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Non-invasive evaluation of myocardial supply-demand balance from the analysis of pulse waveform: from validation to clinical application

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List of publications (2015 – 2018)

Some of the concepts and figures presented in this work have appeared in the publications produced in my years of PhD, which are the following:

1. Noninvasive estimation of central blood pressure and analysis of pulse waves by applanation tonometry. P Salvi, A Grillo, G Parati - Hypertension Research, 2015
2. Validation of noninvasive devices for central blood pressure assessment: a yet unresolved issue. P Salvi, A Grillo, G Parati - Journal of hypertension, 2016
3. Arterial stiffening, pulse pressure, and left ventricular diastolic dysfunction. P Salvi, A Grillo, JE Ochoa, G Parati - European journal of heart failure, 2016
4. Cardio-ankle vascular stiffness index (CAVI) and 24 h blood pressure profiles. A Grillo, LM Lonati, V Guida, G Parati - European Heart Journal Supplements, 2017
5. Influence of carotid atherosclerotic plaques on pulse wave assessment with arterial tonometry. A Grillo, G Simon, P Salvi, M Rovina, C Baldi, I Prearo, et al. - Journal of hypertension, 2017
6. Short-term repeatability of noninvasive aortic pulse wave velocity assessment: comparison between methods and devices. A Grillo, G Parati, M Rovina, F Moretti, L Salvi, et al. - American journal of hypertension, 2017
7. Aortic dilatation in Marfan syndrome: role of arterial stiffness and fibrillin-1 variants. P Salvi, A Grillo, S Marelli, L Gao, L Salvi, M Viecca, et al. - Journal of hypertension, 2018
8. Morning blood pressure surge: pathophysiology, clinical relevance and therapeutic aspects. G Bilo, A Grillo, V Guida, G Parati - Integrated blood pressure control, 2018
9. Cardio–ankle vascular index and carotid–femoral pulse wave velocity: limits and strengths. A Grillo, P Salvi - Journal of hypertension, 2018
10. Systolic time intervals assessed from analysis of carotid pressure waveform. P Salvi, A Grillo, I Tan, G Simon, L Salvi, L Gao, M Rovina, M Butlin, et al. - Physiological measurement, 2018

Abstract

The evaluation of the balance between oxygen supply and demand in the myocardium is useful for predicting and diagnosing myocardial ischemia and type-2 myocardial infarction, conditions that represent a growing part of the health burden of cardiovascular disease, and whose incidence is rapidly increasing due to an ageing population. In its original assessment by invasive registrations, this balance is calculated as the ratio between the oxygen supply, defined as the area between the aortic and left ventricular pressures during diastole (diastolic pressure-time index), and the oxygen consumption, defined as the area under the pressure curve during systole (systolic pressure-time index). This ratio is called SEVR (Subendocardial Viability Ratio) and may also be calculated from the analysis of the non-invasively determined central pressure wave obtained by carotid arterial tonometry, by dividing areas between the diastolic and systolic pressure curves. The conventional non-invasive assessment of SEVR by arterial tonometry is affected by some methodological limitations, that are the exclusion from the calculation of isovolumetric systolic time in the systolic pressure-time index and the exclusion of left ventricular diastolic pressure from diastolic pressure-time index. Moreover, the calibration of central pressure wave derived from carotid tonometry can be affected by the way of calculating mean arterial pressure from brachial cuff blood pressure, which is necessary for scaling the central waveform. This thesis presents a series of studies conducted to overcome the limitations mentioned above, in order to elaborate a corrected form of the SEVR and to validate it against its invasive counterpart and as a clinical predictor.

A methodology to reliably calculate the systolic-time intervals (isovolumetric ejection time and pre-ejection period) from ECG-gated arterial tonometry performed at the carotid and femoral levels, is presented and applied in subjects with or without cardiovascular disease. The issue of calculation of mean arterial pressure from brachial cuff blood pressure was then addressed, as a considerable interindividual and intraindividual variability in brachial pressure form-factor was evidenced in general population of different ages and in hypertensive patients. The best approach for calibration of non-invasive central blood pressure waveform resides in the integration of pressure waveforms, or, when not applicable, in the use of an appropriate algorithm for calculation of brachial form factor. A good correlation of the invasively determined SEVR, in patients undergoing cardiac catheterization, was then demonstrated with the new non-invasive SEVR calculated by arterial tonometry and corrected by considering systolic time intervals and the left ventricular diastolic pressure. An equation for the estimation of left ventricular diastolic pressure was derived from non-invasive parameters of arterial tonometry and the invasive data. The new SEVR was finally applied in the PARTAGE cohort, a large population study of individuals 80 years of age and older living in nursing homes. SEVR was found to be an independent predictor of total mortality in the elderly subjects. A threshold value for SEVR of 100 may be considered in this population.

In summary, a new formulation of an index (SEVR) for the evaluation of myocardial supply-demand balance from non-invasive arterial tonometry was created and clinically validated.

1. Introduction

Cardiovascular disease is the leading clinical condition causing mortality and morbidity worldwide, largely due to the ageing of population [1]. Despite the great improvements in prevention and in therapeutic approaches, in cardiovascular medicine, many clinical situations still cannot be easily categorized. It is frequent that, due to the coexistence of multiple pathologies in a single individual, diagnosing and treating a clinical condition is not an easy task. A thorough analysis of comorbidities and of multiple factors which may affect a patient in a single moment should be performed to achieve a substantial benefit, without causing harm. Therefore, a special effort should be made to improve diagnosis and prevention of complex cardiovascular conditions, with new tools which may adopt a multiparametric approach to the diseased patient.

In a public health perspective, technological improvements led to a considerable progress in diagnosing cardiovascular disease, in a level of detail that was unimaginable until a few decades ago. Technologies like cardiac magnetic resonance imaging or 3-dimensional echocardiography have significantly improved the accuracy in the diagnosis, but with the downside of a steep increase in the costs of treating patients. The highest increase in costs for treating cardiovascular disease is expected to be in the elderly, for ageing of the population and for the treatment improvements in younger patients [2].

The patient with whom the physician treating cardiovascular conditions is more likely to relate is the patient with multiple comorbidities, which undermine the normal cardiovascular compensatory functions. These patients are exposed to a greater risk of developing myocardial ischemia. The concept of myocardial ischemia is often commonly associated with the etiology of coronary atherosclerosis as the only cause. Nevertheless, the expert consensus document on myocardial infarction, in 2012, clarified that type-2 myocardial infarction occurs when the presence of an imbalance between the supply and the demand of oxygenated blood to the heart, even in the absence of coronary heart disease, causes myocardial damage and necrosis [3]. It should be acknowledged that type-2 myocardial infarction is not an uncommon condition, and for the reasons explained above its incidence is currently increasing, particularly in the elder age classes. In patients over 75 years of age type-2 myocardial infarction is much more frequent than the type-1 myocardial infarction, which is due to plaque thrombosis [4]. It should be given considerable importance to this condition, because of the high mortality characterizing type-2 myocardial infarction, which is double compared to myocardial infarction type-1 [5].

Considered the physiopathological basis of type-2 myocardial infarction, there is an unmet need for the diagnosis of this condition, that is currently largely under- or mis-diagnosed. Moreover, even preclinical conditions may be relevant, where an overt myocardial infarction type-2 is not yet manifested, as the imbalance between oxygen supply and demand may lead to the development of myocardial ischemic alterations and expose to the risk of adverse outcomes. A sufficiently accurate and minimally invasive method that can identify a situation of imbalance between the need and the blood supply in the heart is currently lacking.

In the last decades the study of the mechanics of the great arteries has been enriched

by some instruments that, with different modalities, are able to detect through non-invasive method the central arterial pressure, i.e. the pressure within the ascending aorta. From the analysis of the central pressure curve it is possible to derive numerous data about the balance between the supply and demand of oxygen and metabolites at the myocardium. In this regard, the present study proposes the validation of a new form of the Buckberg index or Subendocardial Viability Ratio (SEVR), obtained by the noninvasive evaluation of central blood pressure wave.

The SEVR was conceived in 1972 to diagnose and to quantify the balance between the supply and demand of blood to the heart. Invasive SEVR was able to identify, case by case, any situation with a potential ischemic risk but, for its calculation, it was necessary to record intraventricular and intra-aortic pressures with invasive cardiac catheterization. The advent of non-invasive methods for the measurement of central aortic pressure has led, in the following decades up today, to the reformulation of SEVR obtained from the analysis of the aortic pressure curve alone, with a few substantial limitations due to the differences from invasive and non-invasive measurement of pressure waves.

The new definition of the SEVR formula, which this work proposes to validate and to apply in a clinical scenario, has been rewritten with the aim of deriving a parameter that was as much as possible overlapping the original "old" (but more accurate) Buckberg index, by using only data obtained with a non-invasive method. The present work aims to identify a method that can be useful for everyday clinical practice to diagnose conditions at high risk of the developing myocardial ischemia, in patients with multiple comorbidities which are currently diagnosed and followed-up with expensive imaging modalities. The validation of a new tool could be useful for the cost reduction of diagnosing cardiovascular conditions in the elderly and in patients with multiple comorbidities, in which the increase in costs for treating cardiovascular disease is expected to increase in the near future [2][6].

In our work we first approached the physiopathological basis of SEVR to overcome the limitations of its non-invasive calculation. We considered and validated a new way of calculating the systolic time intervals from non-invasive arterial tonometry, in order to include this parameter in the non-invasive SEVR formulation. We revisited the calibration of non-invasive central pressure waves and proposed a calibration with brachial blood pressure to avoid limits of imperfect estimations of pressure amplification in the arterial tree. We than properly validated the new non-invasive SEVR against its invasive counterpart, the Buckberg index obtained by cardiac catheterization. Lastly, we applied the new SEVR to a population in which its application could be crucial, the PARTAGE cohort, consisting of patients older than 80 years, by exploring the predictive ability for mortality and cardiovascular disease of the new SEVR in this high-risk population.

2. Background

2.1 Principles of vascular hemodynamics

The cardiovascular system is often considered a simple hydraulic circuit, composed of a pump (heart) with a rhythmic activity, that pushes a liquid (blood) into a tube (the aorta), which divides repeatedly in order to reach the peripheral tissues. Its main function is to warrant the transport of oxygen and nutrients to peripheral tissues, in addition to ensure the hormonal regulation and cell signaling mechanisms through the molecules carried by blood. However, this simple view does not consider the prominent role of aorta and the large elastic vessels, which play a major role in the regulation of blood pressure and peripheral blood flow. Aorta and the large arteries have the task of damping the pulsatile output of the left ventricle and of changing the rhythmic, intermittent, and discontinuous activity of the cardiac pump into a continuous one.

The phenomenon for which the rhythmic and intermittent regimen of the heart pump is transformed into a continuous regime is called Windkessel phenomenon, from the system that was used by firefighters until the last century to ensure a continuous and homogeneous flow of water through an air chamber: part of the water was directly expelled outside, while the remainder was stored in a closed tank where it was compressed by the presence of air. The functioning of the vascular system is similar: after the systolic stroke and the closing of the aortic valves, about 60% of the blood is stored at the root of the arterial tree thanks to its distensibility, to be then released during diastole with the transformation of the potential energy in kinetic energy, ensuring the maintenance of adequate pressure levels during the entire duration of the cardiac cycle. The aorta and the large vessels therefore behave like a diastolic phase pump, reducing the work required for the heart muscle.

The composition of aortic and large arteries wall is very important, since they have not only the task of bringing blood to the periphery but also to perform a buffer function. This is allowed by the viscoelastic properties of elastic vessels, made up of large amounts of elastin, a very distensible protein. The component counteracting an excessive relaxation is collagen: the relationship between these two types of fibers (elastin and collagen) is therefore important to allow a good elasticity to the vessels and a good peripheral perfusion.

With aging, the elastin to collagen balance is impaired, as the percentage of collagen fibers increases and the structure of elastic vessels layers change. There is an increase in elastase activity associated with a decreased ability to synthesize elastin, with a decrease of this protein in the aortic wall; this phenomenon is associated with the fragmentation of elastin fibers and the modification of them at the level of the amino acid structure. The reduction in elasticity could also be caused by the deposition of calcium in the medial layer, especially on the elastic fibers located in the median part. At macroscopic level, aortic dilatation in the elderly subject is particularly evident, due to the lack of elastin fibers, and a thickening of the wall for cell hypertrophy and fibrosis, with accumulation of extracellular matrix components, for a greater expression of matrix metalloproteases with less expression of their tissue inhibitors.

In pathological conditions the anatomical, structural and functional characteristics of the large arteries can change, and consequently their mechanical properties. Therefore, arterial stiffening does not happen only during aging, but also in conditions

like arterial hypertension, metabolic alterations and inflammatory states [7]. Similar structural alterations have also been reported in other pathologies, for example in hepatic insufficiency, in calcium dysmetabolism, in oxidative stress, in chronic subclinical inflammation, in vasculitis and in the course of other metabolic diseases[8]. Genetic diseases, such as Ehlers-Danlos syndrome and Marfan syndrome, characterized by the synthesis of mutated fibrillin-1 (FBN1), can also determine arterial stiffness and increase the pulse wave velocity; FBN1 is in fact a structural protein that has an important role in determining the structure of the arterial wall [9]. The result will be: an increase in systolic pressure (with an increase in the left ventricular afterload, which can lead to hypertrophy and predispose to the insufficiency of this chamber, up to heart failure), a reduction in diastolic pressure (with reduction of subendocardial perfusion which occurs in this phase) and consequently an increase in the differential pressure (with possible damage to the kidney and the brain, organs that receive large quantities of the systolic output).

Central blood pressure

The term "central blood pressure" indicates the arterial pressure at the level of the ascending aorta, immediately at the exit from the left ventricle.
Recent studies have shown the importance of this parameter and of the central pulse pressure, obtainable from the difference between central systolic pressure and central diastolic pressure, as cardiovascular prognostic factors. In particular, a substudy of the ASCOT (Anglo-Scandinavian Cardiac Outcomes Trial) trial (which highlighted that patients treated with calcium channel blockers had less cardiovascular events than patients treated with beta-blockers, regardless of the brachial arterial pressure values), that is the CAFE (Conduit Artery Functional Evaluation) study, showed that patients treated with calcium channel blockers presented a reduction in central blood pressure and central pulse pressure, despite having similar brachial pressures to those treated with beta-blockers [10]. From this and other subsequent studies [11][12] [13], it became evident that peripheral arterial pressure is not always the best method to evaluate the efficacy of an antihypertensive drug, since the actual work to which the left ventricle is subjected may be better assessed by central pressures analysis.
Mean arterial pressure and diastolic arterial pressure are relatively constant along the arterial tree, compared to very different systolic blood pressure values [14] (Figure 1). In fact, these are much higher in the periphery than in the aorta, thanks to the phenomenon of arterial pressure amplification.

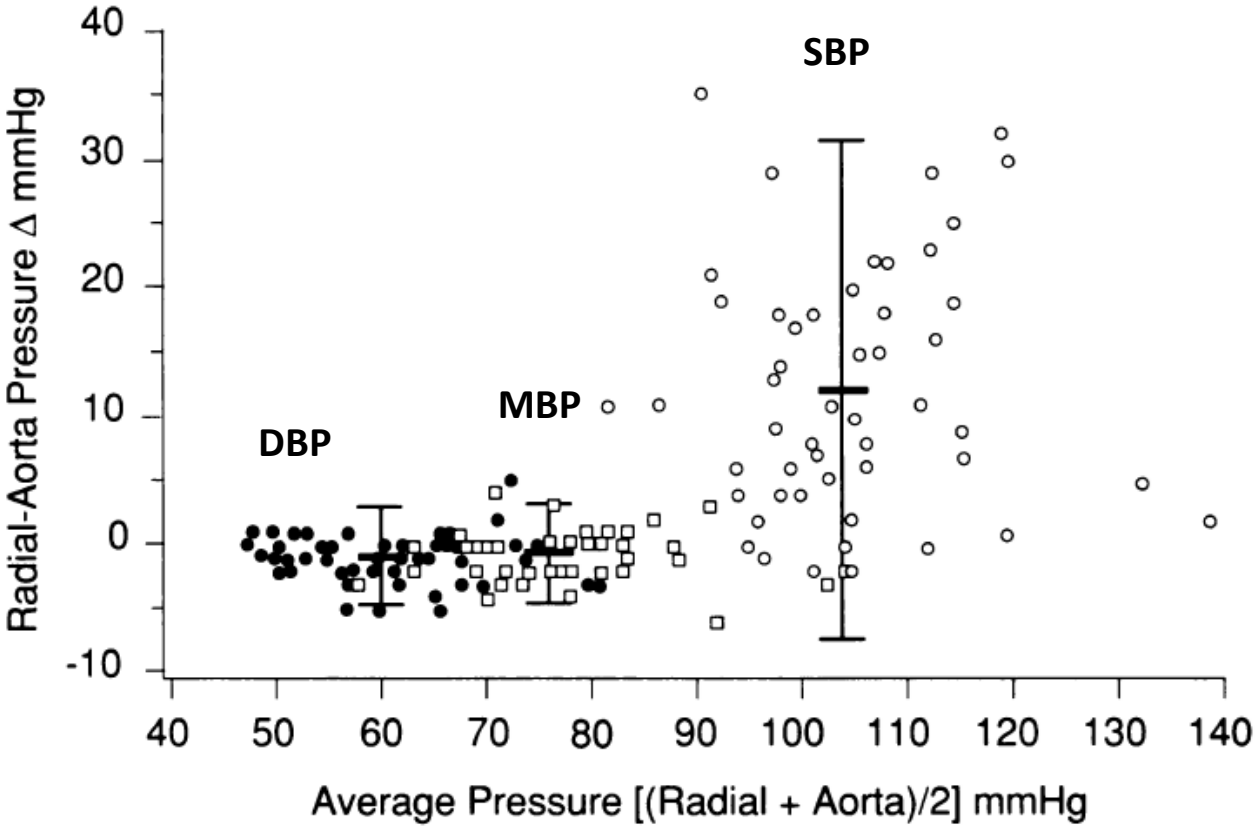

Figure 2.1. Radial and aortic pressures. DBP, MBP, SBP: diastolic, mean and systolic blood pressures. Adapted from Pauca et al., Chest 1992 [14].

This can be explained by the mechanism of wave reflections: in any hydrodynamic circuit, in the presence of an intermittent pump that generates a wave propagating along a conduit, there will be the onset of reflected waves in the reflection sites. These reflected waves are directed towards the centre of the system and sum up to the waves arriving from the centre following the subsequent cardiac systole. In the case of the cardiovascular system, however, these pressure waves are transmitted so quickly that they are also added to the same direct wave that generated them, conditioning the morphology of the entire pressure wave. The arterial pressure is therefore the result of the sum of a direct (centrifugal) wave and of reflected pressure waves (centripetal).
In the cardiovascular system there are many sites of reflection: the bifurcations of the arteries, the atheromatous plaques that cause narrowings and obstructions, and the terminal arterioles, which define the peripheral vascular resistance.
At the peripheral arteries (brachial, radial, femoral) the overlap between the direct pressure wave and the reflected wave, which occurs very early in the diastolic phase due to the proximity of these arteries to the main sites of reflection, is particularly important. As a consequence, the pressure peak will be strongly influenced by the return of the reflected waves.
Consequently, in subjects with normal viscoelastic properties of the large intact arteries there will be a systolic peak not influenced by the return of the reflected waves, and a modified diastolic phase, filled by the effect of these waves. In this way a prolongation of high pressure values during the diastolic phase will be guaranteed, allowing a good coronary flow. In a subject with high arterial stiffness, at the central level, an increase in the values of systolic pressure and of pulse pressure, and

therefore of the post-load of the left ventricle, associated with an reduced diastolic phase without the contribution of the reflected waves. The phenomenon of the "amplification" of blood pressure (i.e. the difference between peripheral and central systolic blood pressure) is influenced by several factors: the viscoelastic properties of the large vessels; the extent and variability of reflected waves; the length of the aorta; heart rate; the phenomenon of pressure wave attenuation.

Central pressure wave measurement

The availability of transcutaneous tonometers able to measure pressure waveform non-invasively, led to in-depth studies about the role of the mechanics of large arteries in the pathophysiology of arterial hypertension and heart disease. The outcomes of several epidemiological clinical trials pointed out some peculiar aspects of vascular hemodynamics, showing aortic stiffness as an independent predictor of cardiovascular mortality, stressing the importance of pulse wave velocity measurement [15][16]. Moreover, some studies have already highlighted the importance of central systolic blood pressure and of central pulse pressure as cardiovascular prognostic factors, more significant than peripheral blood pressure values measured in the brachial artery, as previously mentioned. Therefore, it is very important that central blood pressure values recorded with this method are reliable and that the parameters relative to the central arterial pressure waveform correspond to the ones recorded in ascending aorta. Arterial tonometry is based on the principle of applanation tonometry. It is a noninvasive, reproducible, well tolerated and fast test, which enables evaluation of those arterial districts where the artery runs superficially and where its compression against underlying structures occurs, i.e., at the level of the carotid, brachial, radial, femoral, and posterior tibial and dorsalis pedis arteries.

The "direct method" for evaluating central blood pressure is based on the recording of pulse wave in common carotid artery; it is a surrogate for aortic pressure because of the closely proximity of these arterial sites. Moreover, the carotid artery is generally well accessible and superficial and good quality carotid waveforms can be easily obtained even in obese patients. The technique is reliable for routine high-throughput screening of central pressure.

The validity of transcutaneous tonometry in measuring the aortic central pressure wave is based on two principles, both widely validated and tested.

1) First of all, the sphygmic waves recorded from transcutaneous tonometry are superimposable onto those recorded by means of catheterization. During some hemodynamic sessions, while a catheter, inserted into the origin of the common carotid artery, was recording the pressure wave, carotid transcutaneous tonometry was performed simultaneously; the two pressure waves were absolutely superimposable.

However, the most accurate method to compare two periodic pressure waves is by analyzing the first harmonics of the arterial pressure wave. It is well known that the analysis of the first six harmonics accurately defines the pressure waveform. Moreover, the analysis of each harmonic has confirmed that the two pressure waves, recorded both noninvasively and invasively, are perfectly superimposable.[17].

