



Universitat de Girona

FINAL DEGREE PROJECT

EVOLUTION OF RETINAL MICROVASCULATURE ABNORMALITIES AND
ITS ASSOCIATION WITH CARDIOVASCULAR MORBIMORTALITY

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I am using this opportunity to express my gratitude to Gabriel, who is the kind of doctor and person I would, some day, like to become. To Marc, who is the ultimate responsible for awakening me to the joy of learning. And above all, to my mother, for the things she has always done for me and for everything that is unknown to me.

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1. Introduction

1.1 Demography

Arterial hypertension is a high prevalence disease, not just present in our country but all over the world. The worldwide prevalence estimate of hypertension is 45% and the number of people suffering from this pathology will be increased to a total of 1.56 billion in 2025, as it is demonstrated by different studies(1,2).

Some studies have shown some differences between countries, with a higher proportion of cases in European countries than in the United States and Canada (47%, 28% and 27% respectively)(3).

Stratifying by different variables, we can see a difference in its prevalence.

There is a higher prevalence in low and middle income countries, furthermore, in these regions, half of the burden occurs in the population aged 45-69 years, showing the magnitude of this problem as it corresponds with the productive age(4,5).

According to age, hypertension prevalence correlates proportionally with it, showing an increased value in older people. We can observe a prevalence of 7,3% in those aged 18-39 years, of 32,4% between 40-59 years old and of 65% in those older than 59 years(6).

A global view of the available data shows us that hypertension prevalence values range from 11% in those aged 45-59 years to 82% in those older than 70 years(1). Studies from the United states of America support this data, revealing a prevalence among people older than 65 years of 86,2%(7).

Ethnicity is another important variable, black people present a higher prevalence of arterial hypertension compared to white people (42% and 28% respectively)(6,8) and develop hypertension earlier in life with higher values of blood pressure(9).

Regarding gender, hypertension is more prevalent in women, but we can appreciate a variation depending on the age, prevalence is higher in men among people younger than 45 years, equal between men and women aged 45 to 54 years and finally, more prevalent in women after 54 years(9).

In Girona, there is an estimated prevalence of 31.1% in people aged 45 years and over, and as in other regions, this prevalence increases with age(10).

1.2 Definition

Arterial hypertension can be defined as a condition in which blood pressure values are constantly higher than 140 mmHg and/or 90 mmHg for the systolic and diastolic blood pressure respectively. Establishing the diagnosis requires at least the presence of these values in 2 measures, separated almost 1 minute between them, and their presence in 2 to 3 visits disseminated in time(11). The measure must take place in specific conditions (Annex 1).

Values presented in table 1 are useful to classify hypertension except for the pediatric population, in which, values are associated with percentiles based on height and age(11).

Category	Systolic		Diastolic
Optimal	<120	and	<80
Normal	120–129	and/or	80–84
High normal	130–139	and/or	85–89
Grade 1 hypertension	140–159	and/or	90–99
Grade 2 hypertension	160–179	and/or	100–109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	and	<90

^aThe blood pressure (BP) category is defined by the highest level of BP, whether systolic or diastolic. Isolated systolic hypertension should be graded 1, 2, or 3 according to systolic BP values in the ranges indicated.

Table 1. Definitions and classification of office blood pressure (mmHg)(11)

A relevant aspect of hypertension studies is that most of them are based in office blood pressure; various studies demonstrated that out of office blood pressure correlates better with target organ damage (TOD) and mortality(11–14).

Out of office blood pressure measurements allow us to make the diagnosis of isolated clinical or white coat hypertension, which prevalence in the primary care system has been estimated to be 39,7% between those diagnosed of hypertension in our territory(15).

This kind of hypertension is the one that is only present during the clinical consultation, showing normal values in self-measured blood pressure (SMBP), in which, patients are taught to measure their own blood pressure at home and note the values, and ambulatory blood pressure monitoring (ABPM), in which, blood pressure values are automatically measured at regular intervals by an electronic device(11).

The blood pressure values for the hypertension diagnosis are slightly different and have specific indications (Annex 2,3).

It is important to remark that hypertension follows a unimodal distribution and that diagnostic values are based in the blood pressure levels in which cardiovascular mortality decrease if treated, the reason why being that nowadays some authors prefer to name it treatable blood pressure instead of arterial hypertension(11).

1.3 Mortality and cost

Hypertension is responsible for 7.6 million annual deaths, representing 13,5% of the total deaths and accounts for the loss of 92 million Disability-Adjusted Life Years (DALYs). Not only hypertensive patients are affected, but everyone with blood pressure values superior to the optimal ones(5). Concerning Spain, in people older than 50 years, 54% of all cardiovascular deaths are linked to high blood pressure(16).

Hypertension causes a reduction in life expectancy of 5,1 years and 4,9 years for men and women respectively, and a reduction in cardiovascular disease, myocardial infarction and stroke free time of 7,2 years(17).

In 2008, a multicenter study published the important cost of controlled and uncontrolled high blood pressure in patients older than 30 years, the average cost was 1183,55 € and 1202.13 € per year respectively(18).

