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ARTERIAL STIFFNESS IN A RURAL POPULATION OF CHINA

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BACKGROUND

Principles of physiology

The cardiovascular system is considered to be 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 over and over again to be able to reach the farthest extremes (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 blood-borne molecules.

Mean arterial pressure

Though the circulation is a hydraulic system governed by fluid dynamics laws, is comparable to an electric circuit consisting of a voltage source and a succession of resistors arranged in series and in parallel (Figure 1) [1]. From the difference of electric potential $\Delta V = V_1 - V_2$ originates the motion of the electrons along the mesh, producing a current flow through a resistance; these quantities are linked by a relation of proportionality, enunciated in the first Ohm's law: $\Delta V = I \cdot R$.

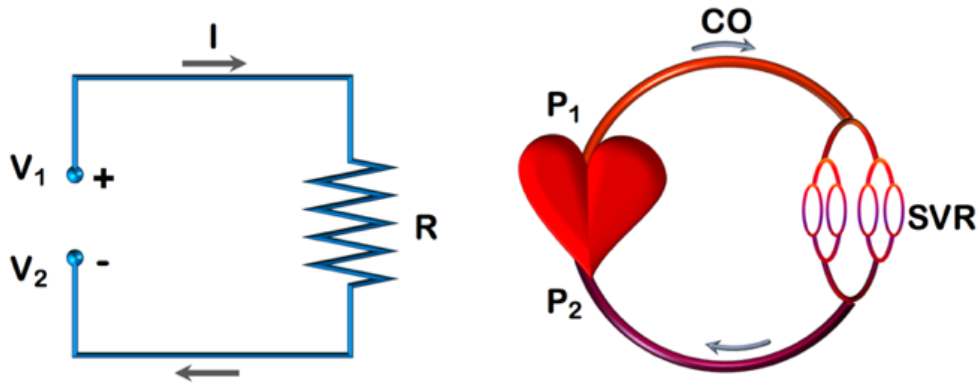


Figure 1. Analogy between an electric circuit and the cardiovascular system.
CO: cardiac output; SVR: systemic vascular resistance

Similarly, the contraction of the left ventricle hesitates in blood dislocation within the aortic bulb, a closed chamber in which the pressure suddenly increases internally, creating a pressure gradient i.e. the driving force supporting the blood flow, better defined as cardiac output (CO). In this case the dipole is represented respectively by the late systolic pressure of the left ventricle (P_1) which, in the absence of aortic valvular defects, matches the aortic pressure (SBP) and the right atrium pressure (P_2) which coincides with the central venous pressure (CVP); the peripheral resistance (SVR) are finally represented by viscous friction developing between the fluid particles and the vessel wall, by blood vessels branching and chiefly by the muscular arterioles.

The formula which include pressure, flow and resistance is written as:

$$\Delta P = CO \cdot SVR$$

whereas the first member of the equation can simply be described as BP, being the value of the venous pressure negligible compared to the magnitude of the systemic BP. Because cardiac output depends on the heart rate (HR) and stroke volume (SV) the first factor can be replaced to obtain:

$$BP = SV \cdot HR \cdot SVR$$

these are the three main determinants of blood pressure on which the vast majority of clinical studies have focused (Figure 2).

In detail, the stroke volume depends on both the ventricular filling or the preload, and the myocardial contractility; heart rate depends on the dominant pacemaker autonomous rate of depolarization in myocardial conduction tissue; the resistances depend on the blood viscosity η, the length l and the fourth power of the vessel radius r, according to the Hagen–Poiseuille equation:

$$SVR = \frac{8\eta l}{\pi r^4}$$

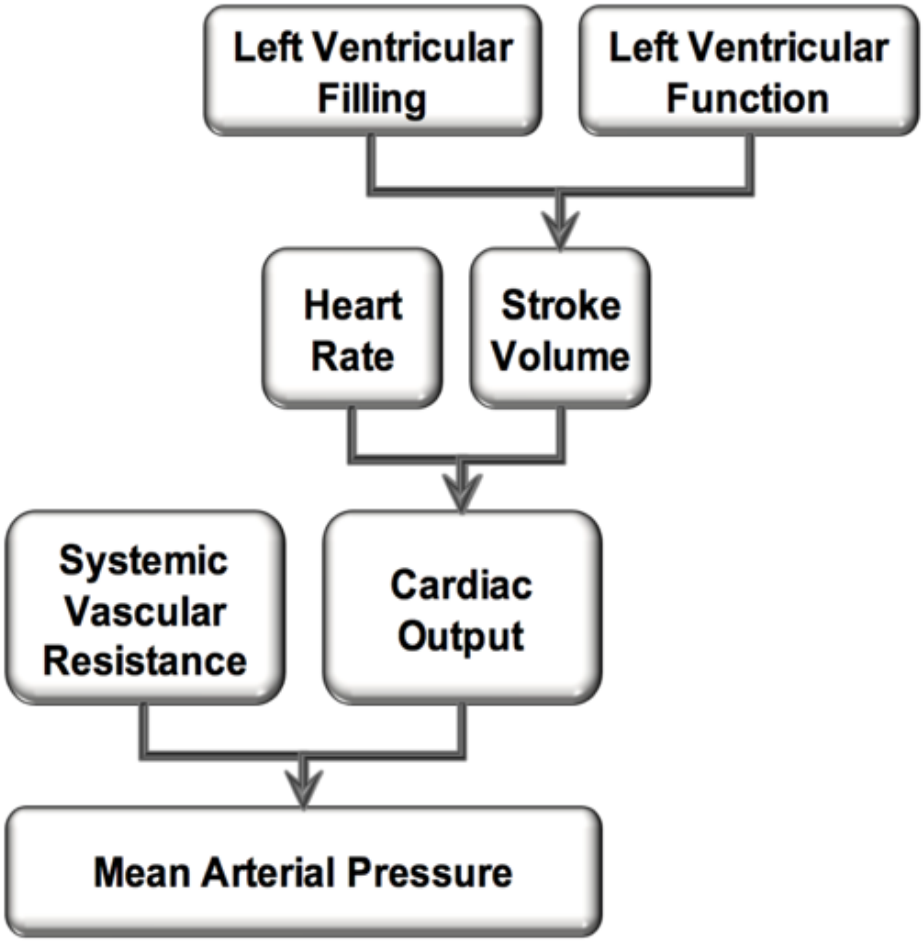


Figure 2. Classic determinants of mean arterial pressure.

Therefore, it is important to point out that Ohm's law applied to the cardiovascular system does not define the systemic systolic pressure but the mean arterial pressure (MAP).

Pulse pressure

To demonstrate that blood pressure cannot be interpreted basing solely on vascular resistance, heart rate and cardiac output, consider two subjects having an identical mean blood pressure of 100 mmHg (Figure 3). The subject (a) has a blood pressure in range while the subject (b) is frankly hypertensive.

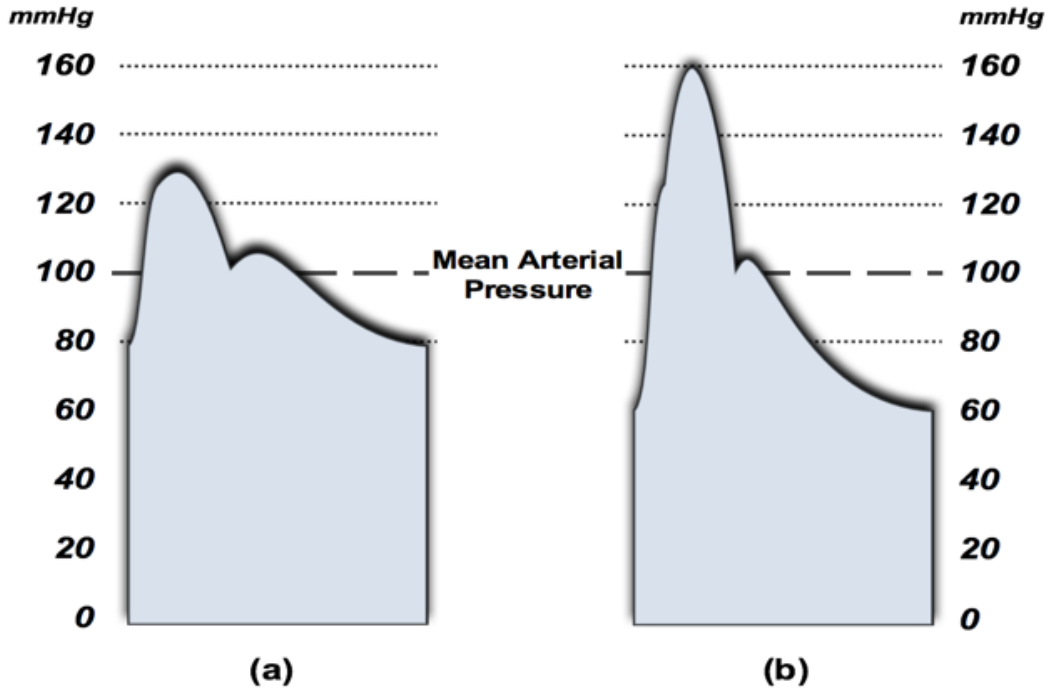


Figure 3. Comparison of two patients with the same mean arterial pressure but different values of systolic and diastolic blood pressure.

Even defining the resistances according to the equation of Poiseuille it's like to assume that blood flow is steady and not pulsated, a simplification absolutely not negligible, barely corresponding to the blood state in the ascending aorta at the end of diastole [2].

In order to fully understand the consequences of the pathological alterations of blood pressure regulation, it's reasonable to consider not only the blood pressure steady components, such as the maximum and minimum values and the MAP, which remains relatively constant along the arterial branches, but it's worth to consider the entire BP curve that conveys the pulsatile component.

For decades, preventive cardiology has focused on systolic, mean and diastolic blood pressure parameters to define individuals at increased risk of developing

cardiovascular events: it is known indeed that each increment of 10-20 mmHg above the baseline value of 115/75 mm Hg doubles the patient risk [3,4]. Though, if in male patients under 60 years the strong association between PAS, DAP, MAP and cardiovascular risk has been confirmed, in older male patients also the pulse pressure, paralleling the systolic, was a major event predictor [5]. A large meta-analysis accomplished by Blacher and colleagues that also included female patients, showed that an increase of 10 mmHg in pulse pressure increased overall cardiovascular mortality in elderly patients [6].

In both sexes there is also a relationship between increased pulse pressure and left ventricular hypertrophy and in particular the PP in women older than 55 years has been proposed as a risk factor independent of static pressure components [7]. Only in younger individuals the diastolic pressure seems to possess a higher predictive value [8].

Such statements are partly justified by pressure changes related to age: starting from 30 years up to old age, is recognizable a linear growth of SBP, joint to that of DBP and MAP. Then, between 50 and 60 years, the latter tends asymptotically to its maximum value and the pulse pressure grows rapidly; on the contrary the diastolic pressure assumes a downward trend [9].

These evidences overcome the traditional model based solely on blood pressure peak values for assessing pressure homeostasis, and legitimize the prognostic value of the pulsatile component, created by the interaction between intermittent contractility of myocardial pump and viscoelastic properties of aorta and large elastic arteries.

Arterial Mechanical Properties

The artery is a viscoelastic tube consisting of three layers: the intima, media and adventitia. The tunica intima is the inner layer, constituted by a single layer of endothelial

cells, resting on a basement membrane, and by a subendothelial layer, which thickens as a homeostatic response to wall stress. The intima structural component, responsible for the tolerance of pressure load is in fact the subendothelial layer, being characterized by a remarkable expression of collagen fibers. The tunica media is a three-dimensional network consisting of a smooth muscle cells, collagen and elastin fibers tangle, parted from adjacent layers by two elastic laminae, internal and external respectively. Also the intermediate layer of the arterial wall contributes to the structural integrity and represents the anatomical substrate from which the rheological properties of the arteries derive. Finally, the tunica adventitia is the sturdier outer layer that provides stability and strength to the vessel, in which densely intertwined collagen fibers, dispersed within the amorphous ground substance are expressed [10].

For simplicity, the arteries can be divided into large caliber elastic arteries, located proximal to the heart with the buffering function towards the stroke volume fluctuations; peripheral muscular conduit arteries responsible for the blood distribution in all the districts of the organism; and arterioles that keep the mean arterial pressure and a steady blood flow to the tissues thanks to caliber (i.e. peripheral resistance) adjustment by means of vasomotor tone.

The subsequent discussion will focus on the large elastic arteries and mainly on aorta. The unique viscoelastic properties of this vessel derive from a quantitative and qualitative relationship between the wall constituent outlined above, with a focus on collagen/elastin ratio [11]; many pathophysiological conditions such as aging, hypertension and diabetes [12] are capable of altering this ratio up to subvert the histological structure and mechanical aortic functions.

The Windkessel Model

The transport function of the arteries is well known, unlike the cardiac output damping function. The cardiac activity cyclically sees a succession of a contraction phase in which the blood is ejected in the ascending aorta, and a relaxation phase that begins when the aortic semilunar valve is closed. However, if there is no myocardial thrust the pressure will not cancel but will descend to a minimum value, defined diastolic indeed: pressure zeroing is prevented by aortic distensibility, a property persisting throughout the vascular tree but with different implications.

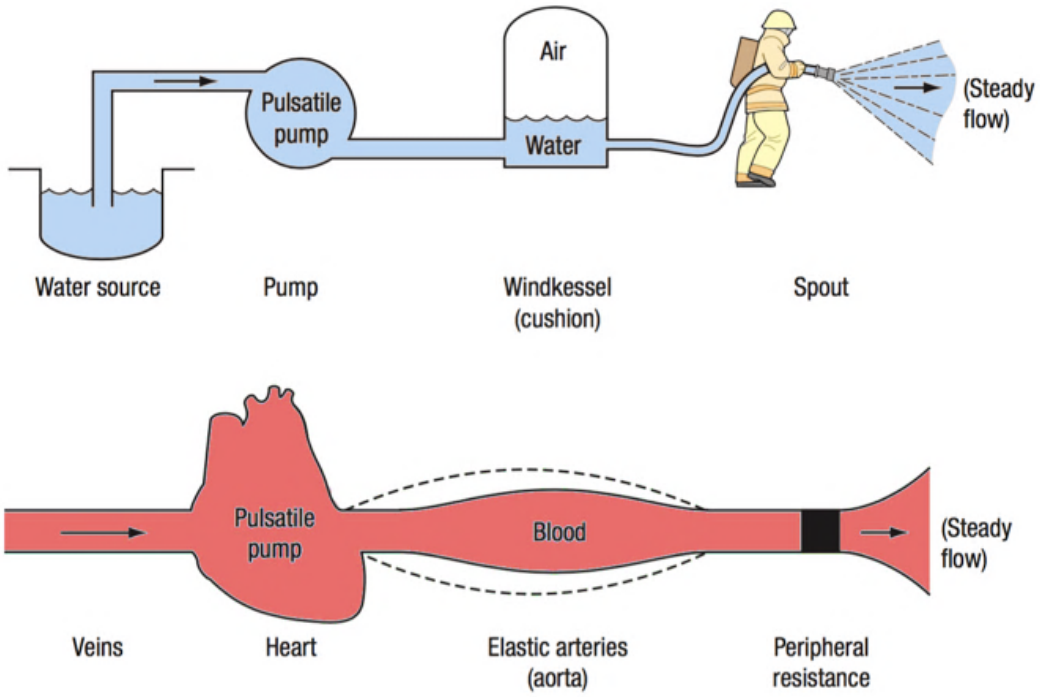


Figure 4. Windkessel Model of aortic distensibility.

At the moment that the cardiac contraction accommodates the blood into the aorta, only a fraction of the stroke volume is pushed directly into periphery, while a larger amount remains into the large arteries next to the heart, dilating the wall; at the end of ejection it tends to return to baseline status thanks to the phenomenon of the elastic

recoiling re-establishing the potential energy that has caused the expansion in the form of kinetic energy. It would therefore be more appropriate to speak of a systolic pump, represented by the cardiac muscle, and a diastolic pump given by the large elastic arteries [2]. This phenomenon that turns the continuous flow into intermittent, is called 'Windkessel effect', from the German word 'air tanker' because in the last century was formerly used by firefighters to extinguish fires (Figure 4).

The ability of the arteries to expand under the thrust of the stroke volume is termed *compliance* or *distensibility*. Truthfully the equivalence of the two terms is incorrect, both from semantic and mathematical point of view: compliance even if it's defined as the change in volume of a hollow structure in relation to transmural pressure changes ($C = \Delta V/\Delta P$), is most used to indicate the adherence to drug therapy of the patient [13]; distensibility is instead derived from the relationship between compliance and initial volume ($D = \Delta V/\Delta P \cdot V_0$). The *elastance* or *elastic modulus* is the reciprocal of compliance ($E = \Delta P/\Delta V$) and corresponds to the *rigidity* or *stiffness*, diction preferably applied instead of compliance or distensibility.

Propagative Model: the pulse wave velocity (PWV)

The Windkessel model has limitations: in the arterial tree is not possible to divide the conduction from the cushioning function as the muscular arteries possess a certain degree of elastance and vice versa the elastic arteries possess resistive properties, albeit negligible [14]; there is an attenuation gradient of the buffer function from the center to the periphery parallel to the progressive increase of the conduction function in distal vessels; the pulse wave velocity (PWV), an arterial stiffness index, has a finite value determined by the structural heterogeneity of vascular branching, in contrast to the model which provides an infinite speed [15]. Then, considering that peripheral arteries are stiffer than centrals and vascular stiffness is modulated both by the autonomic nervous system

[16] and the renin-angiotensin system [17], a propagation model is more functional to interpret the arterial system physiology.

The elastic properties of the arteries influence the transmission of the pulse wave velocity. As every speed, it is defined as the ratio of travelled path length to time taken to travel it and its measurement unit corresponds to the international meter per second (m/s):

$$PWV = \frac{d}{\Delta t}$$

where d represents the distance between two arterial segments and Δt the time delay between the feet of the pressure waves better known as *transit time* (Figure 5).

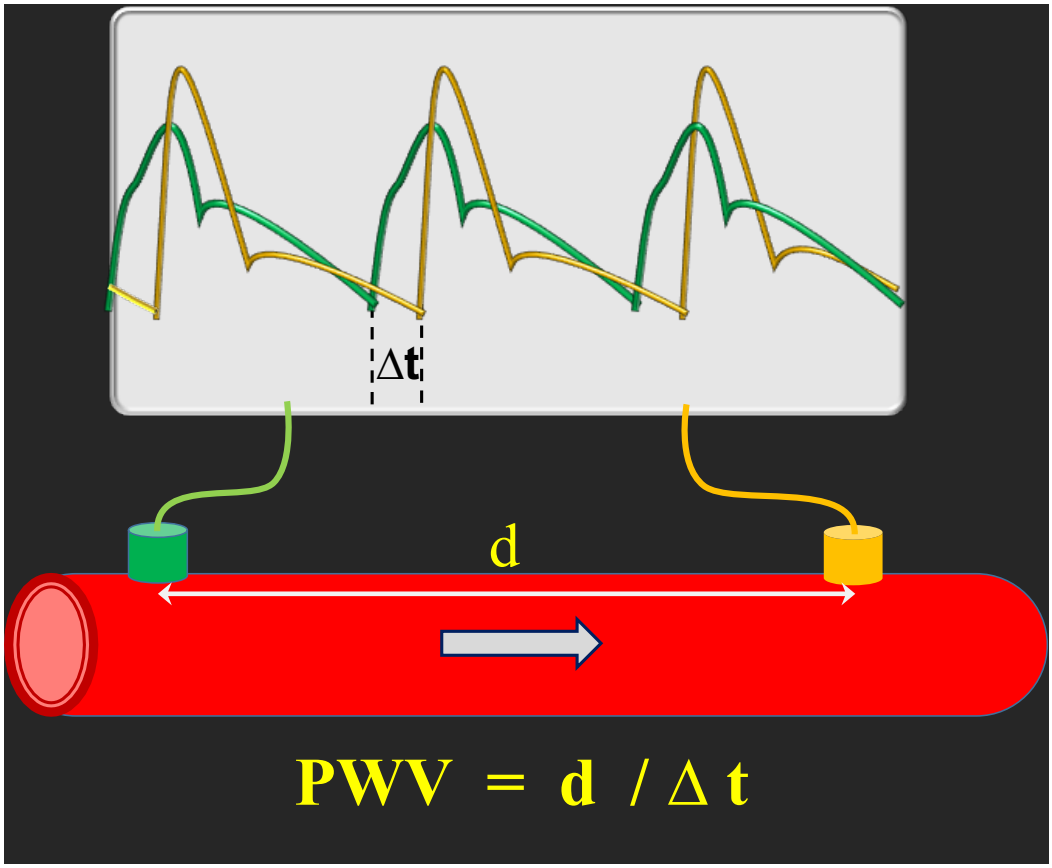


Figure 5. Assessment of the pulse wave velocity

This parameter depends on the arterial distensibility according to the Moens-Korteweg law, empirically derived from Newton's second law:

$$c_0 = \sqrt{Eh/2R\rho}$$

in which c_0 corresponds to the pulse wave velocity, E is the elastic modulus in the circumferential direction, h is the arterial wall thickness, ρ is the blood density, and finally $2R$ is the end-diastolic diameter of the vessel. In 1878 Moens demonstrated the validity of this formula, which greatly simplifies the law of wave propagation, through experiments with elastic rubber tubes filled with water.