2) It is important to note that the sphygmic waves recorded in the carotid artery are similar to the ones recorded in the ascending aorta. While a catheter, placed in the ascending aorta, was recording the pressure wave, carotid transcutaneous tonometry was performed simultaneously: the two pressure waves were nearly superimposable. The analysis of the first six harmonics showed only a slight, and insignificant, difference in the first harmonic of the pressure wave too. However, the difference

between the two pressure wave values was less than 5 mmHg.
The main limitation of applanation tonometry is that it cannot provide absolute values of arterial pressure. A tonometer is able to define pulse pressure values, but it is unable to provide accurate values of diastolic and systolic blood pressure. These are gathered starting from the previously mentioned concept that mean arterial pressure remains constant from the aorta to the peripheral arteries, as does diastolic blood pressure (which tends to decrease, albeit insignificantly, i.e., by less than 1 mmHg, from the center to the periphery). To sum up, a calibration of tonometric pressure wave using brachial arterial pressure values is always required, in particular when central blood pressure is estimated through the analysis of more or less peripheral pulse waves.
Theoretically, the most reliable method to derive calibrated central pressure values from analysis of radial or carotid waveforms should be based on brachial pulse waveform recordings, calibrated by systolic and diastolic blood pressures measured through a validated sphygmomanometer at the same brachial artery level. The mean arterial pressure so calculated from the integral of the brachial pressure waveform can then be used, together with the corresponding diastolic blood pressure, to calibrate pulse waveforms recorded elsewhere.
The analysis of central pulse wave is likely to lead to significant advantages in daily clinical practice. High blood pressure values have been recognized as the main risk factor for cardiovascular mortality and morbidity and the reduction of blood pressure, measured with sphygmomanometer, has significantly reduced cardiovascular mortality in Western countries. However, the suitability and accuracy of traditional blood pressure measurements for cardiovascular risk staging is now a matter of lively debate. Over recent years, pulse waveform analyses have experienced a revival. Indeed, the technique of pulse wave analysis, using noninvasive high fidelity arterial tonometers, has recently become increasingly popular. This method can provide not only quantitative, although indirect, information concerning the levels of central blood pressure, but also qualitative data on the ascending aortic waveform. Analysis of central waveforms can, in fact, define the elastic properties of the arterial wall and offer additional information about the heart physiopathology, which can help in evaluating the cardiovascular profile of the single patient.

2.2 Coronary circulation

Coronary circulation is the circulation of blood in the blood vessels that supply the heart muscle (myocardium). Coronary arteries supply oxygenated blood to the heart muscle, and cardiac veins drain away the blood once it has been deoxygenated.

Structure

Coronary arteries supply blood to the myocardium and other components of the heart. Two coronary arteries originate from the left side of the heart at the beginning (root) of the aorta, just after the aorta exits the left ventricle. There are three aortic sinuses (dilations) in the wall of the aorta just superior to the aortic semilunar valve. Two of these, the left posterior aortic sinus and anterior aortic sinus, give rise to the left and right coronary arteries, respectively. The third sinus, the right posterior aortic sinus, typically does not give rise to a vessel. Coronary vessel branches that remain on the surface of the artery and follow the sulci of the heart are called epicardial coronary arteries.

The left coronary artery distributes blood to the left side of the heart, the left atrium and ventricle, and the interventricular septum. The circumflex artery arises from the left coronary artery and follows the coronary sulcus to the left. Eventually, it will fuse with the small branches of the right coronary artery. The larger anterior interventricular artery, also known as the left anterior descending artery, is the second major branch arising from the left coronary artery. It follows the anterior interventricular sulcus around the pulmonary trunk. Along the way it gives rise to numerous smaller branches that interconnect with the branches of the posterior interventricular artery, forming anastomoses. Anastomosis are areas where vessels unite to form interconnections that normally allow blood to circulate to a region even if there may be partial blockage in another branch. The anastomoses in the heart are very small. Therefore, this ability is somewhat restricted and a coronary artery blockage often results in myocardial infarction causing death of the cells supplied by the particular vessel.

The right coronary artery proceeds along the coronary sulcus and distributes blood to the right atrium, portions of both ventricles, and the heart conduction system. Normally, one or more marginal arteries arise from the right coronary artery inferior to the right atrium. The marginal arteries supply blood to the superficial portions of the right ventricle. On the posterior surface of the heart, the right coronary artery gives rise to the posterior interventricular artery, also known as the posterior descending artery. It runs along the posterior portion of the interventricular sulcus toward the apex of the heart, giving rise to branches that supply the interventricular septum and portions of both ventricles.

Under the most common configuration of coronary arteries, there are three areas of anastomoses. Small branches of the left anterior descending/anterior interventricular branch of the left coronary join with branches of the posterior interventricular branch of the right coronary in the interventricular sulcus. More superiorly, there is an anastomosis between the circumflex artery (a branch of the left coronary artery) and the right coronary artery in the atrioventricular groove. There is also an anastomosis between the septal branches of the two coronary arteries in the interventricular septum. The left and right coronary arteries occasionally arise by a common trunk, or their number may be increased to three; the additional branch being the posterior

coronary artery (which is smaller in size). In rare cases, a person will have the third coronary artery run around the root of the aorta.
The artery that supplies the posterior third of the interventricular septum – the posterior descending artery determines the coronary dominance. If the posterior descending artery is supplied by the right coronary artery, then the coronary circulation can be classified as "right-dominant". If the posterior descending artery is supplied by the circumflex artery, a branch of the left artery, then the coronary circulation can be classified as "left-dominant". If the posterior descending artery is supplied by both the right coronary artery and the circumflex artery, then the coronary circulation can be classified as "co-dominant". Approximately 70% of the general population are right-dominant, 20% are co-dominant, and 10% are left-dominant. A precise anatomic definition of dominance would be the artery which gives off supply to the AV node i.e. the AV nodal artery. Most of the time this is the right coronary artery.

Function

From a functional point of view, the coronary arterial vessels can be divided into two main types: the conductance vessels (large epicardial branches and their main branches) and the resistance vessels (prearterioles and arterioles). Vessels with a diameter of more than 500 microns do not offer significant resistance to blood flow, whereas resistance vessels cause a marked drop in perfusion pressure.
The prearterioles, vessels with a diameter between 100 and 500 microns, perform the function of self-regulation of the coronary flow, keeping it constant in the face of changes in the aortic pressure: they are forced when it increases and dilates when it decreases instead. The tone of these vessels is also influenced by neurohumoral and endothelium-mediated mechanisms.
The arterioles, on the other hand, have a diameter of less than 100 microns and regulate the flow based on the oxygen need of the tissue, dilating when myocardial oxygen consumption increases and forcing itself when it reduces. The tone of these vessels is also influenced by neurohumoral factors.
Under basal conditions, the extraction of oxygen by the myocardiocytes is about 70%; as a result, the only way to increase the oxygen intake under increased conditions need is a proportional increase in coronary flow, given by arteriolar vasodilation. With coronary reserve we indicate the capacity of maximum increase of the flow, with respect to the basal value, in response to a metabolic stimulus.
The main regulating factor of the coronary flow is the metabolic demand of the heart muscle: if it increases, there will be hydrolysis of ATP with release of adenosine in the interstitium and consequent arteriolar vasodilation, with an increase in blood flow proportional to the demand. The other factors involved in the regulation of the coronary circulation are coronary innervation, circulating vasoactive substances (such as catecholamines) and synthesized from the endothelium (such as nitric oxide). During maximal oxygen demand the flow can increase up to 5 times compared to baseline.
The coronary flow mainly depends on the endoluminal perfusion pressure during diastole, due to the occlusion of intramural vessels in systole determined by the ventricular contraction. In fact, in the presence of tachycardia there will be a shortening of the duration of diastole with predisposition to the development of myocardial ischemia (also favored by the increase of myocardial oxygen demand present in this situation). In particular, the septal branches of the anterior descending artery will be more easily compressed by the ventricular contraction of the

surrounding muscle.
Under physiological conditions, the myocardium presents a strictly aerobic metabolism, so it is possible to calculate its overall metabolism by determining its oxygen requirement. During contraction of the ventricular myocardium (systole), the subendocardial coronary vessels (the vessels that enter the myocardium) are compressed due to the high ventricular pressures. This compression results in momentary retrograde blood flow (i.e., blood flows backward toward the aorta) which further inhibits perfusion of myocardium during systole. However, the epicardial coronary vessels (the vessels that run along the outer surface of the heart) remain open. Because of this, blood flow in the subendocardium stops during ventricular contraction. As a result, most myocardial perfusion occurs during heart relaxation (diastole) when the subendocardial coronary vessels are open and under lower pressure. Flow never comes to zero in the right coronary artery, since the right ventricular pressure is less than the diastolic blood pressure.
The heart regulates the amount of vasodilation or vasoconstriction of the coronary arteries based upon the oxygen requirements. Failure of oxygen delivery caused by a decrease in blood flow in front of increased oxygen demand of the heart results in tissue ischemia, a condition of oxygen deficiency. Brief ischemia is associated with intense chest pain, known as angina, and severe ischemia can cause the heart muscle to die from hypoxia, such as during a myocardial infarction. Chronic moderate ischemia causes contraction of the heart to weaken, known as myocardial hibernation. In addition to metabolism, the coronary circulation possesses unique pharmacologic characteristics, as the reactivity to adrenergic stimulation.

Myocardial ischemia

The term ischemic cardiopathy indicates a set of clinical pictures characterized by the development of myocardial ischemia, suffering or damage of myocardial cells resulting from an insufficient supply of oxygen with respect to their metabolic demands.
It represents the heart disease with greater incidence and prevalence in developed countries, with a prevalence of 6.2% in the American population [6]. In Italy it is responsible for 35% of deaths due to cardiovascular diseases, corresponding to about 130000 people per year; has an incidence of about 120,000 new cases per year and a prevalence of around 4% [18].
The most frequent cause of myocardial ischemia is the development of stenosis in the coronary epicardial arterial vessels, due to the presence of atherosclerotic plaques. When a stenosis reduces the lumen by at least 50%, there will be a reduction in the coronary reserve and resistance to the flow at rest, with a pressure drop downstream of the stenosis. If the vessel's caliber is reduced by 80% already at rest, the coronary flow will be insufficient with possible myocardial ischemia. Thrombus formation may also occur in a coronary epicardial branch, often at the level of a complicated atherosclerotic plaque, with subtotal or total vessel occlusion. Another possible cause of coronary reserve reduction is a dysfunction of the coronary microcirculation due to structural alterations (such as hypertrophy or fibrosis) that reduce its capacity for vasodilatation, or functional as an intense and diffuse vasoconstriction of resistance vessels resulting from a spasm coronary.
Myocardial ischemia occurs when coronary flow is inadequate to meet myocardial oxygen consumption. Subsequently, metabolic, mechanical and electrical changes may occur, which constitute the ischemic cascade, accompanied or not by cardiac ischemic pain. In the case of transient ischemia, these changes are reversible; after 20-30

minutes they become irreversible and appears necrosis of the ischemic area, with myocardial infarction.
A clinical classification of myocardial infarction was developed and recently updated [19]. Myocardial infarction is defined as a condition characterized by cardiac cell death following prolonged ischemia, causing a rise in circulating myocardial enzymes (troponins). Myocardial infarction of type 1 is spontaneous, due to the onset of complications on atherosclerotic plaque, and specifically of a coronary thrombus. Type 2 myocardial infarction is characterized by evidence of an imbalance between myocardial oxygen supply and demand unrelated to coronary thrombosis.
Recently, it has been shown that the incidence of type-2 myocardial infarction is as high as the incidence of type-1 myocardial infarction. Patients aged less than 75 years have a higher incidence of type 1 myocardial infarction than type 2 (124 vs 60 per 100,000 people). Conversely, patients aged more than 75 years have a higher incidence of type 2 myocardial infarction or myocardial injury than type-1 myocardial infarction (1008 vs 750 per 100,000 people) [5].

2.3 Cardiac cycle and systolic time intervals

The cardiac cycle is the performance of the human heart from the beginning of one heartbeat to the beginning of the next. It consists of two periods: one during which the heart muscle relaxes and refills with blood, called diastole, followed by a period of robust contraction and pumping of blood, named systole. After emptying, the heart immediately relaxes and expands to receive another influx of blood returning from the lungs and other systems of the body, before again contracting to pump blood to the lungs and those systems. A normally performing heart must be fully expanded before it can efficiently pump again.

There are two atrial and two ventricle chambers of the heart; they are paired as the left heart and the right heart and they work in concert to repeat the cardiac cycle continuously. At the start of the cycle, during ventricular diastole, early, the heart relaxes and expands while receiving blood into both ventricles through both atria; then, near the end of ventricular diastole–late, the two atria begin to contract (atrial systole), and each atrium pumps blood into the ventricle below it. During ventricular systole the ventricles are contracting and vigorously pulsing (or ejecting) two separated blood supplies from the heart—one to the lungs and one to all other body organs and systems—while the two atria are relaxed (atrial diastole). This precise coordination ensures that blood is efficiently collected and circulated throughout the body.

The mitral and tricuspid valves, also known as the atrioventricular valves, open during ventricular diastole to permit filling. Late in the filling period the atria begin to contract (atrial systole) forcing a final crop of blood into the ventricles under pressure. Then, prompted by electrical signals from the sinoatrial node, the ventricles start contracting (ventricular systole), and as back-pressure against them increases the atrioventricular valves are forced to close, which stops the blood volumes in the ventricles from flowing in or out; this is known as the isovolumetric contraction stage.

Due to the contractions of the systole, pressures in the ventricles rise quickly, exceeding the pressures in the trunks of the aorta and the pulmonary arteries and causing the requisite valves (the aortic and pulmonary valves) to open, which results in separated blood volumes being ejected from the two ventricles. This is the ejection stage of the cardiac cycle. After ventricular pressures fall below their peaks and below those in the trunks of the aorta and pulmonary arteries, the aortic and pulmonary valves close again.

Then follows the isovolumetric relaxation, during which pressure within the ventricles begin to fall significantly, and thereafter the atria begin refilling as blood returns to flow into the right atrium (from the vena cavae) and into the left atrium (from the pulmonary veins). As the ventricles begin to relax, the mitral and tricuspid valves open again, and the completed cycle returns to ventricular diastole and a new start of the cardiac cycle.

Throughout the cardiac cycle, blood pressure increases and decreases. The movements of cardiac muscle are coordinated by a series of electrical impulses produced by specialised pacemaker cells found within the sinoatrial node and the atrioventricular node. Cardiac muscle is composed of myocytes which initiate their internal contractions without applying to external stimulus, with the exception of changes in the heart rate due to metabolic demand.

The heart is a volumetric pressure pump, which generates a discontinuous propulsion

on the blood inside the aorta and the pulmonary artery through a continuous alternation of contraction and relaxation phases of the ventricular musculature. The electrical input from the sino-atrial node is transmitted first to atrial myocardiocytes, then, through the conduction system, to the ventricular ones. From the recording in time of the electrical events that accompany the propagation of the excitation wave into the cardiac mass, through surface electrodes, the classical electrocardiographic trace is obtained. The mechanical event, following the arrival of the depolarization wave, is the synchronous contraction of the atrial musculature, and therefore the ventricular one. The beginning of the ventricular systole occurs at the peak of the electrocardiographic R wave: starting from this moment the myocardium is put in tension and the consequent closure of the atrioventricular valves. The intraocavitary pressure then increases rapidly to a pressure such as to equalize the pressure inside the aorta, or in the pulmonary artery, respectively in the left heart and in the right heart.

Timing of mechanical cardiac events, and in particular of systolic time intervals (STI), has been of particular interest for research cardiology in the past decades. The STI as originally described included the left ventricular ejection time (LVET), the isovolumic contraction time (ICT) and the pre-ejection period (PEP). PEP represents the interval from the onset of ventricular depolarization to the beginning of aortic ejection. This first phase of the systole, which is part of PEP, is called electromechanical delay, and is the time lag between onsets of muscle activation and muscle force production and reflects the electro-chemical processes. After the electromechanical delay, the ICT represents in early systole the time during which the ventricles contract with no corresponding volume change (isovolumetrically). This short-lasting portion of the cardiac cycle takes place whilst all heart valves are closed, and causes ventricular pressure to rise. When the aortic pressure is exceeded by the ventricular pressure, the pressure gradient inversion which during the diastole had guaranteed the closing of the semilunar valves; the effect is therefore the opening of these and the propulsion of part of the ventricular content towards the aorta. This second phase of the cardiac cycle is called "isotonic systole", or "ejective period" (left ventricular ejection time, LVET), and is maintained by the total energy gradient (kinetic and pressure), in force within the system; when this gradient is reversed, the semilunar valves will close again and the diastolic phase will start. The ratio between the volume expelled from the ventricle during the isotonic systole and the ventricular telediastolic volume, is called ejection fraction: it is normally used as an index of ventricular contractility and in physiological conditions corresponds to 50-60%. It should be highlighted that, both in the isovolumetric contraction period and in the ejective period, the myocardium it is contracted. ICT and LVET are both moments in which there is oxygen consumption and absence of subendocardial coronary flow. Among the factors influencing PEP and LVET are myocardial contractility, preload, afterload, and heart rate. The ratio between PEP and LVET (PEP/LVET) as well as the ICT and LVET ratio (ICT/LVET) have become useful parameters in left ventricular function evaluation, showing a good correlation with conventional left ventricular systolic performance indices, as ejection fraction [20].

2.4 From the Buckberg index to the Subendocardial Viability Ratio

Myocardial ischemia is often considered synonymous of coronary artery disease, as if coronary atherosclerotic phenomena were the only responsible mechanism of this disease. A more correct approach to ischemic heart disease would need to consider all the factors potentially responsible for an ischemic myocardial injury, beside atherosclerotic coronary stenosis. Among them we should consider myocardial oxygen needs, myocardial (subendocardial) flow supply as well as the arterial oxygen content. In order to better understand the link between arterial stiffness and myocardial ischemia, it is thus necessary to consider ischemic heart disease through a very broad perspective, namely as the complex balance between subendocardial oxygen supply and demand.

It is important to clarify whether the association between myocardial ischemia and aortic stiffness is secondary to an independent action of aortic stiffness on myocardial supply-demand ratio, or rather whether this association depends on the presence of any factors able to affect both coronary flow and arterial wall properties. The question is thus how a stiff aorta might affect myocardial supply flow.

Previous studies clearly described a dysfunction of the myocardium and a reduction in coronary reserve flow after mechanical increase of aortic stiffness. Even in absence of coronary stenosis, decreased aortic compliance was shown to be associated with inadequate subendocardial oxygenation, which could be further deranged in presence of coronary stenosis [21].

Indeed, the aorta and large arteries play a major role in the regulation of blood pressure and peripheral blood flow. It is well known that large arteries not only have a passive function in relation to the transfer of oxygenated blood from the heart to the periphery, but also exert an important buffering function, as they are able to dampen left ventricular stroke volume thanks to their viscoelastic properties.

The reduction in diastolic blood pressure in the aorta may cause a reduction in subendocardial blood flow supply [22]. Actually subendocardial perfusion occurs mostly during diastole because of development of extravascular compressive forces through the systolic phase of the cardiac cycle. During contraction of the left ventricle, the blood vessels going through the myocardial wall are compressed. The compressive force exerted on coronary blood vessels is higher in the subendocardium, where it is similar to the pressure within the left ventricle. Thus, blood flow in subendocardium is virtually withdrawn, even though subepicardial layers remain normally perfused. During the diastolic phase, the whole cardiac muscle is normally perfused again. As a consequence, the subendocardial blood flow is almost exclusively diastolic. In absence of coronary hemodynamically significant stenosis, the diastolic pressure in coronary arteries is equal to diastolic pressure in the ascending aorta. Thus, subendocardial coronary flow depends not only on diastolic time, but also on aortic diastolic pressure and on the pressure gradient in diastole between coronary intravascular pressure and left ventricular pressure.

Blood pressure in the ascending aorta represents the pressure against which the left ventricle has to pump during systolic contraction. If the mean of arterial pressure during the systolic phase in ascending aorta is high, the left ventricle must contract more energetically to maintain adequate stroke volume. Therefore, an increase in systolic blood pressure related to an increase in left ventricular afterload, leads to a rise in cardiac work and consequently to an increase in left ventricular mass and

myocardial oxygen needs. The development of left ventricular hypertrophy, and progression towards the resulting left ventricular failure, predispose to an increase in left ventricular diastolic pressure and to a prolongation in isometric contraction time, further reducing the diastolic subendocardial pressure gradient and perfusion time. The result of all these processes is a decrease in subendocardial oxygen supply.
Moreover, arterial stiffness causes an increased pulse wave velocity through the arterial elastic system. Therefore, if the forward (centrifugal) pressure wave travels faster owing to increased arterial stiffness, similarly, the backward (centripetal) pressure wave goes back to the center at a higher speed. Thus, under reduced arterial viscoelasticity, the earlier superimposition of the two waves, in the protomesosystolic phase of the cardiac cycle, produces a further increase in systolic blood pressure and pulse pressure values.
Another factor affected by arterial stiffness must be considered, however, besides the increase in pulse pressure, i.e. an increase in short term blood pressure variability. Large studies showed a link between increased arterial stiffness and an increase in short term blood pressure variability [23]. Among other mechanisms, such an association may be partly due to a reduced sensitivity of the arterial baroreflex, i.e. of the most important mechanism for short-term control of blood pressure. Arterial baroreceptors respond to the extent of stretching/relaxation of carotid/aortic arterial walls, rather than directly to changes in blood pressure values themselves. Thus, baroreflex responsiveness will be reduced in individuals with increased arterial stiffness, whose vessels distend less than more elastic vessels in response to blood pressure changes. This means that the arterial baroreflex function is significantly affected by the degree of distensibility/stiffness of arterial walls, which in turns determines the degree of stretching/relaxation of carotid/aortic walls in response to blood pressure fluctuations
As a result of the pathophysiological changes mentioned above, aortic stiffness may cause an ischemic heart injury through a reduction in subendocardial oxygen supply and/or an increase in subendocardial oxygen demand. Aortic stiffness indeed appears to be an important factor affecting subendocardial oxygen supply-demand balance, and thus it should be considered more than just an independent risk factor for coronary artery disease. On the other hand, ischemic heart disease should then be considered not only the result of a vascular atherosclerotic process affecting coronary arteries, but rather the result of a complex interaction among local coronary atherosclerotic damage, changes in vascular hemodynamics, oxygen arterial content and the oxygen requirements of myocardial cells. In particular, on the background of these considerations, an imbalance between myocardial flow supply and demand should be always considered in the evaluation of patients with chronic myocardial ischemia.
An useful index in the assessment of cardiac ischemic risk was introduced by Gerald David Buckberg and Julien I.E. Hoffman at the beginning of the 1970s, and named SubEndocardial Viability Ratio (SEVR), also known as Buckberg's index or DPTI:SPTI ratio [24]. This index reflects the subendocardial oxygen supply-demand ratio and can be defined by analyzing left ventricular and aortic pressure curves.
The area under the left ventricular (or aortic) pressure curve in systole (SPTI, systolic pressure-time index), from the onset of ventricular systole to the dicrotic notch, represents the left ventricular afterload and defines cardiac work. If the mean arterial pressure during the systolic phase in the ascending aorta is high, the left ventricle must contract more energetically to maintain adequate stroke volume. Thus SPTI directly correlates with myocardial oxygen consumption, and mainly depends on left

ventricular ejection time, ejection pressure, and myocardial contractility.
The area between the aortic and left ventricular pressure curves in diastole represents the pressure that affects the coronary blood flow and maintains adequate subendocardial blood supply in the diastolic phase of cardiac cycle (DPTI, diastolic pressure-time index).