1.4 Risk evaluation

After establishing the diagnosis, it is necessary to assess the cardiovascular risk as hypertension is associated with important cardiovascular events(5,16,17).

In clinical practice, some tools to assess this risk are available, one of them is the systematic coronary risk evaluation (SCORE). This score uses age, gender, smoking habits, total cholesterol and systolic blood pressure (SBP), based in European cohorts studies, to determine the probability of death by a cardiovascular or cerebrovascular event in the next 10 years in people aged 45 to 64 years(19).

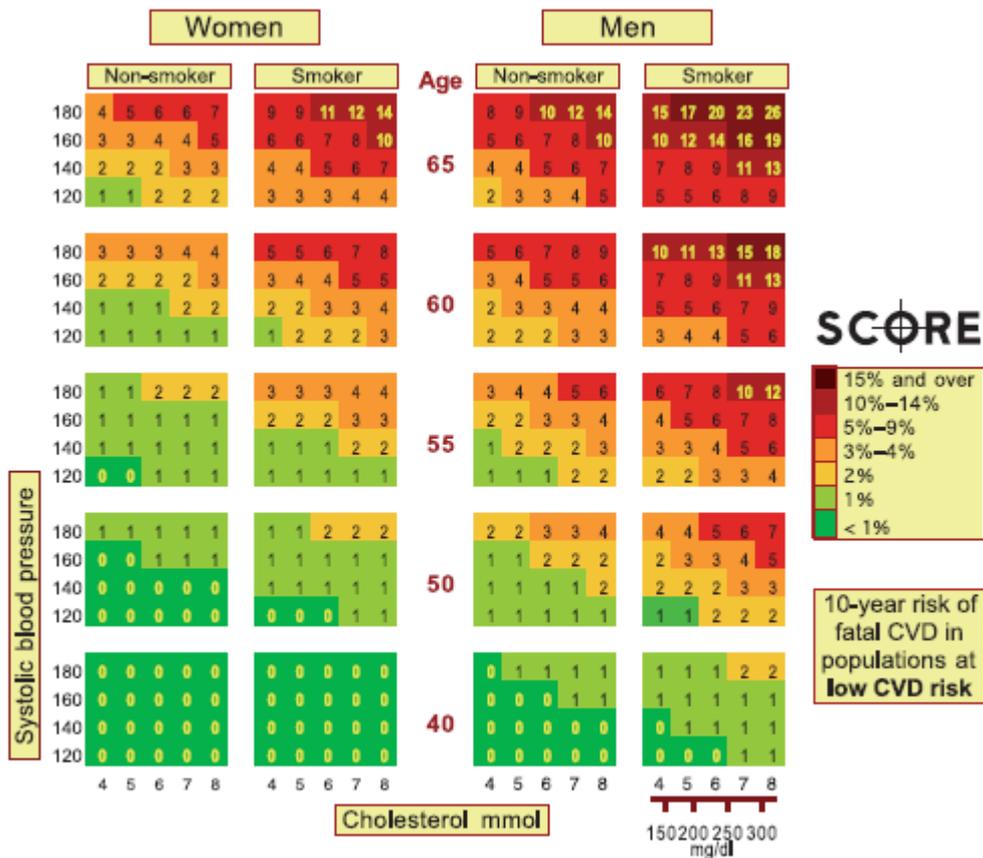


Table 2. Ten-year risk of fatal cardiovascular disease in populations at low cardiovascular risk. Chart based on total cholesterol.(19)

We have other scores that correlate better with our country reality, REGICOR tables, an adaptation from Framingham tables based on Girona’s population, as the use of Framingham tables in our population overestimate the cardiovascular risk.(20)

These tables estimate better the 10-year probability of suffering a coronary event in patients aged from 35 to 74 years(21).

European Society of Hypertension and European Society of Cardiology(ESH-ESC) propose a simpler method to assess the risk; basal risk is based on normal blood pressure and adds risk depending on different factors as target organ damage(11).

Other risk factors, asymptomatic organ damage or disease	Blood pressure (mmHg)			
	High normal SBP 130–139 or DBP 85–89	Grade 1 HT SBP 140–159 or DBP 90–99	Grade 2 HT SBP 160–179 or DBP 100–109	Grade 3 HT SBP ≥180 or DBP ≥110
No other RF		Low risk	Moderate risk	High risk
1–2 RF	Low risk	Moderate risk	Moderate to high risk	High risk
≥3 RF	Low to moderate risk	Moderate to high risk	High risk	High risk
OD, CKD stage 3 or diabetes	Moderate to high risk	High risk	High risk	High to very high risk
Symptomatic CVD, CKD stage ≥ 4 or diabetes with OD/RFs	Very high risk	Very high risk	Very high risk	Very high risk

BP = blood pressure; CKD = chronic kidney disease; CV = cardiovascular; CVD = cardiovascular disease; DBP = diastolic blood pressure; HT = hypertension; OD = organ damage; RF = risk factor; SBP = systolic blood pressure.