The clinical use of this equation would be limited by the presence of two factors not always measurable in situ, such as the Young's modulus and the radius but thanks to the adaptation by Frank (1920) and Bramwell and Hill (1922), it is still used to quantify arterial distensibility and pulse wave velocity [18]:

$$c_0 = 0{,}375\sqrt{\frac{VdP}{dV}} \quad \text{or} \quad c_0 = \frac{3{,}57}{\sqrt{\%\ increase\ in\ volume\ per\ mmHg\ increase\ \ pressure}}$$

According to the previous statement, the PWV is an immediate and easily measurable arterial stiffness index. Besides, it is worth noting that the speed could be obtained either from pressure waves, flow waves or distension waves but the pressure one is technically easier to measure [1].

PATHOLOGICAL BASES OF ARTERIAL STIFFNESS

Anatomical and functional alterations of the arteries are not isolated phenomena but conceal a complex multifactorial and multisystem interaction, being modulated by the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system, the genetic pool, systemic inflammatory states, and multiple cardiovascular risk factors such as diabetes, hypertension, smoking and so on.

The most harmful consequence is certainly the vascular stiffening, an independent prognostic factor for cardiovascular events [19,20], overall [20] and cardiovascular mortality [21] and several extra cardiac diseases such as stroke or end-stage renal diseases [22].

The following paragraphs will describe the arterial stiffness pathogenic mechanisms and their clinical implications.

Arterial Wall Structural Alterations

As repeatedly stressed, the arterial mechanical properties arise from their histological architecture and the ratio between collagen and elastin, kept approximately constant by continuous processes of synthesis and degradation of the extracellular matrix (ECM). Inflammatory stimuli, elevated blood pressure values or metabolic alterations can modify the dynamic balance of these proteins, adjusting the synthesis rate and the tissue spatial conformation: the end result is an boosted expression of structurally abnormal collagen molecules at the expense of elastin ones [23].

Hypertension is, however, the main inducer of collagen synthesis: an experiment conducted on rabbits with iatrogenic thoracic aortic coarctation, has documented a significant increase in wall thickness secondary to the overexpression of the Type I and Type III collagen genes, suggesting a close link between hypertension and reactive

vascular remodeling [24]. In response to the constant mechanical pressure stimulus, mainly the tunica intima and tunica media thicken, attempting to obtain a balanced ratio between tangential forces to the wall and transmural pressures [25], but also ageing influence this process, so much so that the intima-media thickness (IMT) increases from 2 to 3 times between 20 and 90 years. In this sense, although ascertained its value as an independent predictor of cardiovascular events, and the strong association with the development of atherosclerosis, IMT should also be thought as a marker of arterial aging [26].

Aging and hypertension have dissimilar effects on large elastic arteries than on peripheral muscular arteries: the viscoelastic properties of central arteries such as the common carotid, are significantly adulterated by both these issues but there is no clear correlation with distal arteries function such as the femoral artery [27], since, as repeatedly pointed out, distal arteries eminently accomplish the conduction function while the cushioning function gradually falls from heart to distal ends.

Histological changes related to aging are due to disorganization of the extracellular matrix, reduction in the absolute number of structural proteins and cellular elements and in the relative number of elastic fibers compared to collagen fibers, infiltration of polymorphonuclear neutrophils and genetic modulation. By way of example in Figure 6 are portrayed aortic wall histological sections of two subjects, at least 10 years apart: in the older patient is noticeable an impoverished, less organized matrix, with elastic laminae depletion as cardinal finding [28].

At high pressure levels, the collagen fibers of the tunica media predominantly withstand the tensile forces, since are characterized by a relatively high elastic modulus, while elastic fibers rather transmit the energy to the more resistant extracellular matrix proteins [29], but in the senile age the lessened waviness of those fibers implies a lower

tolerance to the load imposed by transmural pressure, with a negative impact on vascular function [28].

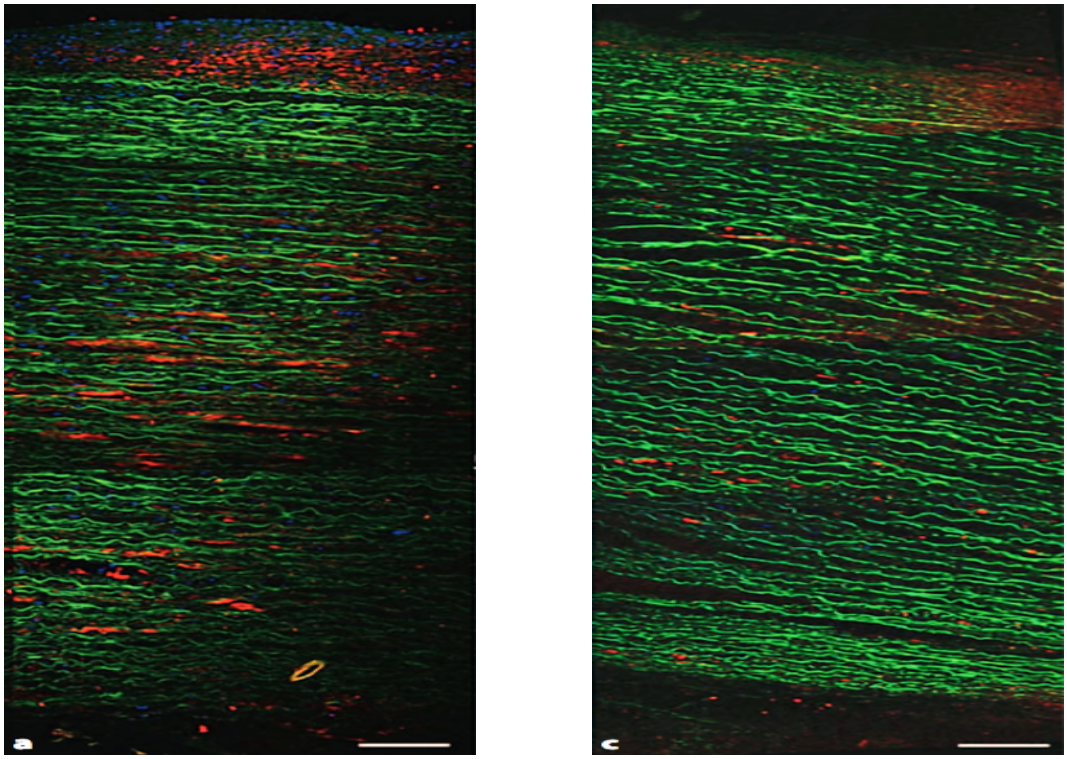


Figure 6. Section of the aortic wall at confocal laser microscopy. Comparison between a young patient (left) and one aged (right) [28].

Like all the other tissue in the human body also the arterial wall undergoes a remodeling process, strictly regulated by the equilibrium between synthesis and degradation rate of the extracellular matrix constituents. Any imbalance of this rate can lead to the loss of the fibers structural integrity: as the concentration or the activity of enzymes such as collagenases, elastases and others matrix metalloproteases (MMPs) rises, a catabolic state develops in the extracellular matrix, there's a recruitment of inflammatory cells that sustain the production of those protease and lessened repair mechanisms. Moreover the cellular elements reaction, chiefly conveyed by the plentiful

smooth muscle cells in the media, synthetizes new scaffold proteins with distorted three dimensional organization and function [30]; the importance of this statement lies on the close association between the spatial architecture and the global mechanical behavior of the arteries [11].

The constitutional activity of tissue MMPs inhibitors, the enzymes responsible for the counter-regulatory response is also impaired, beginning from an abnormal genic expression and a post-transcriptional activation, ending with inappropriate molecular turnover. The MMPs break the elastin fibers and uncoil the collagen ones exposing them to the calcium and phosphate mineralization and to the non-enzymatic protein glycation, an irreversible process bolstered by metabolic syndrome and diabetes that produces the so called advanced glycosylation end-products (AGEs). Along with calcifications, the AGE-linked collagen is mainly responsible for the arterial stiffening and it's barely receptive to hydrolysis and renewal, sustaining the progression of the arterial mechanical dysfunction. Besides the interaction between AGEs and their receptors (RAGE) strengthen the inflammatory response and consequently the activation of MMPs, through a complex molecular pathway triggering the release of inflammatory cytokines, free radicals and reactive oxygen species (ROS) formation and growth factors increase [23]. All those represent an endless supply to the harmful remodeling cascade previously described.

Eventually new perspectives on the potential role of vascular smooth muscle cells (VSMCs) in the development of large arteries stiffness are arising: two recent studies on spontaneously hypertensive rats have shown relevant changes in the intrinsic mechanical properties of the muscular fibers regardless of the ECM alterations [31,32]. Those include localized cellular stiffening and augmented adhesion between cytoskeleton and extracellular proteins, hypertrophy of the muscular layer and dynamic component

dependent on the contractile state of the smooth muscle cells that could offer a potential target for drug treatment, all together paired with hypertension and advancing age. One prior study conducted on monkeys seems to confirm the novel significance recognized to the topic [33].

Functional Arterial Impairment

Alongside anatomical modifications are the dynamical alterations driven by vasomotor tone and endothelium, renin-angiotensin-aldosterone system, dietary salt, metabolic and endocrine disorders, renal diseases [23].

Many endogenous molecules target the muscle layer of the arterial wall attempting to provide the finest blood flow and pressure regulation so that the tissue delivery of oxygen and nutrients is adequate and recent evidences underline that even the ECM is directly involved in the SMCs functional response to various stimuli [11]. The endothelium act as the essential regulator of the vasodilatory response thanks to the nitric oxide synthesis but in an inflammatory contest this molecule is subjected to a sensible concentration drop because of the induction of nitric oxide synthase inhibitor, ROS, peroxynitrite and other molecules related to oxidative stress [23,24]. To stress out the key role of the endothelium in modulating the vascular stiffness it must be said that others not yet known endothelium-secreted substances which help the vascular relaxation exist, as proven by the removal of the endothelium layer from laboratory animals [34].

Nevertheless, the arterial tone is not only affected by endothelial dysfunction but also by the aforementioned factors, for instance the RAA system. This is the main promoter of fibrosis and remodelling since the angiotensin II and the aldosterone have a plethora of effects on many different sites: vasculature, brain, kidneys and heart, the most studied one.

Focusing on the arterial system, the angiotensin binding to its receptor AT_1 elicits

a variety of effects including expansion of the medial mass consequent to the VSMCs hypertrophy and to the boosted ECM protein synthesis, especially fibronectin which mediate the cells-to-matrix adhesion; hypertrophy, hyperplasia and apoptosis of all cell lineages stimulated by connective tissue growth factor (CTGF), vascular-endothelial growth factor (VEGF) and others; inflammation-induced vascular permeability and leukocyte chemotaxis; vasoconstriction; impaired myogenic responsiveness; loss of the endothelial-dependent relaxation accompanying the increased ROS and lessened NO production [35].

Finally, the aldosterone synthesis is directly proportional to the angiotensin blood concentration, being the RAA system end-product and its effects are analogous to those of angiotensin although the vasoconstriction and the fibrosis prevail. Furthermore in hypertensive patients the negative relationship between aldosterone levels and arterial stiffness, mostly noticeable in large proximal arteries is well established and is proven not to be dependent on age, blood pressure, renin or potassium levels [36], suggesting the mineralocorticoid critical impact in modulating arterial compliance.

Arterial Stiffness and Blood Pressure Variability

Blood pressure variability (BPV) is a spontaneous fluctuation of blood pressure values along a defined time period. Depending on the period length, we can classify the BPV in 4 categories: (1) very short term variability, assessed by means of continuous beat-to-beat blood pressure recordings; (2) short term variability, assessed within the 24-h period, by ambulatory blood pressure monitoring (ABPM); (3) mid-term variability assessed by home BP monitoring on a daily basis; (4) long term variability, assessed using repeated office or home BP measurements [37].

As rendered in the Figure 7 below, the whole spectrum of BPV accounts for its

clinical value because not only the cardiovascular risk and mortality, but that of kidney diseases also depends on each of the BPV components to a different extent: Mancia et al. have clearly shown that cardiovascular prognosis is not only related to 24-hour mean blood pressure but to BPV also, inspecting a large population for a long-term follow up [38].

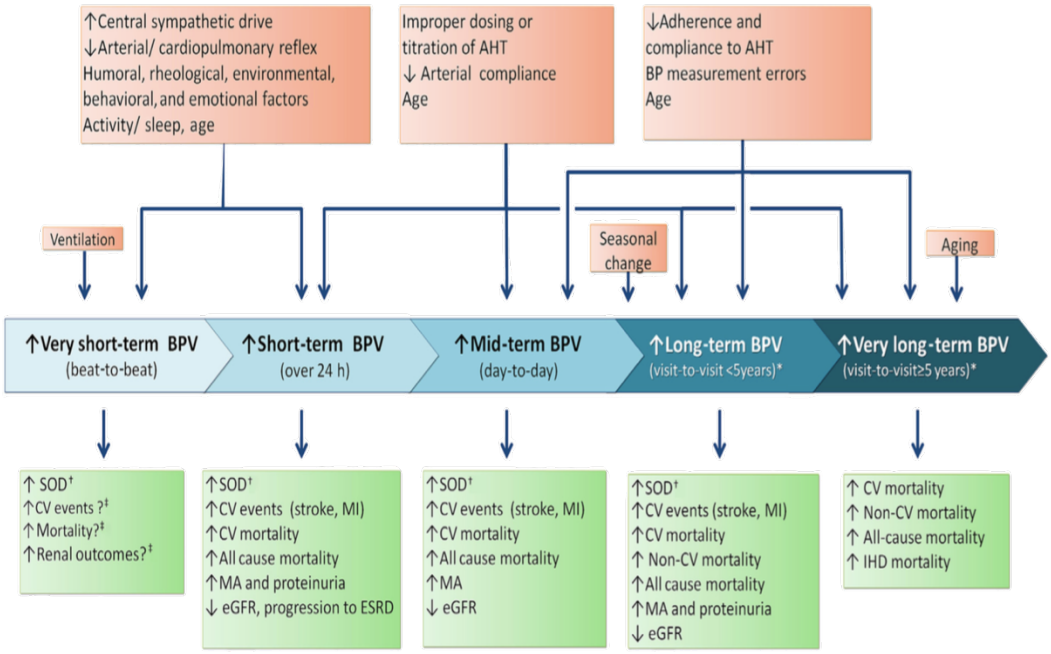


Figure 7. Types, determinants and prognostic relevance of blood pressure variability [37].

In addition to extrinsic influences, represented by environmental stimuli such as exercise, and intrinsic influences such as emotional stress, blood pressure variability is mainly under the control of the sympathetic tone, active on the cardiovascular system. Precisely, one of the blood pressure homeostatic mechanism is the baroreflex, an autonomous regulation of vascular resistance, stroke volume and heart rate that allows a fine, beat-to-beat modulation of the pressure levels. Any pressure stress rise in the internal carotid artery or aortic arch conveys a transmural stretch to the arterial wall and to the within baroreceptors; this stimulation upsurges the receptors firing and produces an

impulse travelling along the vagus and glossopharyngeal nerves to the nucleus of the solitary tract from where the efferent response goes to the heart and vessels, decreasing the sympathetic tone on the cardiovascular system. The opposite happens if the blood pressure falls, i.e. a heartbeat acceleration and peripheral vasoconstriction driven by sympathetic activity.

Arterial stiffening is able to cut the baroreceptor sensitivity (BRS) down, thanks to the vascular remodelling described before: as to elicit the reflex arc a stretching or relaxation should take place, the altered distensibility can weaken the receptors response, eventually leading to blood pressure lability. Also, it's a notable change occurring with age (since the stiffness itself is related to ageing) as confirmed by Monahan et al.: the correlation coefficient between BRS and age was $r = -0.69$ and between BRS and carotid artery compliance was $r = 0.71$. Further, the study proved the arterial compliance independency as a correlate of BRS via multiple regression analysis [39].

Still some uncertainties remain as evidences from animal experiments have revealed an inverse consequentiality between stiffness and blood pressure fluctuations. The development of atheromatous plaques was evaluated in rats underwent sino-aortic denervation and fed with high cholesterol diet; those two causal factors alone produced a slight intima-media thickening but when combined with renal artery clipping induced hypertension produced a relevant atheroma and cellular proliferation [40], suggesting a direct cause-effect relationship.

Only one large multicenter, cross-sectional study has tried to explain the linkage between blood pressure variability and vascular stiffness in two sizeable hypertensive populations. To achieve this goal, all available parameters quantifying the BPV were virtually contemplated: SD of 24h average BP values obtained from ABPM; SD of daytime and SD of night-time; average of daytime and night-time BP SD weighted for the

duration of the day and night periods, called ‘weighted’ 24h BP SD; and finally, ‘average real variability’ (ARV), computed as the average of the absolute differences between consecutive measurements. Then the arterial stiffness was estimated through measurement of carotid-femoral PWV.

The strengths of the study, in addition to the sample size, the subjects recruitment from a real-life context and the generalizability of the results due to the population heterogeneity, were represented by the accuracy of pressor measures acquisitions, the solely selection of the best and most complete pressure profiles by experts in the field, and the quality control carried out on the carotid-femoral pulse wave velocity measurements.

With these premises, the authors were able to incontrovertibly substantiate the actuality of the close independent correlation between short-term blood pressure variability, mainly expressed as ARV, and arterial stiffness, expressed as aortic PWV (the Figure 8 depicts the correlation in the first tested population) but the association was confirmed only for the SBP variability, not for the diastolic [41].

Actually, this is not surprising because the progression of arterial stiffness is expressed through a widening of pulse pressure resulting in a substantial lowering of the DBP. Nevertheless, the study design did not allow to establish the causal link between BPV and stiffness leaving the speculation about who is the prime mover, opened; therefore, the factual possibility that the causality is mutual should be investigated by longitudinal studies.

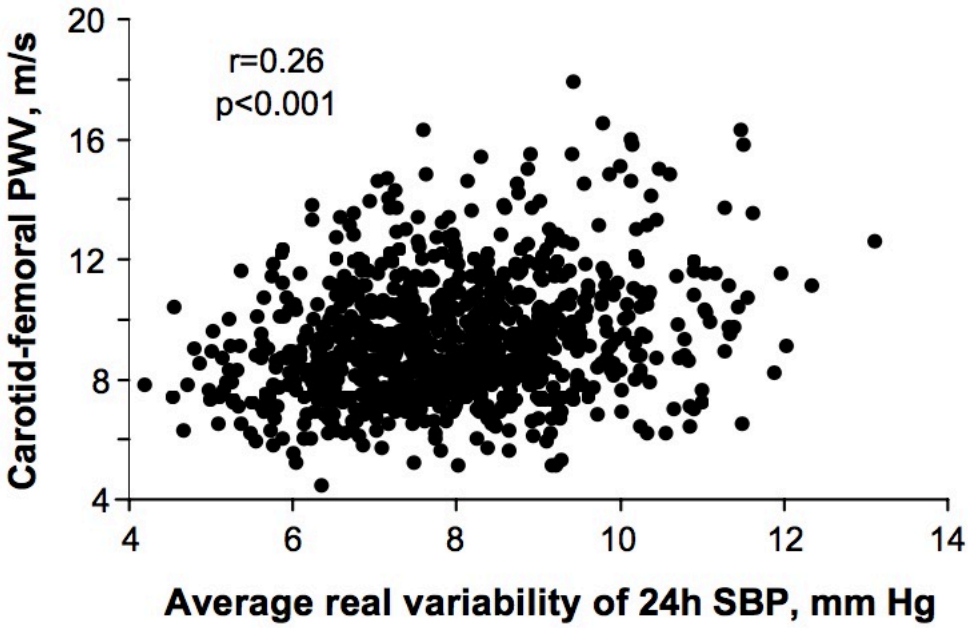


Figure 8. Correlation of carotid-femoral pulse wave velocity with the average real variability of 24-hour systolic blood pressure in 911 untreated hypertensive patients [41].

Role of Passive Large Arteries Mechanics

The baroreceptor sensitivity is an active BPV means of control appreciably altered by arterial stiffening but the passive effects of large elastic arteries on variability is distorted as well. In a rigid tube, the stroke volume cannot be buffered and during the ejection time SBP suddenly rears while DBP is not sufficiently buttressed by elastic recoiling during ventricular relaxation time, producing a broader pressure excursion, thanks to the fluctuations in stroke volume over the 24-hours, too.

The matter is complicated by pressure-dependency of arterial compliance, as tested by Avolio et al. in a lumped parameter arterial model. If the compliance was not pressure-dependent, BPV would decrease exponentially with increasing MAP, i.e. higher mean values keep the blood pressure variability low, and the BPV would depend on beat-to-beat variation of systemic vascular resistances. Yet there's a curvilinear,

U-shaped relation between BPV and MAP, observable in different age groups, since the compliance truly exhibit an age-varying pressure-dependency: with ageing the pressure-dependency property of arterial stiffness is lost (Figure 9), graph on the left) and the minimum pressure for which the oscillations are nearly voided is higher, so that in the youth lower MAP values are required to moderate the variability compared to elderly (right side of Figure 9) [42].

