels of blood pressure, central fatness and low physical activity, with increased carotid stiffness more than 20 years later (39). Finally, other studies have shown indexes of poor vascular function, for the expected age range, in young adults (40, 41).

Thus, the perfect storm for cardiovascular disease manifestations, coursing through the entire lifespan of our population, seems to be setting up: increased prevalence of cardiovascular risk factors in young ages; evolution to CVD in adulthood; increased proportion of older age classes with inherent cardiovascular and cognitive impairment.

The Guimarães / Vizela Study. By the time the Guimarães/Vizela study was being structured (2008 – 2010), many of the above mentioned questions and concerns were lingering, and raising the need to better understand the CV risk phenotype of our population. By then, the prevalence of hypertension had been estimated in 41.2% (42). Importantly, among these, only 11.2% were controlled, and the high stroke incidence was, as is today, a major concern. The CV risk stratification had been reviewed in the meantime (43-45). New “non-traditional” risk factors (RF) had been recognized and there was a reappraisal of the so-called “traditional” RF. Particularly interesting was the definitive assumption of microalbuminuria and decreased glomerular filtration rate (GFR) as two clear CV RF (43-45).

A new concept of *Early Vascular Aging* Syndrome had just been proposed for the early detection of pathological changes in the function and structure of the arterial bed (17, 46). These pathological changes were deemed to be a consequence of genetic, as well as environmental (the action of different CV RF) influences (47). Arterial stiffness evaluation depicted those subjects in which the action of different CVR factors had translated into arterial disease and real risk of cardiovascular event. Also recent, were the first consensus documents on central blood pressure evaluation (48) and clinical significance, stating that these new parameters had higher pathophysiological relevance for the establishment of CVD than peripheral BP measurements (49), especially concerning the brain, the heart and the kidney. With this, came also the discussion on central to peripheral blood pressure amplification, and its clinical importance. New risk stratification strategies using central hemodynamic parameters had just proven to perform with an added predictive value when compared to established algorithms using traditional CVR factors (50).

At the time, one could ascertain that no clear and precise knowledge existed relating to the exact prevalence of CV RF (traditional and non-traditional) in the Portuguese population (lipid profile, kidney function, diabetes). Also unknown was the part of the population that carried higher CVR and/or had developed subclinical organ damage. The standing belief was that the methodologies followed in recent national studies to evaluate the prevalence of CVR factors in Portugal should be reappraised (42, 51-53). More importantly, no national data on a population-based level was available to determine the distribution of pulse wave velocity and central blood pressure values, the prevalence of Early Vascular Aging Syndrome or the existence of CV subclinical organ damage.

Therefore, it seemed crucial to better understand the reasons underlying the high incidence of cardiovascular events and cardiovascular disease in the Portuguese population. Of particular interest was the possibility of determining early markers of CV disease that could lead to a more stringent treatment of the subjects and prevention of CV events or development of subclinical organ damage.

Objectives

Using a representative sample of the population of these two adjacent cities (Guimarães and Vizela), and a methodological approach different from previously national published studies, in this Thesis we aimed to:

1) Determine the prevalence of traditional (hypertension, diabetes, dyslipidemia, overweight, tobacco use, metabolic syndrome) and non-traditional (chronic kidney disease, hyperuricemia, salt consumption, abdominal obesity) CV risk factors.
2) Evaluate the normal distribution of pulse wave velocity and central blood pressure values in the population;
3) Establish the prevalence of Arterial Stiffness signs and Early Vascular Aging in the population;
4) Establish the distribution of the population by the different CV risk classes, following different risk estimation strategies;
5) Identify the fraction of the population that would classically be classified as bearing low added CV risk, and that in fact has high risk levels due to the determination of early vascular aging syndrome and/or subclinical organ damage (as evaluated by arterial stiffness and kidney function studies) – allowing for a precocious clinical intervention or treatment intensification;
6) Define the prevalence of established CV and renal disease in the population;
7) Evaluate the existence of correlation between signs of macrovascular disease (arterial stiffness) and the accelerated development of cognitive deterioration.
8) Evaluate the existence of correlation between the different subtypes of metabolic syndrome and the development of arterial stiffness signs;
9) Evaluate the existence and extension of target organ damage (arterial bed, heart, kidney and brain) in subjects with added CV risk.

References

1. 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. 2013;380(9859):2095-128. Epub 2012/12/19.
2. Nichols M TN, Luengo-Fernandez R, Leal J, Gray A, Scarborough P, Rayner M. . European Cardiovascular Disease Statistics 2012. Brussels: (2012).
3. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. European heart journal. 2014;35(42):2950-9. Epub 2014/08/21.
4. Kim AS, Johnston SC. Global variation in the relative burden of stroke and ischemic heart disease. Circulation. 2011;124(3):314-23. Epub 2011/07/07.
5. Ferreira RC, Neves RC, Rodrigues V, Nogueira PJ, Farinha CS, Soares A, et al. Portugal: Doenças Cérebro-Cardiovasculares em números – 2014. Portugal: Direcção-Geral da Saúde. Direção de Serviços de Informação e Análise, 2014.
6. Correia M, Silva MR, Matos I, Magalhaes R, Lopes JC, Ferro JM, et al. Prospective community-based study of stroke in Northern Portugal: incidence and case fatality in rural and urban populations. Stroke; a journal of cerebral circulation. 2004;35(9):2048-53. Epub 2004/07/17.
7. Muller-Nordhorn J, Binting S, Roll S, Willich SN. An update on regional variation in cardiovascular mortality within Europe. European heart journal. 2008;29(10):1316-26. Epub 2008/02/08.
8. Wang H, Dwyer-Lindgren L, Lofgren KT, Rajaratnam JK, Marcus JR, Levin-Rector A, et al. Age-specific and sex-specific mortality in 187 countries, 1970-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2071-94. Epub 2012/12/19.
9. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. Stroke; a journal of cerebral circulation. 2011;42(9):2672-713. Epub 2011/07/23.
10. Rodriguez-Manas L, Feart C, Mann G, Vina J, Chatterji S, Chodzko-Zajko W, et al. Searching for an operational definition of frailty: a Delphi method based consensus statement: the frailty operative definition-consensus conference project. The journals of gerontology Series A, Biological sciences and medical sciences. 2013;68(1):62-7. Epub 2012/04/19.
11. O'Rourke MF, Safar ME, Dzau V. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. Vasc Med. 2010;15(6):461-8. Epub 2010/11/09.
12. O'Rourke MF. Arterial aging: pathophysiological principles. Vasc Med. 2007;12(4):329-41. Epub 2007/12/01.
13. O'Rourke MF, Hashimoto J. Mechanical factors in arterial aging: a clinical perspective. Journal of the American College of Cardiology. 2007;50(1):1-13. Epub 2007/07/03.
14. Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part III: cellular and molecular clues to heart and arterial aging. Circulation. 2003;107(3):490-7. Epub 2003/01/29.
15. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part II: the aging heart in health: links to heart disease. Circulation. 2003;107(2):346-54. Epub 2003/01/23.
16. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. Circulation. 2003;107(1):139-46. Epub 2003/01/08.
17. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. Journal of hypertension. 2008;26(6):1049-57. Epub 2008/05/14.
18. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. Journal of the American College of Cardiology. 2010;55(13):1318-27. Epub 2010/03/27.
19. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. Journal of the American College of Cardiology. 2014;63(7):636-46. Epub 2013/11/19.
20. Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension. 2002;39(1):10-5. Epub 2002/01/19.

21. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37(5):1236-41. Epub 2001/05/23.
22. Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, et al. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. Stroke; a journal of cerebral circulation. 2003;34(5):1203-6. Epub 2003/04/05.
23. London GM, Blacher J, Pannier B, Guerin AP, Marchais SJ, Safar ME. Arterial wave reflections and survival in end-stage renal failure. Hypertension. 2001;38(3):434-8. Epub 2001/09/22.
24. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, et al. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation. 2006;113(5):664-70. Epub 2006/02/08.
25. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, et al. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. Circulation. 2005;111(25):3384-90. Epub 2005/06/22.
26. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, et al. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. Circulation. 2006;113(5):657-63. Epub 2006/02/08.
27. Waldstein SR, Rice SC, Thayer JF, Najjar SS, Scuteri A, Zonderman AB. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. Hypertension. 2008;51(1):99-104. Epub 2007/11/21.
28. O'Rourke MF. Brain microbleeds, amyloid plaques, intellectual deterioration, and arterial stiffness. Hypertension. 2008;51(3):e20; author reply e1. Epub 2008/02/06.
29. Scuteri A, Tesauro M, Appolloni S, Preziosi F, Brancati AM, Volpe M. Arterial stiffness as an independent predictor of longitudinal changes in cognitive function in the older individual. Journal of hypertension. 2007;25(5):1035-40. Epub 2007/04/07.
30. Zeki Al Hazzouri A, Newman AB, Simonsick E, Sink KM, Sutton Tyrrell K, Watson N, et al. Pulse wave velocity and cognitive decline in elders: the Health, Aging, and Body Composition study. Stroke; a journal of cerebral circulation. 2013;44(2):388-93. Epub 2013/01/17.
31. Webb AJ, Simoni M, Mazzucco S, Kuker W, Schulz U, Rothwell PM. Increased cerebral arterial pulsatility in patients with leukoaraiosis: arterial stiffness enhances transmission of aortic pulsatility. Stroke; a journal of cerebral circulation. 2012;43(10):2631-6. Epub 2012/08/28.
32. Pase MP, Herbert A, Grima NA, Pipingas A, O'Rourke MF. Arterial stiffness as a cause of cognitive decline and dementia: a systematic review and meta-analysis. Internal medicine journal. 2012;42(7):808-15. Epub 2011/12/14.
33. Organisation W-WH. Country profiles on nutrition, physical activity and obesity in the 28 European Union Member States of the WHO European Region. Europe ROf; 2013 ISBN 978 92 890 5009 8.
34. Wijnhoven TM, van Raaij JM, Sjoberg A, Eldin N, Yngve A, Kunesova M, et al. WHO European Childhood Obesity Surveillance Initiative: School nutrition environment and body mass index in primary schools. International journal of environmental research and public health. 2014;11(11):11261-85. Epub 2014/11/02.
35. Maldonado J, Pereira T, Fernandes R, Carvalho M. Blood pressure distribution of a sample of healthy Portuguese children and adolescents: the AVELEIRA registry. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2009;28(11):1233-44. Epub 2010/03/13.
36. Silva D, Matos A, Magalhaes T, Martins V, Ricardo L, Almeida H. [Prevalence of hypertension in Portuguese adolescents in Lisbon, Portugal]. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2012;31(12):789-94. Epub 2012/11/13. Prevalencia de hipertensao arterial em adolescentes portugueses da cidade de Lisboa.
37. Cotter J, Cotter MJ, Oliveira P, Cunha P, Polonia J. Salt intake in children 10-12 years old and its modification by active working practices in a school garden. Journal of hypertension. 2013;31(10):1966-71. Epub 2013/10/11.
38. Bao W, Threefoot SA, Srinivasan SR, Berenson GS. Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. American journal of hypertension. 1995;8(7):657-65. Epub 1995/07/01.
39. Ferreira I, van de Laar RJ, Prins MH, Twisk JW, Stehouwer CD. Carotid stiffness in young adults: a life-course analysis of its early determinants: the Amsterdam Growth and Health Longitudinal Study. Hypertension. 2012;59(1):54-61. Epub 2011/11/10.

40. Oren A, Vos LE, Uiterwaal CS, Grobbee DE, Bots ML. Aortic stiffness and carotid intima-media thickness: two independent markers of subclinical vascular damage in young adults? European journal of clinical investigation. 2003;33(11):949-54. Epub 2003/11/26.
41. Bhuiyan AR, Srinivasan SR, Chen W, Paul TK, Berenson GS. Correlates of vascular structure and function measures in asymptomatic young adults: the Bogalusa Heart Study. Atherosclerosis. 2006;189(1):1-7. Epub 2006/03/30.
42. Macedo ME, Lima MJ, Silva AO, Alcantara P, Ramalhinho V, Carmona J. Prevalence, awareness, treatment and control of hypertension in Portugal: the PAP study. Journal of hypertension. 2005;23(9):1661-6. Epub 2005/08/12.
43. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension. 2007;25(6):1105-87. Epub 2007/06/15.
44. Mancia G, Laurent S, Agabiti-Rosei E, Ambrosioni E, Burnier M, Caulfield MJ, et al. Reappraisal of European guidelines on hypertension management: a European Society of Hypertension Task Force document. Blood pressure. 2009;18(6):308-47. Epub 2009/12/17.
45. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R, et al. European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology. 2007;14 Suppl 2:S1-113. Epub 2007/11/06.
46. Nilsson PM. Early vascular aging (EVA): consequences and prevention. Vascular health and risk management. 2008;4(3):547-52. Epub 2008/10/02.
47. Nilsson PM, Boutouyrie P, Laurent S. Vascular aging: A tale of EVA and ADAM in cardiovascular risk assessment and prevention. Hypertension. 2009;54(1):3-10. Epub 2009/06/03.
48. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European heart journal. 2006;27(21):2588-605. Epub 2006/09/27.
49. Agabiti-Rosei E, Mancia G, O'Rourke MF, Roman MJ, Safar ME, Smulyan H, et al. Central blood pressure measurements and antihypertensive therapy: a consensus document. Hypertension. 2007;50(1):154-60. Epub 2007/06/15.
50. Sehestedt T, Jeppesen J, Hansen TW, Rasmussen S, Wachtell K, Ibsen H, et al. Thresholds for pulse wave velocity, urine albumin creatinine ratio and left ventricular mass index using SCORE, Framingham and ESH/ESC risk charts. Journal of hypertension. 2012;30(10):1928-36. Epub 2012/08/09.
51. Fiuza M, Cortez-Dias N, Martins S, Belo A. Metabolic syndrome in Portugal: prevalence and implications for cardiovascular risk--results from the VALSIM Study. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2008;27(12):1495-529. Epub 2009/03/14.
52. Vinhas J, Gardete-Correia L, Boavida JM, Raposo JF, Mesquita A, Fona MC, et al. Prevalence of chronic kidney disease and associated risk factors, and risk of end-stage renal disease: data from the PREVADIAB study. Nephron Clinical practice. 2011;119(1):c35-40. Epub 2011/06/10.
53. Gardete-Correia L, Boavida JM, Raposo JF, Mesquita AC, Fona C, Carvalho R, et al. First diabetes prevalence study in Portugal: PREVADIAB study. Diabetic medicine : a journal of the British Diabetic Association. 2010;27(8):879-81. Epub 2010/07/27.

Chapter 2

Rational and design of the Guimarães/Vizela study: a multimodal population-based cohort study to determine cardiovascular risk and disease

The Rationale/Design of the Guimarães/Vizela Study: A Multimodal Population-Based Cohort Study to Determine Global Cardiovascular Risk and Disease

Pedro Guimarães Cunha, MD,†‡ Jorge Cotter, PhD, MD,*†‡ Pedro Oliveira, PhD,§ Isabel Vila, BSN,* and Nuno Sousa, PhD, MD†‡*

Background: Cardiovascular disease and dementia are growing medical and social problems in aging societies. Appropriate knowledge of cardiovascular disease and cognitive decline risk factors (RFs) are critical for global CVR health preventive intervention. Many epidemiological studies use case definition based on data collected/measured in a single visit, a fact that can overestimate prevalence rates and distant from clinical practice demanding criteria. Portugal displays an elevated stroke mortality rate. However, population's global CV risk characterization is limited, namely, considering traditional/nontraditional RF and new intermediate phenotypes of CV and renal disease. Association of hemodynamic variables (pulse wave velocity and central blood pressure) with global CVR stratification, cognitive performance, and kidney disease are practically inexistent at a dwelling population level.

Study Design and Methods: After reviewing published data, we designed a population-based cohort study to analyze the prevalence of these cardiovascular RFs and intermediate phenotypes, using random sampling of adult dwellers living in 2 adjacent cities. Strict definition of phenotypes was planned: subjects were observed twice, and several hemodynamic and other biological variables measured at least 3 months apart.

Results: Three thousand thirty-eight subjects were enrolled, and extensive data collection (including central and peripheral blood pressure, pulse wave velocity), sample processing, and biobank edification were carried out. One thousand forty-seven cognitive evaluations were performed.

Conclusions: Seeking for CV risk reclassification, early identification of subjects at risk, and evidence of early vascular aging and cognitive and renal function decline, using the strict daily clinical practice criteria, will lead to better resource allocation in preventive measures at a population level.

Key Words: blood pressure, cardiovascular risk, chronic kidney disease, cognitive impairment, arterial stiffness, early vascular aging

(*J Investig Med* 2014;62: 813–820)

From the *Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Internal Medicine Department, Guimarães–Centro Hospitalar do Alto Ave, and †Life and Health Science Research Institute (ICVS), School of Health Science, University of Minho; ‡ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães; and §Departamento de Estudo de Populações, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

Received November 4, 2013, and in revised form January 23, 2014.
Accepted for publication January 23, 2014.

Reprints: Pedro Guimarães Cunha, MD, Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Serviço de Medicina Interna do Hospital de Guimarães, Centro Hospitalar do Alto Ave, Life and Health Science Research Institute (ICVS), University of Minho, Rua dos Cutileiros, Creixomil, 4810, Guimarães. Portugal.
E-mail: pedrogcunha@netcabo.pt; pedrocunha@ecsaude.uminho.pt.

All authors declare no conflict of interest.
Supplemental digital content is available for this article. Direct URL citation appears in the printed text and is provided in the HTML and PDF versions of this article on the journal's Web site (www.jinvestigativemed.com).

ISSN: 1081-5589
DOI: 10.231/JIM.0000000000000069

Growing scientific evidence progressively changed clinical management of cardiovascular (CV) risk patients. Currently, the CV risk profile of the subject based on a wider picture of concurrent CV risk factors (CVRFs) must be considered. Tailoring CVRF control to the CV risk profile of the individual is, therefore, the current recommended strategy to prevent and delay the establishment of CV disease (CVD) and the progression on the CV continuum, as defined by Dzau[1,2] and recently reviewed by O'Rourke et al.[3] Still, and despite growing knowledge and adjustment of clinical management of CVRFs, death by CVD is still increasing and the leading cause of mortality in the world.[4]

Effective prevention of CVRFs and CVD is dependent on the establishment of appropriate public health policies and health care resource allocation based on the correct knowledge of the local epidemiological reality. Therefore, it is of relevance to gather information related to the following questions: Is the Portuguese reality concerning CVD and cognitive decline similar to what has been registered in different European countries? If not, can knowledge gathered in populations with different CVD manifestations and risk profile open new doors in CVD research and be applicable elsewhere? Is there a correct knowledge concerning the prevalence of the different CVRFs and inherent risk profile? Are there different contributors to the establishment of CVD and dementia? Can new concepts on risk of CVD development be applied to recognize subjects at risk?

To answer these questions, we propose to carry out an epidemiological study evaluating the CV risk profile of the population of 2 adjacent cities in the north of Portugal, not only analyzing the prevalence of traditional and nontraditional RFs, but also identifying in this population the prevalence of new intermediate markers of risk and/or new intermediate phenotypic expressions of progression to CVD or target-organ disease (heart, brain, and kidney). Ultimately, we seek to find a global CV risk and subclinical CVD picture of the population, which will guide future clinical search of individuals at risk through early reclassification of their CV and cognitive risk status.

Objectives

A cohort study was designed to evaluate a representative sample of the adult population of 2 adjacent cities in the north of Portugal: Guimarães and Vizela. Using a different methodology of approach and strict definition of criteria, the main objectives of this study are as follows:

1. to establish the prevalence of traditional (hypertension, diabetes, dyslipidemia, overweight, tobacco use, metabolic syndrome) and nontraditional (chronic kidney disease [CKD], hyperuricemia, salt consumption, abdominal obesity) CVRFs.
2. to evaluate the normal distribution of pulse wave velocity (PWV) and central blood pressure (BP) values in the

population and establish the prevalence of arterial stiffness and early vascular aging (EVA) signs.
3. to define the prevalence of established CV and renal disease and evaluate the existence and extension of target-organ damage (TOD) (arterial bed, heart, kidney, and brain) in subjects with added CV risk.
4. to establish the population's risk profile (using the European Society of Hypertension [ESH][5] risk classes and the SCORE [Systematic COronary Risk Evaluation] classification)[6,7] and identify the fraction that would be reclassified in terms of risk assessment due to the determination of EVA syndrome and/or subclinical organ damage (as evaluated by arterial stiffness and kidney function studies), allowing for a precocious clinical intervention or treatment intensification.
5. to evaluate the existence of an association between signs of macrovascular disease (arterial stiffness) with both different subtypes of metabolic syndrome and the accelerated development of cognitive deterioration.

MATERIALS AND METHODS

Study Consortium and Ethical Issues

To achieve the proposed goals, a protocol of collaboration was established between 3 different institutions: The Centro Hospitalar do Alto Ave (hospital reference center); The Agrupamento de Centros de Saúde (ACES–Group of Community Primary Health Care) Guimarães/Vizela (coordinating the activity of all the 13 primary community health care centers [PCHCCs] that operate in the area); and the Life and Health Sciences Research Institute, Minho University. The project was submitted to and approved not only by the ethics committee of the Administração Regional de Saúde do Norte (Health Administration for the North Region) but also by the National Commission for Data Protection.

Study Type, Sample Selection, and Subject Recruitment

We designed a cohort study (prepared for a longitudinal evaluation) evaluating a representative sample of the population of the 2 previously mentioned cities. We did not intend to obtain data that would be extrapolated to the national population as some characteristics of the population of these 2 cities (age and socioeconomic distribution) are different from the rest of the country (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/JIM/A21).

There is no direct clinical intervention by the investigators, and no invasive procedure beyond blood sample collection in a peripheral venous access was programmed.

Participants have been randomly appointed through a random sampling method performed based on the list of citizens currently living in Guimarães and Vizela. In Portugal, every citizen must be registered in the PCHCC of his/her residence area. We first compared the information concerning the characteristics of citizens living in Guimarães/Vizela (INE, 2006) and the same information of those with an actual registry in one of the primary care facilities operating in the 2 cities, finding that the difference between both lists was inferior to 2%, therefore allowing us to state that, for practical use, the populations enrolled in PCHCC and living in Guimarães/Vizela are virtually the same. Portugal, like several European countries, does not collect information on ethnicity of the resident population; the overwhelming majority of its population is white. Ethnicity was not therefore considered as a factor in the study. Foreign residents in these 2 cities represent 3.6% of the population, and most of them are of European origin (Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/JIM/A21).

Bearing in mind the available information regarding the characteristics of the population of Guimarães and Vizela and the characteristics that we intend to study, a representative sample of that adult population (≥18 years of age) stratified by age was defined previewing the necessity of including 4000 individuals (95% confidence interval with an estimation error inferior to 2%, considering a 25% safety margin to cope with nonadherence and dropout rate between visits). At the same time, and anticipating a higher nonadherence and dropout rates on younger and professionally active individuals, the number of randomized individuals to enroll was divided unevenly according to their age (2000 individuals would be <35 years of age, 1000 subjects would have 35 and 65 years of age, and 1000 subjects would be >65 years). Individuals registered in the local PCHCC and corresponding to the stratification characteristics were randomized as previously mentioned. Family doctors in charge of the randomized individuals have then been contacted, explained the goals of the project, and equipped with information material—they were afterward responsible to contact the subjects and obtain their written consent form to participate. It was therefore clear that only randomly assigned subjects could be enrolled and that no volunteers or physician-selected subjects would be included. If the subject refused, the general practitioner could not replace him/her with a volunteer or with someone from his/her practice. Only randomized subjects were accepted. Pregnant women and subjects unable to move or bedridden were excluded.

Criteria Definition

In accordance with the strict methodology already described, the following criteria to define conditions were chosen:
1. blood pressure categorization and hypertension definition according to the ESH 2007 guidelines[5] or whenever a subject was taking antihypertensive medication; 3 measurements under recommended conditions were taken using a validated device (Omron 705-IT; OMRON Healthcare Europe B.V., Hoofddorp, The Netherlands), and the mean of the last 2 measurements was used;
2. diabetes mellitus and glucose metabolism conditions according to the American Diabetes Association,[8] or whenever a subject was taking antidiabetic medication;
3. dyslipidemia, metabolic syndrome, abdominal obesity, and family history of premature CVD according to ESH 2007 definition[5] of CVRFs;
4. glomerular filtration rate categorization, microalbuminuria, proteinuria, and CKD definitions according to the National Kidney Foundation[9];
5. overweight and obesity according to criteria established by the fifth European Joint Task Force on Cardiovascular Disease Prevention[10];
6. salt consumption will be estimated using the renal excretion of sodium measured in a valid 24-hour urine collection;
7. pulse wave velocity and central BP will be measured using the Sphygmocor device (AtCor Medical Pty Ltd, New South Wales, Australia) and calibrated using the individual's sitting brachial BP;
8. the definition of hypertension, diabetes, CKD, microalbuminuria, or proteinuria will respect the existence of 2 agreeing measurements taken at least 3 months apart; and
9. cognitive function evaluation and cutoff definitions were done using different tools, as detailed elsewhere,[11] and allowing for the analysis of global, executive, and memory functions.

Research Team

A team of 88 researchers (medical doctors, cardiopneumology technicians, psychologists, and nurses) was assembled to observe the subjects and collect the data. A software tool was built to function as an electronic case record form, containing prevalidated and standardized questions and/or compulsory recording items to all the subjects observed. All the researchers involved were engaged in training sessions before beginning subject's observation, and standardization of procedures, measurements, and recording were ensured. A calendar of activities was designed to be followed for 2 years (86 observation dates).

Subject's Observations

Every subject was considered as enrolled after his/her family doctor had contacted him/her and was explained the study goals and procedures, and provided a signed written consent form. Subjects are to be observed twice at their PCHCC, on Saturday mornings (the 2 observations are programmed to occur at least 3 months apart).

Whenever the team of researchers was scheduled to visit a particular PCHCC, the enrolled subjects were contacted by telephone during the preceding week, remembering the time schedule and instructing them to bring their prescribed medication, to observe fasting for at least 8 hours (including alcohol and caffeine/caffeinated beverages), and to refrain from smoking until clinical evaluation, BP measurement, and blood/urine samples collection were finished. All the data/all subject observations were collected/performed in the morning, following the same protocol during the entire study period.

At their first visit, subjects were submitted to a clinical interview collecting information regarding relevant socioeconomic, clinical, and family information; clinical parameters measurement—weight, height, abdominal perimeter, and BP (3 measurements in optimal conditions); PWV and central BP measurements (using Sphygmocor device); electrocardiogram; and biologic specimen collection—blood and occasional urine samples (Fig. 1).

At their second visit, subjects were asked to repeat the clinical parameters measurement (above described), collect biological specimen: blood and occasional and 24-hour urine samples, and perform a neurocognitive evaluation (in individuals >50 years of age). Prescription drugs were once more recorded for every participant (Fig. 2).

Follow-up information will be gathered through contact with the subjects themselves and through their family doctors, as scheduled and previewed in the written consent form. Subjects older than 50 years, and after providing written consent, will be invited to perform a brain magnetic resonance imaging study, as a supplementary evaluation.

Central Laboratory Workup

All the collected blood and urine samples (occasional and 24-hour collections) will be processed in 1 central laboratory (the Clinical Pathology Department of the Centro Hospitalar do Alto Ave), ensuring standardized measurement techniques and result outputs. On the week preceding each evaluation, a kit containing prelabeled collection tubes was produced for every subject to be evaluated on the upcoming Saturday. Upon arrival, blood and urine samples were processed, and plasma, serum, and urine samples of each participant (in each visit) were frozen and subsequently stored at −82°C, allowing for the progressive edification of a sample bank.

Statistical Analysis

All the collected information will be included in a database that will be subjected to a predefined statistical analysis to provide answers to the initial queries. The database was constructed in due respect of the national and international guidelines to ensure the protection of the clinical data. This cross-sectional study will be based on a representative sample collected by stratified random sampling from the database comprising all the subjects listed in all the health centers of the 2 cities, comprising a total of 183,146 citizens. Prevalence of different studied characteristics will be estimated and calculated by demographic characteristics (age group, sex, education level) and RFs. The resulting database will be a very large one, comprising more than 2 million cells. Statistical data analysis will explore the associations between variables, using regression models so that predictive models can be built for the relevant variables. Moreover, similarities between subjects will be studied so that the possible existence of clusters can be identified. Also, other multivariate analysis, such as principal component analysis will be studied.

RESULTS

Of the 4000 subjects randomly selected, 3038 accepted to participate in the study. During the last 2 years, they have been observed according to the established and already described plan. A team of 88 researchers from 17 different clinical institutions performed more than 5580 patient observations, blood and urine sample collections, and predetermined examinations (electrocardiogram, PWV, central BP), including 1047 cognitive evaluations (in subjects >50 years of age). A biobank, containing more than 10,000 samples of plasma, 10,000 samples of serum, and 5000 samples of urine, is appropriately accommodated in our University. In Supplemental Table 3 (Supplemental Digital Content 1, http://links.lww.com/JIM/A21), the number of subjects observed, distributed by gender and age strata are presented for visits 1 and 2.

DISCUSSION

CVRFs and CVD in Portugal

Portugal is the only Western European state in the top 10 countries of the world where stroke exceeds ischemic heart disease's (IHD's) standardized mortality rate.[12] The incidence of transient ischemic attacks and stroke is known to be very high in Portugal and particularly in the northern region (up to 3 strokes and 0.67 TIAs per year/1000 inhabitants[13,14]). Ischemic heart disease has a complete different pattern of incidence in Portugal; the country bears the second lowest standard mortality rate by IHD in Europe[15] and a particular geographic pattern of distribution of its incidence,[16] with the northern coastal area recording the lowest admission and mortality rates—data that enhance the contrast with stroke incidence. With the strict CVR phenotyping methodology here presented, we aspire to uncover unclear contributions to this paradoxical CVD manifestation.

The prevalence of different CVRFs and the incidence of CV events (CVEs) in the Portuguese population have received greater attention in the last decade. Studies with different measurement and population sampling methodologies have found a national prevalence of hypertension ranging from 41.2% to 42.6%,[17–19] with the diagnosis of isolated systolic hypertension estimated to affect 34.7% of Portuguese individuals older than 55 years[20] and prehypertension afflicting 39.5% of the adult population.[17] However, the use of BP values measured in a single visit and the recruitment strategies used raised criticisms, arguing an overestimation of hypertension prevalence and/or underestimation of its awareness and treatment rates. Overestimation of hypertension and mean BP values with single visit strategies have been reported[21–23] (ranging from 12.6% to 35% for hypertension in Portugal). Surprisingly, the number of

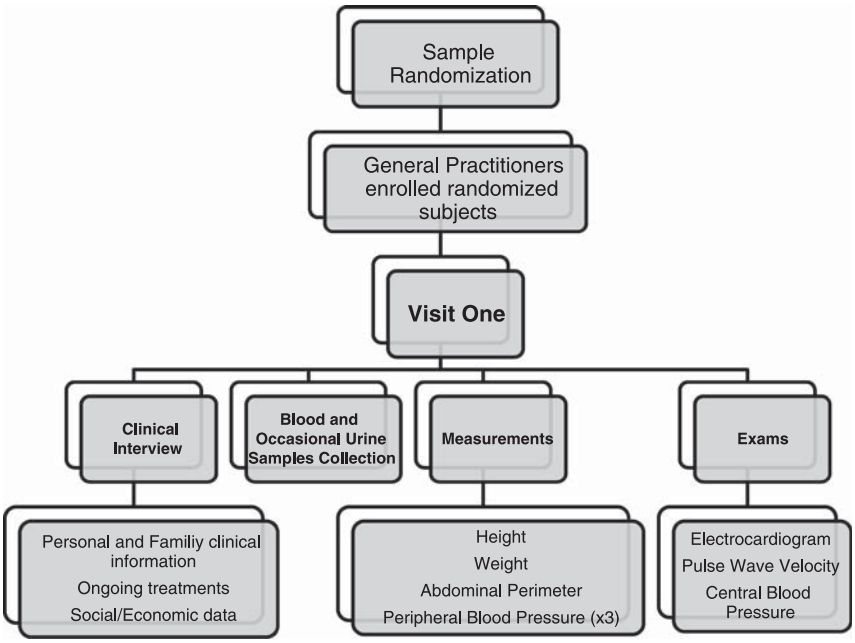


FIGURE 1. Study procedures outline (visit 1).

studies using at least a 2-visit strategy is very scarce worldwide.[24,25] The prevalence, awareness, treatment, and control rates of hypertension in Portugal are not different from those in other southern and western European countries.[25] The paradox lies on the concept that the Portuguese hypertension prevalence as well as BP mean values have been trending down in the last decades,[26] at a rate that is reported to be superior to that observed in other Western European countries,[27] and still the incidence of CVE and particularly stroke has kept disproportionally elevated, even if presenting a tendency to decline.[28,29] We aim to produce more precise estimation of BP levels and hypertension prevalence as well as the quantification of overestimation of BP and hypertension that results from single BP evaluation (with the consequent underestimation of BP treatment and control).

Other CVRFs have been less studied in Portugal. The worldwide trend in mean fasting plasma glucose (FPG) has slowly increased, and the age-standardized prevalence of diabetes ranges from 9.8% in men to 9.2% in women.[30] Portuguese estimations of the prevalence of diabetes (single visit strategy) range from 11.7% to 13% of the adult population,[31,32] evidencing a higher prevalence of the disease than globally recorded, especially concerning men; the prevalence of prediabetes was estimated in 23.3%.[31] According to the International Diabetes Federation, the prevalence of diabetes in Portugal was the highest recorded in the European countries analyzed.[33] We could not find any national trend analysis of diabetes and FPG levels. The relevance of mean FPG levels increases with evidence linking growing levels with CVD, independently of the existence of diabetes.

Chronic kidney disease and microalbuminuria are well recognized CVR factors.[34–37] An estimation of CKD (based on a single measurement of serum creatinine levels and on information from a national registry of patients under renal replacement therapy) reported a 6.1% prevalence in the Portuguese adult inhabitants,[38] similar to reports in other European countries.[39] However, more

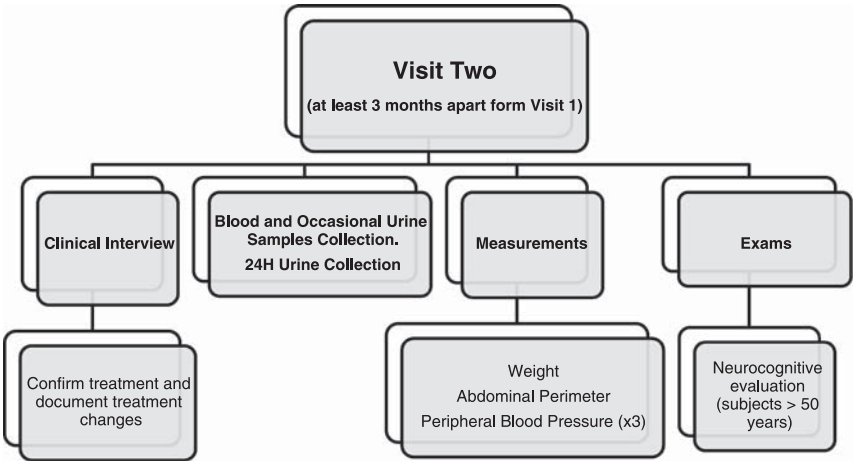


FIGURE 2. Study procedures outline (visit 2).

accurate methods of staging CKD have been established, combining glomerular filtration rate estimates and albuminuria[40,41] and exhibiting a better association both to the risk of progression to end-stage renal disease and to the CVR in the population, throughout all age classes.[10,42,43] Therefore, a more accurate definition of CKD prevalence in Portugal, using both markers, is missing. Only for Portuguese hypertensive subjects[44] can a report of prevalence of microalbuminuria (24%) be found.

Regarding dyslipidemia, a systematic review of the literature found several difficulties in aggregating results from different studies in Portugal, with different population groups, measurement methodologies, selection bias, and cutoff values.[45–47] Main extractable results revealed mean serum total cholesterol values greater than 200 mg/dL, 20% of the population recording cholesterol levels greater than 240 mg/dL, and low high-density lipoprotein and high triglyceride levels affecting 25% and 30.7% of the population, respectively.[48] In Portuguese subjects treated with statins, 68% and 62.9% did not have their total cholesterol and low-density lipoprotein cholesterol levels controlled, respectively.[49] Worldwide, a slow and small global trend of decreasing levels of total cholesterol has been recorded in the last 3 decades, estimating current mean levels in Western Europe as 212.7 mg/dL.[50]

Metabolic syndrome has been estimated to afflict 27.5% of the national population and 23.9% of a regional urban sample of adult dwellers[48,51]; male sex was predominantly presenting the syndrome from 18 to 49 years, with females being more afflicted in older age groups.[48] Overweight and obesity prevalence are also growing in the Portuguese population, with a composite prevalence of 66.6% and 57.9% for men and women, respectively.[52,53]

In summary, we believe that it is important to look simultaneously at strictly determined BP and FPG mean values, lipid serum levels, estimated glomerular filtration rate, and albuminuria when trying to understand the different CVE rates registered in Portugal (more stroke than IHD), and to look for other explanations of these same CVE that go beyond individual prevalence of hypertension, diabetes, and CKD (cumulative RFs, intermediate CV phenotypes, CV risk reclassification strategies—global CVR stratification).

Recent Concepts of Cardiovascular Risk and CVD

New methods of evaluating CVD and CVR have been proposed. Measurement of arterial stiffness (through PWV) has been accepted as portraying the reflex of different CVRFs in the arterial structure, becoming itself a marker of CVR with independent predictive power of CVE beyond and above traditional CVRFs, allowing for risk restratification of significant proportions of the population; the predictive ability of arterial stiffness is higher in high-risk groups, but it retains its discriminative power both in the general population and hypertensive subjects independently of age.[54–56] Recent European guidelines establish PWV values greater than 10 m/s as TOD,[57] and reference values for the European population have been published.[58]

In Portugal, arterial stiffness has been raising growing interest. In a pioneer study, a concurrent determination of salt intake and PWV in a convenience sample of 426 subjects could record a high salt intake and its independent correlation with PWV values (r = 0.256, after adjustment for age and BP).[59] Another project has determined PWV values to subjects referred by their clinicians to 3 clinical centers[60]; a predictive value of PWV for CVE could be defined for values of PWV greater than the 95th percentile. Interestingly, the unadjusted risk ratio for stroke was 6.68, and that for myocardial infarction was 5.4. In a subsample of 668 young subjects (mean age, 40 years) with low CV risk and significant male predominance (60%), the authors statistically extrapolated "normal" PWV values for 16 different age classes (8 per sex).[61]

Central blood pressure and central hemodynamic indexes are another aspect of vascular evaluation that can be determined safely and noninvasively. Its association with the development of TOD and CVD has been well documented.[62] A recent meta-analysis has established their values as independent predictors of CVE and all-cause mortality,[63] and interest has been increasing as CBP is thought to better reflect the pressure load sustained by vascular circulation of the brain, kidney, and coronary arteries.[64–67]

Early vascular aging is a functional and clinical concept[68] in fast development: the normal age-dependent process of vascular change in structure and function can, in susceptible subjects or under the influence of different factors (including CVRFs), be accelerated and/or be replaced by a pathologic process of vascular remodeling, leading to premature atherosclerosis and CVD.[69–73] The definition of EVA is dependent on arterial stiffness measurements, but debate is ongoing to better refine the criteria to use. The question remains if by identifying subjects with early vascular changes, one could reclassify low CV risk subjects in higher CV risk classes and treat early and more intensively to prevent CVD.[72,74] No evaluation of EVA, PWV, or CBP has ever been attempted in the Portuguese population. The CVR reclassification value of these variables in the Portuguese CVD setting is something we aim to establish with the current study.

Vascular Aging and Brain Aging

Subclinical organ damage is one important parameter to classify subjects concerning their total CVR. The brain is particularly challenging (when it comes down to evaluate TOD induced by CVRFs or CVD) as there is no simple way to identify subjects with subclinical manifestations, and TOD is defined only when a documented cerebrovascular event has been registered. Much has been debated concerning the influence and contribution of vascular disease, CVRFs, or hemodynamic variables to the acceleration of the brain aging process and/or the establishment of cognitive decline, and the concept of vascular cognitive impairment is well established.[75,76]

With an aging population, progression to dementia and the increase in the number of dependent subjects are clearly a concern; estimation of the prevalence of mild cognitive impairment ranges from 10% to 20% in adults older than 65 years,[77] which obviates the need to early identification of subjects at risk of cognitive decline and control of factors that contribute to dementia. The LADIS (Leukoaraiosis and Disability) study[78–80] has shown that older independent subjects with white matter hyperintensities progress to disability and functional decline with the more severe changes relating to higher risk of decline.[78] An association of lacunar infarcts with cognitive decline and white matter hyperintensities has also been considered (hinting for a spectrum of different expression of small vessel disease),[79] and an independent effect of CVRFs in cognitive decline has also been reported.[80] In Portugal, the prevalence of cognitive impairment has been studied in a regional sample of the population, and a report of 12.0% to 16.8% (urban-rural areas, respectively) has been described.[81]

Evidence fueling the merit of a precocious and preventive identification of subjects at risk of progression to dementia or cerebrovascular disease comes from the Framingham study, where changes in white matter microstructure and reduction in gray matter volume could be seen in association with growing levels of BP in young subjects.[82] With the knowledge that

arterial stiffness measurements can be associated and predict cognitive decline[83–85] and silent cerebral small vessel disease,[86] one could aspire to an early recognition of subjects at risk using PWV measurement.

CONCLUSIONS

Here we reviewed the literature reporting the state of knowledge concerning CVD, CVRF prevalence, and cognitive decline estimates of the Portuguese population, comparing it with what is known abroad. Based on this evidence, we developed the rationale of the Guimarães/Vizela Study, a multimodal population-based cohort study to determine global CV risk and disease, a comprehensive approach to determination of global CV risk and risk reclassification perspectives, with the ultimate goal of early detection of subjects at risk for CVD, renal disease, cognitive impairment, and dementia.

ACKNOWLEDGMENTS

The authors thank the following institutions and departments: Agrupamento de Centros de Saúde de Guimarães e Vizela, Serviço de Patologia Clínica do Centro Hospitalar do Alto Ave, Centro de Saúde da Amorosa, USF Afonso Henriques, USF Ara Trajano, USF Duo Vida, USF Novos Rumos, USF de Pevidém, USF Physis, USF de Ponte, USF de Ronfe, USF S. Nicolau, USF de S. Torcato, USF de Serzedelo e USF Vimaranes. They thank all physicians working in the PHCCCs, whose work enrolling subjects was vital.

The Guimarães Study Group is also composed by the following researchers, responsible for data collection: Helena Sarmento, Gloria Alves, Sara Freitas, Ana Sofia Alves, Sofia Gomes, Marta Gonçalves, Rui Fernandes, António Pedro Fonte, José Miguel Sá, Clarisse Neves, Andreia Sampaio, Cristina Cunha, Sílvia Sousa, Filipe Gonçalves, Joana Malheiro, João Silva, Carlos Fernandes, Estefânia Bustabad, Laura Castro, Nuna Vieira, Filipa Ramos, Ana Catarina Marques, Joana Monteiro, Ana Sofia Silva, Margarida Rocha, Mafalda Jordão Abreu, Margarida Dias, Magda Fernandes, Paula Felgueiras, Francisca Castro, Vânia Gomes, Ana Luísa Novo, Carla Pereira, Ana Cristina Ramalho, Fernando Esculcas, Dina Fernandes, Ricardo Rodrigues, Rafael Velho, Diana Coimbra, Joana Pimenta, João Pedro Teixeira, Ana Luísa Neves, Joana Leitão, Sara Pereira, Carla Ferreira, Clarinda Neves, Samuel Pedreira, Pedro Neves, Elisabete Lima, Carla Mendes, Orlanda Barbosa, Ana Catarina Martins, Célia Lemos, Eduarda Macedo, Sílvia Azevedo, Ana Rita Ribeiro, Elsa Salgado, Joana Dias, Eduarda Piairo, Marisa Carneiro, Ismael Costa, Lucília Miranda, Ana João Gonçalves, Natália Rodrigues, Manuela Morais, Rosário Santos, Odete Rodrigues, Alexandra Barreira, Ana Rita Romano, Catarina Marques, Fernanda Marisa Santos, Sónia Coelho Pereira, Elisabete Pinto Teixeira, and Joana Andrade Pinto

REFERENCES

1. Dzau VJ, Antman EM, Black HR, et al. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part I: pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). *Circulation*. 2006;114:2850–2870.
2. Dzau VJ, Antman EM, Black HR, et al. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part II: clinical trial evidence (acute coronary syndromes through renal disease) and future directions. *Circulation*. 2006;114:2871–2891.
3. O'Rourke MF, Safar ME, Dzau V. The cardiovascular continuum extended: aging effects on the aorta and microvasculature. *Vasc Med*. 2010;15:461–468.
4. Lozano R, Naghavi M, Foreman K, 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*. 2013;380:2095–2128.
5. Mancia G, de Backer G, Dominiczak A, et al. 2007 Guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2007;25:1105–1187.
6. Conroy RM, Pyorala K, Fitzgerald AP, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J*. 2003;24:987–1003.
7. Reiner Z, Catapano AL, de Backer G, et al. ESC/EAS guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J*. 2011;32:1769–1818.
8. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2012;35(suppl 1):S64–S71.
9. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis*. 2004;43:S1–S290.
10. Perk J, de Backer G, Gohlke H, et al. European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2012;33:1635–1701.
11. Santos NC, Costa PS, Cunha P, et al. Mood is a key determinant of cognitive performance in community-dwelling older adults: a cross-sectional analysis. *Age (Dordr)*. 2013;35:1983–1993.
12. Kim AS, Johnston SC. Global variation in the relative burden of stroke and ischemic heart disease. *Circulation*. 2011;124:314–323.
13. Correia M, Silva MR, Matos I, et al. Prospective community-based study of stroke in Northern Portugal: incidence and case fatality in rural and urban populations. *Stroke*. 2004;35:2048–2053.
14. Correia M, Silva MR, Magalhaes R, et al. Transient ischemic attacks in rural and urban northern Portugal: incidence and short-term prognosis. *Stroke*. 2006;37:50–55.
15. Muller-Nordhorn J, Binting S, Roll S, et al. An update on regional variation in cardiovascular mortality within Europe. *Eur Heart J*. 2008;29:1316–1326.
16. Ferreira-Pinto LM, Rocha-Goncalves F, Teixeira-Pinto A. An ecological study on the geographic patterns of ischaemic heart disease in Portugal and its association with demography, economic factors and health resources distribution. *BMJ Open*. 2012;2. pii:e000595.
17. Macedo ME, Lima MJ, Silva AO, et al. Prevalence, awareness, treatment and control of hypertension in Portugal: the PAP study. *J Hypertens*. 2005;23:1661–1616.
18. Cortez-Dias N, Martins S, Belo A, et al. Prevalence and management of hypertension in primary care in Portugal. Insights from the VALSIM study. *Rev Port Cardiol*. 2009;28:499–523.
19. de Macedo ME, Lima MJ, Silva AO, et al. Prevalence, awareness, treatment and control of hypertension in Portugal. The PAP study. *Rev Port Cardiol*. 2007;26:21–39.
20. Clara JG, de Macedo ME, Pego M. Prevalence of isolated systolic hypertension in the population over 55 years old. Results from a national study. *Rev Port Cardiol*. 2007;26:11–18.
21. Figueiredo D, Azevedo A, Pereira M, et al. Definition of hypertension: the impact of number of visits for blood pressure measurement. *Rev Port Cardiol*. 2009;28:775–783.