Table 3. Stratification of total cardiovascular risk categories of low, moderate, high and very high risk according to SBP and DBP and prevalence of risk factors, asymptomatic organ damage, diabetes, chronic kidney disease or asymptomatic cardiovascular disease. (11)

1.5 Target organ damage

Hypertension is a systemic disease responsible for a general damage to the body, more specifically, to the heart, brain, kidneys, vessels and eyes. ESH-ESC recommends the evaluation of these organs in order to detect any subclinical damage, once the hypertension diagnosis is made.

The heart's main condition is left ventricular hypertrophy, it is recommended to proceed to an electrocardiogram (ECG) to evaluate this condition. The more common methods to evaluate it are Cornell and Sokolov criteria. In order to have better results, a new test based in ECG voltage is used, the Cornell product, since it has increased the sensitivity of this procedure, (22,23). Some studies have demonstrated that echocardiography can reclassify 29% of the patients into a higher risk grade, but due to economical and functional problems, is not systematically carried out(11,24).

Renal damage is evaluated by renal function and the presence of elevated excretion of albumin. In order to evaluate kidneys, European guides recommend to test their function, both at the moment of the diagnosis and annually. This evaluation is based on serum creatinine levels, the estimation of glomerular filtration rate (GFR) calculated by different formula as Modification of Diet in Renal Disease (MDRD), Cockcroft- Gault and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), and proteinuria, which presence supports an established damage in those organs(11).

Vessel damage can be assessed by different methods. The first method is the evaluation of carotid intima-media-thickness (IMT) which is related to myocardial infarction and stroke independently of the cardiovascular risk. Values greater than 0,9 mm are considered as an alteration, even if for older people this value could be higher(11,25,26).

Pulse wave velocity is another marker of vessel damage and gold standard in the diagnosis of aortic stiffness, some recent studies estimate a value of 10 m/s to

establish a high risk patient. It is an independent marker of cardiovascular events in hypertensive patients(11,27).

Finally, ankle brachial index evaluate peripheral vessel disease with a cutoff point of 0,9, lower values double the 10 years mortality risk(28).

Hypertension is responsible for the presence of elevated intensity, white matter lesions, silent infarctions, higher incidence of lacunes and micro bleeds in the brain; these abnormalities are related to cognition problems and dementia. Silent lesions are more prevalent in cerebral tissue than in heart and kidneys but MRI's availability restricts the diagnose(11,29,30).

1.6 Eye evaluation

European guidelines suggest analyzing this organ, especially in patients suffering from diabetes or arterial hypertension type 2, in order to discover the presence of any alteration and to classify the retinopathy, if present, according to the Keith, Wagener and Barker classification(11).

Grade 1	Mild generalized retinal arterial narrowing or sclerosis
Grade 2	Focal narrowing and arteriovenous nipping Moderate to marked sclerosis of the retinal arterioles Exaggerated arterial light reflex
Grade 3	Retinal hemorrhages, exudates and cotton wool spots Sclerosis and spastic lesions of retinal arterioles
Grade 4	Severe grade 3 and papilledema

Table 4. Keith Wagener Barker classification of hypertensive retinopathy source: own elaboration based on (31)

1.6.1 Pathophysiology

Hypertension acts in the eye by vessel disruption, during first stages, a vasoconstrictive phase appears as an adaptive mechanism to high blood pressure; due to a local autoregulatory process, tone in arterioles is increased.

These changes can be observed by the clinician as an arteriolar spasm and/or an arteriolar narrowing. In this phase, a process of hyperplasia of the media wall as well as a deposit of hyaline products in the intima wall starts.

If high blood pressure persists over time or a sudden high elevation of blood pressure appear, an exudative phase shows up as a result of a blood-retina barrier disruption, along with lipid and blood extravasations and retinal ischemia. This phase is characterized by lesions, such as hard exudates, cotton-wool spots and microaneurysms. The end of this process, if not treated, is a sclerotic phase resulting in a necrosis of the media wall and a hyaline degeneration. This phase is characterized by a high degree of arteriovenous nipping and end-stage disruptions in the arteriolar light reflex(32).

1.6.2 Retinal abnormalities

Retinal alterations can be classified into 2 groups, abnormalities affecting the microvasculature and abnormalities affecting the retinal tissue.

Microvasculature alteration:

Arteriolar light reflex:

When we observe the retinal vessels, what we really see is the blood inside them. With persistent high blood pressure, media wall hyperplasia and hyaline deposits in retinal arterioles, a light reflection increase is caused, which is seen as copper wires during first stages. Subsequently, because of this process of evolution, blood is no longer visible and a light reflection increases, giving the arterioles a silver wire appearance(31,32).

Arteriovenous nipping:

In the arteriovenous crossing point, the adventitia is common for both vessels. Long hypertensive states produce a widening and hardening of this external coat, causing a constriction in both vessel, but due to arteriolar resistance, venule is more affected. These changes are measured in 4 grades (Gunn’s Sign)(31,32).

Grade 1	The arteriole covers and hides the venule
Grade 2	Compression of the venule causing a lack of blood proximal and distal to the arteriole
Grade 3	Distal dilatation of the venule
Grade 4	Different kind of lesions, such as exudates, appear around the arteriovenous nipping

Table 5. Gunn’s sign grades source: own elaboration based on(31)

Arteriolar narrowing and spasms:

Hypertension causes an arteriole vasoconstriction by a local regulatory mechanism, this is responsible for a generalized arteriolar narrowing whenever this autoregulatory mechanism is present.