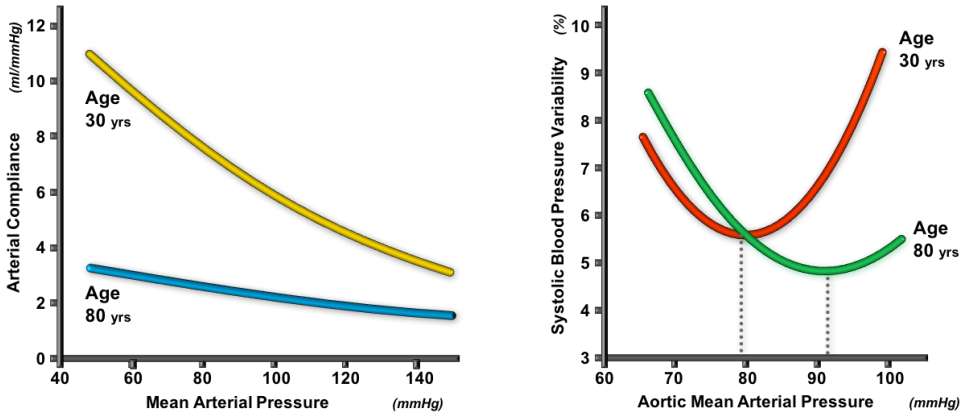


Figure 9. Exponential relationship between arterial compliance and mean arterial pressure on the left; Systolic blood pressure variability as function of mean arterial pressure with advancing age on the right [42].

The results of this modelling study clearly indicate that the hemodynamic effect of age-related artery stiffening affects the blood pressure variability, inasmuch as for any given pressure level the elder has greater fluctuations than the young.

Pathogenesis of Cardiovascular Complications

As previously pointed out by the Windkessel model, the arterial viscoelastic properties buffer the ejected volume from the heart in order to maintain an adequate diastolic pressure. This capability can be described using the compliance C, already defined as

$\Delta V/\Delta P$ and because the aorta is the main elastic artery, the total arterial compliance can be estimated as the stroke volume-pulse pressure ratio ($C = SV/PP$); according to the equation, any stroke volume rise or compliance drop implies an enlargement of the pulse pressure. Few studies have tested the validity of this approach, emphasising the relevance of the SV in the youth and of the compliance in the elderly [9,43,44].

Anyway, the Windkessel model has to be incorporated in the propagative one, taking into account the wave reflections. The Moens-Korteweg formula highlighted the relationship between PWV and the arterial wall elastic modulus so that a decline in vascular compliance match a pulse wave speeding up. Such assumption explains why the reflected waves travel back faster from the periphery to the centre, superimposing to the systolic phase instead of the diastolic; meanwhile the forward wave diastolic component is deprived of the reflection contribution. Hence, the shape of the aortic pressure wave displays a remarkable PP spread, resulting from the widened difference between the augmented SBP and the lessened DPB.

The consequences reverberate on the heart and the coronary arteries above all compromising their mechanical efficiency: to maintain the same stroke volume in spite of the increased arterial impedance, depicted by the high SBP, the left ventricle pump up his wall tension, his aerobic work and lastly his oxygen consumption but cannot improve its vascular supply, because the coronary pressure perfusion relies on the DBP. Thus, the subendocardium became liable to ischemia, furthered by the LV hypertrophy, a compensatory reaction to the higher afterload. Eventually the addition of those detrimental factors is the LV diastolic dysfunction prelude (Figure 10) [1,45].

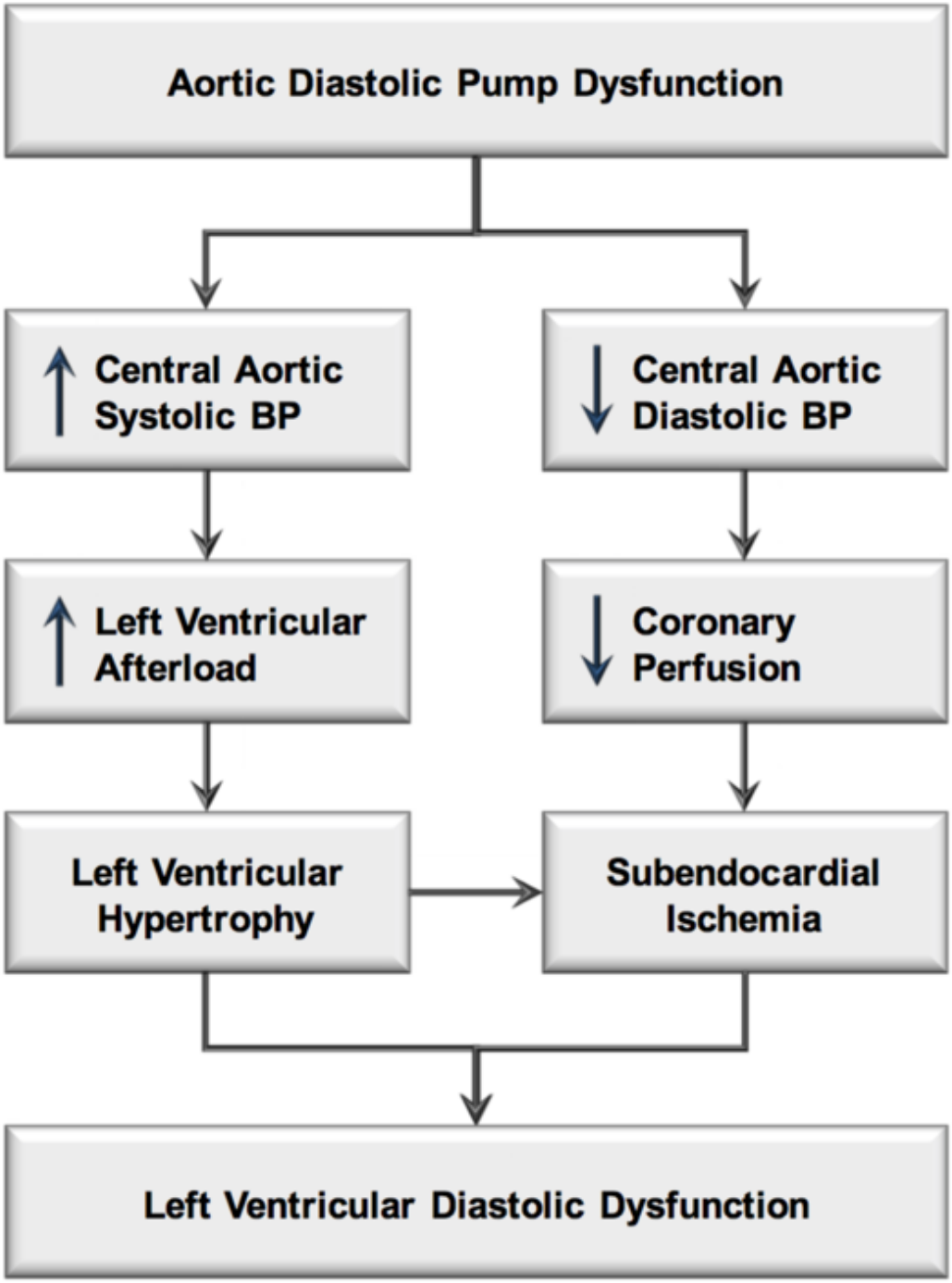


Figure 10. The pathway from aortic stiffening to left ventricular diastolic dysfunction.

The senescence pattern provides the large artery stiffening progression, the PP widening and the amplification phenomenon fading. Those changes have been under

investigation in the well-known Framingham Heart Study, a prospective cohort study of cardiovascular disease and the risk factors causing them. By following-up a large male and female middle-aged and old population, the the relation of coronary heart disease (CHD) hazard ratios (HRs) to blood pressure component was inspected. The mean follow-up time was 14,3 years and all the recruited individuals had no clinical evidence of CHD or were on antihypertensive drug therapy.

The results revealed that the blood pressure pulsatile component alone was superior or equal to the SBP or DBP in predicting the CHD occurrence: the HRs per 1 standard deviation (SD), after adjustment for traditional CV risk factors, were 1.38 for PP; 1.35 for SBP and 1.14 for DBP, respectively. Besides the association of two blood pressure components yielded a modest incremental contribution for DBP when combined with SBP and no added value of SBP or DBP if joint with PP. Lastly, a negative correlation between DBP and CHD risk at any given SBP over 120 mmHg have been established, suggesting that PP derived from arterial stiffening is an essential CV risk determinant [46].

The figure 11 presents the two component model of blood pressure: on the left for a given systolic pressure of 170 mmHg the coronary events HR nearly doubles if the DBP descends from 110 mmHg to about 70 mmHg; on the right at the same SBP as the pulse pressure rises the CHD risk follows but for any given PP having a higher SBP doesn’t contribute so much in the risk elevation.

These outcomes emphasise the greater risk connected to isolated systolic hypertension (ISH) opposed to systolic-diastolic hypertension, assuming a superior damage coming from pulsatile stress more than continual stress imputable to SVR strengthening.

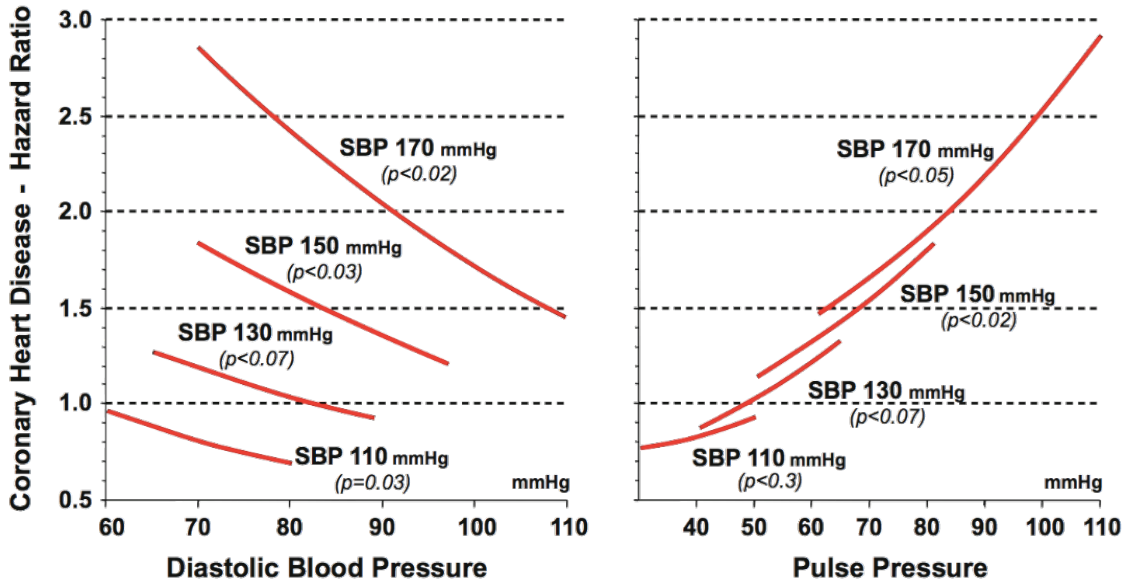


Figure 11. Joint influence of SBP, DBP and PP on the CHD risk. The Framingham Heart Study [46].

As said before, several experimental model of arterial stiffening in laboratory animals support the association between viscoelastic properties alteration and ventricular function impairment. In 1992 Kelly et al. tested the efficiency and contractile function of a canine heart after aortic bypass implantation. The left ventricle was coupled with a stiff plastic tube draining into the infra-renal abdominal aorta and several cardiac function indexes were assessed, like pressure-volume relation; myocardial oxygen consumption was also measured in situ by placing a catheter in the coronary sinus. After baseline recording, the blood flow was redirected into the plastic conduit and the compliance subsequently fell by about 70% compared to the native aorta, the pulse pressure rose by about 65 mmHg and the myocardial oxygen demand increased by 32%. Although there was no reduction in left ventricular stroke volume, delivering the same blood flow despite the stiffener outflow extents the energetic cost to the heart [47]. This is a predisposing factor for the development of stress-induced ischemia or for a fractional flow reserve constraint in the occurrence of coronary stenosis.

Similar conclusions are claimed by further studies on dogs [48] and rats [49], but the former demonstrates a threefold decline in the ejection fraction, a SBP drop and an enlargement of LV end-systolic volume after 2 minutes of anterior descending artery occlusion compared to animals with normal compliant aorta; the latter confirm the development of ventricular hypertrophy in rats chronically exposed to aortic elastocalcinosis as proved by LV weight/body weight ratio imbalance and augmented LV collagen content.

The binomial cardiac hypertrophy-hypertension in certainly not knew [50] but the findings in the rat model postulates that the structural modifications occurring in the heart attached to a stiffer vessel, is an intermediate stage of compensated cardiomyopathy probably evolving into full-blown heart failure [49].

Another worth mentioning harmful effect of augmented pressure pulsatility is microvascular damage. Physiologically the noblest organs like brain and kidneys deserve a high steady blood flow, independently of potentially oscillating MAP values; to prevent deleterious imbalances, many local autonomous regulatory responses exist, i.e. myogenic tone, vascular wall remodeling, local and humoral vasoactive molecules, nervous impulses. If there's a progressive aortic stiffening, the central pulse pressure became enlarged, exposing the high-demanding tissues to injury.

The underlying mechanism is the transmission and dissipation of the pulsatility into microvessels and capillaries, due to the match or even exceed of aortic and peripheral vascular impedance: the forward-travelling pulse wave reflexion coefficient is diminished and the pulsatile energy conveyed by the forward wave is not sufficiently damped, allowing the accomplishment of the pulsatile barotrauma [51].

Indeed, the high flow organs have a low impedance vascular bed that already permit a greater pulsatility transfer when compared to low flow, high impedance vessel as

Ageing
Other physiological conditions
Low birth weight
Menopausal status
Lack of physical activity
Genetic background
Parental history of hypertension
Parental history of diabetes
Parental history of myocardial infarction
Genetic polymorphisms

CV risk factors Obesity
Smoking
Hypertension
Hypercholesterolemia
Impaired glucose tolerance
Metabolic syndrome
Type 1 diabetes
Type 2 diabetes
Hyperhomocysteinemia
High CRP level

CV diseases
Coronary heart disease
Congestive heart failure
Fatal stroke
Primarily non-CV diseases
ESRD
Moderate chronic kidney disease
Rheumatoid arthritis
Systemic vasculitis
Systemic lupus erythematosus

those found in resting skeletal muscles. The tissue counteracts enhancing the vascular resistance and maintaining the same MAP but reducing the blood flow, being the last calculated as the ratio between the two previous elements; this causes repeated episodes of ischemia followed in the long run by stable ischemic damages [52].

Arterial Stiffness Clinical Implication

Table 1. Clinical conditions associated with increased arterial stiffness and/or wave reflections [15].

In addition to ageing, many pathophysiological conditions are associated with arterial stiffness, as reviewed by Laurent and colleagues (Table 1) [15]: low birth weight children, born at term after normotensive pregnancy, exhibit an aged large arteries phenotype with increase in indexes of wave reflections [53]; during the ovulatory phase of the menstrual cycle the radial artery stiffens, the opposite of what happens in the luteal phase, suggesting an hormone-dependency of VSMCs tone [54]; there's no significant progression of age-dependent arterial stiffening in highly physically active women [55]; in habitual smokers, just one cigarette cause a short-term increase in arterial stiffness and plaque rupture [56]; patients with hypercholesterolemia have higher pulse pressure and vascular stiffness compared to control subjects [57]; in a young adult population the metabolic syndrome is significantly associated with carotid artery stiffening [58]; impaired glucose metabolism and type 2 diabetes mellitus are related to peripheral and

central arterial stiffness [59]; the genetic background such as parental history of hypertension, diabetes or myocardial infarction, the presence of multiple CV risks factor or CV disease and even non CV disease like lupus and rheumatoid arthritis, all are strictly related to arterial stiffness [15] and finally, numerous other correlations with different conditions exist, not yet investigated.

Nevertheless, the following discussion will merely focus on the strongest evidence indicating vascular stiffness as an excellent prognostic factor in the wide spectrum of cardiovascular diseases.

Arterial Stiffness and Cardiovascular Events

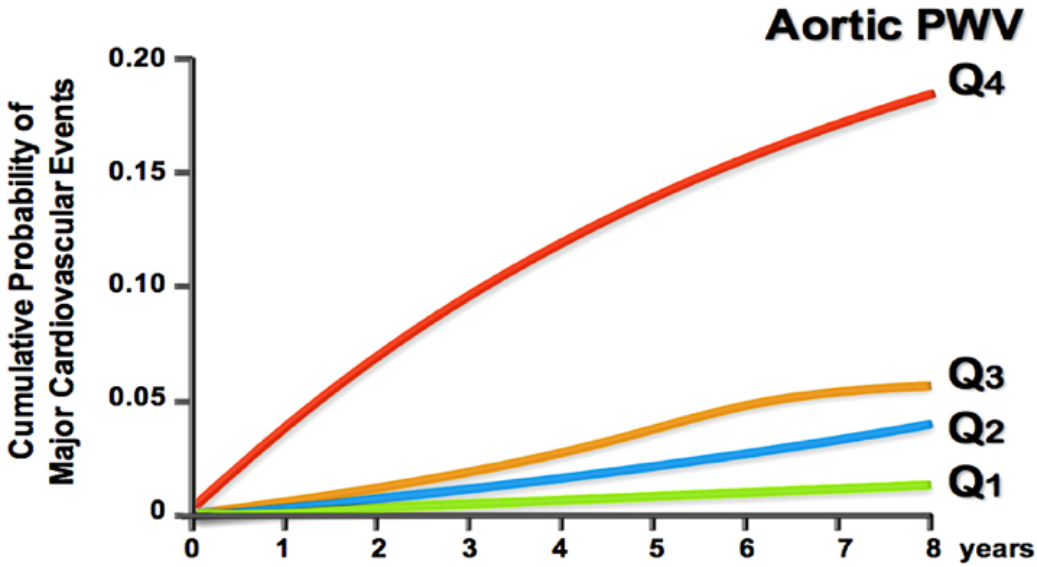
The clinical importance of arterial stiffness resides in its capability to predict cardiovascular events and mortality independently of others risk factors. Furthermore, the availability on the market of many devices at a relative low cost made the interest in this field grow over the past few years.

As recommended by the European guidelines on for the management of hypertension the PWV is the gold standard for measuring aortic stiffness [60] and even if many others index of arterial stiffness exist, almost all of the studies that are going to be reviewed, have considered this method because the aortic stiffness rather than arterial stiffness best correlates with CV accidents and the PWV is a direct, non-invasive, reproducible measure when compared to the others techniques.

To evaluate the power of arterial stiffness as cardiovascular risk marker, from the community-based observational Framingham Heart Study a large sample of 2232 subjects composed of middle aged and older individuals (mean age 63 years, 58% females) was inspected for a median follow up of 7.8 years. Being the outcomes defined as fatal or non-fatal myocardial infarction, unstable angina with documented ST-segment

changes, heart failure and ischemic or hemorrhagic stroke, the inclusion criteria required a silent history of those major cardiovascular events.

At the end of the study 6.8% of patients experience a CV accident and the PWV was strongly associated to disease development, with a hazard ratio of 1.48 adjusted for traditional risk factors; the association persisted unchanged even after adjustment for brachial pulse pressure, central pulse pressure or pulse pressure amplification. Besides the addition of PWV to traditional risk factor permitted a reclassification of 15.7% for individuals at intermediate risk and an overall improvement in risk discrimination of 0.7% in the full sample. As depicted in the figure 12, the estimated cumulative probability of



developing a CV event rises along with aortic PWV value and when compared to the lowest quartile (PWV $<$7.8 m/s), the highest (PWV $\geq$11.8 m/s) yielded an adjusted HR of 3.4.

Figure 12. Kaplan-Meier plot of cumulative probability of major CV event. The subjects are grouped according to quartiles of PWV.

Actually, this association does not apply to carotid radial pulse wave velocity, nor pulse pressure, nor augmentation index, nor pulse pressure amplification advising that only carotid-femoral artery, a measure of aortic stiffness, is unfavorably linked to cardiovascular diseases. Possible explanations rely upon (1) the strong correlation between central and peripheral pulse pressure in middle aged and older adults who are at highest risk; (2) the reduction of wave reflection due to aortic and muscular arteries impedance match; (3) sample selection [19].

Another longitudinal study evaluated the PWV predictive value for cardiovascular events but further focusing on coronary diseases: the primary end point was a first fatal or non-fatal CHD, i.e. myocardial infarction, sudden death, coronary revascularization and angina; the secondary end point was any primary CV event, i.e. stoke, AAA, PAD. The population sample was composed of 1045 essential hypertensive patients (36% women) aged 51 years on average, without any evidence of disease attributable to heart or vessels and was followed up for a mean time of 5.7 years. The typical patient presenting CV pathologies was male, 4.5 years older and had higher PWV and Framingham Risk Score compared to individuals without incident events. Because cholesterol, smoke, age and others are PWV modifiers as well as conditions of increased CV risk, a multivariate model that included those elements made possible the adjustment of the PWV predictive value for CHD and CV events: in the Table 2 below, are summarized the relative risks (RR) divided into two groups, the ‘all patient’ one including the whole sampled population, and the ‘patients at low risk’ one paralleling both the first and the second tertile of the FRS stratification; additionally, the columns distribute for comparison the values in unadjusted, adjusted for FRS and adjusted for standard CV risk factors.