22. Modesti PA, Rapi S, Bamoshmoosh M, et al. Impact of one or two visits strategy on hypertension burden estimation in HYDY, a population-based cross-sectional study: implications for healthcare resource allocation decision making. *BMJ Open*. 2012;0:e001062.
23. Bovet P, Gervasoni JP, Ross AG, et al. Assessing the prevalence of hypertension in populations: are we doing it right? *J Hypertens*. 2003;21:509–517.
24. Kearney PM, Whelton M, Reynolds K, et al. Worldwide prevalence of hypertension: a systematic review. *J Hypertens*. 2004;22:11–19.
25. Pereira M, Lunet N, Azevedo A, et al. Differences in prevalence, awareness, treatment and control of hypertension between developing and developed countries. *J Hypertens*. 2009;27:963–975.
26. Pereira M, Carreira H, Vales C, et al. Trends in hypertension prevalence (1990–2005) and mean blood pressure (1975–2005) in Portugal: a systematic review. *Blood Pressure*. 2012;21:220–226.
27. Danaei G, Finucane MM, Lin JK, et al. National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet*. 2011;377:568–577.
28. Leal J, Luengo-Fernandez R, Gray A, eds. *European Cardiovascular Disease Statistics 2012*. ed. Brussels: European Heart Network, Sophia Antipolis: European Society of Cardiology; 2012.
29. Saúde DGd. *Statistic Elements—General Health Information/2008*. Saúde DGd, ed. Lisbon, Portugal: Direcção Geral de Saúde; 2010:159.
30. Danaei G, Finucane MM, Lu Y, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*. 2011;378:31–40.
31. Gardete-Correia L, Boavida JM, Raposo JF, et al. First diabetes prevalence study in Portugal: PREVADIAB study. *Diabet Med*. 2010;27:879–881.
32. Cortez-Dias N, Martins S, Belo A, et al. Prevalence, management and control of diabetes mellitus and associated risk factors in primary health care in Portugal. *Rev Port Cardiol*. 2010;29:509–537.
33. Whiting DR, Guariguata L, Weil C, et al. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract*. 2011;94:311–321.
34. Sarnak MJ, Levey AS, Schoolwerth AC, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Hypertension*. 2003;42:1050–1065.
35. de Zeeuw D, Hillege HL, de Jong PE. The kidney, a cardiovascular risk marker, and a new target for therapy. *Kidney Int Suppl*. 2005:S25–S29.
36. Hillege HL, Fidler V, Diercks GF, et al. Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation*. 2002;106:1777–1782.
37. Ruilope L, Kjeldsen SE, de la Sierra A, et al. The kidney and cardiovascular risk–implications for management: a consensus statement from the European Society of Hypertension. *Blood Pressure*. 2007;16:72–79.
38. Vinhas J, Gardete-Correia L, Boavida JM, et al. Prevalence of chronic kidney disease and associated risk factors, and risk of end-stage renal disease: data from the PREVADIAB study. *Nephron Clin Pract*. 2011;119:c35–c40.
39. Zhang QL, Rothenbacher D. Prevalence of chronic kidney disease in population-based studies: systematic review. *BMC Public Health*. 2008;8:117.
40. Gansevoort RT, de Jong PE. The case for using albuminuria in staging chronic kidney disease. *J Am Soc Nephrol*. 2009;20:465–468.
41. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011;80:17–28.
42. Hallan SI, Matsushita K, Sang Y, et al. Age and association of kidney measures with mortality and end-stage renal disease. *JAMA*. 2012;308:2349–2360.
43. Fox CS, Matsushita K, Woodward M, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *Lancet*. 2012;380:1662–1673.
44. Polonia J, Carmona J, Mendes E, et al. Prevalence of microalbuminuria in non-diabetic hypertensive patients attended by Portuguese GPs. *Rev Port Cardiol*. 2007;26:637–644.
45. Costa J, Borges M, Oliveira E, et al. Incidence and prevalence of hypercholesterolemia in Portugal: a systemic review. Part II. *Rev Port Cardiol*. 2003;22:683–702.
46. Costa J, Borges M, Oliveira E, et al. Incidence and prevalence of hypercholesterolemia in Portugal: a systematic review. Part I. *Rev Port Cardiol*. 2003;22:569–577.
47. Costa J, Borges M, Oliveira E, et al. Incidence and prevalence of hypercholesterolemia in Portugal: a systematic review. Part III. *Rev Port Cardiol*. 2003;22:829–836.
48. Fiuza M, Cortez-Dias N, Martins S, et al. Metabolic syndrome in Portugal: prevalence and implications for cardiovascular risk—results from the VALSIM Study. *Rev Port Cardiol*. 2008;27:1495–1529.
49. da Silva PM, Cardoso SM. Persistent lipid abnormalities in patients treated with statins: Portuguese results of the Dyslipidemia International Study (DYSIS). *Rev Port Cardiol*. 2011;30:47–63.
50. Farzadfar F, Finucane MM, Danaei G, et al. National, regional, and global trends in serum total cholesterol since 1980: systematic analysis of health examination surveys and epidemiological studies with 321 country-years and 3.0 million participants. *Lancet*. 2011;377:578–586.
51. Santos AC, Lopes C, Barros H. Prevalence of metabolic syndrome in the city of Porto. *Rev Port Cardiol*. 2004;23:45–52.
52. Sardinha LB, Santos DA, Silva AM, et al. Prevalence of overweight, obesity, and abdominal obesity in a representative sample of Portuguese adults. *PLoS One*. 2012;7:e47883.
53. do Carmo I, Dos Santos O, Camolas J, et al. Overweight and obesity in Portugal: national prevalence in 2003–2005. *Obes Rev*. 2008;9:11–19.
54. Mitchell GF, Hwang SJ, Vasan RS, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation*. 2010;121:505–511.
55. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol*. 2010;55:1318–1327.
56. Laurent S, Cockcroft J, van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006;27:2588–2605.
57. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2013;31:1281–1357.
58. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J*. 2010;31:2338–2350.
59. Polonia J, Maldonado J, Ramos R, et al. Estimation of salt intake by urinary sodium excretion in a Portuguese adult population and its relationship to arterial stiffness. *Rev Port Cardiol*. 2006;25:801–817.
60. Maldonado J, Pereira T, Polonia J, et al. Arterial stiffness predicts cardiovascular outcome in a low-to-moderate cardiovascular risk

population: the EDIVA (Estudo de DIstensibilidade VAscular) project. *J Hypertens*. 2011;29:669–675.

61. Pereira T, Maldonado J, Polonia J, et al. A statistical definition of aortic pulse wave velocity normality in a Portuguese population: a subanalysis of the EDIVA project [Portuguese]. *Rev Port Cardiol*. 2011;30:691–698.
62. Agabiti-Rosei E, Mancia G, O'Rourke MF, et al. Central blood pressure measurements and antihypertensive therapy: a consensus document. *Hypertension*. 2007;50:154–160.
63. Vlachopoulos C, Aznaouridis K, O'Rourke MF, et al. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J*. 2010;31:1865–1871.
64. Roman MJ, Devereux RB, Kizer JR, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension*. 2007;50:197–203.
65. Roman MJ, Devereux RB, Kizer JR, et al. High central pulse pressure is independently associated with adverse cardiovascular outcome the strong heart study. *J Am Coll Cardiol*. 2009;54:1730–1734.
66. Briet M, Pierre B, Laurent S, et al. Arterial stiffness and pulse pressure in CKD and ESRD. *Kidney Int*. 2012;82:388–400.
67. O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension*. 2005;46:200–204.
68. Nilsson PM, Boutouyrie P, Cunha P, et al. Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. *J Hypertens*. 2013;31:1517–1526.
69. Nilsson PM. Early vascular aging (EVA): consequences and prevention. *Vasc Health Risk Manag*. 2008;4:547–552.
70. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. *Circulation*. 2003;107:139–146.
71. Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part III: cellular and molecular clues to heart and arterial aging. *Circulation*. 2003;107:490–497.
72. Laurent S. Defining vascular aging and cardiovascular risk. *J Hypertens*. 2012;(suppl 30):S3–S8.
73. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. *J Hypertens*. 2008;26:1049–1057.
74. Kotsis V, Stabouli S, Karafillis I, et al. Early vascular aging and the role of central blood pressure. *J Hypertens*. 2011;29:1847–1853.
75. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–2713.
76. Gorelick PB, William M. Feinberg lecture: cognitive vitality and the role of stroke and cardiovascular disease risk factors. *Stroke*. 2005;36: 875–879.
77. Petersen RC. Clinical practice. Mild cognitive impairment. *N Engl J Med*. 2011;364:2227–2234.
78. Inzitari D, Pracucci G, Poggesi A, et al. Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (Leukoaraiosis and Disability) study cohort. *BMJ*. 2009;339:b2477.
79. Poggesi A, Pantoni L, Inzitari D, et al. 2001–2011: A decade of the LADIS (Leukoaraiosis And DISability) study: what have we learned about white matter changes and small-vessel disease? *Cerebrovasc Dis*. 2011;32:577–588.
80. Verdelho A, Madureira S, Ferro JM, et al. Differential impact of cerebral white matter changes, diabetes, hypertension and stroke on cognitive performance among non-disabled elderly. The LADIS study. *J Neurol Neurosurg Psychiatry*. 2007;78:1325–1330.
81. Nunes B, Silva RD, Cruz VT, et al. Prevalence and pattern of cognitive impairment in rural and urban populations from Northern Portugal. *BMC Neurol*. 2010;10:42.
82. Maillard P, Seshadri S, Beiser A, et al. Effects of systolic blood pressure on white-matter integrity in young adults in the Framingham Heart Study: a cross-sectional study. *Lancet Neurol*. 2012;11:1039–1047.
83. Waldstein SR, Rice SC, Thayer JF, et al. Pulse pressure and pulse wave velocity are related to cognitive decline in the Baltimore Longitudinal Study of Aging. *Hypertension*. 2008;51:99–104.
84. Scuteri A, Tesauro M, Appolloni S, et al. Arterial stiffness as an independent predictor of longitudinal changes in cognitive function in the older individual. *J Hypertens*. 2007;25:1035–1040.
85. Pase MP, Herbert A, Grima NA, et al. Arterial stiffness as a cause of cognitive decline and dementia: a systematic review and meta-analysis. *Intern Med J*. 2012;42:808–815.
86. Henskens LH, Kroon AA, van Oostenbrugge RJ, et al. Increased aortic pulse wave velocity is associated with silent cerebral small-vessel disease in hypertensive patients. *Hypertension*. 2008;52:1120–1126.

The rational /design of the Guimarães/Vizela study: a multimodal population based cohort study to determine global cardiovascular risk and disease

Pedro G. Cunha[(a)(b)(c)], Jorge Cotter[(a)(b)(c)], Pedro Oliveira[(d)], Isabel Vila[(a)], Nuno Sousa[(b)(c)]

Supplemental Material

Table 1

Comparison of the age distribution of the population of two cities (Guimarães/Vizela) with the one verified in the Northern region and the entire country

	Portugal	Northern Region of Portugal	Guimarães/Vizela
Age < 30	3375720 (32%)	1216153 (33%)	64147 (35.3%)
Age 30 – 39	1598250 (15.1%)	557849 (15.1%)	28963 (15.9%)
Age 40 – 49	1543392 (14.6%)	567015 (15.4%)	29723 (16.3%)
Age 50 – 59	1400011 (13.3%)	502790 (13.6%)	25131 (13.8%)
Age 60 – 69	1186442 (11.2%)	392718 (10.6%)	16935 (9.3%)
Age >= 70	1458363 (13.8%)	453157 (12.8%)	16961 (9.3%)
Total	10562178 (100%)	3689682 (100%)	181860 (100%)

Source – National Statistics Institute / Census 2011; Values between brackets are percentages (%)

Table 2

Comparison of foreigners residing in Portugal, in the northern region of Portugal and in the two cities included in this project (Guimarães/Vizela)

	Portugal	Northern Region of Portugal	Guimarães/Vizela
% of foreigners	8.3	4.7	3.6
% of foreigners of European origin	32	45	68

Source – National Statistics Institute / Census 2011

Table 3

Subjects observed during each visit

	Visit 1			Visit 2		
	Female	**Male**	**Total**	**Female**	**Male**	**Total**
Age < 30	391 (54.2)	330 (45.8)	721	311 (55.3)	251 (44.7)	562
Age 30 – 39	400 (58.9)	279 (41.1)	679	336 (58.3)	240 (41.7)	576
Age 40 – 49	185 (55.4)	149 (44.6)	334	160 (55.9)	126 (44.1)	286
Age 50 – 59	174 (51.2)	166 (48.8)	340	162 (53.3)	142 (46.7)	304
Age 60 – 69	220 (55.7)	175 (44.3)	395	189 (54.6)	157 (45.4)	346
Age >= 70	304 (53.4)	265 (46.6)	569	242 (51.7)	226 (48.3)	468
Total	1674 (55.1)	1364 (44.9)	3038	1400 (55.1)	1142 (44.9)	2542

Values between brackets are percentages (%)

Chapter 3

An epidemiological study determining blood pressure in a Portuguese cohort: the Guimarães/Vizela study

Journal of Human Hypertension (2014), 1–8

www.nature.com/jhh

ORIGINAL ARTICLE

An epidemiological study determining blood pressure in a Portuguese cohort: the Guimarães/Vizela study

PG Cunha[1,2,3], J Cotter[1,2,3], P Oliveira[4], I Vila[1] and N Sousa[2,3]

Surveying the evolution of blood pressure (BP) levels and hypertension (HTN) prevalence is important. A stringent strategy was utilized in a population cohort study. The BP was measured at two visits at least 3 months apart, and the results were analyzed using the following two methods: the Surveillance method (three BP measurements were performed in one visit, and the results were compared with those published previously for the identical method) and the Clinical method (three measurements per visit for two visits, and the concordant results in both visits were used to determine the BP classification). A total of 2542 subjects completed the evaluation. Using the Clinical method, an average systolic/diastolic BP value of 129.8/76.8 mm Hg was obtained, and the prevalence of HTN was 31.6%. Of the hypertensive patients, 74.3% were aware of his/her condition; 69.1% were treated and 40.8% of those treated had adequate BP control. A total of 24.7% of subjects changed his/her BP classification between visits, and 13.7% misreported HTN. Using the Surveillance method, we determined that the average global SBP has been maintained, with HTN prevalence increasing in this region, drifting from reported trends nationally and worldwide. There has been improvement in the proportion of treated and controlled subjects; however, the Surveillance method overestimated the HTN prevalence and underestimated the proportion of treated and controlled subjects. The BP levels were higher than observed worldwide in high-cardiovascular (CV) risk countries as well as higher than the minimum risk exposure level for developing CV disease.

Journal of Human Hypertension advance online publication, 31 July 2014; doi:10.1038/jhh.2014.61

INTRODUCTION

The methodology used to measure blood pressure (BP) is crucial.[1,2] The implications of a single-visit BP measurement in epidemiological studies and the value of BP for establishing the prevalence of hypertension (HTN) and other peripheral BP-based classifications have been the focus of extensive debate.[3–7] This issue has gained further relevance in light of the recent emphasis on the relevance of BP variability to cardiovascular (CV) risk.[8] In Portugal, published data on the average values and classification of BP have been derived from measurements performed in single-visit studies.[9,10]

Collecting the data during a single visit affects the definitions of the awareness, treatment and control of BP, frequently leading to incorrect estimations. To properly evaluate the evolution and trends of the BP levels and HTN prevalence over time as well as to audit the effect of BP control measures, it is fundamental that the BP measurements and data analysis techniques are performed with the exact same methodology,[1] avoiding the creation of multiple data sources that cannot be compared.[11]

In a country in which the citizens are generally considered as belonging to the low-risk CV group but the average systolic BP (SBP) values rank as the highest recorded in high-income countries,[12] exact methodological approaches for measuring and categorizing BP are needed. We designed an epidemiological study in which we observed each enrolled subject two times (at least 3 months apart) to accurately determine the peripheral BP average values and peripheral BP-derived classifications as well as to determine the proper estimates of HTN awareness, treatment and control. To investigate the effect of different analytical methods, we present the results from the following two methods: (a) the Surveillance method (one visit/three BP measurements), performed as in a national survey 10 years ago[9] and recommended elsewhere[1] and (b) the Clinical method (two visits with three BP measurements per visit), in which concordant results in both visits are used to classify the subjects.

METHODS

The methodology in this study has been described in detail elsewhere.[13] A representative sample of the adult population (⩾18 years) in two adjacent cities in northern Portugal (Guimarães and Vizela) was randomly selected and evaluated on two occasions at least 3 months apart after the participants signed a written consent form approved by an Ethics Committee. On both visits, clinical and biological information was gathered. The individuals were instructed to arrive in the morning, with their usual prescription medication; caffeinated beverages and tobacco use were not allowed before the BP measurements. Trained physicians performed the BP evaluations on every occasion, and standardization of the BP measurement was ensured by the use of training sessions before the beginning of the study. BP was measured three times (on each visit) with the subjects in a seated position after at least 15 min of rest; BP measurements were collected 2 min apart using a validated OMRON-705IT device (Omron Healthcare Europe B.V, Hoofddorp, The Netherlands). In the Surveillance method of data analysis, average of three measurements in one visit was used to determine the average BP and BP classification of each subject; subjects where considered hypertensive if the SBP/diastolic BP (DBP) was ⩾140/90 mm Hg and/or if they were treated with

[1]Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Internal Medicine Department, Guimarães—Centro Hospitalar do Alto Ave/Minho University, Guimarães, Portugal; [2]Life and Health Science Research Institute (ICVS), School of Health Science, University of Minho, Braga, Portugal; [3]ICVS/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal and [4]Departamento de Estudo de Populações, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal. Correspondence: Dr PG Cunha, Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Serviço de Medicina Interna do Hospital de Guimarães, Centro Hospitalar do Alto Ave, Life and Health Science Research Institute (ICVS), University of Minho, Rua dos Cutileiros, Creixomil, Guimarães 4810, Portugal. E-mail: pedrogcunha@netcabo.pt
Received 27 January 2014; revised 10 June 2014; accepted 16 June 2014

antihypertensive medication. Age stratification was performed using the following three age groups: < 35 years, 35–64 years, and ⩾ 65 years. In the Clinical method, the average of the last two measurements in each visit was used to define the average BP values of the visit, and the final average BP values were calculated using the two last measurements of each visit (average of four values); subjects were considered hypertensive if the SBP/DBP ⩾ 140/90 mm Hg in both visits and/or if they were treated with an antihypertensive medication. The age stratification was performed using the following seven age groups: < 30 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years, and ⩾ 80 years. We did not include self-reporting of HTN as a criterion for the definition of HTN in either method.

The BP categorization and definitions used were according to the 2007 European Society of Hypertension guidelines.[14]

Statistical analysis

The collected information was included in a database and subjected to a predefined statistical analysis. The database followed national and international guidelines to ensure protection of the clinical data. This cross-sectional study was based on a representative sample collected by stratified random sampling from a database comprising subjects in all the health centers of the two cities (a total of 183, 146 citizens living in both cities). The age and gender distribution of the database differed by < 2% from the age and gender distribution of the population of the two cities for the year 2006, as estimated by the National Institute of Statistics. The prevalence of the studied characteristics was estimated and calculated by the demographic characteristics (age group, gender and education level) and the risk factors.

RESULTS

Four thousand subjects were randomly selected. A total of 3038 subjects agreed to enroll in the clinical observation plan (Supplementary Figure S1—Supplementary Material). These 3038 subjects were evenly distributed by gender (55.0% female), with a mean age (s.d.) of 45.1 (19.5) years (men: 44.2 (19.6) years; women: 46.1 (19.4) years). The age distribution is shown in Table 1. A total of 83.9% of the enrolled subjects completed both visits (2550 subjects); 488 subjects attended only the first visit, with a dropout rate of 16.1%. A full dropout analysis was performed, and the results are available in the Supplementary Material.

For the BP analysis, we considered 2542 subjects who completed the two-visit plan and whose BP measurement records were complete; their biological characteristics are described in Table 1.

In Table 2, we present the average BP values from each visit, stratified by gender and adjusted by age and gender, according to the two analytical methods chosen for this study.

Results obtained using the Clinical method

Overall, the difference between the SBP and DBP in the visits was < 1 mm Hg (Table 2). In both the visits, the average SBP and DBP values were significantly higher in men across all age groups, although for ages > 60 years, these differences were no longer statistically significant (data not shown).

Figure 1 displays the average BP values according to the age class in the 2542 subjects who completed the study.

Both visits were analyzed individually and included in the final calculation of the prevalence of HTN (concordant results in both visits); males registered a significantly higher prevalence of HTN ($X^2 = 10.216$; $X^2 = 25.272$; $X^2 = 9.244$, $P < 0.01$). Supplementary Figure S2 and Supplementary Table H in (Supplementary Material) display the prevalence of HTN according to age class and gender.

In the 2542 subjects studied, 35.7% (crude rate) were considered hypertensive (in both visits), and 52.8% were considered normotensive (in both visits). A total of 11.6% were labeled as either normotensive or hypertensive at one of the two visits.

Anti-hypertensive drug treatment was offered to 69.1% of the hypertensive subjects (verified by a physician in both visits). In Supplementary Table S4 (Supplementary Material), we present the percentage of the treated hypertensive subjects according to their gender and age class. Females were more likely to be treated than males ($X^2 = 13.904$; $P < 0.001$), particularly in the younger age groups; older subjects were more likely to be treated than younger subjects ($X^2 = 75.999$, $P < 0.001$).

After identifying the subjects undergoing treatment, we presented the average BP values according to the BP status (normotensive and treated/untreated hypertensive) and the global average BP values according to age and gender stratification—Supplementary Tables I and J (Supplementary Material).

HTN awareness. A total of 74.3% of the hypertensive subjects were aware of his/her diagnosis and reported themselves as having HTN; women were more aware of their disease status than men (81.1% vs 67.3%, respectively) ($X^2 = 22.126$, $P < 0.001$). A total of 73.4% of the subjects unaware of having HTN were not undergoing treatment. Of the subjects who reported themselves as having HTN, 13.7% were not classified as hypertensive subjects.

HTN control. A total of 29.5% of the hypertensive subjects had controlled BP (< 140/90 mm Hg) (Figure 2; Supplementary Graphic M). Of the hypertensive subjects undergoing treatment, 40.8% had controlled BP (Supplementary Graphic N). In the Supplementary Graphics M and N, we present the distribution of these subjects by age class and gender (Supplementary Material).

In the hypertensive subjects, 34.3% of women had controlled BP vs 24.5% of men ($P < 0.01$); the BP control rates vary with age and according to gender, showing a statistically significant variation for age > 30 years in men and not in women (data not shown). In the treated hypertensive subjects, although the BP control rates were higher in women, these numbers are not statistically significant. With increasing age, the BP control rate in treated hypertensive subjects was progressively lower ($X^2 = 38.2$, $P < 0.001$).

Normal/high normal BP (or prehypertension). Table 2 displays the prevalence of prehypertension[15] observed at each visit and the final prevalence of only those with persistently recorded values that are consistent with the diagnosis in both visits. In Supplementary Graphic P, we present the distribution according to age and gender. There was a significant prevalence in males (27.5% vs 12.3%) ($X^2 = 77.133$, $P < 0.001$), and 76.7% of the cases of prehypertension occurred before the age of 50 years; the prevalence in males was higher until the age of 50 years, after

Table 1. Biological characteristics of 2542 subjects studied for blood pressure

	Total	*Female*	*Male*
Number of cases	2542	1400	1142
Mean age (years)	45.5	46.1 (18.9)	44.2 (19.4)
Age < 30 years (*n*)	562	311	251
Age 30–39 years (*n*)	576	336	340
Age 40–49 years (*n*)	286	160	126
Age 50–59 years (*n*)	304	162	142
Age 60–69 years (*n*)	346	189	157
Age 70–79 years (*n*)	388	203	185
Age ⩾ 80 years (*n*)	80	39	41
Mean BMI (Kg m^{-2})	26.6 (4.6)	26.5 (5.1)	26.6 (4.0)

Abbreviation: BMI, body mass index.

Table 2. Average (s.d.) BP values, hypertension and prehypertension prevalence according to gender distribution and using different analysis methods (adjusted for gender and age, according to study population characteristics in Portuguese National Institute, 2006)

	Clinical method			*Surveillance method*		
	Male (n = *1142*)	*Female* (n = *1400*)	*Total* (n = *2542*)	*Male* (n = *1142*)	*Female* (n = *1400*)	*Total* (n = *2542*)
Visit one						
Systolic BP (mm Hg)	133.5 (18.4)	126.7 (20.4)	130.0 (20)	135.1 (18.6)	126.8 (20.4)	130.4 (20.2)
Diastolic BP (mm Hg)	78.8 (10.9)	75.9 (10.4)	77.3 (10.7)	79.6 (10.7)	77.0 (10.3)	78.5 (10.6)
Hypertension (%)	40.3	37.2	38.7	43.7	37.6	40.5
Prehypertension (%)	42.7	22.8	32.5	42.0	23.4	33.5
Visit two						
Systolic BP (mm Hg)	133.8 (19.5)	124.7 (20.6)	129.1 (20.7)			
Diastolic BP (mm Hg)	77.7 (10.8)	74.7 (10.0)	76.2 (10.4)			
Hypertensive (%)	40.4	33.6	36.9			
Prehypertensive (%)	40.3	23.6	31.7			
Both visits						
Systolic BP (mm Hg)	133.6 (17.5)	126.2 (19.1)	129.8 (19.0)			
Diastolic BP (mm Hg)	78.2 (9.9)	75.3 (9.3)	76.8 (9.7)			
Hypertensive (%)	33.0	30.2	31.6			
Prehypertensive (%)	27.5	12.3	19.7			

Abbreviation: BP, blood pressure.

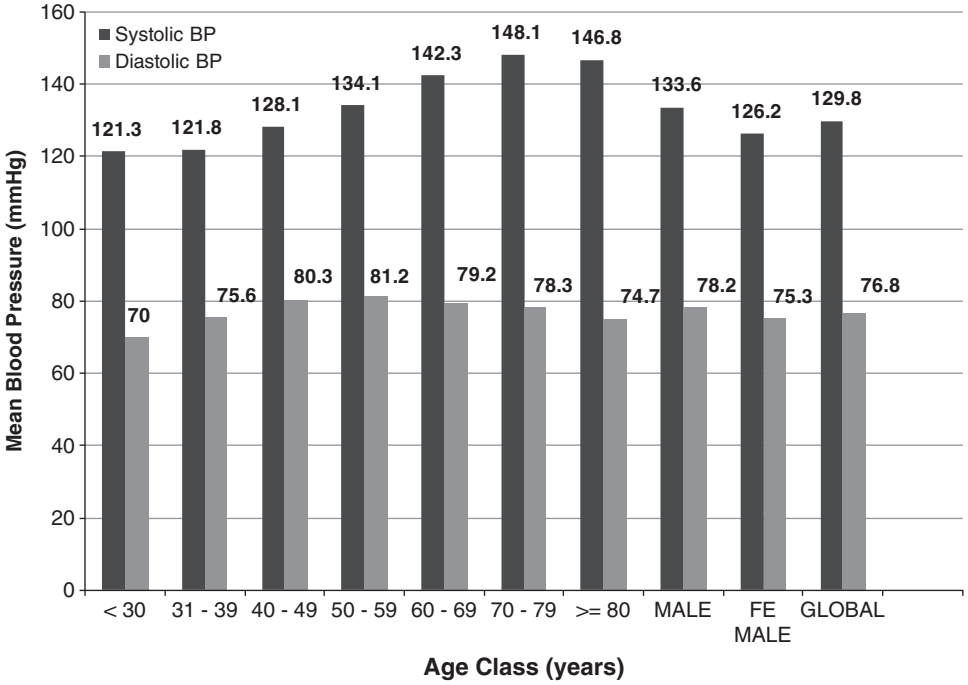


Figure 1. Average BP values according to age class (2542 subjects) (Clinical method).

which the distribution of the prehypertension prevalence was similar in males and females.

Results obtained using the Surveillance method

This method analyzes the data from BP readings obtained three times during a single visit. The data collected during visit 1 was used to perform the analysis. The BP values, stratified by age, gender, BP classification and treatment status, (normotensive and untreated/treated hypertensive subjects) are presented in Table 2 and Table 3A. The average BP values from each visit overlap and are nearly identical, which supports the reproducibility of this method (data not shown). Consistent with the Clinical method, males had higher average BP values and prevalence of HTN (Tables 2 and 3B).

The record of HTN treatment was 63.2%; in the hypertensive subjects, controlled BP was recorded in 25.1%; of those treated, 38.0% of the subjects had controlled BP (Table 3B). With this method, we verified that women were more likely to be treated than men and had higher control rates.

DISCUSSION

This study is an epidemiological evaluation in Portugal of BP that was based on a two-visit methodology, with the visits separated

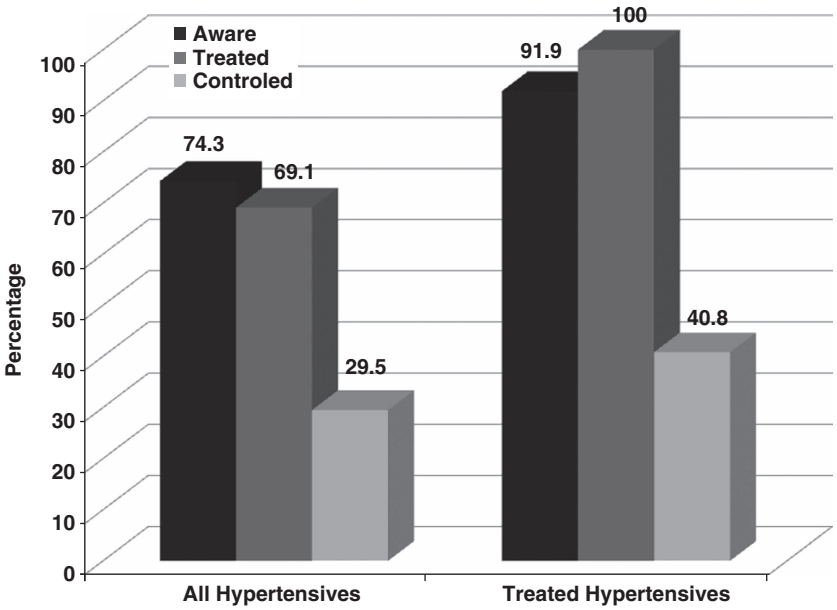


Figure 2. Awareness, treatment and control in hypertensive subjects (Clinical method).

by at least 3 months, using a representative sample of the population and following a standardized measurement method (including the time of the day). The implications of using this methodology are considerable; we showed that 24.7% of the subjects shifted their classification as hypertensive, prehypertensive or normotensive with optimal BP between visits. These results are based on objective measurement and verification of ongoing therapy; we did not include self-reported HTN as a criterion for defining BP, which could cause a considerable overestimation of the prevalence of HTN and decrease the variability of the classification between visits. We analyzed the data using two methods, allowing for the numbers to be compared with the data produced with similar methods in Portugal and abroad; on the other hand, the differences in the results of the two methods have implications from the epidemiological/clinical and public health viewpoints.

A sample size of at least 2339 subjects was required to achieve 2% precision in an estimated prevalence of HTN of 41.2%,[16] with a 95% confidence level. This sample size was surpassed even after accounting for the subjects who dropped out (16.1%), which was within the expected range.[17] The subjects who dropped out of the study did not significantly change the representative character of the sample population.

Difference in the results obtained using the Surveillance or Clinical method

With each method, we found no significant differences in the observed average BP values in a comparison of the two visits. In the comparison of the two analytical methods, there were no significant differences in the global average BP values; however, males consistently registered lower average SBP values in analysis by the Clinical (more stringent) method (Table 2—the absolute differences in the average SBP values is 0.6 mm Hg for the global average BP; 0.6 mm Hg for females and 1.5 mm Hg for males). The Clinical method stratifies age into seven groups, whereas the Surveillance method uses three groups. The known effect of the use of a smaller number of age groups as well as excluding the first BP reading from the analysis has been addressed[1] and could be fully reproduced here.

Compared with the Clinical method, the Surveillance method overestimated the prevalence of HTN in 8.9% (approximately 11% in men) of the cases and underestimated the proportion of treated, controlled and treated/controlled hypertensive subjects by 3.1, 4.4 and 2.8%, respectively.

Comparing the BP values with the previously available regional data

Previous data on BPs collected and analyzed with the identical methods in this region are scarce. The only published study that used the Surveillance method describes national characteristics; the study was conducted approximately 10 years ago and contained few data on the region in which this local study was conducted, which is summarized in Tables 3A and B, (including unpublished data of the PAP study, courtesy of Macedo *et al.*[9,16]

The standardized global average values of SBP/DBP obtained with both methods (Clinical and Surveillance) were 129.8/76.8 and 130.4/78.5 mm Hg, respectively. These values contrast with the last published (2003) national figures, in which the average values were 134.7/80.5 mm Hg nationally and 130.5/79.9 in the northern region.[9,16] It becomes apparent that, in this region, the global average SBP has been maintained, whereas the DBP has decreased. A more thorough analysis revealed that the average SBP was slightly higher (than 10 years ago, in this region) in subjects >35 years of age (Table 3A). These regional/local data do not support the so-called decreasing BP trend that has been published regarding Portugal and other countries.[11,12]

Comparing the BP values with the national and international data

Compared with the national data by Macedo *et al.*, the local results obtained 10 years later show that, in the normotensive group, the pulse pressure is slightly increased compared with that in previous national reports (approximately 2 mm Hg). In the untreated and treated hypertensive subjects, the SBP and pulse pressure are lower, particularly in the treated hypertensive subjects. Because local data from 10 years ago are not available for these groups, it is not possible to establish a regional trend in the BP evolution from these results. The normotensive subjects in our region today register BP values that are globally similar to those published 10 years ago nationwide except in the age groups >65 years (globally), in which the current local values are higher. These findings are not in agreement with those of previous studies.[11]

Compared with the national data reported 10 years ago, the difference in the average BP in the hypertensive-treated

Table 3A. Average BP values, stratified by age gender and BP/treatment of BP status, using the Surveillance method and comparing with results from 10 years ago

	Surveillance method—Visit 1 (Guimarães/Vizela, 2012)				*PAP Study (2003) from Macedo* et al.[9,16]			*PAP Study (2003) (unpublished)*[a]
	Average SBP/mean DBP (mm Hg)				*National results, average SBP/DBP (mm Hg)*			*North Region*
	Normotensive	*Untreated HTN*	*Treated HTN*	*Total*	*Normotensive*	*Untreated HTN*	*Treated HTN*	*Total*	*Total*
All	121.2/74.0	149.4/88.8	136.0/82.2	130.4/78.5	120.5/75.1	151.9/88.9	152.1/85.3	134.7/80.4	130.49/79.96
< 35 years	117.5/71.7	146.0/87.8	121.0/79.2	119.6/73.0	120.5/73.7	145.2/86.7	135.1/88.2	125.1/76.2	125.2/77.4
35–64 years	121.2/75.6	149.6/90.9	141.4/85.0	132.0/81.0	122.1/76.7	150.8/91.6	146.1/88.2	134.6/83.0	129.9/82.3
⩾ 65 years	128.7/73.1	155.7/83.5	148.8/78.6	147.4/78.8	125.6/72.9	161.1/84.2	158.4/82.1	152.1/80.8	145.9/78.8
Males	125.5/75.0	150.8/86.6	142.4/85.1	135.1/79.6	125.3/74.6	153.0/84.6	154.4/85.7	139.1/80.8	
< 35 years	124.0/72.2	147.3/86.2	138.3/86.9	127.1/74.2	125.2/72.8	147.8/84.6	135.5/85.7	131.1/75.9	132.5/78.3
35–64 years	125.5/77.2	151.0/91.1	142.9/85.6	136.7/83.1	125.2/76.8	152.2/91.4	148.8/88.6	139.2/84.2	135.8/84.2
⩾ 65 years	129.6/73.4	159.2/84.5	150.6/78.5	149.2/78.9	125.3/72.8	161.4/84.1	160.7/81.4	153.4/80.7	147.3/78.4
Females	118.4/73.2	146.6/90.0	134.0/81.2	126.8/77.0	119.4/75.4	150.3/91.1	150.7/89.1	131.1/80.1	
< 35 years	113.1/71.3	141.9/93.4	115.8/76.9	114.0/72.1	116.9/74.4	139.7/91.1	135.0/89.1	119.6/76.4	118.6/76.5
35–64 years	118.4/74.5	147.6/90.5	140.3/84.5	128.0/79.3	120.2/76.7	148.9/91.8	145.5/87.9	130.9/82.0	125.3/80.8
⩾ 65 years	127.6/72.8	152.0/82.5	147.3/78.8	145.8/78.7	125.5/73.1	160.7/84.3	156.8/82.5	151.1/80.9	144.5/79.2

[a]Courtesy of Macedo *et al.*[9,16]

group across all age groups and, particularly, in women is remarkable.

Considering the national trend analysis proposed by Danaei *et al.*,[12] the SBP average values (Clinical—Surveillance methods) for males (133.6–135.1 mm Hg) and females (126.2–126.8 mm-Hg) are within the average BP uncertainty interval proposed as the mean BP values for this country in 2008. The values presented here are higher than those in the national trend analysis[11] reported until 2005 in the subjects with an average age of 30, 50 and 70 years.

It is safe to state that, in 2012, the Guimarães/Vizela age-and gender-standardized average SBP values were higher than those recorded worldwide,[12] regardless of the method used to analyze the data (Surveillance vs Clinical). The later values stem from recent publications in the USA,[17] Italy (Sardinia),[18] Switzerland,[19] Spain,[20–23] Latin America[24] and other European countries (Austria, Belgium, Denmark, France, Germany, Greece, Iceland, Italy, Luxembourg, The Netherlands, Spain, Sweden and United Kingdom) on the average values reported until 2008.[25] In these studies of different populations, whenever data on the average BP are presented according to the age groups, the values recorded in our population are higher for men and women across all the age groups. Few studies worldwide have conducted a double-visit strategy to phenotype the BP status.[5,7]

Considering that the theoretical minimum risk exposure for the development of CV disease (CVD) is a SBP of between 110 and 115 mm Hg,[26,27] significant exposure is present for the CV risk factor in this population (beginning at a very young age, particularly in men) with an expected inherent increase in the CV morbidity and mortality.[26]

HTN prevalence, awareness, treatment and control

Using stringent methodology (the Clinical method), we showed that 31.6% of the population is persistently labeled as hypertensive; men have a significantly higher prevalence than women, and the increasing prevalence with age is well documented, reaching 83.1% in the older age group. To compare our data with previously published reports in Portugal,[9,16] we used the Surveillance method and found that in the past 10 years, the prevalence of HTN in this region increased 7.1% (see Table 3B).

In our study, a surprising number of hypertensive subjects (74.3%) were aware of their diagnosis; of those treated (69.1% of the hypertensive patients), 40.8% had controlled BP. Men were significantly more unaware of their diagnosis than women, and higher percentages of women underwent treatment and presented with controlled BP across all the age groups. These numbers contrast with the findings reported 10 years ago[9] nationwide and in northern Portugal (Tables 3A and B); using the Surveillance method, we observed significant improvement in the treatment (29.2% more treated subjects) and control of HTN (13.3% more controlled subjects) in this region in the past 10 years. Although the number of treated hypertensives has increased (by 29%), it is disturbing that only an increase of approximately 5.5% in those who are concurrently treated and controlled was observed (Table 3B). It is also confirmed herein that, with increasing age, the level of BP control decreases; after 60 years of age, control rates fall well below 50%. The control rates registered in our population are within those registered in similar recent population based studies, where the treatment rates vary between 33.3% and 72% and the control rates (among treated subjects) range from 32.8% to 64.4%.[17,18,21–23,28–32] In the nearby province of Galiza, Spain, the prevalence of HTN is 25.5%, and treatment was offered to 72% of subjects, 36.4% of which had their BP controlled;[22] the best control rate among treated subjects was recorded in the United States of America.[17]

Table 3B. Prevalence of hypertension, treatment and control, stratified by age and gender, using the Surveillance method and comparing with results from 10 years ago

	Surveillance method—Visit 1 (Guimarães/Vizela, 2012)				*PAP Study (2003) from Macedo* et al.[9,16]			
	Prevalence (%)				*National results, prevalence (%)*			
	HTN	*Treated HTN*	*Controlled HTN*	*Treated/Controlled HTN*	*HTN*	*Treated HTN*	*Controlled HTN*	*Treated/Controlled HTN*
All	40.5	66.0	25.1	38.0	42.1	38.9	11.2	28.6
Males	43.7	58.5	19.3	33.0	49.5	30.6	7.2	23.4
Females	37.6	73.4	30.8	41.9	38.9	48.1	15.4	32.1
					North Region results, prevalence (%)			
				All	33.4	36.8	11.8	32.5

Abrreviations: BP, blood pressure; DBP, diastolic BP; HTN, hypertension; SBP, systolic BP.

Prehypertension

The observed prevalence of the subjects who were persistently labeled as prehypertensive in both visits was 19.7% (age and gender adjusted), with a significant predominance of men, occurring until the 50–59 years of age class. Earlier national studies reported a prevalence of 39.5% for prehypertension using the debated one visit/average of three measurements strategy.[9] The rates of progression to HTN in 4 years in the subjects with normal and high-normal BP at baseline were described in the Framingham study[33] and varied (according to the age class) from a minimum of 17.6% to 49.5%. Similar rates have been obtained in the Flemish Study on Environment, Genes and Health Outcomes.[34] These numbers have not been studied in the Portuguese population; however, applying the lowest progression rate to the data presented and reported for the Guimarães/Vizela population could indicate that approximately 30 000 new diagnoses of HTN would be expected in the next 4 years. As important as the ability to screen subjects who are at risk of developing HTN earlier is knowing that the prevalence of prehypertension is fundamental, because these subjects are at a higher risk of left ventricular concentric remodeling and diastolic dysfunction,[35] CV events (stroke or coronary heart disease)[36–38] and chronic kidney disease.[39]

Variability in the BP classification

The most persuasive argument for the use of an objective two-visit measurement of BP for classifying subjects according to their BP status (as we did in the Clinical method, excluding the self-reporting of HTN) is that we identified 24.7% of the subjects who had a different label concerning their BP class (from 'optimal' to 'prehypertensive' or from 'hypertensive' to 'normotensive') between the two visits. Equally important is that 13.7% of the subjects who self-reported as hypertensive could not be labeled as such based on objective BP measurement and clinical evaluation. Therefore, the overestimation of the prevalence of HTN is understandable; we could identify these effects (overestimation of HTN, especially in men, and underestimation of treatment and control) when comparing the results with the Surveillance method analysis and one-visit method (Table 2).

These significant numbers present a strong argument for the careful categorization of subjects and the inherent subsequent CV risk attribution in clinical practice or research studies. We placed subjects in the BP classes in which they objectively and persistently belonged during the two-visit schedule. This says very little about the prognosis of those with variable BP values, because variability *per se* is associated with an adverse CV prognosis.[8]

Limitations

The limitations of this study include the gathering of data from a regional setting, with limited previously published results that could be used for better and more accurate comparisons and evolution evaluations over time.