Physiological values of the ratio arteriolar and venular diameter are $\frac{3}{4}$ and $\frac{2}{3}$, inferior values are considered as pathological(31,32).

Arteriole-venule angle (Salus's sign)

The arteriole-venule angle increases in the arteriovenous crossing point, this angle can reach 90° or wider, representing a return way of the venule(31).

Tortuosity (Guist's sign)

This sign represents the tortuosity of retinal vessels around the macula(31).

Hemorrhages

Due to vessel damage and a junction disruption between endothelial cells (31)

Retinal tissue alterations:

Mycoaneurysms

They appear in grade 3 and 4 of hypertensive retinopathy, even if they can appear at anywhere in the retina, they are more commonly found in the bifurcation of the arterioles, generally they are thrombosed(31,32).

Cotton-wool spots:

Spots with no defined edges, the physiopathological mechanism of these abnormalities is an occlusion of terminal arterioles, causing an inner retinal ischemia(31,33).

Hard exudates:

These lesions derive from a degenerative neural process or an exudative mechanism, they are seen as white or yellowed delimited elements, these alterations correspond to a lipid deposit(31,34).

Papilledema:

Optic disk edges are erased and can be elevated, it is caused by a high optic nerve hydration due to a high arteriolar pressure or a low venular drainage(31).

1.6.3 Evaluation process

The retinal microvasculature evaluation poses a problem all around the world, depending on the guides, lesions can be classified into target organ damage or not.

The seventh report of the Joint National committee (JNC)(35) considers arterial spasms and arteriovenous ratio (AVR) disturbance as a target organ damage, however, in Europe, for the ESH-ESC, and since 2003, for the WHO and the International Society of Hypertension (ISH), lesions corresponding with grade 1 and 2 of Keith-Wagener-Barker classification of hypertensive retinopathy, are considered as early stage of retinopathy and lesions corresponding with grade 3 and 4 (microaneurysms, hemorrhages, hard exudates, cotton-wool spots and optic disc's swelling) are considered retinal disease(11,36,37).

In the past, in order to evaluate this organ, a direct fundoscopy was used. This method has some disadvantages, one of the most important being that it is operator dependent. This technique, as indicated in studies, has a high interobserver(20%-42%) and intraobserver (10%-33%) variability when applied to people suffering from mild hypertension(38).

The natural next step in the fundus evaluation was the implementation of retinal photographs made by a retinograph, this method could solve some of the problems mentioned above. Images could be evaluated at any moment by any specialist, it was no longer an operator dependent test, besides, it allowed a better comparison of the same lesions in different moments or disease stages, although a high interobserver and intraobserver variability were still present(39).

In order to estimate some parameters such as the caliber of the vessels, some programs were developed, this kind of software has given rise to semiautomatic retinography, in which, arterioles and venules are selected by the user, and the program calculates the caliber of the different vessels and arteriovenous ratios.

Nowadays, new generation softwares are able to make the difference between arterioles and venules by their selves; the automatic retinography. As there is no operator that selects and describes the type of vessel, both the intraobserver and interobserver variability in the vessel evaluation disappear. The calculated concordance with experts is 87,68% (40,41).

1.6.4 Utility

Fundus eye examination can reclassify to a higher risk grade 10% to 15% of hypertensive patients(42,43).

Hypertension is related to different retinal alterations, some of them are focal arterial narrowing, generalized arteriolar narrowing, retinopathy and signs of arteriovenous nicking. It is important to remark that initial abnormalities are related to different conditions and morbimortality, some of these alterations, for example, have been related to high blood pressure in the past, incident stroke and diabetes(44,45).

Retinal abnormalities prevalence estimates vary from 2 to 14% as it is affirmed by Wong in a revision of different studies, among them, Blues mountain study, the Atherosclerosis Risk in Communities study (ARIC), Framingham study and Beaver Dam Eye Study. These abnormalities have a higher prevalence among hypertensive patients(46).

1.6.5 Retinal abnormalities associations

In general terms, retinal abnormalities have been associated with different diseases or alterations by different studies all around the world(47). Some of these alterations are intracerebral hemorrhages and thrombosis(48,49), presence of cerebral infarcts(50), cerebral atrophy, evaluated as higher sulca widening and ventricular grade of dilatation(51),and higher prevalence of white matter lesions(52). If we analyze specific retinal alterations we can observe they are associated with different results:

Arteriovenous ratio

It has been demonstrated that low values are related to a higher risk of developing hypertension in the future (60% more risk than people with normal AVR)(53,54). Other studies established a higher incidence of coronary disease and acute myocardial infarction for each reduction of standard deviation(55), and a higher incidence of stroke(56).

Even if arteriovenous ratio is related to a wide number of abnormalities, there are still some questions about its association with mortality(57,58).

It is important to emphasize that in the past, most of the studies defined arteriolar narrowing as low arteriovenous ratio, however, it has been established that not just arteriolar abnormalities are important as prognostic value, increased venular caliber influences and it is related to different alterations.