Globally, the unadjusted relative risk of CHD predicted by 1 SD of PWV (3.5 m/s) was 1.42; likewise, the FRS was associated with a significant 51% upsurge in coronary events. Even when placed in a multivariate analysis the PWV retain its correlation with CHD whereas the FRS didn't. The adjustment for FRS returned a RR of 1.34 per SD increase in PWV while correcting for traditional CV risk factors an overall RR of 1.39 turned out.

Table 2 Rates and RRs of CHD and all CV events as a function of PWV expressed as tertiles [61].

	Events, n (%)	Unadjusted (95% CI)	Adjusted for FRS (95% CI)	Adjusted for Age, Sex, BP, and Other CV Risk Factors (95% CI)
All patients				
CHD events				
PWV, m/s				
<10.0 m/s	10/348 (2.9)	1.00	1.00	1.00
10.0–12.3 m/s	14/349 (4.0)	1.67 (1.16–2.42)	1.67 (1.16–2.42)	1.63 (1.13–2.36)
>12.3	29/348 (8.3)	2.80 (1.33–5.87)	2.80 (1.33–5.87)	2.66 (1.27–5.56)
CV events				
PWV				
<10.0 m/s	17/348 (4.9)	1.00	1.00	1.00
10.0–12.3 m/s	38/349 (10.9)	1.59 (1.21–2.08)	1.38 (1.03–1.84)	1.22 (0.91–1.65)
>12.3	54/348 (15.5)	2.53 (1.47–4.34)	1.90 (1.07–3.39)	1.49 (0.82–2.71)
Patients at low risk*				
CHD events				
PWV				
<10.0 m/s	5/289 (1.7)	1.00	1.00	1.00
10.0–12.3 m/s	10/242 (4.1)	2.37 (1.45–3.86)	2.37 (1.45–3.86)	2.43 (1.49–3.96)
>12.3	17/166 (10.2)	5.60 (2.10–14.93)	5.60 (2.10–14.93)	5.90 (2.22–15.68)
CV events				
PWV				
<10.0 m/s	10/289 (3.5)	1.00	1.00	1.00
10.0–12.3 m/s	23/242 (9.5)	1.85 (1.28–2.69)	1.85 (1.28–2.69)	1.82 (1.25–2.65)
>12.3	24/166 (14.5)	3.44 (1.65–7.25)	3.44 (1.63–7.25)	3.31 (1.56–7.05)

*Patients belonging to the first and second FRS tertiles.

Aiming at the division into population tertiles (Figure 13), the incidence of CHD grows parallel to the PWV tertiles and to the FRS tertile though the rise is steeper moving along the PWV axis for the first two FRS tertiles: the patients considered at low risk for the Framingham risk score have a number of coronary diseases nearly 6 times higher if they belong to the upper end of the distribution according to PWV (RR of 5.90 adjusted

for classical CV risk factors). The same trend occurs for CVDs although less dramatically (RR of 3.31).

Comparing the tabulated values, in the 'all patients' category the PWV seems an indipendent predictor of CHD regardless the adjustmen while its predictive value for CV events loses significance after full adjustment for all CV risk factors (the RR decreases from 1.59 unadjusted to 1.22 and from 2.53 to 1.49 in the second and third tertile respectively).

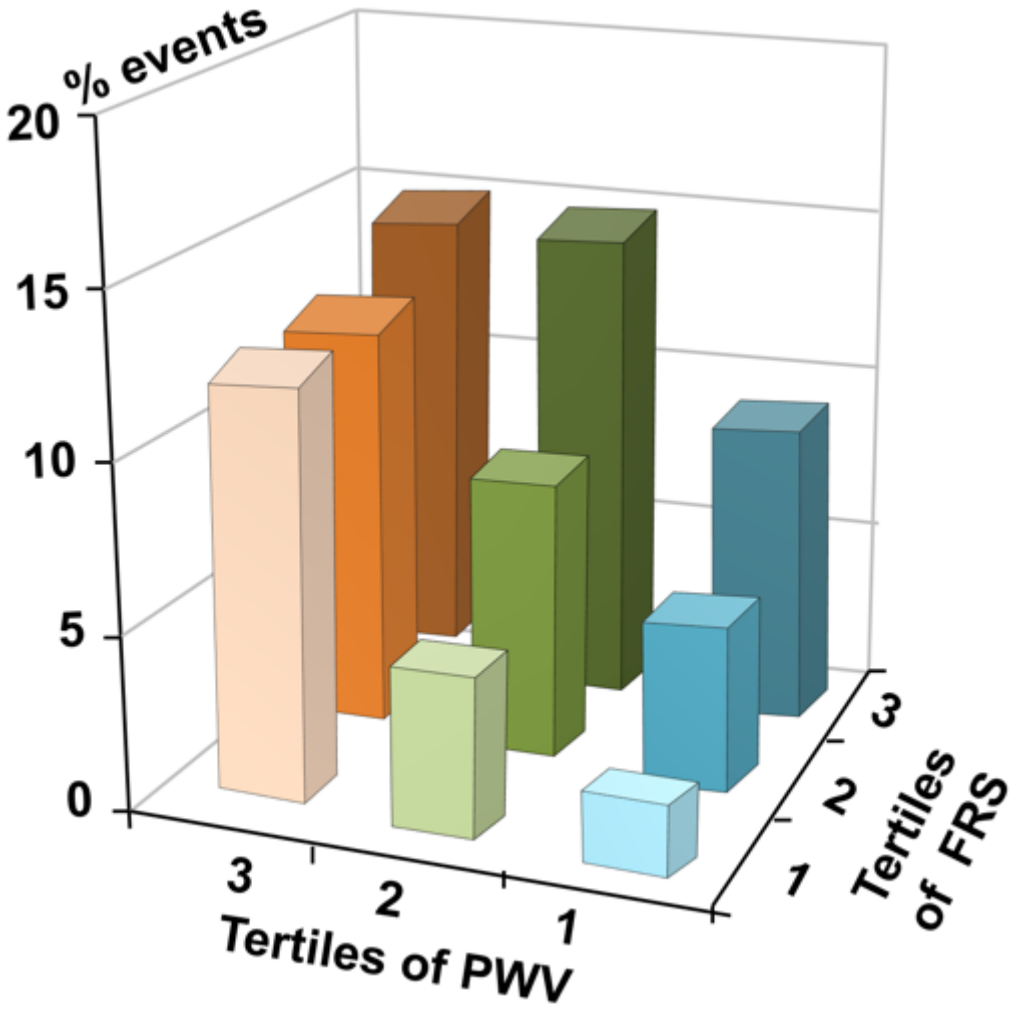


Figure 13. Cumulative incidence of fatal or non-fatal CHD divided according to tertiles of PWV and FRS [61].

Conclusively, in hypertensive patients the aortic stiffness assessed with pulse wave velocity predicts the occurrence of primary coronary heart disease and of primary cardiovascular accidents to a lesser extent, especially in patients classified as low risk by FRS [61].

Many studies validate the clinical significance of aPWV as index of arterial stiffness in different patients group: hypertensive, middle aged and older, with end-stage renal disease, etc. while others try to sample the widest population allowing results generalization. The Rotterdam Study for example is a large cohort study comprising 7983 individuals which provided a strong correlation between arterial stiffness and risk of CHD and stroke but even working on large numbers has only recruited subjects aged over 55 years [62].

Differently, Hansen et al. studied a Danish population stratified by sex and age groups from 30 up to 60 years creating a model of the general population. The achieved results are not surprising: once again the significant predictive capability of PWV has been confirmed for a composite of cardiovascular outcomes, i.e. CHD and CV mortality, above and beyond traditional risk factors, including MAP, office pulse pressure and 24-hour ambulatory pulse pressure [63]. The Figure 14 on the side abstract the study findings: virtually in each percentile the aortic PWV has an equal or superior hazard ratio to the general population, excepting the first quintile; all the plotted data are adjusted for sex and age.

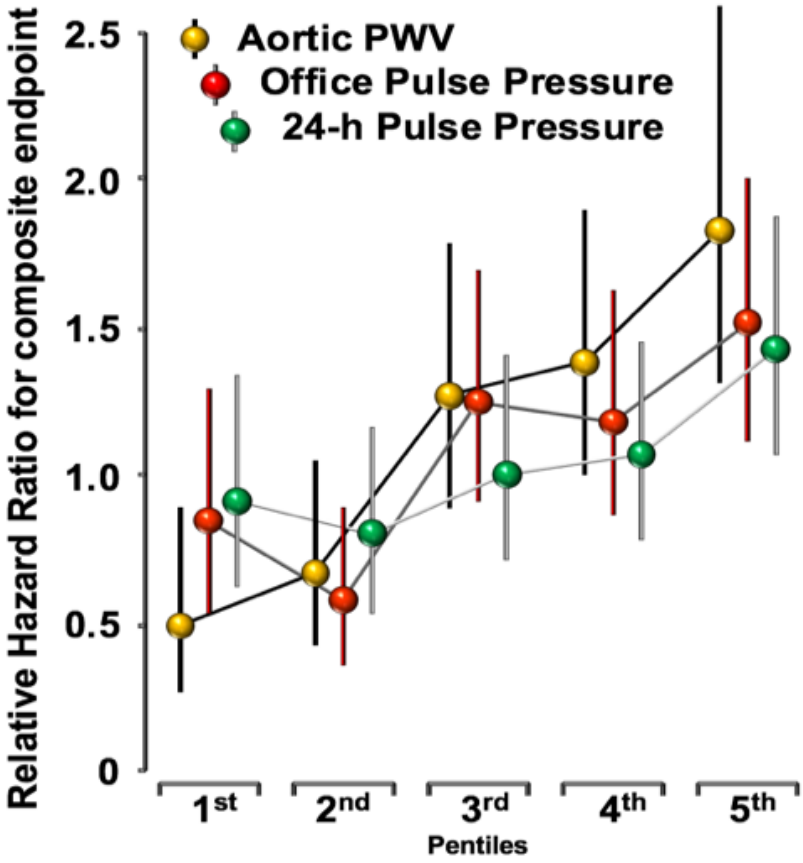


Figure 14. Relative hazard ratios for the composite CV end point by quintiles of the distribution of aPWV, office pulse pressure and 24-hour pulse pressure [63].

The Impact of Aortic Stiffness on Survival

According to the WHO, cardiovascular diseases are the leading cause of deaths worldwide but unfortunately they are not straightforwardly predictable on using just traditional risk factors. In these circumstances the arterial stiffness offers a new powerful approach not only to high risk, high mortality patients but maybe also to the general population, as demonstrated by Laurent et al. in the study discussed below.

In a cohort of 1980 patients no more than mildly hypertensive, the all-cause and cardiovascular mortalities were investigated during a mean follow up of 9.3 years,

starting from a mean age of 50 and with a low prevalence of antihypertensive drug usage. As usual the arterial stiffness was calculated using the PWV measurement and to test its correlation with deaths, was included in several models, using univariate and multivariate analysis. The PWV was significantly associated to all-cause and mainly cardiovascular mortality in both statistical analysis, yielding an odds ratio (OR) of 1.34 and 1.51 per 5 m/s increase in PWV for all-cause and cardiovascular mortality, respectively. Surprisingly, in the univariate models, an acceleration of the pulse wave by 5 m/s parallels the risk acquired with 10 years of ageing, as shown in Table 3 for cardiovascular mortality, but this statement is true for all-cause mortality also.

Table 3. Relative risk of cardiovascular mortality according to various parameters: univariate analysis [64].

Parameters	OR	Lower 95% CI	Higher 95% CI	*P*
PWV, 5 m/s	2.35	1.76	3.14	<0.0001
Previous CVD, yes/no	14.81	7.98	27.47	<0.0001
Age, 10 y	2.32	1.78	3.01	<0.0001
PP, 10 mm Hg	1.53	1.31	1.80	<0.0001
SBP, 10 mm Hg	1.26	1.12	1.42	<0.001
Diabetes, yes/no	4.23	1.96	9.15	<0.001

Regarding the pulse pressure, in the multivariate model it was not appreciably related to all-cause mortality and was poorly related to CV deaths: once again only the PWV held its independent predictive value as index of arterial stiffness [64].

Talking about categories at greater risk of death, Blacher et al. enrolled a cohort of patients with end-stage renal disease (ESRD) undergoing hemodialysis from at least 3 months, to prove the actuality of a correlation between PWV and mortality using a Doppler ultrasonography technique. The population under investigation is a specific category of patients in whom cardiovascular diseases develop rapidly due to the

underlying uremic state: the main outcomes are indeed cardiac ischemia, stroke, LV hypertrophy, heart failure and sudden death.

As a result of the statistical analysis the only meaningful covariates impacting on the group mortality were age, PWV, duration of hemodialysis and DBP (with an inverse relation). After standardization PWV was the best predictors of mortality above all: the patients in the third tertile (PWV <12 m/s) had a relative odds ratio of 5.4 compared to the first tertile for all-cause mortality and for each increase of 1 m/s in the pulse speed there is an escalation of deceases by 39%; analogously, for cardiovascular deaths patient in the upper tertile had an OR of 5.9 [22]. The Kaplan-Meier survival curves according to tertiles (Figures 15-16) report a very remarkable difference between the three clusters: starting from 80 patients at highest risk, 51 died regardless the *causa mortis,* a considerable mortality of 64%; contrariwise the low risk category experience only a 17% reduction of survival.

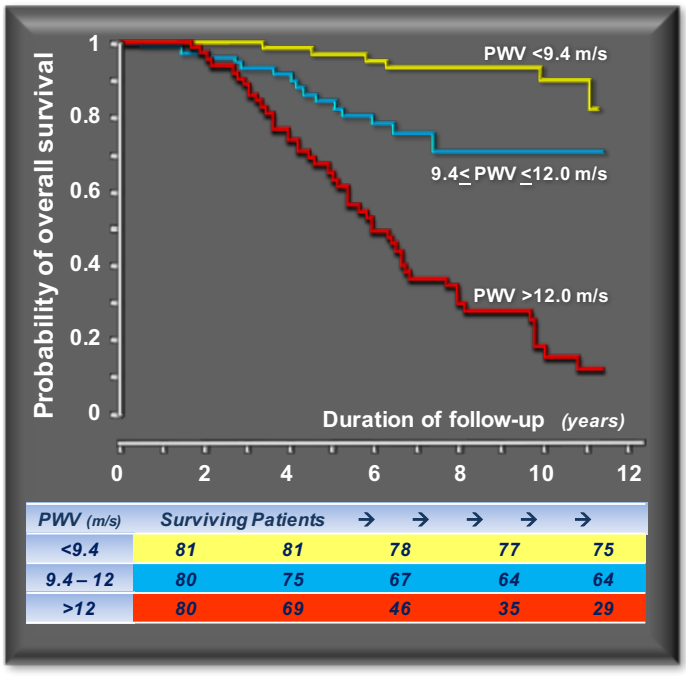


Figure 15. Probabilities of overall survival. Under the diagram are listed the residual numbers of survived subject as function of follow up months.

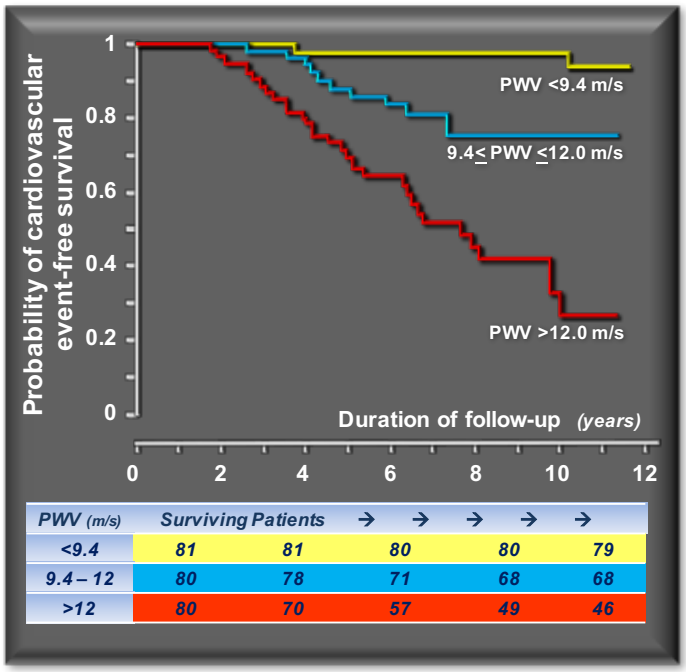


Figure 16. Probabilities of event-free survival. Under the diagram are listed the residual numbers of survived subject as function of follow up months.

The same researchers group tested the positive impact of PWV reduction on survival in patient with end-stage renal failure (ESRF): the absence of PWV decrease in response to blood pressure decrease was an independent predictor of mortality with a 2.6-fold adjusted relative risk of all-cause mortality and a 2.4-fold adjusted risk of cardiovascular mortality [65]. As illustrated in the ROC curve (Figure 17), the sensitivity of ΔPWV was 56% and its specificity was 84%, giving a quite good negative predictive value of 70% and a positive one of 74%, i.e. 74% of patient with a decline in pulse wave velocity survived during the follow up. The clinical import of those statistical indicators reverberates on the chance of early poor-prognosis patient identification, being able to promptly intervene.

Aortic stiffness measured through PWV and mortality are a well-established duo yet in others selected populations: stroke patients, geriatric patients and type 2 diabetes patients.

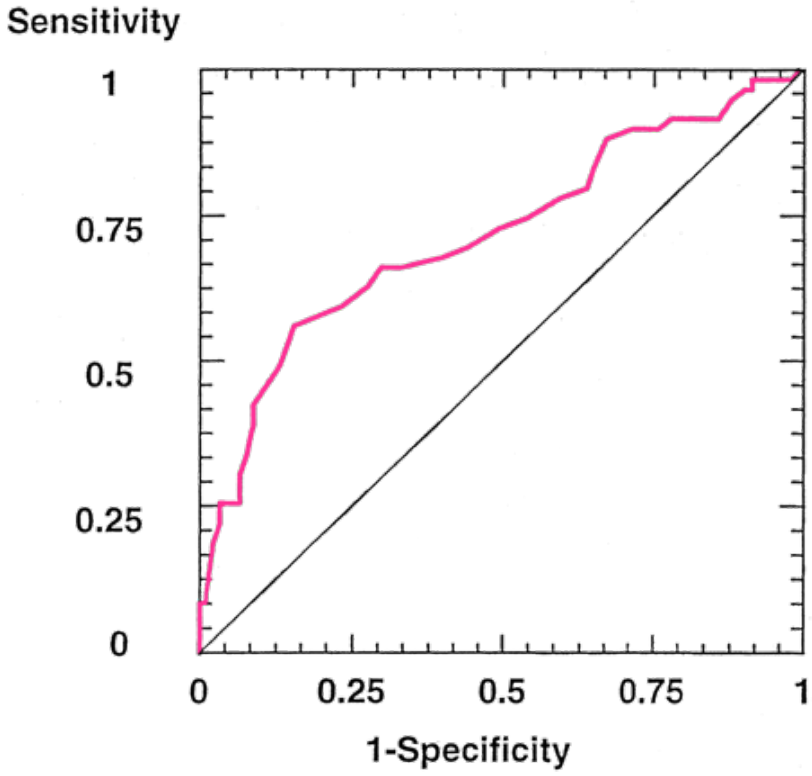


Figure 17. Ability of adjusted aPWV change to predict death.

The incidence of stoke deaths was appraised in essential hypertensive subjects appreciating a close relation with high PWV: in univariate analysis for each SD increase (4 m/s) of PWV the stoke risk rose by 72% while in multivariate analysis the percentage descent to 39, still maintaining a strong predictive value and independency of classical risk factors [66].

In three French geriatric departments, about one hundred and half patients were enrolled to assess the determinants of old age mortality. Beyond age and loss of autonomy which predicted superbly the overall mortality, the aortic PWV was the major joint with cardiovascular deceases, having an OR equal to 1.19 [67].

Lastly, knowing that diabetes and glucose intolerant patients have stiffer arteries [12], a comparison with a control group has been made endorsing this hypothesis: aortic PWV was consistently greater in subject with impaired glucose metabolism than in

controls and the mortality risk was doubled, as evidenced by the HR of 2.34 and 2.12 for type 2 diabetics and glucose intolerant respectively. However the PWV capability to forecast a fatality was less conspicuous: per 1 m/s the hazard ratio is a little but still noteworthy 1.08 [68].

In conclusion, arterial stiffness is firmly associated to many clinical conditions if evaluated using the measurement of pulse wave velocity along the aorta, therefrom the heightened thoughtfulness about this stiffness quantifier that occurred in the last few years.

ARTERIAL STIFFNESS ASSESSMENT METHODS

Not only many physical quantities and terminologies defining arterial stiffness exist, but also many different techniques and devices are commercially available. Even more the arterial tree can be considered altogether or by a segmental district or a single vessel, determining systemic, regional or local stiffness measurements, respectively.

Since the systemic stiffness can only be estimated resorting to circulation models than actually measuring someway related parameters its use in clinical practice has gradually been reduced. Nevertheless, it can represent the total hemodynamic load and the opposition of large arteries to the pulsatile effects of ventricular ejection: its clinical values comes from this and its immediate simplicity [69]. In the past it was calculated by the ratio of stoke volume and pulse pressure (SV/PP), a very simplistic approximation, or basing on the Windkessel model and performing a pulse contour analysis, or analogies with electrical models combining capacitance and resistance in series.