Applying routine clinical methodology and criteria (measurement on two occasions; excluding self-reporting of disease) to an epidemiological study has a clear effect on the estimates of the prevalence, awareness, treatment and control of HTN and prehypertension. The average SBP values in our study population are markedly higher than those representing the minimum exposure risk for the development of CV disease and higher than the average values recorded worldwide and in high-CV risk countries. The reported national and worldwide trend of the BP decrease was not observed in the region in which this study was conducted; the global average BP values have been maintained in the past 10 years, but for those aged >35 years the SBP and pulse pressure are slightly higher than previously recorded in this region.

These data suggest an increase in the CV risk over the past 10 years in the studied population and should encourage larger dimension studies to verify whether the same is happening in other regions of this so-called low-CV risk country.

What is known about the topic

- Most hypertension and prehypertension estimates are based on the measurements of BP performed on a single occasion with subsequent influence on the estimates of blood pressure treatment and control.
- The average population BP levels have been reported as decreasing, especially in Western and high-income countries.

What this study adds

- Applying routine clinical methodology and criteria (measurement on two occasions and excluding self-report of disease) to an epidemiological study has a clear effect on the estimates of prevalence, awareness, treatment and control of hypertension and prehypertension.
- In this region, the reported national and worldwide trend of decreasing BP was not observed.
- The SBP average values in a low-CV risk area are markedly higher than the minimum exposure risk for the development of CV disease as well as higher than the average values recorded worldwide and in high-CV risk countries.
- The data suggest an increase in the CV risk over the past 10 years in the studied population and should encourage larger studies to verify whether an identical increase is occurring in other regions of this so-called low-CV risk country.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the following institutions and departments who collaborated in this project: Agrupamento de Centros de Saúde de Guimarães e Vizela, Serviço de Patologia Clínica do Centro Hospitalar do Alto Ave, Centro de Saúde da Amorosa, USF Afonso Henriques, USF Ara Trajano, USF Duo Vida, USF Novos Rumos, USF de Pevidém, USF Physis, USF de Ponte, USF de Ronfe, USF S. Nicolau, USF de S. Torcato, USF de Serzedelo e USF Vimaranes. A special word is addressed to thank all the physicians working in those different Primary Health Care Community Centers, whose work of enrolling subjects was vital to the successful prosecution of our goals. We also thank Professor ME Macedo and co-authors of the PAP Study, for providing access to previously unpublished results of that project. The Guimarães Study Group is also composed of the following researchers responsible for data collection: Helena Sarmento, Gloria Alves, Sara Freitas, Ana Sofia Alves, Sofia Gomes, Marta Gonçalves, Rui Fernandes, António Pedro Fonte, José Miguel Sá, Clarisse Neves, Andreia Sampaio, Cristina Cunha, Sílvia Sousa, Filipe Gonçalves, Joana Malheiro, João Silva, Carlos Fernandes, Estefânia Bustabad, Laura Castro, Nuna Vieira, Filipa Ramos, Ana Catarina Marques, Joana Monteiro, Ana Sofia Silva, Margarida Rocha, Mafalda Jordão Abreu, Margarida Dias, Magda Fernandes, Paula Felgueiras, Francisca Castro, Vânia Gomes, Ana Luísa Novo, Carla Pereira, Ana Cristina Ramalho, Fernando Esculcas, Dina Fernandes, Ricardo Rodrigues, Rafael Velho, Diana Coimbra, Joana Pimenta, João Pedro Teixeira, Ana Luísa Neves, Joana Leitão, Sara Pereira, Carla Ferreira, Clarinda Neves, Samuel Pedreira, Pedro Neves, Elisabete Lima, Carla Mendes, Orlanda Barbosa, Ana Catarina Martins, Célia Lemos, Eduarda Macedo, Sílvia Azevedo, Ana Rita Ribeiro, Elsa Salgado, Joana Dias, Eduarda Piairo, Marisa Carneiro, Ismael Costa, Lucília Miranda, Ana João Gonçalves, Natália Rodrigues, Manuela Morais, Rosário Santos, Odete Rodrigues, Alexandra Barreira, Ana Rita Romano, Catarina Marques, Fernanda Marisa Santos, Sónia Coelho Pereira, Elisabete Pinto Teixeira, and Joana Andrade Pinto.

REFERENCES

1 Crim MT, Yoon SS, Ortiz E, Wall HK, Schober S, Gillespie C *et al.* National surveillance definitions for hypertension prevalence and control among adults. *Circ Cardiovasc Qual Outcomes* 2012; **5**(3): 343–351.
2 Ikeda N, Sapienza D, Guerrero R, Aekplakorn W, Naghavi M, Mokdad AH *et al.* Control of hypertension with medication: a comparative analysis of national surveys in 20 countries. *Bull World Health Organ.* 2014; **92**(1): 10–9C.
3 Bovet P, Gervasoni JP, Ross AG, Mkamba M, Mtasiwa DM, Lengeler C *et al.* Assessing the prevalence of hypertension in populations: are we doing it right? *J Hypertens* 2003; **21**(3): 509–517.
4 Modesti PA, Rapi S, Bamoshmoosh M, Baldereschi M, Massetti L, Padeletti L *et al.* Impact of one or two visits strategy on hypertension burden estimation in HYDY, a population-based cross-sectional study: implications for healthcare resource allocation decision making. *BMJ Open* 2012; **2**(4): e001062.
5 Kearney PM, Whelton M, Reynolds K, Whelton PK, He J. Worldwide prevalence of hypertension: a systematic review. *J Hypertens* 2004; **22**(1): 11–19.
6 Figueiredo D, Azevedo A, Pereira M, de Barros H. Definition of hypertension: the impact of number of visits for blood pressure measurement. *Rev Port Cardiol* 2009; **28**(7-8): 775–783.
7 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; **27**(5): 963–975.
8 Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B *et al.* Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* 2010; **375**(9718): 895–905.
9 Macedo ME, Lima MJ, Silva AO, Alcantara P, Ramalhinho V, Carmona J. Prevalence, awareness, treatment and control of hypertension in Portugal: the PAP study. *J Hypertens* 2005; **23**(9): 1661–1666.
10 Cortez-Dias N, Martins S, Belo A, Fiuza M. Prevalence and management of hypertension in primary care in Portugal. Insights from the VALSIM study. *Rev Port Cardiol* 2009; **28**(5): 499–523.
11 Pereira M, Carreira H, Vales C, Rocha V, Azevedo A, Lunet N. Trends in hypertension prevalence (1990-2005) and mean blood pressure (1975-2005) in Portugal: a systematic review. *Blood Press* 2012; **21**(4): 220–226.
12 Danaei G, Finucane MM, Lin JK, Singh GM, Paciorek CJ, Cowan MJ *et al.* National, regional, and global trends in systolic blood pressure since 1980: systematic analysis of health examination surveys and epidemiological studies with 786 country-years and 5.4 million participants. *Lancet* 2011; **377**(9765): 568–577.
13 CunhaPG CJ, Oliveira P, Vila I, Sousa N. The rational/design of the Guimarães/Vizela study: a multimodal population based cohort study to determine global cardiovascular risk and disease. *J Investig Med* 2014; **62**(5): 813–820.
14 Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G *et al.* 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**(6): 1105–1187.
15 Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr *et al.* Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; **42**(6): 1206–1252.
16 De Macedo ME, Lima MJ, Silva AO, Alcantara P, Ramalhinho V, Carmona J. Prevalence, awareness, treatment and control of hypertension in Portugal. The PAP study. *Rev Port Cardiol* 2007; **26**(1): 21–39.
17 Guo F, He D, Zhang W, Walton RG. Trends in prevalence, awareness, management, and control of hypertension among United States adults, 1999 to 2010. *J Am Coll Cardiol* 2012; **60**(7): 599–606.
18 Scuteri A, Najjar SS, Orru M, Albai G, Strait J, Tarasov KV *et al.* Age- and gender-specific awareness, treatment, and control of cardiovascular risk factors and subclinical vascular lesions in a founder population: the SardiNIA Study. *Nutr Metab Cardiovasc Dis* 2009; **19**(8): 532–541.
19 Guessous I, Bochud M, Theler JM, Gaspoz JM, Pechere-Bertschi A. 1999-2009 Trends in prevalence, unawareness, treatment and control of hypertension in Geneva, Switzerland. *PLoS ONE* 2012; **7**(6): e39877.
20 Huerta JM, Tormo MJ, Gavrila D, Navarro C. Cardiovascular risk estimated after 13 years of follow-up in a low-incidence Mediterranean region with high-prevalence of cardiovascular risk factors. *BMC Public Health* 2010; **10**: 640.
21 Ortiz Marron H, Vaamonde Martin RJ, Zorrilla Torras B, Arrieta Blanco F, Casado Lopez M, Medrano Albero MJ. [Prevalence, degree of control and treatment of hypertension in the adult population of Madrid, Spain]. *Rev Esp Salud Publica* 2011; **85**(4): 329–338.
22 Perez-Fernandez R, Marino AF, Cadarso-Suarez C, Botana MA, Tome MA, Solache I *et al.* Prevalence, awareness, treatment and control of hypertension in Galicia (Spain) and association with related diseases. *J Hum Hypertens* 2007; **21**(5): 366–373.
23 Felix-Redondo FJ, Fernandez-Berges D, Fernando Perez J, Zaro MJ, Garcia A, Lozano L *et al.* [Prevalence, awareness, treatment and control of cardiovascular risk factors in the Extremadura population (Spain). HERMEX study]. *Aten Primaria* 2011; **43**(8): 426–434.
24 Hernandez-Hernandez R, Silva H, Velasco M, Pellegrini F, Macchia A, Escobedo J *et al.* Hypertension in seven Latin American cities: the Cardiovascular Risk Factor Multiple Evaluation in Latin America (CARMELA) study. *J Hypertens* 2010; **28**(1): 24–34.
25 Nichols MTN, Luengo-Fernandez R, Leal J, Gray A, Scarborough P, Rayner M. *European Cardiovascular Disease Statistics 2012*. Brussels, Belgium, 2012.
26 Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H *et al.* A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**(9859): 2224–2260.
27 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* 2002; **360**(9349): 1903–1913.
28 Gupta R, Deedwania PC, Achari V, Bhansali A, Gupta BK, Gupta A *et al.* Normotension, prehypertension, and hypertension in urban middle-class subjects in India: prevalence, awareness, treatment, and control. *Am J Hypertens* 2013; **26**(1): 83–94.
29 Tocci G, Rosei EA, Ambrosioni E, Borghi C, Ferri C, Ferrucci A *et al.* Blood pressure control in Italy: analysis of clinical data from 2005-2011 surveys on hypertension. *J Hypertens* 2012; **30**(6): 1065–1074.
30 Cifkova R, Skodova Z, Bruthans J, Holub J, Adamkova V, Jozifova M *et al.* Longitudinal trends in cardiovascular mortality and blood pressure levels, prevalence, awareness, treatment, and control of hypertension in the Czech population from 1985 to 2007/2008. *J Hypertens* 2010; **28**(11): 2196–2203.
31 Efstratopoulos AD, Voyaki SM, Baltas AA, Vratsistas FA, Kirlas DE, Kontoyannis JT *et al.* Prevalence, awareness, treatment and control of hypertension in Hellas, Greece: the Hypertension Study in General Practice in Hellas (HYPERTENSHELL) national study. *Am J Hypertens* 2006; **19**(1): 53–60.
32 Falaschetti E, Chaudhury M, Mindell J, Poulter N. Continued improvement in hypertension management in England: results from the Health Survey for England 2006. *Hypertension* 2009; **53**(3): 480–486.
33 Vasan RS, Larson MG, Leip EP, Kannel WB, Levy D. Assessment of frequency of progression to hypertension in non-hypertensive participants in the Framingham Heart Study: a cohort study. *Lancet* 2001; **358**(9294): 1682–1686.
34 Zhang H, Thijs L, Kuznetsova T, Fagard RH, Li X, Staessen JA. Progression to hypertension in the non-hypertensive participants in the Flemish Study

on Environment, Genes and Health Outcomes. *J Hypertens* 2006; **24**(9): 1719–1727.
35 Markus MR, Stritzke J, Lieb W, Mayer B, Luchner A, Doring A *et al*. Implications of persistent prehypertension for ageing-related changes in left ventricular geometry and function: the MONICA/KORA Augsburg study. *J Hypertens* 2008; **26** (10): 2040–2049.
36 Fukuhara M, Arima H, Ninomiya T, Hata J, Yonemoto K, Doi Y *et al*. Impact of lower range of prehypertension on cardiovascular events in a general population: the Hisayama Study. *J Hypertens* 2012; **30**(5): 893–900.
37 Zhang Y, Galloway JM, Welty TK, Wiebers DO, Whisnant JP, Devereux RB *et al*. Incidence and risk factors for stroke in American Indians: the Strong Heart Study. *Circulation* 2008; **118**(15): 1577–1584.
38 Lee M, Saver JL, Chang B, Chang KH, Hao Q, Ovbiagele B. Presence of baseline prehypertension and risk of incident stroke: a meta-analysis. *Neurology* 2011; **77** (14): 1330–1337.
39 Kanno A, Kikuya M, Ohkubo T, Hashimoto T, Satoh M, Hirose T *et al*. Prehypertension as a significant predictor of chronic kidney disease in a general population: the Ohasama Study. *Nephrol Dial Transplant* 2012; **27**(8): 3218–3223.

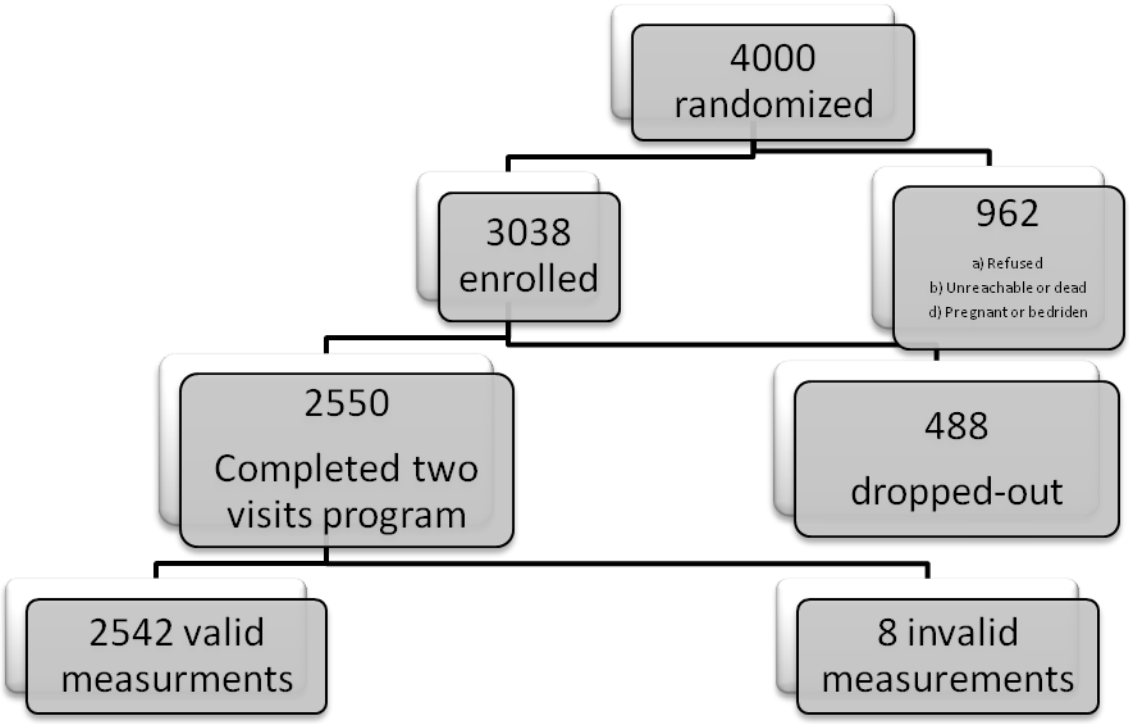
Supplementary Information accompanies this paper on the Journal of Human Hypertension website (http://www.nature.com/jhh)

An epidemiological study determining blood pressure in a Portuguese cohort. The Guimarães/Vizela Study

Supplemental Material

In this section we present data supporting the drop out analysis of all the subjects enrolled, details concerning prevalence, awareness, treatment and control of hypertension stratified by gender, global average BP values of the population stratified by age and gender and similar analysis for pre-hypertension.

Figure Sup.1 - Flow chart of the enrollment and participation of 4000 subjects in the Guimarães/Vizela Study



Drop-out analysis

83.9% of enrolled subjects completed both visits (2550 subjects), and 16.1% attended only the first visit (488 subjects) - drop out rate = 16.1% (Table **Sup.A**). Analyzing the global gender distribution, there were no significant differences between the group of drop outs (55.3% women) and the group that completed the study (55.1% women) (Table **Sup.B**), but drop-outs were slightly but significantly younger (44.5 years) than those who completed both visits (47.4 years) – Table **Sup.C**. Looking into subject's distribution by age classes, it is clear that drop-out subjects were distributed by the different age groups in numbers that vary between 10% and 26.6% - the higher percentages of drop-out were observed in the extreme age groups (i.e - <30 years (22.1%) and >80 years (26.6%)). Table **Sup.D**.

Education degree was similar between both groups; the lowest rate of drop out was recorded amongst those who completed primary instruction (4 years of schooling). Drop-out subjects were mainly recorded in the classes that were, on one hand, younger and with higher education, and on the other, older without any education (Table **Sup.E**). Using the National Health Statistics Professional classification, we could record that the drop - out rate according to professional class varied between 13.6 and 22.1%. The level of participation of every professional group (subjects who completed 2 visits) was approximately 80% (data not shown). Both in what concerns the distribution by age classes and education degree, the effect of drop-out subjects in the stratification of the population that completed the two visits is largely compensated by the type of sample randomization that was chosen and that defined a priori a larger recruitment in the younger age classes. When comparing the two groups (Drop out vs Complete evaluation) by age class, one cannot find differences concerning gender, professional group and education degree in all age classes, except for the age class from 30-39 years, where a difference concerning professional group distribution was found - drop-out subjects belonged mainly to two groups of professions: "Technicians and Intermediate Level Professionals" (25%; 16 out of 64 individuals) and "Salesmen and Tertiary Service staff" (25.6%; 23 out of 90 individuals) (Data not shown).

In the age class 50-59 years, the percentage of drop-out individuals of the female sex (6.3%; 11 out of 174 subjects) was significantly inferior to the male sex (13.9%; 23 out of 166 subjects) – this was the only Age class where this difference could be verified (Table **Sup.F**).

Comparing Mean Age within each Age Class for both groups (Drop-outs vs Complete evaluation) we could only find significant differences in the age class < 30 years (23.6 years average age in the Drop-out group vs 24.4 years average age in those that completed full evaluation) – a difference with probably no biological meaning. (Tables **Sup.G**)

Table Sup. A

Number of subjects attending planned Study Visits

	Frequency	Percent	Valid Percent	Cumulative Percent
1 Visit	**488**	**16.1**	**16.1**	**16.1**
2 Visits	**2550**	**83.9**	**83.9**	**100.0**
Total	**3038**	**100.0**	**100.0**	

From the initial 3038 subjects, 488 did not complete the 2 visit protocol (drop out rate=16.1%)

Table Sup. B

Gender distribution by number of visits completed

			Sex		Total
			Female	Male	
	1 Visit	Count	270	218	488
		% within Visits	55.3%	44.7%	100.0%
	2 Visits	Count	1404	1146	2550
		% within Visits	55.1%	44.9%	100.0%
Total		Count	1674	1364	3038
		% within Visits	55.1%	44.9%	100.0%

No significant difference in gender distribution of subjects participating in 1 or 2 visits.

(X^2=0.012; p>0.05)

Table Sup. C: Mean age according to number of visits completed

	Visits	N	Mean	Std. Deviation	Std. Error Mean
Age	1 Visit	488	44.53	21.062	.953
	2 Visits	2550	47.40	19.138	.379

Subjects participating in both visits were slightly but significantly older

(Mann-Whitney test: Z= -3.754 ; p<0.01)

Table Sup. D: Distribution of drop – out rates by age groups

			Visits		Total
			1 Visit	2 Visits	
Age Classes	<30	Count	159	562	721
		% within Age (classes)	22.1%	77.9%	100.0%
	30 -39	Count	102	577	679
		% within Age (classes)	15.0%	85.0%	100.0%
	40-49	Count	48	286	334
		% within Age (classes)	14.4%	85.6%	100.0%
	50-59	Count	34	306	340
		% within Age (classes)	10.0%	90.0%	100.0%
	60-69	Count	46	349	395
		% within Age (classes)	11.6%	88.4%	100.0%
	70-79	Count	70	390	460
		% within Age (classes)	15.2%	84.8%	100.0%
	>=80	Count	29	80	109
		% within Age (classes)	26.6%	73.4%	100.0%
Total		**Count**	**488**	**2550**	**3038**
		% within Age Classes	**16.1%**	**83.9%**	**100.0%**

(X^2=44.655; p<0.01)

Table Sup. E: Years of Education by number of visits completed

			Visits		Total
			1 Visit	2 Visits	
Shool Years	<4 years	Count	92	418	510
		% within School Education	18.0%	82.0%	100.0%
	4 years	Count	124	954	1078
		% within School Education	11.5%	88.5%	100.0%
	5 to 9 years	Count	137	568	705
		% within School Education	19.4%	80.6%	100.0%
	10 to 12 years	Count	91	401	492
		% within School Education.	18.5%	81.5%	100.0%
	> 12 years	Count	44	208	252
		% within School Education	17.5%	82.5%	100.0%
Total		Count	488	2549	3037
		% within School Education	16.1%	83.9%	100.0%

(X^2 = 26.559; p<0.01)

Table Sup. F

Gender distribution in the Age Class 50 – 59 years

		Visits		Total
		1 Visit	2 Visits	
Female	**Count**	**11**	**163**	**174**
	% within Sex	**6.3%**	**93.7%**	**100.0%**
	% within Visits	**32.4%**	**53.3%**	**51.2%**
Male	**Count**	**23**	**143**	**166**
	% within Sex	**13.9%**	**86.1%**	**100.0%**
	% within Visits	**67.6%**	**46.7%**	**48.8%**
Total	**Count**	**34**	**306**	**340**
	% within Sex	**10.0%**	**90.0%**	**100.0%**
	% within Visits	**100.0%**	**100.0%**	**100.0%**

(X^2= 5.357; p<0.05)

Table Sup. G : Average age in the Age class < 30 years

	Visits	N	Mean	Std. Deviation	Std. Error Mean
Age	**1 Visit**	**159**	**23.57**	**3.022**	**.240**
	2 Visits	**562**	**24.36**	**3.206**	**.135**

(t=-2.777; p<0.01)

Figure Sup.2 - Hypertension prevalence within each age class (Clinical Method)(n=2542)

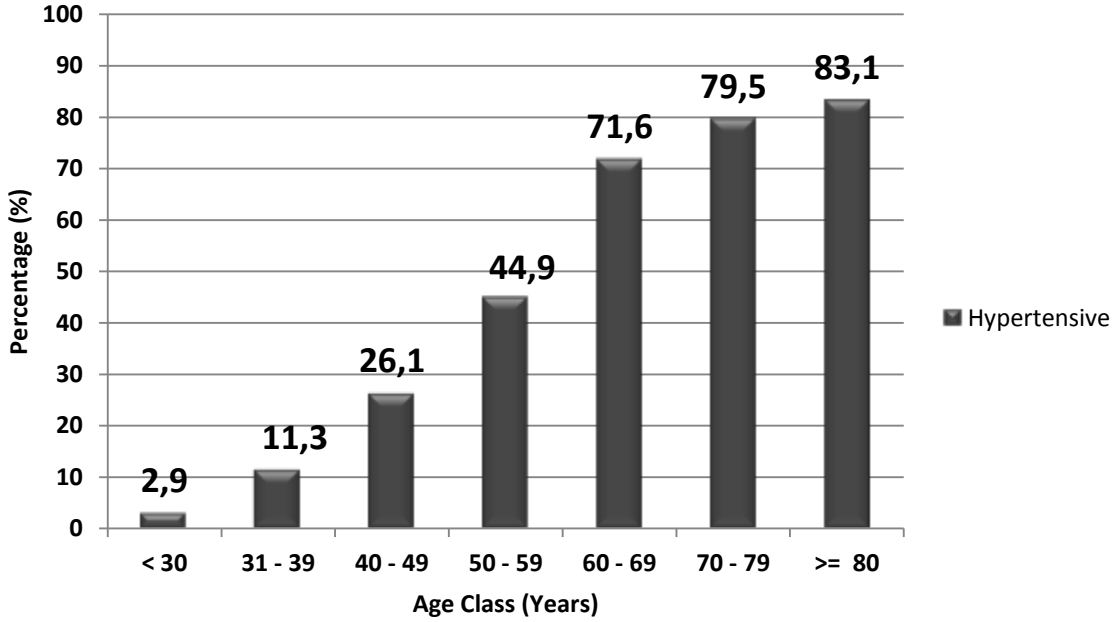
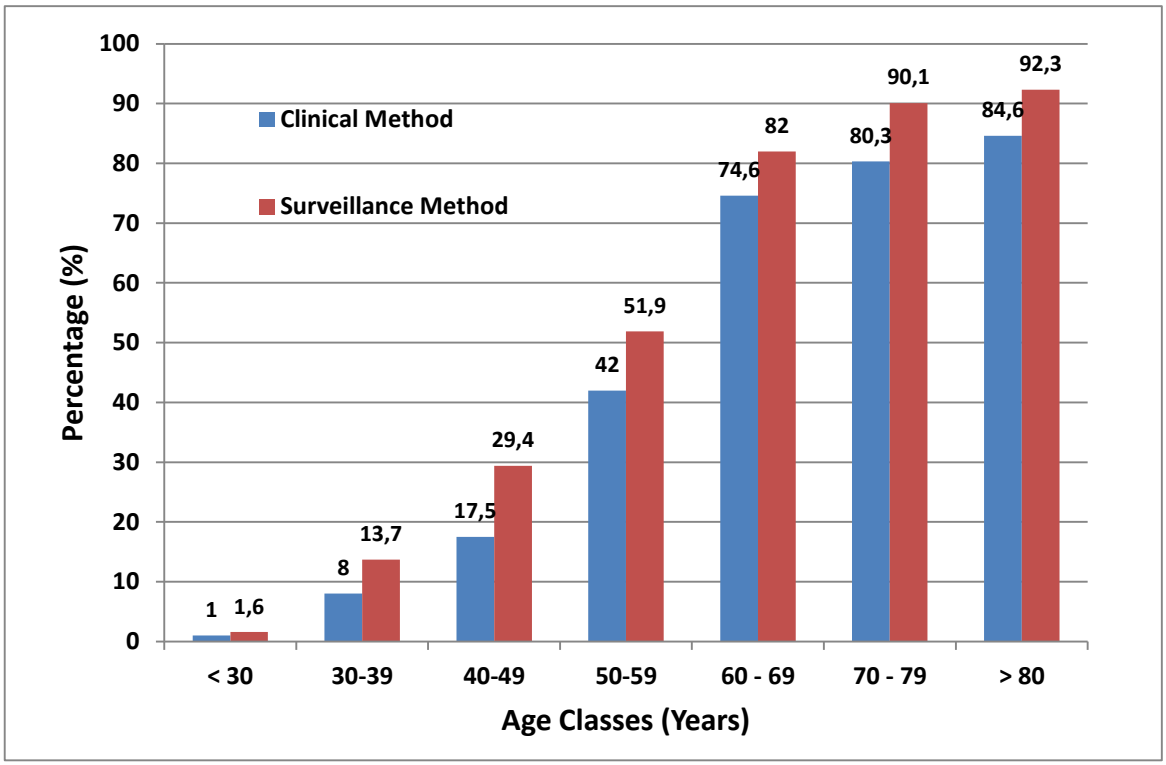


Table Sup. H – Prevalence of Hypertension, according to gender

Female Gender



Male Gender

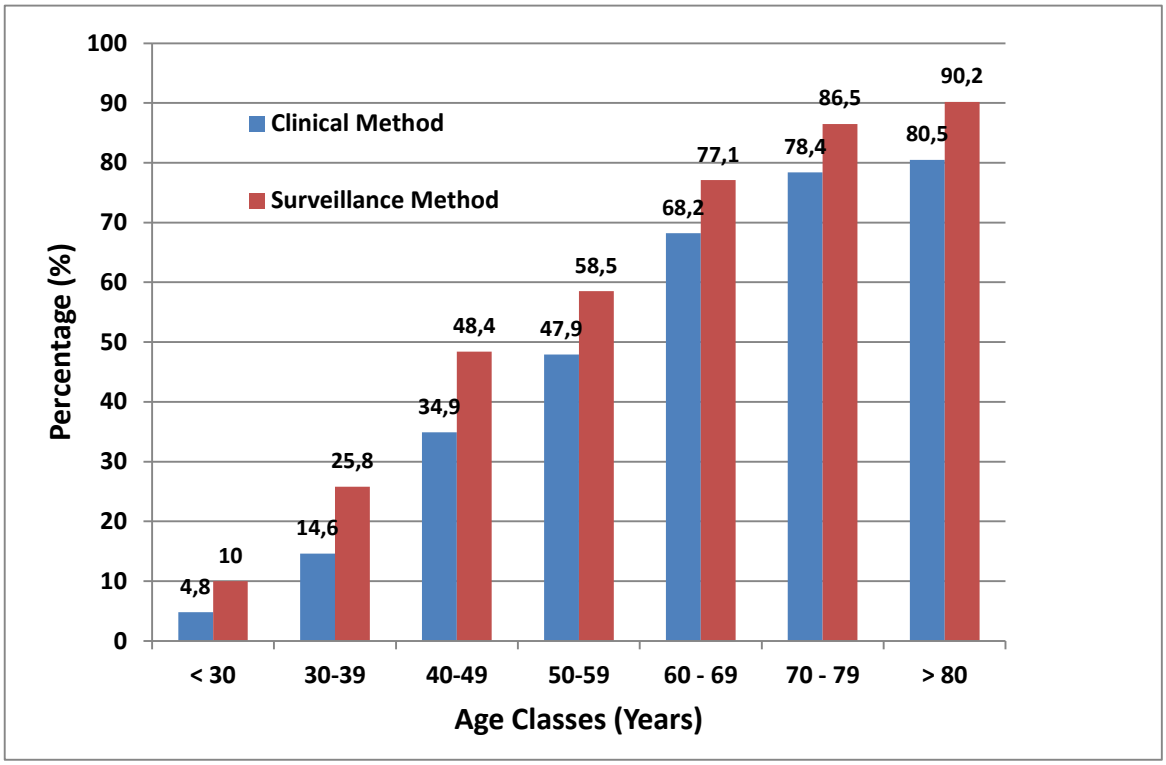

100
90
80
70
60
50
40
30
20
10
0
Percentage (%)
Clinical Method
Surveillance Method
4,8
10
14,6
25,8
34,9
48,4
47,9
58,5
68,2
77,1
78,4
86,5
80,5
90,2
< 30
30-39
40-49
50-59
60 - 69
70 - 79
> 80
Age Classes (Years)

Table Sup. 4 - Percentage of treated hypertensives, according to age and gender (Clinical method)

	Nr. of HTN	Treated HTN	Nr. of Male HTN	Treated Male HTN	Nr. of Female HTN	Treated Female HTN
Age < 30 *	15	4(26.6%)	12	1(8.13%)	3	3(100%)
Age 30 – 39 *	62	21(33.9%)	35	7(20.0%)	27	14(51.9%)
Age 40 – 49	72	36(50%)	44	19(43.2%)	28	17(60.7%)
Age 50 – 59	136	97(71.3%)	68	44(64.7%)	68	53(77.9%)
Age 60 – 69	248	184(74.2%)	107	78(72.9%)	141	106(75.2%)
Age 70 – 79	308	229(74.4%)	145	105(72.4%)	163	124(76.1%)
Age >= 80	66	56(84.8%)	33	27(81.8%)	33	29(87.8%)
Total	907	627(69.1%)	444	281(63.3%)	463	346(74.7%)

HTN – Hypertensives**; *** $p<0.01$

Table SUP.I – Average BP values according to BP status, age and gender (*Clinical* Method)

	Normotensive			Untreated Hypertensives			Treated Hypertensives		
	nr.	SBP	DBP	nr.	SBP	DBP	nr.	SBP	DBP
TOTAL	1635	120.7(12.1)	73.2(8.1)	280	153.6 (14.0)	86.6 (9.6)	627	145.1 (17.2)	79.6(9.2)
Age <30	547	116.8(10.3)	69.5(7.3)	11	148.3 (4.1)	87.5 (6.1)	4	128.6 (21.6)	85.0 (18.6)
Age 30-39	514	118.4(11.1)	73.6(7.6)	41	146.6 (9.3)	92.7 (8.0)	21	127.3 (13.9)	80.6 (9.1)
Age 40-49	214	122.1(12.4)	76.8(8.1)	36	150.2 (18.2)	93.0 (8.1)	36	136.0 (9.2)	85.2 (7.3)
Age 50-59	168	126.3(10.8)	77.9(7.5)	39	152.5 (9.0)	88.5 (6.6)	97	139.7 (14.3)	83.6 (7.8)
Age 60-69	98	129.7(11.7)	75.1(7.8)	64	156.8 (15.1)	84.6 (10.0)	184	144.0 (15.4)	79.4 (8.5)
Age 70-79	80	133.9 (11.4)	74.6(6.9)	79	157.0 (14.7)	82.2 (9.1)	229	150.3 (18.6)	78.3 (8.9)
Age >=80	14	127.5 (11.9)	71.2(8.2)	10	157.0 (8.9)	77.9 (7.3)	56	150.9 (15.6)	74.3 (10.6)
MEN	698	126.4(9.9)	74.3 (8.1)	163	154.7 (13.7)	87.8 (9.9)	281	147.9 (17.8)	80.0(9.3)
Age <30	239	123.3(8.7)	69.8 (7.6)	11	148.3 (4.1)	87.5 (6.1)	1	158.8	107.5
Age 30-39	205	125.1(8.8)	75.2(7.1)	28	148.3 (7.4)	92.7 (9.0)	7	136.9 (8.8)	83.2 (6.2)
Age 40-49	82	128.8(9.7)	80.4(5.9)	25	152.9 (20.6)	94.5 (8.9)	19	137.1 (7.8)	86.4 (6.4)
Age 50-59	74	129.4(10.0)	79.3(6.8)	24	152.8 (7.9)	88.8 (6.9)	44	140.6 (12.6)	84.0 (7.1)
Age 60-69	50	130.4(12.6)	75.6(8.5)	29	161.2 (16.2)	85.6 (10.2)	78	146.3 (16.8)	78.9 (9.4)
Age 70-79	40	135.3(9.7)	73.7(6.5)	40	157.9 (12.4)	83.6 (9.6)	105	152.9 (20.5)	79.3 (8.8)
Age >=80	8	127.0(10.7)	68.8(8.8)	6	157.9 (8.4)	73.8 (4.5)	27	154.9 (14.8)	73.5 (10.1)
WOMEN	937	116.4(11.8)	72.4(8.0)	117	152.0 (14.4)	84.8 (8.9)	346	142.9 (16.4)	79.3 (9.1)
Age <30	308	111.7 (8.5)	69.2 (7.1)	0	-	-	3	118.5 (9.6)	77.5 (13.5)
Age 30-39	309	114.0(10.3)	72.5 (7.7)	13	143.1 (12.2)	92.7 (5.6)	14	122.5 (13.7)	79.3 (10.2)
Age 40-49	132	117.9(12.1)	74.5 (8.5)	11	144.0 (9.1)	89.6 (4.6)	17	134.8 (10.7)	83.9 (8.2)
Age 50-59	94	123.9(10.9)	76.8(8.0)	15	152.0 (11.0)	87.9 (6.3)	53	138.9 (15.7)	83.3 (8.4)
Age 60-69	48	128.9(10.6)	74.5(7.0)	35	153.2 (13.3)	83.7 (9.9)	106	142.3 (14.1)	79.8 (7.8)
Age 70-79	40	132.4(12.9)	75.4(7.2)	39	156.0 (16.9)	80.8 (8.5)	124	148.0 (16.7)	77.5 (8.9)
Age >=80	6	128.0(14.5)	74.3(6.8)	4	155.6 (10.8)	84.3 (6.2)	29	147.2 (15.8)	75.1 (11.1)

Table Sup. J – Global Average BP values according to age and gender (Clinical Method)

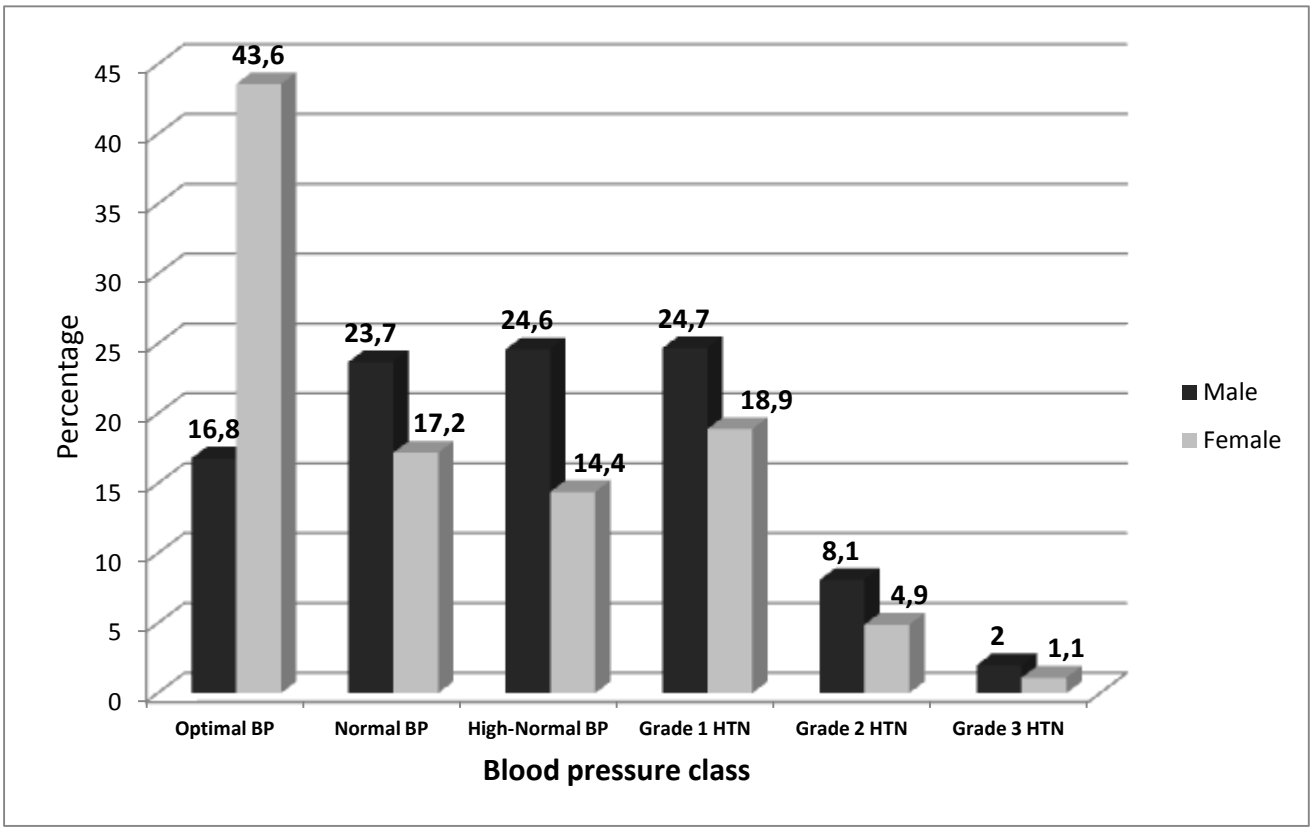
GLOBAL			
	nr.	**SBP (mmHg ± SD)**	**DBP (mmHg ± SD)**
TOTAL	2542	129.8 (19.0)	76.8 (9.7)
Age <30 years	562	121.3(11.3)	70.0(7.9)
Age 30-39 years	576	121.8(13.3)	75.6(9.1)
Age 40-49 years	286	128.1(16.2)	80.3(9.8)
Age 50-59 years	304	134.1(15.1)	81.2(8.4)
Age 60-69 years	346	142.3(17.1)	79.2(9.1)
Age 70-79 years	388	148.1(18.3)	78.3(8.9)
Age >=80 years	80	146.8(17.1)	74.7(9.9)
MEN TOTAL	**1142**	**133.6(17.5)**	**78.2(9.9)**
Age <30 years	251	124.5(10.2)	70.7(8.6)
Age 30-39 years	240	128.1(11.5)	77.5(9.3)
Age 40-49 years	126	134.8(15.5)	84.1(8.7)
Age 50-59 years	142	136.8(13.7)	82.3(7.7)
Age 60-69 years	157	143.9(18.8)	79.1(9.8)
Age 70-79 years	185	150.2(18.8)	79.0(9.1)
Age >=80 years	41	149.9(17.4)	72.6(9.3)
WOMEN TOTAL	**1400**	**126.2(19.1)**	**75.3(9.3)**
Age <30 years	311	111.8(8.6)	69.3(7.1)
Age 30-39 years	336	115.5(12.0)	73.6(8.7)
Age 40-49 years	160	121.5(14.3)	76.5(9.4)
Age 50-59 years	162	131.4(15.8)	80.0(8.9)
Age 60-69 years	189	140.9(15.4)	79.2(8.6)
Age 70-79 years	203	146.5(17.7)	77.7(8.7)
Age >=80 years	39	145.1(16.8)	75.9(10.4)

Table Sup. K – Distribution of BP categories according to age class (*Clinical* Method)

Age	Gender	Optimal BP	Normal BP	Normal – High BP	Grade I	Grade II	Grade III	Total
< 30 years	**Male**	91 (36%)	84(33.5%)	58(23.1%)	17(6.8%)	1(0.4%)	0 (0%)	**562**
	Female	250(80.4%)	48(15.4%)	11(3.5%)	2(0.6%)	0(0%)	0(0%)	
30 – 39 years	**Male**	56(23.3%)	86(35.8%)	61(25.4%)	32(13.3%)	4(1.7%)	1(0.4%)	**576**
	Female	224(66.7%)	61(18.2%)	34(10.1%)	16(4.8%)	1(0.3%)	0 (0%)	
40 – 49 years	**Male**	16(12.7%)	28(22.2%)	36(28.6%)	40(31.7%)	4(3.2%)	2(1.6%)	**286**
	Female	77(48.1%)	34(21.2%)	21(13.1%)	28(17.5%)	0(0%)	0(0%)	
50 – 59 years	**Male**	12(8.5%)	29(20.4%)	47(33.1%)	44(31%)	10(7.%)	0(0%)	**304**
	Female	35(21.6%)	38(23.5%)	39(24.1%)	42(25.9%)	6(3.7%)	2(1.2%)	
60 – 69 years	**Male**	10(6.4%)	23(14.6%)	38(24.2%)	59(37.6%)	21(13.4%)	6(3.8%)	**346**
	Female	13(6.9%)	26(13.8%)	52(27.5%)	73(38.6%)	22(11.6%)	3(1.6%)	
70 – 79 years	**Male**	5(2.7%)	17(9.2%)	37(20.0%)	71(38.4%)	41(22.2%)	14(7.6%)	**388**
	Female	8(3.9%)	28(13.8%)	41(20.2%)	83(40.9%)	33(16.3%)	10(4.9%)	
>= 80 years	**Male**	2(4.9%)	4(9.8%)	4(9.8%)	19(46.3%)	12(29.3%)	0(0%)	**80**
	Female	3(7.7%)	6(15.4%)	4(10.3%)	20(51.3%)	6(15.4%)	0(0%)	
	Total	**802**	**512**	**483**	**546**	**161**	**38**	**2542**