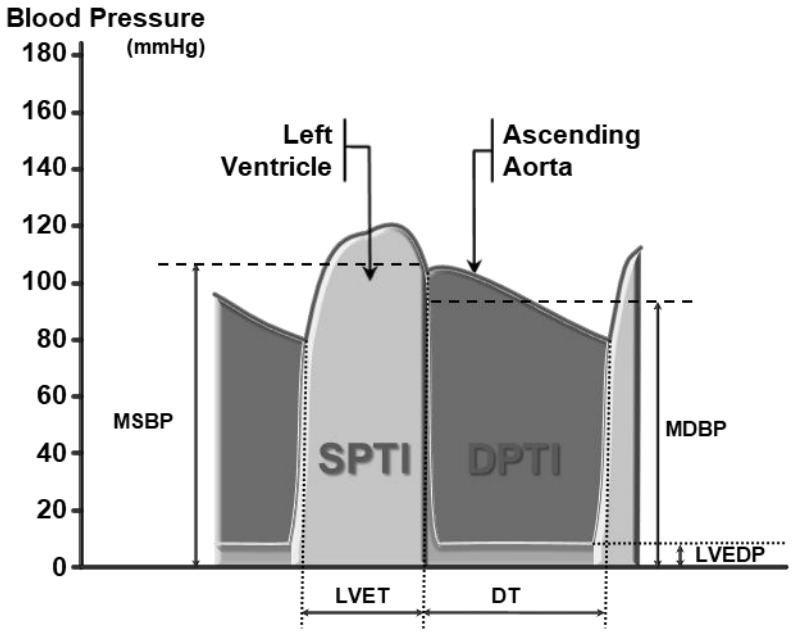

Figure 2.2 The invasive Buckberg's index or subendocardial viability ratio (SEVR).

During the systolic phase, blood supply to the subendocardial layers is not allowed, owing to the presence of two extravascular compressive forces: the first force is left ventricular intracavitary pressure, which is fully transmitted to the subendocardial layers, but which falls off to almost zero at the epicardium; the second one is the force developed during left ventricular contraction itself leading to coronary vascular occlusion. The overall compressive force exerted on coronary blood vessels is higher in the subendocardium, where it is similar to the pressure within the left ventricle. Thus, blood flow to subendocardial fiber layers is virtually absent in systole, even though subepicardial layers remain normally perfused. Along this line, experimental studies clearly showed that a reduction in aortic distensibility alters the transmural myocardial blood flow distribution of left ventricle and decreases the subendocardial/subepicardial flow ratio.
During the diastolic phase, the degree of myocardial perfusion largely depends on DPTI, which in turn is a function of the coronary arterial diastolic pressure, the pressure gradient in diastole between coronary arteries and left ventricular pressure and the duration of diastole. DPTI is obtained by subtracting the left ventricular mean diastolic blood pressure (LVDP) from the area, in diastole, of the aortic pressure curve. LVDP can be, substantially and successfully, replaced by an estimate of the left ventricular (or atrial) end-diastolic pressure, which is more easily assessed, non-invasively, by echocardiography.
To sum up, the subendocardial viability ratio describes the relationship between supply and demand, i.e., between the myocardial blood supply and myocardial oxygen requirement.
The DPTI/SPTI ratio represents thus the balance between oxygen subendocardial

supply and demand. In the elderly and in presence of aortic stiffness these two parameters significantly change (SPTI increases and DPTI decreases) and the subendocardial oxygen supply-demand ratio (DPTI:SPTI ratio) is greatly reduced, a finding which highlight the possible usefulness of subendocardial oxygen supply-demand ratio assessment in clinical practice. However, the need in the past of an invasive arterial catheterization for its estimate, has for a long time represented a major limitation for its application in a clinical setting.

A condition of arterial stiffness is accompanied by a decrease in DPTI and by an increase in SPTI, not only owing to the reduced "buffer" function of the aorta due to the altered Windkessel effect but also because of the earlier return of wave reflection. The Rotterdam Study demonstrated that an increase in pulse wave velocity (PWV) is accompanied by an increase in SPTI and by a decrease in DPTI, and therefore by a significant reduction in SEVR [25]. The increase in oxygen consumption, generated by the increase in afterload owing to arterial stiffness, and the reduction in oxygen supply to the myocardium are elements which can help us understand the association between aortic stiffness (as shown by PWV) and cardiovascular mortality and morbidity.

A critical value has been proved for SEVR; it corresponds to 0.45, below which the ratio between subendocardial flow and subepicardial flow, per gram, is reduced in the left ventricle as a signal of insufficient subendocardial vascularization [26]. However, even thanks to the coronary autoregolation, over this critical value, a linear relationship between SEVR and coronary blood flow does not exist.

In other words, for values greater than 0.45, the degree of subendocardial vascularization remains almost constant and the ratio between subendocardial flow and subepicardial flow, per gram, remains within the reference range.

Actually, oxygen supply to the subendocardium depends not only on coronary blood flow but also on the oxygen content of blood. It is worth considering in this regard that, for the same coronary blood flow, the oxygen supply to the subendocardium can decrease significantly in the presence of anemia or hypoxemia (e.g., with respiratory insufficiency or at high altitude).

Under these conditions, it is advisable to adapt the formula defining the subendocardial viability ratio (SEVR) by multiplying DPTI by the arterial oxygen content:

The introduction of transcutaneous arterial tonometry has provided a new approach to non-invasively assessment of the subendocardial oxygen supply-demand ratio through a simple and easily implementable test. Actually, applanation tonometry is, at present, considered the reference method for non-invasive estimation of central blood pressure and for central pulse wave analysis. The devices using this method, which are currently available, provide values of Buckberg's index on the basis of the morphological analysis of the central arterial pressure wave recorded by the tonometer itself. In the context of DPTI:SPTI ratio assessment by transcutaneous tonometry, DPTI represents the area under the diastolic portion of the blood pressure wave, and is obtained by multiplying the mean value of blood pressure during the diastolic phase of cardiac cycle by the diastolic time. Conversely, SPTI represents the area under the systolic portion of the pressure wave, obtained by multiplying the mean value of blood pressure during the systolic phase of cardiac cycle by the left ventricular ejection time. So,

Traditional SEVR by Arterial Tonometry= DPTI/SPTI= MDBP/MSBP · DT/LVET

emerging as the result of the ratio between two pressures (MDBP and MSBP) and two

time measures (DT and LVET).
At present, the assessment of DPTI:SPTI ratio is only based on pulse waveforms recorded by arterial tonometers in daily clinical practice and is therefore affected by a number of important limitations:

- First, only left ventricular ejection time is taken into account when analyzing tonometric pulse waveforms to calculate this ratio whereas left ventricular isovolumic contraction time is not considered in the determination of systolic left ventricular function, with a consequent underestimation of SPTI. On the other hand, left ventricular isovolumic contraction time is considered in DPTI determination, even though this parameter should actually be considered as a component of cardiac workload and not as an oxygen supply time, with the consequent result of an overestimation of DPTI. Since the isovolumic contraction time/ejection time ratio increases significantly in the elderly and in heart failure, the tonometry method may overestimate the DPTI:SPTI ratio in these patients by even 80-100% with regard to its real value.
- Second, left ventricular diastolic pressure is not taken into account by this approach. In subjects with heart failure or with cardiac valve disease, characterized by increase in left ventricular diastolic pressure, this limitation leads to overestimate the DPTI value when only focusing on tonometry data.
- Finally, a correct estimation of the oxygen demand by the myocardium cannot ignore the muscle mass or contractility. Actually, an increase in muscular mass, such as in left ventricular hypertrophy, increases the oxygen needs. Thus, in order to improve the estimation of SPTI, recently Hoffmann and Buckberg suggested to multiply SPTI by the relative left ventricular mass as determined by echocardiography.

These methodological aspects may have affected the studies performed so far on SEVR. Despite SEVR being calculated in all epidemiological studies estimating arterial stiffness by arterial tonometry, there are indeed very few papers reporting data on the relationship between SEVR and cardiovascular disease. Likely, this could be at least in part the consequence of the above reported inaccuracies affecting the measurement of DPTI:SPTI ratio.
Overall, on the background of the above methodological issues related to the estimation of Buckberg's index only based on arterial tonometry data, the current approach seems to provide a relatively unreliable surrogate assessment of the real subendocardial oxygen supply-demand ratio.

3. Systolic time intervals assessed from analysis of carotid pressure waveform

Timing of mechanical cardiac events, and in particular of systolic time intervals (STI), was described 50 years ago, using phonocardiogram, electrocardiogram and carotid arterial pulse tracings [27] as a non-invasive indirect parameter for the assessment of cardiovascular performance [28]. The STI as originally described included the left ventricular ejection time (LVET), the isovolumetric contraction time (ICT), the electromechanical delay and the pre-ejection period (PEP), which is the sum of ICT and electromechanical delay. This non-invasive methodology was validated against invasive measurements [29], and extensively used for the quantitative estimation of left ventricular performance and in studying effects of pharmacologic therapies [28]. More recently, the echocardiographic approach has become the reference method in the study of STI in daily clinical use. The ratio between PEP and LVET (PEP/LVET) as well as that between ICT and LVET (ICT/LVET) have become useful parameters in left ventricular function evaluation, showing a good correlation with a conventional left ventricular systolic performance index, such as ejection fraction (EF) [20], and a good prognostic power in predicting cardiovascular outcomes in general population [30], after myocardial infarction [30] and in chronic heart failure [31]. Several different methods have been proposed for the noninvasive estimation of STI, from oscillometric brachial blood pressure measurements [32], finger blood pressure tracking [33], wearable accelerometers [34] and impedance cardiography [35], but their use is currently confined to a research setting.

Arterial applanation tonometry is a validated methodology that is increasingly applied in clinical setting [7]. Although used mainly for evaluation of arterial stiffness, other hemodynamic variables have been obtained from analysis of the carotid pressure waveform, such as central blood pressure, augmentation index and subendocardial viability ratio (SEVR), which allow a more detailed description of mechanical and functional properties of the vascular system. The ability to offer an evaluation of left ventricular systolic function could be an important additional feature of arterial tonometry, a noninvasive technique available in a clinic setting, without the use of complex, time-consuming and user-dependent methodologies. Moreover, the possibility to provide a reliable estimate of STI from arterial applanation tonometry could improve the calculation of other variables derived from the pulse wave analysis, such as SEVR, which at present does not consider the isovolumetric contraction of the heart in the assessment of myocardial supply-demand balance [36].

As the ability of carotid tonometry to reliably measure LVET [28][37] is already established, the aim of this study is to verify the possibility to measure the other STI indices (ICT, PEP) directly from the analysis of pulse waveform recorded at the carotid artery level. The evaluation of STI could be of value in giving a global quantitative index of ventricular performance, and could improve the calculation of pulse wave analysis-derived variables, such as SEVR. In this work, we present the methodology to derive STI from ECG-gated arterial tonometry and we compare STI derived from tonometry with STI measured from echocardiography, in a general population and in patients with systolic heart failure. Furthermore, we investigate the relationship of the measured parameters with left ventricular systolic function assessed by conventional echocardiography.

Methods

A total of 111 subjects were enrolled in this study, including both healthy volunteers and heart failure patients. Sixty-four apparently healthy individuals, free from overt cardiovascular disease, showing normal echocardiography and ECG and not taking medications, were recruited by cardiology university centers in Sydney (Australia) and Trieste (Italy), amongst students, university workers and their relatives. In order to account for age effects, these subjects were recruited with an even distribution across age subgroups from 20 to 75 years. Additionally, heart failure patients with reduced ejection fraction (HFrEF) were recruited by cardiology university centers in Beijing (China) and Milan (Italy). Forty-seven patients periodically followed at heart failure center, with EF ≤40 at the last check, were enrolled in this study. Exclusion criteria were: age <18 years, atrial fibrillation, body mass index ≥35 and severe valve disease as well as presence of any severe systemic disease.

Participants were admitted to a quiet, temperature controlled room (21±2 °C), and were invited to rest on a bed in the supine position for 15 minutes before commencing data collection. Patients were recommended to abstain from coffee, smoking and from taking any medications in the 3 hours before the test. The study protocol was approved by institutional Ethics Committees and was conducted in accordance with the Helsinki Declaration, with subjects giving informed consent to all procedures.

Echocardiographic data analysis

For the echocardiographic evaluation, each of the four research groups used its own cardiac ultrasound system [Philips EnVisor® C (Milan and Sydney), GE Vivid® E9 (Beijing), GE Vivid® Q (Trieste)], with the same trained operator being responsible for the echocardiographic examinations in each center. All cardiac ultrasound recordings were sent to the core echocardiographic laboratory at Milan center and subjected to centralized analysis. A standard echocardiogram was performed in all individuals enrolled in this study. EF was calculated using the modified biplane Simpson's rule. Left ventricular end diastolic volume and left ventricular end systolic volume were obtained from apical four- and two-chamber views. STI were assessed based on pulsed Doppler acquisitions on left ventricular outflow tract (Figure 3.1).

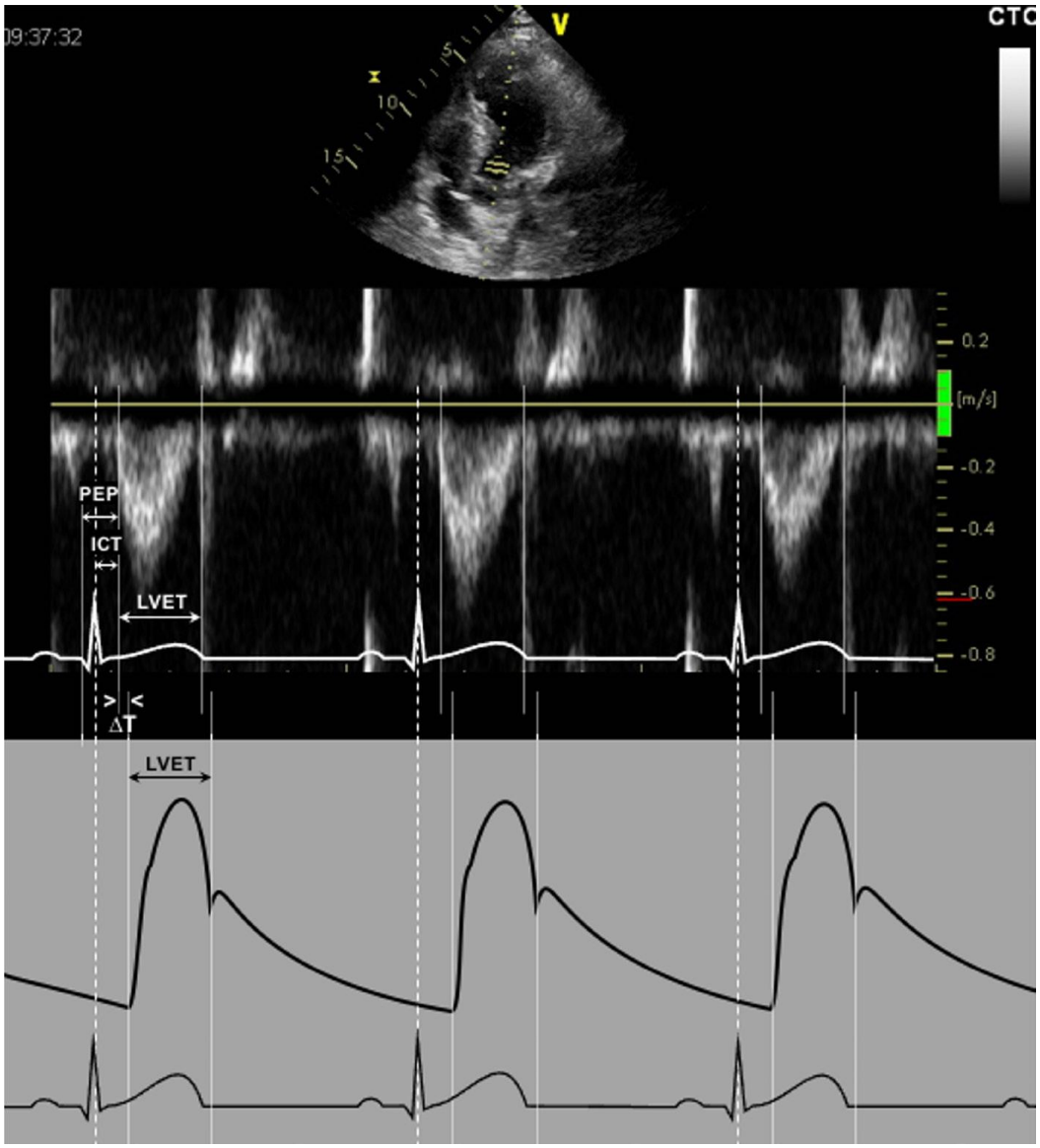

Figure 3.1. Upper panel shows echocardiographic evaluation of systolic time intervals based on pulsed Doppler. ICT, isovolumic contraction time; LVET, left ventricular ejection time; PEP, pre-ejection period. Lower panel shows pulse pressure waveforms recorded in common carotid artery by arterial tonometry, synchronized by ECG. ΔT is the transit time of the pressure wave between the left ventricle to the common carotid artery.

Aortic PEP was calculated as the interval from the onset of ventricular depolarization to the beginning of aortic ejection. PEP was assessed by measuring the delay from the 'Q' wave of the QRS complex to aortic valve opening. ICT was calculated as the delay between the 'R' wave of the ECG and the start of the ejection period. LVET represents the interval from the beginning and the end of aortic flow. PEP to LVET ratio (PEP/LVET) was also calculated. All measurements were performed offline, by the same trained operator blinded to clinical data. Echocardiographic measurements were averaged from three cardiac cycles on digital stored images (DICOM format) with dedicated software RadiAnt® DICOM Viewer (Medixant, Poland).

Carotid pressure wave analysis

Blood pressure waveforms recorded at the common carotid artery were taken as a surrogate for ascending aortic pressure waveforms. Carotid pulse waves were recorded

by means of validated transcutaneous arterial tonometers. A PulsePen® tonometer (DiaTecne, Milan, Italy) [38] was used by the Italian and Chinese research groups, whereas a SpygmoCor® XCEL device (AtCor Medical, Sydney, Australia) [39]was used by the Australian group. An excellent concordance between these two tonometers was widely demonstrated in previous papers [40][41][42]. The main parameters derived from the analysis of pulse waveform were assessed, especially by separately analyzing data obtained during the systolic and diastolic phases of the cardiac cycle. Pulse waves obtained during 10 cardiac cycles at the common carotid artery site by applanation tonometry and during 4 seconds of aortic transvalvular flow by echocardiographic ultrasound system were simultaneously recorded. An electrocardiogram was recorded simultaneously with both carotid pulse wave and ultrasound recordings, allowing for synchronization of the two sets of measurements by ECG gating. Finally, ICT values measured by echocardiography were compared with the corresponding ICT values derived using applanation tonometry. To check the reproducibility of the data, a second synchronous tonometric and ultrasound data acquisition was repeated after 5 minutes in a subgroup of 43 patients.

Estimation of systolic time intervals

The time delay between the peak of the 'R' wave of the ECG and the foot of the carotid pulse wave (RcW) was considered as the sum of the time taken by the pressure wave arising from the left ventricle to reach the common carotid artery (Δt) and the time of isovolumetric contraction, i.e. $RcW = \Delta t + ICT$. Therefore, $ICT = RcW - \Delta t$. Δt can be calculated by knowing the distance between the carotid site where the pulse wave was recorded and the origin of the aorta, and the pulse wave velocity (PWV, in m/s) in the ascending aorta, according to the formula: Δt = distance/PWV. In the present study, the 'distance' was considered as the sum of the tape-measured distance from the carotid site to the suprasternal notch and the length of the ascending aorta. We considered an ascending aorta length of 74 mm, corresponding to the average value described in literature on a population of 256 apparently healthy adults [43]. Carotid-femoral PWV, a surrogate measure for aortic PWV, was measured in all participants in this study. However, it is well known that PWV in the ascending aorta is significantly lower than carotid-femoral PWV, and the ratio between PWV in the ascending aorta and in the whole aorta has been shown to be related to age [44]. Thus, the carotid-femoral PWV values were corrected with coefficients derived from the work of Hickson et al. [44]: PWV in ascending aorta = carotid–femoral PWV (-0.0034 age +0.9627). Finally, ICT values measured by echocardiography were compared with the corresponding ICT values derived using applanation tonometry. The duration of the interval between the "Q" and the "R" wave of the ECG was automatically computed by the PulsePen® software (WPP 2.0.1 version). For the data acquired by SphygmoCor, the "QR" interval was manually measured on the ECG tracing. This time was added to tonometry-derived ICT for the calculation of tonometry-derived PEP.

Measurement of carotid-femoral pulse wave velocity

Carotid-femoral PWV is considered the non-invasive gold-standard method for assessing aortic distensibility [8] with a good repeatability [45]. PWV was measured as the time delay between the foot of the central (carotid) arterial waveform and the foot of the simultaneously recorded peripheral pulse waveform. An arterial tonometer was placed on the common carotid artery, considered as central detection site, and the second transducer recorded the pulse wave curve simultaneously in the femoral artery.

PulsePen® records femoral pulse using a second arterial tonometer, whereas SphygmoCor® XCEL records femoral pulse using the volume displacement waveform measured in a cuff placed around the upper thigh, inflated to sub-diastolic pressure [39]. PulsePen® and SphygmoCor® XCEL devices are both able to measure the carotid-femoral pulse-wave velocity (PWV) following current recommendations [46] and by considering the direct pulse transit time between the carotid and femoral arteries. The distance between the carotid and femoral arteries was measured, and the PWV was automatically determined by dividing the distance, multiplied for 0.8, by the pulse transit time.

Statistical methods

Values were presented as mean ± standard deviation (SD) or as absolute numbers (percentages). The relationship between variables was determined with linear regression (coefficient of determination, r^2). Assumptions of data normality and homoscedasticity were evaluated with Shapiro test and Bartlett's test respectively. T-test was used to compare the differences between the measurements. Multiple comparisons were accounted for by using a false discovery rate adjustment at the $\alpha=0.05$ level. When two sets of measures were compared, the results were analyzed in two steps [47]: first the degree of correlation between the two sets of measures was rated by simple linear regression (r^2); subsequently, in the presence of a significant correlation, the difference between the paired data has been correlated with the relative average values (Bland-Altman plot). The level of agreement between two measurements was assessed by the mean difference and the 95% confidence interval (CI), calculated as mean difference ± 1.96 SD of differences. The coefficient of variation was assessed to define intra-observer repeatability. To facilitate the comparison between healthy subjects and heart failure patients, the former was subdivided into two groups with age lower than 45 years and equal to or higher than 45 years, respectively. The sample size was computed by considering a SD of differences between the ICT measurements of 9.5 ms [27] on 100 patients; the 95% confidence interval of the mean difference is 1.88 ms and the 95% confidence interval of the limits of agreement is 3.22 ms. Assuming a dropout rate of about 10%, the final sample size was computed as 111 subjects. Statistical analysis was performed with SPSS version 20.0 (IBM Corp., IBM Corp., Armonk, New York, USA).