Inversely regional and local stiffness are detected using direct measurements of parameters linked to arterial mechanical properties, wherefrom the widespread adoption in several clinical settings and studies.

Indexes of Arterial Stiffness and Definitions

The general term 'arterial stiffness' simply refers to the rigidity of the arterial wall and can be assessed using one of the following indexes [1,69,70]:

a. *Arterial distensibility*: relative diameter (or area) change for a pressure increment; it corresponds to the inverse of elastic modulus.

   $\Delta D/(\Delta P \cdot D)$ (mmHg^{-1})

b. *Arterial compliance*: absolute diameter (or area) change for a given pressure step at fixed vessel length

   $\Delta D/\Delta P$ (cm/mmHg) or (cm^2/mmHg)

c. *Volume elastic modulus*: pressure step required for (theoretical) 100% increase in volume where there is no change in length

   $\Delta P/(\Delta V/V)$ or $\Delta P/(\Delta D/D)$ (mmHg)

d. *Elastic modulus*: pressure step required for theoretical 100% stretch from resting diameter at fixed vessel length

   $(\Delta P \cdot D)/\Delta D$ (mmHg)

e. *Young's modulus*: elastic modulus per unit area; the pressure step per square centimetre required for theoretical 100% stretch from resting length

   $(\Delta P \cdot D)/(\Delta D \cdot h)$ (mmHg/cm)

f. *Pulse wave velocity*: speed of travel of the pulse along an arterial segment

   $Distance/\Delta t$ (m/s) or (cm/s)

g. *Augmentation index*: difference between the second and first systolic peaks as a percentage of pulse pressure

   $(P_2 - P_1)/PP$ (%)

h. *Characteristic impedance*: relationship between pressure change and flow velocity in the absence of wave reflections

   $\Delta P/\Delta v$ (mmHg · s/cm)

i. *Stiffness index* β: ratio of natural logarithm (systolic/diastolic pressures) to (relative change in diameter)

$$\beta = \frac{\ln(P_s/P_d)}{(D_s - D_d)/D_d}$$

j. *Compliance coefficient (CC)*: compliance per unit length, which is the change in cross-sectional area per unit of pressure

$$CC = \frac{\Delta V/L}{\Delta P} = \frac{\Delta A}{\Delta P} = \frac{\pi D \cdot \Delta D}{2\Delta P}$$

k. *Distensibility coefficient (DC)*: relative change in cross-sectional area per unit of pressure

$$DC = \frac{\Delta A/A}{\Delta P} = \frac{2(\Delta D/D_d)}{\Delta P}$$

In the scientific world, these terms are often used interchangeably but in truth they have slightly different meanings; it must also be said that these indexes are difficult to interpret in clinical practice and that many depend on the pressure because the stiffness itself is pressure-dependent. The stiffness index is the only one partially decoupled from pressure.

Apart from augmentation index (Aix) that relies on pressure waveforms, all the aforementioned indexes can be measured ultrasonographically or using MRI (excluding the stiffness index). Instead the PWV can be obtained from pressure, distension (volume) or Doppler (flow) waves and again with MRI.

Regional Stiffness Measurements

The rationale of measuring segmental arterial stiffness is based upon direct methods and involves some arterial sites of specific interest such as thoraco-abdominal aorta, carotid artery and brachial artery. The aorta is the first interface to which the pulse wave is compared and mainly expresses the buffering properties of large elastic arteries; the aging employs its effect prominently on it and many population studies have proved it to be the best district correlated with cardiovascular prognosis [20,61,62,65-68,71,72]. Besides,

	Device	Methods	parameters
stemic stiffness	Area method	Diastolic decay	
	HDI PW CR-2000®	Modif. Windkessel	
	CVMS PWV	Stroke volume, pulse pressure	
gional stiffness	Complior®	Mechanotransducer	aPWV
	Sphygmocor	Tonometer	aPWV
	PulsePen®	Tonometer	aPWV
	SwanTrack®	Echotracking	aPWV
	Ultrasound systems	Doppler probes	aPWV
cal Stiffness	WallTrack®	Echotracking	CCA[a], CFA, BA
	NIUS®	Echotracking	RA
	Ultrasound systems	Echotracking	CCA[a], CFA, BA
	MRI device	Cine-MRI	Aorta

CCA, common carotid artery; CFA, common femoral artery; BA, brachial artery; RA, radial artery

Table 4. Devices and methods used for determining systemic, regional, and local arterial stiffness and wave reflections [15]

brachial artery is the elective seat for peripheral pressure valuation using an arterial cuff, while the lower limb arteries are extremely susceptible to the formation of atherosclerotic plaques as well as the carotid artery.

Although with different arrangements and devices, the only technique implemented for evaluating the regional stiffness is the measurement of aortic pulse wave velocity, as indicated in Table 4.

Pulse Wave Velocity

The gold standard for measuring aortic stiffness is the carotid-femoral PWV, a position statement assumed, among others [15], also by the European Society of Cardiology [60]. Usually it is calculated using the foot-to-foot velocity method form various waveform

acquired non-invasively with transcutaneous probes, placed on the common carotid artery and the femoral artery on the same patient side (normally the right).

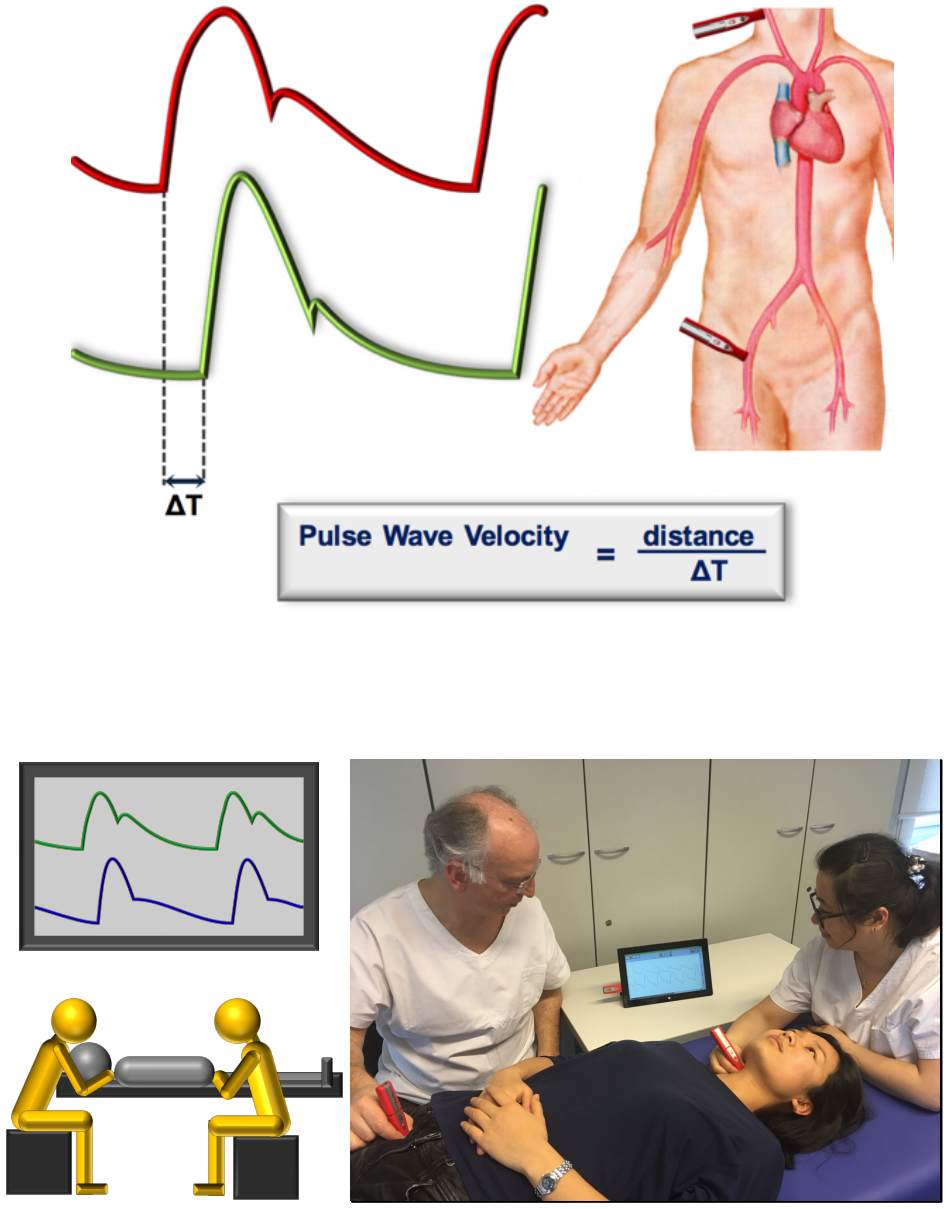


Figure 18. Simultaneous recording of carotid and femoral pulse wave using two probes.

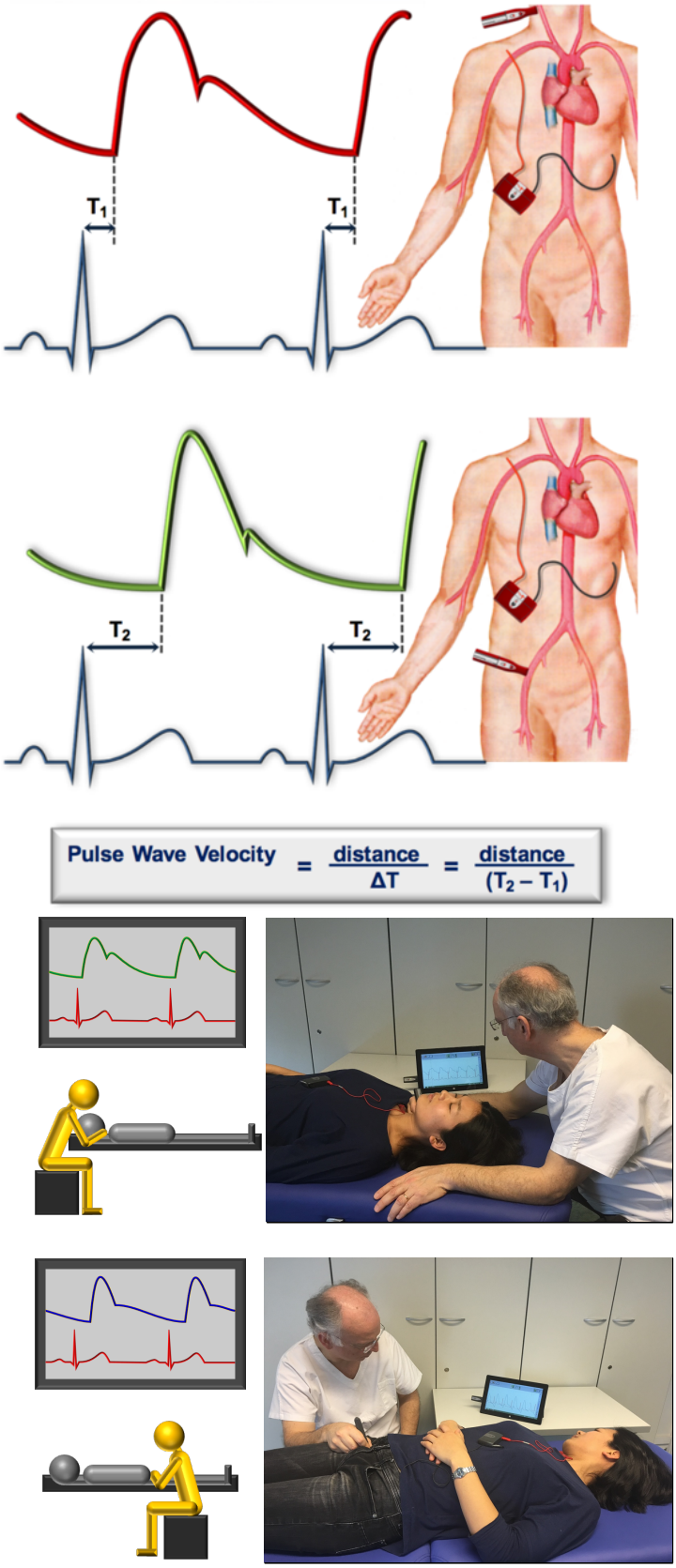


Figure 19. Sequential wave recording at the carotid and femoral arteries with the aid of the electrocardiographic signal.

The recording can be simultaneous for appliances equipped with two probes, but it is possible to perform two sequential measurements using the electrocardiogram (ECG) as a reference for single probe devices. In the first case the transit time Δt is calculated from the femoral pressure wave foot delay compared to the carotid pressure wave foot (Figure 18), both obtained with an algorithm chosen by the device manufacturer, although the most commonly used, validated and less influenced by alterations of the wave-front or reflection phenomenon, is the intersecting tangent algorithm, currently become the reference standard [73].

In the second case the signals registration takes place in two stages in a very short time, so that the transit time is hardly influenced by changes in the isovolumic contraction period of the left ventricle or heart rate variability [15]; the recordings are synchronized with the ECG and the temporal distance between the foot of each wave and the R wave peak of the QRS complex is calculated in order to obtain two time intervals (t_1 and t_2) that return desired the transit time when subtracted ($\Delta t = t_2 - t_1$; see Figure 19).

The other required parameter for deriving the pulse wave velocity is the travelled distance. It's possible to estimate the arterial length with surface measurements that consider as reference points those where the probe is placed, i.e. the points of greatest pulsation of the carotid and femoral vessels. With a tape measure the two points are joined on a straight line and the value expressed in meters is the distance travelled by the pulse wave.

This is not the only way to estimate the length of the aorta and is still an approximation of the vascular anatomy: one MRI study on about a hundred of healthy subjects, representative of various classes of age (from 21 to 76 years), has shown that carotid-femoral distance overestimates the real distance of 25% and the result more closely aligned to the actual aortic path length is the carotid-femoral distance multiplied

by 0.8 [74]. According to the expert consensus group on arterial stiffness it is the standard distance to use in daily practice [75].

Currently the distances between measurement sites more widely used are: (1) the total distance between the carotid and femoral pulses; (2) the result of subtracting from the total carotid-femoral distance the segment between carotid pulse and sternal notch; (3) the result of the subtraction between sternal notch to femoral pulse distance and sternal notch to carotid pulse distance (Figure 20); each of these approximations showed no significant differences in interventional studies with repeated measures [76].

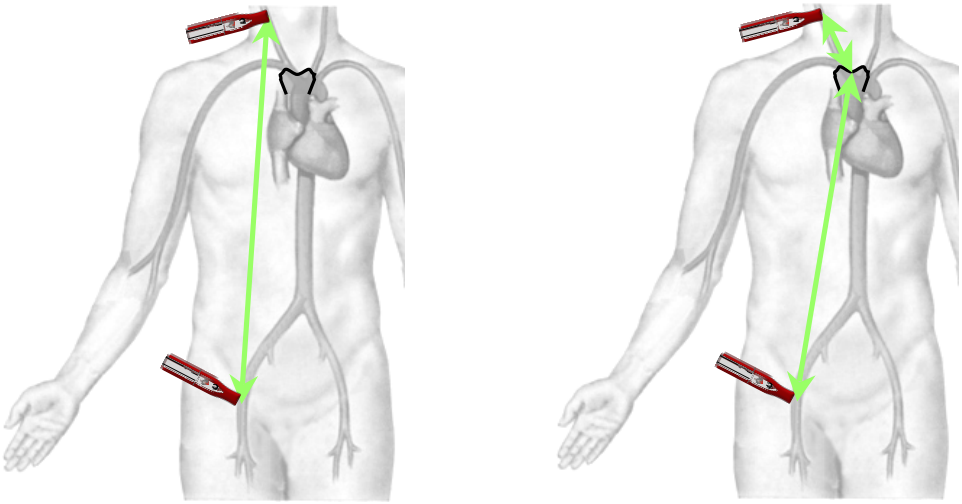


Figure 20. Carotid to femoral distance measured by direct (left) and subtractive method (right)

Since even small inaccuracies can alter greatly the value of PWV the accuracy is the main problem with the superficial measure of carotid-femoral distance: obese subjects with very high BMI prevent the femoral artery palpation and it's very likely to overestimate the distance because of abdominal fat; busty women have the same issue. Furthermore, subjects with stocky physique worsen the measurement as the accuracy

improves with the increase of the measured length and shorter distances extent the absolute error in determining transit time. Likewise diabetes, metabolic syndrome, arterial stenosis located near the measurement site and PAD, can interfere with the careful acquisition of the femoral waveform [15,76].

Devices

Tools for measuring PWV using pressure waveforms utilizing pressure sensors are the most common and usually require a low level of expertise to be employed.

The Complior Analyze® System (Alam Medical, Vincennes, France; http://www.complior.com/) measures simultaneously the PWV with up to 4 dedicated mechanotransducers (piezoelectric sensors) so that up to 3 arterial segments can be assessed at once: carotid-femoral, carotid-brachial and femoral-dorsalis pedis. Registration is also possible in case of atrial fibrillation or other arrhythmias and is generally very fast: with 15 seconds of pressure waves acquisition it's possible to obtain at least 10 wave complex, enough for a quality measure that covers the respiratory cycle fluctuations. An advantage for health professionals is the ability to secure the carotid sensor with a firm collar in order to avoid the probe dislodgment which could happen if it were hand-held. While recording, the curves are displayed on the screen to be validated before confirming their acquisition and the software performs quality checks on the data retaining only the adequate pressure waves automatically.

The PulsePen® (DiaTecne srl, Milan, Italy; http://www.pulsepen.com/) is a pocket-size device. It is available on the market in two forms: two tonometers for simultaneous recording or a single tonometer paired with a 2-lead ECG for sequential recording. The probes, designed with ergonomics in mind, are shockproof and wireless connected. The instrument's software allows direct quality-check on waveforms, displaying on the video, instant-by-instant, curve-by-curve, the variability index between

each pressure wave and the next one. Once 10 good quality curves are registered, the data can be captured and also edited offline: any ectopic beats or aberrant wave can be excluded from the final evaluation.

The SphygmoCor® System (AtCor Medical Pty. Ltd., West Ride, Australia; http://www.atcormedical.com/) exploits a single hand-held high-fidelity tonometer (Millar, Houston, Texas) for determining the pressure wave at the carotid and femoral arteries in rapid succession, using a 3-leads ECG as a reference. The software acquires pressure waveforms in a range of 10 seconds and basing on the electrocardiographic and tonometric data variability returns an 'operator index' that specifies the records quality, possibly suggesting a data recapture if the index is too low.

Reference values of carotid-femoral PWV

Reference values for cf-PWV have been established in 1,455 healthy subjects and in a larger population of 11,092 subjects with cardiovascular risk factors [19]. These are reported according to age and blood pressure levels. Although the relationship between cf-PWV and cardiovascular events is continuous, a threshold of 12 m/s has been initially suggested as a conservative estimate of significant alterations of aortic function in middle aged hypertensive patients [7], and included in the ESH/ESC guidelines for the management of hypertension. This threshold was based on cf-PWV using the full direct carotid-to-femoral distance and has been revised in a recent consensus document down to 10 m/s [5], in order to normalize PWV values according to the arterial pathway. The new threshold is included in the 2013 ESH/ESC guidelines for the management of hypertension [20].

Figure 21 shows the reference values obtained in 3208 apparently healthy adults, with no cardiovascular risk factors and without manifest cardiovascular disease using the "direct" method to measure distance [carotid to femoral artery] x 0.8, as suggested by a

recent expert consensus document on the measurement of aortic stiffness.

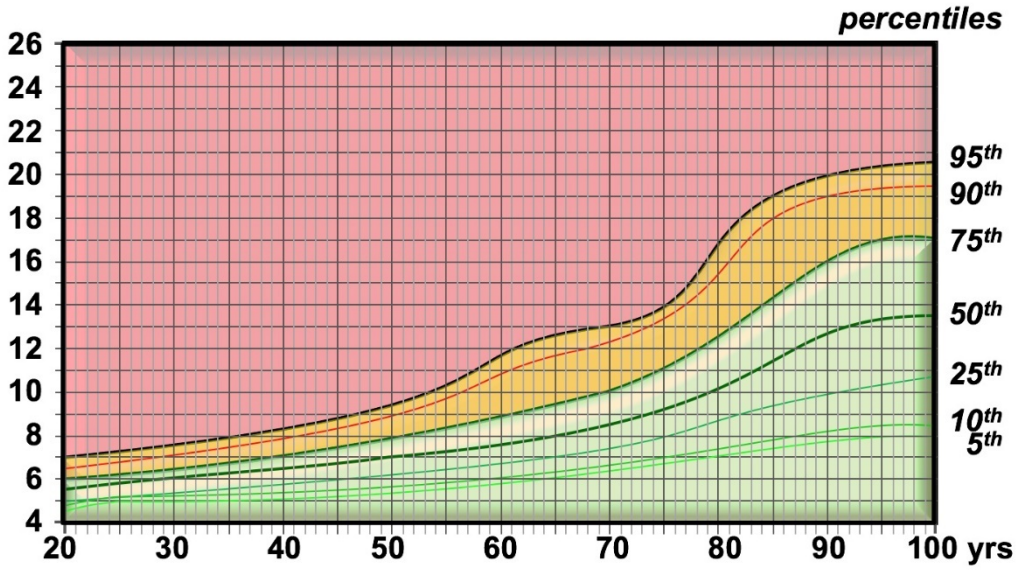


Figure 21. Aortic pulse wave velocity reference values: percentile curves in adults according to age. Data observed in healthy population, free from overt cardiovascular diseases and cardiovascular risk factors, using the PulsePen® tonometer.