Arterial narrowing

Arteriolar caliber reduction has been related to current and past hypertension(59), carotid plaques(60), increasing values of blood pressure and left ventricular hypertrophy(61–63), a higher risk of developing diabetes mellitus(64) and can predict a higher risk, more than double, for coronary heart disease in men(65). Arteriolar narrowing, as arteriovenous nipping, is related to prevalent and incident stroke and stroke mortality(66).

Venular diameter:

Venular caliber has been associated with different parameters. A caliber alteration is related to an increased carotid plaques score, increased number of aortic calcifications and a reduction in ankle-arm index(67). In the Rotterdam study, venular caliber has been related to a higher risk of stroke and cerebral infarction, this risk was independent of other variables and independent of arteriolar caliber(68). The importance of the venular caliber in stroke prediction has been established by other studies(69) and as arterial narrowing, it is related to a higher risk of developing hypertension(70).

2. Justification

Target organ damage is related to cardiovascular mortality(71), in the case of the eye, we have seen that its evaluation can reclassify 10% to 15% of the hypertensive patients to a higher risk grade(42,43) and that some abnormalities, as for example arteriovenous ratio changes, are related to a higher incidence of coronary disease, myocardial infarction and stroke(55,56).

A recent study tried to show the association between changes in retinal microvasculature and the evolution of TODs, showing a relation between them(72). However, we still don't know if the evolution of these lesions is important in clinical practice.

As with new automatic retinographies, the classification and evaluation of these lesions are easier to allocate and can be done in primary care centers, which implies a more accurate, faster and cheaper method to reclassify the risk than an ophthalmologist consultation; as the important end-point is to know if the evolution of retinal microvasculature abnormalities, as arteriovenous ratio and arteriolar and venular caliber, is related to the cardiovascular morbimortality; as hypertension is a high prevalence disease with important consequences in the population health and as there is no published information about it, it is important to continue the research on this field.

3. Hypothesis

Evolution of retinal microvasculature abnormalities (arteriovenous ratio, arteriolar caliber and venular caliber) measured by automatic retinography, in patients suffering from essential hypertension of new onset and never treated, is associated with 5 years cardiovascular morbimortality.

4. Objectives

The main objective is to verify if there is an association in newly-diagnosed and never treated hypertensive patients between:

Arteriovenous ratio evolution measured by automatic retinography and 5 years cardiovascular morbimortality.

Arteriolar caliber evolution measured by automatic retinography and 5 years cardiovascular morbimortality.

Venular caliber evolution measured by automatic retinography and 5 years cardiovascular morbimortality.

The secondary objective is to verify if there is an association between arteriovenous ratio baseline values and cardiovascular mortality.

5. Patients and methods

5.1 Study design

Multicenter prospective cohorts study.

5.2 Definition of the participants

The target population is composed by patients with essential hypertension of new onset and never treated, according to the ESH-ESC criteria, aged 35 to 74 years.

Inclusion criteria:

Patients with essential hypertension of new onset and never treated and patients aged 35 to 74 years.

Exclusion criteria:

Patients with secondary hypertension, patients that follows or followed antihypertensive treatment, patients with clinical isolated hypertension, inability, in the health professional's opinion, to perform self-measured blood pressure, major cardiovascular complications (as symptomatic cardiovascular disease, symptomatic cerebrovascular disease, acute myocardial infarction and stroke), serious endocrine or hematological illness or other illness or limitation that the physician considered to be a motive for exclusion, diabetes mellitus, patients who had clouded eyes or ophthalmologic diseases that could affect the interpretation of optic fundus, alcoholism or serious psychological illness and renal or hepatic insufficiency .

5.3 Sample selection and sample size

Calculated by an statistical software property of PhD Marc Saez, based on Sample size Library of the R environment version 3.1.1, accepting an alpha risk of 0,05, a beta risk of 0,2 and a coefficient of variation of 100% from an infinite population, we need 600 patients to demonstrate a difference of 2% in the 5-year cardiovascular morbimortality with and anticipated drop-out rate of 20%.

The sampling method selected will be consecutive, patients who meet our inclusion criteria will be suggested to participate in the study as they come to the consultation at primary care centers.

5.4 Variable definition

Independent variables: Arteriovenous ratio, arteriolar caliber measured in pixels and venular caliber measured in pixels.

Dependent variables: Cardiovascular morbimortality.

Any of the following alterations will be considered morbidity and measured as presence or absence: acute myocardial infarction, angor pectoris, cardiac insufficiency, stroke (hemorrhagic, ischemic and transient ischemic attack), renal insufficiency (GFR <30 ml/min/1,73 m²), dialysis, renal transplantation, Fontaine classification ≥2, amputation, ocular venous thrombosis and grade ≥3 of the Keith, Wagener and Barker classification. For the patients that will experience multiples nonfatal events, the analysis will include just the first event. In the case of nonfatal events and fatal events, just the fatal event will be included in the statistical analysis.