Results

Five enrolled patients with heart failure and two healthy volunteers had an inadequate ultrasound window and were excluded from the analysis. Thus, 62 healthy volunteers (32 males, age±SD: 47.4±17.3 years) and 42 patients with heart failure and reduced heart failure (33 males, age: 65.8±14.0) were considered for our analysis. General and echocardiographic characteristics of the patients from the 4 centers are shown in Table 3.1.

Table 3.1. Anthropometric parameters, clinical features and hemodynamic parameters in healthy volunteers (aged <45 years and ≥45 years) and heart failure patients with reduced ejection fraction.

Parameters	A Healthy Volunteers Aged <45 years	B Healthy Volunteers Aged ≥45 years	A vs B *P*	C Heart Failure	B vs C *P*
Gender (M/F)	17 / 15	15 / 15		33 / 9	
Age, years	32.9 ± 6.5	62.9 ± 10.3	<0.001	65.8 ± 14.0	0.334
Height, cm	172.9 ± 12.0	170.6 ± 8.6	0.391	167.0 ± 8.4	0.168
Weight, kg	67.5 ± 13.4	69.2 ± 10.5	0.611	73.3 ± 16.5	0.484
BMI, m/kg^2	22.7 ± 3.7	23.7 ± 2.8	0.254	26.1 ± 4.6	0.034
BSA, m^2	1.79 ± 0.22	1.81 ± 0.16	0.722	1.83 ± 0.24	0.722
NYHA (1/2/3/4), %	–	–		5 / 62 / 33 / 0	
CAD, n (%)	0	0		29 (69%)	
Hypertension, n (%)	0	0		17 (40%)	
Mitral Insufficiency, n (%)	0	0		13 (31%)	
Aortic stenosis, n (%)	0	0		10 (24%)	
Pace-maker, n (%)	0	0		3 (7%)	
ICD, n (%)	0	0		11 (26%)	
Diabetes Mellitus, n (%)	0	0		13 (31%)	
Bundle Branch Block, n (%)	0	0		14 (33%)	
Treatment:					
Beta-blockers, n (%)	0	0		19 (45%)	
RAS blocker, n (%)	0	0		30 (71%)	
Ca-antagonist, n (%)	0	0		10 (24%)	
Diuretics, n (%)	0	0		25 (60%)	
Brachial Systolic BP, mmHg	119.9 ± 11.1	131.3 ± 18.0	0.010	121.4 ± 18.9	0.031
Carotid Systolic BP, mmHg	111.9 ± 8.2	122.1 ± 16.9	0.050	113.8 ± 17.7	0.072
Mean BP, mmHg	90.9 ± 8.3	95.7 ± 11.4	0.067	84.7 ± 11.1	<0.001
Diastolic BP, mmHg	76.4 ± 8.2	77.9 ± 9.6	0.532	66.3 ± 9.5	<0.001
Heart Rate, bpm	65.8 ± 10.6	68.3 ± 10.6	0.738	68.3 ± 10.9	0.979
LV Ejection Fraction, %	63.5 ± 5.1	62.7 ± 6.3	0.559	35.4 ± 9.7	<0.001
Carotid-Femoral PWV, m/s	7.2 ± 1.1	10.7 ± 3.7	<0.001	10.2 ± 2.7	0.482
PWV in Ascending Aorta, m/s	6.1 ± 0.9	8.0 ± 2.5	<0.001	7.5 ± 1.8	0.046
'R' to Carotid Wave TT, ms	100.2 ± 11.0	90.4 ± 11.7	0.001	104.0 ± 20.9	<0.001
Aortic Valve to Carotid TT, ms	30.3 ± 4.7	24.2 ± 5.5	<0.001	24.1 ± 6.0	0.954
LVET by Tonometry, ms	305.9 ± 20.6	294.2 ± 19.4	0.050	294.4 ± 36.3	0.980
LVET by Echo, ms	311.9 ± 21.8	299.8 ± 20.5	0.056	300.1 ± 37.3	0.965
ICT by Tonometry, ms	69.8 ± 11.6	66.2 ± 13.1	0.266	79.4 ± 20.3	0.001
ICT by Echo, ms	69.7 ± 8.9	69.0 ± 12.0	0.783	77.0 ± 20.6	0.044
ICT/LVET by Tonometry, %	22.9 ± 4.4	22.6 ± 4.6	0.759	27.6 ± 8.5	0.002
ICT/LVET by Echo, %	22.9 ± 3.7	22.9 ± 4.2	0.546	26.2 ± 8.5	0.040
PEP by Tonometry, ms	111.5 ± 15.6	101.5 ± 12.5	0.007	133.1 ± 22.8	<0.001
PEP by Echo, ms	113.0 ± 11.5	106.7 ± 11.5	0.035	126.2 ± 20.4	<0.001
PEP/LVET by Tonometry, %	36.7 ± 6.5	34.6 ± 4.6	0.145	46.2 ± 10.9	<0.001
PEP/LVET by Echo, %	36.5 ± 5.2	35.8 ± 4.5	0.550	44.0 ± 9.6	<0.001

Data are mean ±SD or percentage. BMI, body mass index; BP, blood pressure; BSA, body surface area; CAD, coronary artery disease; ICD, implantable cardioverter-defibrillator; F, females; M, males; NYHA, New York Heart Association functional classification of patients' heart failure; BP, blood pressure; ICT, isovolumic contraction time; LV, left ventricle; LVDP, left ventricular diastolic pressure; LVET, left ventricular ejection time; PEP, pre-ejection period; PWV, pulse wave velocity; RAS, renin angiotensin system; TT, transit time.

Systolic time intervals

In Table 3.1 the main parameters derived from arterial tonometry and echocardiography are reported separately for the healthy group (subdivided into two age subgroups) and the heart failure group.

In the entire study population, ICT derived from carotid pulse wave analysis by applanation tonometry showed a very strong correlation with ICT measured from echocardiography: r=0.90, p<0.001 (Figure 3.2).

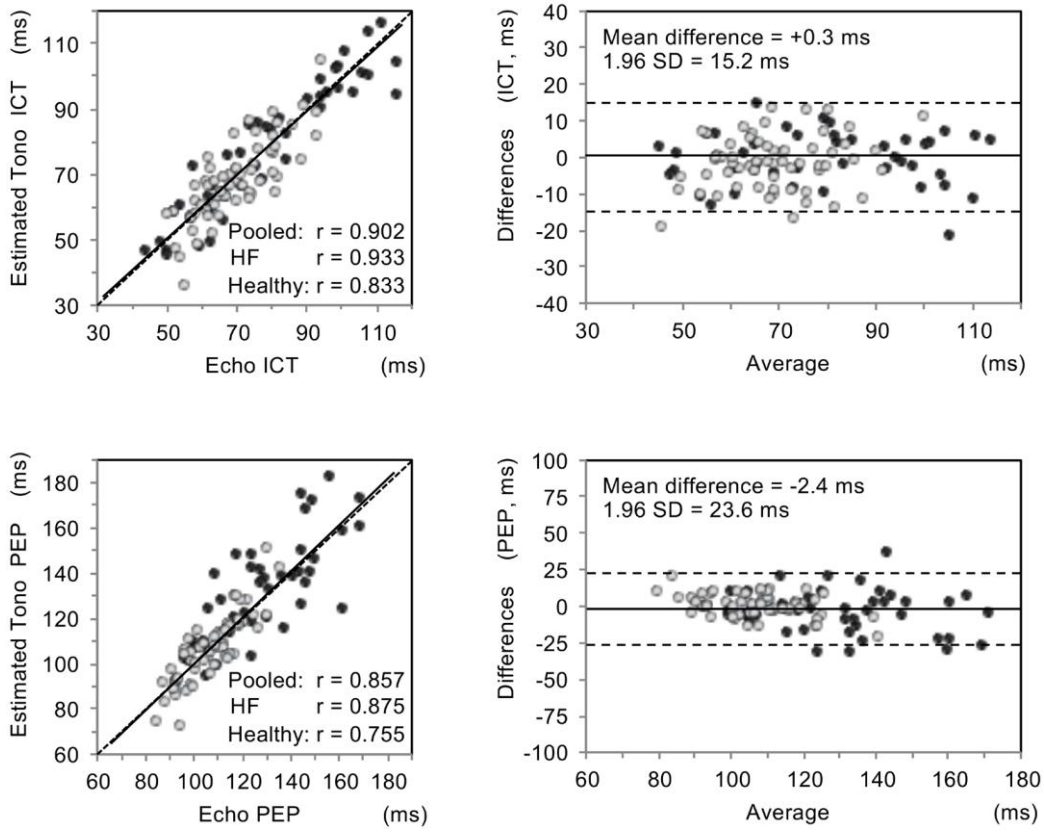

Figure 3.2. Relationship between isovolumic contraction time (ICT, upper panels) and pre-ejection period (PEP, lower panels) measured by echocardiography (Echo ICT and Echo PEP) and estimated values by arterial tonometry (Tono ICT and Tono PEP), taking into account aortic pulse wave velocity and carotid to suprasternal notch distance. White dots: healthy volunteers. Black dots: heart failure patients (HF) with reduced ejection fraction. Left panels: the scatterplots show linear correlation between the values where the dashed line represents the identity line. Right panels; the Bland-Altman analysis shows differences observed between the values obtained by the two methods as a function of their mean values. SD, standard deviation.

This close relationship was maintained in both the healthy and heart failure group, analyzed separately: r=0.83, p<0.001, in healthy volunteers and r=0.93, p<0.001, in heart failure group for ICT. Bland-Altman analysis comparing the two methods showed a homogenous distribution of data and no skewed tendencies: mean difference (95%CI) = 0.2 (-14.2 to 14.5) ms. Also in the case when the time delay between

ascending aorta and carotid artery is not taken into account, a strong correlation between PEP measured by tonometry and by echocardiography was present (r=0.85, p<0.001), with an average overestimation of 25.6 ms by the tonometric measurement. The time delay between ascending aorta and carotid artery, calculated as the difference between the delay between the 'R' of ECG to foot of pulse wave in carotid artery measured by applanation tonometry and the delay between the 'R' wave of the ECG and the start of the ejection period measured by echocardiography, was 23.6±7.8 ms. In multivariate regression analysis, PWV in ascending aorta and distance between carotid and suprasternal notch were the only factors significantly affecting the aortic-carotid time delay.

When comparing PEP derived either from tonometry or from echocardiography (Figure 3.2), a strong correlation was found in the whole group (r=0.86, p<0.001) as well as in healthy (r=0.76, p<0.001) and heart failure individuals (r=0.88, p<0.001) separately considered, with a homogenous distribution and no skewed tendencies in the Bland-Altman analysis: mean difference (95%CI) = 0.5 (-22.2 to 22.8) ms.

PEP/LVET derived from tonometry (Figure 3.3) showed a significant inverse relationship with EF, in the pooled population (r=-0.56) (Figure 3.4).

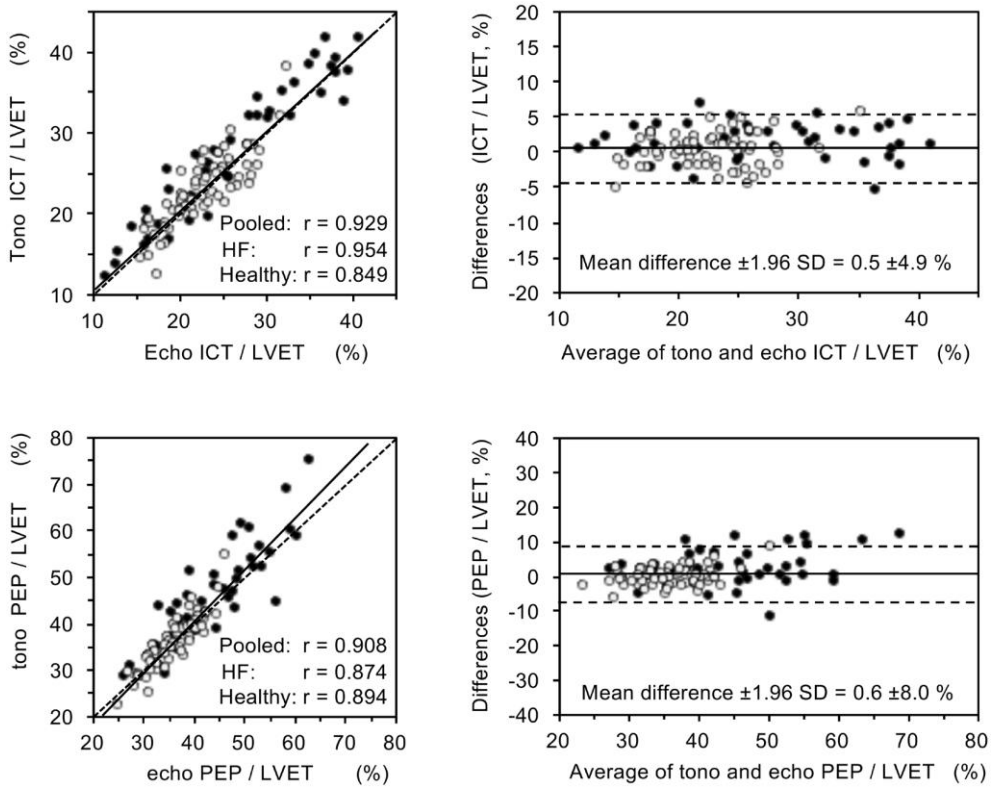

Figure 3.3. Isovolumic contraction time / left ventricular ejection time ratio (ICT/LVET, upper panels) and pre-ejection period / left ventricular ejection time ratio (PEP/LVET, lower panels) measured with carotid tonometry (tono ICT/LVET and tono PEP/LVET) versus the standard method, measured by echocardiograph. White dots: healthy volunteers. Black dots: heart failure patients with reduced ejection fraction. Left panels: the scatterplots show linear correlation between the values where the dashed line represents the identity line. Right panels: the Bland-Altman analysis shows differences observed between the values obtained by the two methods as a function of their mean values. Data are expressed in percentage. SD, standard deviation.

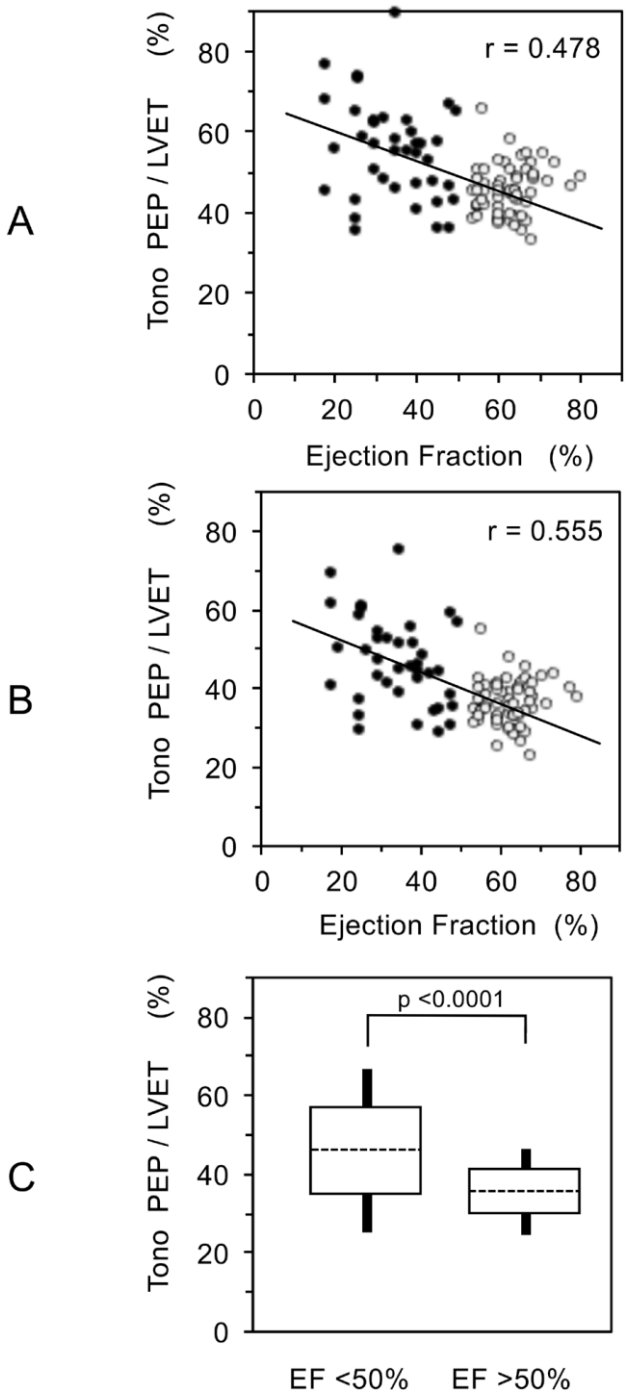

Figure 3.4. Correlation between pre-ejection period - left ventricular ejection time ratio (PEP/LVET) measured by carotid tonometry and ejection fraction measured by echocardiography (in percentage). Upper panel (A) shows data without any correction. Middle panel (B) shows the relationship between tonometric and echocardiographic measurement when the time delay between ascending aorta and carotid artery was estimated by aortic pulse wave velocity and carotid to suprasternal notch distance. Lower panel (C) presents these latter data as a boxplot, showing mean values (dashed line), standard deviation (box) and 2SD (solid line). White dots: healthy volunteers. Black dots: heart failure patients with reduced ejection fraction.

Discussion

Our study demonstrates for the first time the possibility of obtaining a measurement of the cardiac STI by computing ICT and PEP from carotid pulse waveform and carotid-femoral PWV, evaluated with arterial tonometry. The STI assessed with this approach showed a good agreement with measurements performed with conventional

echocardiography and provided reliable indexes for the estimation of left ventricular systolic function.

Previous investigations, conducted more than 30 years ago [37], showed that the STI evaluated invasively in the left ventricle and externally in the carotid artery were highly correlated, thus suggesting the possibility to use external pulse tracings recorded from the right and left carotid arteries for the study of left ventricular STI. The measurements were originally obtained from simultaneous carotid pulse tracing, electrocardiogram and phonocardiogram. This non-invasive methodology was gradually abandoned despite its proven value in the non-invasive evaluation of left ventricular performance in favor of more recent methodologies, like echocardiography, which can assess a wide range of morphological and functional parameters. The present study demonstrates that the assessment of ICT by arterial tonometry is possible through the calculation of pulse transit time from the heart to the carotid artery, with ECG-gated recordings. In our study, ICT derived from the carotid pulse waveform obtained by arterial tonometry and PWV was compared to the ICT measured by echocardiography, and a very close correlation was shown both for healthy controls and patients with heart failure. We subsequently aimed to evaluate the PEP measured by ECG-gated arterial tonometry by adding the electromechanical delay, approximated as the QR interval of the electrocardiogram, to the ICT. A good agreement was found also for this parameter, thus confirming the reliability of arterial tonometry in the assessment of both ICT and PEP.

A reliable evaluation of STI recording carotid pulse waveform should take into account the time delay between ascending aorta and carotid artery, to avoid a significant overestimation of the measurements. In our study, an option to estimate accurately this time delay was provided by considering aortic PWV and carotid to suprasternal notch distance. The upper panel of figure 2 suggests that a fixed values of aortic-carotid time delay could be also used, even in the absence PWV measurement. Nevertheless, considering that aortic-carotid time delay may undergo a large inter-subject variability, it may be necessary to define this delay as accurately as possible. In certain clinical scenarios associated with abnormal changes in PWV values, such as genetic disorders [9], extreme environments [48], extremely high or low blood pressure values or concurrent cardiovascular risk factors [49]) a fixed estimate of this timing may not be sufficient for a reliable calculation of STI in each individual case.

The evaluation of left ventricular systolic function is one of the main challenges in clinical cardiology, requiring an instrumental assessment of the patient. The measurement of EF by conventional echocardiography remains the cornerstone of left ventricular function evaluation because more sophisticated methodologies, such as echocardiographic speckle tracking of the myocardium and cardiac magnetic resonance imaging, are expensive and not very commonly used, and other, as cardiac angiography, are invasive methods. The STIs are related to the normal and abnormal functioning of cardiac systole and change significantly in heart disease. PEP prolongation is closely related to the deterioration of left ventricular function, given that a diseased left ventricle requires a longer time to achieve a ventricular pressure equal to that of the aorta. Furthermore, in heart failure the left ventricle is not able to maintain an adequate pressure during systole, leading to a reduction of LVET. Therefore, a significant increase in PEP associated with a decrease in LVET results in a significantly increased PEP/LVET. Evaluation of systolic times by echocardiography has already been proposed as a useful and accurate measure of cardiac function in clinical practice [20], with a good agreement with echocardiographic EF, representing

a practical alternative to conventional left ventricular function evaluation when the quality of the acoustic window is suboptimal. Measurement of systolic cardiac times also provides clinical prognostic information, with good performance as an independent predictor of future cardiovascular events [30]. Our study confirms that the PEP/LVET ratio is the best index among STI for the quantification of overall left ventricular performance, because of the close association between PEP/LVET and left ventricular EF, which is in line with findings of previous investigations [27][30][50]. The assessment of STI by arterial tonometry could therefore become a simple and reliable tool for the evaluation of cardiac function, whereas other methodologies require complex and expensive technologies and more time for the analysis.

Apart from being useful in the cardiac evaluation, an accurate timing of cardiac events could improve some of the indexes which can be extrapolated from the analysis of arterial pulse wave by applanation tonometry. As an example, the application of STI could readily and significantly improve the non-invasive estimation of the subendocardial oxygen supply and demand ratio, by the inclusion of ICT in this calculation. The subendocardial viability ratio (SEVR) or Buckberg index is a useful parameter which was originally introduced by analyzing left ventricular and aortic pressure curves by catheterization [26][51], and was later reconsidered from the non-invasive evaluation of the central aortic pressure by tonometric devices. This method has been applied in several clinical studies [52][53][54], albeit being affected by an important limitation. In the evaluation of the cardiac workload, defined from the area under the aortic pressure curve, only the LVET is taken into account whereas ICT is excluded, despite being part of the cardiac systole. Furthermore, left ventricular ICT is erroneously included in the tonometric diastolic time, and is thus considered in determination of oxygen supply to the heart, with the consequent result of an overestimation of SEVR. Therefore, an accurate measurement of ICT is necessary for the correct estimation of non-invasive SEVR, especially in conditions where ICT is prolonged, as in the elderly and in heart failure [16]. Our study, by showing the possibility of measuring STI by arterial tonometry, offers a means to improve non-invasive SEVR assessment, free from previous methodological limitations. Further studies are needed to demonstrate the improvement in accuracy and in the diagnostic and prognostic power of the SEVR corrected by the inclusion of ICT.