The data shown in the graphics can be interpreted as follows:

- the values below the 75th percentile may be considered as "normal values";
- between the 75th and 95th percentile: "border-line values". It is advisable to repeat the test periodically and to try to identify the causes of increased arterial stiffness;
- above the 95th percentile: evidence for "arterial stiffness". High risk for cardiovascular disease.

The Complior Analyse®, the PulsePen® and the SphygmoCor® use similar algorithm systems for measuring the time delay of the femoral pulse wave with respect to the carotid pulse wave, and their outcomes are superimposable. Therefore, these reference values can be applied, in clinical application and research, when these devices are used.

oke change in diameter	Change in diameter during systole: $\boldsymbol{D_s - D_d}$ (mm)
oke change in lumen area	Change in lumen area during systole: $\Delta \boldsymbol{A} = \boldsymbol{\pi}(\boldsymbol{D_s}^2 - \boldsymbol{D_d}^2)/\boldsymbol{4}$ (mm^2) with $\boldsymbol{D}$ = internal diameter
ll cross-sectional area	Surface of a cross-section of the arterial wall: $\boldsymbol{WCSA} = (\boldsymbol{D_e}^2 - \boldsymbol{D_i}^2)/\boldsymbol{4}$

Local Stiffness Measurements

Table 5 Indexes of arterial stiffness applied to geometrical measurements of large arteries with ultrasounds [15].

stic properties of the artery as a whole	
oss-sectional distensibility efficient (DC)	Relative change in lumen area during systole for a given pressure change: $\boldsymbol{DC} = \Delta \boldsymbol{A}/\boldsymbol{A} \cdot \Delta \boldsymbol{P}$ (kPa^{-1})
oss-sectional compliance efficient (CC)	Absolute change in lumen area during systole for a given pressure change: $\boldsymbol{CC} = \Delta \boldsymbol{A}/\Delta \boldsymbol{P}$ (m^2kPa^{-1})

Da *De* *Di*

Taking advantage of an ultrasound probe, it is possible to estimate the local stiffness of all superficial arteries with geometrical investigation. Although many echotracking devices exist, in order to explore the viscoelastic properties of deeper arteries a different imaging technique must be applied: the considerably more expensive cine magnetic resonance. Even using simply one type of device, various indexes can be calculated: Table 5 lists the most commonly used methods. Anyway, the normally required parameters are the value of late diastolic vascular diameter and the stroke change in diameter.

The local stiffness assessment is a method offering many benefits but unfortunately some limitations too [15]. First of all, there is no need of circulation models nor approximation to derive measures of compliance or distensibility but the pressure

variation that induces the change of volume is directly quantified; in second place a specific diameter-pressure curve for each particular vessel can be figured, which corresponds to the compliance curve of the vessel itself; thirdly it is possible to calculate the PWV along the arterial segment simply considering the delay time between two contiguous distention waves; finally, recalling that the stiffness exhibits a pressure dependence, arterial rigidity is evaluated parallel to time-varying pressure and, from the same apparatus and the same acquired images, IMT can be inferred; therefore the Young elastic modulus can be computed, being the intima-media thickness a surrogate of the whole arterial wall thickness.

Regarding the limitations, many of the ultrasonography devices make use of video-assisted calipers and the screens often have an inadequate spatial resolution for estimating sub-millimetric variations such as those induced by the pulse wave transit. Only high-resolution instruments employing radiofrequencies or with high pixels per inch (PPI) video-image analyzer can reach a sensitivity of 1 micron being suitable for diameter changes analysis. The need to measure the local arterial pressure is another technical issue. This can be fixed with a bloody technique, i.e. arterial catheterization, or with a non-invasive technique, through transcutaneous arterial tonometry. The latter returns a value virtually identical to that intra-arterially measured, but requires further blood pressure calibration; since the mean blood pressure value is nearly constant along thorough arterial tree and the diastolic pressure value changes negligibly, the pulse pressure at the measurement site can be derived [76]. Lastly for the wall properties assessment it is assumed that the arterial cross sectional area is circular.

Given that local arterial stiffness adds nothing in prognostic terms when compared to regional stiffness, the expert consensus document suggests to restrict the application of this index to pathophysiological, interventional or pharmacological studies

[15].

Considering all the theoretical and methodological limitations, Giannattasio et al. [77] implemented in collaboration with the Universities of Milan-Bicocca and Nancy (A. Benetos) and company DiaTecne srl a technique that would eliminate many of the uncertainties arising from the use of inadequate equipment, models and approximations, or indirect indexes of stiffness.

Relying on continuous echography sampling of a common carotid artery diameter changes by WallTrack® System (Pie Medical Imaging, Maastricht, Netherlands; http://www.piemedicalimaging.com/), paired with a continuous pulse wave sampling through arterial tonometry on the contralateral carotid by PulsePen® device, they reported the following benefits: (1) the change in the vascular diameter has been directly linked to the variation of the current internal pressure; (2) the acquisition of multiple pressure and diameter values throughout the cardiac cycle rather than just 2 representative values of systole and diastole, allowed to trace the pressure-diameter relationship as a slope; (3) obtaining of multiple slope admits a beat-to-beat and cycle-to-cycle comparison of arterial compliances; (4) systolic and diastolic compliance can be stated separately [77].

With echotracking device two curves representing the movement of the front and rear wall, respectively, are acquired, which add up to shape the diameter variation curve (Figure 22); then the ultrasound track is synchronized with the tonometry track via ECG to plot the pressure diameter relationship.

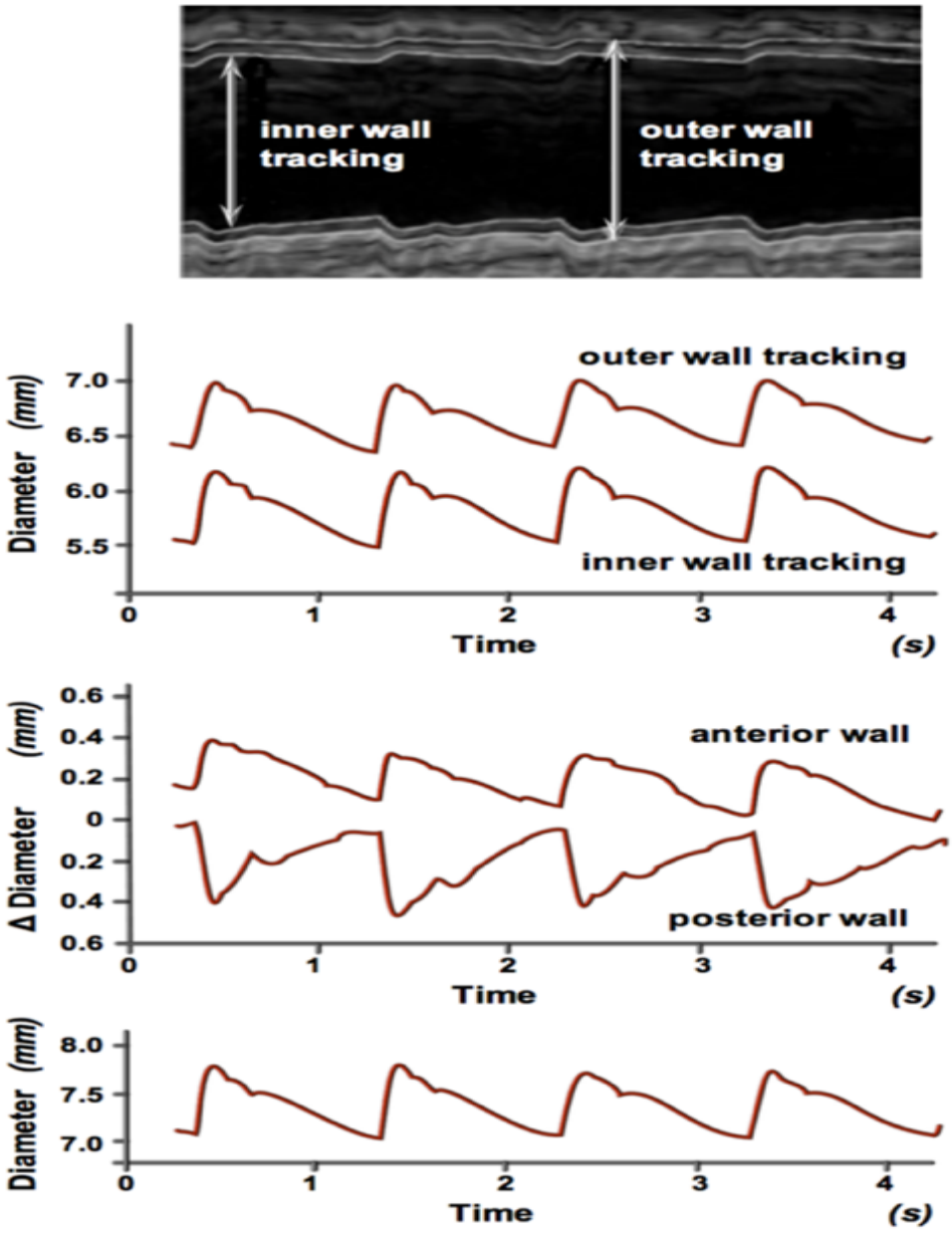


Figure 22. Wall Track System: assessment of the arterial diameter change during a cardiac cycle by means of an ultrasound device.

Looking at the graph of the compliance slopes, that summarizes the results of the study, there's a momentous divergence between young (age <45 years) and elderly (>45 y.o.): as expected older people have a less inclined curve because arterial stiffness is the aging process hallmark (Figure 23).

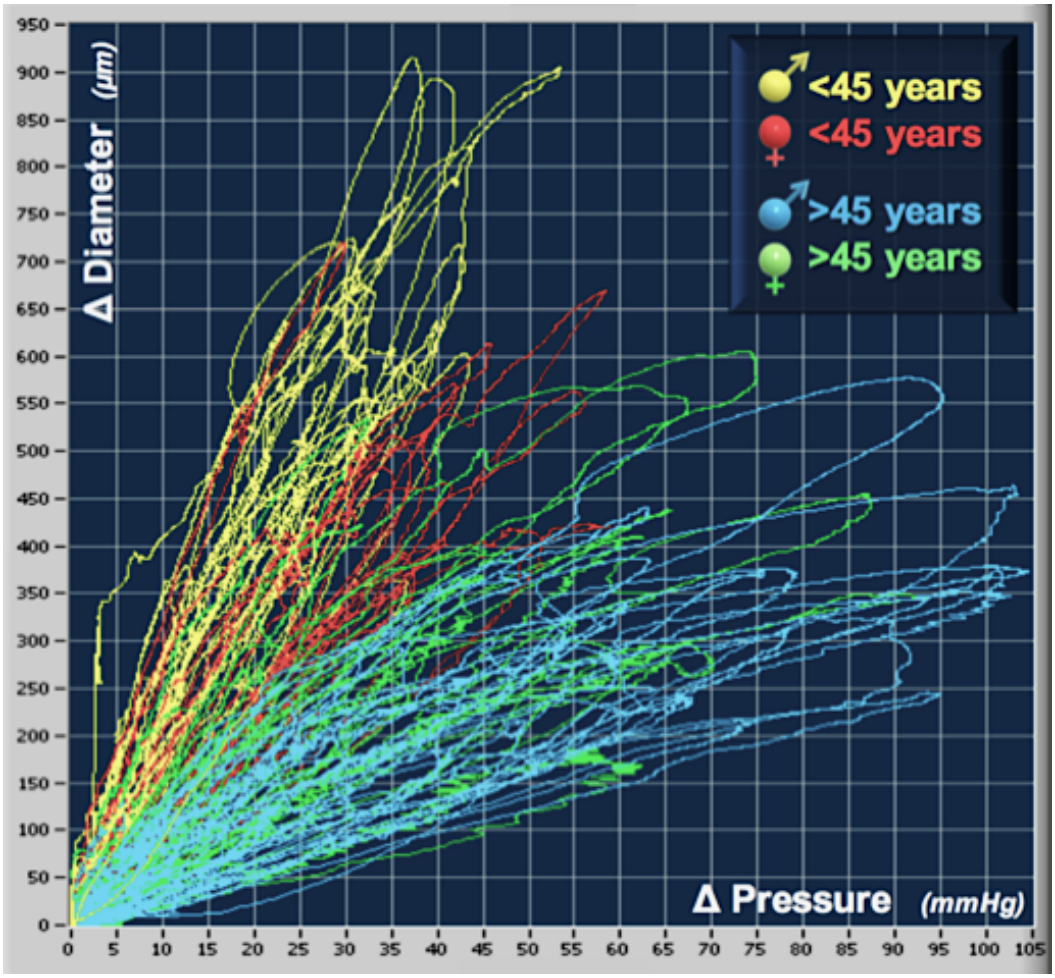


Figure 23. Diameter-pressure relationship in young and old subject [1].

ARTERIAL STIFFNESS IN A RURAL POPULATION OF CHINA

Rationale of the study

Previous studies show that cf-PWV can predict cardiovascular events and mortality in both the general population and the patients with various diseases, such as hypertension, diabetes mellitus and end-stage renal disease. Current hypertension guidelines recommend the use of cf-PWV for the assessment of arterial stiffness and cardiovascular risk. It is of note that brachial-ankle PWV (ba-PWV) has been criticized due to the involvement of muscular arteries and the estimation of wave path from body height instead of real measurement. But ba-PWV is still extensively being used in China for some reasons. For example, exposure of inguinal region could be ethically unacceptable for a majority of patients. Therefore, the primary aim of this study is 1) to measure the index of arterial stiffness, especially cf-PWV, in a rural population of China; 2) to assess the correlation between different measures of arterial stiffness.

Study cohort

The study was conducted in a rural population of China with patients aged over 35 years old. Patients were recruited in 22 villages and towns in Lianyungang, Jiangsu Province (Figure 24). Written informed consents were obtained from all subjects.

Figure 24. Chinese province of Lianyungang

Methods

The cf-PWV was measured by means of PulsePen device (DiaTecne, Milan, Italy), a validated and easy-to-use arterial tonometer [78,79]. In the PulsePen system, waveforms of carotid artery and femoral artery were recorded sequentially within 3 minutes and cf-PWV was calculated from the transit time between the two feet of the waveforms from the two arterial sites, which were determined by their relation to the R-wave of the ECG. The time between the ECG and the pulse of carotid artery was subtracted from the time between ECG and the pulse of femoral artery to obtain the pulse transit time (Δt). The foot of the pressure waveform was used as a reference point. The distance (d) covered by the waves was estimated to be 80% of the direct carotid-femoral distance. The cf-PWV was calculated as PWV ＝d (meters)/ Δt (seconds).

Brachial-ankle PWV was measured by the Vascular Profiler-1000 device (Omron, Kyoto, Japan), with an oscillometric cuff technique. After the subjects had rested in the supine position for 10 min, the cuffs were placed on both arms and ankles. The device automatically measured the pulse waves of the brachial and ankle arteries, and estimated the path length of the pulse wave according to the body height of the subjects. The

ba-PWV was then calculated as the ratio of the path length to the time interval between the ankle and brachial pulse waveforms for both sites.

Statistical Analysis

The categorical variables were described as number (percent) and the continuous variables as mean ± standard deviation. Means and proportions were compared with the Student t test and the χ 2 test, respectively. The interrelationship was analyzed by correlation analysis with Pearson's method and by the Bland-Altman approach after normalization of the two PWV measurements. Single and multiple regression analyses were performed to study the associations of the cf-PWV with cardiovascular risk factors.

Results

From 9 July 2016 to 24 August 2016, totally 19,784 participants were enrolled in this study and 36.9% of them were male. The mean age was 61.1± 9.5 years. The characteristics of the participants who completed the study are reported in Table 6.

Table 6. Characteristics of all participants at the study, subdivided in quartiles of carotid-femoral PWV.

Parameters	**Pooled**	**Q1**	**Q2**	**Q3**	**Q4**	**P**
Cf-PWV, m/s		<7.91	7.91-8.98	8.99-10.47	>10.47	
N	19,784	4,934	4,927	4,971	4,952	
Age, years	61.1 ± 9.5	55.9 ± 8.7	59.1 ± 8.8	62.3 ± 8.5	67.0 ± 8.1	<0.001
Female, N	12479	3222	3128	3078	3051	<0.001
(%)	(63.1%)	(65.3%)	(63.5%)	(61.9%)	(61.6%)	

Parameters	**Pooled**	**Q1**	**Q2**	**Q3**	**Q4**	**P**
Cf-PWV, m/s		<7.91	7.91-8.98	8.99-10.47	>10.47	
BMI, kg/m^2	26.0 ± 3.8	26.1 ± 3.7	26.1 ± 3.7	26.0 ± 3.9	25.7 ± 3.9	<0.001
Homocysteine, μmol/l	13.9 ± 6.6	13.2 ± 6.2	13.4 ± 6.3	13.9 ± 6.3	15.0 ± 7.2	<0.001
Glucose, mmol/l	5.8 ± 1.8	5.4 ± 1.4	5.6 ± 1.5	5.8 ± 1.9	6.2 ± 2.3	<0.001
Total cholesterol, mmol/l	4.8 ± 1.2	4.7 ± 1.3	4.8 ± 1.2	4.8 ± 1.2	5.0 ± 1.3	<0.001
Triglyceride, mmol/l	1.9 ± 1.6	1.8 ± 1.4	1.9 ± 1.5	2.0 ± 1.8	2.0 ± 1.7	<0.001
Urine protein negative, N (%)	14539 (91.7%)	3743 (92.8%)	3662 (91.7%)	3667 (91.9%)	3467 (90.3%)	0.008
Smoking, N (%)	2557(14.2%)	573(12.6%)	630(14.0%)	657(14.4%)	697(15.7%)	<0.001
Hypertension, N (%)	17661 (95.9%)	4201 (91.8%)	4381 (95.2%)	4560 (97.9%)	4519 (98.8%)	<0.001
Diabetes, N (%)	1541 (8.5%)	231 (5.1%)	294 (6.5%)	409 (9.0%)	607(13.7%)	<0.001
Stroke, N (%)	2860 (15.9%)	568 (12.5%)	611 (13.5%)	787 (17.3%)	894(20.2%)	<0.001
Coronary heart dis., N (%)	1299 (7.2%)	265 (5.8%)	312 (6.9%)	321 (7.0%)	401 (9.1%)	<0.001
Chronic kidney dis., N (%)	246 (1.4%)	51 (1.1%)	67 (1.5%)	64 (1.4%)	64 (1.4%)	0.437
SBP, mmHg	143.5 ± 13.7	136.2 ± 13.6	141.9 ± 12.6	145.9 ± 12.0	150.0 ± 12.6	<0.001
DBP, mmHg	91.9 ± 12.2	88.3 ± 11.0	91.3 ± 11.5	93.1 ± 12.1	94.8 ± 13.2	<0.001
Heart rate, bpm	72.6 ± 11.6	65.6 ± 9.6	67.5 ± 9.9	69.1 ± 10.5	72.6 ± 11.6	<0.001
Ba-PWV, m/s	17.1 ± 3.6	14.4 ±2.1	15.9 ± 2.2	17.6 ± 2.8	20.5 ± 4.0	<0.001
Cf-PWV, cm/s	9.4 ± 2.2	7.2 ±0.6	8.4 ± 0.3	9.7 ± 0.4	12.4 ± 1.9	<0.001

The cf-PWV was 9.4± 2.2 m/s for all the subjects. The quartiles of cf-PWV were 7.91, 8.98and 10.48(m/s). Form table 6 we also can find that participants with higher cf-PWV were more likely to be older and with higher blood pressure, homocysteine, fasting glucose, total cholesterol, and triglyceride levels. Single factor analysis showed that cf-PWV was associated with age (p<0.001, 95%CI: (0.1, 0.1)) , gender (p=0.001, 95%CI: (-0.2, 0.0)), blood pressure level (MAP(p<0.001, 95%CI: (0.1, 0.1)), SBP (p<0.001, 95%CI: (0.1, 0.1)), DBP (p<0.001, 95%CI: (0.0, 0.0))), BMI (p<0.001, 95%CI: (0.0, 0.0)), homocysteine (p<0.001, 95%CI: (0.0, 0.0)), fasting glucose (p<0.001, 95%CI: (0.2, 0.2)), total cholesterol (p<0.001, 95%CI: (0.1, 0.2)), triglyceride levels (p<0.001, 95%CI: (0.0, 0.1)). The value of cf-PWV were also associated with hypertension (p<0.001, 95%CI: (1.2, 1.5)), diabetes mellitus (p<0.001, 95%CI: (0.9, 1.1)), history of stroke (p<0.001, 95%CI: (0.4, 0.6)), history of coronary heart disease (p<0.001, 95%CI: (0.2, 0.5)), taking anti-hypertensive medicine (p<0.001, 95%CI: (0.3, 0.4)), taking hypoglycemic agents (p<0.001, 95%CI: (0.6, 1.0)), and smoking (p<0.001, 95%CI: (0.1, 0.3)). After adjusted for baseline characteristics: age, gender, BMI, SBP, homocysteine, fasting glucose, total cholesterol, triglyceride, smoke status, history of stroke, history of coronary heart disease, chronic kidney disease, cancer and the medication of anti-hypertension, hypoglycemic and lipid-lowering, the multiple regression analysis showed that cf-PWV still correlated with age (p<0.001, 95%CI: (0.1, 0.1)), history of stroke(p=0.003, 95%CI: (0.0, 0.2)), history of cancer(p=0.028, 95%CI: (0.0, 0.6)), homocysteine (p<0.001, 95%CI: (0.0, 0.0)), fasting glucose (p<0.001, 95%CI: (0.1, 0.1)), total cholesterol (p=0.002, 95%CI: (0.0, 0.1)), triglyceride levels (p<0.001, 95%CI: (0.0, 0.1)), SBP (p<0.001, 95%CI: (0.0, 0.1)), taking anti-hypertensive medicine (p=0.012, 95%CI: (0.0, 0.1)), and taking hypoglycemic

agents (p=0.001, 95%CI: (0.1, 0.5)).