Graphic Sup. L

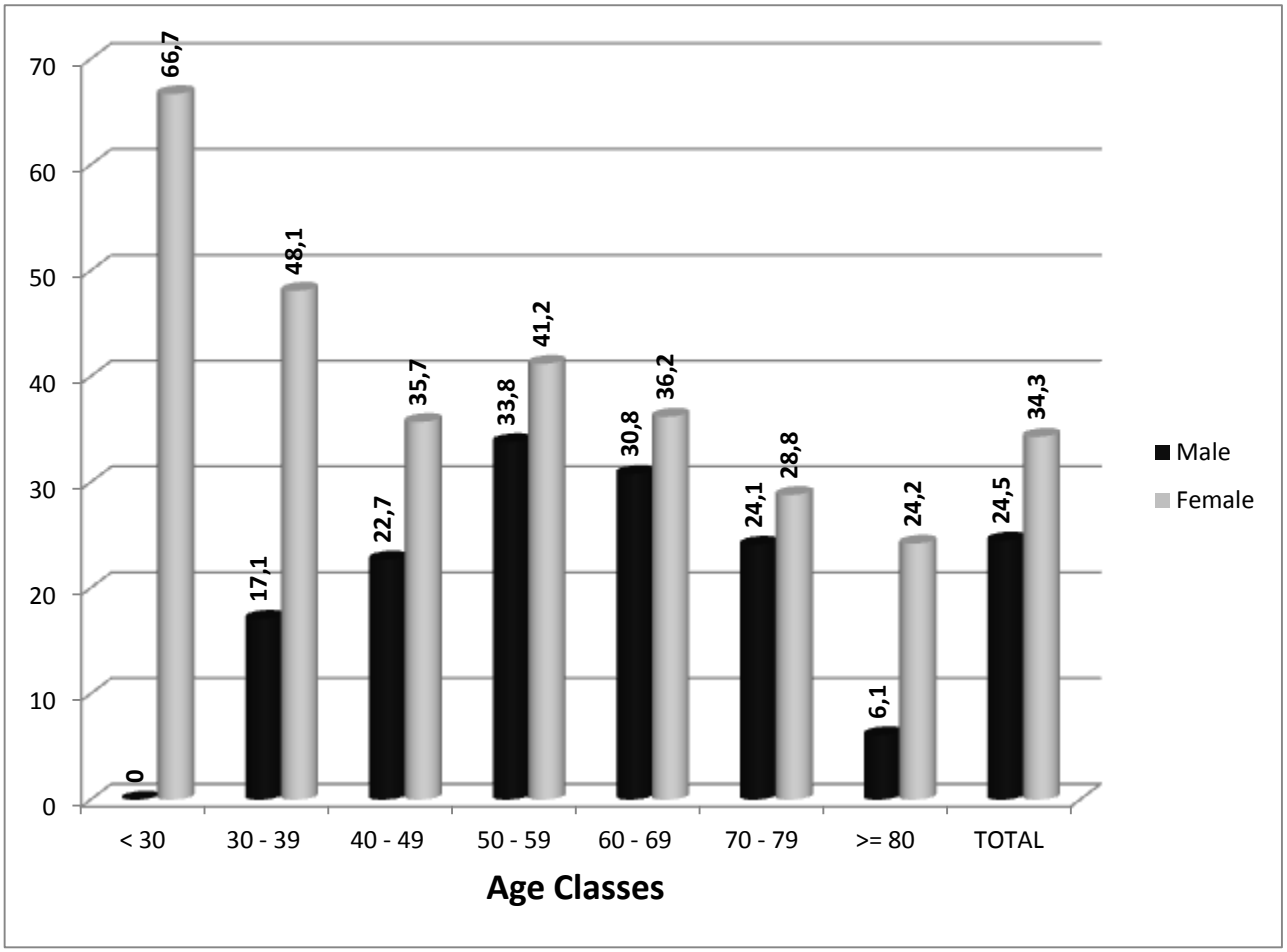
Distribution of 2542 subjects according to BP class and gender (*Clinical* Method)



Considering gender differences, it is important to point out that women and men have different distribution by blood pressure classes (X^2 = 214.7, p< 0.001). After 60 years of age, distribution by BP class is independent of sex; however with respect to subjects below 60 years of age the BP class distribution is not independent of sex, with women exhibiting a much larger prevalence on the classes of "optimal" and "normal BP" in comparison with men; that is to say that somehow the BP class distribution in men is shifted to the right when compared to women ($\mathbf{X^2}$ values for age classes < 30, 30 – 39, 40 – 49 and 50 – 59 years are respectively: 123.8, 107.1, 45.5, 12.1, **p** < 0.01)

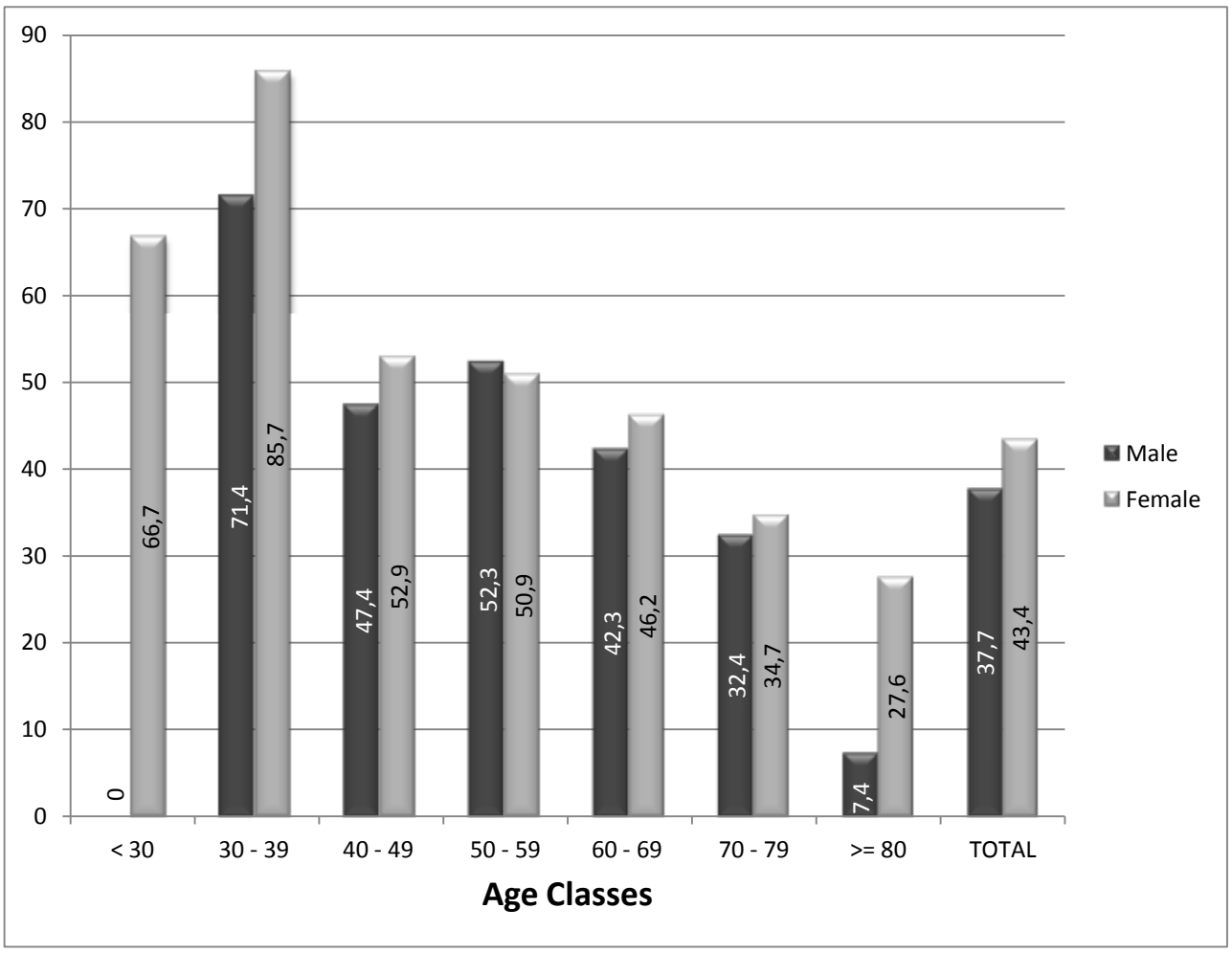
Graphic Sup. M

Distribution of controlled hypertensive subjects by age class /gender (*Clinical* Method) (all hypertensive subjects, n=907)



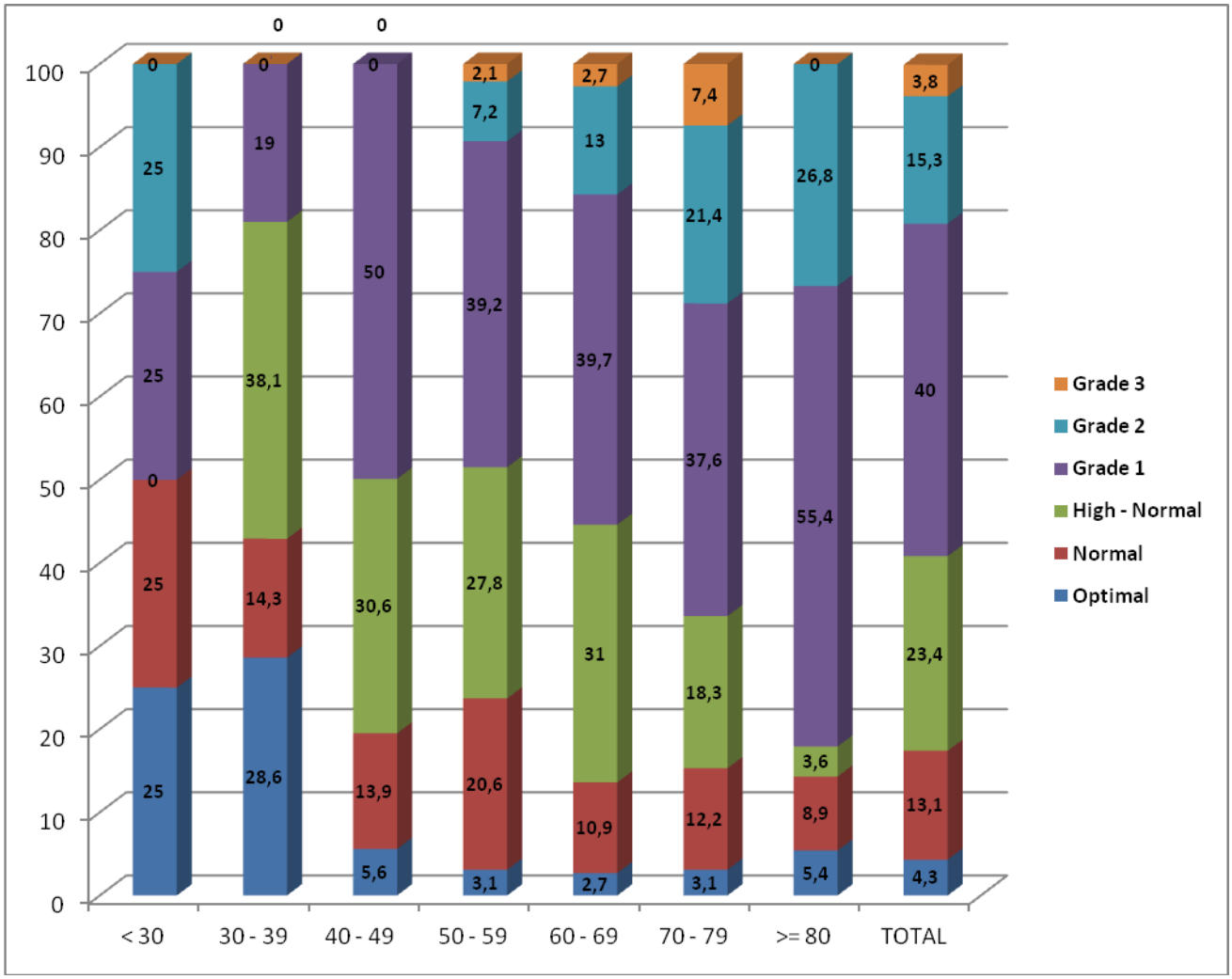
Graphic Sup. N:

Distribution of treated and controlled hypertensive subjects by age class and gender (only treated hypertensive subjects, n= 627) (*Clinical* Method)



Graphic Sup.O

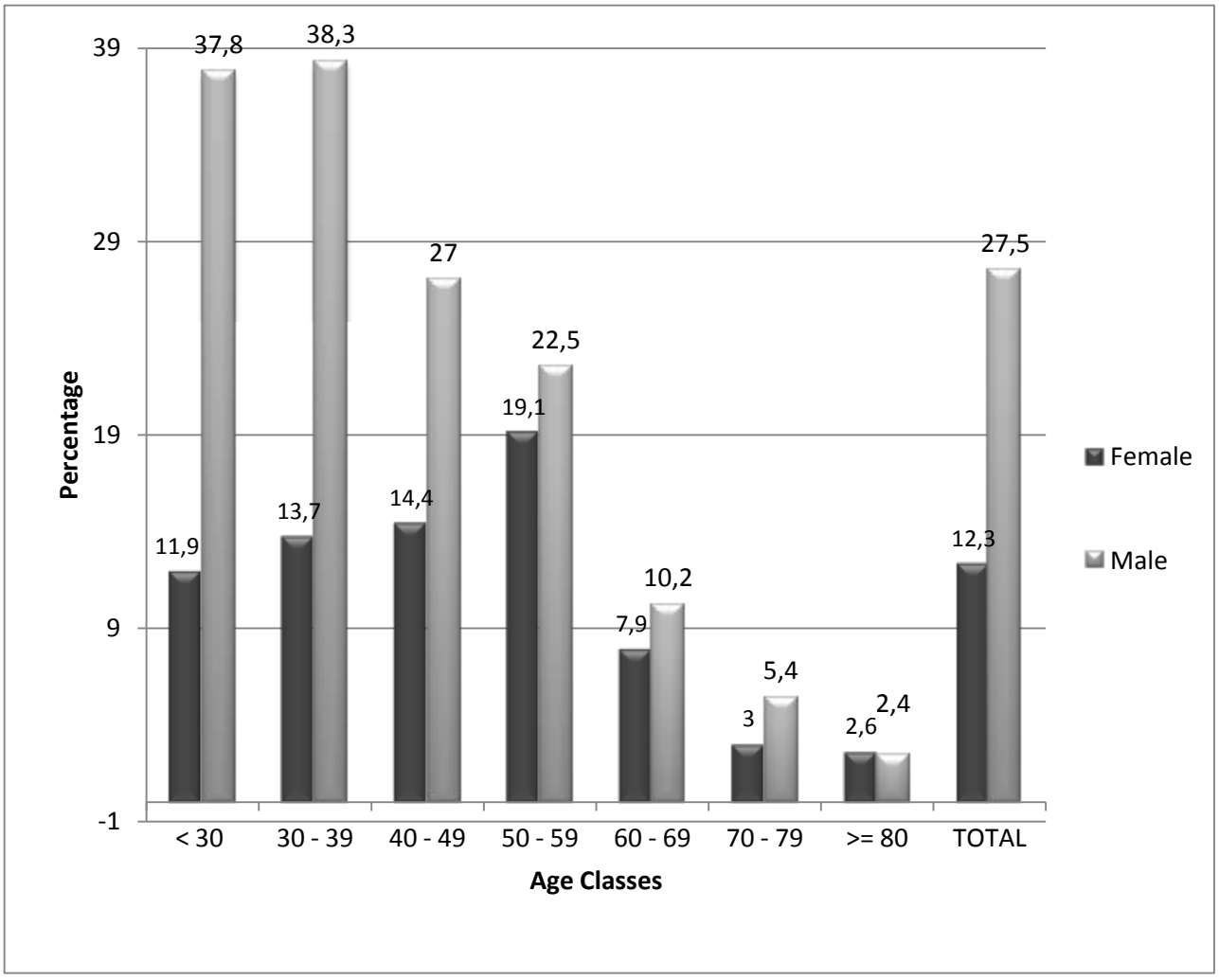
Distribution of treated hypertensive subjects by ESH BP class (n= 627) (*Clinical* Method)



With increasing age, in treated subjects, and in spite of treatment, an increasing number of subjects elude BP control and an increasing number of subjects bears higher BP levels. (X^2= 74.9, p< 0.001). After 50 years of age, the percentage of uncontrolled treated subjects is always greater than 48.5%)

Graphic Sup. P

Distribution of Pre – Hypertension prevalence according to age and gender (n=2542) (*Clinical* Method)



Chapter 4

Cardiovascular risk estimation: significant reclassification through rigorous phenotyping in a low-cardiovascular risk area

(manuscript under submission)

Cardiovascular risk estimation: significant reclassification through rigorous phenotyping in a low-cardiovascular risk area

Pedro G. CUNHA [(a)(b)(c)], Jorge COTTER[(a)(b)(c)], Pedro OLIVEIRA[(d)(e)], Isabel VILA[(a)], Nuno SOUSA[(b)(c)]

(a) Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Internal Medicine Department, Guimarães – Centro Hospitalar do Alto Ave / Minho University. Portugal; (b) Life and Health Science Research Institute (ICVS), School of Health Science, University of Minho; (c) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; (d) Departamento de Estudo de Populações, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto; (e) Instituto Saúde Pública Universidade Porto, EPIUnit;

Corresponding author:

Pedro Guimarães Cunha. Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk. Serviço de Medicina Interna do Hospital de Guimarães, Centro Hospitalar do Alto Ave; Life and Health Science Research Institute (ICVS), Minho University. Rua dos Cutileiros, Creixomil. 4810 – Guimarães, Portugal. pedrocunha@ecsaude.uminho.pt

Conflicts of Interest and Source of Funding: no conflicts of interest to declare; funding by the Life and Health Research Institute, Minho University

Word count: 3516; **Number of tables**: 7; **Number of figures**: 1;

ABSTRACT

Introduction. Widespread use of cardiovascular (CV) risk predicting tools in clinical practice is mandatory, but CV mortality and morbidity are still the highest. **Objectives**. To determine: i) the distribution of subjects by SCORE risk groups and understand how a parallel rigorous phenotypic evaluation would impact their initial SCORE classification; ii) the risk stratification of hypertensive subjects and its clinical implications.

Methods. A population based and representative sample of two adjacent cities in the north of Portugal has been evaluated twice, phenotyping subjects with a specific cardiovascular risk characteristic whenever results were confirmatory in both visits. Different risk estimation strategies were followed, comparing the widespread version of the SCORE algorithm with an evaluation with SCORE modulated by specific high-risk attributing phenotypes. Hypertensive subjects were additionally studied with the ESC/ESH risk charts.

Results. 18.2% of the population were reclassified into higher risk classes with the SCORE Modulated strategy, especially coming from the original Moderate Risk class (38.5% of these Moderate risk subjects were reclassified); the Very High Risk class increased its dimension by 32 times. 62.7% of hypertensives were classified with High or Very High risk of a CV event in 10 years according to the ESC/ESH risk charts; only 43% of hypertensives are receiving appropriate anti-lipidic treatment and only 14.3% of the Very High Risk group achieve expected treatment targets.

Conclusions. Approximately 1/5 of the population could benefit from more stringent phenotypic characterization of CV risk; aggressive and effective CV risk factor control should be in place for 21.7% of the population and 62.7% of hypertensives.

Key words: Cardiovascular risk; Risk reclassification; SCORE; Hypertension; Epidemiology; Portugal; Target Organ Damage

I – INTRODUCTION

Several tools are nowadays used in clinical practice to predict the 10-year risk of sustaining a cardiovascular event (CVE), fatal or fatal and non-fatal (1-3). The results have implications mainly in the need and/or intensification of intervention (pharmacologic or non-pharmacologic) to reduce the foreseeable risk and avoid cardiovascular disease (CVD).

In Europe the SCORE (1) algorithm has been extensively disseminated and, after progressive changes (4), is currently widely used to support the initiation or intensification of primary prevention measures (5). It is designed to ascertain the 10-year risk of fatal CVE in asymptomatic adult subjects without evidence of CVD (3). The algorithm uses two different versions to estimate the 10-year risk of fatal CVE: one for low and another for high-cardiovascular risk countries (1). The definition of high or low risk is determined by the annual cardiovascular (CV) mortality in subjects between 45–74 years registered in the country. Whenever it exceeds 220 deaths/100 000 for men or 160/100 000 for women, the classification of high risk is awarded (5). Portugal is one of the countries using the low-risk version of the SCORE algorithm, since its institution (1). In Hypertensive subjects, however, the ESC/ESH guidelines have recommended the use of another risk algorithm, that estimates the 10-year risk of fatal and non-fatal CVE, according to blood pressure class and cumulative risk characteristics, classifying subjects as bearing Very High, High, Moderate or Low added risk (6).

Many authors have dedicated their efforts to improve the predictive power of the model, by showing how, using different phenotypic characteristics of the individual, one can reclassify them into different risk classes, with different recommended intervention strategies. This has been documented by Sehestedt and colleagues, showing that adding evidence of target organ damage to the SCORE algorithm would improved its performance (7, 8). In fact, the last European joint recommendation in cardiovascular risk (CVR) prevention lists a number of

conditions or characteristics that should immediately modulate risk evaluation of a subject. For example, a known history of CVD, severe chronic kidney disease (CKD) or Diabetes Mellitus with one or more CV risk factors and/or target organ damage (TOD) should immediately confer the Very High Risk classification, irrespective of the score obtained in the algorithm (5).

Nevertheless, much has still to be achieved to improve not only risk classification, but especially to implement the more adequate prevention strategy in conformity with the obtained risk class. The Eurika study (9), using more than 7000 subjects attending primary care facilities and outpatient clinics in 12 European countries, has shown that the average 10-year risk of fatal CVD (using the SCORE equation) was 8.2%; 30% of this risk was attributable to a lack of control of 4 CVR factors: hypertension, diabetes, dyslipidemia and smoking.

Working in a so-called Low-CVR area, we observe subjects with the highest western European annual stroke incidence (10, 11) and with a standardized mortality rate by CVD that exceeds the ones caused by other pathologic entities, and still far from the World Health Organisation's goal for 2020 (12, 13). In this setting, we decided to study a population-based cohort of two adjacent cities in this area, with the following purposes: i) to determine the distribution of subjects by SCORE risk groups and understand how considering a rigorous phenotypic evaluation of those same subjects would impact/modulate their initial SCORE classification; ii) to determine the risk stratification of hypertensive subjects using both the SCORE and the ESH/ESC proposed methodologies.

II – SUBJECTS AND METHODS

The methodology employed has already been detailed elsewhere (14, 15). In a few words, a randomly selected and representative sample of the population from two adjacent cities in the north of Portugal was evaluated on two different occasions at least three months

apart, after signing a written consent form approved by an Ethics Committee. The sample selection was based considering the estimated prevalence of hypertension in Portugal (16, 17). A sample size of at least 2339 subjects was required to achieve 2% precision. Thus, a sample of the adult population (**>**18 years of age) stratified by age was defined previewing the necessity of including 4000 individuals (95% confidence interval with an estimation error inferior to 2%, considering a 25% safety margin to cope with non-adherence and dropout rate between visits).

The randomized subjects were enrolled after signing a written consent to participate. It was clear that only randomly assigned subjects could be enrolled and that no volunteers or physician-selected subjects would be included. If the subject refused, he/she could not be replaced with a volunteer. Only randomized subjects were accepted (15, 18).

On both visits, individuals were observed in the morning, fasting over-night and carrying their usual drug prescriptions. No intake of caffeinated beverages or tobacco use was allowed. Subjects underwent a standardized workup including medical history, biologic and arterial measurements. Trained physicians performed BP measurements at every occasion; training sessions performed prior to the beginning of the study insured standardization of blood pressure (BP) (peripheral and central) and pulse wave velocity measurements as well as electrocardiogram (EKG) performance. Peripheral BP was measured three times (at each visit) with subjects in a sitting position, after a 15 minutes resting period, taken with 2 minutes interval, using the validated OMRON-705IT© device (Omron Healthcare Europe B.V, Hoofddorp, The Netherlands). During their first visit, patients were then placed in the supine position where an EKG was performed with a Schiller Cardiovit AT101 device (SCHILLER AG, Altgasse 68

CH-6341 Baar, Switzerland), and Central BP and PWV measured using the Sphygmocor© device (Atcor Medical Pty Ltd ,West Ryde, NSW, Australia).

Subject phenotyping and diagnostic criteria – establishment of specific phenotypes was performed whenever the characteristics that define it were verified as concordant in the evaluation performed in both visits (separated at least 3 months apart). The definitions of CVR factors, diagnosis of hypertension, dyslipidemia, TOD and established CV and renal disease were performed in agreement with the ESC/ESH guidelines (6); The diagnosis of Diabetes was performed in agreement with the ADA criteria (2 measurements of fasting glucose ≥ 126 mg/dl or 1 measurement above 200 mg/dl or HbA1C ≥ 6.5 % or patient under anti-diabetic drugs) (19).

Risk Estimation using the SCORE algorithm – using the equations of the more recently adjusted SCORE algorithm (including HDL levels)(4), we first calculated the 10-year probability of a fatal CVE for all our subjects, and according to this result we classified them as: Low risk – score < 1%; Moderate Risk – 1% ≥ score < 5%; High Risk – 5% ≥ score < 10%; Very High Risk – score ≥ 10%; afterwards we reclassified subjects into those same risk classes, but using the following criteria: High Risk – score ≥ 10% or established CVD, or Diabetes with one or more CVR factors/TOD, or severe CKD; High Risk – score ≥ 5 and < 10%, or Total Cholesterol > 305 mg/dl, or BP > 180 mmHg, or Diabetes without complications/associated risk factors, or moderate CKD; Moderate and Low risk retained the same characteristics as above. TOD was defined whenever a subject presented with: left ventricular hypertrophy (LVH), grade II albuminuria, PWV > 10 m/s or estimated glomerular filtration rate (eGFR) > 30 and < 60 ml/min (CKD_EPI formula) (20).

Risk Estimation using the SCORE algorithm for subjects below 40 years of age – The relative risk of developing fatal CVE in 10-years was calculated using the relative risk chart proposed by SCORE authors (1, 5).

Risk Estimation using the ESH/ESC risk charts for hypertensive subjects – in hypertensive subjects the 10-year risk of fatal and non-fatal CVE was determined by establishing the risk category of an individual according to his/her blood pressure category and cumulative risk characteristics (6).

Statistical analysis. Statistical methodology concerning population sampling, database elaboration and management, as well as predefined statistical analysis have already been addressed elsewhere (15). Prevalence of different studied characteristics was estimated and adjusted by demographic characteristics (age group, sex, education level) agreeing to the known distribution of these characteristics in the population of the two cities, according to Statistics Portugal for the year 2006.

III – RESULTS

2542 subjects (of the initially enrolled 3038) completed the two clinical observation plan (drop-out rate of 16.1%). Drop-out subjects were not significantly different from those who were present in the two visits, as discussed in previous publications (14). In Table 1 we present the relevant biologic characteristics of the subjects that completed the observations, pointing out the mean age (45.5 years) with slight female prevalence (55.1%) – a reflection of the demographic distribution in the studied population; using the criteria of only defining a phenotypic characteristic whenever it was confirmed in two distinct measurements (when applicable), we recorded the following prevalence of CVR factors: Hypertension – 31.6% (14); Diabetes – 9.1%, Dyslipidemia 75.1% and Smoking – 18.8%.

Electrocardiogaphic evaluation detected a prevalence of 8.0% for LVH (in subjects above 35 years) and 2.4% for auricular fibrillation.

SCORE evaluation: simple vs modulated risk estimation. In Figure 1 we present a summary of the differences found when we applied to the same subjects a different strategy of risk estimation: either using the SCORE equations and translating the results into risk classes; or using the SCORE risk equations but also adding to risk classification phenotypic characteristics that modulate it (SCORE modulated). The distribution of the two risk strategies exhibit a degree of agreement of K(Cohen)=0.681. We observed significant changes in risk classification particularly concerning the Moderate and Very High classes: the first decreasing and the latter increasing when the SCORE modulated strategy was applied (Figure 1). 464 (18.2%) subjects were reclassified into higher risk classes with the SCORE modulated strategy (Table 2). 38.5% of the subjects initially classified as Moderate Risk, are reclassified into High and Very High Risk; 45.7% of those initially placed in a High Risk class, are moved to the Very High Risk group. Overall, the Very High Risk class defined by the SCORE Modulated strategy increases by 32 times its initial dimension with the simple SCORE equation.

Table 3 presents the population adjusted distribution of risk classes stratified by age and gender. The distribution of the subjects by the risk classes is not independent of the sex (X^2= 83.9; $p<0.001$), observing a higher prevalence of men in the High and Very High risk classes, as opposed to what is observed in the Low risk class, where female predominance is recorded. The male predominance is seen throughout all age groups in the High and Very High classes, but is significant only for age classes above 40 years; In the Moderate risk class, males are significantly predominant only in the age group 40 – 49, above which females are significantly more prevalent.

SCORE evaluation: Relative risk in subjects below 40 years of age. In Table 4 the relative risk of a 10-year fatal CVE is presented for subjects under 40 years, when compared with individuals of the same age with no added CV risk. 39.9% have at least twice as much risk as an average healthy individual of his/her age; approximately 9.5% have at least 3 times the risk and 2.5% have four or more times the risk of fatal CVE.

Risk estimation in hypertensive subjects: SCORE vs ESH/ESC charts. Using the two different algorithms to estimate risk of CVE in hypertensive subjects yielded disturbing results (Tables 5 and 6). Comparing the results in the two tables, there are two distinctive differences that can be pointed out: the fact that the ESH/ESC charts classify more hypertensive subjects in the High and Very High risk groups, and the coincidence of recorded subjects in the Very High group for both strategies. Only 43.0% of all hypertensives are medicated with a statin; only 14.3%/21.7% of Very High/High risk hypertensives, respectively, attain the recommended LDL-Cholesterol levels for their risk group (Table 7). It is also very important to look at the information provided by the risk classification of hypertensives performed using the ESC/ESH charts: 62.7% of hypertensives belong to High of Very High risk classes, and 80.4% are classified as having at least a "moderate to high risk".

Discussion

We present a distinctive approach to risk stratification, by phenotyping subjects in a population-based and population-representative study in Portugal. The distinctive results herein recorded were: i) the very significant reclassification, with a SCORE algorithm modulated by distinctive risk characteristics, of subjects to Very High Risk classes (32 times increase in the number of subjects originally classified as very high risk), especially coming

from the original Moderate Risk class (38.5% of these subjects are reclassified to higher risk classes); ii) the extremely elevated percentage of hypertensives with High or Very High risk of a CVE in 10 years – 62.7%, according to the ESC/ESH risk charts; iii) the disappointing percentage of hypertensives that is not receiving appropriate treatment nor achieving treatment targets in correspondence with their actual risk class.

Different risk models, different risk information. The SCORE, Framingham and ESC/ESH charts give different information concerning CVE risk (3). SCORE estimates risk of CV deaths, Framingham includes major CVE (fatal and non-fatal); and recently the ESC/ESH charts have modified its risk prediction, abandoning the older WHO-ISH and Framingham criteria, to include estimation of cardiovascular mortality in 10 years, using the same terminology as in SCORE, but analyzing subjects differently (6, 21). Zambon and coworkers have compiled all the different information and applied it to the subdivisions of the ESC/ESH chart, allowing the user to obtain information of risk prediction performed by the different models (21). The SCORE model has also an age limitation as it was initially designed to analyze risk of subjects between 40 and 65 years of age (1). The use of the Weibull model and the survival equations allows for estimates to be executed especially for subjects in older age groups, even if one must be aware that their confidence level is not the same as for the 40 – 65 year old range. This is clearly one of the limitations of its use. This was also why a relative risk chart was devised for subjects under 40 (1), and the main reason why we used it in our younger population. Looking carefully, 97 – 98.6% of our subjects under 40 are placed in the Low-risk category, even after modulation by higher-risk conferring characteristics. Still when applying the relative risk charts, more than 30 % have at least the double of the expected risk for their age (approximately 10%, with 3 to 8 times as much risk). These risk predictions could be indicative of an early vascular decline (22), and the recent findings of a high prevalence of early vascular aging in our youngest population (18) raises the question of whether these findings are linked.

The need for better risk prediction and concurrent treatment policies in risk reduction are clear, especially in a country like Portugal where CVD is still the leading cause of standardized death (11, 23). To this purpose, we could not refrain from remembering the prevalence of two other CVR factors: although not included in the risk algorithm atrial fibrillation conveys increased risk of CVE (especially stroke), and it was documented in 2.4% of our subjects, a number in line with those reported in previous studies (24); LVH, on the other hand, was already included in the SCORE modulated risk evaluation strategy, but the record of it afflicting 8% of the population above 35 years of age is worrisome (even if it should be confirmed by echocardiographic methods), as it is significantly more prevalent than registered in neighbor countries, using the same methodology (25). Importantly, the unsuccessful implementation of risk reduction policies is generalized, and the overall benefit of achieving low CVR cannot be overemphasized (26).

Widespread use of SCORE risk algorithms. The SCORE algorithm is being widely used in all primary care centres across the country. However, herein we show that the application of its simple score-dependent risk attribution would classify incorrectly 18.2% of the population, and, in particularly, 35% of subjects placed in the moderate risk class. As the Moderate risk class was the second largest in the initial simple SCORE risk evaluation (820 subjects, Table 2), this could mean a high absolute number of CVE occurring later on without being given the opportunity of aggressive CVR factor control and limitation of disease progression. These facts highlight the need to search for higher risk attributing characteristics (history of previous CV or renal disease, complications of diabetes, presence of reduced eGFR, grade III albuminuria or LVH) especially in the subjects that the initial SCORE evaluation classifies as Moderate Risk, in any healthcare setting, including primary care setting. The subsequent, non-invasive search of TOD could be done in reference centers, whenever necessary (PWV, Carotid intima-media

thickness, echocardiogram). Our results are not only supporting the adoption of such strategy, but in line with the increase in risk prediction that it would confer (7, 21, 27).

Risk prediction in Hypertensives and therapeutic implications. The evidence that 62.7% of our hypertensives would be classified as High/Very High risk subjects, or that 80.2% would have at least a moderate – to high risk classification by the ESH/ESC charts is another part of the problem concerning risk reduction policies in our country. This number is different from those previously published for Portugal (28), despite differences in methodologies and population-base. We have previously shown that, in this same population, only 29.5% of all hypertensives have controlled BP (14); herein we show the poor lipid level control achieved and the undertreated number of subjects at risk. These results are in line with reports of insufficient CVR factor control and the need of addressing multiple cardiovascular risk issues when approaching the hypertensive patient (29).

The difference in allocation of hypertensive subjects to higher risk classes between the SCORE modulated and the ESC/ESH charts that we report here, have also been described elsewhere (30). This is explained by the parameters that each algorithm has chosen to define High/Very High risk. This is a subtle but important aspect, even if the ESC/ESH chart should be preferred in hypertensive subjects.

Strengths and limitations. This approach decided to phenotype more intensively subjects in a population cohort study, approaching clinical standards to epidemiological research. On top of this multiple risk-variable analysis, our sample of an homogenous population living in adjacent cities in the same region of northern Portugal and representative of all adult age classes, levels of education and professional status merits notice. In spite of our effort, some variables could only be measured once, and this might still introduce bias in our analysis.

Conclusions. Significantly high/very high risk estimation of 10-year CVE are presented for both the general population and hypertensives. Approximately one fifth of the population could benefit from a more thorough risk evaluation, as they would be reclassified to a higher risk status. Alarming levels of unsuccessful control of CVRF are concurrently and consequently occurring, in spite of evidence accumulating recently on the benefits of lowering BP, smoking (31) and lipid levels (32, 33). Several attempts have been made to help the physician translate risk status to his patient (Heart Age(34), Heart/Vascular Age(2)); they are based in the SCORE and the Framingham risk algorithms (respectively), and their impact on better CVR prevention and therapeutic compliance is still being scrutinized. The need of better identification of subjects at risk and treatment in conformity is evident, especially taking into account the natural evolution of CVR factor prevalence and CVD incidence in modernizing countries (35).

Acknowledgments. The authors would like to thank Prof Ian Graham and Prof. Marie Therese Cooney for sharing the SCORE algorithm equations and for being available to discuss aspects of the algorithm applicability. They also wish to show their acknowledgment to the following institutions and departments who collaborated in this project: Agrupamento de Centros de Saúde de Guimarães e Vizela, Serviço de Patologia Clínica do Centro Hospitalar do Alto Ave, Centro de Saúde da Amorosa, USF Afonso Henriques, USF Ara Trajano, USF Duo Vida, USF Novos Rumos, USF de Pevidém, USF Physis, USF de Ponte, USF de Ronfe, USF S. Nicolau, USF de S. Torcato, USF de Serzedelo e USF Vimaranes. A special word is addressed to thank all the physicians working in those different Primary Health Care Community Centers, whose work enrolling subjects was vital to the successful prosecution of our goals.

The Guimarães Study Group is also composed by the following researchers, who were involved in data collection: Helena Sarmento, Gloria Alves, Sara Freitas, Ana Sofia Alves, Sofia Gomes, Marta Gonçalves, Rui Fernandes, António Pedro Fonte, José Miguel Sá, Clarisse Neves, Andreia Sampaio, Cristina Cunha, Sílvia Sousa, Filipe Gonçalves, Joana Malheiro, João Silva, Carlos Fernandes, Estefânia Bustabad, Laura Castro, Nuna Vieira, Filipa Ramos, Ana Catarina Marques, Joana Monteiro, Ana Sofia Silva, Margarida Rocha, Mafalda Jordão Abreu, Margarida

Dias, Magda Fernandes, Paula Felgueiras, Francisca Castro, Vânia Gomes, Ana Luísa Novo, Carla Pereira, Ana Cristina Ramalho, Fernando Esculcas, Dina Fernandes, Ricardo Rodrigues, Rafael Velho, Diana Coimbra, Joana Pimenta, João Pedro Teixeira, Ana Luísa Neves, Joana Leitão, Sara Pereira, Carla Ferreira, Clarinda Neves, Samuel Pedreira, Pedro Neves, Elisabete Lima, Carla Mendes, Orlanda Barbosa, Ana Catarina Martins, Célia Lemos, Eduarda Macedo, Sílvia Azevedo, Ana Rita Ribeiro, Elsa Salgado, Joana Dias, Eduarda Piairo, Marisa Carneiro, Ismael Costa, Lucília Miranda, Ana João Gonçalves, Natália Rodrigues, Manuela Morais, Rosário Santos, Odete Rodrigues, Alexandra Barreira, Ana Rita Romano, Catarina Marques, Fernanda Marisa Santos, Sónia Coelho Pereira, Elisabete Pinto Teixeira, Joana Andrade Pinto

References

1. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, et al. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. European heart journal. 2003;24(11):987-1003. Epub 2003/06/06.
2. D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008;117(6):743-53. Epub 2008/01/24.
3. Cooney MT, Dudina A, D'Agostino R, Graham IM. Cardiovascular risk-estimation systems in primary prevention: do they differ? Do they make a difference? Can we see the future? Circulation. 2010;122(3):300-10. Epub 2010/07/21.
4. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). European heart journal. 2011;32(14):1769-818. Epub 2011/06/30.
5. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). European heart journal. 2012;33(13):1635-701. Epub 2012/05/05.
6. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European heart journal. 2013;34(28):2159-219. Epub 2013/06/19.
7. Sehestedt T, Jeppesen J, Hansen TW, Wachtell K, Ibsen H, Torp-Pedersen C, et al. Risk prediction is improved by adding markers of subclinical organ damage to SCORE. European heart journal. 2010;31(7):883-91. Epub 2009/12/26.
8. Sehestedt T, Jeppesen J, Hansen TW, Rasmussen S, Wachtell K, Ibsen H, et al. Thresholds for pulse wave velocity, urine albumin creatinine ratio and left ventricular mass index using SCORE, Framingham and ESH/ESC risk charts. Journal of hypertension. 2012;30(10):1928-36. Epub 2012/08/09.
9. Guallar E, Banegas JR, Blasco-Colmenares E, Jimenez FJ, Dallongeville J, Halcox JP, et al. Excess risk attributable to traditional cardiovascular risk factors in clinical practice settings across Europe - The EURIKA Study. BMC public health. 2011;11:704. Epub 2011/09/20.
10. Correia M, Silva MR, Matos I, Magalhaes R, Lopes JC, Ferro JM, et al. Prospective community-based study of stroke in Northern Portugal: incidence and case fatality in rural and urban populations. Stroke; a journal of cerebral circulation. 2004;35(9):2048-53. Epub 2004/07/17.
11. Nichols M TN, Luengo-Fernandez R, Leal J, Gray A, Scarborough P, Rayner M. . European Cardiovascular Disease Statistics 2012. Brussels: (2012).
12. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. European heart journal. 2014;35(42):2950-9. Epub 2014/08/21.
13. Organisation WH. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. 2013.
14. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. An epidemiological study determining blood pressure in a Portuguese cohort: the Guimaraes/Vizela study. Journal of human hypertension. 2014. Epub 2014/08/01.

15. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. The rationale/design of the guimaraes/vizela study: a multimodal population-based cohort study to determine global cardiovascular risk and disease. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2014;62(5):813-20. Epub 2014/03/04.
16. Macedo ME, Lima MJ, Silva AO, Alcantara P, Ramalhinho V, Carmona J. Prevalence, awareness, treatment and control of hypertension in Portugal: the PAP study. Journal of hypertension. 2005;23(9):1661-6. Epub 2005/08/12.
17. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. Journal of hypertension. 2014;32(6):1211-21. Epub 2014/03/29.
18. Cunha PG, Cotter J, Oliveira P, Vila I, Boutouyrie P, Laurent S, et al. Pulse wave velocity distribution in a cohort study: from arterial stiffness to early vascular aging. Journal of hypertension. 2015;33(7):1438-45. Epub 2015/04/02.
19. (2) Classification and diagnosis of diabetes. Diabetes care. 2015;38 Suppl:S8-S16. Epub 2014/12/30.
20. Summary of Recommendation Statements. Kidney international supplements. 2013;3(1):5-14. Epub 2013/01/01.
21. Zambon A, Arfe A, Corrao G, Zanchetti A. Relationships of different types of event to cardiovascular death in trials of antihypertensive treatment: an aid to definition of total cardiovascular disease risk in hypertension. Journal of hypertension. 2014;32(3):495-508. Epub 2014/01/01.
22. Nilsson PM, Boutouyrie P, Cunha P, Kotsis V, Narkiewicz K, Parati G, et al. Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. Journal of hypertension. 2013;31(8):1517-26. Epub 2013/06/08.
23. Ferreira RC, Neves RC, Rodrigues V, Nogueira PJ, Farinha CS, Soares A, et al. Portugal: Doenças Cérebro-Cardiovasculares em números – 2014. Portugal: Direcção-Geral da Saúde. Direção de Serviços de Informação e Análise, 2014.
24. Bonhorst D, Mendes M, Adragao P, De Sousa J, Primo J, Leiria E, et al. Prevalence of atrial fibrillation in the Portuguese population aged 40 and over: the FAMA study. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2010;29(3):331-50. Epub 2010/07/20.
25. Felix-Redondo FJ, Fernandez-Berges D, Calderon A, Consuegra-Sanchez L, Lozano L, Barrios V. Prevalence of left-ventricular hypertrophy by multiple electrocardiographic criteria in general population: Hermex study. Journal of hypertension. 2012;30(7):1460-7. Epub 2012/05/11.
26. Stamler J, Stamler R, Neaton JD, Wentworth D, Daviglus ML, Garside D, et al. Low risk-factor profile and long-term cardiovascular and noncardiovascular mortality and life expectancy: findings for 5 large cohorts of young adult and middle-aged men and women. JAMA : the journal of the American Medical Association. 1999;282(21):2012-8. Epub 1999/12/11.
27. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001;37(5):1236-41. Epub 2001/05/23.
28. Polonia J, Mesquita-Bastos J, Pessanha P, Bertoquini S, Martins L, Silva JA, et al. Global cardiovascular risk stratification of hypertensive patients followed in Portugal in primary care or in hospital care according to the 2007 ESH/ESC guidelines. Revista portuguesa de cardiologia : orgao oficial da Sociedade Portuguesa de Cardiologia = Portuguese journal of cardiology : an official journal of the Portuguese Society of Cardiology. 2010;29(11):1685-96. Epub 2011/02/12.

29. Tocci G, Valenti V, Sciarretta S, Volpe M. Multivariate risk assessment and risk score cards in hypertension. Vascular health and risk management. 2007;3(3):313-20. Epub 2007/08/21.
30. Sehestedt T, Jeppesen J, Hansen TW, Rasmussen S, Wachtell K, Ibsen H, et al. Risk stratification with the risk chart from the European Society of Hypertension compared with SCORE in the general population. Journal of hypertension. 2009;27(12):2351-7. Epub 2009/11/17.
31. Mons U, Muezzinler A, Gellert C, Schottker B, Abnet CC, Bobak M, et al. Impact of smoking and smoking cessation on cardiovascular events and mortality among older adults: meta-analysis of individual participant data from prospective cohort studies of the CHANCES consortium. BMJ. 2015;350:h1551. Epub 2015/04/22.
32. Cannon CP, Blazing MA, Giugliano RP, McCagg A, White JA, Theroux P, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. The New England journal of medicine. 2015;372(25):2387-97. Epub 2015/06/04.
33. Bangalore S, Breazna A, DeMicco DA, Wun CC, Messerli FH. Visit-to-visit low-density lipoprotein cholesterol variability and risk of cardiovascular outcomes: insights from the TNT trial. Journal of the American College of Cardiology. 2015;65(15):1539-48. Epub 2015/04/18.
34. Cooney MT, Vartiainen E, Laatikainen T, De Bacquer D, McGorrian C, Dudina A, et al. Cardiovascular risk age: concepts and practicalities. Heart. 2012;98(12):941-6. Epub 2012/05/26.
35. Danaei G, Singh GM, Paciorek CJ, Lin JK, Cowan MJ, Finucane MM, et al. The global cardiovascular risk transition: associations of four metabolic risk factors with national income, urbanization, and Western diet in 1980 and 2008. Circulation. 2013;127(14):1493-502, 502e1-8. Epub 2013/03/14.