Covariables:

Age, gender, systolic and diastolic office BP measured in mm Hg, systolic and diastolic SMBP measured in mmHg, tobacco consumption defined as patients who had consumed at least 1 cigarette per day in the last 6 months, body mass index (BMI) measured in Kg/m², fasting glucose measured in mg/dL, total cholesterol measured in mg/dL, HDL-cholesterol measured in mg/dL, LDL-cholesterol measured in mg/dL, triglycerides measured in mg/dL, creatinine measured in mg/dL, glomerular filtration rate measured in ml/min/1,73 m² according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), high urinary albumin excretion rate defined as two out of three consecutive test results were required to be positive in order to make the diagnosis, left ventricular hypertrophy defined by Cornell's product, months of evolution, pharmacological treatment, treatment intensity (number of drugs) and use of angiotensin converting enzyme inhibitor(73) and angiotensin II receptor blockers(74).

5.5 Measure instruments

Omron 705 CP (HEM 759 E2, Tokyo, Japan) as blood pressure monitor.

Canon CR6-45NM, Camera EOS D30 (Canon, Melville, New York, USA) as retinograph.

SIRIUS as computer program to analyze retinographies.(40,41)

Weighing machine Atlántida S-11 (Año Sayol, Barcelona, Spain)

Gem Heart One+ as electrocardiograph (GEM-MED, S.L., Barcelona, Spain)

J. Trueta's laboratories will be in charge of analyzing blood and urine samples by standard and validated methods.

5.6 Data collection

Data will be collected in specific formularies that could be found in a specific web site created for this study.

5.7 Work plan

This study will be carried out in 20 urban and rural primary care centers in Girona, the cohort will be recruited during 1 year and hypertension will be diagnosed based on measurements taken by nurses, following the standard conditions recommended by European society of hypertension (Annex 1).

Isolated clinical hypertension will be dismissed by self-measured blood pressure, each patient will be instructed by a trained nurse, the patients would have to perform the process twice in presence of a trained nurse to check his ability to do so and that the measurement is correct.

Two readings will be performed for three consecutive days, first one in the morning before breakfast and the second one in the evening before dinner. First day's readings will be discarded. If the mean BP values are inferior to 135/85 mmHg (systolic and diastolic BP respectively) the diagnosis of isolated clinical hypertension will be made.

At the start of the study, every patient will follow a physical examination, fasting blood and urine analysis, standard 12-lead electrocardiogram, and retinography. A morning

urine sample will be analyzed for the detection of urine albumin excretion rate (UAER), if positive, a reactive strip will look for the presence of leukocytes, red blood cells or nitrites. Two out of three consecutive test results will be required to be positive in order to make the diagnosis of high UAER. During the study, different test will be applied following the Intitut Català de Salut (ICS)(75) recommendations.

SMBP will be measured every year, taking profit of the annual renal examination.

Retinographies will be acquired using a retinograph equipped with a non-mydratiac digital camera, centered at the disc, one per eye.

After 1 year and at the end of the study, a new retinography will be acquired for each patient. All images will be evaluated by an experienced physician and processed using SIRIUS application to automatically obtain the AVR, arteriole and venular caliber.

Patients will be treated using Guidelines of the Health Region (based on the European Society of Hypertension Guidelines) as a reference.

6. Statistical analysis

We will compare the baseline and final values of the variables of interest, at the end of the first year and at the end of the study, both non-stratified and stratified by quartiles of AVR and quartiles of the difference between the final and baseline values of AVR (AVR Qdif). We will use paired-samples t tests and Wilcoxon test for quantitative variables and χ^2 tests for qualitative variables. The linear-by-linear associations between cardiovascular morbimortality and the quartiles of both AVR and the

difference of final and baseline values of AVR, will be tested by means of χ^2 tests; in the case of the expected frequencies where less than 5 the Fisher correction will be applied. Finally, we will adjust the relationship between the fourth quartile of the difference of final and baseline values of retinal abnormalities (AVR, arteriolar and venular caliber) and cardiovascular morbimortality by means of logistic regression. The model will be adjusted for age, gender, baseline clinic BP, difference of final and baseline values of clinic BP, baseline SMBP, difference of final and baseline values of SMBP, body mass index, smoking status, fasting glucose, LDL-cholesterol, months of evolution, treatment, treatment intensity and use of angiotensin converting enzyme inhibitor and angiotensin II receptor blockers.

7. Ethical aspects

This project will be presented for validation to Clinical Research Ethics Committee (CEIC) of the J.Trueta Hospital for its approval. We will guarantee the anonymity of the data in order to preserve patient's confidentiality, the patient's autonomy and its rights based on the Organic Law 15/1999 of 13 December on the Protection of Personal Data, the Basic Law 41/2002 on the autonomy of the patient and the rights and obligations with regard to clinical information and documentation and the Law 14/2007, of 3 July, on Biomedical Research.

Signature of informed consent will be required before the incorporation to the study.

A copy, in Spanish, of this document could be found at Annex 4.

8. Clinical implications

Positive results of this study will approach retinography to the primary care centers as an accessible and low-cost method to routinely monitor hypertensive patients.

This study could redefine the initial alterations of the retinal microvasculature as target organ damage and become retinography an easy and fast method to reclassify into a different cardiovascular risk category and therefore, propose a better treatment plan to the patient.

This study could confirm arteriovenous ratio as a marker of cardiovascular morbimortality.