Figures 25 to 37 show the cf-PWV values related to age and to blood pressure categories. All the 19,784 participants were included. The correlation of PWV with age is less strong than the European population and confirms a better quadratic correlation rather than linear. The correlation of PWV with MAP tends to decrease with increasing age and it is weaker in patients with systolic blood pressure >140 mmHg.

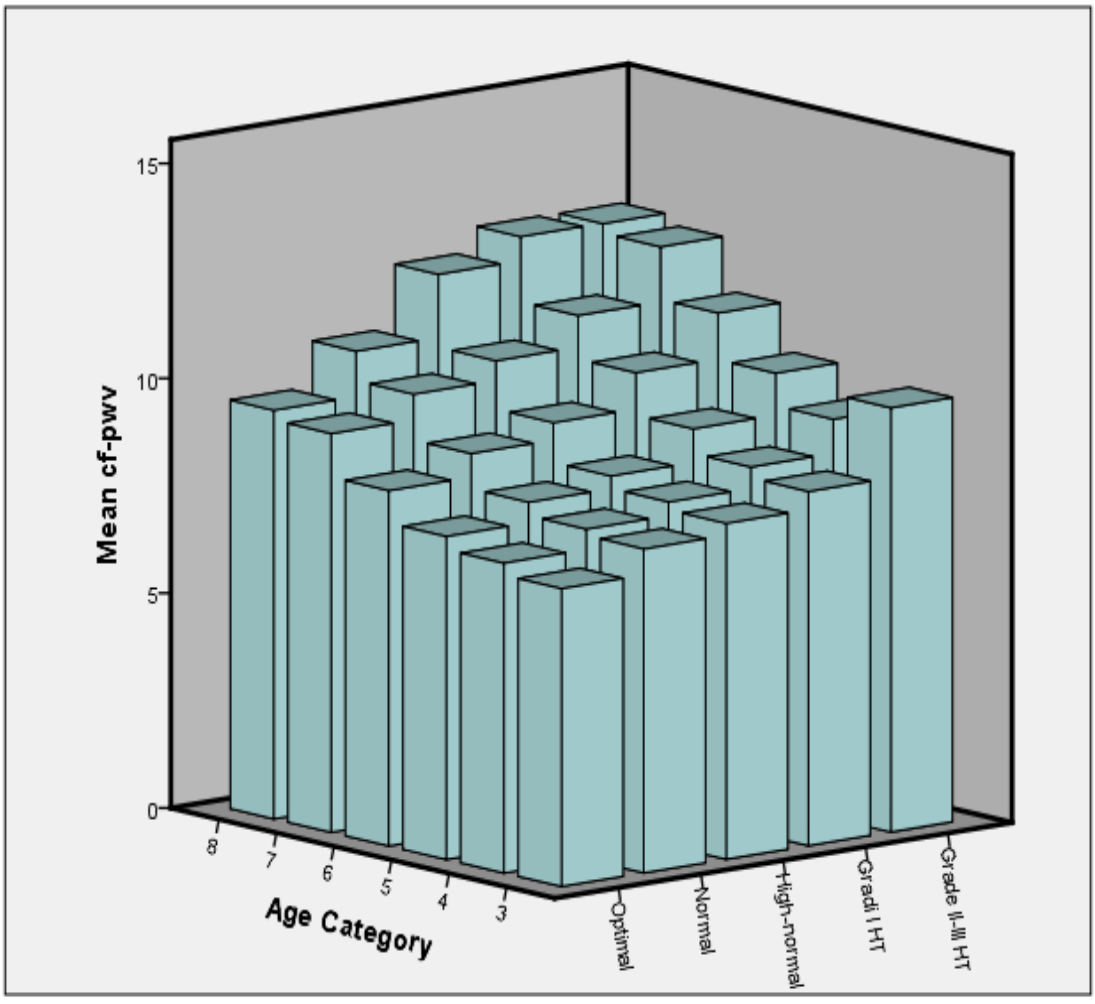


Figure 25. Carotid-femoral pulse wave velocity (cf-PWV) values related to age (3=30-40 years old, 4=40-50 years, and so on) and to blood pressure categories. All the 19,784 participants were included.

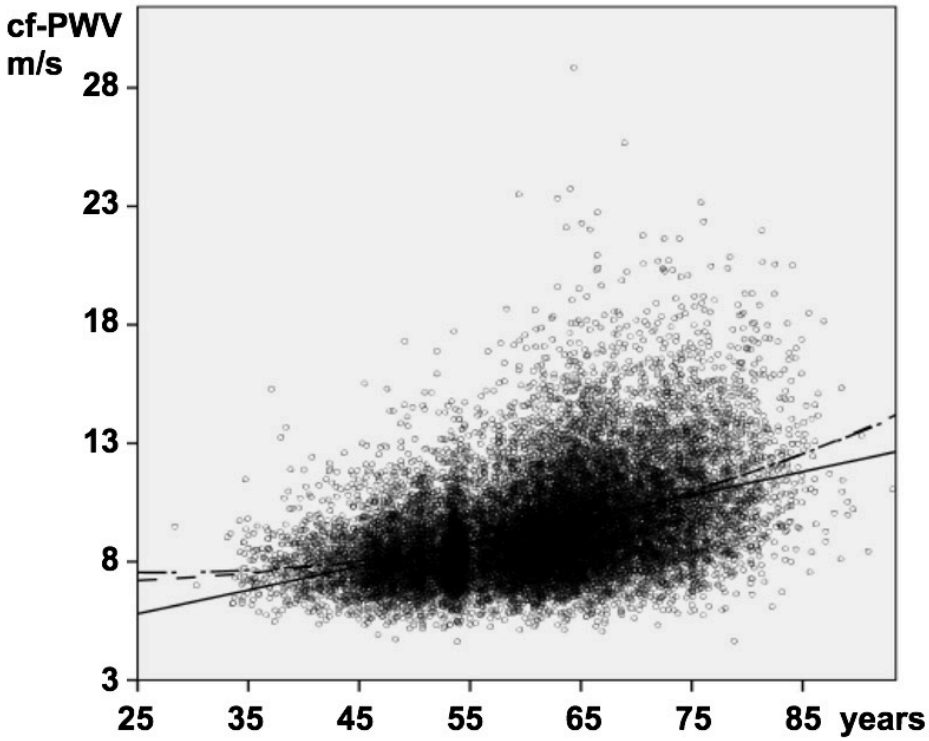


Figure 26. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age. All the 19,784 participants were included. Linear (solid line), quadratic (dash dot line) and cubic (dashed line) relationship are shown.

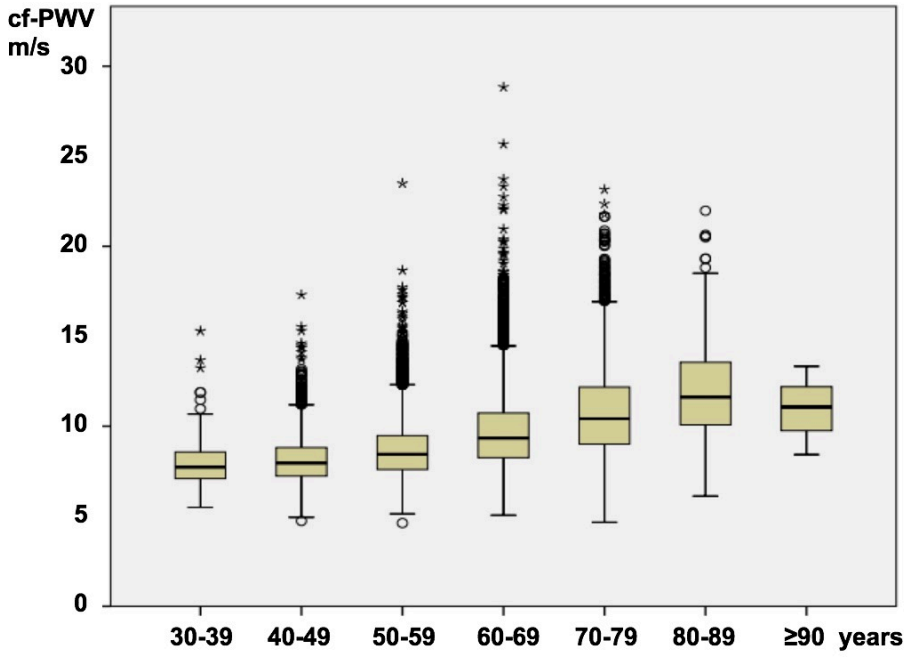


Figure 27. Carotid-femoral pulse wave velocity (cf-PWV) values at the different age categories. All the 19,784 participants were included.

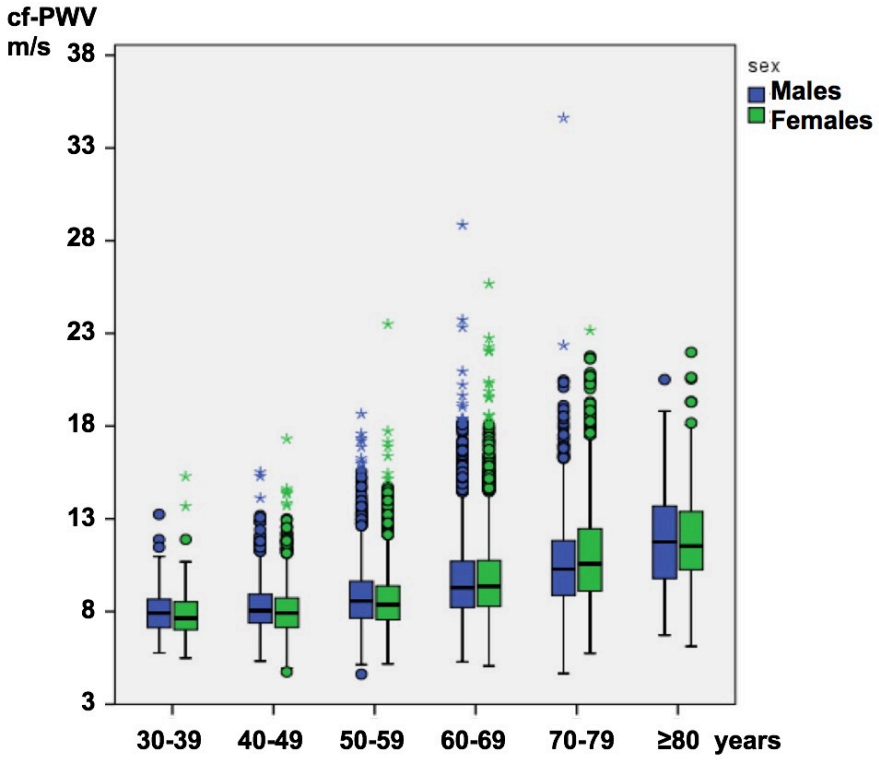


Figure 28. Carotid-femoral pulse wave velocity (cf-PWV) values at the different age categories, subdivided for gender. All the 19,784 participants were included.

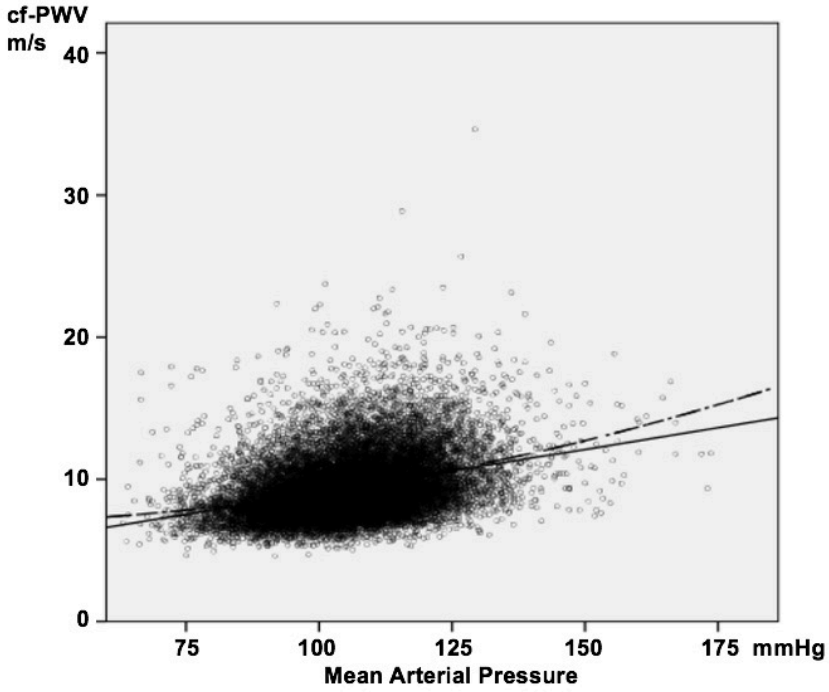


Figure 29. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and mean arterial pressure values. All the 19,784 participants were included. Linear (solid line) and quadratic (dash dot line) relationship are shown.

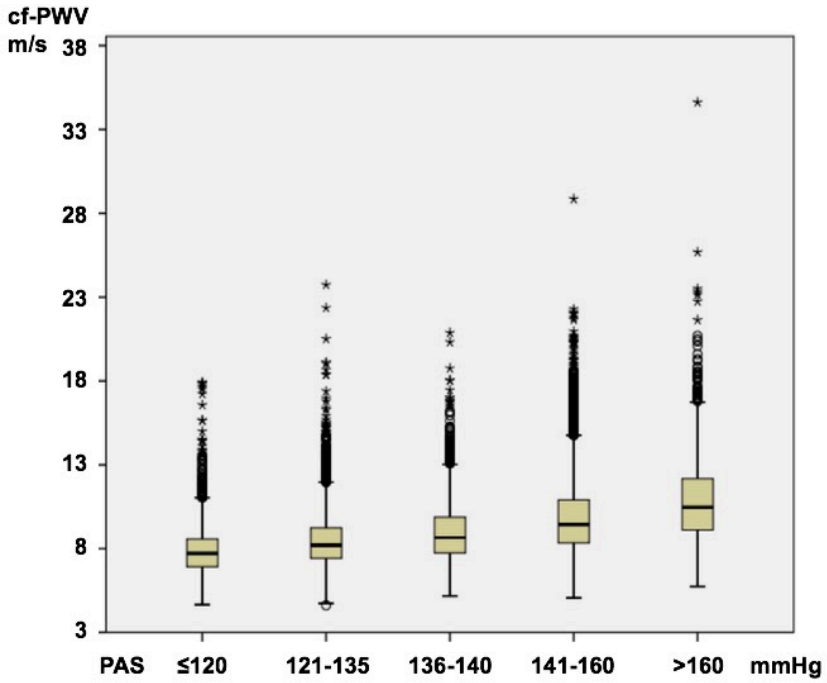


Figure 30. Carotid-femoral pulse wave velocity (cf-PWV) values at the different blood pressure categories (related to systolic blood pressure values). All the 19,784 participants were included.

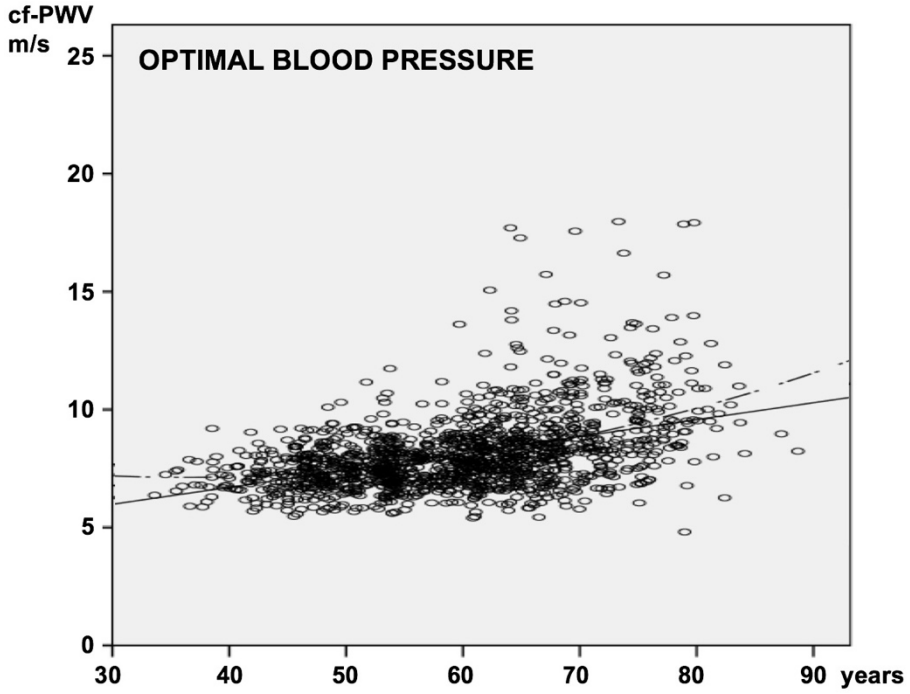


Figure 31. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age in subjects with optimal blood pressure values. Linear (solid line) and quadratic (dash dot line) relationship are shown.

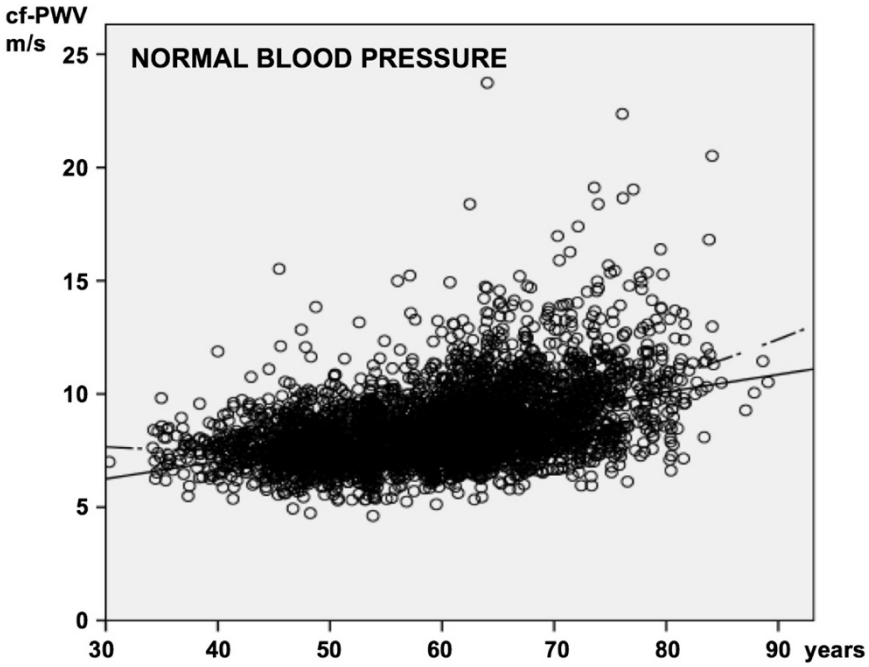


Figure 32. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age in subjects with normal blood pressure values. Linear (solid line) and quadratic (dash dot line) relationship are shown.

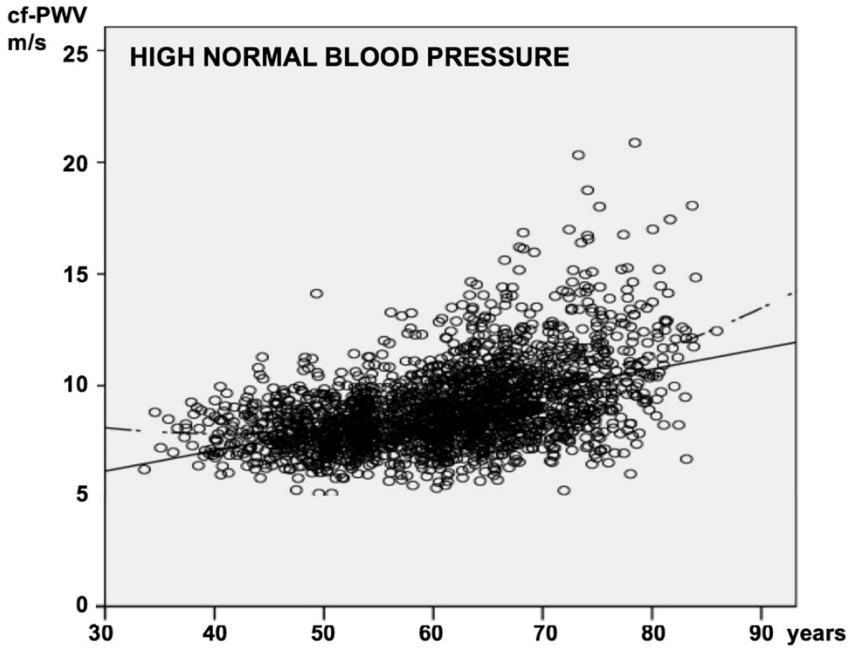


Figure 33. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age in subjects with high-normal blood pressure values. Linear (solid line) and quadratic (dash dot line) relationship are shown.