Figure and Table Legend

Table 1 – Clinical characteristics of 2542 subjects studied

Table 2 – Absolute reclassification values observed between the two phenotyping strategies

Table 3 – SCORE Risk Distribution by age and gender, adjusted to the studied population

Table 4 – Relative Risk evaluation according to SCORE charts for subjects below 40 years of age

Table 5 – 10-years risk of fatal CVE using SCORE modulated methodology in hypertensive subjects (n=907)

Table 6 – Risk classification of hypertensive subjects according to the ESC/ESH Charts (n=907)

Table 7 – Dyslipidemia, anti-lipidic treatment and treatment goals achieved, stratified by SCORE modulated risk group, in hypertensive subjects (n=907)

Figure 1 – SCORE classification of the studied population before and after reclassification

Table 1 – Clinical characteristics of 2542 subjects who completed the visit plan

Variables	Final Population (2542)
Gender (M/F)%	44.9/55.1
Age (Years)	45.5 ± 19.1
BMI > 30 Kg/m2 (%)	21.3
Abdominal Obesity (%)	46.6
Hypertension (%)	31.6
Diabetes (%)	9.1
Dyslipidemia (%)	75.1
PWV > 10m/s (%)	18.7
Grade II Albuminuria (%)	7.3
Grade III Albuminuria (%)	1.2
Left Ventricular Hypertrophy (%)	8.0
eGFR 30 – 60 ml/min (%)	4.3
eGFR < 30 ml/min (%)	0.7
Smokers (%)	18.8
Non – Smokers (%)	65.1
Ex – Smokers (%)	16.1
Known CVD (%)	5.0

CVD – Cardiovascular Disease; **eGFR** – estimated glomerular filtration rate; **PWV** – Pulse Wave Velocity

Table 2 – Absolute reclassification values observed between the two phenotyping strategies

	Modulated SCORE Low Risk	Modulated SCORE Moderate Risk	Modulated SCORE High Risk	Modulated SCORE Very High Risk	Total
Simple SCORE Low Risk	**1485**	0	9	75	**1569**
Simple SCORE Moderate Risk	0	**504**	53	263	**820**
Simple SCORE High Risk	0	0	**76**	64	**140**
Simple SCORE Very High Risk	0	0	0	**13**	**13**
Total	**1485**	**504**	**138**	**415**	**2542**

Table 3 – SCORE Risk Distribution by age and gender, adjusted to the studied population

	Very High Risk			High Risk			Moderate Risk			Low Risk		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
< 30y	1.2	1.6	1.4	0	0	0	0	0	0	98.8	98.4	98.6
30 – 39y	2.9	2.4	2.7	0.4	0.3	0.4	0	0	0	96.7	97.3	97
40 – 49y	11.1	7.5	9.3	4.8	0	2.4	27.8	0.6	14.1	56.3	91..9	74.2
50 – 59y	24.6	17.9	21.2	2.8	1.9	2.3	59.9	8.0	33.5	12.7	72.2	43
60 – 69y	38.2	28.0	32.8	17.2	3.7	10	44.6	59.3	52.4	0	9	4.8
≥70 y	45.6	35.5	39.6	27.0	11.6	17.8	27.4	52.1	42.1	0	0.8	0.5
Total	15.0	12.6	13.7	5.7	2.2	3.9	22.2	14.2	18.1	57.2	70.9	64.2

Table 4 – Relative Risk evaluation according to SCORE charts for subjects below 40 years of age (n= 1138 subjects)

Relative Risk	Percentage of subjects (%)
1x	60.1
2x	30.1
3x	7.4
4x	1.4
5x	0.6
6x	0.2
7x	0.2
8x	0.1

Table 5 – 10-years risk of fatal CVE using SCORE modulated methodology in hypertensive subjects (n=907)

	Very High Risk			High Risk			Moderate Risk			Low Risk		
	Males	Females	Total	Males	Females	Total	Males	Females	Total	Males	Females	Total
TOTAL (%)	**41.4**	**32.2**	**36.7**	**17.8**	**6.7**	**12.1**	**26.8**	**40.4**	**33.7**	**14**	**20.7**	**17.4**
n	**184**	**149**	**333**	**79**	**31**	**110**	**119**	**187**	**306**	**62**	**96**	**158**

Table 6 – Risk classification of hypertensive subjects according to the ESC/ESH Charts (n=907)

	Optimal BP	Normal BP	High Normal BP	HTN grade I	HTN grade II	HTN grade III
No RF	0.1%	0%	0%	0.3%	0%	0%
1 – 2 RF	0.6%	1.1%	1.5%	5.3%	1%	0.2%
≥ 3 RF	0.2%	2.3%	4.2%	11.9%	2.6%	0.4%
TOD, DM or moderate CKD	0.7%	3.1%	4.8%	15.8%	7.2%	1.5%
CVD, DM + OD/RF or Severe CKD	1.4%	2.8%	6.7%	15.4%	6.7%	2.0%

Very High Risk – 35% | High Risk –27.7% | Moderate to High Risk – 17.7%

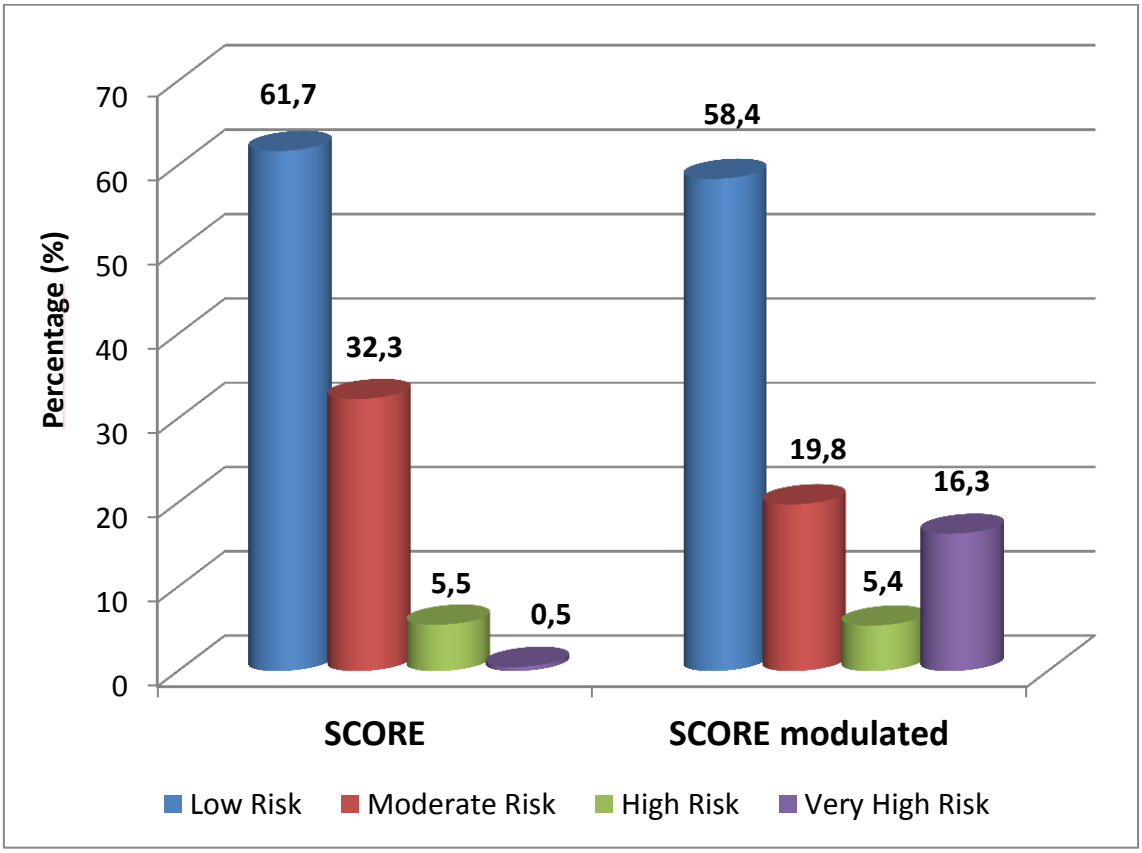
Moderate Risk –5.3% | Low to Moderate Risk – 4.2% | Low Risk – 1.8%

BP – Blood Pressure; **CVD** – Cardiovascular Disease; **CKD** – Chronich Kidney Disease; **DM** – Diabetes; **RF**– Risk factors; **TOD** – Target Organ Damage; **HTN** - Hypertension

Table 7 – Dyslipidemia, anti-lipidic treatment and treatment goals achieved, stratified by SCORE modulated risk group, in hypertensive subjects (n=907)

SCORE Modulated algorithm	Very High Risk	High Risk	Moderate Risk	Low Risk
Dyslipidemia prevalence (%)	91,1	92	89,5	64,5
Anti-lipidic Treatment (%)	50,4	34,1	34,1	4,2
Total Cholesterol >190 mg/dl (%)	41,1	66,7	60,8	46,9
LDL Cholesterol >115 mg/dl (%)	39,4	65,2	58	45,3
LDL cholesterol < 70 mg/dl (%)	14,3	4,3	3,6	6,1
LDL Colesterol <100 mg/dl (%)	44.5	21.7	25.1	35.0

Figure 1 – SCORE classification of the studied population before and after reclassification (crude rates)



Chapter 5

Pulse Wave Velocity Distribution in a cohort study

– from arterial stiffness to early vascular aging (EVA)

Original Article

Pulse wave velocity distribution in a cohort study: from arterial stiffness to early vascular aging

Pedro G. Cunha[a,b,c], Jorge Cotter[a,b,c], Pedro Oliveira[d,e], Isabel Vila[a], Pierre Boutouyrie[f] Stéphane Laurent[f], Peter M. Nilsson[g], Angelo Scuteri[h], and Nuno Sousa[b,c]

Background: By contrast with other southern European people, north Portuguese population registers an especially high prevalence of hypertension and stroke incidence. We designed a cohort study to identify individuals presenting accelerated and premature arterial aging in the Portuguese population.

Method: Pulse wave velocity (PWV) was measured in randomly sampled population dwellers aged 18–96 years from northern Portugal, and used as a marker of early vascular aging (EVA). Of the 3038 individuals enrolled, 2542 completed the evaluation.

Results: Mean PWV value for the entire population was 8.4 m/s (men: 8.6 m/s; women: 8.2 m/s; $P < 0.02$). The individuals were classified with EVA if their PWV was at least 97.5th percentile of *z*-score for mean PWV values adjusted for age (using normal European reference values as comparators). The overall prevalence of EVA was 12.5%; 26.1% of individuals below 30 years presented this feature and 40.2% of individuals in that same age strata were placed above the 90th percentile of PWV; and 18.7% of the population exhibited PWV values above 10 m/s, with male predominance (17.2% of men aged 40–49 years had PWV > 10 m/s). Logistic regression models indicated gender differences concerning the risk of developing large artery damage, with women having the same odds of PWV above 10 m/s 10 years later than men.

Conclusion: The population PWV values were higher than expected in a low cardiovascular risk area (Portugal). High prevalence rates of EVA and noteworthy large artery damage in young ages were found.

Keywords: arterial stiffness, cardiovascular risk, early vascular aging, epidemiology, large artery, Portugal, pulse wave velocity

Abbreviations: BP, blood pressure; c-f PWV, carotid–femoral pulse wave velocity; CVD, cardiovascular disease; CVRFs, cardiovascular risk factors; ERVC, European reference values collaboration; EVA, early vascular aging; HR, heart rate; TOD, target organ damage

INTRODUCTION

Changes in arterial wall structure are part of the aging process and occur as a result of mechanical, biochemical, or metabolic insults. These changes are attenuated by local repair mechanisms [1]. Arterial stiffness progresses with age and can be accelerated by different factors, high blood pressure (BP) being one of the most relevant [2]. The impact of these factors on arterial stiffness is significant in younger and older individuals as well as in men and women, and across different countries [3,4]. Other contributors to this unsuccessful aging process can be related to early-life determinants (fetal programming, intrauterine growth retardation, low birth weight, postnatal growth pattern) [5] and genetic determinants, concerning different aspects of arterial stiffness heritability that range from arterial wall composition to transcriptional pathways related to gene expression [6–9].

Early vascular aging (EVA) is a concept with growing interest and relevance [1,5,10–14]. It corresponds to unsuccessful aging: the normal aging process is accelerated and arteries display characteristics typically observed at older (chronological) ages. The early identification of individuals at increased absolute [15] and relative cardiovascular risk when compared with individuals with the same age is critical; measurement of arterial stiffness through pulse wave velocity (PWV) fulfills part of this issue [16]. This group of individuals would benefit from clinical intervention to reduce risk through cardiovascular risk factor (CVRF) control [17] and healthy lifestyle behaviors: exercise [18], diet [19], and lower salt consumption [20]. This is called the aggressive decrease of atherosclerosis modifiers strategy [11].

Arterial stiffness measurement is particularly useful to obtain a comprehensive insight of the accumulated effect of different CVRF through an extended time window, in the

Journal of Hypertension 2015, 33:000–000

[a]Department of Internal Medicine, Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Guimarães – Centro Hospitalar do Alto Ave, [b]Life and Health Science Research Institute (ICVS), School of Health Science, University of Minho, Guimarães, [c]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, [d]EPI Unit, [e]Instituto de Saúde Pública, Instituto de Ciências Biomédicas Abel Salazar da, Universidade do Porto, Porto, Portugal, [f]Department of Pharmacology and INSERM U970, Assistance Publique Hôpitaux de Paris, Université Paris Descartes, Paris, France, [g]Department of Clinical Sciences, Lund University, Skane University Hospital, Malmö, Sweden and [h]San Raffaele Pisana IRCCS, Rome, Italy

Correspondence to Pedro Guimarães Cunha, Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Serviço de Medicina Interna do Hospital de Guimarães, Centro Hospitalar do Alto Ave; Life and Health Science Research Institute (ICVS), University of Minho, Rua dos Cutileiros, Creixomil, Guimarães 4810, Portugal. E-mail: pedrocunha@ecsaude.uminho.pt

Received 30 September 2014 **Revised** 10 February 2015 **Accepted** 10 February 2015

DOI:10.1097/HJH.0000000000000565

vascular aging process [11]. Longitudinal follow-up of this measurement could also identify those whose clinical intervention has failed to achieve CVRF control and regression/stabilization of early arterial stiffness signs [21]. Carotid–femoral (c-f)PWV reference values adjusted to age, sex, and influencing CVRF have been recently published [16], allowing the clinician to compare an individual's measure of arterial damage against the expected value for that same age.

By contrast with other southern European people, the northern Portuguese population registers an especially high stroke incidence [22]. Previous reports published on this population have related increased arterial stiffness to salt intake and prevalence of hypertension (HTN); however, no study was designed to assess EVA patterns. Assessment of arterial wall damage as a marker of increased risk to the development of cardiovascular disease (CVD), cardiovascular events, and all-cause mortality [15,23] is therefore of particular interest, in this geographic area.

We designed a large-scale population-based cohort study to accurately establish the prevalence of several CVRF and determine the distribution of PWV values and signs of pathological arterial stiffness. The aim was to identify individuals presenting accelerated and premature arterial aging, through age-adjusted analysis, or those who have PWV values above the 10 m/s threshold [24].

PATIENTS AND METHODS

The methodology employed in this study has already been detailed elsewhere [25,26]. Briefly, a representative sample of the population from two adjacent cities (Guimarães and Vizela) was randomly selected and evaluated on two different occasions at least 3 months apart, after signing a written consent form approved by an Ethics Committee. In Portugal, every citizen must be registered in the Primary Community Healthcare Centre (PCHCC) of his/her residence area. Comparing the characteristics of citizens living in Guimarães/Vizela (Statistics Portugal for the year 2006) and the same information of those with an actual registry in one of the PCHCC facilities operating in the two cities, the difference between both the lists was inferior to 2%, therefore allowing the consideration that, for practical use, the populations enrolled in PCHCC and living in Guimarães/Vizela are virtually the same. Therefore, participants have been randomly selected from the list of citizens currently living in Guimarães and Vizela.

Considering that there was no available estimate of the prevalence of EVA or large artery damage for the Portuguese population, the sample selection was based considering the estimated prevalence of HTN in Portugal. A sample size of at least 2339 participants was required to achieve 2% precision in an estimated prevalence of HTN of 41.2%. Thus, a sample of the adult population (>18 years of age) stratified by age was defined previewing the necessity of including 4000 individuals (95% confidence interval with an estimation error inferior to 2%, considering a 25% safety margin to cope with nonadherence and dropout rate between visits).

Anticipating a higher nonadherence and dropout rates on younger and professionally active individuals, the number of randomized individuals to enroll was stratified unevenly according to their age (2000 individuals would be <35 years of age, 1000 individuals would have 35–65 years of age, and 1000 individuals would be >65 years).

The estimation of PWV values for the population was adjusted by age and sex agreeing to the known distribution of these characteristics in the population of the two cities, according to Statistics Portugal for the year 2006.

The lists of randomized individuals were delivered to the corresponding family doctors so that they could contact and enroll them, and obtain the written consent to participate. It was therefore clear that only randomly assigned individuals could be enrolled and that no volunteers or physician-selected individuals would be included. If the individual refused, the general practitioner could not replace him/her with a volunteer or with someone from his/her practice. Only randomized individuals were accepted.

Individuals were observed in the morning, fasting overnight, and carrying their usual drug prescriptions. No intake of caffeinated beverages or tobacco use was allowed. The participants underwent a standardized workup including medical history, and biologic and arterial measurements. Trained physicians performed BP measurements at every occasion; training sessions performed prior to the beginning of the study insured standardization of BP and PWV measurements. BP was measured three times (at each visit) with participants in a sitting position, after a 15-min resting period, taken with 2 min interval, using the validated OMRON-705IT device (Omron Healthcare Europe B.V, Hoofddorp, The Netherlands). During their first visit, patients were then placed in the supine position and c-fPWV was measured using the Sphygmocor device (Atcor Medical Pty Ltd ,West Ryde, NSW, Australia).

Measured PWV values were standardized and normalized to allow us to compare the obtained values with the ones published as European reference [16] (details in Supplemental Material, http://links.lww.com/HJH/A474).

Definition of early vascular aging

The primary definition of EVA was based upon the age-adjusted normal population of the European reference values collaboration (ERVC) [16]. Accordingly, EVA was defined as a PWV of at least 97.5th percentile of *z*-score for mean PWV values adjusted for age, using the normal population PWV values as comparator. The normal population in the ERVC included the so-called normotensive 'healthy' individuals, as they did not have any known CVRF or CVD and presented optimal or normal BP [16]. In a separate exercise, we also tested another definition of EVA wherein we included individuals with PWV values 2 standard deviation (SD) above the mean PWV value determined as reference for someone with his/her age and BP class; as comparator, we used the reference population values also presented by the ERVC [16] – the total reference population in that collaboration included individuals with high–normal to Grade 3 BP (untreated), dyslipidemia (untreated), smokers, and no diabetes or established CVD [16].

We also considered the existence of arterial target organ damage by dichotomizing PWV values above 10 m/s or below [24].

Statistical analysis

Statistical methodology concerning population sampling, database elaboration, and management, as well as predefined statistical analysis have already been addressed elsewhere [25]. Prevalence of different studied characteristics was estimated and calculated by demographic characteristics (age group, sex, education level) and risk factors. Linear regression models were studied with PWV as the dependent variable and with independent variables that included age, age^2, sex, SBP (mean of four measurements), heart rate (HR), BMI, years of education, tobacco use, family history of premature CVD, antihypertensive medication use, fasting glucose, lipid profile, mean estimated glomerular filtration rate, C-reactive protein, antidiabetic treatment, antilipidic treatment, and known CVD.

To avoid collinearity problems, age and SBP were standardized by their mean and SD. Logarithmic transformation of PWV values was performed to guarantee a normal homocedastic distribution. Because of detected interactions between sex and major risk factor, we created separate models for men and women. Logistic regression models were constructed to study contributing variables to both the development of large artery damage (PWV $>$ 10 m/s) and EVA. Again, as men and women presented a different discriminative value of age for large artery damage, two different models, based on gender, are presented (Figure SM1 and Supplemental Material, http://links.lww.com/HJH/A474).

RESULTS

From the initial 4000 randomized individuals, 962 could not participate (these individuals either refused to participate, were no longer residents, or were either pregnant or bedridden). From the 3038 enrolled, the dropout rate was 16.1%, between the two clinical observations defined in the study [25,26]. The individuals who dropped out of the study were similar in terms of age, sex, and years of education when compared with the adherent individuals [26].

The distribution of population by age class and sex, their clinical characteristics, and mean values of PWV distribution stratified and adjusted for age/sex are summarized in Table 1. In Table 2, we present the prevalence of relevant biologic characteristics (CVRFs, CVD, pharmacologic treatments used), stratified by age class. The adjusted mean PWV value for the entire population was 8.4 m/s (8.2 m/s for women; 8.6 m/s for men). Men had an average 0.6 m/s higher PWV than women ($P < 0.001$). The sex difference in PWV values was observable in any age class ($P < 0.02$) and it increased with advancing age: from 0.4 m/s in individuals below 30 years to 1.3 m/s in individuals of at least 70 years. Regression analysis showed that in males, the increase in PWV depended quadratically on age. In females, a linear model best described this association and in addition to age, SBP and HR were significantly associated with PWV ($R^2 = 0.44$). In males, in addition to age and age^2, SBP, HR, and fasting glucose were also significant variables associated with PWV ($R^2 = 0.49$). Stepwise regression showed that the main associations, by order of importance, were with age, SBP, and HR. The observed differences between genders justify the adoption of gender-specific regression models (Table 3).

TABLE 1. Clinical characteristics of 2542 individuals studied

	Total
Number of cases/female %	2542 / 55
Mean age (years)	45.5
Mean BMI (kg/m^2) (SD)	26.6 (4.6)
Mean SBP/DBP (mmHg)	129.8/76.8
Hypertension (%)	31.6
Diabetes (%)	9.1
Dyslipidemia (%)	75.1
Years of education: ≤4/5–9/10–12/>12 (%)	53.7/22.3/15.7/7.8
Current/former/no smoker (%)	18.8/16.1/65.1
Antihypertensive treatment (%)	22.0
Antidiabetic treatment (%)	6.7
Use of lipid-lowering drugs (%)	17.7
Established cardiovascular disease (%)	5.0

PWV mean values (m/s)	Total	Male	Female
<30 years (249/311)	7.1	7.3	6.8
30–39 years (236/334)	7.7	7.9	7.4
40–49 years (122/154)	8.3	8.5	8.1
50–59 years (136/160)	8.5	8.6	8.5
60–69 years (141/169)	9.9	10.3	9.5
70–79 years (145/157)	10.8	11.6	10.2
≥80 years (22/25)	11.4	12.1	11.0
All (1051/1310)	8.4	8.6	8.2

Numbers in brackets correspond to the number of male (M) and female (F) individuals (M/F) in each age class. PWV, pulse wave velocity; SD, standard deviation.

Early vascular aging

Using the above-mentioned *z*-scores methodology, every individual was ranked within his/her age strata. We present the percentage of individuals placed above the 90th, 95th, and 97.5th percentile of predicted PWV for their age class in Fig. 1.

EVA was recorded in 12.5% of the total population. Strikingly, 19.3% of individuals below 40 years and 26.1% of individuals below 30 would be classified as EVA individuals, that is, one out of every four young adults. In Supplemental Material, http://links.lww.com/HJH/A474, the calculation of EVA prevalence using the 2SD above the mean PWV value adjusted for age and BP class is presented (using the European 'reference' population values) – Figure SM2, http://links.lww.com/HJH/A474. With this alternative method of calculation, observations of the same nature can be made regarding the high prevalence of EVA, notably at ages below 50 years. With any of the methods applied, there was a significantly higher prevalence of EVA individuals of the male gender in age classes below 40 years.

In Table SM3, http://links.lww.com/HJH/A474, we present the biologic characteristics of individuals below 50 years of age classified as having EVA, by comparison with those classified as normal arterial aging within the same age strata. The EVA individuals presented higher male prevalence, mean BP, HTN prevalence, and a slight increase in mean HR, but lower high-density lipoprotein cholesterol levels. In Table SM4, http://links.lww.com/HJH/A474, we show the results of the logistic regression

TABLE 2. Prevalence (percent) of relevant biological characteristics stratified by age group (crude rates)

Age (years)	Current smokers	Former smokers	Diabetes	Dyslipidemia	HTN	CKD	CVD	HTN treatment	Diabetes treatment	Lipid treatment
<30	30.2	7.8	0.4	49.5	2.7	0.4	0.2	0.7	0.2	0.7
30–39	25.9	9.7	1.2	68.1	10.8	0.7	0.5	3.6	0.5	1.9
40–49	26.6	19.6	6.6	81.8	25.2	1.4	1.7	12.6	4.9	8.4
50–59	13.8	21.1	14.8	88.5	44.7	1.7	7.9	31.9	11.2	31.3
60–69	8.7	20.9	25.1	91.3	71.7	4.4	9.0	53.2	20.2	43.1
≥70	2.4	25.1	23.3	90.8	79.9	13.7	21.2	60.9	14.1	44.4

CKD, chronic kidney disease; CVD, cardiovascular disease (including ischemic stroke, hemorrhagic stroke, acute coronary syndromes, coronary heart disease, coronary artery bypass surgery, and peripheral artery disease); HTN, hypertension.

analysis of EVA. Identified variables were age below 30 years [odds ratio (OR) = 3.2], male sex (OR = 1.4), BP above or equal to the high–normal category ($OR_{optimal\ BP} = 1$; $OR_{high-normal\ BP} = 1.7$; $OR_{Grade\ 1\ BP} = 3.1$, and $OR_{Grade\ 2/3\ BP} = 9.4$), HR above 75 bpm (OR = 1.7), and diabetes (OR = 2.8).

Large artery damage

We also aimed to know how many individuals presented PWV values over the established high-risk cutoff of 10 m/s. Figure 2 exhibits the age and sex-adjusted estimate of the prevalence of individuals with measurements above this value.

Men had a higher prevalence of high-risk arterial stiffness both globally ($X^2 = 32.4$, $P < 0.001$) and particularly in the age groups 30–39 ($X^2 = 4.5$, $P < 0.05$), 60–69 ($X^2 = 8.9$, $P < 0.01$), and at least70 years ($X^2 = 16.9$, $P < 0.001$). Overall, a prevalence rate of 18.7% was observed in the population (after adjustment for age and sex). Our attention was drawn to the estimates obtained for the age classes below 50 years, where prevalence can reach as high as 14.1% (ages 40–49 years), with higher figures being recorded in men (17.2%). The fact that 7.2% of men in the age class of 30–39 years exhibited large artery damage is a noticeable finding. Logistic regression analysis models showed that age above 40 years and BP are variables increasing the risk of developing large artery damage; the logistic models for PWV above 10 m/s also evidence the difference between genders. Observing Figure SM1, http://links.lww.com/HJH/A474, it becomes clear that for a 50% probability of developing PWV above 10, and for the same BP level, the corresponding chronological age for males and females differs approximately by 10 years (higher for men); for a given age, the difference in probability of developing PWV above 10, within the same BP class, is approximately 15% (higher for men). HR is also a contributor, especially for women (OR = 2.2), and diabetes status contributes to the development of arterial stiffness in men (OR = 1.9) (Table SM5, http://links.lww.com/HJH/A474).

Finally, putting side by side both the prevalence of individuals that contributed PWV values above the median and the expected 90th percentile (using the normal 'healthy' population as comparator) and the prevalence of individuals with PWV above 10 m/s (Fig. 3), we were able to describe the coexistence of both early and late signs of vascular aging in this population.

DISCUSSION

We present for the first time in a population-based study in Portugal, the mean values of PWV distributed by age category and sex. The main finding is that PWV values are markedly higher than expected from the European reference values, especially in younger individuals and males. We sought to describe how individuals' PWV values from a so-called low CVR area rank relative to European references [16]. We confirmed a clear increase of PWV with age, as well as higher mean levels of PWV in men, similar to descriptions in other reports [27].

Only few studies have characterized the distribution of PWV values using comprehensive population-based samples. A recent publication from Uruguay [28] reports population reference values higher than those published here and in the ERVC, hinting (as previously suggested [27]) that ethnic and geographic differences could explain different CVD geographic patterns. The Bogalusa Heart study

TABLE 3. Linear regression models for pulse wave velocity as dependent variable, following logarithmic transformation

Variables	Males				Females			
	Coefficients (SE)	Standardized coefficients	*P* values	R^2 incremental	Coefficients (SE)	Standardized coefficients	*P* values	R^2 incremental
Age	0.117 (0.007)	0.461	<0.001	0.411	0.113 (0.007)	0.474	<0.001	0.392
Age^2	0.034 (0.007)	0.109	<0.001	0.421	–	–	–	–
SBP	0.059 (0.007)	0.216	<0.001	0.466	0.058 (0.007)	0.239	<0.001	0.425
HR	0.003 (0.001)	0.134	<0.001	0.485	0.003 (0.001)	0.123	<0.001	0.439
Fasting glucose	0.001 (0.0003)	0.069	0.005	0.492	–	–	–	–
Constant	1.847 (0.039)	–	<0.001	–	1.904 (0.035)	–	<0.001	–
R^2		0.492				0.439		

Explanatory variables studied in the regression model: years of schooling, smoking, antihypertensive treatment, antidiabetic treatment, use of lipid-lowering drugs, prevalence of established cardiovascular disease, mean fasting glucose, family history of premature cardiovascular disease, BMI, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, mean estimated glomerular filtration rate, and C-reactive protein. Age2, quadratic transformation of age; HR, heart rate; PWV, pulse wave velocity; SE, standard error.

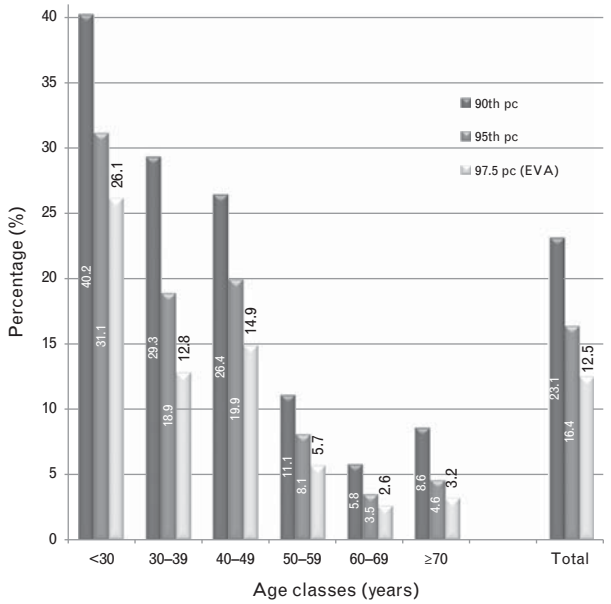


FIGURE 1 Prevalence of early vascular aging (EVA) stratified by age and showing percentage of individuals above the 90th and 95th percentile of pulse wave velocity (PWV) *z*-score values, using normal 'healthy' European individuals as comparators. These high values above expected percentiles in the population are complementary explained by analyzing distribution histograms of PWV (Figure SM6) and PWV *z*-scores (Figure SM7) as well as the difference in mean PWV values between the healthy European reference values collaboration population and the Guimarães/Vizela population (Table SM8), presented in Supplementary Material, http://links.lww.com/HJH/A474. pc, percentile.

[29], the Enigma study [30], and the ARYA study [31] reported mean PWV values for individuals below 40 years that are lower than those found in the city of Guimarães/Vizela. Most of these studies were performed in either healthy or lower CVRF populations (when compared with our cohort).

Other population studies have been conducted in older individuals [32,33], normotensives, and untreated hypertensive patients [34], as well as a subset of 998 healthy normotensive individuals in the Anglo-Cardiff Collaborative Trial trial [35]. In this latter study, as well as in the Baltimore Longitudinal Study of Aging [36] and again in the data we are presenting here, the existence of a nonlinear progression of PWV with age is registered, evidencing a much steeper increase from mid-life onward, more pronounced in men.

Noticeably, in our (so-called) low-risk population, significantly higher than expected PWV values are being recorded: 80–84% of individuals under 50 years have a PWV value above the expected median value for their age class in the normal ('healthy') population of the ERVC (Fig. 3) [16]. The male gender prevalence was expected, and it also reflects either the higher prevalence of CVRF or increased mean levels of several variables that are known to influence PWV (mainly BP) in males (data not shown).

The independent predictive value of PWV concerning cardiovascular events/mortality in population-based studies has been well established [37], and goes beyond the CVR stratification achieved by use of well accepted risk scores [38,39]. Therefore, when comparing the mean values of our population with those of the normal 'healthy' population [16], it is relevant to state that, compared with individuals with no CVRF or established CVD, our individuals below 50 years present mean PWV values that are overall 1 m/s higher. Knowing that an increase in 1 m/s in PWV is related to an increase in cardiovascular events, cardiovascular mortality, and overall mortality by 14, 15, and 15%, respectively [15], our findings raise apprehension. We cannot exclude that part of the difference between our population and the ERVC could be attributed to methodological differences even if standardization and staff training took place before screening. Still, recent evidence has emphasized that c-fPWV improves CVR prediction and reclassifies

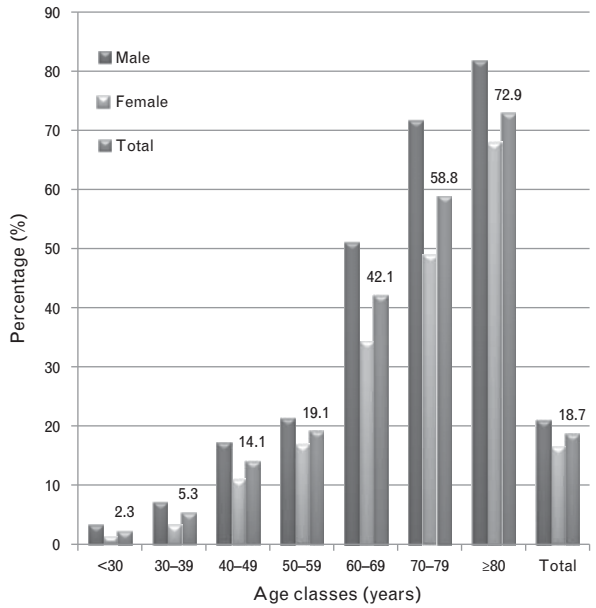


FIGURE 2 Percentage of individuals presenting carotid–femoral pulse wave velocity value above the 10 m/s high-risk cutoff, stratified by age and sex.

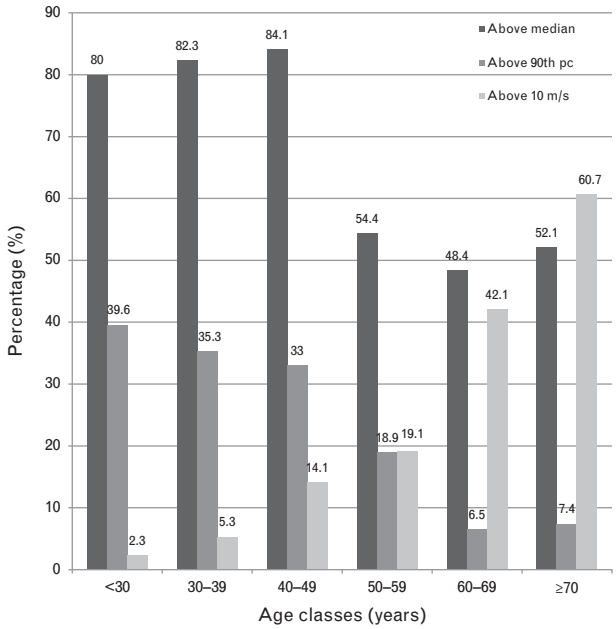


FIGURE 3 Percentage of individuals above the median and above the 90th percentile of the normal pulse wave velocity value expected for age, and above the 10 m/s cutoff (stratified by age decade). pc, percentile.

individuals at risk for CVD events, especially concerning individuals below 61 years of age and particularly regarding stroke [23].

The presented regression models allowed an explanation of 44–49% of the recorded PWV values in our population. Associations with age and SBP were not surprising, as they have been extensively documented [40]. For HR, the known strength of association is positive [33,40–42] but weaker [40], a fact also reproduced here, with HR only increasing significantly the risk of large artery damage in women. The association of fasting glucose with PWV and the increased risk of development of large artery damage with diabetes were only observed for men, results that are partially in line with data from the Framingham and the Whitehall studies [33,42] but conflicting with the majority of findings in the literature [40], a fact that cannot be dissociated from the differences in prevalence of this condition for both genders.

Early vascular aging

These results have a significant relevance for cardiovascular prevention and raise several questions about not only the CVR profile of our population but also concerning the need for emergent clinical and public health measures. First, we have shown that, whatever the method used to determine its prevalence, there is an overwhelming higher prevalence of EVA in younger age classes, especially below the age of 40. As expected, EVA has proven to be a useful concept to identify individuals at risk in younger age classes where it was more prevalent; the relative decline in EVA prevalence after the age of 50 years accompanies the expected steeper increase of PWV after that age, as has been shown in different trials [35]. This could mean that differences according to EVA prevalence will become more visible in younger age groups, reflecting differential biological aging of the arterial tree, but that convergence applies to older age groups when the impact of chronological aging in general overrides the differential biological aging, more visible in younger individuals. This is in line with epidemiological findings that PWV is a relatively stronger cardiovascular risk marker in middle-aged individuals than in the elderly [23]. The other complementary explanation is survival bias, a phenomenon related to selective better survival in individuals with low PWV.

We observed that males were particularly afflicted by EVA across all age classes, a fact that cannot be dissociated from the overall higher CVRF levels/prevalence in males. Estimation of EVA prevalence is difficult in the absence of consensus on its definition. In addition, the analysis is not focused in outliers in a distribution, but rather with a global shift of the distribution of real values compared with predicted values. It is more conservative to say that the real EVA prevalence should lie within the values determined by both definitions used here (>97.5th percentile of normal mean PWV values stratified by age or >2SD of the mean PWV adjusted to BP class – see Supplemental Materials, http://links.lww.com/HJH/A474). This would provide a global prevalence of EVA between 8.7 and 12.5%, and the following prevalence rate intervals could be determined for every age class: 20.1–26.2% (<30 years), 10.0–12.8% (30–39 years), 7.2–14.9% (40–49 years), 2–5.7% (50–59 years), 1.6–2.6% (60–69 years), and 1.1–3.2% (≥70 years). In addition, we found that 40.2% of individuals in the age class below 30 years and 26.4–29.3% of individuals in the age classes 40–49 and 30–39 years, respectively, are above the 90th percentile of PWV *z*-score. This impressive result means that even if not strictly considered as EVA individuals, over 34.7% of individuals below 40 years are above the 90th percentile of PWV expected for their age: these individuals also have significant added risk for the development of CVD (pointing out the fact that their mean PWV value is at least 1 m/s above their normal/reference counterparts [15,16]). They deserve a particularly strict follow-up, as proposed elsewhere [12].

HR appears as one of the variables associated with EVA. The frequency of individuals exhibiting HR above 75 bpm is slightly higher across all age groups, in particular in the 30–39 years group. Looking at Table SM3, http://links.lww.com/HJH/A474, the absolute difference in HR between EVA and non-EVA individuals is rather small (2 bpm in the whole population, 3.1 and 3.6 bpm in the age groups 30–39 and 40–49 years, respectively). This raises the question of the real biological explanation of this statistical finding, for example, a relatively increased activity of the sympathetic nervous system. On the other hand, could specific genetic alterations [9] associated with early, prolonged 'environmental' stressors (mediated through sympathetic nervous system) result in stiffer than average arteries? The significance of HR as a determinant of EVA may support this hypothesis.

To explain this increased EVA prevalence in our population, one cannot forget the importance of increased average BP and pulse pressure. Individuals with EVA have significantly higher SBP (Table SM3, http://links.lww.com/HJH/A474), way above the minimum exposure risk for the development of CVD.

Other authors have proposed a role of stress hormones acting on the endothelium as well as media smooth muscle layer for contraction, suggesting that PWV increases not only as a reflection of arterial wall structure modification (elastin degeneration, collagen material increase) [6,17] but also with increased vascular smooth muscular cell tonus [43]. At this point, we cannot offer an explanation for the influence on these results of early-life determinants or other proposed contributors to accelerated vascular aging as (short-term) BP variability [44] or salt consumption (recent studies in Portugal have reported the average individual salt consumption as reaching 10.7 g/day in adults, and 7.8 g/day in 10–12-year-old children [45,46]).

Large artery damage (pulse wave velocity above 10 m/s)

The observation that 18.7% of our population (mean age of 47 years) displays large artery damage is novel and surprising. In Copenhagen [39], Framingham [38], and Vobarno [47], significantly older aged populations registered the prevalence of large artery damage between 23.6 and 31.7%. In our population, for the age class 60–70 years, 42% of the individuals would have large artery damage. We have not seen data including the prevalence of increased PWV in the adult population stratified by age class and sex presented elsewhere. Still, values observed in younger age

classes are striking. Logistic regression models concerning PWV above 10 m/s have been discussed previously, mainly obviating the role of age and BP in the development of large artery damage, with differences between genders observed only in the magnitude of risk increase.

Strengths and limitations

The strength of our approach resides on having set up an evaluation of an homogenous population living in adjacent cities in the same region of northern Portugal and using a very large random sample representative of all adult age classes, levels of education, and professional status. The study also has clear limitations. Only one measurement of PWV was performed per individual. A white-coat effect cannot be excluded, particularly in younger individuals. The precise definition of EVA is questionable in the absence of a general definition. PWV might not be sufficient for characterizing EVA, and it could also include other cardiovascular or age-related target organ damage variables [48]. We also used the reference value data to characterize EVA. This set of data is valuable, but not immune to bias as it was collected over a variety of locations, using various techniques, and included individuals defined as healthy or bearing risk factors on a post-hoc basis; on another perspective, we cannot exclude that a historic cohort effect could have some influence in our results, when we compare our data with the data from the ERVC (that included cohorts analyzed over 20 years ago).

In conclusion, consistently, whether looking at mean absolute PWV values, prevalence of EVA based on distribution (>97.5 percentile or >2SD over mean for age categories) or prevalence of large artery damage (PWV > 10 m/s), we observed a pattern of increased signs of arterial damage, portraying a cardiovascular risk picture different from what could be expected in a low cardiovascular risk country as Portugal. As Portugal has paradoxically one of the highest rates of stroke in Europe, finding that the general population has skewed distribution of PWV with much higher values might be particularly meaningful when recent evidence shows that c-fPWV improves CVR prediction, especially for stroke [23]. At the same time, the fact that c-fPWV has a higher predictive value in younger ages suggests that we cannot disconnect the high prevalence of EVA from large artery damage in young ages, and stroke (or other CVD) at older age. Only the longitudinal follow-up of this cohort will provide answers to these questions.

The high prevalence of EVA together with the impressive percentage (34.7%) of individuals below 40 years who are above the 90th percentile of the expected PWV value for their age and BP class is clearly worrisome, increasing also the need for better knowledge of prevalence and early control of CVRF as well as tighter risk stratification and inherent clinical intervention.

ACKNOWLEDGEMENTS

Please refer to the Supplemental Material, http://links.lww.com/HJH/A474.