Some limitations have to be acknowledged for our study. First, the enrollment of two groups of cases with selected characteristics, healthy volunteers and patients with reduced EF, could limit the generalizability of our results. The selection of patients with a reduced EF was motivated by the opportunity to find a population with pathological values of STI. In particular, the importance of ICT would be reduced in patients with heart failure with preserved EF given the prevailing pathological impact of the prolongation of relaxation times. Nevertheless, we have no reason to doubt that the estimation of ICT and PEP would be different in the healthy or in those who have heart disease with preserved EF or those with cardiac diseases. Another methodological limitation is that electromechanical delay was approximated to be equal to the QR interval of the electrocardiogram. This approximation was introduced with the aim of obtaining the PEP from the ICT and motivated by the fact that electromechanical delay, the time between the onset of Q-wave and the onset of the left ventricular systolic pressure rise, is fairly constantly related to the considered electrical interval, except in the case of left bundle branch block [55]. The use of the present methodology of estimation of the STI should be applied with caution in patients with left bundle branch block or with significant arrhythmia, as atrial

fibrillation. These latter patients were excluded from this study.
In the present work, we present the methodology to derive ICT and PEP from ECG-gated arterial tonometry, performed at the carotid and femoral levels. Measurements of ICT and PEP obtained from arterial tonometry were compared to those of the echocardiographic reference method, showing a good agreement for healthy subjects and for patients with structural heart disease. We confirmed the significant relationship of the ratio PEP/LVET with conventional measure of left ventricular systolic function. Arterial tonometry provides a reliable estimate of STI in health and disease. Measurement of STI by arterial tonometry could be considered a useful and non-invasive method for the evaluation of left ventricular performance and could improve the calculation of indexes derived from pulse wave analysis, such as SEVR.

4. How to improve the calculation of mean blood pressure: implications for the calibration of central pressure wave

Mean arterial pressure (MAP) is considered the main driving force for the perfusion of vital organs, as it represents the steady component along which BP fluctuates between the systolic and diastolic values, and a relevant determinant of the alterations caused by elevated blood pressure in the cardiovascular system. MAP has been used as a target for blood pressure control, as it retains clinical prognostic power in predicting the risk for cardiovascular events, which may be even superior to systolic and diastolic pressures in young subjects [56]. Given its major pathophysiological importance, MAP is commonly considered in many clinical circumstances, as in the critically ill patient to determine hemodynamic stability, or in the calculation of peripheral vascular resistances.

Furthermore, as MAP is relatively stable from the ascending aorta to peripheral arteries and may be evaluated non-invasively in the arm, it is commonly used for the calibration of non-invasive central blood pressure measurement. Many devices, using different methodological approaches, calibrate the central pressure with mean and diastolic peripheral pressures to obtain the central systolic blood pressure value, as also recommended by current consensus on validation of non-invasive central blood pressure devices [57]. Nevertheless, the accuracy of this estimation may be affected by how MAP is measured.

The true MAP is defined as the average blood pressure along the entire cardiac cycle and may be calculated as the integral of the pressure curve. At the brachial artery level, it can be measured invasively by catheterization, although relevant artifacts may affect the acquisition of pressure signal, or non-invasively using arterial applanation tonometry. The shape of the pressure wave, relatively to the MAP, is commonly quantified by the so-called "form factor", which is the ratio of the difference between the mean diastolic values of the pressure wave over the pulse amplitude [58]. Alternatively, the "form-factor" can be viewed as the contribution in percentage of the pulse pressure to the diastolic pressure to obtain the MAP (PP%).

Several equations have been proposed to quantify the MAP from systolic and diastolic pressure values. Traditionally, MAP is calculated by adding a 1/3 of pulse pressure to diastolic pressure, as proposed to Gauer et al. in 1960 [59]. Other formulas have been proposed to quantify the "form-factor", which were tested in different populations [60]. Currently, there is no consensus on which MAP formula should be used for the most accurate MAP estimation. The approach of calculating MAP by considering a fixed "form-factor" or PP% may lead to a correct estimate at a population level but may not be appropriate to estimate the MAP in the single patient, if significant interindividual variations occur.

Aim of our study is to examine interindividual variations in MAP, which was calculated from the integration of pressure waveform measured at the brachial artery level by applanation tonometry, from measurements obtained in 3 large populations. Furthermore, we sought to derive from these data an equation to provide an individualized and improved estimate of MAP in the single patient. A reliable estimation of MAP is a fundamental piece of the calculation of indexes derived from the central blood pressure analysis, such the subendocardial viability ratio (SEVR).

Methods

Populations

Data from 3 populations were considered in the present study. Population 1 consisted of a large database of individuals undergoing applanation tonometry recordings in the frame of a general population study. Individuals were mainly healthy subjects without overt cardiovascular disease or hypertension, residents in Europe. Population 2 were patients with hypertension enrolled from reference clinics for treating hypertensive outpatients, in Italy. Arterial applanation tonometry was performed for the evaluation of hypertensive organ damage, as recommend by current guidelines [61]. Population 3 came from baseline data of the PARTAGE study, a multicenter, longitudinal study aimed at determining predictive value of blood pressure and arterial functional parameters on mortality and cardiovascular outcomes in a population of individuals 80 years of age and older living in nursing homes. Patients were enrolled in nursing homes in Italy and France. Only data from patients with a brachial arterial tonometry of sufficient quality were used in the current study. Quality of data was reviewed by an independent operator who was blinded to other patient's data.
In a group of 18 healthy controls, selected from peers and colleagues, we assessed inter-observer repeatability of brachial waveform, and variations after mental stress test. The subject performed forced mental arithmetic with serial subtractions of 7 from 700 or of 13 from 1012, trying to keep pace with a metronome at a rate of approximately 90 beats/min. Informed consent was obtained from each subject
before inclusion in the study. The protocol was approved by the Ethics Committee of the IRCCS Istituto Auxologico Italiano, and the study was performed according to the Declaration of Helsinki.

Brachial applanation tonometry

A validated, easy to use and high-fidelity PulsePen tonometer (DiaTecne srl, Milan, Italy), was used to acquire the brachial pulse waveform. According to this method, the sensor is placed over the skin were the artery is found, applying a moderate pressure so that the artery comes slightly compressed (applanation tonometry) with a balance of the circumferential forces inside the vessel. In this way the sensor records the pressure in the middle of the compressed artery. These pulse waves do not differ significantly from pulse waves recorded by intraarterial measurements. The tip of the tonometer was placed in the point of maximum pulsation of the brachial artery in the cubital fossa, detected by finger palpation. Tonometer signal acquisition lasted 10 cardiac cycles, with a simultaneous ECG performed, while the patient was laying supine in complete rest. Brachial tonometry was performed by trained observers. Data concerning intra-operator and inter-operator reproducibility of arterial tonometry variables were published in previous papers from our group [62][63].
Repeatability was assessed interobserver in 18 healthy controls, in a single session. In these 18 subjects variations during mental stress test were assessed, by measuring brachial waveform after 3 and 6 minutes from the start of arithmetic calculations. Results obtained in these subjects were averaged from two measurements obtained at rest and two during stress.

Mean arterial pressure measurement

The integral of the brachial pulse waveform was calculated by proprietary software to define the percentage of pulse pressure that has to be added to diastolic blood pressure

(DAP) to obtain the MAP (PP%). MAP was defined from systolic (SAP) and DAP brachial oscillometric blood pressure measurements, by the following equation:
MAP = DAP + PP% (SAP-DAP)
An example of brachial pressure wave measured by arterial tonometry and measurement of the relative MAP is provided in Figure 1.
Other empirical formulas which are very popular in medical literature or used in clinical trials were used to calculate MAP, and tested against the reference:
MAP^1 = DBP + (SAP-DAP)/3
MAP^2 = DBP + 0.4 (SAP-DAP)
MAP^3 = DBP + 0.412 (SAP-DAP)
MAP^4 = DBP + (SAP-DAP)/3 + 5 mmHg

SAP and DAP measurements were performed at the brachial artery level using validated oscillometric devices, immediately after the measurement by tonometry

Statistical analysis

Descriptive statistics are presented as mean±SD. Continuous variables were tested to detect deviations from normality by computing the Shapiro-Wilk test. Qualitative data were compared with Pearson's chi-square or Fisher's exact test when appropriated, continuous variables with t-test or ANOVA. Stepwise multiple linear regression models were constructed to elucidate independent determinants of PP%. Variance inflation factor was computed to check multicollinearity between independent variables in linear regression models, with a cut-off value of 5. Separate analyses were conducted for the pooled population and for subpopulations. The degree of correlation was expressed as Pearson's R. The agreement between different MAP estimates (MAP^n) and the reference (MAP) was assessed using the Bland Altman plot. The differences were defined as significant in the presence of $p < 0.05$. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, Release 20.0; SPSS, Chicago, IL).

Results

A total of 1517 subjects were considered in the study. General characteristics of the pooled population and of the 3 populations are presented in Table 1. Figure 1 shows the distribution of PP% in the pooled population. PP% ranged from 23% to 58%. PP% was found to be significantly higher in females compared to males in all the populations ($p<0.001$).

Table 4.1. General characteristics of the 1517 subjects. PP%: brachial form factor.

	Population 1	Population 2	Population 3	Pooled
Number of subjects	490	283	744	1517
Age (years)	49.6 ± 12.7	87.5 ± 4.6	59.3 ± 14.4	61.4 ± 18.2
Gender (m/f)	193/297	71/212	406/339	670/848
Systolic BP (mmHg)	127.0 ± 18.6	138.5 ± 20.7	135.7 ± 19.7	133.4 ± 20.1
Diastolic BP (mmHg)	73.9 ± 9.7	69.4 ± 10.9	80.8 ± 10.8	76.4 ± 11.4
Heart rate (s^{-1})	67.3 ± 11.3	68.8 ± 9.9	65.3 ± 10.2	66.6 ± 10.6
Mean BP (mmHg)	96.7 ± 13.8	97.9 ± 15.4	104.0 ± 13.7	100.5 ± 14.5
PP %	42.8 ± 6.0	40.9 ± 5.3	42.4 ± 4.8	42.2 ± 5.4
Hypertensive (%)	0%	66.4%	100%	61.5%

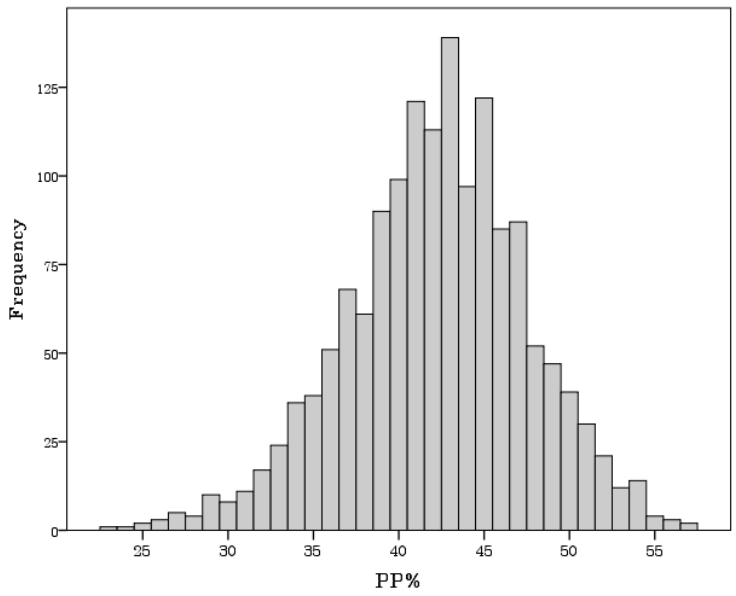

Figure 4.2. Distribution of the brachial form factor (PP%), or the percentage of pulse pressure required to be added to diastolic pressure to obtain mean arterial pressure, in 1517 subjects.

Comparison of different MAP estimates

Average bias for different MAP^n estimates from reference MAP, in the pooled population, was -5.04±3.43 mmHg for MAP^1, -1.24±3.11 mmHg for MAP^2, -0.55±3.10 mmHg for MAP^3, -0.35±3.43 mmHg for MAP^4. Average absolute bias, defined as the average of absolute values of differences, was 5.21±3.16 mmHg for MAP^1, 2.61±2.10 mmHg for MAP^2, 2.41±2.01 mmHg for MAP^3, 2.60±2.23 mmHg for MAP^4. Absolute difference from the reference MAP ranged from 0 to up 19.48 mmHg (for MAP^1). Bland-Altman plots (Figure 2) comparing MAP^n with reference MAP showed a clear skewed tendency toward an overestimation of MAP^n for low MAP values, and an

underestimation for high MAP values. Results were consistent in the single 3 populations and when stratifying the population by gender.

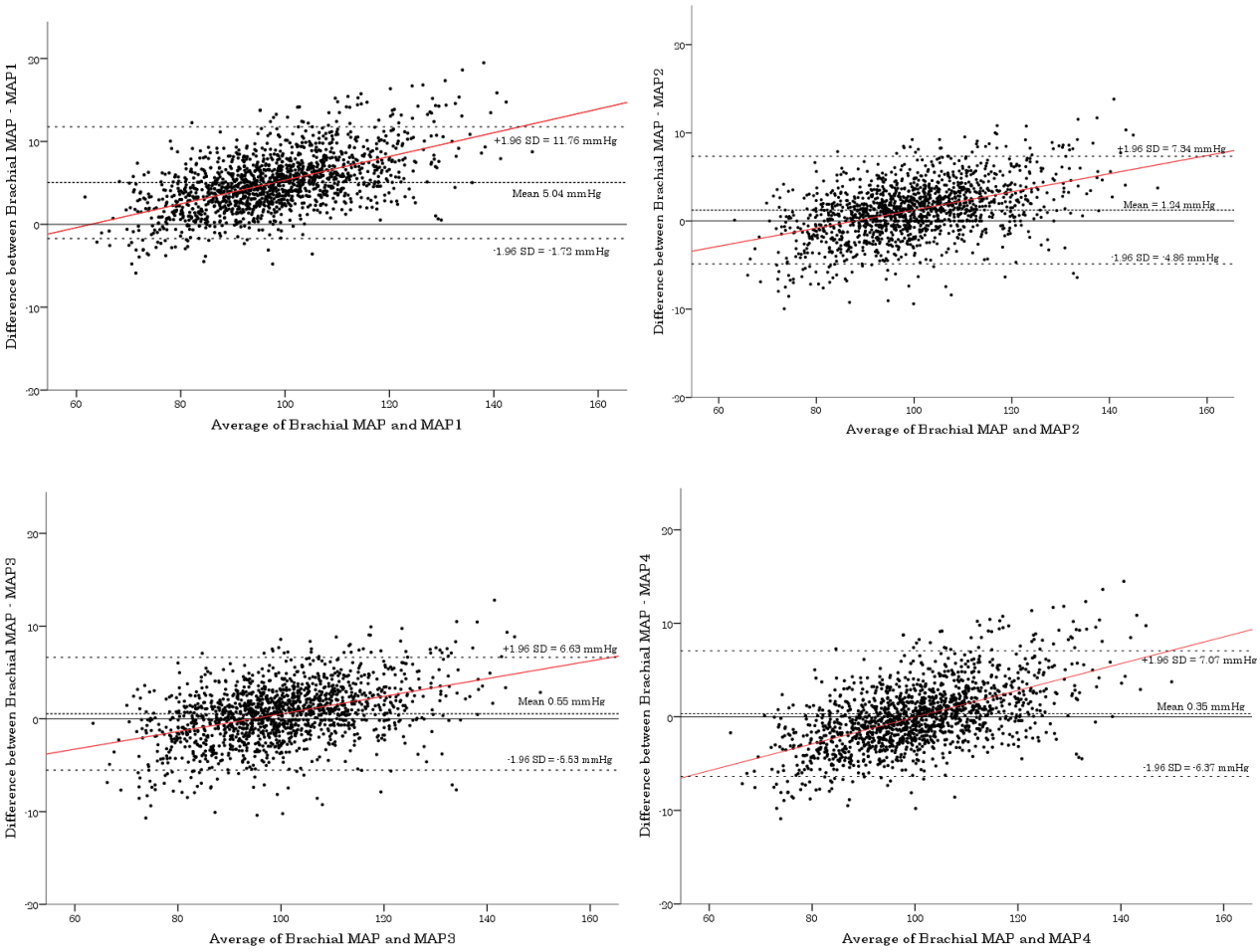

Figure 4.2. Bland-Altman plots of different estimates of mean arterial pressure (MAP^n) compared to reference MAP obtained by brachial tonometry. See text for definitions of MAP^n.

Determinants of brachial form factor

Multivariate regression models were created to identify independent determinants of PP% in the three populations and in the pooled population. Variables identified from stepwise approach and results of the regression procedure are reported in Table 2.

Table 4.2. Results of stepwise regression analysis to identify predictors of PP% (percentage of pulse pressure required to be added to diastolic blood pressure to obtain the mean arterial pressure).

		Unstandardized Coefficients		Standardized Coefficients	Significance
		B	Std. Error	Beta	
Population 1	(Constant)	26.791	1.995		<0.001
	Diastolic BP	0.194	0.026	0.314	<0.001
R^2 = 0.136	Gender	2.721	0.518	0.222	<0.001
Population 2	(Constant)	16.987	2.746		<0.001
	Systolic BP	0.070	0.017	0.272	<0.001
	Heart rate	0.089	0.029	0.165	0.002
	Diastolic BP	0.102	0.032	0.207	0.002
R^2 = 0.242	Gender	1.301	0.658	0.106	0.049
Population 3	(Constant)	25.070	1.540		<0.001
	Diastolic BP	0.169	0.017	0.381	<0.001
	Gender	1.983	0.313	0.207	<0.001
	Heart rate	0.082	0.016	0.174	<0.001
R^2 = 0.215	Systolic BP	-0.019	0.009	-0.079	0.038
Pooled	(Constant)	26.142	1.131		<0.001
	Diastolic BP	0.180	0.012	0.385	<0.001
	Gender	2.075	0.257	0.193	<0.001
	Heart rate	0.036	0.012	0.072	0.002
R^2 = 0.174	Population	-0.601	0.149	-0.100	<0.001

Estimation of a predictive model for brachial form factor

Among variables found to be significant predictors of PP% in the multivariate model, DAP and gender were selected to identify a predictive model for PP% estimation, because these were the only two significant predictors in the 3 considered populations. The influence of heart rate in the multivariate model in the pooled population was, although significant, only marginally contributing to the model predictivity.

Coefficients of regression model considering DAP and gender were calculated from a training set consisting in a subset of randomly selected 70% of the subjects:

$$PP\% = 27.070 + 0.181 * DAP \ (+ 2.303 \text{ if female}) \quad [1]$$

Performance of equation [1] was tested against the remaining 30% of dataset, considered as validation set. Coefficient of determination of regression was 0.173 in the training set and 0.128 in the validation set.

Mental stress test

Mental stress was administered to 18 healthy young subjects (mean age: 30.6 years, 50% males, 44% smokers). Mental stress caused significant a rise in SAP of 13.2±6.2 mmHg, in DAP of 12.1±6.2 mmHg, and in HR of 13.7±7.9 s^{-1}. PP% increased from 39.9±3.2 to 43.0±4.0 ($p<0.0001$) (Figure 3). Variations in PP% between baseline and mental stress were significantly correlated to variations in SAP, DAP and HR (respectively R=0.711, p=0.001; R=0.629, p=0.005; R=0.633, p=0.001).

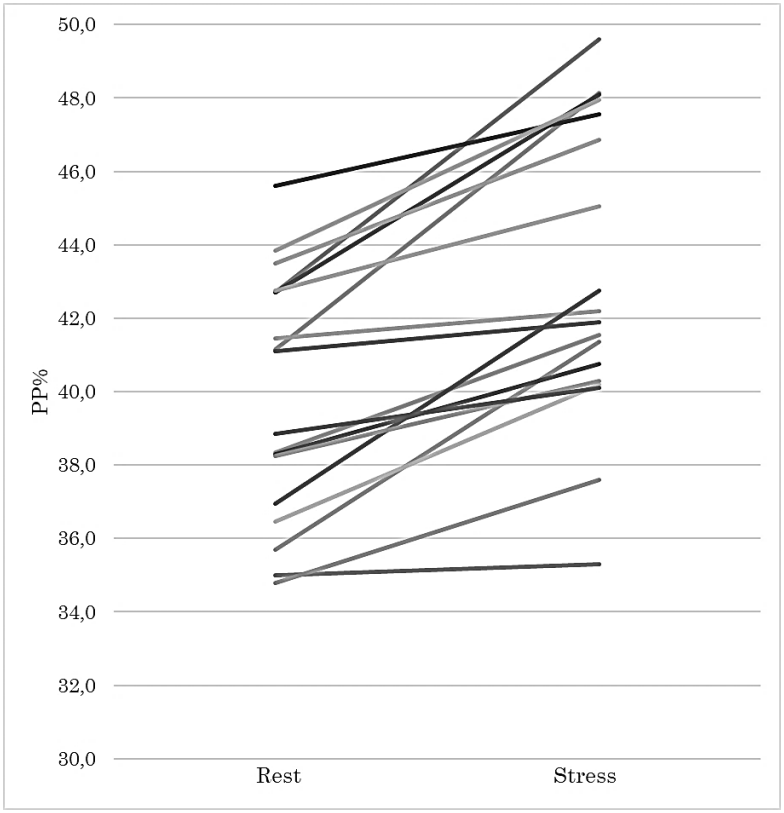

Figure 4.3. Variations in brachial form factor (PP%) at rest and during mental stress test in 18 healthy subjects.