9. Study limitations

We will use a consecutive sampling method making possible a selection bias, as not all the general population use public health system as first choice.

As we will use new cases of hypertension, we will dispose of a young cohort and our mortality study will be limited as this outcome is more common in older people.

Eye alterations are another important limitation as they could interfere with our retinographic study, mild alterations of the ocular transparency as mild retinal detachment, mild cataract or uveitis, could make retinal microvasculature abnormalities impossible to measure.

10. Budget

The collaboration of 40 medical doctors and their assigned nurses will be required for this study. No extra money will be needed for the majority part of the clinical techniques necessary for the study, since all of them will be made following the current protocol(75), just retinography, as it is not included in the routine evaluation protocol.

The retinograph is already available from previous studies, therefore, no extra money will be required.

Assuming that 40% of the blood pressure monitors are available in the primary care centers, needing 2 monitors per physician, we will have to acquire 48 new monitors, with a cost of 70€ per unit.

A qualified physician will be hired, he will be in charge of retinographies and will be paid 27€ per hour, the estimated time per retinography is 10 minutes and 3 retinographies will be done per patient during the study.

He will also work as a common contact; he will telephone every physician every year until the end of the study, he will be in charge of answering any doubt about the protocol with an estimated duration of 10 minutes per phone call.

A qualified statistician will be hired for the data analysis, he will be paid 35€ per hour, with an estimated time for this work of 30 hours.

A website designer will be hired, he will be in charge of creating (500€) and updating (100€ per year) a specific web site to introduce the data.

An annual meeting will be scheduled to share data and comments about the study, for renting a local and aperitifs 300€ per year will be needed.

English translation of the final article (500€), its presentation by 2 of our physicians in Catalan, Spanish and European congress (500€ for each national congress and person, and 1000€ for the European congress and person) and for its diffusion as open access article (500€).

Cost of the study:

Technician and contact person	9.000 €
Statistician	1.050€
Website	1.000€
Blood pressure monitors	3.360€
Local and meetings	1.500€
Translation and diffusion	5.000€
Total	20.910€

11. Annexes

Annex 1: conditions for blood pressure measurement(11)

When measuring BP in the office, care should be taken:
<ul style="list-style-type: none">• To allow the patients to sit for 3–5 minutes before beginning BP measurements.
<ul style="list-style-type: none">• To take at least two BP measurements, in the sitting position, spaced 1–2 min apart, and additional measurements if the first two are quite different. Consider the average BP if deemed appropriate.
<ul style="list-style-type: none">• To take repeated measurements of BP to improve accuracy in patients with arrhythmias, such as atrial fibrillation.
<ul style="list-style-type: none">• To use a standard bladder (12–13 cm wide and 35 cm long), but have a larger and a smaller bladder available for large (arm circumference >32 cm) and thin arms, respectively.
<ul style="list-style-type: none">• To have the cuff at the heart level, whatever the position of the patient.
<ul style="list-style-type: none">• When adopting the auscultatory method, use phase I and V (disappearance) Korotkoff sounds to identify systolic and diastolic BP, respectively.
<ul style="list-style-type: none">• To measure BP in both arms at first visit to detect possible differences. In this instance, take the arm with the higher value as the reference.
<ul style="list-style-type: none">• To measure at first visit BP 1 and 3 min after assumption of the standing position in elderly subjects, diabetic patients, and in other conditions in which orthostatic hypotension may be frequent or suspected.
<ul style="list-style-type: none">• To measure, in case of conventional BP measurement, heart rate by pulse palpation (at least 30 s) after the second measurement in the sitting position.

Annex 2: Definitions of hypertension by office and out-of-office blood pressure levels(11)

Category	Systolic BP (mmHg)		Diastolic BP (mmHg)
Office BP	≥140	and/or	≥90
Ambulatory BP			
Daytime (or awake)	≥135	and/or	≥85
Nighttime (or asleep)	≥120	and/or	≥70
24-h	≥130	and/or	≥80
Home BP	≥135	and/or	≥85

Annex 3: Clinical indications for out-of-office blood pressure measurement(11)

Clinical indications for HBPM or ABPM
• Suspicion of white-coat hypertension
- Grade I hypertension in the office
- High office BP in individuals without asymptomatic organ damage and at low total CV risk
• Suspicion of masked hypertension
- High normal BP in the office
- Normal office BP in individuals with asymptomatic organ damage or at high total CV risk
• Identification of white-coat effect in hypertensive patients
• Considerable variability of office BP over the same or different visits
• Autonomic, postural, post-prandial, siesta- and drug-induced hypotension
• Elevated office BP or suspected pre-eclampsia in pregnant women
• Identification of true and false resistant hypertension
Specific indications for ABPM
• Marked discordance between office BP and home BP
• Assessment of dipping status
• Suspicion of nocturnal hypertension or absence of dipping, such as in patients with sleep apnoea, CKD, or diabetes
• Assessment of BP variability

HOJA DE INFORMACIÓN PARA EL PACIENTE

TÍTULO DEL ESTUDIO: “Evolución de las alteraciones de la microvasculatura retiniana y su asociación a la morbilidad cardiovascular”

Lugar de realización: Centros de atención primaria de Girona

Finalidad: Determinar si la evolución de las alteraciones de la microvasculatura retiniana, están relacionadas con la morbilidad cardiovascular.