This work was funded by the Life and Health Research Institute, Minho University, Guimarães, Portugal.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nilsson PM, Boutouyrie P, Cunha P, Kotsis V, Narkiewicz K, Parati G, *et al*. Early vascular ageing in translation: from laboratory investigations to clinical applications in cardiovascular prevention. *J Hypertens* 2013; 31:1517–1526.
2. O'Rourke MF, Safar ME, Dzau V. The Cardiovascular Continuum extended: aging effects on the aorta and microvasculature. *Vasc Med* 2010; 15:461–468.
3. Scuteri A, Najjar SS, Orru M, Usala G, Piras MG, Ferrucci L, *et al*. The central arterial burden of the metabolic syndrome is similar in men and women: the SardiNIA Study. *Eur Heart J* 2010; 31:602–613.
4. Scuteri A, Cunha PG, Rosei EA, Badariere J, Bekaert S, Cockcroft JR, *et al*. Arterial stiffness and influences of the metabolic syndrome: A cross-countries study. *Atherosclerosis* 2014; 233:654–660.
5. Nilsson PM, Lurbe E, Laurent S. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. *J Hypertens* 2008; 26:1049–1057.
6. Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. *Hypertension* 2005; 45:1050–1055.
7. Tarnoki AD, Tarnoki DL, Stazi MA, Medda E, Cotichini R, Nistico L, *et al*. Heritability of central blood pressure and arterial stiffness: a twin study. *J Hypertens* 2012; 30:1564–1571.
8. Mitchell GF, Verwoert GC, Tarasov KV, Isaacs A, Smith AV, Yasmin. *et al*. Common genetic variation in the 3'-BCL11B gene desert is associated with carotid-femoral pulse wave velocity and excess cardiovascular disease risk: the AortaGen Consortium. *Circ Cardiovasc Genet* 2012; 5:81–90.
9. Tarasov KV, Sanna S, Scuteri A, Strait JB, Orru M, Parsa A, *et al*. COL4A1 is associated with arterial stiffness by genome-wide association scan. *Circ Cardiovasc Genet* 2009; 2:151–158.
10. Nilsson PM. Early vascular aging (EVA): consequences and prevention. *Vasc Health Risk Manag* 2008; 4:547–552.
11. Nilsson PM, Boutouyrie P, Laurent S. Vascular aging: a tale of EVA and ADAM in cardiovascular risk assessment and prevention. *Hypertension* 2009; 54:3–10.
12. Kotsis V, Stabouli S, Karafillis I, Nilsson P. Early vascular aging and the role of central blood pressure. *J Hypertens* 2011; 29:1847–1853.
13. Laurent S. Defining vascular aging and cardiovascular risk. *J Hypertens* 2012; 30 (Suppl):S3–S8.
14. Cockcroft J, Mancia G. Vascular aging: shifting the paradigm of risk assessment and reduction in hypertension. *J Hypertens* 2012; 30 (Suppl):S1–S2.
15. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; 55:1318–1327.
16. The Reference Values for Arterial Stiffness' Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference, values'. *Eur Heart J* 2010; 31:2338–2350.
17. O'Rourke MF. Arterial aging: pathophysiological principles. *Vasc Med* 2007; 12:329–341.
18. Kokkinos P, Manolis A, Pittaras A, Doumas M, Giannelou A, Panagiotakos DB, *et al*. Exercise capacity and mortality in hypertensive men with and without additional risk factors. *Hypertension* 2009; 53:494–499.
19. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, *et al*. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013; 368:1279–1290.
20. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *BMJ* 2013; 346:f1325.
21. Nilsson PM, Khalili P, Franklin SS. Blood pressure and pulse wave velocity as metrics for evaluating pathologic ageing of the cardiovascular system. *Blood Pressure* 2014; 23:17–30.
22. Correia M, Silva MR, Matos I, Magalhaes R, Lopes JC, Ferro JM, *et al*. Prospective community-based study of stroke in Northern Portugal: incidence and case fatality in rural and urban populations. *Stroke* 2004; 35:2048–2053.
23. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, *et al*. Aortic pulse wave velocity improves cardiovascular event

prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *J Am Coll Cardiol* 2014; 63:636–646.
24. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, *et al.* Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* 2012; 30:445–448.
25. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. The Rationale/Design of the Guimaraes/Vizela Study: a multimodal population-based cohort study to determine global cardiovascular risk and disease. *J Investig Med* 2014; 62:813–820.
26. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. An epidemiological study determining blood pressure in a Portuguese cohort: the Guimaraes/Vizela study. *J Hum Hypertens* 2015; 29:190–197.
27. Avolio AP, Chen SG, Wang RP, Zhang CL, Li MF, O'Rourke MF. Effects of aging on changing arterial compliance and left ventricular load in a northern Chinese urban community. *Circulation* 1983; 68:50–58.
28. Farro I, Bia D, Zocalo Y, Torrado J, Farro F, Florio L, *et al.* Pulse wave velocity as marker of preclinical arterial disease: reference levels in a uruguayan population considering wave detection algorithms, path lengths, aging, and blood pressure. *Int J Hypertens* 2012; 2012:169359.
29. Bhuiyan AR, Srinivasan SR, Chen W, Paul TK, Berenson GS. Correlates of vascular structure and function measures in asymptomatic young adults: the Bogalusa Heart Study. *Atherosclerosis* 2006; 189:1–7.
30. McEniery CM, Yasmin. Wallace S, Maki-Petaja K, McDonnell B, Sharman JE, *et al.* Increased stroke volume and aortic stiffness contribute to isolated systolic hypertension in young adults. *Hypertension* 2005; 46:221–226.
31. Oren A, Vos LE, Uiterwaal CS, Grobbee DE, Bots ML. Aortic stiffness and carotid intima-media thickness: two independent markers of subclinical vascular damage in young adults? *Eur J Clin Investig* 2003; 33:949–954.
32. Mattace-Raso FU, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, *et al.* Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; 113:657–663.
33. Mitchell GF, Guo CY, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, *et al.* Cross-sectional correlates of increased aortic stiffness in the community: the Framingham Heart Study. *Circulation* 2007; 115:2628–2636.
34. Wang KL, Cheng HM, Sung SH, Chuang SY, Li CH, Spurgeon HA, *et al.* Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. *Hypertension* 2010; 55:799–805.
35. McEniery CM, Yasmin. Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol* 2005; 46:1753–1760.
36. AlGhatrif M, Strait JB, Morrell CH, Canepa M, Wright J, Elango P, *et al.* Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore Longitudinal Study of Aging. *Hypertension* 2013; 62:934–941.
37. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, *et al.* Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006; 113:664–670.
38. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, *et al.* Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010; 121:505–511.
39. Sehestedt T, Jeppesen J, Hansen TW, Wachtell K, Ibsen H, Torp-Pedersen C, *et al.* Risk prediction is improved by adding markers of subclinical organ damage to SCORE. *Eur Heart J* 2010; 31:883–891.
40. Cecelja M, Chowienczyk P. Dissociation of aortic pulse wave velocity with risk factors for cardiovascular disease other than hypertension: a systematic review. *Hypertension* 2009; 54:1328–1336.
41. McEniery CM, Spratt M, Munnery M, Yarnell J, Lowe GD, Rumley A, *et al.* An analysis of prospective risk factors for aortic stiffness in men: 20-year follow-up from the Caerphilly prospective study. *Hypertension* 2010; 56:36–43.
42. Johansen NB, Vistisen D, Brunner EJ, Tabak AG, Shipley MJ, Wilkinson IB, *et al.* Determinants of aortic stiffness: 16-year follow-up of the Whitehall II study. *PloS One* 2012; 7:e37165.
43. Najjar SS, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 2005; 46:454–462.
44. Schillaci G, Bilo G, Pucci G, Laurent S, Macquin-Mavier I, Boutouyrie P, *et al.* Relationship between short-term blood pressure variability and large-artery stiffness in human hypertension: findings from 2 large databases. *Hypertension* 2012; 60:369–377.
45. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. *J Hypertens* 2014; 32:1211–1221.
46. Cotter J, Cotter MJ, Oliveira P, Cunha P, Polonia J. Salt intake in children 10-12 years old and its modification by active working practices in a school garden. *J Hypertens* 2013; 31:1966–1971.
47. Muiesan ML, Salvetti M, Paini A, Monteduro C, Rosei CA, Aggiusti C, *et al.* Pulse wave velocity and cardiovascular risk stratification in a general population: the Vobarno study. *J Hypertens* 2010; 28:1935–1943.
48. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. *Eur Heart J* 2010; 31:1865–1871.

Reviewers' Summary Evaluations

Reviewer 1

This paper is focused on the study of the north Portuguese population with a known high prevalence of HTN and stroke. The author studied pulse wave velocity (PWV) in 3038 subjects. The results show that in this population PWV values were higher than expected. Strengths of this work are certainly the large population evaluated, the state of the art method and the correct methodologic approach, including the statistical analysis. A weakness is the absence of other important clinical parameters, which would have helped in mechanistic explanations.

Reviewer 2

The authors assessed early vascular aging (EVA) by measuring pulse wave velocity (PWV) in subjects aged 18–96 years. They compared the measured PWV values with those published in the European Reference Values Project (RVP). They considered a subject to be at risk for EVA when the PWV value exceeded the 97.5th percentile of the value found in the subgroup of RVP called 'healthy subjects'. The values were stratified according to age decades. The overall prevalence of EVA was 12.5%, the highest prevalence being in the youngest subjects aged below 30 years; on the contrary, when the absolute cut-off value of PWV >10 m/s was taken, the prevalence of pathologic values increased steeply with age.

Studying the relative increase of PWV, i.e. comparing the measured value with the reference value at the same age (and blood pressure) group, seems a sound approach to discover arterial pathological changes at a young age. This may be a good approach for early prevention in the future.

Pulse Wave Velocity distribution in a cohort study - from Arterial Stiffness to Early Vascular Ageing (EVA)

Pedro G. Cunha [(1)(2)(3)], Jorge Cotter[(1)(2)(3)], Pedro Oliveira[(4)], Isabel Vila[(1)], Pierre Boutouyrie[(5)], Stéphane Laurent[(5)], Peter M Nilsson[(6)], Angelo Scuteri[(7)], Nuno Sousa[(2)(3)]

SUPPLEMENTAL MATERIAL

Methodology of Pulse Wave Velocity measurement and standardization

All PWV values were obtained using the subtracted distance method for path length; for standardization and comparison purposes they were uniformly transformed to the value obtained using the direct path length (also measured during patient evaluation), through the equation proposed by Boutouyrie *et al* (16):

$$X_{direct} = 0.45 \times Distance_{subtracted} + 0.21 \times Height + 0.08 \text{ (m)}$$

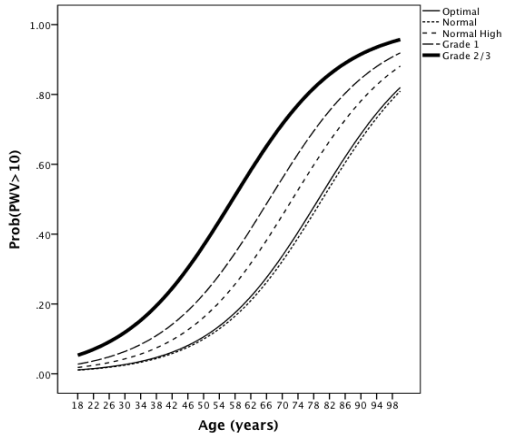
The PWV values obtained using the calculated direct path length were normalized (multiplied by 0.8). In a separate analysis (not shown), both PWV measures (direct path vs. subtracted path length) were highly correlated (r = 0.96) and using an Bland-Altman plot methodology of comparison evidenced no substantial difference (if anything, the direct path method would, in average, underestimate PWV values by 0.2 m/s). Using the normalized direct distance calculated PWV will now allow us to compare the obtained values with the ones published as European reference (16).

Acknowledgments. The authors wish to show their acknowledgment to the following institutions and departments who collaborated in this project: Agrupamento de Centros de Saúde de Guimarães e Vizela, Serviço de Patologia Clínica do Centro Hospitalar do Alto Ave, Centro de Saúde da Amorosa, USF Afonso Henriques, USF Ara Trajano, USF Duo Vida, USF Novos Rumos, USF de Pevidém, USF Physis, USF de Ponte, USF de Ronfe, USF S. Nicolau, USF de S. Torcato, USF de Serzedelo e USF Vimaranes. A special word is addressed to thank all the physicians working in those different Primary Health Care Community Centers, whose work enrolling subjects was vital to the successful prosecution of our goals.

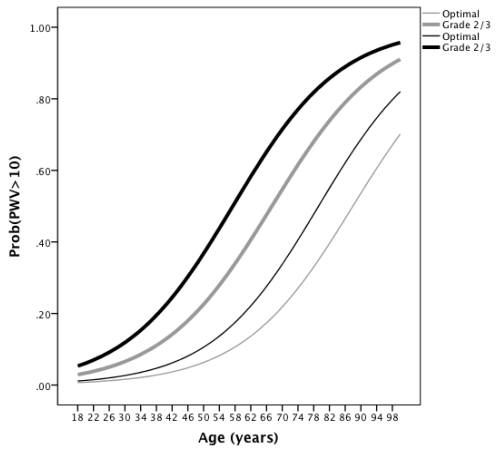
The Guimarães Study Group is also composed by the following researchers, who were involved in data collection: Helena Sarmento, Gloria Alves, Sara Freitas, Ana Sofia Alves, Sofia Gomes, Marta Gonçalves, Rui Fernandes, António Pedro Fonte, José Miguel Sá, Clarisse Neves, Andreia Sampaio, Cristina Cunha, Sílvia Sousa, Filipe Gonçalves, Joana Malheiro, João Silva, Carlos Fernandes, Estefânia Bustabad, Laura Castro, Nuna Vieira, Filipa Ramos, Ana Catarina Marques, Joana Monteiro, Ana Sofia Silva, Margarida Rocha, Mafalda Jordão Abreu, Margarida Dias, Magda Fernandes, Paula Felgueiras, Francisca Castro, Vânia Gomes, Ana Luísa Novo, Carla Pereira, Ana Cristina Ramalho, Fernando Esculcas, Dina Fernandes, Ricardo Rodrigues, Rafael Velho, Diana Coimbra, Joana Pimenta, João Pedro Teixeira, Ana Luísa Neves, Joana Leitão, Sara Pereira, Carla Ferreira, Clarinda Neves, Samuel Pedreira, Pedro Neves, Elisabete Lima, Carla Mendes, Orlanda Barbosa, Ana Catarina Martins, Célia Lemos, Eduarda Macedo, Sílvia Azevedo, Ana Rita Ribeiro, Elsa Salgado, Joana Dias, Eduarda Piairo, Marisa Carneiro, Ismael Costa, Lucília Miranda, Ana João Gonçalves, Natália Rodrigues, Manuela Morais, Rosário Santos, Odete Rodrigues, Alexandra Barreira, Ana Rita Romano, Catarina Marques, Fernanda Marisa Santos, Sónia Coelho Pereira, Elisabete Pinto Teixeira, Joana Andrade Pinto

Figure SM1 - Logistic curves for the development of PWV>10m/s as a function of age and BP class for men and women without Diabetes, and with normal Heart Rate < 75 bpm

Male



Female



Overlapp Men (black lines) / Women (grey lines)

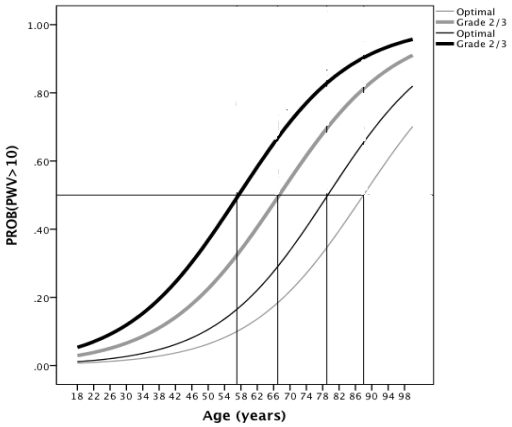


Figure SM2 - Prevalence of EVA stratified by age using the 2SD above expected mean PWV value category for age and BP class, comparing with "Reference" population subjects

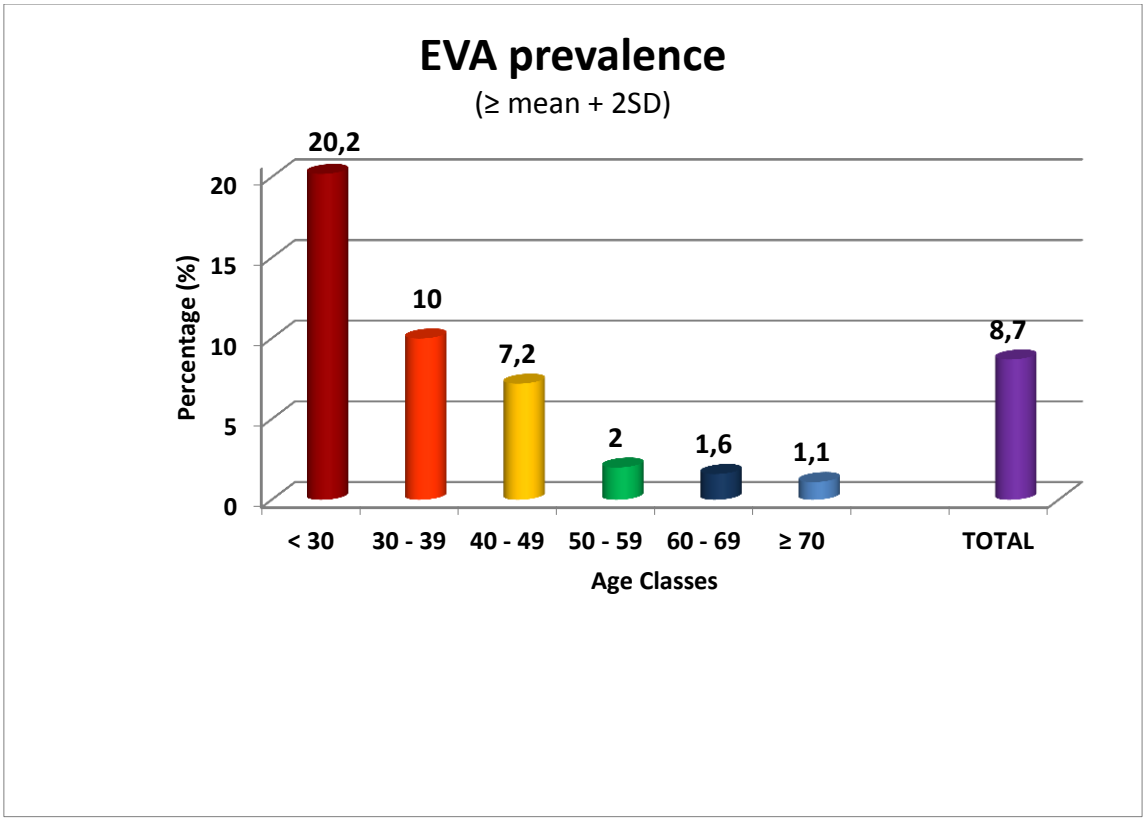


Table SM3 – Biologic characteristics of EVA and non-EVA subjects below 50 years of age

	EVA (using normal reference) Subjects < 50 years of age	General population Subjects < 50 years of age	p – value
Number subjects	260	1146	-
Male gender	54.6%	40.6%	< 0.001
Age (years)	30.8 (8.1)	32.4 (8.0)	< 0.01
PWV (m/s)	9.4 (1.4)	7.1 (1.0)	0.001
SBP (mmHg)	125.2 (15.2)	119.6 (13.0)	0.001
DBP (mmHg)	76.8 (10.3)	73.3 (9.2)	0.001
BMI (Kg/m^2)	25.0 (4.4)	25.3 (4.2)	ns
Glycaemia (mg/dl)	75.0 (11.6)	75.7 (12.3)	ns
Total Cholesterol (mg/dl)	188.3 (39.4)	188.8 (37.0)	ns
HDL Cholesterol (mg/dl)	53.1 (14.9)	55.7 (14.4)	0.011
Triglycerides (mg/dl)	105.6 (74.6)	100.3 (68.2)	ns
LDL Cholesterol (mg/dl)	111.1 (31.2)	109.8 (30.1)	ns
HR (bpm)	67.8 (11.9)	65.5 (9.8)	0.022
Diabetes	3.5%	1.6%	0.07
Dyslipidemia	61.5%	63.6%	ns
Hypertension	16.5%	8.6%	< 0.001
BP Treatment	6.2%	3.7%	0.08
Optimal BP	40%	52.9%	< 0.001
Normal BP	23.5%	24.0%	
High Normal BP	19.2%	14.7%	
Grade 1 HT	14.6%	7.9%	
Grade 2/3 HT	2.7%	0.4%	
Family History Premature CVD	8.8%	9,6%	ns
HR > 75 bpm	21.5%	15.3%	0.02

Table SM4 – Logistic Regression model for EVA

	B- Coefficient (SE)	Odds ratio (95% CI)	p value
Age <30 years	1.173 (0.225)	3.232 (2.078-5.026)	<0.001
Age 30-39 years	0.114 (0.228)	1.121 (0.717-1.752)	0.618
Normal BP	0.227 (0.193)	1.255 (0.859-1.834)	0.240
High-Normal BP	0.562 (0.220)	1.754 (1.1.40-2.698)	0.011
Grade 1 HT	1.134 (0.260)	3.107 (1.865-5.174)	0.010
Grade 2/3 HT	2.235 (0.630)	9.350 (2.719-32.149)	<0.001
HR > 75 bpm	0.510 (0.187)	1.665 (1.154-2.403)	0.006
Diabetes	1.042 (0.444)	2.832 (1.188-6.789)	0.019
Male Sex	0.363(0.165)	1.438 (1.041-1.986)	0.028

EVA – Early Vascular Aging**; HR –** Heart Rate**; BP –** Blood Pressure**; HT –** Hypertension**;** **bpm –** beats per minute**; SE-**Standard Error**; CI –** Confidence Interval

Using Age 40-49 years, Optimal BP, and female sex as reference class;

Table SM5 – Logistic Regression model for Arterial Stiffness (PWV >10m/s)

Variables	Males			Females		
	B- Coefficient (SE)	Odds ratio (95% CI)	p value	B- Coefficient (SE)	Odds ratio (95% CI)	p value
Age 30-39	0.687 (0.444)	1.988 (0.832-4.548)	0.122	0.711 (0.595)	2.036 (0.634-6.536)	0.232
Age 40 – 49	1.355 (0.448)	3.876 (1.610-9.330)	0.003	1.911 (0.579)	6.762 (2.175-21.024)	0.001
Age 50 – 59	1.412 (0.440)	4.106 (1.734-9.725)	0.001	2.099 (0.577)	8.160 (2.632-25.297)	<0.001
Age 60 – 69	2.725 (0.419)	15.258 (6.707-34.709)	<0.001	2.923 (0.570)	18.600 (6.084-58.863)	<0.001
Age >= 70	3.582 (0.426)	35.945 (15.591-82.869)	<0.001	3.613 (0.570)	37.072 (12.118-113.409)	<0.001
Normal BP	-0.072 (0.401)	0.931 (0.424-2.040)	0.858	0.311 (0.337)	1.364 (0.705-2.642)	0.357
High-Normal BP	0.544 (0.373)	1.723 (0.829-3.581)	0.145	0.786 (0.328)	2.194 (1.154-4.171)	0.017
Grade 1 HT	0.976 (0.372)	2.654 (1.280-5.504)	0.009	1.134 (0.316)	3.108 (1.674-5.771)	<0.001
Grade 2/3 HT	1.593 (0.428)	4.916 (2.123-11.384)	<0.001	1.605 (0.403)	4.979 (2.261-10.962)	<0.001
HR > 75 bpm	0.458 (0.267)	1.580 (0.937-2.655)	0.086	0.788 (0.219)	2.199 (1.433-3.375)	<0.001
Diabetes	0.650 (0.254)	1.915 (1.164-3.152)	0.011	-	-	ns
Smoking	0.356 (0.189)	1.427 (0.986-2.068)	0.060	-	-	ns

PWV – Pulse Wave Velocity; **HR** - Heart Rate; **BP** – Blood Pressure; **HT** – Hypertension; **bpm** – beats per minute; **SE**-Standard Error; **CI** – Confidence Interval

Using Age <30 years and Optimal BP class as reference.

Figure SM6 - Histogram of PWV values distribution by age class

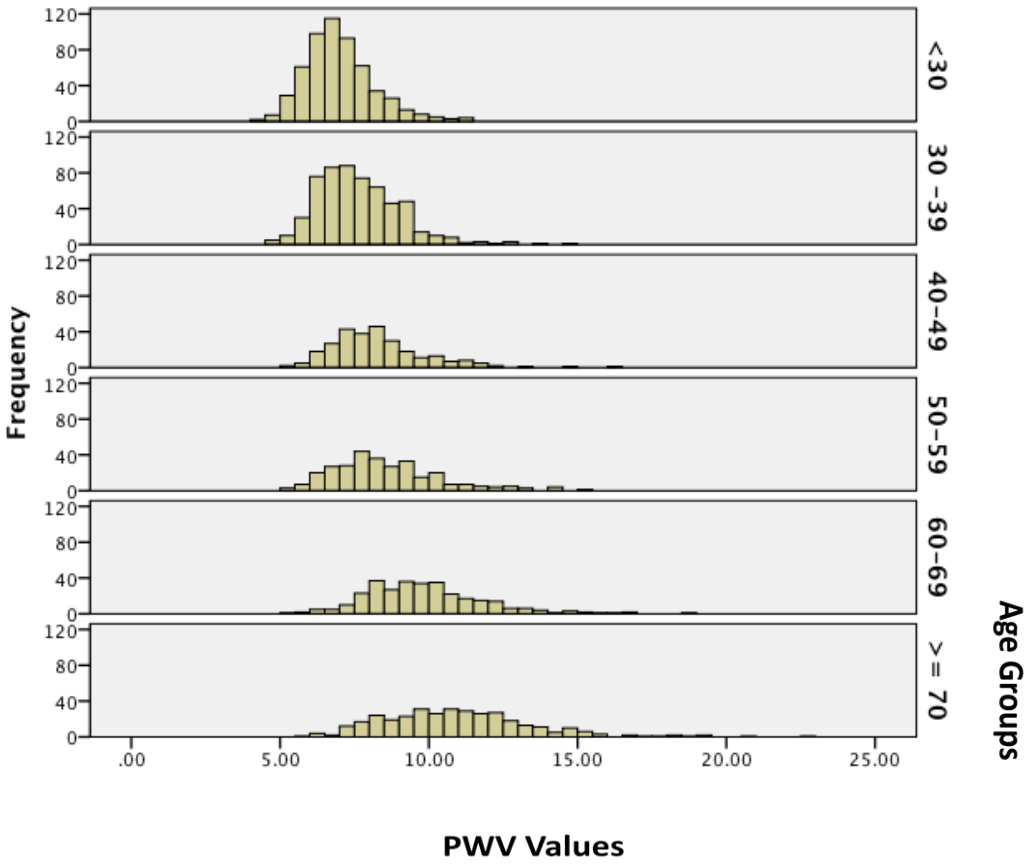


Figure SM7 - Distribution of PWV Z-score values by age class

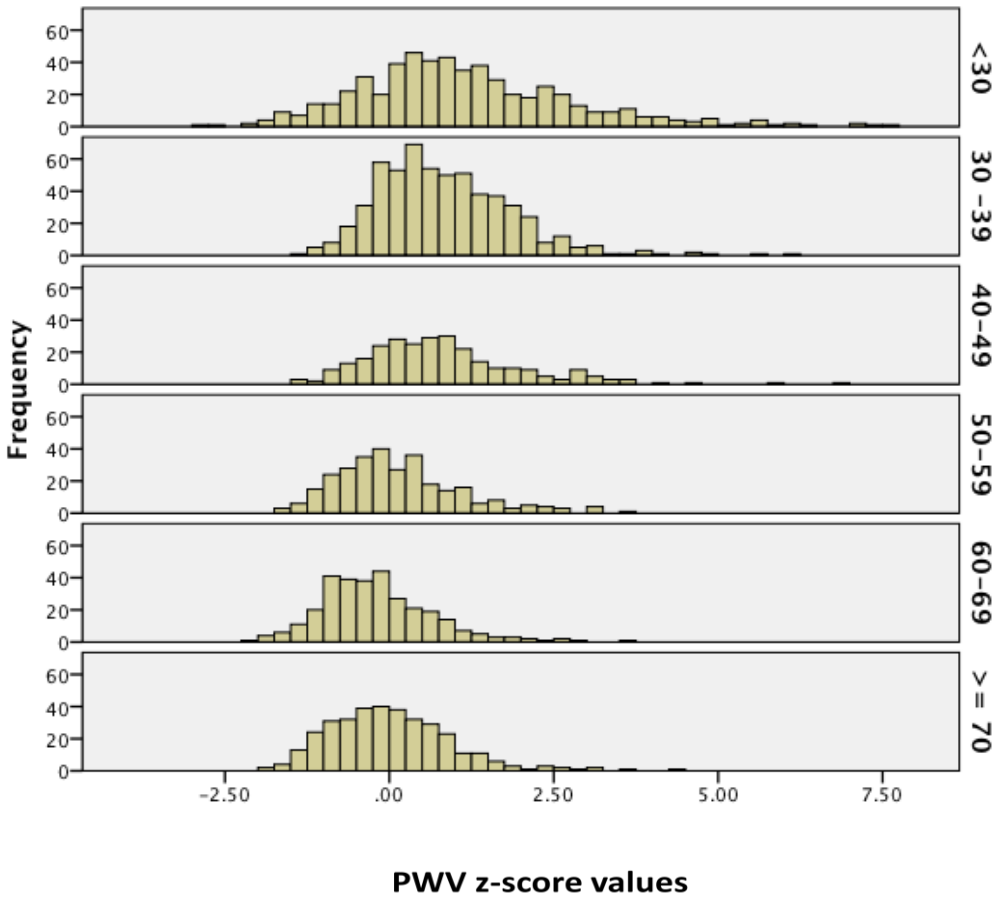


Table SM8 - Comparing age class mean PWV value for the Healthy population in the ERVC and for the population in Guimarães/Vizela

	Mean PWV Values in the Healthy population of the ERVC (ref. 16)	Mean PWV Values in the Guimarães/Vizela population
< 30 years	6.2	7.1
30-39 years	6.5	7.7
40 – 49 years	7.2	8.3
50 – 59 years	8.3	8.5
60 – 69 years	10.3	9.9
≥ 70 years	10.9	10.8

Chapter 6

Central blood pressure and salt in a population cohort

from a high stroke incidence area

(manuscript under submission)

Central blood pressure and salt in a population cohort from a high stroke incidence area

Pedro G. CUNHA [(a)(b)(c)], Jorge COTTER[(a)(b)(c)], Pedro OLIVEIRA[(d)(e)], Isabel VILA[(a)], Pierre BOUTOUYRIE[(f)], Stéphane LAURENT[(f)], Peter M NILSSON[(g)], Angelo SCUTERI[(h)], Nuno SOUSA[(b)(c)]

(a) Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk, Internal Medicine Department, Guimarães – Centro Hospitalar do Alto Ave / Minho University. Portugal; (b) Life and Health Science Research Institute (ICVS), School of Health Science, University of Minho; (c) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; (d) Departamento de Estudo de Populações, Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto; (e) Instituto Saúde Pública Universidade Porto, EPIUnit; (f) Department of Pharmacology and INSERM U970, Assistance Publique Hôpitaux de Paris, Université Paris Descartes, Paris, France; (g)Department of Clinical Sciences, Lund University, Skane University Hospital, Malmö. Sweden; (h) San Raffaele Pisana IRCCS, Rome. Italy

Corresponding author:

Pedro Guimarães Cunha. Center for the Research and Treatment of Arterial Hypertension and Cardiovascular Risk. Serviço de Medicina Interna do Hospital de Guimarães, Centro Hospitalar do Alto Ave; Life and Health Science Research Institute (ICVS), Minho University. Rua dos Cutileiros, Creixomil. 4810 – Guimarães, Portugal. pedrocunha@ecsaude.uminho.pt

Conflicts of Interest and Source of Funding: no conflicts of interest to declare; funding by the Life and Health Research Institute, Minho University

Word count: 4115 ; **Number of tables**: 5; **Number of figures**: 4;

Number of supplementary digital content files: 1.docx

ABSTRACT

Introduction: The value of central blood pressure (CBP) over peripheral blood pressure, concerning its independent predictive power for cardiovascular (CV) events / target organ damage (TOD) and its use as a therapeutic target, is under debate.

Objectives: Characterize the distribution of CBP values in a population residing in an area of high stroke incidence through comparison with published reference values for healthy subjects, and identifying subjects at higher CV risk; analyze the influence of salt on CBP, and assess whether CBP plays a role in risk stratification and treatment.

Methods: A longitudinal cohort study, evaluated a representative random sample of the adult population of two adjacent cities, in a two visit study plan, including CBP measurements, TOD evaluation and 24h urine sampling.

Results: 2542 subjects completed the study (average 45.5 years; 55% female). Central systolic blood pressure (CSBP) and Central Pulse Pressure (CPP) presented mean values of 119.2 and 41.8 mmHg, respectively; several age groups registered mean values of CSBP/CPP exceeding 10 - 20 mmHg the expected for healthy subjects. Central Pulse Pressure Amplification (CPPA), registered a mean ratio of 1.32. 37.5% of the population/72.4% of hypertensives presented CSBP >90th percentile (90thp); CPP >90thp was registered in 23.7/51.5% of the population/hypertensives. In treated and controlled hypertensives, 33.9/20.5% of the subjects persist with CSBP/CPP values >90thp. TOD was significantly more prevalent in subjects above CSBP/CPP 90thp (2 to 4 times). Salt was an independent explanatory variable of CSBP (p=0.038) and CPPA (p=0.026) in multivariate regression analysis, and it increased the risk of having CSBP >90thp (p=0.048).

Conclusions: High records of CBP, and associated TOD, were found in an area of high stroke incidence. 33.9% of controlled hypertensives maintained significantly elevated CSBP.

Key words: Central Blood Pressure; Salt; Stroke; Cardiovascular Risk; Epidemiology; Portugal; Large Artery

I – INTRODUCTION

Central Blood Pressure (CBP) components have been discussed as having a better predictive value of future cardiovascular events and target organ damage than peripheral blood pressure (PBP)(1, 2). Recent meta-analysis have shown its modest added value as independent predictor of cardiovascular events (9% and 11.5 % augmented risk per each increase of 10 mmHg in Central Systolic BP and Central Pulse Pressure respectively), when compared to peripheral measurements (1, 3, 4). However, in the Anglo-Cardiff Collaborative Trial 2(5), 70% of individuals with normal-high PBP had CBP values overlapping with those of subjects classified as having grade I Hypertension by PBP measurement; similar observations with central pulse pressure have been recorded in the Strong Heart Study (6). This has demonstrated that it is not possible to accurately estimate CBP from PBP measurements.

The debate has also been carried on the basis that central blood pressures correspond more realistically to the pressure and pulsatility transmitted to central organs and low resistance vascular systems (like the ones in the brain and kidney), thus providing a better correlation with target organ damage (7-9). In fact, the recently published SAFAR study has supported this view, by showing that the development of left ventricular hypertrophy was better predicted by 24h CBP than other peripheral measurements (10). Cheng and colleagues (11) have derived and validate prospectively (10 year follow-up) CBP values that had a significantly predictive power for cardiovascular events, and especially stroke: subjects with CBP above 130/90 mmHg had an 8-fold increased hazard ratio of stroke. Central Pulse Pressure Amplification (CPPA) has also been looked into with great interest, as it is inversely correlated with total arterial compliance, peripheral arterial resistance and reflection wave time (12). Finally, new scientific evidence has emerged, showing different effects of anti-hypertensive drugs on central and PBP, as well as hinting that therapeutic decisions based on CBP rather than PBP may be more precise (13-15).

These facts have spurred our interest to study central blood pressure in the northern region of Portugal, where the incidence of stroke is the highest of western European countries (16), and where standardized stroke death rates are higher than those related to coronary heart disease (17). Herein, we studied a population-based cohort of two adjacent cities in the north of Portugal, aiming to: i) characterize the distribution of CBP values and its determinants in this high stroke incidence population; ii) identify higher risk subjects due to elevated central blood pressure; iii) understand if CBP could play a role in risk stratification and management of hypertensive subjects.

II – SUBJECTS AND METHODS

The methods used have been described in detail in previous reports (18, 19). Briefly, a representative sample of the population from two northern adjacent cities (Guimarães and Vizela) was randomly selected and evaluated on two different occasions, after signing a written consent form approved by an Ethics Committee. We observed that the difference between the list of individuals registered in the Primary Community Health Care Centre (PCHCC) of his/her residence area, and the list of citizens living in Guimarães/Vizela (Statistics Portugal, 2006) was inferior to 2%; therefore, for practical purposes, participants have been randomly selected from the list of citizens currently living in Guimarães and Vizela.

Taking into account that there was no available estimate of the prevalence of increased central blood pressure for the Portuguese population, the sample selection was based considering the estimated prevalence of hypertension in Portugal. A sample size of at least 2339 subjects was required to achieve 2% precision in an estimated prevalence of HTN of 41.2%. Thus, a sample of the adult population (**>**18 years of age)

stratified by age was defined previewing the necessity of including 4000 individuals (95% confidence interval with an estimation error inferior to 2%, considering a 25% safety margin to cope with non-adherence and dropout rate between visits).

Anticipating a higher non-adherence and dropout rates on younger and professionally active individuals, the number of randomized subjects to enroll was stratified unevenly according to their age (2000 individuals would be **<**35 years of age, 1000 subjects would have 35 and 65 years of age, and 1000 subjects would be **>**65 years)(20, 21).

The list of randomized subjects was delivered to the corresponding family doctors that contacted and enrolled them, obtaining their written consent to participate. It was therefore clear that only randomly assigned subjects could be enrolled and that no volunteers or physician-selected subjects would be included. If the subject refused, the general practitioner could not replace him/her with a volunteer or with someone from his/her practice. Only randomized subjects were accepted (20, 21).

Individuals were observed in the morning, fasting over-night and carrying their usual drug prescriptions. No intake of caffeinated beverages or tobacco use was allowed. Subjects underwent a standardized workup including medical history, biologic and arterial measurements. Trained physicians performed BP measurements at every occasion; training sessions performed prior to the beginning of the study insured standardization of PBP and CBP measurements. PBP was measured three times (at each visit) with subjects in a sitting position, after a 15 minutes resting period, taken with 2 minutes interval, using the validated OMRON-705IT© device (Omron Healthcare Europe B.V, Hoofddorp, The Netherlands). During their first visit, patients were then

placed in the supine position and CBP was measured using the Sphygmocor© device (Atcor Medical Pty Ltd ,West Ryde, NSW, Australia), calibrated with systolic and diastolic peripheral blood pressure. Measured CBP values were standardized in order to allow us to compare the obtained values with the ones published as European reference. They were adjusted by age and sex agreeing to the known distribution of these characteristics in the population of the two cities, according to Statistics Portugal for the year 2006. As measurements were made by the Sphygmocor© device, the absolute value of central systolic blood pressure of each individual was corrected by adding 0.135 mmHg to the obtained value, as proposed in the European Reference Values for Central Blood Pressure (22). Central Pulse Pressure Amplification (CPPA) was calculated as the ratio between Peripheral pulse pressure / Central Pulse Pressure (12).

Salt: sample validation – On the second visit subjects were asked to gather a 24h urine collection, where sodium and creatinine would be measured. Total measured urinary creatinine was used to validate the 24h urine sample, according to the expected value of creatinine excretion in urine for each individual's gender, weight and age (Male: 18.5 to 25.0 mg/kg if age < 50 years; 15.7 to 20.2 mg/kg if age ≥ 51 and < 70 years of age; 10 mg/kg if age $\geq$ 70 years of age. Female: 16.5 to 22.4 mg/kg if age < 50 years; 11.8 to 16.1 mg/kg if age ≥ 51 and < 70 years of age; 10 mg/kg if age $\geq$ 70 years of age) (23). Only subjects whose measured urinary excretion of urine were above the expected value for their weight and age, were validated and included for analysis. The value of excreted salt in the urine was obtained using the following equation: 1mmol of measured 24H urinary sodium = 0.05844 g of salt/24h (24).

Definition of CBP_90 – Subjects whose central systolic blood pressure or central pulse pressure were above the 90th percentile of the values described as normal for healthy subjects with the same age, according to the European Reference Values collaboration (22), were identified as bearing higher risk for target organ damage and/or cardiovascular events.

Statistical analysis. Statistical methodology concerning population sampling, database elaboration and management, as well as predefined statistical analysis have been reported (18). Prevalence of different studied characteristics was estimated and calculated by demographic characteristics (age group, sex, education level) and risk factors. Linear regression models were studied with CBP as the dependent variable and with independent variables that included: age, sex, height, heart rate (HR), Body Mass Index (BMI), years of education, tobacco use, fasting glucose, lipid profile, mean estimated glomerular filtration rate (eGFR).

Logistic regression models were constructed to study contributing variables to the development of CBP measurements above the expected 90th percentile as defined above.

III – RESULTS

Three thousand and thirty eight subjects were enrolled (out of the 4000 subjects initially randomized), and 2542 completed the two clinical observation plan initially proposed (drop-out rate of 16.1%)(19). There was no significant difference between subjects who dropped out and those who completed the study, as discussed elsewhere (19). 2472 had valid central blood pressure measurements. **Table 1** shows the biologic

characteristics of the subjects that completed the observations. **Table 2** summarizes the observed values of Central Blood pressure components and 24h salt consumption.

Central Blood Pressure and Amplification. Central Systolic BP (CSBP) (mean: 119.2 mmHg) and Central Pulse Pressure (CPP) (mean: 41.8 mmHg) increased progressively with age, registering higher values for men than women (122.7 vs 116.4 mmHg – $t=7.593$; $p<0.001$; 43.7 vs 40.2 mmHg – $t= 5.68$; $p<0.001$, respectively) Figures A and B - Supplementary Material. CSBP mean values of our cohort are 10 to 22 mmHg higher than the expected value for healthy subjects in the ERVC, throughout the different age groups (with higher differences found in males). CPP values in our study also rank higher than expected in healthy subjects, especially after the age of 60 years, and again more in men. Central Diastolic BP (CDBP) increased until the age of 40-49 years and descended from then onward. Amplification of CSBP overall decreases from the younger age groups until 40-49 years after which it keeps a considerably stable value, except for women and for subjects over 70 years of age (the latter recording a slight increase in amplification when compared to precedent age groups; Figure C and Table F in Supplementary Materials). Men registered higher values of Amplification than women (12.7 vs 9.7 mmHg – $t=10.1$; $p<0.001$), and the lowest mean amplification values was recorded in women at the age group of 40-49 years (6.6 mmHg). Central Pulse Pressure Amplification (CPPA) on the other hand, has a progressive and constant decline from younger to older ages, with men registering higher values than women.

Salt. 2017 subjects were delivered a 24h urine sample for salt excretion analysis. Only 82% (1642) were validated according to the above mentioned criteria. Thus, of the

2542 subjects who completed the study, only 64.6% had a valid urinary 24h sample collection – 539 did not present a sample, and 361 had an incorrectly collected one. The biologic characteristics of subjects with valid 24h urine samples are presented in **Table 1**. When comparing these 1641 subjects with the ones that did not have a valid 24h urine sample (n=900), we could see that they were significantly older (by average 2 years), and had a higher male prevalence than those not presenting valid urine samples; they were also lighter (70.0 vs 72.2 Kg), had lower abdominal perimeter (93.2 vs 94.6 cm), BMI (26.5 vs 27 Kg/m2) and mean heart rate (65.5 vs 66.9 bpm); on the other hand they presented significantly higher total and LDL cholesterol levels (193.5 vs 189.8 and 117.0 vs 112.6 mg/dl, respectively).

82.0% of the population was ingesting more than 6 grams of salt per day, 23.4% more than 12 grams/day and 1.7% ingests more than 20 grams of salt per day (maximum value of 30 g/24h). Men ingested more salt (11.0g/24h) than women (8.4 g/24h) across all the age groups ($t=13.6$, $p<0.001$), and older individuals have a lower salt consumption than younger ones ($F=9.4$, $p<0.001$). Figure 1 displays the association of CSBP with salt (divided in 3 classes : < 6g salt /day, 6 – 9g salt/day and > 9g salt/day) stratified by age. Within each age class, higher levels of salt consumption are associated with higher CSBP values, particularly when using as reference the lowest class of salt consumption.

Linear Regression Models. Tables 3 and 6 present the linear regression models using Central Systolic Blood Pressure (CSBP), Central Pulse Pressure (CPP) and Central Pulse Pressure Amplification as dependent variables. Due to the difference in the number of

subjects with valid 24h urine samples we decided to present 2 different models for each of these dependent variables: one model without the 24h urinary salt, and another where this variable has been considered. As shown, the models for CSBP offer 46.8% of explanation for the variability found. The introduction of the variable "24h Salt" into the model allows to add some explanation power (p=0.038), without greatly altering the absolute values of the other significant variables. Thus, we found as explanatory variables for CSBP: age, sex, triglicerydes, fasting glucose, LDL cholesterol, uric acid and 24H excreted salt.

For the model regarding CPP, we could find no effect of salt as an explanatory variable. Thus, a single model is presented, offering 53.6% of explanation of the variability, and including, age, heart rate, sex, fasting glucose and triglycerides as explanatory variables.

The Absolute Amplification linear regression model could explain less than 20% of the variability (R^2 = 0.199) (data not shown). On the contrary, we could build two models for CPPA, reaching an explanatory value of 43 – 47% and having as explanatory variables: sex, age, height, triglycerides, LDL-cholesterol, smoking, heart rate and 24h-Urine Salt (p=0.026)

Prevalence of subjects above the 90th percentile of central blood pressure measurements. Males registered higher proportions of individuals with CSBP and CPP above the 90th percentile, than females (42.9% vs 33.2% - X^2= 24.8; p<0.001; and 20.2% vs 28% - X^2 = 25.2; p<0.001; respectively). The distribution of subjects by age was not independent of these two variables (CSBP > 90th percentile - X^2 =367.5; p<0.001. CPP >

90^{th} percentile - X^2 = 493.7; p<0.001). The prevalence of CSBP and CPP above the normal 90th percentile, increased with age. 37.5% and 23.7% of the population had CSBP and CPP, respectively, above the 90th percentile expected for normal individuals of the same age, according to the ERVC(22). Figure 2 displays the distribution of subjects above the 90^{th} percentile for these two variables, stratified by age and sex. It is worthwhile to note the percentages observed for males for the age groups < 30 and 30 – 39 years: approximately 1 out of 5, and 1 out of 3 respectively have high-risk levels of CSBP when compared to other subjects.

Logistic Regression Models. In Tables 4 and 5 we present the logistic regression models for CSBP and CPP above the 90^{th} percentile. Once more, and when appropriate, we constructed two models (1 without 24h urinary salt and another with 24h urinary Salt entered). For CSBP, variables increasing the risk of achieving a level above the 90^{th} percentile were: age, sex, BMI, 24h urinary Salt (OR – 1.6; CI – 1.1 to 2.3, in the 6-9g/day class, hypertension and anti-hypertensive treatment. For CPP, variables influencing the risk of achieving levels above the 90^{th} percentile were: age, sex, hypertension, anti-hypertensive treatment, anti-lipidic treatment, heart rate and previous cardiovascular disease.