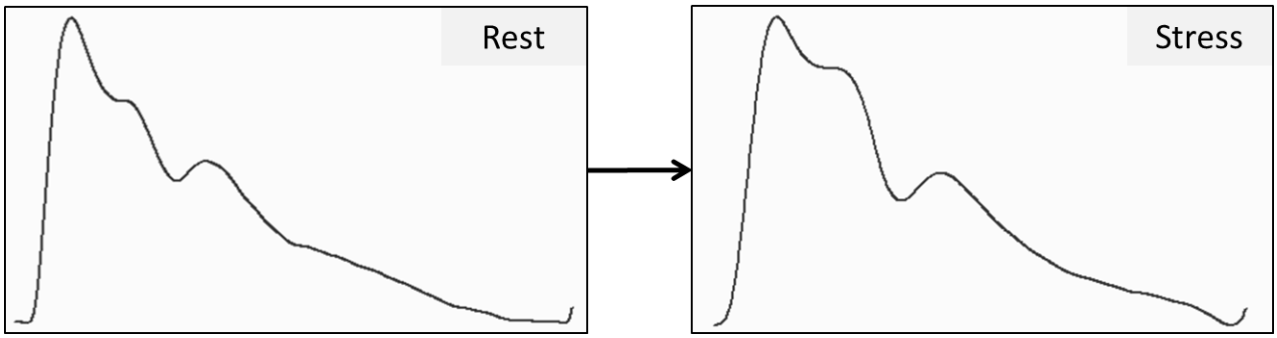

Figure 4.4. Example of brachial pressure wave in a male subject at rest (Blood pressure 123/72 mmHg, heart rate 50, PP% 34,2) and during mental stress (Blood pressure 136/92 mmHg, heart rate 63, PP% 38,4).

Repeatability

PP% presented a good interobserver repeatability at rest and during mental stress test. At rest, repeated measurements of PP% by two different operators were highly correlated (R^2=0.81), with a mean difference of 0.13 and a mean absolute difference of 1.33, and an intraclass correlation coefficient of 0.91. During mental stress test, intraobserver repeatability of PP% was also good, with R^2=0.71), a mean difference of -0.13, a mean absolute difference of 1.56, and an intraclass correlation coefficient of 0.85.

Discussion

The present study showed, for the first time in large populations of both normal and hypertensive individuals of different age classes, a marked interindividual variability in the brachial pressure wave form-factor or PP%, measured by brachial arterial tonometry and defined as the percentage of pulse pressure required to be added to diastolic pressure to calculate MAP. PP% showed a significant gender difference, an association with DAP values, and a marginal association with heart rate and SAP in elderly and in hypertensive groups. We showed that PP% increased significantly after mental stress in healthy individuals, in parallel to blood pressure and heart rate rise. We calculated from our data an improved formula to calculate MAP that minimizes interindividual variability, by considering pressure levels and gender.

MAP is one of the most important parameters in cardiovascular physiology, representing the driving pressure for organ perfusion, and is evaluated in several situations in clinical practice, most commonly by measuring it non-invasively by brachial cuff. As examples of current use, peripheral vascular resistances can be calculated in systemic calculation by dividing MAP per the cardiac output, and MAP is monitored during the treatment of sepsis, to titrate fluids and vasopressor. Moreover, some evidence suggest that MAP may be more accurate in predicting cardiovascular outcomes than SAP or DAP, although current guidelines on arterial hypertension consider only systolic and diastolic pressure values as targets for treatment.

The recent improvements in the measurement of central blood pressure by non-invasive devices has also raised the interest on the evaluation of MAP. Calibration of pressure signals is commonly made by peripheral brachial cuff blood pressure. Given that MAP and DAP are relatively stable from center to the periphery [14], their measurement from brachial cuff is the preferred method for the non-invasive calibration of the central pressure signal [57]. Nevertheless, the method used to calculate MAP can significantly modify the estimation of central systolic blood pressure, and therefore alter its prognostic value in clinical studies [64]. A reliable measurement of MAP from peripheral cuff measurement is a mandatory step to improve the measurement of central blood pressure and its use in future clinical trials and for the calculation of indexes derived from the central pressure waveform.

Our findings, supported by the evaluation of MAP in a large number of patients, show that a fixed formula for the evaluation of MAP may not be appropriate for everyone. The form factor, or PP%, of the brachial arterial wave presents a marked inter-individual variability, ranging from 23% to 58%. If one of the fixed formulas for MAP calculation are used, mean average bias from brachial MAP is up to 5 mmHg. And, most importantly, the bias from the real MAP is greater for subjects with higher blood pressure values, in which an underestimation of MAP is more common. The prognostic value of MAP could be therefore overlooked in the subjects at higher risk for cardiovascular events.

Diastolic blood pressure seems to be the most relevant determinant of brachial form factor. The role of this parameter is statistically more relevant than systolic blood pressure and other variables, such heart rate of age. A possible explanation of this phenomenon resides on the functional changes induced by an increased diastolic blood pressure in brachial artery geometry. Vascular tone in resistance arterioles is the main drive of peripheral vascular resistances and may increase diastolic blood pressure, more than systolic, Moreover, brachial form factor showed a marked gender difference, with higher value in females compared to males. Estrogens relevantly influence the

vascular function in women, by inducing vasodilation and by inhibiting vascular remodeling [65]. Gender differences on the vascular wall are canceled by menopause, as testified by the steep increase in vascular stiffness after menopausal transition. The less marked gender difference in PP% among elderly patients (Population 2) supports the hypothesis of a role of gonadal hormones in the shape of the brachial pressure wave.

The formula indicating the PP% from DAP and gender (reported as equation [1]) could represent an opportunity to easily calculate MAP from readily accessible variables. Despite the expected accuracy of this model in practice is suboptimal, as evidenced by the cross-validation procedure, it could be better than a fixed estimate of PP%. Our data encourage the direct measurement of the brachial PP% in the practice to obtain an accurate estimate of MAP, but, if this is not possible, the application of the above-mentioned formula is a realistic possibility to improve the calculation of MAP. As the formula was validated with an internal analysis, and not with an independent sample this could represent a possible limitation causing an overestimation of the true replicability of the formula. Further independent validation studies are needed to examine the predictive ability of the formula.

Our study may be also limited by the adopted non-invasive methodological approach. An invasive approach is also possible to estimate MAP, despite systematic errors and inaccuracies also commonly affect invasive blood pressure measurements obtained by catheterization. These issues are frequently observed in the catheterization of peripheral arteries, as the brachial artery, because the low sectional area may interfere with measurements. Therefore, the non-invasive approach is the only option to evaluate unbiased brachial pressure waveforms in a large population.

The proposed approach for the calculation of MAP permits a reliable estimation of this parameter from the available non-invasive measurements of systolic and diastolic blood pressure. Since MAP, along with DAP, are necessary for the calibration of central blood pressure wave acquired non-invasively (with arterial tonometry), a more reliable of true blood pressure values in the central is expected. The improvement in MAP calculation is therefore an important piece for the methodological improvement of central blood pressure wave acquisition and for the indexes derived from it, as the SEVR.

5. In-vivo comparison of invasive and non-invasive Subendocardial Viability Ratio

In the last years the group involved in research about large artery mechanics of the cardiological research laboratory of the Istituto Auxologico Italiano has improved the evaluation of the SEVR by arterial tonometry, correcting the important limitations that characterized the previous method of measurement of this index. The proposed equation, which redefines the SEVR on the basis of the left ventricular diastolic pressure (LVDP) and of the time of cardiac isovolumetric contraction and relaxation (ICT and IRT), and applies a calibration of central pressure wave on the real mean brachial blood pressure, required a validation towards invasive gold standard method.
The two main purposes of the present study were:
- to verify the greater accuracy of the improved formulation of SEVR by arterial tonometry compared to the previous one (SEVRold), through a comparison to invasive gold standard method, obtained by cardiac catheterization;
- to refine, on the basis of the collected data, the non-invasive definition of the left ventricular diastolic pressure (LVDP), a relevant element of the new equation.

Methods

The study was conducted at the Hemodynamics Service of the Policlinico di Monza, where the registration of the aortic and left ventricular pressure curves is included in each coronary angiographic examination, performed for clinical reasons. Both the non-invasive measurements, carried out through arterial tonometry, and the statistical analysis of the results, were conducted by the group of the cardiological research laboratory of the Italian Auxological Institute. The invasive measurements were collected by cardiologists expert in interventional procedures who were performing the exams for diagnostic or interventional purposes. The study protocol was approved by institutional Ethics Committees and was conducted in accordance with the Helsinki Declaration, with subjects giving informed consent to all procedures.
Participation in the study was proposed to all patients undergoing coronary diagnostic examination at the Hemodynamics Service of the Policlinico di Monza, which did not fall within the exclusion criteria from the study; patients enrolment was conducted until the expected sampling number was reached. Of the 65 total patients enrolled, all those presenting potential confounding biases were excluded during the analysis. In particular, all the hypertrophic cardiomyopathies with hemodynamically significant gradient for outflow obstruction (4 cases) and all severe valvulopathies (4 mitral insufficiencies, 1 aortic insufficiency, 1 aortic stenosis) were discarded. 1 further case was excluded from the analysis for technical problems related to the invasive measurement (ineffective catheter washing). The analysis was therefore performed on 54 patients.
Exclusion criteria were for patients undergoing angiographic examination in emergency conditions; conditions of heart failure in labile hemodynamic compensation; atrial fibrillation; presence of pace-maker.

Experimental protocol

First, the patient's medical records were assessed, to highlight the presence of any exclusion criteria from the study.
The candidate patient was conducted in an outpatient room, where the objective of the

research was clearly explained and the willingness to participate in the study was requested. The patient was asked to carefully read the document of the informed consent to the study, and to sign it only in case of consent. Subsequently the main anthropometric parameters were collected.
The patient was made to lie down on the couch and left in absolute rest for 5 minutes. In the meantime the operator has collected from the clinical data the main anamnestic details, with particular reference to drug therapy.
The electrodes were then placed for the electrocardiogram and the cuff for pressure measurement, with subsequent recording of the carotid, femoral and brachial arterial pressure curves using a validated PulsePen® tonometer (DiaTecne, Milan). From the registration of the carotid and femoral arteries the measurement of the velocity of the carotid-femoral pulse wave (carotid-femoral PWV), indicative of the viscoelastic properties of the aorta, was obtained. Carotid-femoral PWV was then used in the definition of the systolic times, in particular for the quantification of the isovolumetric contraction time (see previous chapter), necessary for the correct definition of the SEVR.
The patient then had access to the hemodynamics room, where the introduction of the intraarterial catheter was performed for the angiographic procedure, all in accordance with the routine procedure of the host structure.Subsequently, invasive pressure was recorded in the left ventricle cavity for 10 seconds.
The tip of the catheter was then removed until it reached the ascending aorta, 3 cm above the valve plane: it was then allowed the entry of the "tonometer" operator inside the hemodynamic room, for the transcutaneous carotid registration of central pressure, carried out simultaneously to intraaortic recording.
Invasive and non-invasive measurements were compared only at the end of the study.
Two custom designed softwares for specific data processing (SPEGL, Milan, Italy) were used to obtain the invasive SEVR. The first software perfomed the identification of the invasive registration and paired it with the tonometric signal (Fig. 5.1). By bridging the gap between the two signal (usually a few milliseconds), it permitted the exact overlap of the R waves of the respective ECG tracks and therefore of the pressure curves.
Using a second software, the average invasive mean curves, both intraventricular and aortic, were calculated; then the overlap was performed, by pairing the wave feet and the systodiastolic transition point. The areas defining the DPTI: SPTI ratio were thus obtained, and the gold-standard invasive SEVR, as described by Buckberg and Hoffmann, was then calculated from invasive aortic and left ventricular pressure waves.

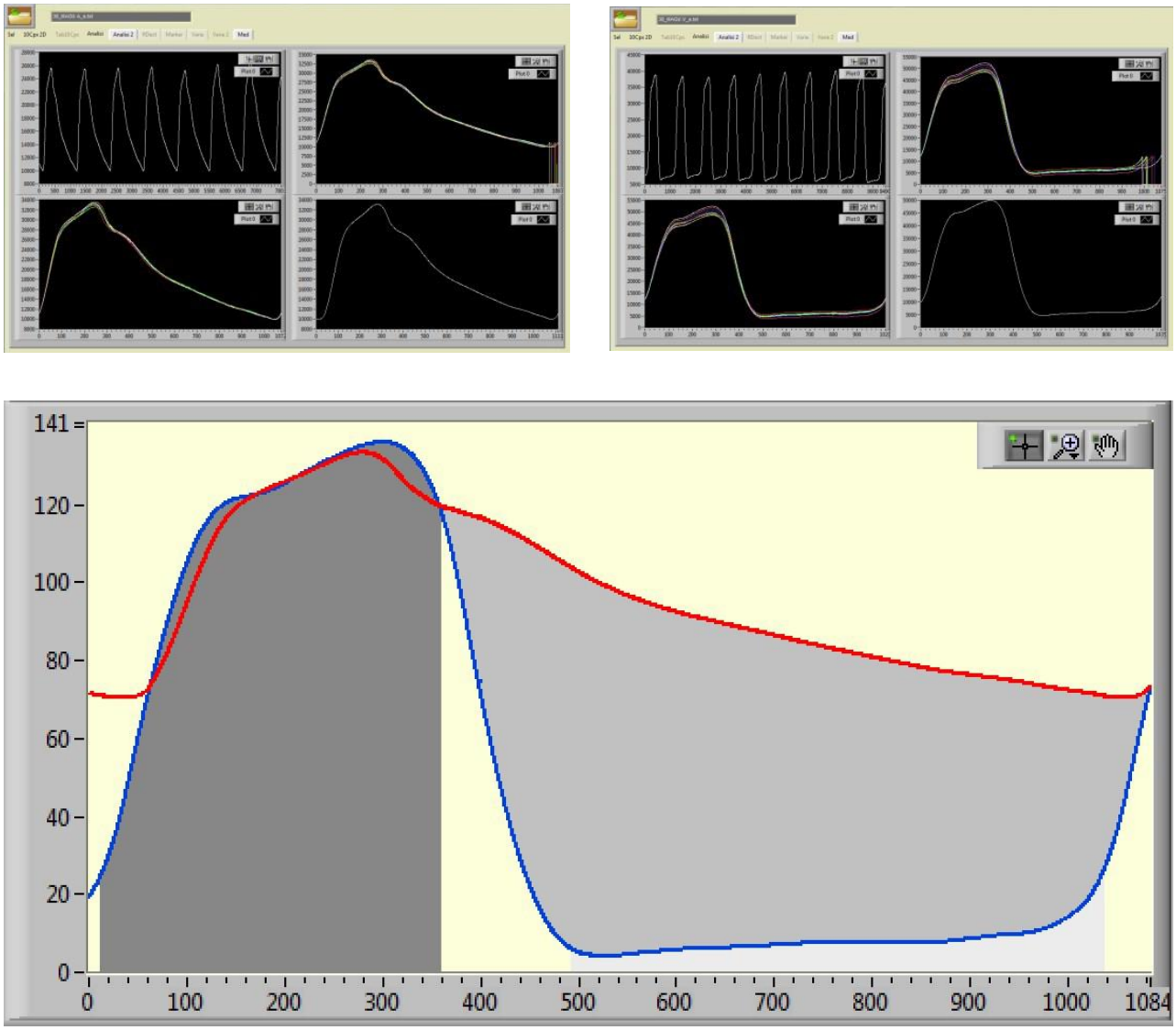

Fig. 5.1. Pressures detected with invasive catheterization, respectively aortic (upper left panel) and left intra ventricular (upper right panel). Lower panel: Superimposition of the two waves and determination of SPTI and DPTI areas for Buckberg index calculation.

The traditional SEVRold is basically calculated from different tonometers on the market today, including the Pulsepen (the device used in this study), by calculating the ratio of diastolic and systolic integrals of the pressure waves (Figure 5.2):

$$\text{SEVRold} = \frac{\text{DPTI}}{\text{SPTI}} = \frac{\text{MDBP}}{\text{MSBP}} \cdot \frac{\text{DT}}{\text{LVET}}$$

, where MDSP and MSBP are mean diastolic and systolic pressures.

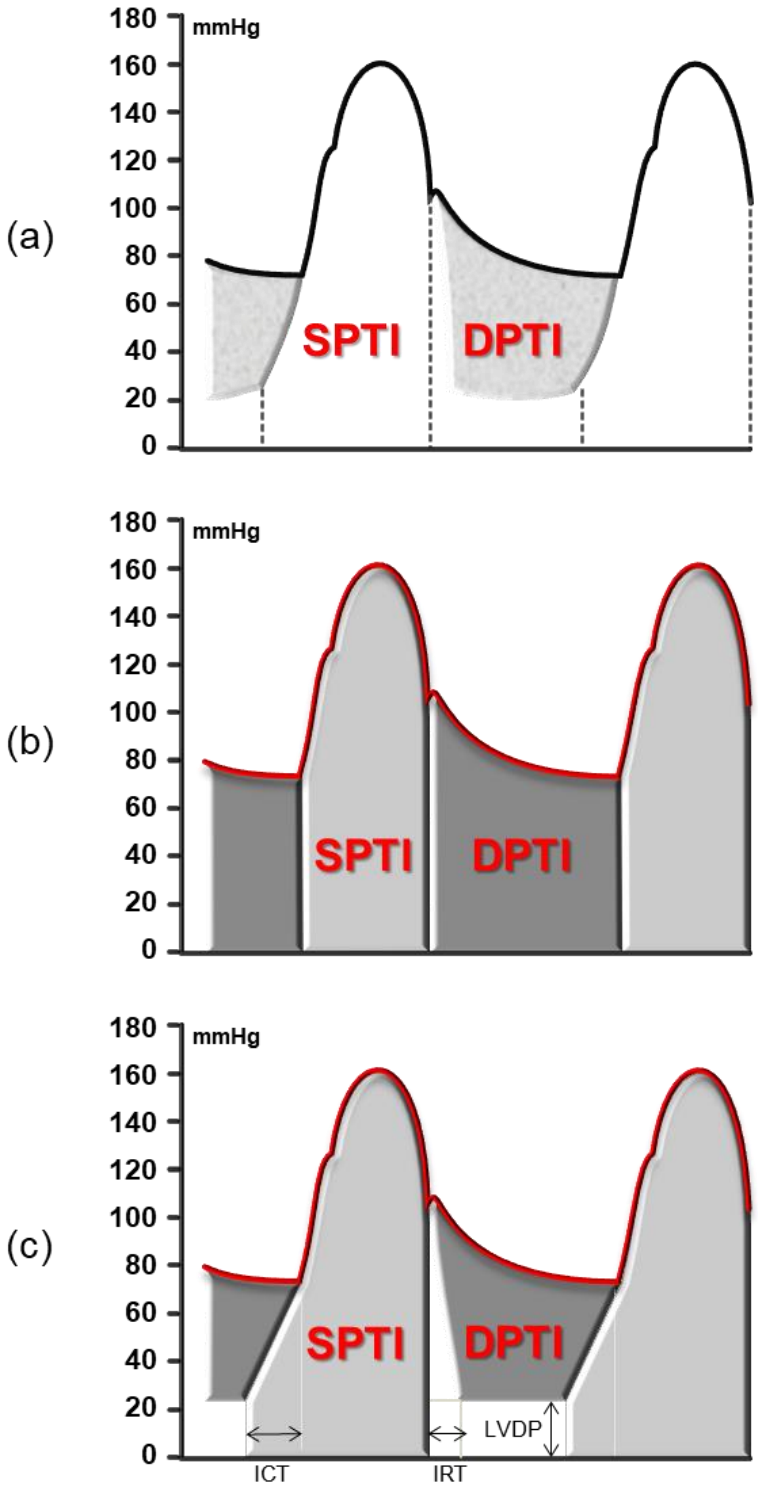

Figure 5.2. a) The Buckberg index as derived from invasive pressure curves. b) The SEVR old. SPTI: systolic pressure time index. DPTI: diastolic pressure time index. c) The revised version of non invasive SEVR. ICT: isovolumic contraction time. IRT: isovolumic relaxation time. LVDP: left ventricular diastolic pressure.

For the revised definition of the SEVR, instead, several corrections are needed, as shown in Figure 5.2, panel c. The isovolumic contraction time (ICT) is needed to adjust the calculation of SPTI, because the ICT represent a cardiac interval that should be included in systole, rather than in diastole. The isovolumic relaxation time (IRT) and the average left ventricular diastolic pressure (LVDP) are also needed to refine the calculation of DPTI, as they can significantly affect this parameter.
For the definition of non-invasive values of LVDP, a multivariate analysis was performed considering the LVDP obtained from invasive catheterization of left ventricle as the outcome variable. Parameters that can be entirely obtained using a non-invasive method were considered namely: heart rate, left ventricular ejection time (LVET), diastolic time (DT), left ventricular myocardial function (expressed by the PEP / LVET ratio), central pulse pressure (CPP), mean arterial pressure (MAP). The algorithm for ICT calculation is described in chapter 3, and has already been validated in a previous study [66]. Considering the value of ICT, an estimate of IRT was then obtained on the basis of the value of the aortic end-of-systole pressure (ESBP).
The following are the equations for the calculation of the revised SPTI and DPTI:

$$SPTI = (LVET * MSBP) + \frac{(LVDP + DBP) * ICT}{2}$$

$$DPTI = (DT * MDBP) - \frac{(LVDP + DBP) * ICT}{2} - \frac{(LVDP + ESBP) * IRT}{2} - ((DT - ICT - IRT) * LVDP))$$

The new SEVR resulted from the ratio of revised DPTI and SPTI.
Furthermore, the calibration of the non-invasive central pressure wave was obtained by calibration with mean arterial pressure obtained from the integral of brachial pressure wave, as described in Chapter 4.

Statistical analysis

Prior to the study calculation of the required sample size was performed. Based on the method described by Bland and Altman, with a standard deviation of the differences in the measurement of the SEVR between the invasive and non-invasive method of 0.20, the 95% confidence interval of the mean difference between approximately ± 0.057 and a 95% confidence interval of the limits of agreement of about 0.194, assuming a 10% dropout, it was necessary to enroll 65 patients in this study.
For the values thus obtained of the SEVR and of the SEVRold, the degree of agreement with the Buckberg index obtained invasively by simple linear regression was initially assessed. Subsequently, in the presence of a significant correlation, the difference between the paired data was related to the relative average values, according to the method of Bland and Altman [47]. The equation to calculate LVDP was derived from multiple regression analysis including important determinants obtained by arterial tonometry. Data are expressed as mean ± standard deviation (SD). The differences were defined as significant in the presence of $p < 0.05$. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS for Windows, Release 20.0; SPSS, Chicago, IL).

Results

The analysis was performed on 54 patients (40 men, 14 women) of average age 66±12 years and average BMI 27±3 kg/m2. 24% of patients had type 2 diabetes mellitus, 85% hypertension and 67% dyslipidemia.
In the following table the assessed hemodynamic parameters are reported, including the systolic and diastolic times, of the measured invasive ventricular diastolic of the invasively obtained Buckberg index, as well as of the SEVR and of the $SEVR_{old}$.

Table 5.1. Hemodynamic parameters evaluated in the 54 patients in the study. BP: blood pressure. SEVR: subendocardial viability ratio.