Beneficios para el paciente: La realización de estudio conllevará un mejor conocimiento de la patología hipertensiva, lo que se traducirá en una posible mejora en el tratamiento de ésta. Durante el estudio, el paciente será controlado de manera continua con uno de los métodos que se ha demostrado, está mejor relacionado con la mortalidad que la presión arterial clínica, la automedida de la presión arterial.

Riesgos asociados: El paciente seguirá el protocolo general del Institut Català de Salut (ICS) y además, realizará una retinografía, que al no usar elementos midriáticos carece de riesgos para el paciente, y la automedida de la presión arterial, la cual no está asociada a ningún efecto adverso.

Extensión y duración: El paciente seguirá el protocolo de actuación del ICS y llevará acabo 3 retinografías, una al inicio, otra transcurrido un año y una final, al quinto año del estudio. La automedida de la presión arterial se realizará a lo largo de los 5 años de estudio.

¿Por qué hacemos este estudio? Determinados sujetos pueden presentar alteraciones a nivel de la microvasculatura retiniana asociadas a la hipertensión. Nuestro objetivo es identificar estas lesiones y su evolución, para comprobar si están asociadas con la morbilidad cardiovascular.

¿Por qué han pensado en incluirle en el estudio? Su médico ha detectado que sufre una enfermedad llamada hipertensión, al ser un nuevo diagnóstico y no haber sido

nunca tratado de esta patología, usted sería un candidato idóneo ya que cumple todos los criterios inclusión y ninguno de exclusión establecidos para el estudio.

¿Qué le pedimos que haga? Su participación en el estudio es totalmente voluntaria. Le pedimos que asista a los controles que se le realizarían igualmente de forma rutinaria para el control de su hipertensión arterial y que realice dos pruebas más, las cuales no están dentro del protocolo estándar de la atención a su patología.

Una retinografía, la cual consiste en un proceso indoloro en el cual se le realizará una fotografía de la retina, que corresponde a la estructura situada en el fondo del ojo, y que haga un seguimiento de su presión arterial desde su propia casa a través de un dispositivo que le proporcionaremos, y anote los valores, los cuales nos deberá entregar en las visitas correspondientes.

¿Cómo se protegerá su intimidad, autonomía y derechos inherentes? Este estudio sigue las leyes actuales del estado en lo referente a materia de investigación, en este caso la Ley Orgánica 15/1999, de Protección de Datos de Carácter Personal, la Ley 41/2002 básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica y la Ley 14/2007 de investigación biomédica.

Usted tiene derecho a revocar el consentimiento en cualquier momento, sin perjuicio de su tratamiento médico, a decidir el destino de sus muestras y datos personales en caso de decidir retirarse del estudio.

Este estudio ha sido aprobado por el Comité Ético de Investigación Clínica (CEIC) del Hospital Josep Trueta de Girona. Tanto si finaliza el estudio como si no, sus datos serán confidenciales, y se le garantiza que su nombre no saldrá en ninguna publicación o informe relativo al estudio.

CONSENTIMIENTO INFORMADO POR ESCRITO

Título del estudio: “Evolución de las alteraciones de la microvasculatura retiniana y su asociación a la morbimortalidad cardiovascular”

Centro:

Datos del participante/paciente:

Persona que proporciona la información y la hoja de consentimiento:

Yo.....

(nombre y apellidos del paciente escritos por él)

Doy mi pleno consentimiento, de manera libre, para participar en este estudio. He leído la hoja informativa sobre el proyecto. He comprendido que la investigación es una contribución a los conocimientos médicos. Sé que puedo retirar mi consentimiento en cualquier fase del procedimiento.

Estoy de acuerdo en que se utilicen mis datos para el estudio indicado y los posibles estudios que se deriven de él.

Doy mi permiso para que los datos de mi historia clínica, sean utilizados por el equipo de investigación para fines relacionados con este estudio, entendiendo que tras su comprobación se eliminará del registro toda información que pudiera identificarme.

Se me ha entregado una copia de la Hoja de Información al Participante y una copia de este Consentimiento Informado, fechado y firmado.

Se me han explicado las características y el objetivo del estudio y los posibles beneficios y riesgos del mismo.

Se me ha dado tiempo y oportunidad para realizar preguntas. Todas las preguntas fueron respondidas a mi entera satisfacción.

Sé que se mantendrá la confidencialidad de mis datos.

El consentimiento lo otorgo de manera voluntaria y sé que soy libre de retirarme del estudio en cualquier momento del mismo, por cualquier razón y sin que tenga ningún efecto sobre mi tratamiento médico futuro.

He podido hacer preguntas sobre el estudio.

He recibido suficiente información sobre el estudio y he comprendido que este trabajo es una contribución a los conocimientos médicos .

Comprendo que mi participación es voluntaria.

Fecha Nombre y Apellidos del participante Firma del participante

Confirmando que he explicado al participante el carácter y el propósito del proyecto de investigación.

Firmado _____ (miembro del equipo del proyecto)

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