Hypertension and the prevalence of subjects above the 90^{th} percentile of central blood pressure measurements. 31.6% of these subjects were hypertensive (19). In the entire hypertensive population, 72.4% and 51.5% have a CSBP and a CPP, respectively, above the normal 90^{th} percentile; more importantly, when looking at those hypertensives that are under anti-hypertensive treatment and are confirmed twice (in

two distinct occasions) to have their peripheral BP controlled, we found that 33.9% and 20.5% still have their CSBP and CPP above the normal 90th percentile, respectively (Figure D – Supplementary Material). 20.1% of treated and controlled hypertensives have CSBP > 130 mmHg, and 30.1% have CPP > 50 mmHg. (Figure E – Supplementary Material

Prevalence of Target Organ Damage (TOD) in subjects with Central blood pressure components above or below the 90th percentile. In Figure 4 we compared the existence of target organ damage between subjects above and below the 90th percentile for CSBP and CPP. We found that grade II albuminuria (microalbuminuria), large artery damage (PWV > 10 m/s) and eGFR below 60 ml/min can be 2 to 4 times more prevalent in the subjects with CSBP and CPP above the 90th percentile. Of note, all studied TOD (including Left Ventricular Hypertrophy) are significantly more prevalent in the CPP above the 90th percentile group.

Discussion

The main findings of the present work are that: i) central blood pressure components (like CSBP and CPP) in our cohort (a geographic area presenting the highest stroke incidence of Western Europe) are significantly higher than those expected for the same age and gender in healthy subjects (22); ii) the prevalence of highly increased (> 90th percentile for healthy subjects) CSBP and CPP is surprising, especially when looking into treated and controlled hypertensives; iii) subjects with CBP values above the 90th percentile bear significantly more target organ damage than the remainder of the population; iv) an overwhelming amount of salt is consumed amongst the citizens

of these two cities, and salt itself is registered both as an independent explanatory variable of the values of CSBP and CPPA in multivariable linear regression analysis, and as risk factor for the development of extremely high CSBP values (above the 90th percentile) – as determined in multivariable logistic regression analysis.

CBP components in a cohort from a high stroke incidence area – Both CPP and CSBP in our cohort are distinctively elevated when compared to the expected value for "normal/healthy" subjects with the same age and gender in the European Reference Values Collaboration (ERCV)(22). Although these parameters have been measured in few cohorts, the published data (mainly in healthy subjects) (25, 26) revealed values that are lower than the ones herein presented; furthermore, the prevalence of subjects above the "normal/healthy" 90th percentile (as determined by the ERVC) in our study is remarkably high, particularly if we take into account the percentages recorded for men in the early age groups (30 – 39 and 40 to 49 years). This data is even more significant when we recall the evidence concerning central blood pressure: CPP has been determined as a better predictor of left ventricular mass, restenosis after coronary angioplasty, carotid intima-media thickness, coronary artery disease severity, and mortality in end-stage renal disease (27). Moreover, it has been independently associated with cardiovascular events and subclinical vascular disease in the Strong Heart Study (6, 28), especially for CPP values above 50 mmHg (of notice, 28% of our population registers levels of CPP above 50 mmHg, and the mean CPP for age groups above 60 is over 50 mmHg) ; in older community dwelling individuals (over 65 years of age), CSBP and CPP retained an independent predictive power for future CV events, whereas peripherally measured blood pressure did not (29).

Cheng and colleagues established a pathologic cut-off value for central systolic and diastolic blood pressure (130/90 mmHg)(11); with extremely higher risk for the development of stroke during follow up. A similar proposal has also been formulated (using different methodology) by McEniery and colleagues in the ACCT trial, where a cut-off value 125/90 mmHg was proposed (5). Using a conservative approach, we determined that more than 30% of our population is above 130/90 mmHg for central blood pressure, and recorded that approximately 10% of subjects in the 30 to 50 age groups are above this cut-off. Altogether, remembering the increased risk associated with 10mmHg increase in CSBP and CPP above mentioned, it is crucial to underline the fact that subjects in our population are, in average, 10 – 22 mmHg (male) and 5 – 17 mmHg (female) higher than the expected age specific 50^{th} percentile for CSBP, and 4 – 17 mmHg (male) and 4 – 12 mmHg higher than the specific 50^{th} percentile for CPP.

There are no reference values for CPPA, but comparing our data to the one presented with the ACCT trial (that enrolled healthy subjects), we could clearly see that our values per age decade are also lower than the ones there presented.(5)

The impact of salt consuption. Much has been published about the influence of salt in peripheral blood pressure and the risk of stroke. Still, only a few studies have linked salt consumption with an effect in increasing central blood pressure (mainly CPP) in hypertensives (30, 31) and in the general population (32). Our results concerning salt consumption mirror recent analysis performed in a nationwide study (24), with men eating more salt than women. It is also worthy of note that salt consumption is higher in younger groups. Our findings of a positive effect of salt in CSBP in linear regression

models could not be reproduced in CPP, as evidenced in other studies (30, 31). The novelties in our findings, however, are two: a) salt is an explanatory variable for CPPA, a fact that we have not found previously in the literature; b) salt consumption above 6g/day increases the likelihood of achieving CSBP above the "normal" 90th percentile. These findings should be read with care and their true meaning should not be over-interpreted before other studies have been performed to confirm its validity. Many of the physiologic explanation of salt effect in central blood pressure comes from an influence in its pulsatile component via increased arterial stiffness (33), vascular remodeling and up-regulation of the renin-angiotensin-aldosterone system, increasing peripheral resistance (31). Still, these are also arguments in favor of an increase in central systolic blood pressure, as we have documented herein; furthermore, other colleagues have found CSBP to have a higher correlation with cardiovascular mortality than other CBP components (34). Shortcomings in our analysis are related to a more reduced sample size for valid 24h urine collections associated with the above mentioned fact that those who did deliver valid urine samples were slightly older and lighter (characteristics associated to lower salt consumption). The main issue is still the coincidence of a high salt intake (more than 80% above the recommended (35)), high stroke incidence (16), the high levels of both CBP and CBP above the normal 90th percentile in our cohort and the continuous trend of increased CSBP with age and higher level salt intake classes (Figure 1).

Central Blood Pressure, target organ damage and variability in hypertensives. Previous studies have established a relationship between CBP components and TOD in normotensives (9), normotensives and untreated hypertensives (34), hypertensives

(10), older subjects (7) and in population wide studies (36). Still, no previous study evaluated the association between CBP values above the new 90th percentile (as determined by the ERVC for normal/healthy subjects) and the development of target organ damage. The results presented herein offer support to the fact that subjects above the 90th percentile of their CSBP and CPP could be considered as high risk patients, as the prevalence of distinct forms of TOD (left ventricular hypertrophy, albuminuria, pulse wave velocity and eTFG below 60 ml/min) is significantly higher than those below this percentile. Our data are also in line with the body of evidence produced concerning TOD and central blood pressure (37), and produces ground to the concern with TOD at the brain level, which is not described in this study.

Finally, it is fundamental to discuss the variability of central blood pressure, and its inconsistent relationship with peripheral BP (37). This detail has been well documented in the ACCT Trial in healthy individuals (5). Herein we present for the first time, data that illustrates two different worrisome findings: firstly, a high prevalence of CSBP and CPP above the 90th percentile (for healthy/normal subjects) in hypertensive subjects; secondly, and more importantly, hypertensive subjects under treatment and considered to be controlled in two separate occasions have CSBP and CPP > 90th percentile in approximately 1/3 and 1/5 of cases, respectively.

Strengths and limitations. The stronger aspects of our approach lie on the fact that we have observed a very large random sample of a population geographically contained and homogeneous, representative of all adult age classes, levels of education and

professional status. This sample has been evaluated with the same methodology and CBP measurements were made with the same device, under the same protocol.

However, this study also has clear limitations. Only one measurement of CBP was performed per subject. A white coat effect cannot be excluded, particularly in younger subjects. We used the reference value data to compare CBP; this set of data is valuable, but not immune to bias since it was collected over a variety of locations, using various techniques and devices and included subjects defined as healthy or bearing risk factors on a post-hoc basis.

Conclusions. The present results provide an overview of the characterization of central blood pressure parameters in a population afflicted by a high incidence of stroke. Our data reveals that: i) subjects with high levels of CSBP and CPP have higher risk of cardiovascular disease; ii) subjects with elevated CBP parameters have a significantly higher prevalence of target organ damage; iii) treated and controlled hypertensives remain with high values of CBP in spite of treatment and peripheral blood pressure reduction and iv) high salt intake could partially explain these results, and, for the first time, is associated with CPPA.

Acknowledgments. Please refer to Supplemental Material.

References

1. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. European heart journal. 2010;31(15):1865-71. Epub 2010/03/04.
2. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. European heart journal. 2006;27(21):2588-605. Epub 2006/09/27.
3. Nichols WW, McDonald DA. McDonald's blood flow in arteries : theoretic, experimental, and clinical principles. 6th ed. London: Hodder Arnold; 2011. xiv,755 p. p.
4. Vlachopoulos C. Progress towards identifying biomarkers of vascular aging for total cardiovascular risk prediction. Journal of hypertension. 2012;30 Suppl:S19-26. Epub 2012/11/21.
5. McEniery CM, Yasmin, McDonnell B, Munnery M, Wallace SM, Rowe CV, et al. Central pressure: variability and impact of cardiovascular risk factors: the Anglo-Cardiff Collaborative Trial II. Hypertension. 2008;51(6):1476-82. Epub 2008/04/23.
6. Roman MJ, Devereux RB, Kizer JR, Okin PM, Lee ET, Wang W, et al. High central pulse pressure is independently associated with adverse cardiovascular outcome the strong heart study. Journal of the American College of Cardiology. 2009;54(18):1730-4. Epub 2009/10/24.
7. Verger A, van der Gucht A, Guedj E, Marie PY, Hossu G, Mandry D, et al. Central pulse pressure is a determinant of heart and brain remodeling in the elderly: a quantitative MRI and PET pilot study. Journal of hypertension. 2015;33(7):1378-85. Epub 2015/04/02.
8. Chirinos JA, Kips JG, Jacobs DR, Jr., Brumback L, Duprez DA, Kronmal R, et al. Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multiethnic Study of Atherosclerosis). Journal of the American College of Cardiology. 2012;60(21):2170-7. Epub 2012/10/30.
9. Booysen HL, Norton GR, Maseko MJ, Libhaber CD, Majane OH, Sareli P, et al. Aortic, but not brachial blood pressure category enhances the ability to identify target organ changes in normotensives. Journal of hypertension. 2013;31(6):1124-30. Epub 2013/04/05.
10. Protogerou AD, Argyris AA, Papaioannou TG, Kollias GE, Konstantonis GD, Nasothimiou E, et al. Left-ventricular hypertrophy is associated better with 24-h aortic pressure than 24-h brachial pressure in hypertensive patients: the SAFAR study. Journal of hypertension. 2014;32(9):1805-14. Epub 2014/07/08.
11. Cheng HM, Chuang SY, Sung SH, Yu WC, Pearson A, Lakatta EG, et al. Derivation and validation of diagnostic thresholds for central blood pressure measurements based on long-term cardiovascular risks. Journal of the American College of Cardiology. 2013;62(19):1780-7. Epub 2013/07/16.
12. Avolio AP, Van Bortel LM, Boutouyrie P, Cockcroft JR, McEniery CM, Protogerou AD, et al. Role of pulse pressure amplification in arterial hypertension: experts' opinion and review of the data. Hypertension. 2009;54(2):375-83. Epub 2009/07/01.
13. Sharman JE, Marwick TH, Gilroy D, Otahal P, Abhayaratna WP, Stowasser M. Randomized trial of guiding hypertension management using central aortic blood pressure compared with best-practice care: principal findings of the BP GUIDE study. Hypertension. 2013;62(6):1138-45. Epub 2013/09/26.
14. Asmar RG, London GM, O'Rourke ME, Safar ME. Improvement in blood pressure, arterial stiffness and wave reflections with a very-low-dose perindopril/indapamide combination in hypertensive patient: a comparison with atenolol. Hypertension. 2001;38(4):922-6. Epub 2001/10/20.

15. Boutouyrie P, Achouba A, Trunet P, Laurent S. Amlodipine-valsartan combination decreases central systolic blood pressure more effectively than the amlodipine-atenolol combination: the EXPLOR study. Hypertension. 2010;55(6):1314-22. Epub 2010/04/21.
16. Correia M, Silva MR, Matos I, Magalhaes R, Lopes JC, Ferro JM, et al. Prospective community-based study of stroke in Northern Portugal: incidence and case fatality in rural and urban populations. Stroke; a journal of cerebral circulation. 2004;35(9):2048-53. Epub 2004/07/17.
17. Kim AS, Johnston SC. Global variation in the relative burden of stroke and ischemic heart disease. Circulation. 2011;124(3):314-23. Epub 2011/07/07.
18. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. The Rationale/Design of the Guimaraes/Vizela Study: A Multimodal Population-Based Cohort Study to Determine Global Cardiovascular Risk and Disease. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2014. Epub 2014/03/04.
19. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. An epidemiological study determining blood pressure in a Portuguese cohort: the Guimaraes/Vizela study. Journal of human hypertension. 2014. Epub 2014/08/01.
20. Cunha PG, Cotter J, Oliveira P, Vila I, Sousa N. The rationale/design of the guimaraes/vizela study: a multimodal population-based cohort study to determine global cardiovascular risk and disease. Journal of investigative medicine : the official publication of the American Federation for Clinical Research. 2014;62(5):813-20. Epub 2014/03/04.
21. Cunha PG, Cotter J, Oliveira P, Vila I, Boutouyrie P, Laurent S, et al. Pulse wave velocity distribution in a cohort study: from arterial stiffness to early vascular aging. Journal of hypertension. 2015;33(7):1438-45. Epub 2015/04/02.
22. Herbert A, Cruickshank JK, Laurent S, Boutouyrie P. Establishing reference values for central blood pressure and its amplification in a general healthy population and according to cardiovascular risk factors. European heart journal. 2014. Epub 2014/08/13.
23. Taal MWB, Barry M; Rector, Floyd C. Brenner & Rector's the kidney. 9th ed. United States: Philadelphia, PA : Elsevier/Saunders, c2012.; 2012.
24. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. Journal of hypertension. 2014;32(6):1211-21. Epub 2014/03/29.
25. McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). Journal of the American College of Cardiology. 2005;46(9):1753-60. Epub 2005/11/01.
26. Wojciechowska W, Staessen JA, Nawrot T, Cwynar M, Seidlerova J, Stolarz K, et al. Reference values in white Europeans for the arterial pulse wave recorded by means of the SphygmoCor device. Hypertension research : official journal of the Japanese Society of Hypertension. 2006;29(7):475-83. Epub 2006/10/19.
27. Nichols WW. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. American journal of hypertension. 2005;18(1 Pt 2):3S-10S. Epub 2005/02/03.
28. Roman MJ, Devereux RB, Kizer JR, Lee ET, Galloway JM, Ali T, et al. Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. Hypertension. 2007;50(1):197-203. Epub 2007/05/09.
29. Pini R, Cavallini MC, Palmieri V, Marchionni N, Di Bari M, Devereux RB, et al. Central but not brachial blood pressure predicts cardiovascular events in an unselected geriatric population: the ICARe Dicomano Study. Journal of the American College of Cardiology. 2008;51(25):2432-9. Epub 2008/06/21.

30. Polonia JJ, Magalhaes MT, Senra D, Barbosa L, Silva JA, Ribeiro SM. Association of 24-h urinary salt excretion with central haemodynamics and assessment of food categories contributing to salt consumption in Portuguese patients with hypertension. Blood pressure monitoring. 2013;18(6):303-10. Epub 2013/11/07.
31. Park S, Park JB, Lakatta EG. Association of central hemodynamics with estimated 24-h urinary sodium in patients with hypertension. Journal of hypertension. 2011;29(8):1502-7. Epub 2011/06/15.
32. Redelinghuys M, Norton GR, Scott L, Maseko MJ, Brooksbank R, Majane OH, et al. Relationship between urinary salt excretion and pulse pressure and central aortic hemodynamics independent of steady state pressure in the general population. Hypertension. 2010;56(4):584-90. Epub 2010/08/25.
33. Avolio AP, Deng FQ, Li WQ, Luo YF, Huang ZD, Xing LF, et al. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. Circulation. 1985;71(2):202-10. Epub 1985/02/01.
34. Wang KL, Cheng HM, Chuang SY, Spurgeon HA, Ting CT, Lakatta EG, et al. Central or peripheral systolic or pulse pressure: which best relates to target organs and future mortality? Journal of hypertension. 2009;27(3):461-7. Epub 2009/03/31.
35. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European heart journal. 2013;34(28):2159-219. Epub 2013/06/19.
36. Roman MJ, Okin PM, Kizer JR, Lee ET, Howard BV, Devereux RB. Relations of central and brachial blood pressure to left ventricular hypertrophy and geometry: the Strong Heart Study. Journal of hypertension. 2010;28(2):384-8. Epub 2010/01/07.
37. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. European heart journal. 2014;35(26):1719-25. Epub 2014/01/25.

Figure and Table Legend

Table 1 – Clinical characteristics of 2542 subjects who completed the visit plan, and 1642 subjects with valid 24H urine samples

Table 2 – Central Hemodynamic variables and 24h salt consumption.

Table 3 – Linear Regression models for Central Systolic Blood Pressure and Central Pulse Pressure as dependent variables.

Table 4 - Logistic Regression model for Central Systolic BP above the 90th percentile

Table 5 - Logistic Regression model for Central Pulse Pressure above the 90th percentile

Table 6 – Linear Regression models for Central Pulse Pressure Amplification as a dependent variable

Figure 1 – Central Systolic BP relationship with salt and age

Figure 2 - Percentage of subjects presenting CSBP and CPP values above the expected 90th percentile stratified by age and sex**.**

Figure 3 - Percentage of subjects presenting CSBP value above the 90th percentile of the ERVC, salt consumption above 6g/day and CBP above 130/90 mmHg, stratified by age and sex

Figure 4 - Prevalence of Target Organ Damage in subjects with Central blood pressure components above or below the 90th percentile

Table 1 – Clinical characteristics of 2542 subjects who completed the visit plan, and 1642 subjects with valid 24H urine samples

Variables	Final Population (2542)	Valid 24H Urine Population (1642)
Gender (M/F)%	44.9/55.1	47.1/52.9
Age (Years)	45.5 ± 19.1	48.0 ± 18.9
Abdominal Perimeter (cm)	93.7 ± 11.8	93.2 ± 11.2
Height (cm)	163.4 ± 9.7	163.3 ± 9.5
Weight (Kg)	71.2 ± 13.4	70.6 ± 12.8
BMI (Kg/m2)	26.7 ± 4.6	26.5 ± 4.3
Fasting Glucose (mg/dl)	84.4 ± 23.8	84.4 ± 22.9
Serum Creatinine (mg/dl)	0.84 ± 0.5	0.83 ± 0.2
eTFG (ml/min)	96.9 ± 20.6	96.8 ± 19.7
Total Cholesterol (mg/dl)	192.2 ± 37.6	193.5 ± 38.1
LDL – Cholesterol (mg/dl)	115.4 ± 33.1	117 ± 33.4
HDL – Cholesterol (mg/dl)	54.7 ± 14.3	54.6 ± 14
Triglycerides (mg/dl)	111.7 ± 71.4	110.8 ± 71.7
Systolic BP (mm/Hg)	129.8 ± 20	130.7 ± 19.9
Diastolic BP (mm/Hg)	76.8 ± 10.7	76.2 ± 9.4
Heart rate (bpm)	66 ± 10.3	66 ± 10
Uric Acid (mg/dl)	4.4 ± 1.4	4.4 ± 1.4
Hypertension (%)	31.6	35.5
Smokers (%)	18.8	16.8
Non – Smokers (%)	65.1	67.2
Ex – Smokers (%)	16.1	16
Known CVD (%)	5.0	6.5
Diabetes Medication (%)	6.7	7.1
Lipid Medication (%)	17.7	19.5
HTN Medication (%)	22.0	24.3
Daily Exercise (%)	7.0	7.5

BP – Blood Pressure; **CVD** – Cardiovascular Disease; **eTFG** – estimated glomerular filtration rate; **HTN** - Hypertension

Table 2 – Central Hemodynamic variables (n=2472) and 24h salt consumption (=1642).

Central Hemodynamic Variables and 24H salt consumption								
		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Central Systolic BP (mmHg)	Total	101.4 ± 10.9	109.5 ± 13.9	120 ± 16.5	125.4 ± 15.1	133.5 ± 18.6	138.8 ± 20.7	119.2 ± 21
	Males	105.7 ± 10.5	114.4 ± 12.7	125.3 ± 14.7	126.8 ± 14	134.9 ± 20	139.4 ± 21	122.7 ± 20
	Females	97.9 ± 9.9	106 ± 13.7	115.8 ± 16.7	124.3 ± 16	132.4 ± 17.2	138.2 ± 20.2	116.4 ± 21.4
Central Diastolic BP (mmHg)	Total	70.8 ± 8.8	76.7 ± 10	81.5 ± 11.1	82.3 ± 10	80.2 ± 10.5	78 ± 11	77.3 ± 10.9
	Males	71.7 ± 9.6	79 ± 10.1	85.9 ± 10.2	83.9 ± 9.2	80.3 ± 11	78.4 ± 11.7	78.8 ± 11.3
	Females	70 ± 8.1	75.1 ± 9.5	78 ± 10.6	80.8 ± 10.4	80 ± 10	77.7 ± 10.4	76.1 ± 10.4
Central Pulse Pressure (mmHg)	Total	30.5 ± 6.5	32.7 ± 7.5	38.4 ± 9.9	43.2 ± 11.1	53.1 ± 14.8	60.6 ± 16.3	41.8 ± 15.9
	Males	34 ± 6.2	35.5 ± 6.9	39.4 ± 9.3	42.9 ± 9.9	54.5 ± 16.3	70 ± 17.5	43.7 ± 15.6
	Females	27.8 ± 5.3	30.9 ± 7.3	37.8 ± 10.3	43.5 ± 11.6	52.3 ± 13.3	60.4 ± 15.1	40.2 ± 16
Central Pulse Pressure Amplification	Total	1.6 ± 0.26	1.39 ± 0.2	1.24 ± 0.16	1.21 ± 0.14	1.18 ± 0.13	1.17 ± 0.14	1.32 ± 0.24
	Males	1.6 ± 0.21	1.4 ± 0.18	1.26 ± 0.14	1.25 ± 0.15	1.19 ± 0.15	1.19 ± 0.16	1.34 ± 0.23
	Females	1.53 ± 0.28	1.37 ± 0.21	1.23 ± 0.17	1.17 ± 0.11	1.17 ± 0.11	1.15 ± 0.13	1.3 ± 0.24
24H Salt excretion (g/day)	Total	9.8	10.0	10.7	9.9	9.1	8.5	9.6
	Males	11.3	11.4	11.8	11.7	10.2	9.6	11
	Females	8.5	8.8	9.5	8.3	8.2	7.2	8.4

Table 3 – Linear Regression models for Central Systolic BP and Central PP as a dependent variable

Variables	Model 1 - Central Systolic BP (24H Urinary salt entered)			Model 2 - Central Systolic BP (24H Urinary salt not entered)			Model for Central PP		
	Coefficients (SE)	Standardized coefficients	p values	Coefficients (SE)	Standardized coefficients	p values	Coefficients (SE)	Standardized coefficients	p values
Sex	2.8 (0.94)	0.067	0.003	3.8 (0.73)	0.090	<0.0001	1.34 (0.46)	0.042	0.004
Age	0.676 (0.02)	0.604	<0.0001	0.67 (0.018)	0.600	<0.0001	0.57 (0.013)	0.678	<0.0001
Fasting Glucose	0.05 (0.02)	0.054	0.008	0.052 (0.015)	0.058	<0.0001	0.038 (0.010)	0.057	<0.0001
Triglycerides	0.028 (0.006)	0.096	<0.0001	0.03 (0.005)	0.096	<0.0001	0.009 (0.003)	0.042	0.004
LDL Cholesterol	0.022 (0.012)	0.034	0.067	0.039 (0.009)	0.060	<0.0001	-	-	-
Uric Acid	1.0 (0.352)	0.065	0.005	0.62 (0.28)	0.041	0.025	-	-	-
Heart Rate	-	-	-	-	-	-	-0.26 (0.022)	-0.171	<0.0001
24H Salt	0.21 (0.1)	0.041	0.038	n.a.	n.a.	n.a.	-	-	-
Constant	69.8 (2.4)	-	<0.0001	71.5 (1.8)	-	<0.0001	27.7 (1.64)	-	<0.0001
R^2	0.468			0.468			0.536		

Explanatory variables studied in the regression models: Age, Sex, BMI, mean fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, Heart rate, 24H Salt.

Table 4 - Logistic Regression models for Central Systolic BP and Central Pulse Pressure above the 90th percentile

Variables	Model 1 – CSBP (without 24H Urinary Salt; n=2542)			Model 2 – CSBP (with 24H Urinary Salt; n=1642)		
	B-Coefficient (SE)	Odds ratio (95% CI)	p value	B-Coefficient (SE)	Odds ratio (95% CI)	p value
Age REF	-	-	<0.0001	-	-	0.005
Age 30-39	-0.005(0.17)	0.1(0.7 – 1.4)	0.98	-0.051 (0.22)	0.95(0.62 – 1.46)	0.817
Age 40 – 49	0.55(0.19)	1.7(1.2 – 2.5)	0.004	0.54 (0.25)	1.7(1.0 – 2.8)	0.032
Age 50 – 59	0.37(0.2)	1.5(1.0 – 2.1)	0.062	0.446 (0.25)	1.6(1.0 – 2.5)	0.069
Age 60 – 69	0.61(0.21)	1.8(1.2 – 2.8)	0.003	0.48 (0.25)	1.6(1.0 – 2.7)	0.058
Age >= 70	0.7(0.2)	2(1.4 – 3.0)	0.001	0.75(0.25)	2.1(1.3 – 3.4)	0.003
Sex	0.337(0.1)	1.4(1.1 – 1.7)	0.001	0.43(0.14)	1.5(1.2 – 2.0)	0.002
Salt Ref	-	-	-	-	-	0.048
Salt (6-9g/day)	-	-	-	0.446(0.19)	1.6(1.1 – 2.3)	0.022
Salt > 9g/day	-	-	-	0.181(0.19)	1.2(0.8 – 1.7)	0.34
BMI REF	-	-	<0.001	-	-	<0.0001
BMI 25 - 30	0.54(0.12)	1.7(1.4-2.2)	<0.0001	0.66(0.15)	1.9(1.4 – 2.6)	<0.0001
BMI > 30	0.55(0.15)	1.7(1.3 – 2.3)	<0.0001	0.63(0.19)	1.9(1.3 – 2.7)	0.001
Anti – HTN Tx	-1.5(0.22)	0.2(0.2 – 0.4)	<0.001	-1.6(0.27)	0.21(0.1 – 0.4)	<0.0001
HTN	3.1(0.2)	22.1(14.6 – 33.3)	<0.0001	3.176(0.23)	23.9(14.3 – 40.0)	<0.0001

BMI – Body Mass Index; **CSBP**– Central Systolic Blood Pressure; **CPP** – Central Pulse Pressure; **CVD** – Cardiovascular Disease; **HR** - Heart Rate; **BP** – Blood Pressure; **HTN** – Hypertension; **REF** – reference; **SE**-Standard Error; **CI** – Confidence Interval; **Tx** – Treatment

Table 5 - Logistic Regression models for Central Pulse Pressure above the 90th percentile

Variables	Model 3 – CPP		
	B- Coefficient (SE)	Odds ratio (95% CI)	p value
Age REF	-	-	<0.0001
Age 30-39	0.143(0.26)	1.2(0.7 – 1.9)	0.6
Age 40 – 49	1.0(0.26)	2.8(1.7 – 4.7)	<0.0001
Age 50 – 59	1.5(0.25)	4.4(2.7 – 7.2)	<0.0001
Age 60 – 69	1.8(0.25)	6.2(3.8 – 10.2	<0.0001
Age >= 70	2(0.25)	7.7(4.7 – 12.5)	<0.0001
Sex	0.37(0.11)	1.4(1.2 – 1.8)	<0.0001
Anti – HTN Treatment	-0.47(0.17)	0.67(0.5 – 0.9)	0.006
Anti-lipidic Treatment	-0.40(0.14)	0.7(0.5 – 0.9)	0.005
Heart Rate	-0.54(0.16)	0.6(0.4 – 0.8)	0.001
Known CVD	0.47(0.2)	1.6(1.1 – 2.4)	0.018
HTN	1.9(0.17)	6.9(5.0 – 0.6)	<0.0001

BMI – Body Mass Index; **CSBP**– Central Systolic Blood Pressure; **CPP** – Central Pulse Pressure; **CVD** – Cardiovascular Disease; **HR** - Heart Rate; **BP** – Blood Pressure; **HTN** – Hypertension; **REF** – reference; **SE**- Standard Error; **CI** – Confidence Interval; **Tx** – Treatment

Table 6 – Linear Regression models for Central Pulse Pressure Amplification as a dependent variable

Variables	Model 1 - Central Pulse Pressure Amplification (24H Urinary salt entered)			Model 2 – Central Pulse Pressure Amplification (24H Urinary salt not entered)		
	Coefficients (SE)	Standardized coefficients	p values	Coefficients (SE)	Standardized coefficients	p values
Sex	0.076 (0.012)	0.169	<0.0001	0.07 (0.011)	0.152	<0.0001
Age	-0.007(0.0001)	-0.579	<0.0001	-0.007(0.0001)	-0.559	<0.0001
Height	0.191 (0.068)	0.081	0.005	0.164 (0.059)	0.066	0.005
Triglycerides	0.0001(0.0001)	-0.084	<0.0001	0.0001(0.0001)	-0.073	<0.0001
LDL Cholesterol	0.001(0.0001)	-0.064	0.001	-0.001(0.0001)	-0.077	<0.0001
Smoking	-0.040 (0.012)	-0.066	0.001	-0.040 (0.010)	-0.065	<0.0001
Heart Rate	0.007 (0.0001)	0.300	<0.0001	0.006 (0.0001)	0.276	< 0.0001
24H Salt	-0.002 (0.001)	-0.044	0.026	n.a.	n.a.	n.a.
Constant	0.971 (0.121)	-	<0.0001	1.03 (0-105)	-	<0.0001
R^2	0.474			0.432		

Figure 1. Central Systolic BP relationship with salt and age

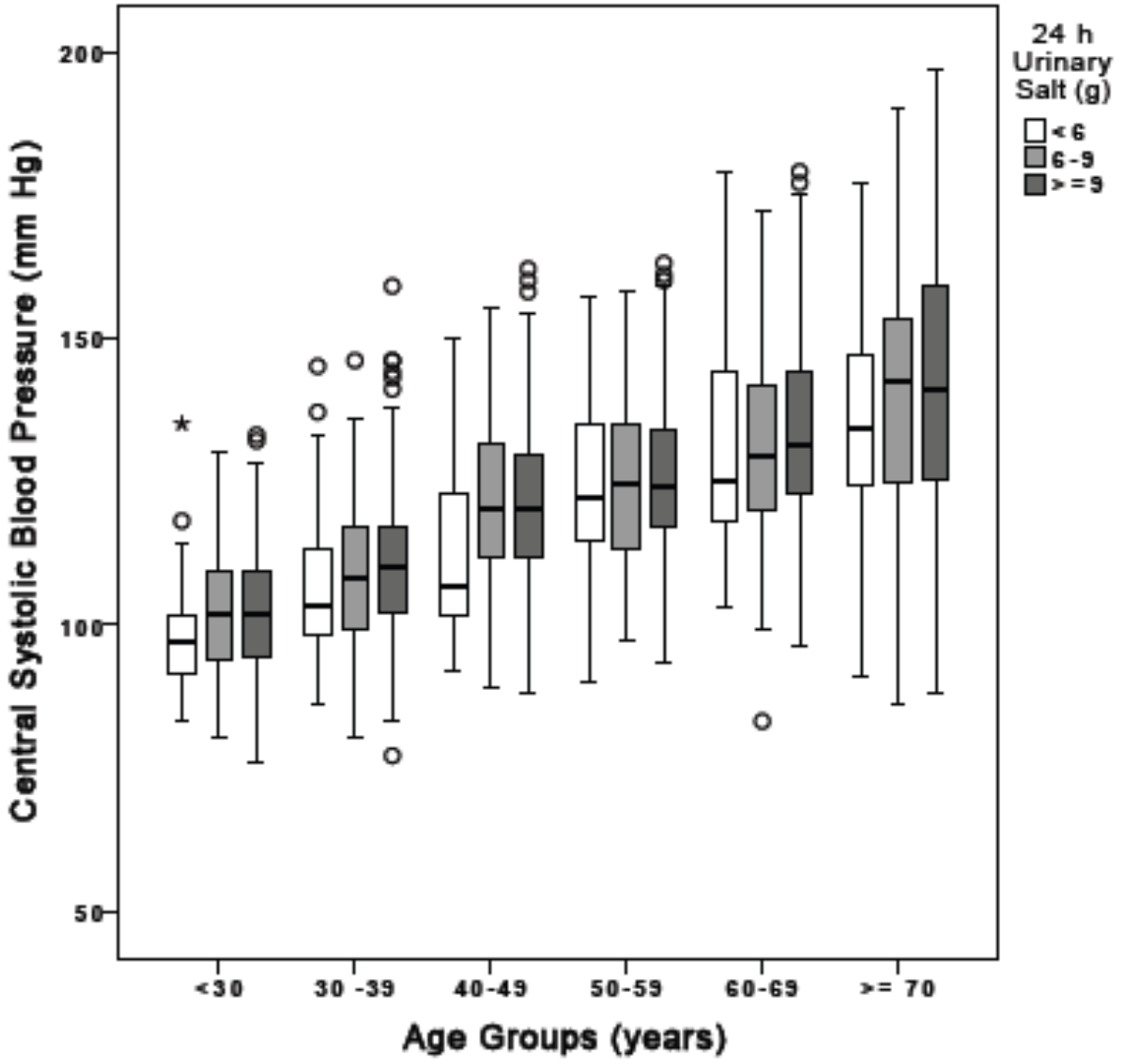


Figure 2 - Percentage of subjects presenting CSBP and CPP values above the expected 90th percentile stratified by age and sex**.**

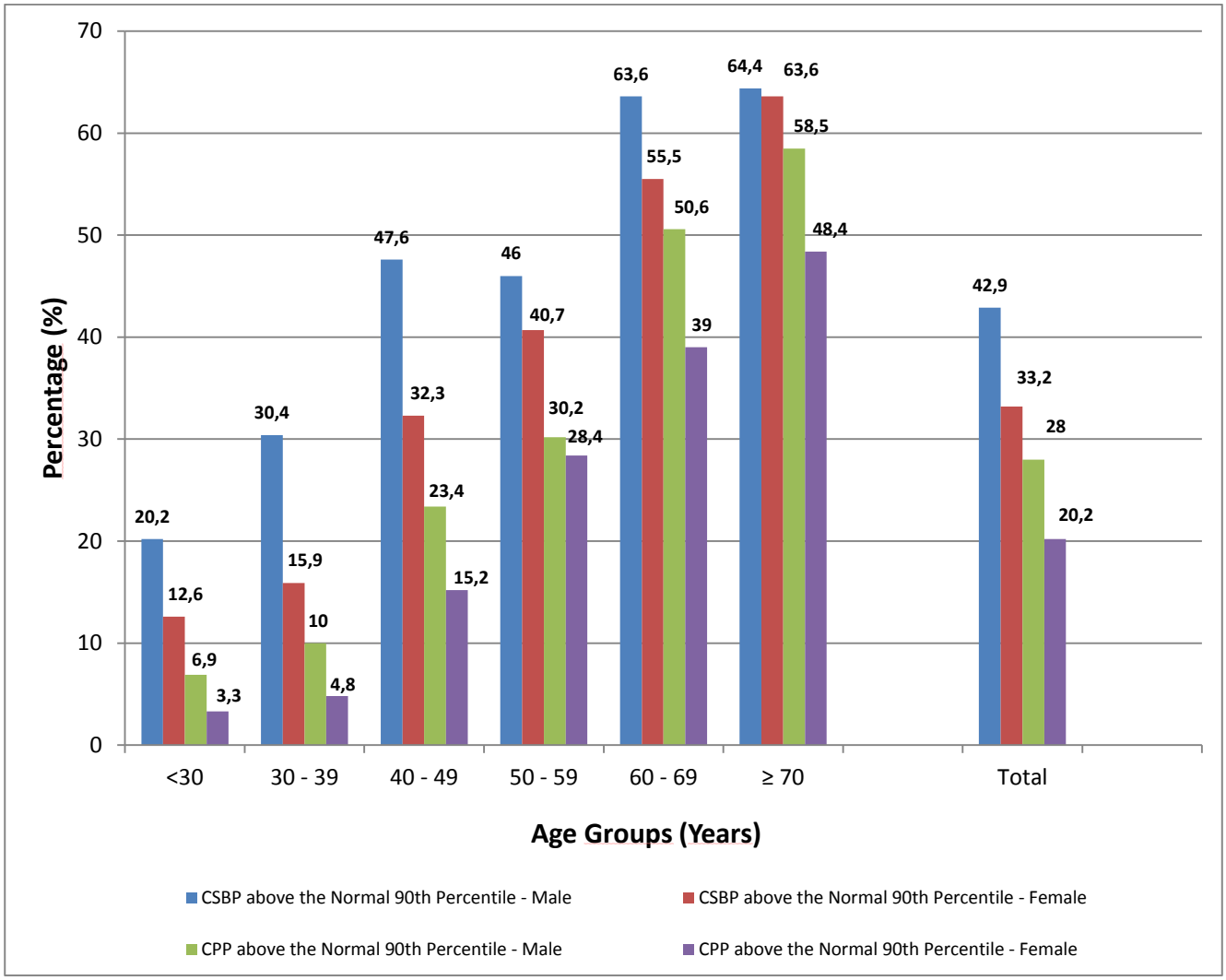


Figure 3 - Percentage of subjects presenting CSBP value above the 90th percentile of the ERVC, salt consumption above 9g/day and CBP above 130/90 mmHg, stratified by age and sex

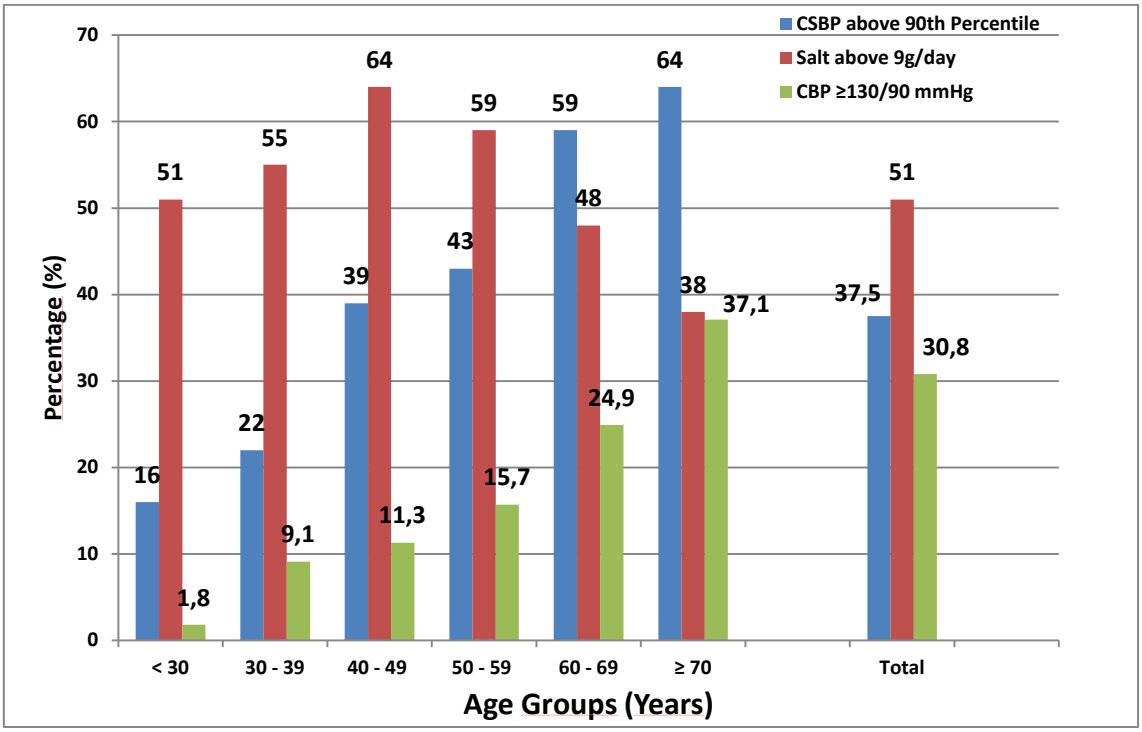
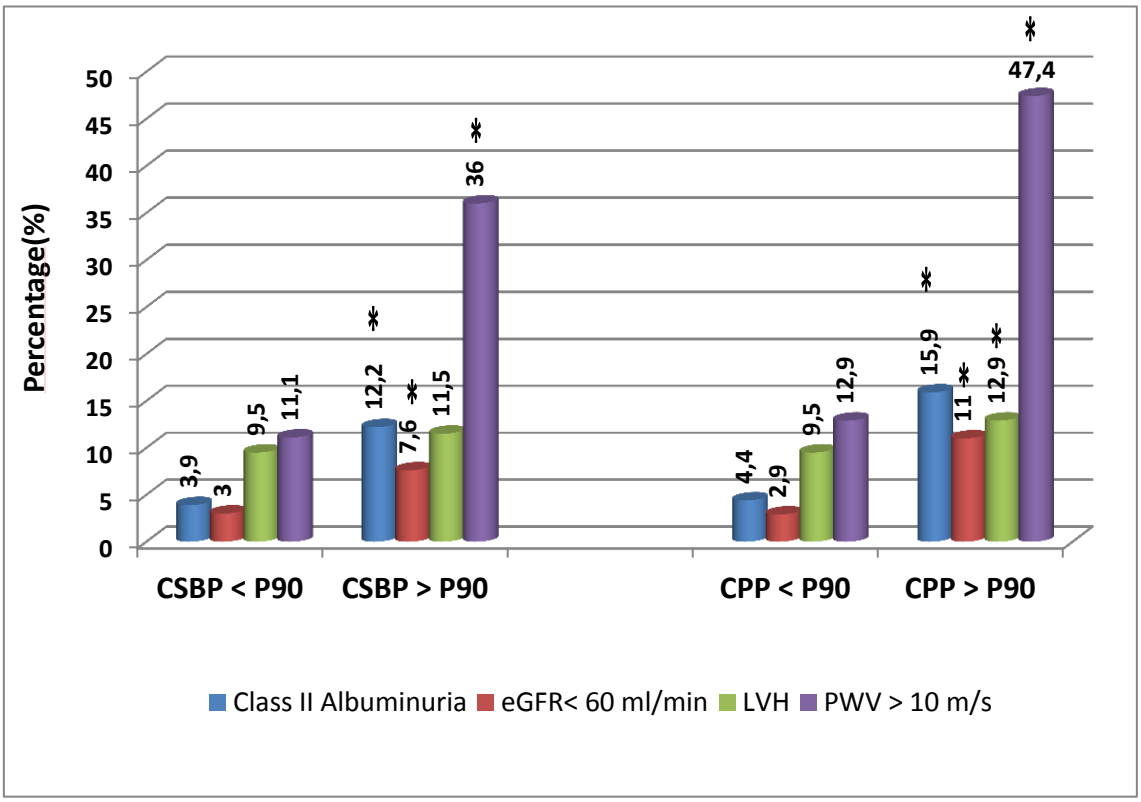


Figure 4 - Prevalence of Target Organ Damage in subjects with Central blood pressure components above or below the 90th percentile

*** - significant when compared with subjects below P90**



CSBP – Central Systolic Blood Pressure; CPP – Central Pulse Pressure; P90 – 90th Percentile LVH – Left Ventricular Hypertrophy; eGFR – estimated Glomerular Filtration rate; PWV – Pulse Wave Velocity

Supplemental Material

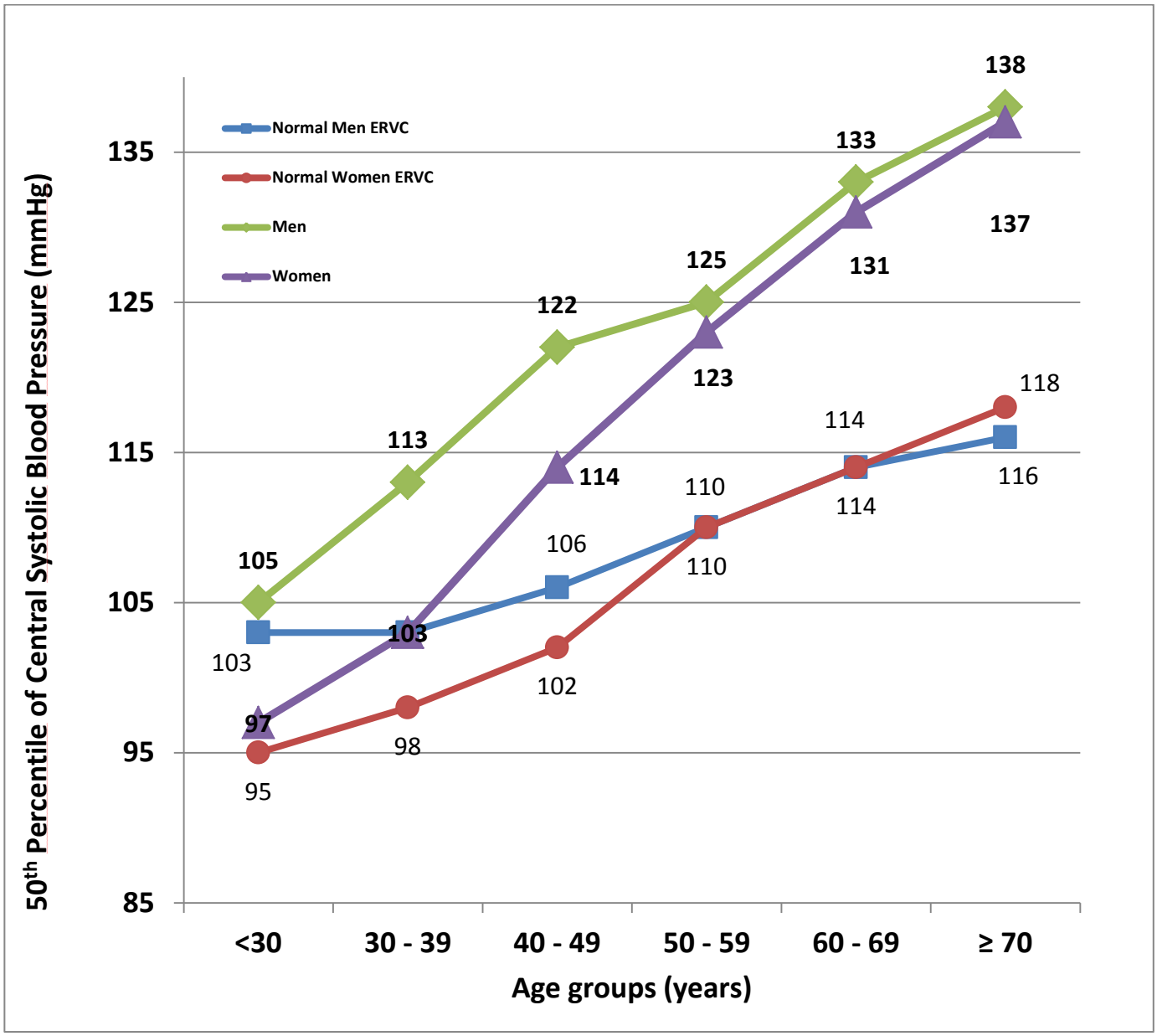
Central blood pressure and salt in a population cohort from a high stroke incidence area

Pedro G. CUNHA [(a)(b)(c)], Jorge COTTER[(a)(b)(c)], Pedro OLIVEIRA[(d)(e)], Isabel VILA[(a)], Pierre BOUTOUYRIE[(f)], Stéphane LAURENT[(f)], Peter M NILSSON[(g)], Angelo SCUTERI[(h)], Nuno SOUSA[(b)(c)]

Acknowledgments. The authors wish to show their acknowledgment to the following institutions and departments who collaborated in this project: Agrupamento de Centros de Saúde de Guimarães e Vizela, Serviço de Patologia Clínica do Centro Hospitalar do Alto Ave, Centro de Saúde da Amorosa, USF Afonso Henriques, USF Ara Trajano, USF Duo Vida, USF Novos Rumos, USF de Pevidém, USF Physis, USF de Ponte, USF de Ronfe, USF S. Nicolau, USF de S. Torcato, USF de Serzedelo e USF Vimaranes. A special word is addressed to thank all the physicians working in those different Primary Health Care Community Centers, whose work enrolling subjects was vital to the successful prosecution of our goals.