Brachial Systolic BP	145±22 mmHg	Left Ventricular Ejection time	322±33 msec
Brachial Diastolic BP	80±8 mmHg	Diastolic Time	617±153 msec
Heart rate	66±12 bpm	Isovolumic Contraction Time	74±20 msec
Central Systolic BP	134±21 mmHg	Isovolumic Relaxation Time	112±33 msec
Central Diastolic BP	80±8 mmHg	Pulse Wave Velocity	11±3 m/sec
Central Pulse Pressure	55±20 mmHg	Invasive Systolic Pressure Time Index	42527±9212
Mean blood pressure	101±11 mmHg	Invasive Diastolic Pressure Time Index	37407±11693
Mean Systolic BP	122±17 mmHg	Buckberg Index	92±35
Mean Diastolic BP	90±8 mmHg	Left ventricular diastolic pressure	15±6 mmHg
End-systolic BP	110±13 mmHg	Revised Systolic Pressure Time Index	43220±8611
Augmentation index	21±14	Revised Diastolic Pressure Time Index	41310±13151
Systolic Pressure-Time Index	39581±8776	Pre-Ejection Period	122±27 msec
Diastolic Pressure-Time Index	55486±13884	PreEjection/LeftVentricular Time ratio	0,4±0,1
$SEVR_{old}$	140±34	SEVR	98±33

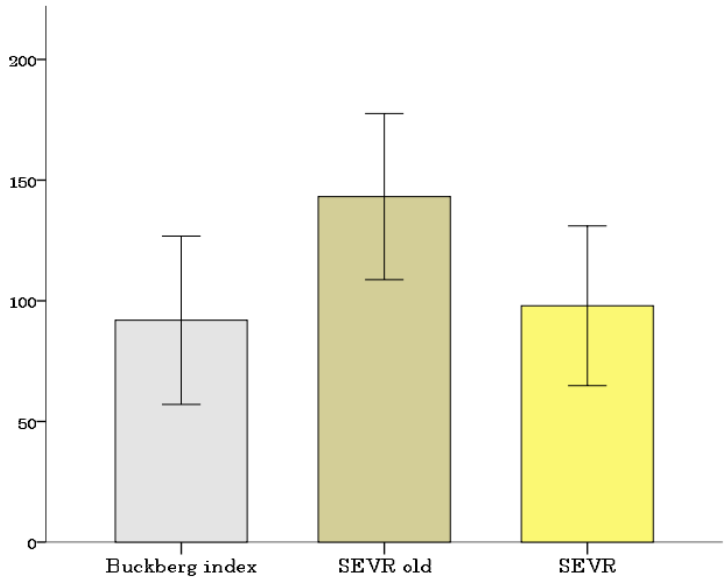

Figure 5.3. Comparison among the invasive Buckberg index and the old and revised versions of SEVR.

The new SEVR was significantly lower ($p<0.0001$) when compared to the $SEVR_{old}$, as shown in Figure 5.3. The good agreement between the Buckberg Index and the new SEVR was confirmed in the linear correlation and Bland Altman analysis (Figure 5.4).

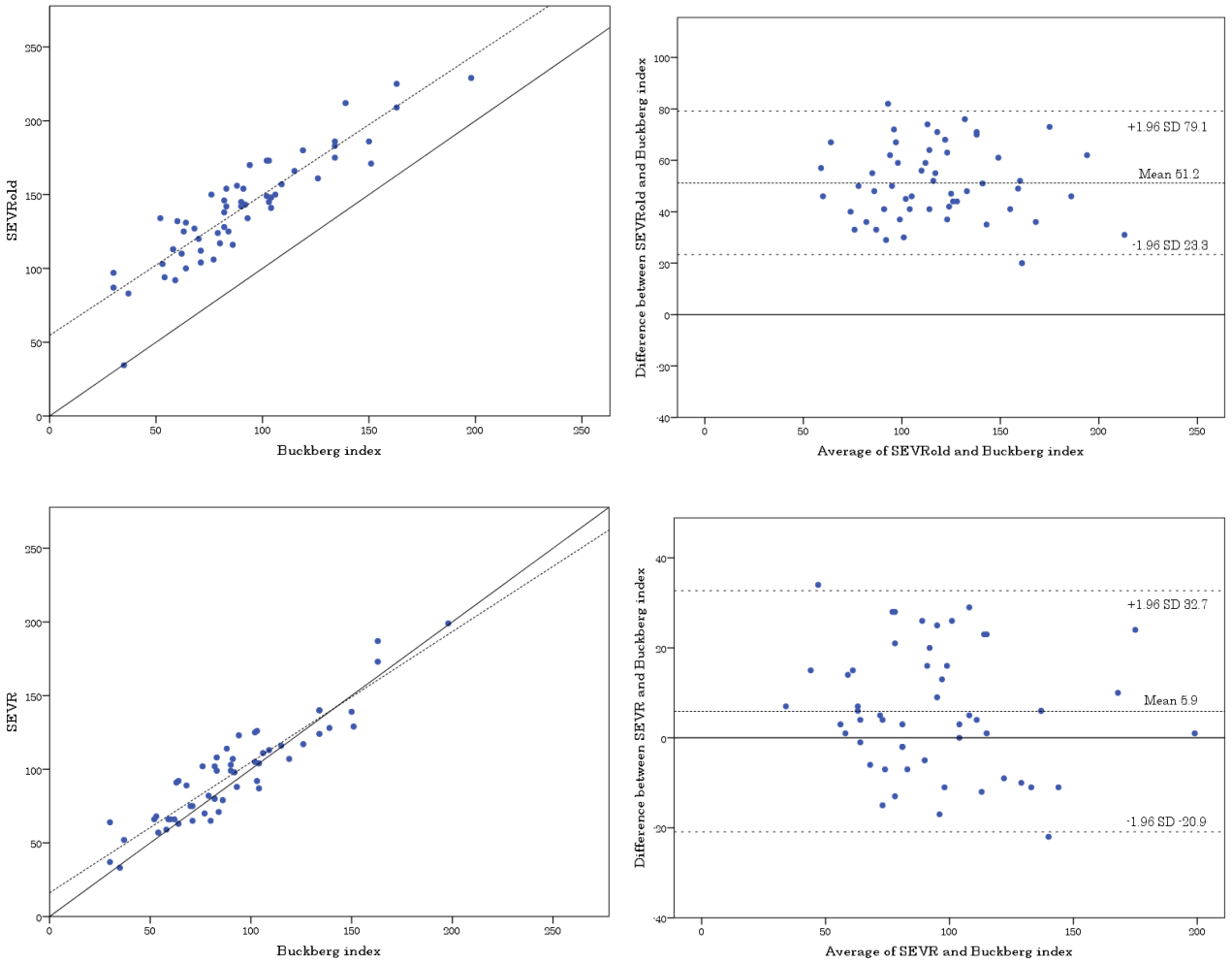

Figure 5.4 Linear correlation and Bland-Altman plots between $SEVR_{old}$ and the invasively determined Buckberg index and between the new SEVR and the Buckberg index.

The $SEVR_{old}$ presented a good correlation with the Buckberg Index ($R^2 = 0.84$) but clearly overestimated compared to the invasive Buckberg index. The revised SEVR, maintained a strong linear correlation with its invasive counterpart, ($R^2 = 0.85$) was located near the line of identity. The overestimation of the $SEVR_{old}$ was also shown in the Bland-Altman plot (Figure 5.4).

LVDP was defined by a multivariate regression analysis from physiological determinants that can be non-invasively estimated by the arterial tonometry. The following equation was elaborated:

LVDP = (-0,36 x HR) + [12,3 x (cPP / MAP)] + [-15,6 x (DT / LVET)] + [48,3 x (PEP / LVET)] + 43,8

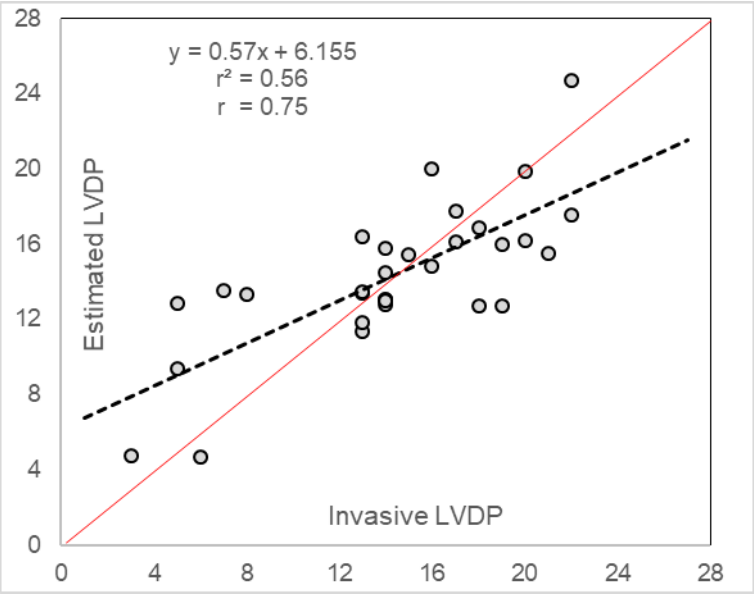

Figure 5.5. Correlation between left ventricular diastolic pressure (LVDP) measured by invasive catheterization and LVDP estimated by equation (see text).

Discussion

Several studies demonstrated the accuracy of the Buckberg index in defining the imbalance between oxygen supply and demand in the subendocardium, and showed variations of this balance according to physiological or pathological conditions [24][67][68][69][70][71][26]. These same studies have also suggested a critical threshold for the SEVR, below which the subendocardial blood flow is insufficient and the onset of electrocardiographic, clinical and laboratory signs of ischemic suffering is more likely [72]. However, it has never been possible to introduce the SEVR in everyday clinical practice: for its definition, in fact, the invasive procedure of aortic and ventricular catheterization is necessary. The introduction of transcutaneous arterial tonometry provided a new approach to the non-invasive evaluation of the index, which, however, had some major drawbacks in its first formulation. The traditional tonometric $SEVR_{old}$ does not take into account isovolumetric contraction and relaxation times, nor the left ventricular diastolic average pressure, with a consequent overestimation of the index and, probably, a less accurate evaluation and predictivity myocardial ischemia and cardiovascular outcomes. In clinical studies evaluating the $SEVR_{old}$, no solid clinical correlates were identified that would suggest practical use of this non-invasive parameter [36].

The new method for the calculation SEVR which was formulated and validated in our laboratory provides a value of non-invasive SEVR which is substantially identical to the parameter obtained from invasive gold standard method, making the old formula out to date. A slight tendency to overestimation of invasively determined values is still present in the new SEVR, although not comparable to the amount of overestimation of the previous formula, which was on average 50%.

The procedure of invasive validation conducted in the present investigation was mandatory in order to understand if the index is sufficiently reliable to be used in clinical practice. Our experimental procedure allowed to meet the validity criteria of the new non-invasive SEVR. The invasive evaluation of left ventricular and aortic pressures was obtained in the best possible conditions, with fluid-filled catheters with

correct calibration. The patients which were enrolled in the study were only cardiac patients with confirm coronary disease or under evaluation for that, because of the impossibility to enrol healthy controls for invasive procedures for ethical, as well as practical, reasons. This may not be a limitation since the SEVR is designed to be applied mainly to cardiac patients. Nevertheless, a validation on healthy subjects, as well as in other clinical conditions, is desirable.

Another possible limitation of the SEVR evaluated in our study possibly resides in the calculation of LVDP, which was estimated by multivariate analysis of some relevant hemodynamic variables derived from arterial tonometry, and not directly measured. Clearly, LVDP is not an easy parameter to be estimated non-invasively, and even echocardioghic methods to assess LVDP show insufficient accuracy[73][74]. We aimed to find an easier method to estimate this parameter, which is necessary to reliably evaluate SEVR at the bedside of the patient with a single, well tolerated, easily repeatable examination. A good level of approximation was found for the estimated LVDP against the invasive data. Clearly, further studies to validate the equation of the SEVR calculation are needed in the forthcoming studies.

The last aspect to consider is the absence, at the present time, of an univocal threshold value for the non-invasive SEVR, to predict subendocardial ischemia. In a clinical study involving healthy subjects performing strenuous exercise on a treadmill, the investigators found that all subjects with a invasively determined SEVR <0.45 had ischemic changes on the electrocardiogram, whereas the electrocardiogram was normal whenever DPTI:SPTI exceeded 0.45 [70]. This value is significantly lower than what was found in animal experimental conditions (anesthetized [51] or conscious [75] dogs) and in patients with aortic stenosis [76][77], in which a threshold value for subendocardial ischemia of 0.8 was found.

Moreover, a further adjustment of the SEVR for the arterial oxygen content and for the relative left ventricular mass and tension may be necessary to overcome the intrinsic imperfections of this index, as suggested by Buckberg [26]. However, this was not the aim of the present validation study, which was focused on the pressure-time derived indexes in the comparison between non-invasive and invasive measurements.

The validation of the new formulation of SEVR offers new opportunities for this index and is the first step to introduce this non-invasive diagnostic tool in the clinical practice. For how it was conceived, the non-invasive SEVR has several application possibilities. Only future studies will be able to clarify the validity of new SEVR formulation, by applying this index in different clinical settings and exploring the issue of the critical threshold for predicting myocardial ischemia.

6. Predictivity of the Subendocardial Viability Ratio: application in the PARTAGE study

The PARTAGE (Predictive Values of Blood Pressure and Arterial Stiffness in Institutionalized Very Aged Population) is a multicentre longitudinal study aimed at determining the predictive value of blood pressure and arterial functional parameters on total mortality and on major cardiovascular and cognitive outcomes in a large population of individuals 80 years of age and older living in nursing homes [78]. Several studies were conducted from perspective data of this large database. A higher mortality was found among elderly patients using multiple BP-lowering agents [79], as well as among subjects with orthostatic hypotension [80]. Regarding the role of arterial tonometry, the PARTAGE study demonstrated the role of the amplification of blood pressure (from the central arteries to the periphery) as a predictor of mortality among elderly patients [81].
The application of SEVR in elderly subjects, at high risk for myocardial ischemia, may represent an interesting opportunity to analyse with a non-invasive tool the myocardial supply-demand balance, which otherwise would require invasive procedures not always feasible in this category of patients. The health burden of ischemic heart disease in the elderly is very high, considered that incidence and prevalence of most cardiovascular diseases rise progressively with age, and cardiovascular diseases is the leading cause of death in those older than 75 years [82]. However, although the high cardiovascular burden in the oldest patients, they have been markedly underrepresented in most cardiovascular prevention studies. Challenges to implement cardiovascular prevention strategies in older adults are related with difficulty in estimating life expectancy and physician's discomfort with such prediction, although benefit for preventive strategies would be higher in older patients [83].
The SEVR may overcome the current knowledge gap in the elderly patients, an age class with the highest burden of cardiovascular disease that cannot be tested with invasive examinations or with costly imaging modalities, for economical, ethical and practical reasons. With this study, we aimed to evaluate the prognostic value of the new formulation of SEVR in the PARTAGE study, both for total mortality and for cardiovascular events. Additionally, we aimed to find the most appropriate value of SEVR to maximize the discrimination for cardiovascular events/mortality in this population.

Methods

Existing data from the PARTAGE study, a multicentre, longitudinal study, were collected from the principal investigators of the study. The PARTAGE study aimed at determining the 2-year predictive value of BP and arterial functional parameters on total mortality (primary endpoint) and on major CV outcomes and cognitive decline (secondary endpoints), in a large population of individuals 80 years of age and older living in nursing homes. Participants were enrolled in 4 French (Nancy, Dijon, Paris, Toulouse) and 2 Italian (Cesena and Verona) university hospital centres between January 2006 and June 2008. A total of 72 nursing homes participated in this study in France and Italy [78].
Participants were included if they were 80 years of age and over, living in nursing homes, and signed the informed consent. Subjects were excluded if they had severe

dementia (Mini-Mental Status Examination score <12 out of 30), a low level of autonomy (Activity of Daily Living [ADL] scale score ≤2 out of 6) or were under guardianship or some measure of legal protection. No other exclusion criteria were applied. The family and/or the physician of the patient were informed of the study and gave their approval.

According to the inclusion criteria, 1,259 individuals living in the nursing homes participating in this study were eligible. Among them, 1,130 (89%) agreed to participate and were enrolled in this study.

This study was approved by the respective regional ethics committees in France (Comité de Protection des Personnes) and in Italy (Comitato Etico Area Vasta Romagna and Comitato Etico della Provincia di Verona), and all participants gave written informed consent before the study.

Patients were followed for 10 years in the Nancy centre in France and from the Cesena centre in Italy. Data from Verona are yet not available after the 2-years follow-up. Follow-up was made by patients visits, electronic data collection and telephonic interviews of patients or relatives. In the present analysis only data from the Nancy and Cesena centres were used, because of the more extensive follow-up.

All geriatric assessment instruments and arterial measurements were performed in the nursing homes by a trained medical research investigator present at each university hospital centre participating in the study.

Arterial functional parameters

Central blood pressure values and aortic pressure waveforms were obtained directly from the common carotid artery using an applanation tonometer. Arterial tonometry was performed on right common carotid artery and femoral artery using a PulsePen device (DiaTecne srl, Milan, Italy). As previously demonstrated, the pressure waves recorded noninvasively by the PulsePen tonometer at the site of the common carotid artery are similar to pressure waveforms obtained invasively by means of an intra-arterial catheter [38] and central BP values and pulsed wave analysis recorded in the common carotid artery are a reliable surrogate of analysis recorded in the aorta by invasive methods [84]. Central BP values were obtained by the carotid curve integral after calibration with brachial mean and diastolic diastolic blood pressure measured noninvasively by a validated oscillometric sphygmomanometer at the brachial artery (Omron 705IT, Omron Co., Kyoto, Japan). Mean blood pressure was defined from the form factor of the brachial artery pressure wave, obtained by applanation tonometry performed with the same tonometer (PulsePen), in 291 patients. Where brachial tonometry was not performed for technical reasons or of not sufficient quality for analysis (778 patients), a form factor of 40% was used to obtain mean arterial pressure. The PulsePen device was also used for measuring carotid-femoral PWV, which is considered the gold standard for measuring arterial stiffness. The procedure was described in detail previously [46].

Subendocardial viability ratio (SEVR) was defined according to procedures described in the previous chapter of this work, by considering the isovolumetric contraction time and the left ventricular diastolic pressure calculated by the previously shown algorithm. Along with the redefined SEVR, the previous definition of SEVR (without corrections) was also calculated ($SEVR_{old}$).

The primary endpoint was overall mortality during the follow-up period. The secondary pre-specified endpoints were major CV events. Major CV events (CV morbidity and mortality) included both nonfatal CV events that led to hospitalization or a specific long-term new treatment as well as death from cardiac, cerebrovascular,

and other vascular causes.

Statistical analysis

Descriptive values are expressed as mean ± SD or number and percentage. The Wilcoxon rank sum test was used to compare continuous variables, the chi-square test for discrete variables, and the log-rank test for mortality and major CV events. Univariate correlations were made with Pearson's parametric test. The Kolmogorov-Smirnov test was used to check the normality of distribution of the analysed variables. Variables not fulfilling criteria for normality were log-transformed when appropriate. Univariable and subsequent multivariable linear regression was used to assess likely determinants of SEVR. The occurrence of total mortality and major CV events according to cutoff of SEVR was estimated using Kaplan-Meier curves for graphic representation and compared by the log-rank test. Cox regression multivariate models were used to assess the relative risk (hazard ratio and 95% confidence interval) of total mortality and of major CV events, according to SEVR as a continuous variable and as a discrete variable categorized by its median value. In the multivariate Cox models, age and gender variables were considered in the model. Additional adjustments for MAP and heart rate were made. The proportional hazards assumption was assessed on the basis of a test of Schoenfeld residuals with the Cox regression. A p value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS v.20 (IBM corporation) statistical software package.

Results

Among subjects enrolled in the PARTAGE study, 703 subjects (78% females) were selected as having an arterial tonometry at the baseline of sufficient quality for the analysis of SEVR calculated according to the revised formulation presented in the previous chapter. SEVR showed a skewed not-normal distribution in the population and was therefore log-transformed (LogSEVR, Figure 1) to be used in the Cox models. Baseline characteristics of these patients according to gender are shown in Table 1.

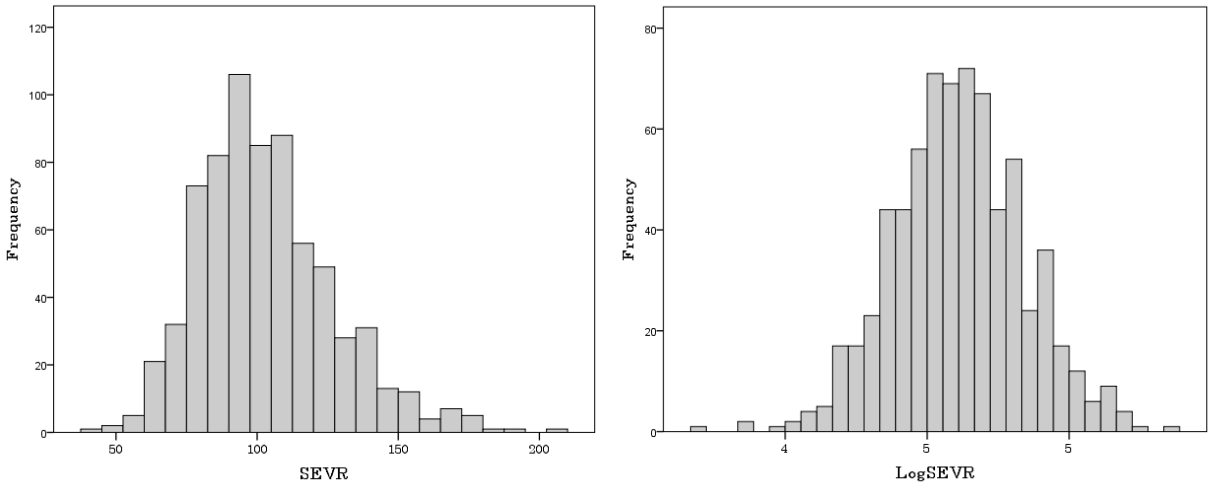

Figure 6.1. Distribution of SEVR and LogSEVR in the population.

Table 6.1. Characteristics of patients at baseline.

Parameters	All	Men	Women	p Value
n (%)	703	146 (79.2)	557 (20.8)	
Age, yrs	88 ± 5	87 ± 5	88 ± 5	0.22
Weight, kg	64 ± 13	70 ± 12	62 ± 13	<0.0001
Height, cm	158 ± 9	167 ± 7	155 ± 7	<0.0001
Smoking (past + current), %	162 (23.0)	102 (70.8)	60 (10.8)	<0.0001
History of CV disease, %	355 (50.5)	77 (53.1)	270 (48.5)	0.32
Diabetes, %	120 (17.1)	21 (14.4)	99 (17.8)	0.39
Dyslipidemia, %	191 (27.2)	29 (19.9)	162 (29.2)	0.03
History of hypertension, %	502 (71.4)	88 (60.3)	414 (74.3)	0.001
Current hypertension treatment, %	562 (79.9)	103 (80.5)	459 (82.4)	0.002
MAP, mmHg	98 ± 14	95 ± 14	98 ± 15	0.01
Heart rate, beats/min	69 ± 11	71 ± 13	69 ± 11	0.042
SEVR, %	103 ± 24	110 ± 27	101 ± 23	<0.0001
LogSEVR	4.6 ± 0.2	4.67 ± 0.24	4.59 ± 0.22	<0.0001
$SEVR_{old}$, %	138 ± 24	147 ± 27	135 ± 27	<0.0001

Determinants of SEVR were analysed in a multivariate regression analysis which is shown in Table 2. Among general characteristics of subjects, age and gender were chosen as variable to be included in the Cox model because of their significant

correlation with LogSEVR in the multivariate analysis. Hemodynamic determinants (mean arterial pressure and heart rate) were also included in the model, although only heart rate was significantly correlated with SEVR, as expected.

Table 6.2. Multivariate analysis of clinical variables associated with LogSEVR

	Beta	t	P-value
Costant		4.410	0.0001
Age	-0.472	-2.295	0.022
Gender	-6.238	-1.988	0.047
Weight	-0.008	-0.103	0.918
Height	0.128	0.920	0.358
Diabetes	-4.670	-1.890	0.059
Hypertension	1.083	0.531	0.595
Dyslipidemia	1.289	0.618	0.537
Tobacco use	0.248	0.093	0.926

At the 5 year follow-up, 287 among the 703 enrolled subjects were alive, 297 died, while the remaining were lost to follow-up. 203 subjects showed a major CV event, while 481 did not. Cut-off value of SEVR was chosen by performing a receiver operating characteristics (ROC) curve for mortality at 5-years of follow-up (Figure 2). By point analysis of the curve, a cut-off of 100 was chosen, because it showed the best sensitivity/specificity for mortality. The value of SEVR = 100 matches the median value of SEVR in the analysed population. Area under the ROC curve was 0.550 for SEVR, while it was slightly lower (0.540) for the $SEVR_{old}$.

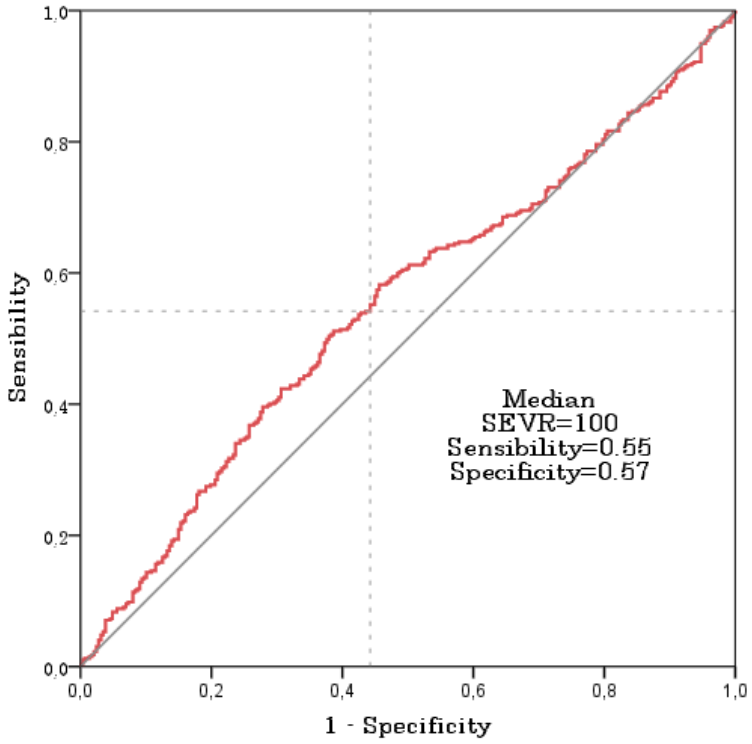

Figure 6.2. Receiver operating characteristics curve for mortality prediction at 5-years follow-up according to SEVR.

At the 10 years follow-up of the 703 enrolled subjects, only 18 were still alive, while 195 were lost to follow-up. Cox-regression models were built to elucidate variables significantly associated with mortality and major CV events at the 5 and 10-years follow-up. LogSEVR as a continuous variable was associated with mortality in the unadjusted model, in the model corrected for age and gender (Model 1), which were also determinants of total mortality, and in the fully adjusted model further corrected for mean arterial pressure and heart rate (Model 2). Heart rate was log-transformed to normalize values. Coefficients of uncorrected and corrected models and hazard ratios for total mortality are shown in Table 3. Coefficients and hazard ratio for mortality at 5 years were similar to 10 years, with slightly reduced significance (not shown). No significant association of LogSEVR with the outcome of major CV events was found neither at 5 nor at 10 years.

Table 6.3. Relationship between mortality and logSEVR at 10 years of follow-up.

	B	Significance	Hazard ratio	Lower 95% CI	Lower 95% CI
LogSEVR (unadjusted)	-0.656	0.001	0.519	0.348	0.773
Model 1					
LogSEVR	-0.750	<0.0001	0.472	0.315	0.709
Age	0.044	<0.0001	1.045	1.026	1.065
Gender	-0.592	<0.0001	0.553	0.447	0.685
Model 2					
LogSEVR	-0.463	0.061	0.629	0.388	1.022
Age	0.047	<0.0001	1.048	1.028	1.068
Gender	-0.549	<0.0001	0.578	0.465	0.718
MAP, 1 mmHg	-0.002	0.456	0.998	0.992	1.004
Log Heart rate, 1 min^{-1}	0.777	0.030	2.176	1.078	4.391

A similar analysis was then conducted to show the predictive ability for 10-years mortality of $SEVR_{old}$, by considering the same covariates in the Cox models. A similar predictivity, with lower significance levels, was found, as shown in Table 4.

Table 6.4. Relationship between mortality and the uncorrected formulation of SEVR ($SEVR_{old}$)at 10 years of follow-up.

	B	Significance	Hazard ratio	Lower 95% CI	Lower 95% CI
$LogSEVR_{old}$ (unadjusted)	-0.637	0.006	0.529	0.336	0.832
Model 1					
$LogSEVR_{old}$	-0.766	0.001	0.465	0.292	0.741
Age	0.044	<0.0001	1.045	1.026	1.065
Gender	-0.589	<0.0001	0.555	0.448	0.688
Model 2					
$LogSEVR_{old}$	-0.467	0.094	0.627	0.363	1.082
Age	0.047	<0.0001	1.048	1.028	1.068
Gender	-0.544	<0.0001	0.581	0.467	0.722
MAP, 1 mmHg	-0.003	0.318	0.997	0.991	1.003
Log Heart rate, 1 min^{-1}	0.849	0.015	2.338	1.181	4.626

As SEVR should not forcibly considered a continuous variable, but rather as mean to diagnose an imbalance between oxygen supply and demand, and therefore values higher or lower to a certain threshold may be balanced by coronary reserve or autoregulation, we decided to considered also SEVR as a discrete variable, by

considering the previously identified cut-off of 100.
Therefore, the same Cox models were considered by entering SEVR a binary variable (higher or lower than the 100 cut-off), as shown in Table 5.

Table 6.5. Relationship between mortality and SEVR higher than the 100 cut-off at 10 years of follow-up.

	B	Significance	Hazard ratio	Lower 95% CI	Lower 95% CI
SEVR> 100 (unadjusted)	-0.370	<0.0001	0.726	0.607	0.869
Model 1					
SEVR > 100	-0.369	<0.0001	0.691	0.576	0.829
Age	0.047	<0.0001	1.048	1.028	1.068
Gender	-0.576	<0.0001	0.562	0.455	0.694
Model 2					
SEVR > 100	-0.271	0.009	0.763	0.623	0.934
Age	0.048	<0.0001	1.049	1.030	1.069
Gender	-0.547	<0.0001	0.579	0.467	0.717
MAP, 1 mmHg	-0.003	0.404	0.997	0.991	1.003
Log Heart rate, 1 min^{-1}	0.753	0.024	2.124	1.104	4.087

According to the cut-off value of 100, patients were categorized in two groups and Kaplan-Meier curves were considered for graphical representation of survival. Log-rank test was used to compare mortality at 5 years and 10 years of follow-up, as shown in Figure 3.

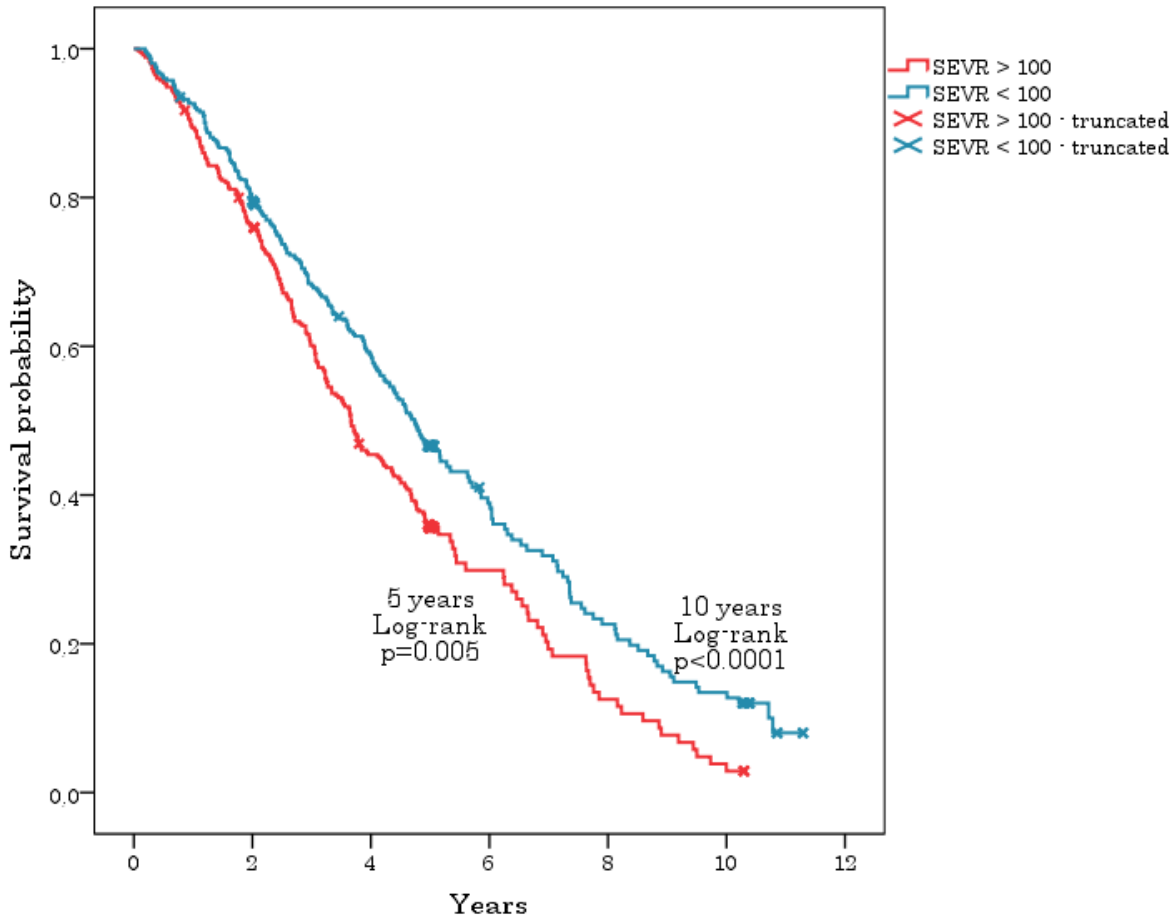

Figure 6.3. Survival curves (Log-rank analyses) for totality mortality in 703 subjects according to SEVR cutoff of 100.

Discussion

This study highlights the importance of the new SEVR formulation as an independent predictor of mortality in the geriatric population in the PARTAGE study. The best threshold of SEVR for prediction of total mortality was identified in the study. Performance of new SEVR for prediction of mortality was superior to the old SEVR calculated by arterial tonometry, even after correction for covariates.

This longitudinal analysis shows that SEVR was predictive for total mortality, with an inverse relationship. As SEVR showed a skewed distribution in the PARTAGE population, the logarithmic transformation of the variable was used in the anto analyse it as a continuous variable. A reduction in logSEVR was associated with an increase in total mortality with significant hazard ratios for mortality at 5 and 10 years, even in the model corrected for important covariates as age and gender. As the use of logSEVR is not easily understandable in the daily clinical use, the use of a threshold to use it as a predictor for outcome may be more useful. Moreover, since SEVR is used to describe the balance between oxygen supply and demand in the myocardium, and predict subendocardial ischemia, the use of a critical value is more logical, below which autoregulation of the coronary diastolic flow ceases and

myocardial blood flow becomes pressure dependent. Once autoregulation fails, a decreased perfusing pressure cannot be compensated for by coronary vasodilatation, or an increased myocardial oxygen need cannot increase flow, resulting in myocardial ischemia. Thus, another aim of our study was to find the optimal cut-off value of SEVR to discriminate mortality in the cohort. This threshold was identified in 100, and subjects with a SEVR lower than 100 were more likely to die in the 5 years and 10 years follow-up, and this finding was highly significant, even in the fully adjusted model corrected for age, gender and hemodynamic covariates. The 100 threshold is far from the critical value identified by Buckberg in healthy subjects [70], but is closer to the threshold found by the same author in patients with aortic stenosis [85]. The finding of our study could mean that the cardiovascular system in the elderly is closer to the pathological state of patient with overt cardiovascular disease. This is also in-line with findings of validated mathematical models, which evidenced a physiological age-induced supply/demand imbalance, which can augment the risk of myocardial ischemia [86]. Clearly, more studies are needed to define predictivity of SEVR values for myocardial ischemia, cardiovascular outcomes and mortality in different populations at risk for ischemic heart disease.

An unclear result of our study is why SEVR was associated with total mortality, and not to cardiovascular events, neither in the short- nor in the long-term longitudinal follow-up. Unfortunately, after two years of follow-up the recognition of cardiovascular events became less accurate in the PARTAGE study, because patients were not routinely visited in the clinic by investigators, but only occasional recall of clinical data was made by contacting patients, nursing homes or family physicians. Therefore, the only data to reliably trust in the study became total mortality in the 5-year or 10-year follow-up, as death is an indisputable endpoint which can be assessed by patient documentation. Moreover, in the very elderly, as patients in the PARTAGE study are, major cardiovascular events, as type-1 myocardial infarction, may become less clinically manifest, and harder to diagnose because of the lack of symptoms or to the impossibility to perform clinical exams.

Previous studies in the very elderly have shown an absent or even negative association of mortality with blood pressure values [87][88], as well as other classical major cardiovascular events, as overweight or lipid levels. These paradoxical results can be explained by the fact that in these very elderly frailty is the most important syndrome leading to comorbidities in the cardiovascular system. The SEVR, for how it was designed, permit a comprehensive evaluation of the balance in the myocardium, which is of importance in the geriatric population. New information concerning the assessment of mortality risk in this population is hypothesis generating for the physiopathology of frailty and the loss of cardiovascular reserves in the very elderly. Moreover, SEVR permits a bedside cardiovascular examination which can be very useful in the elderly patients, needing a titration of cardiovascular drugs, considered that drugs can variably affect the subendocardial supply-demand balance [89]. However, a limitation of our study is that no biological marker of frailty was analysed in the association with SEVR. Therefore, our finding is only speculative for the causative role of myocardial imbalance between the oxygen supply and demand to the mortality of patients followed in the study.

Clearly, more studies are needed to elucidate the predictive ability of SEVR in different populations, and possibly in the association with myocardial ischemia, although studies conducted with invasive indexes can be borrowed for this purpose. The comparison of the old SEVR formulation with the new one in our analysis showed

that the modifications in the new SEVR (measurement of systolic times, non-invasive evaluation of left ventricular diastolic pressure) are useful to improve the predictive ability of this index.
In conclusion, this study validated in a clinical study the new formulation of SEVR by showing its independent predictive ability in a longitudinal study. A cut-off value was identified to predict mortality in the elderly population. The findings of the present study pave the way to the use of SEVR in the clinical context of geriatric population and in further research settings.

7. Conclusions and perspectives

Cardiovascular diseases are commonly recognized as the leading cause of death globally [1], and represent a costly adverse health condition in both social and economic terms. Coronary artery disease and stroke account for 80% of deaths for cardiovascular diseases, and older adults are the mostly affected age class. Ageing is also associated with changes in the mechanical and structural properties of the vascular wall, which leads to the loss of arterial elasticity and reduced arterial compliance [8]. Strong evidence suggests that the stiffness of large arteries is a strong predictor of cardiovascular events and all-cause mortality both in general population and in disease sub-populations [16][7][90][91]. The degree of pressure amplification and the magnitude of wave reflections are closely linked to the structural alterations in the large arteries, and these parameters were identified as strong predictors of events and mortality, especially in the elderly population [81]. The onset of myocardial ischemia, which is closely linked both to coronary artery disease and to large artery stiffness [92], could be predicted by non-invasive methods which analyse the central blood pressure wave and the properties of large arteries, as arterial tonometry [93]. This is a point of particular importance in the elderly, a population presenting a very high incidence of cardiovascular morbidity, especially of type-2 myocardial infarction [5], and requiring simple and non-invasive approaches for the stratification of the risk of cardiovascular disease.
In the light of this evidence, this thesis presents a series of experiments and population studies aiming to the design, the validation and the application of a new index for the quantification of the imbalance between myocardial supply and demand: the revised subendocardial viability ratio, or SEVR. The findings of this work can be summarized in the following points:

1) The old formulation of SEVR derived from arterial tonometry is affected by some methodological limitations significantly affecting its validity and applicability. These limitations are the exclusion from the calculation of isovolumetric systolic time in the systolic pressure-time index and the exclusion of left ventricular diastolic pressure from diastolic pressure-time index. Moreover, the calibration of central pressure wave derived from carotid tonometry can be affected by the way of calculating mean arterial pressure from brachial cuff blood pressure, which is necessary for scaling the central waveform.
2) We demonstrated the possibility of calculating the systolic-time intervals (isovolumetric ejection time and pre-ejection period) in subjects with or without cardiovascular disease from ECG-gated arterial tonometry, performed at the carotid and femoral levels. Methodology for this calculation is presented in Chapter 3.
3) The issue of calculation of mean arterial pressure from brachial cuff blood pressure was then addressed. We evidenced a considerable interindividual and intraindividual variability in brachial pressure form-factor, in general population of different ages and in hypertensive patients. The best approach for calibration of non-invasive central blood pressure waveform resides in the

integration of pressure waveforms, or, when not applicable, in the use of an appropriate algorithm for calculation of brachial form factor.

4) We demonstrated a good correlation of the invasively determined SEVR, in patients undergoing cardiac catheterization, with the new non-invasive SEVR calculated by arterial tonometry and corrected by considering systolic time intervals and the left ventricular diastolic pressure. An equation for the estimation of left ventricular diastolic pressure was derived from non-invasive parameters of arterial tonometry and the invasive data.
5) The new SEVR was applied in the PARTAGE cohort. SEVR was found to be an independent predictor of total mortality in the elderly subjects. A threshold value of 100 may be considered in this population.

In summary, we created and clinically validated a new formulation of an index (SEVR) for the evaluation of myocardial supply-demand balance from non-invasive arterial tonometry.

The SEVR can open up new perspectives in the management of patients with established heart disease and also in primary cardiovascular prevention. This index can reliably identify an imbalance in the heart circulation and evaluate the risk of developing myocardial ischemia, without the need of performing invasive procedures or expensive and complex imaging exams. The direct and indirect costs of a heart catheterization, and the procedural risks for the patient, make unfeasible the extensive use in all the patients who may probably benefit of this diagnostic procedure. The arterial tonometry, which is an easy and non-invasive exam, can be performed at the bedside of the patient and does not require expensive equipment or long execution time. Even the learning curve for the operators is very quick, and can be performed by physicians or trained nurses, with good repeatability [62].

These features are of utmost importance in a public heart perspective, as the application of SEVR could significantly reduce costs of the management of cardiac patients, without increasing the economic burden of heart disease, which is already high and steeply increasing. This may be particularly relevant in patients with multiple comorbidities, high procedural risks or frailty. Therefore, an optimal setting for the use of SEVR is that of the elderly patient, in whom invasive procedures are not indicated for comorbidities and a shorter life expectancy make difficult to calculate any cost/efficacy balance for a diagnostic exam. The SEVR may provide additional information in these patients, not only for the prognostic evaluation of mortality, as evidences in our analysis, but also for the management of medications. In fact, since polypharmacy is considered a poor prognostic factor in the geriatric patients [79], the physician should rationally consider the number of drugs affecting the cardiovascular balance, as anti-hypertensive drugs or beta-blockers, and the SEVR may offer a ready way to objectively quantify this balance in the follow-up [89].

There are many possible applications of non-invasive SEVR and future studies will shed light in the value of this index in different clinical settings. The improved reliability in diagnosing an imbalance between oxygen supply and demand in the myocardium will allow new studies for diagnosing ischemic changes in the heart non-invasively, in different scenarios and with repeated measurements. Our non-invasive methodology permits the monitoring of short-term changes in the

myocardial balance brought by modifications of cardiac therapies, of volume status or of heart rate and blood pressure. A particularly interesting setting is the peri-operative management of patients undergoing non-cardiac surgery, in whom a high incidence of silent heart ischemia detected by troponin increase is associated with a high mortality [94]. Another interesting application will be the possibility to improve the appropriateness of prescription of blood transfusion, which are routinely administered to cardiac patients in order to avoid ischemic consequences in the heart. The possibility to assess the imbalance between oxygen supply and demand by SEVR, which can be readily corrected with haemoglobin levels and oxygen saturation (following the corrections suggested by Buckberg et al. [26]) might improve treatment of patients requiring blood transfusions. Moreover, our study demonstrated that the new SEVR is a strong and independent predictor for mortality in elderly subjects. This finding should be replicated in different populations and in primary and secondary prevention studies, but the low cost and ease to perform of the SEVR make this index an interesting candidate for cardiovascular prevention studies.

Considered that these suggestions are not the only ones for new studies about the SEVR, and further possibilities will be explored in future, this work is an encouraging first step toward a non-invasive, cost-effective and above all reliable diagnostic procedure, bearing in mind that the improvement of patient care is the necessary way for advancing quality in public health.

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