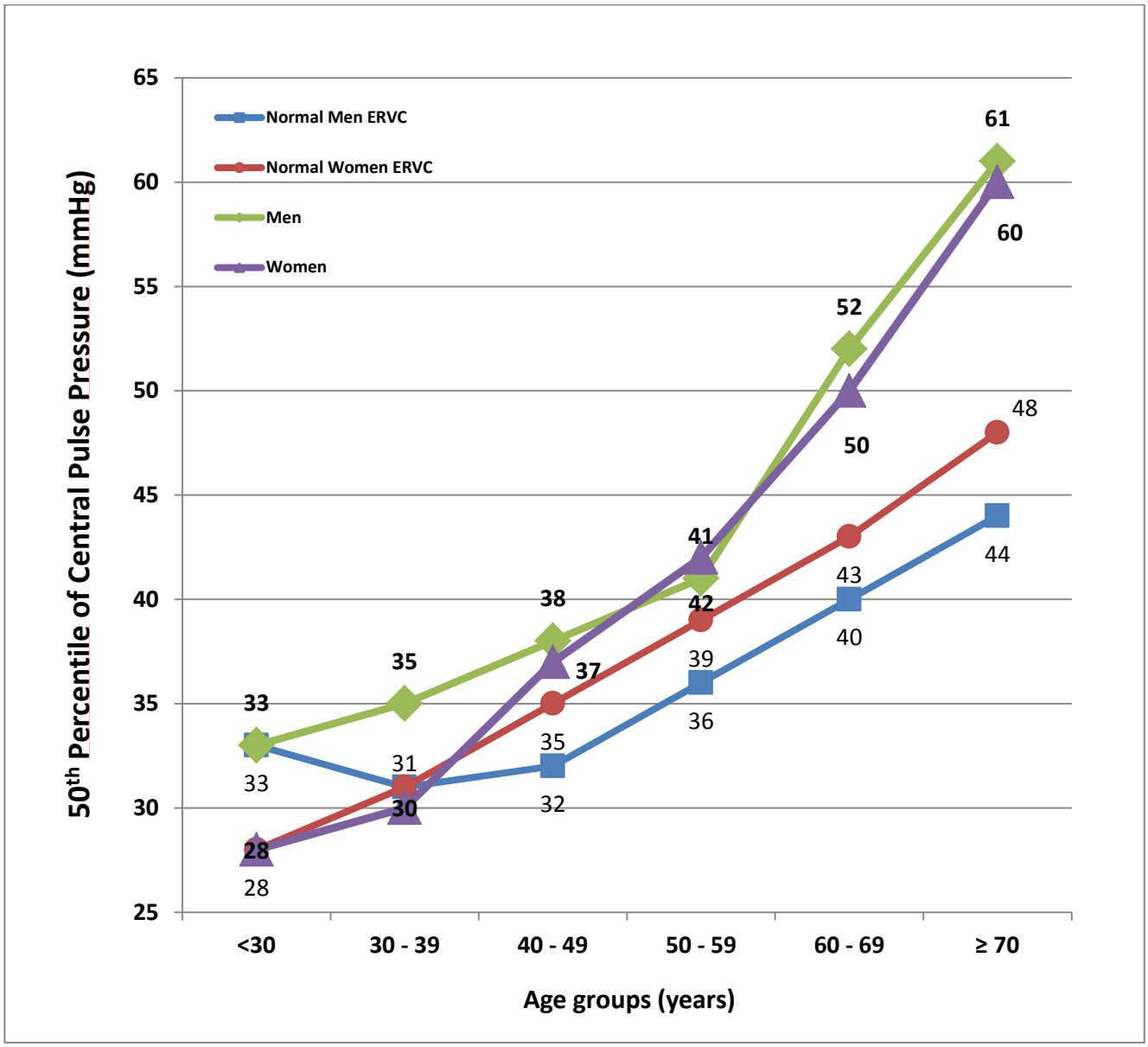
The Guimarães Study Group is also composed by the following researchers, who were involved in data collection: Helena Sarmento, Gloria Alves, Sara Freitas, Ana Sofia Alves, Sofia Gomes, Marta Gonçalves, Rui Fernandes, António Pedro Fonte, José Miguel Sá, Clarisse Neves, Andreia Sampaio, Cristina Cunha, Sílvia Sousa, Filipe Gonçalves, Joana Malheiro, João Silva, Carlos Fernandes, Estefânia Bustabad, Laura Castro, Nuna Vieira, Filipa Ramos, Ana Catarina Marques, Joana Monteiro, Ana Sofia Silva, Margarida Rocha, Mafalda Jordão Abreu, Margarida Dias, Magda Fernandes, Paula Felgueiras, Francisca Castro, Vânia Gomes, Ana Luísa Novo, Carla Pereira, Ana Cristina Ramalho, Fernando Esculcas, Dina Fernandes, Ricardo Rodrigues, Rafael Velho, Diana Coimbra, Joana Pimenta, João Pedro Teixeira, Ana Luísa Neves, Joana Leitão, Sara Pereira, Carla Ferreira, Clarinda Neves, Samuel Pedreira, Pedro Neves, Elisabete Lima, Carla Mendes, Orlanda Barbosa, Ana Catarina Martins, Célia Lemos, Eduarda Macedo, Sílvia Azevedo, Ana Rita Ribeiro, Elsa Salgado, Joana Dias, Eduarda Piairo, Marisa Carneiro, Ismael Costa, Lucília Miranda, Ana João Gonçalves, Natália Rodrigues, Manuela Morais, Rosário Santos, Odete Rodrigues, Alexandra Barreira, Ana Rita Romano, Catarina Marques, Fernanda Marisa Santos, Sónia Coelho Pereira, Elisabete Pinto Teixeira, Joana Andrade Pinto

Figure A – Comparing the 50th Percentile of CSBP from the European Reference Value Collaboration with the ones registered in the Guimarães/Vizela Study



ERVC – European Reference Values Collaboration

Figure B – Comparing the 50th Percentile of CPP from the European Reference Value Collaboration with the ones registered in the Guimarães/Vizela Study



ERVC – European Reference Values Collaboration

Figure C – Comparing the 50th Percentile of Amplification from the European Reference Value Collaboration with the ones registered in the Guimarães/Vizela Study

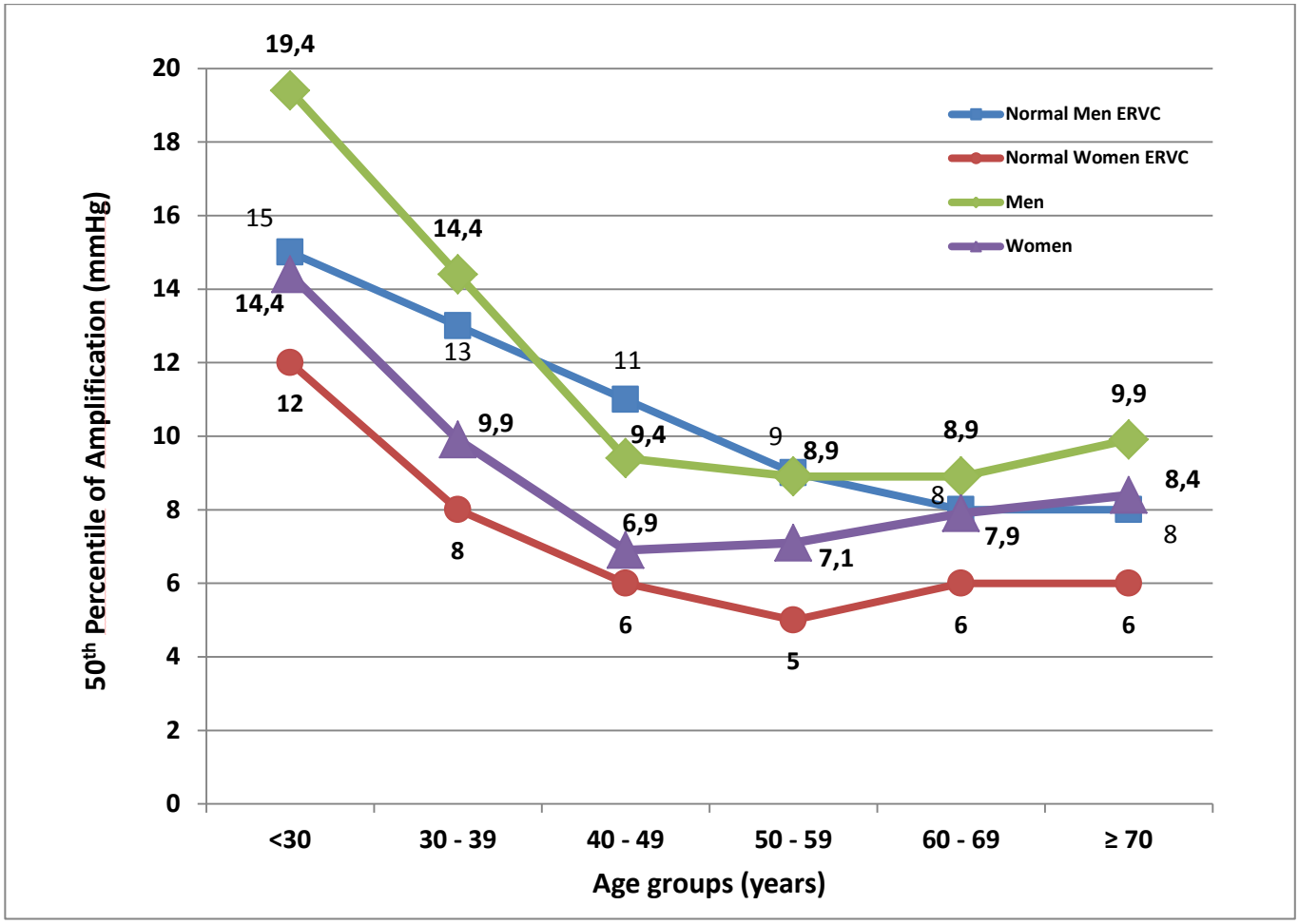


Figure D – Prevalence of CSBP and CPP above the 90th percentile in the general population, in hypertensives and in treated and controlled hypertensives

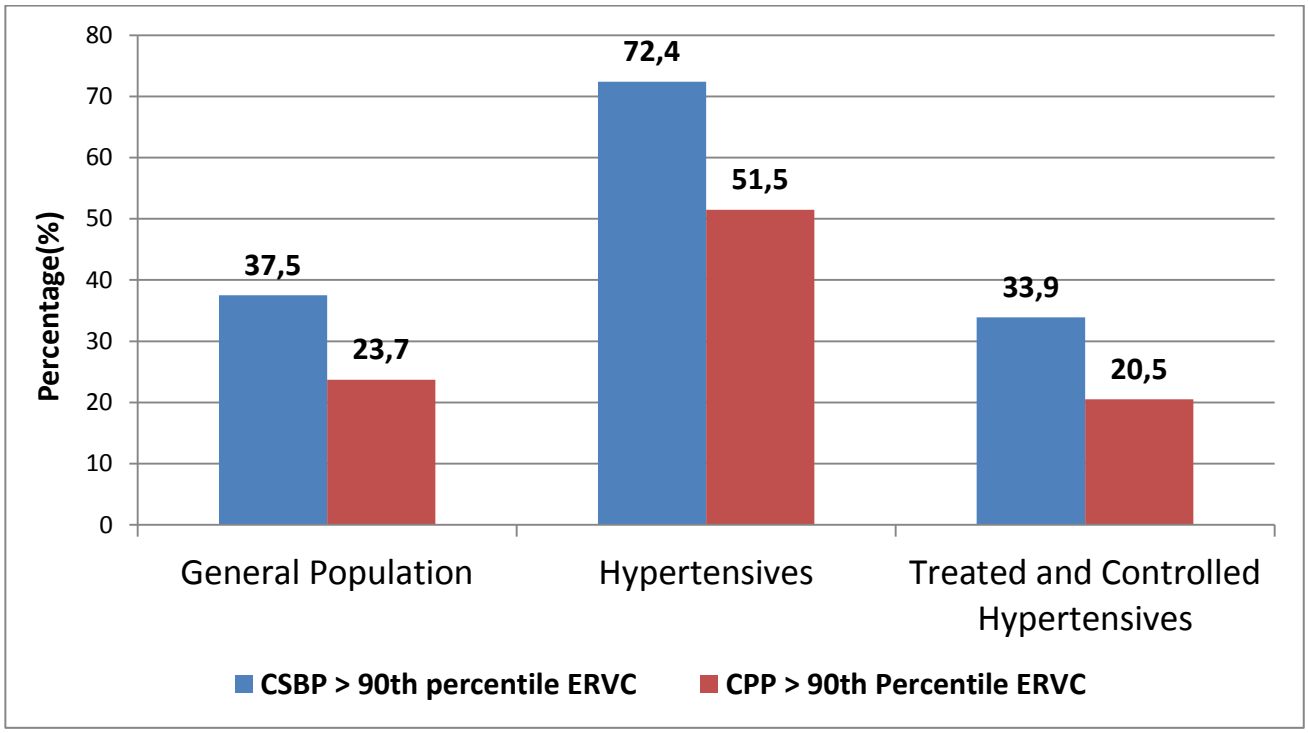


Figure E – CSBP and CPP mean values and prevalence of CSBP >130 mmHg or CPP > 50 mmHg in hypertensives and in treated and controlled hypertensives

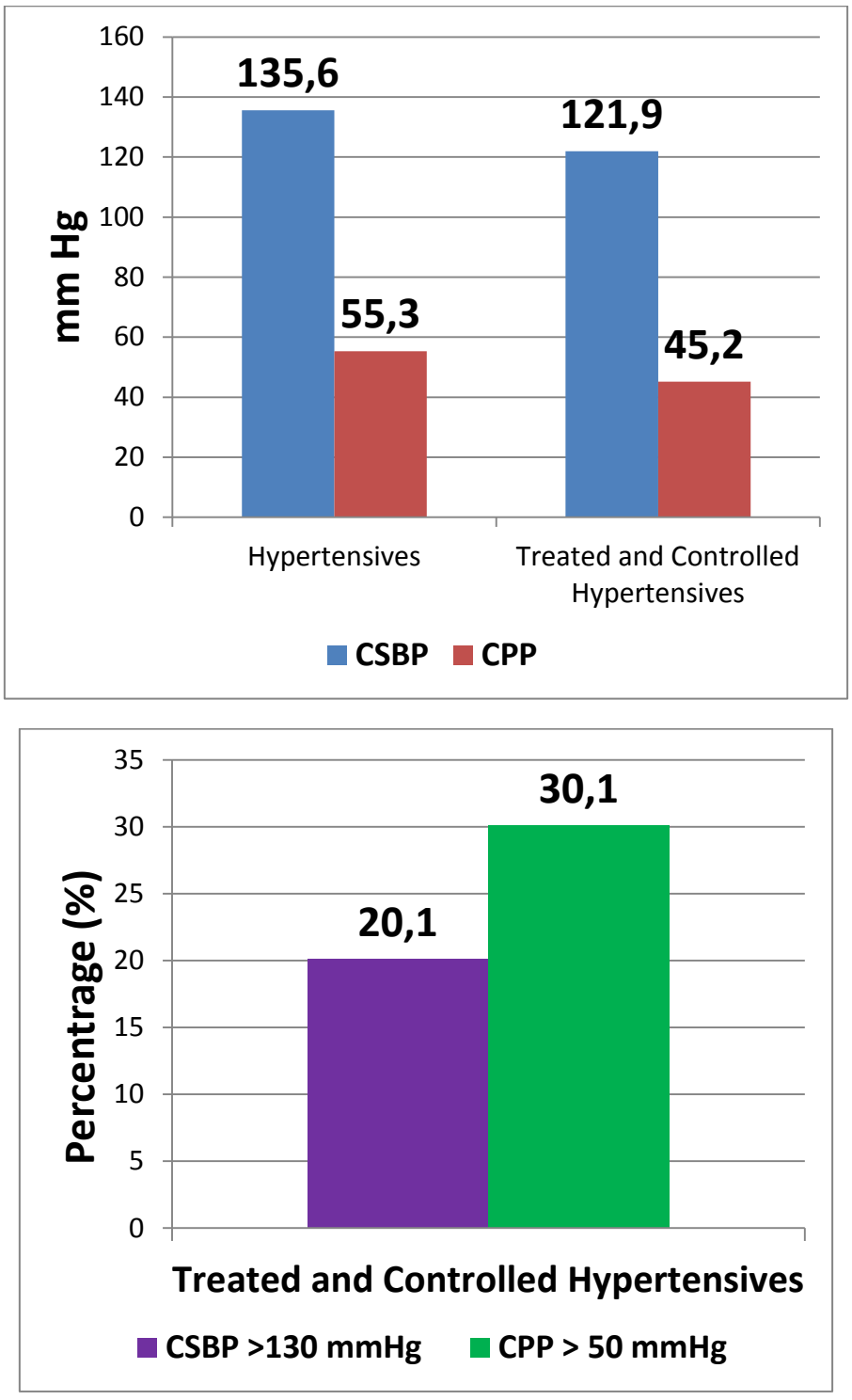


Table F – Absolute Amplification of systolic blood pressure.

Absolute Systolic BP Amplification								
		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Amplification (mmHg)	Total	16.3 ± 6.7	11.7 ± 6.2	7.9 ± 7.1	8.6 ± 5.7	8.7 ± 6.5	9.2 ± 8.4	11 ± 7.5
	Males	19 ± 6.3	14.3 ± 5.8	9.6 ± 6.5	9.6 ± 6.1	9.1 ± 7.4	10.1 ± 9.8	12.7 ± 8
	Females	14 ± 6.2	9.7 ± 5.8	6.6 ± 7.4	7.7 ± 5.2	8.3 ± 5.7	8.4 ± 6.8	9.7 ± 6.7

Chapter 7

Discussion and Future Perspectives

Discussion and Future Perspectives

The focused discussion of the results achieved so far has already been detailed in each chapter of this document. Our goal, with this final section, is to expose how we have integrated the knowledge collected, to structure both a scientific and social contribution.

Methodology. The first reflection must be done on the methodology that was herein used. The option to perform a multimodal approach to each subject, evaluating different dimensions of the cardiovascular, renal and cognitive functions has allowed a more integrative evaluation and understanding of the interaction between them; on the other hand, the choice to use clinical criteria to define pathologic characteristics of these systems (establishing a diagnostic when confirmed on two distinctive evaluations), has created a more accurate image of the individual's phenotype, and consequently permits the partial elimination of bias. This approach had already been used in hypertension studies (1, 2), but we had not seen it applied to different dimensions in an integrative evaluation. Within the limitations of a cohort study, this methodology produced two different sets of information: i) a more reliable baseline characterization of a population based study on the different components of its programmed evaluation; ii) the quantification of over/underestimation attributed to several risk characteristics, when compared to the use of standard epidemiologic criteria (3) (please see also Tables 2 – 4, chapter 8). Recently published information on a national dimension study has also tackled this problem, with a different method, concerning hypertension (4).

Some limitations are also present in our methodology: not all the measurements could be reproduced twice, as we would have wanted. Practical issues concerning the organisation of the visits (always performed at the primary care centers of the subjects) have forced us to compromise and obtain the best possible protocol; our process of thinking has also been to establish a proof-of-concept of such multimodal and more thorough phenotyping approach, and move forward to its full range of implementation in the near future.

Minimum risk exposure. The most complex set of information (and subsequent need for action) is not coming from the well defined pathologies, with fully established characteristics and diagnostic criteria that we have described so far. In our view, the most problematic information is gathered on those who are not considered "diseased", and that, therefore are excluded from any kind of intervention, independently of the divergence from "normality" that they may present. The truth to the matter is that an overwhelmed primary care system has little space for integrated interventions targeting subjects who still have not crossed the established threshold (or consensus criteria) of disease, but who are already at added risk for the development of pathology.

A great extent of our work has set out to pinpoint subjects at higher than expected risk for the development of cardiovascular, renal or cognitive ailment. The basis for this reasoning is supported on the notion that there is a continuous relationship of risk that starts not only at the designated "disease thresholds" but much earlier, for several variables: blood pressure(5), fasting glucose(6), lipids (6, 7), body mass index (8, 9), abdominal obesity(8), pulse wave velocity (10), central blood pressure (11), amongst others (please see Tables 1A – D, Chapter 8) . These generally recognized "minimum risk exposure" (12) are all surpassed for all the above mentioned variables in this studied population, meaning that the exposure to factors that will contribute to the development of CV is very significant, without triggering any effective action both educational or clinical by those who could perceive it.

The preservation of an ideal health profile from the CVR standpoint (and inherently, from the brain damage perspective) cannot be overemphasized. Much work has been published not only evidencing the disparity between the "ideal CV health attitudes" to preserve and the real world compliance with them (13), but also evidencing the value of preserving a low CVR profile throughout the years (14-16).

As presented herein, we did not verify the existence of ideal CV health characteristics in our population, but also (and more dramatic), we find several main CVR variables that are significantly above the theoretical minimum risk exposure in young ages – meaning a potential higher period of time of exposure.

Central hemodynamics and early vascular aging. The above expressed facts, led us to focus on the study of central hemodynamics and early vascular aging – a construct that comes in the footsteps of William Osler's (1898) and James Mackenzie's (1902) body of work, but that has now gained a new visibility with the possibility of non-invasive measurements permitted by new devices and algorithms.

In this Thesis, a possible connection between the surpassing of several minimum exposure risks for the development of CVD and the elevated prevalence of both early vascular aging and large artery damage (pulse wave velocity above 10m/s) is suggested based on the data obtained. Information that fills in the blank spaces could come from the results of the MARE project, showing that different combinations of the components of the so-called metabolic syndrome have a different association with arterial stiffness magnitude (17).

The higher levels of pulse wave velocity that we registered in our population, have also drawn our attention to the effect that this could have in promoting the increase in blood pressure and hypertension prevalence; several longitudinal studies have shown that: a) blood pressure and pulse wave velocity have different rates of change over time – divergent and indicative that the pulse wave velocity increase is not only dependent on blood pressure (18); b) pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and hypertension (19-21). We can only speculate if this effect has played a role in the increased prevalence of hypertension during the last decade in our region.

Concurrently, we report associations of salt consumption with central blood pressure parameters, and especially central pulse pressure amplification, in a population based study from a high-stroke incidence area, with a high salt consumption; at the same time, we register disparities in the concept of controlled hypertension when we use the existing central blood pressure parameters and thresholds (22, 23).

Call for action. Multimodal evaluation – Multifaceted intervention. The information gathered so far has deep implications in the clinical care and follow-up plan of our population. Obviously, significant improvement in blood pressure control, lipid levels and salt consumption must be promoted immediately. In spite of the significant

progress achieved in the last 10 years concerning the number of treated and controlled hypertensives, much has still to be done. On another level, primary and secondary prevention therapeutic attitudes need revision in agreement with CVR status and unachieved therapeutic goals (24-26).

Being attentive to the possibility of including the central hemodynamic parameters as variables to consider when treating hypertensive subjects at higher risk (i.e with higher pulse wave velocity), the therapeutic range, in terms of drug classes, would become narrower. Pulse wave velocity reduction beyond blood pressure reduction has been proven feasible in several studies (27-29), but the issue still to demonstrate is if reduction beyond blood pressure carries with it proven CV benefit in terms of morbid-mortality (30).

For Central Blood Pressure, the road to a full blown use in clinical practice (as a decision tool in therapy) is still being travelled and at its early stages. There is no clear indication, that one could define a strategy for blood pressure control based on CBP measurements alone. Despite this previous point, some publications have already contributed to this issue partially (31-33).

Yet, the most challenging goal is the reversal of the attitude towards health that needs to operate in several levels: on the educational plan, promoting ideal health behaviours as part of the school programs (34); on the primary health care setting, alerting physicians for the need of identifying early subjects at risk for the development of CVD, and to inherently promote lifestyle changes, therapeutic adjustments as well as a more stringent follow-up plan; at the governmental arena: a) empowering health officials and learned societies with the tools to reduce exposure to toxic substances (like salt or glucose) and, b) adapting official school programs and activities to fit the expected lifestyle being taught to younger generations. This multi-level intervention including several players of a community has recently been described as successful elsewhere (35).

Future Perspectives. There are two domains in which we foresee future developments, stemming from this body of work: a) the first one is the continuous analysis and publication of other results that still have not seen daylight, namely associating our central hemodynamic parameters with the cognitive evaluations and

functional imaging, performed in subjects over 50 years of age; b) the second one is the need to proceed to a longitudinal evaluation of subjects, re-evaluating them, observing evolution and defining causal associations whenever possible. This process is in due time of preparation, as 5 years after the beginning of the observation of the first subjects are now being completed.

On the scientific domain, many are the expectations regarding this field: will the early vascular aging construct prove its clinical utility? Will there be evidence concerning the CV benefit of targeted therapies for increased arterial stiffness? Will central blood pressure prove its independent worth in clinical practice beyond peripheral blood pressure measurements?

Much of the advances and research effort being put forward today in the pulse wave analysis are focusing on the components of the central wave form, and its relation with CV events (36-41). The ventriculo-vascular coupling is another area where we expect developments (42). The pitfall of these new potential markers of risk is, on one hand, the primal position they occupy at present in their route to acknowledgement as CVR markers with utility benefits (in terms of risk stratification) that go beyond the other established an used markers; on the other, and after clinical utility proven, the posterior challenge to find an easy way of disseminating its clinical use.

The most pressing matter for future achievements is more down to earth and seems much straightforward – the reduction of the CVD burden and progression of cognitive decline. The road to this goal does not need many new gadgets or many new biomarkers. It definitely needs drive and coordinated actions from the scientific community, the health care administrators, the education program planners, the law-makers and the living-forces in the community, looking to achieve as many ideal cardiovascular health behaviours as possible, in order to help the next generation.

References

1. Bovet P, Gervasoni JP, Ross AG, Mkamba M, Mtasiwa DM, Lengeler C, et al. Assessing the prevalence of hypertension in populations: are we doing it right? Journal of hypertension. 2003;21(3):509-17. Epub 2003/03/18.
2. Modesti PA, Rapi S, Bamoshmoosh M, Baldereschi M, Massetti L, Padeletti L, et al. Impact of one or two visits strategy on hypertension burden estimation in HYDY, a population-based cross-sectional study: implications for healthcare resource allocation decision making. BMJ open. 2012;2(4). Epub 2012/08/10.
3. Crim MT, Yoon SS, Ortiz E, Wall HK, Schober S, Gillespie C, et al. National surveillance definitions for hypertension prevalence and control among adults. Circulation Cardiovascular quality and outcomes. 2012;5(3):343-51. Epub 2012/05/03.
4. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. Journal of hypertension. 2014;32(6):1211-21. Epub 2014/03/29.
5. 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. 2002;360(9349):1903-13. Epub 2002/12/21.
6. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet. 2010;375(9733):2215-22. Epub 2010/07/09.
7. Danesh J, Erqou S, Walker M, Thompson SG, Tipping R, Ford C, et al. The Emerging Risk Factors Collaboration: analysis of individual data on lipid, inflammatory and other markers in over 1.1 million participants in 104 prospective studies of cardiovascular diseases. European journal of epidemiology. 2007;22(12):839-69. Epub 2007/09/19.
8. Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, et al. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet. 2011;377(9771):1085-95. Epub 2011/03/15.
9. Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet. 2009;373(9669):1083-96. Epub 2009/03/21.
10. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. Journal of the American College of Cardiology. 2010;55(13):1318-27. Epub 2010/03/27.
11. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Baou K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with central haemodynamics: a systematic review and meta-analysis. European heart journal. 2010;31(15):1865-71. Epub 2010/03/04.
12. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2224-60. Epub 2012/12/19.
13. Bambs C, Kip KE, Dinga A, Mulukutla SR, Aiyer AN, Reis SE. Low prevalence of "ideal cardiovascular health" in a community-based population: the heart strategies concentrating on risk evaluation (Heart SCORE) study. Circulation. 2011;123(8):850-7. Epub 2011/02/16.
14. Yano Y, Stamler J, Garside DB, Daviglus ML, Franklin SS, Carnethon MR, et al. Isolated systolic hypertension in young and middle-aged adults and 31-year risk for cardiovascular mortality: the Chicago Heart Association Detection Project in Industry study. Journal of the American College of Cardiology. 2015;65(4):327-35. Epub 2015/01/31.
15. Chomistek AK, Chiuve SE, Eliassen AH, Mukamal KJ, Willett WC, Rimm EB. Healthy lifestyle in the primordial prevention of cardiovascular disease among young women. Journal of the American College of Cardiology. 2015;65(1):43-51. Epub 2015/01/13.
16. Stamler J, Stamler R, Neaton JD, Wentworth D, Daviglus ML, Garside D, et al. Low risk-factor profile and long-term cardiovascular and noncardiovascular mortality and life expectancy: findings for 5 large cohorts of young adult and middle-aged men and women. JAMA : the journal of the American Medical Association. 1999;282(21):2012-8. Epub 1999/12/11.
17. Scuteri A, Cunha PG, Rosei EA, Badariere J, Bekaert S, Cockcroft JR, et al. Arterial stiffness and influences of the metabolic syndrome: A cross-countries study. Atherosclerosis. 2014;233(2):654-60. Epub 2014/02/25.

18. Scuteri A, Morrell CH, Orru M, Strait JB, Tarasov KV, Ferreli LA, et al. Longitudinal perspective on the conundrum of central arterial stiffness, blood pressure, and aging. Hypertension. 2014;64(6):1219-27. Epub 2014/09/17.
19. Najjar SS, Scuteri A, Shetty V, Wright JG, Muller DC, Fleg JL, et al. Pulse wave velocity is an independent predictor of the longitudinal increase in systolic blood pressure and of incident hypertension in the Baltimore Longitudinal Study of Aging. Journal of the American College of Cardiology. 2008;51(14):1377-83. Epub 2008/04/05.
20. Kaess BM, Rong J, Larson MG, Hamburg NM, Vita JA, Levy D, et al. Aortic stiffness, blood pressure progression, and incident hypertension. JAMA : the journal of the American Medical Association. 2012;308(9):875-81. Epub 2012/09/06.
21. AlGhatrif M, Strait JB, Morrell CH, Canepa M, Wright J, Elango P, et al. Longitudinal trajectories of arterial stiffness and the role of blood pressure: the Baltimore Longitudinal Study of Aging. Hypertension. 2013;62(5):934-41. Epub 2013/09/05.
22. Cheng HM, Chuang SY, Sung SH, Yu WC, Pearson A, Lakatta EG, et al. Derivation and validation of diagnostic thresholds for central blood pressure measurements based on long-term cardiovascular risks. Journal of the American College of Cardiology. 2013;62(19):1780-7. Epub 2013/07/16.
23. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. European heart journal. 2014;35(26):1719-25. Epub 2014/01/25.
24. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). European heart journal. 2012;33(13):1635-701. Epub 2012/05/05.
25. Cook NR, Cutler JA, Obarzanek E, Buring JE, Rexrode KM, Kumanyika SK, et al. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: observational follow-up of the trials of hypertension prevention (TOHP). BMJ. 2007;334(7599):885-8. Epub 2007/04/24.
26. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ. 2013;346:f1325. Epub 2013/04/06.
27. Ong KT, Delerme S, Pannier B, Safar ME, Benetos A, Laurent S, et al. Aortic stiffness is reduced beyond blood pressure lowering by short-term and long-term antihypertensive treatment: a meta-analysis of individual data in 294 patients. Journal of hypertension. 2011;29(6):1034-42. Epub 2011/04/27.
28. Shahin Y, Khan JA, Chetter I. Angiotensin converting enzyme inhibitors effect on arterial stiffness and wave reflections: a meta-analysis and meta-regression of randomised controlled trials. Atherosclerosis. 2012;221(1):18-33. Epub 2012/01/03.
29. Boutouyrie P, Achouba A, Trunet P, Laurent S. Amlodipine-valsartan combination decreases central systolic blood pressure more effectively than the amlodipine-atenolol combination: the EXPLOR study. Hypertension. 2010;55(6):1314-22. Epub 2010/04/21.
30. Van Bortel LM, De Backer T, De Buyzere M. How to treat arterial stiffness beyond blood pressure lowering? Journal of hypertension. 2011;29(6):1051-3. Epub 2011/05/06.
31. Asmar RG, London GM, O'Rourke ME, Safar ME. Improvement in blood pressure, arterial stiffness and wave reflections with a very-low-dose perindopril/indapamide combination in hypertensive patient: a comparison with atenolol. Hypertension. 2001;38(4):922-6. Epub 2001/10/20.
32. Sharman JE, Marwick TH, Gilroy D, Otahal P, Abhayaratna WP, Stowasser M. Randomized trial of guiding hypertension management using central aortic blood pressure compared with best-practice care: principal findings of the BP GUIDE study. Hypertension. 2013;62(6):1138-45. Epub 2013/09/26.
33. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. Circulation. 2006;113(9):1213-25. Epub 2006/02/16.
34. Cotter J, Cotter MJ, Oliveira P, Cunha P, Polonia J. Salt intake in children 10-12 years old and its modification by active working practices in a school garden. Journal of hypertension. 2013;31(10):1966-71. Epub 2013/10/11.
35. Record NB, Onion DK, Prior RE, Dixon DC, Record SS, Fowler FL, et al. Community-wide cardiovascular disease prevention programs and health outcomes in a rural county, 1970-2010. JAMA : the journal of the American Medical Association. 2015;313(2):147-55. Epub 2015/01/15.

36. Chirinos JA, Kips JG, Jacobs DR, Jr., Brumback L, Duprez DA, Kronmal R, et al. Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multiethnic Study of Atherosclerosis). Journal of the American College of Cardiology. 2012;60(21):2170-7. Epub 2012/10/30.
37. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension. 2004;43(6):1239-45. Epub 2004/05/05.
38. Mitchell GF, Wang N, Palmisano JN, Larson MG, Hamburg NM, Vita JA, et al. Hemodynamic correlates of blood pressure across the adult age spectrum: noninvasive evaluation in the Framingham Heart Study. Circulation. 2010;122(14):1379-86. Epub 2010/09/22.
39. Cooper LL, Rong J, Benjamin EJ, Larson MG, Levy D, Vita JA, et al. Components of hemodynamic load and cardiovascular events: the Framingham Heart Study. Circulation. 2015;131(4):354-61; discussion 61. Epub 2014/11/25.
40. Wang KL, Cheng HM, Sung SH, Chuang SY, Li CH, Spurgeon HA, et al. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. Hypertension. 2010;55(3):799-805. Epub 2010/01/13.
41. Cecelja M, Jiang B, McNeill K, Kato B, Ritter J, Spector T, et al. Increased wave reflection rather than central arterial stiffness is the main determinant of raised pulse pressure in women and relates to mismatch in arterial dimensions: a twin study. Journal of the American College of Cardiology. 2009;54(8):695-703. Epub 2009/08/15.
42. Torjesen AA, Sigurethsson S, Westenberg JJ, Gotal JD, Bell V, Aspelund T, et al. Pulse pressure relation to aortic and left ventricular structure in the Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. Hypertension. 2014;64(4):756-61. Epub 2014/07/16.

Chapter 8

Annex Tables

Table 1A – Prevalence of non-traditional cardiovascular risk factors (n= 2542)

Traditional cardiovascular risk factors								
		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Smoking (%)	Total	30.2	25.9	26.6	13.8	8.7	2.4	18.8
	Males	41-0	35-6	34.1	25.4	17.2	4.4	26.7
	Females	21.5	19.0	20.6	3.7	1.6	0.4	12.4
Diabetes Mellitus (%)	Total	0.4	1.2	6.7	14.9	25.2	22.6	9.1
	Males	0.4	1.2	7.9	16.9	25.5	26.1	9.5
	Females	0.3	1.2	5.6	13.0	24.9	20.7	8.7
Dislypidemia (%)	Total	49.0	69.2	82.0	88.5	91.3	90.2	75.1
	Males	45.8	74.9	83.2	88.6	88.6	85.3	74.9
	Females	51.9	61.3	80	89.1	93.6	94.7	75.3
Obesity (BMI≥30 Kg/m2) (%)	Total	7.7	15.1	18.5	31.6	30.8	33.5	21.3
	Males	8	15	18.3	20.4	24.2	24.3	17.6
	Females	7.1	14	17.5	40.1	32.8	38.4	22.6

Table 1B – Prevalence of non-traditional cardiovascular risk factors (n= 2542)

Non – Traditional cardiovascular risk factors								
		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Hyperuricemia	Total	5.5	8.7	12	11.6	16.2	20.1	11.8
	Males	12	19.6	26.4	22	28.7	26.1	21.5
	Females	0.3	0.9	0.6	2.5	5.8	14.5	3.9
Abdominal Obesity (%)	Total	23.7	33.2	39.9	49.5	61.6	64.9	43.5
	Males	8.8	15.0	17.5	21.8	32.3	38.9	21.9
	Females	35.7	46.3	57.5	73.9	85.7	89.2	61.1
Chronic Kidney Disease (%)	Total	2.5	2.0	7.5	8.1	16.1	27.2	8.4
	Males	2.4	2.1	8.7	12.7	22.3	31.4	9.9
	Females	2.6	1.8	6.3	3.7	10.6	24.4	7.0
Grade II Albuminuria (%)	Total	2.4	0.8	7.0	6.4	12.6	18.5	7.2
	Males	1.9	1.0	7.3	10.7	14.8	20.1	8.7
	Females	2.8	0.7	6.8	2.7	10.7	16.7	5.9

Table 1C — Prevalence of other biologic characteristics(n= 2542)

		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Pre-diabetees	Total	1.4	3.5	12.5	22.6	22.3	25.6	13.0
	Males	2.4	5.4	16.9	20.9	17.9	21.4	12.8
	Females	0.7	2.1	8.9	24.2	25.9	29.5	13.1
Total Cholesterol > 190 mg/dl (%)	Total	30.5	50.4	63.9	62.8	56.7	50.3	49.4
	Males	21.9	55.9	59.5	57	53.1	44.9	45.7
	Females	37.9	46.5	67.4	68.4	59.5	54.9	52.3
Triglycerides > 150mg/fl (%)	Total	8.2	15.2	22.3	22.4	24.6	24.8	18.1
	Males	7.9	21.9	36.5	32.7	29.7	23	22.6
	Females	8.5	10.5	10.9	12.6	20.5	26.3	14.5
Low HD (%)	Total	15.1	17.8	21.1	16.8	18.7	20.9	18.1
	Males	19.5	24.4	25.7	17.6	18.3	17.0	20.3
	Females	11.3	13.3	17.4	16.1	19.1	24.3	16.3
LDL Cholesterol > 115 mg/dl (%)	Total	30.3	48.5	60.5	60.5	53.9	47.3	47.3
	Males	31.3	60.2	62.8	55.8	53.1	41.9	48.5
	Females	29.4	40.3	58.7	64.9	54.5	52	46.3

Table 1D — Mean values of other biologic characteristics(n= 2542)

		< 30 y	30 – 39 y	40 – 49 y	50 – 59 y	60 – 69 y	≥ 70 y	All Years
Uric Acid (mg/dl)	Total	4.1	4.2	4.3	4.4	4.6	4.9	4.4
	Males	4.9	5.1	5.4	5.0	5.3	5.4	5.2
	Females	3.4	3.5	3.5	3.9	4.0	4.5	3.8
Abdominal Perimeter (cm)	Total	86.5	90.7	93.2	96	98.5	101.3	93.7
	Males	88	93.3	95.7	96.6	98.8	100.7	95.0
	Females	85.4	88.9	91.3	95.6	98.3	101.8	92.6
Glucose (mg/dl)	Total	72.4	75.7	82.5	92	95.9	94.5	83.9
	Males	73.7	78.2	86.7	95.1	98.2	95.9	86.5
	Females	71.3	73.9	79.3	89.3	94	93.1	81.8
Body Mass Index (Kg/m^2)	Total	24	25.8	26.9	28	28.5	28.8	26.7
	Males	24.6	26.4	27.2	27.2	27.6	28.0	26.7
	Females	23.5	25.4	26.7	28.7	29.1	29.6	26.8

Table 2 — eGFR < 60 ml/min, according to study visit

	Total
Visit 1	5.1%
Visit 2	5.4%
Concordant in both visits	3.8%

Table 3 — Albuminuria according to Study Visit

	Albuminuria grade II (30 – 300 mg/g)	Albuminuria grade III (> 300 mg/g)
Visit 1	11.8 %	1.6 %
Visit 2	10.3 %	1.7 %
Concordant in both visits	7.3 %	1.2 %

Table 4A – Classification of chronic kidney disease according to KDIGO in visit 1 (n= 2397)

	Albuminuria grade I (< 30 mg/g)	Albuminuria grade II (30 – 300 mg/mol)	Albuminuria grade III (> 300 mg/g)	Total
G1 (≥90 ml/min)	59.7	5.2	0.7	65.5%
G2 (60 – 89 ml/min)	24.1	5.1	0.4	29.5%
G3a (45 – 59 ml/min)	2.1	0.8	**0.2**	3.1%
G3b (30 – 44 ml/min)	0.6	**0.5**	**0.1**	1.2%
G4 (15 – 29 ml/min)	**0.1**	**0.2**	**0.1**	0.4%
G5 (< 15 ml/min)	**0.04**	**0.04**	**0.2**	0.2%
Total (%)	86.6	11.8	1.6	100%

Very High Risk – 1.5% | High Risk –2.5% | Moderate Risk –12.4% | Low Risk – 83.8 %

Table 4B – Classification of chronic kidney disease according to KDIGO in visit 2 (n= 2347)

	Albuminuria grade I (< 30 mg/g)	Albuminuria grade II (30 – 300 mg/mol)	Albuminuria grade III (> 300 mg/g)	Total
G1 (≥90 ml/min)	59.8	4.2	0.6	64.7%
G2 (60 – 89 ml/min)	24.9	4.8	0.6	30.3%
G3a (45 – 59 ml/min)	2.5	0.9	**0.04**	3.4%
G3b (30 – 44 ml/min)	0.6	**0.4**	**0.2**	1.2%
G4 (15 – 29 ml/min)	**0.3**	**0**	**0.1**	0.3%
G5 (< 15 ml/min)	**0**	**0.04**	**0.2**	0.3%
Total (%)	88.0	10.3	1.7	100%

Very High Risk – 1.3% | High Risk –2.7% | Moderate Risk –11.5% | Low Risk – 84.7 %