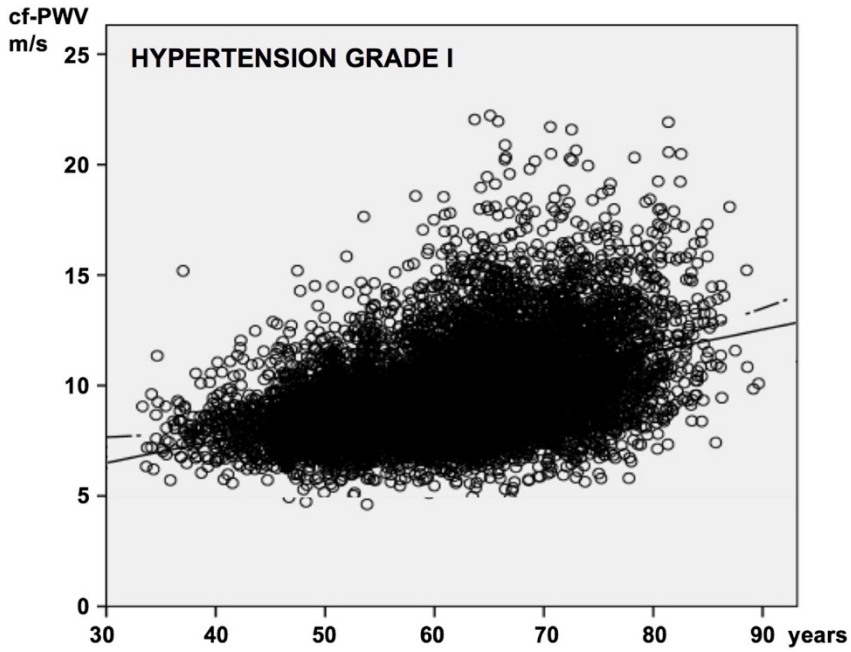


Figure 34. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age in subjects with "grade I" hypertension. Linear (solid line) and quadratic (dash dot line) relationship are shown.

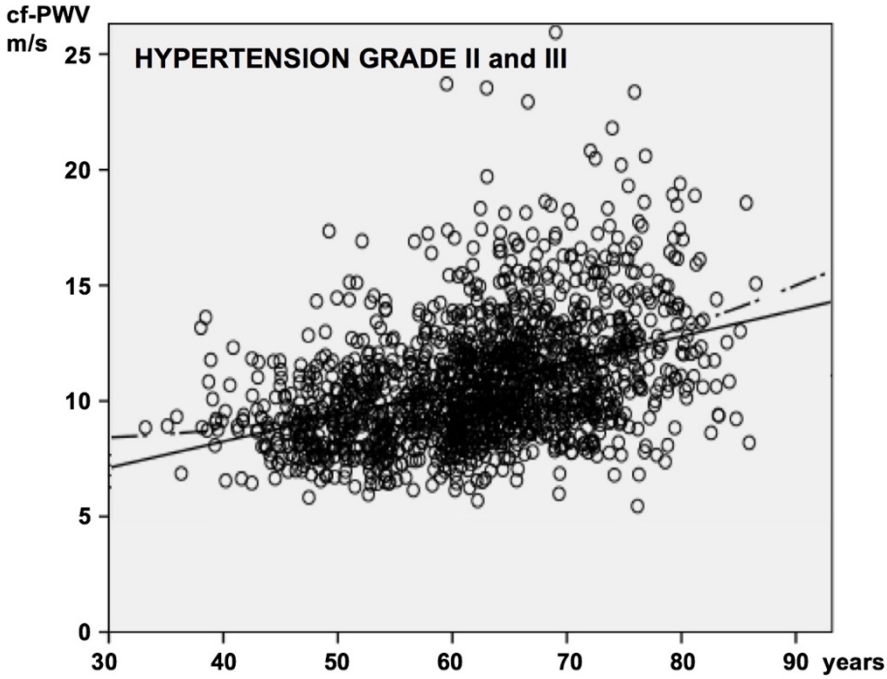


Figure 35. Relationship between carotid-femoral pulse wave velocity (cf-PWV) and age in subjects with "grade II and grade III" hypertension. Linear (solid line) and quadratic (dash dot line) relationship are shown.

Brachial-ankle PWV (ba-PWV) was also measured on 18,185 participants. The ba-PWV and cf-PWV were weakly correlated (R^2=0.421, p = 0.01) (Figure 36).

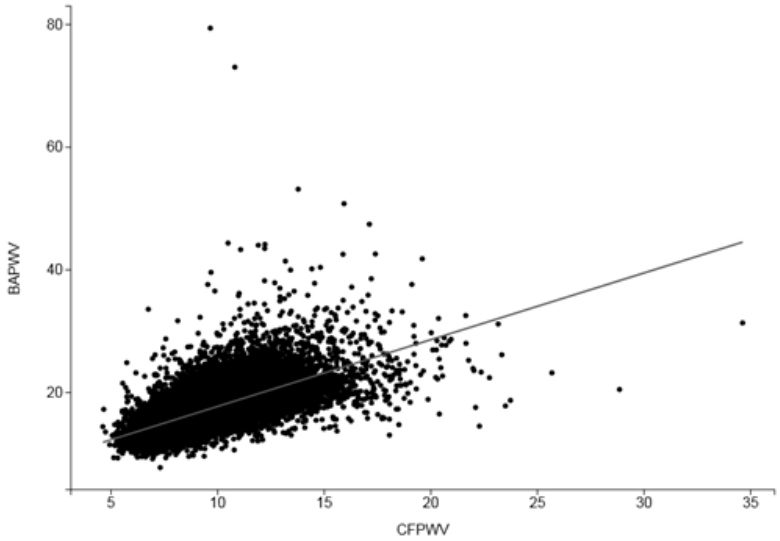


Figure 36. Correlation between cf-PWV and ba-PWV.

The augmentation index (AIx) could be measured with carotid tonometry during the measurements of cf-PWV. The instrument used to assess ba-PWV provides also an indirect evaluation of AIx. In this study the AIx measured from two ways were only weakly related (R^2=0.099, p = 0.01) (Figure 37).

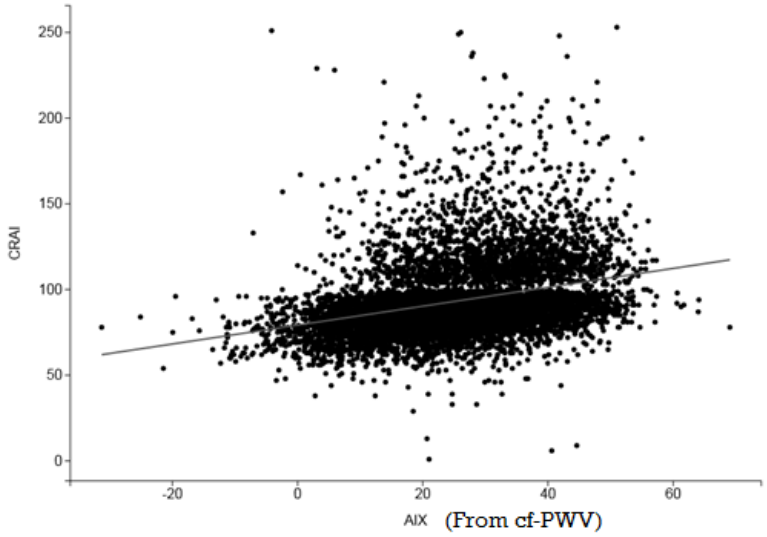


Figure 37. Correlation between CRAI (AIx measured by ba-PWV) and AIx (AIx measured with carotid tonometry).

This study also explored the relationship between homocysteine and cf-PWV. Totally 16,649 participants have been tested the level of homocysteine (Table 7).

Table 7. Characteristics of the patients who tested homocysteine.

Homocysteine, μmol/L	Pooled	<10	10 - 15	>15	P-value
Parameters					
N	16649	3254	8377	5018	
Age, years	61.1 ± 9.5	58.2 ± 9.4	60.9 ± 9.1	63.6 ± 9.7	<0.001
Female, N (%)	12479 (63.1%)	2521 (77.5%)	5665 (67.6%)	2350 (46.8%)	<0.001
BMI, kg/m^2	26.0 ± 3.8	26.3 ± 4.1	26.0 ± 3.7	25.7 ± 3.7	<0.001
SBP, mmHg	143.5 ± 13.7	143.1 ± 14.0	143.6 ± 13.9	143.3 ± 13.6	0.233
DBP, mmHg	91.9 ± 12.2	91.5 ± 12.0	91.7 ± 12.2	92.1 ± 12.5	0.123
Heart rate, bpm	76.2 ± 11.5	76.7 ± 11.3	76.0 ± 11.3	76.3 ± 11.8	0.005
Homocysteine, μmol/l	13.9 ± 6.6	7.7 ± 2.3	12.4 ± 1.4	20.5 ± 8.0	<0.001
Fasting glucose, mmol/l	5.8 ± 1.8	5.8 ± 2.1	5.8 ± 1.8	5.6 ± 1.7	<0.001
Total cholesterol, mmol/l	4.8 ± 1.2	4.8 ± 1.3	4.8 ± 1.2	4.8 ± 1.3	<0.001
Triglyceride, mmol/l	1.9 ± 1.6	2.0 ± 1.7	1.9 ± 1.4	2.0 ± 1.8	0.214
Urine protein negative, N (%)	15220 (93.4%)	2459 (94.5%)	6614 (94.2%)	3905 (91.3%)	<0.001
Smoking, N (%)	2156 (14.2)	273 (9.0%)	958 (12.54)	925 (20.3)	<0.001
Hypertension, N (%)	14836 (95.7%)	2897 (95.1%)	7488 (96.1%)	4551 (97.7%)	0.057
Diabetes, N (%)	1278 (8.4%)	290 (9.7%)	655 (8.6%)	333 (7.3%)	<0.001
Stroke, N (%)	2861 (15.9%)	399 (13.3%)	1143 (15.0%)	873 (19.2%)	<0.001
Coronary heart dis., N (%)	1299 (7.2%)	198 (6.6%)	563 (7.4%)	329 (7.2%)	0.368
Chronic kidney dis., N (%)	246 (1.4%)	42 (1.4%)	104 (1.4%)	80 (1.8%)	0.193

Adjusted age, gender and blood pressure levels, a spline curve can show the relationship between homocysteine and cf-PWV (Figure 38).

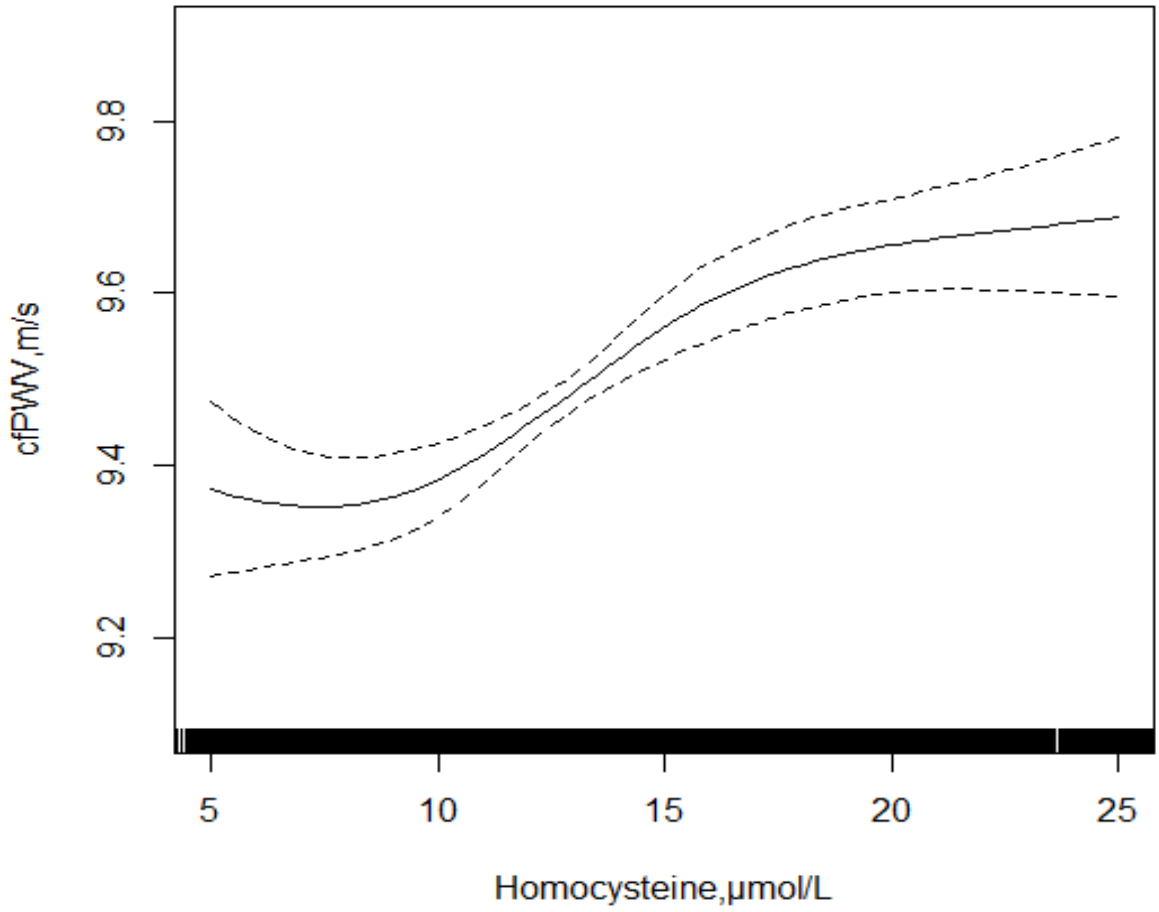


Figure 38. The spline curve between homocysteine and carotid-femoral PWV.

The Logistic Regression Analysis showed that the homocysteine was independently related with the cf-PWV (Table 3).

Table 8. The logistic regression analysis of cf-PWV and homocysteine.

		Unadjusted		Adjusted	
Homocysteine	N (%)	OR (95%CI)	P	OR (95%CI)	P
Continuous	5296	1.03 (1.03, 1.04)	<0.001	1.02 (1.01, 1.03)	<0.001
Logarithm transformation		3.48 (2.89, 4.18)	<0.001	1.94 (1.57, 2.41)	<0.001
Categories					
<10	755 (23.2)	1.0		1.0	
10 - 15	2541 (30.3)	1.44 (1.31, 1.58)	<0.001	1.16 (1.04, 1.30)	0.007
>15	2000 (39.9)	2.19 (1.99, 2.42)	<0.001	1.49 (1.33, 1.68)	<0.001
P for trend			<0.001		<0.001
Quartiles					
Q1 (<10.2)	839 (23.6)	1.0		1.0	
Q2 (10.2-12.4)	1121 (27.9)	1.26 (1.13, 1.39)	<0.001	1.06 (0.94, 1.20)	0.316
Q3 (12.4-15.2)	1445 (33.4)	1.63 (1.47, 1.80)	<0.001	1.20 (1.07, 1.35)	0.003
Q4 (>15.2)	1891 (39.9)	2.15 (1.96, 2.37)	<0.001	1.46 (1.30, 1.65)	<0.001
P-for trend			<0.001		<0.001

Adjust for: Age, Sex, SBP, DBP, BMI, FPG, TC, TG, Smoke

EFFECT OF INTENSIVE SYSTOLIC BLOOD PRESSURE CONTROL ON THE PROGRESSION OF CAROTID-FEMORAL PULSE WAVE VELOCITY AMONG HYPERTENSIVE PATIENTS IN CHINA

105 hypertensive patients aged over 60 years old without cardiovascular or cerebrovascular diseases were enrolled and randomized into three groups with different systolic blood-pressure goals of 140 ~ < 150 mmHg (Group A), 130 ~ < 140 mmHg (Group B) and less than 130 mmHg (Group C). cf-PWV was measured in all 105 patients at the entry of the study (Table 9).

Table 9 Baseline characteristics

Variables	All Populations	Group A	Group B	Group C	P
N	92	31	31	30	
Age,mean(SD),y	68.2±5.4	67.2±4.6	68.4±5.9	68.9±5.8	0.465
Male,No. (%)	25 (29.2%)	8 (25.8)	9 (29.0)	8 (26.7)	0.957
Body mass ndex,(mean± SD)	26.5±3.4	25.7±3.3	26.9±3.7	27.0±3.2	0.254
Fasting glucose,mg/dl	6.5±1.6	6.6±1.6	6.5±2.0	6.3±1.2	0.414
Triglycerides,mg/dl	1.8±1.5	2.1±2.3	1.5±1.0	1.7±0.8	0.305
HDL-C,mg/dl	1.5±0.3	1.5±0.3	1.5±0.3	1.5±0.3	0.984
LDL-C,mg/dl	4.0±0.9	4.0±1.0	4.0±0.9	4.1±0.9	0.692
Homocysteine,umol/ml	10.2±2.7	10.7±3.3	9.8±2.0	10.1±2.6	0.398
Smoking, No. (%)					0.737
Never	76 (82.6)	25 (80.6)	25 (80.6)	26(86.7)	
Former	8 (8.7)	4(12.9)	2 (6.5)	2 (6.7)	
Current	8 (8.7)	2 (6.5)	4 (12.9)	2 (6.7)	
Alcohol drinking, No. (%)					0.652
Never	80 (87.0)	28 (90.3)	26(83.9)	26 (86.7)	
Former	2 (2.2)	1 (3.2)	0(0.0)	1 (3.3)	
Current	10 (10.9)	2 (6.5)	5 (16.1)	3(10.0)	
Diabetes, No. (%)	16 (17.4)	9 (29.0)	3 (9.7)	4 (13.3)	0.103

Another two cf-PWV measurements were successful obtained in 92 patients in the 2nd and the 6th month after the antihypertensive treatment started. At the 6th month post-treatment,

the mean systolic blood pressure was 136.5 mmHg in Group A, 130.0 mmHg in Group B and 124.7 mmHg in Group C (Table 10).

Table 10 Change of blood pressure

Variables	Pooled	Group A	Group B	Group C	P
N	**92**	**31**	**31**	**30**	
SBP in 2-month, mmHg	**138.0±12.9**	**142.5±14.0**	**136.9±10. 4**	**134.6±13.1**	**0.049**
DBP in 2-month, mmHg	**80.5±7.3**	**83.6±7.3**	**79.3±6.0**	**78.7±7.7**	**0.013**
SBP in 6-month, mmHg	**130.5±14.9**	**136.5±14.1**	**130.0±14.7**	**124.7±13.9**	**0.007**
DBP in 6-month, mmHg	**74.2±8.7**	**77.4±7.7**	**74.1±9.8**	**71.0±7.6**	**0.016**

The change rate of cf-PWV [(cf- PWV in 6-month—cf- PWV in 2-month)/CF PWV in 2-month] was 0.08±0.20 in Group C, and -0.01±0.12 and 0.01±0.14 in Group A and Group B, respectively. The multivariate analysis showed that, compared to the other two groups, participants in Group C had a significantly greater change rate of cf-PWV (p=0.003).

DISCUSSION

This study provides a large amount of data collected in mostly untreated hypertensive patients from 22 villages in the Jiangsu Province, a rural area in China, where this population study was conducted. Aim of the study was to collect data about cardiovascular risk factors, blood pressure and arterial stiffness from a large population in a rural area. In this study, arterial stiffness parameters obtained from different devices (cf-PWV with PulsePen and ba-PWV with Vascular Profiler-1000) were evaluated.

The most relevant findings of this study regard the trends in arterial stiffness along the age and blood pressure spectrum, measured by the gold standard measure of cf-PWV. Cf-PWV appears a suitable tool to identify cardiovascular risk in this mostly untreated hypertensive population, as a confirm of previous findings, conducted in the general and hypertensive population [64,80]. Mean values of arterial stiffness are presented in our population by age decades and blood pressure categories. As expected, the main factors affecting aortic stiffness are age and mean BP. The age-related increase in cf-PWV is not influenced by gender. Interestingly, the best fit for the relationship between cf-PWV and age is not simply linear, but quadratic. This confirms the previous findings in a European population [80], but a lower coefficient of determination (R^2) between cf-PWV and age is present in our population when compared to the European one (R^2=0.19 in Chinese population, R^2 >0.3 in the European population). This is probably due to the cardiovascular burden of the Chinese population, which was mainly a population of hypertensive patients, while the European study was conducted in the general population. Therefore, the main factors affecting arterial stiffness, in the Chinese population, were not only age, but also blood pressure levels and cardiovascular risk factors. To prove this hypothesis, we separately analyzed the subjects which had a good control of BP levels, in which the R^2 with age was stronger

than the subjects with uncontrolled blood pressure levels. In the latter, a lower R^2 of the relation between age and cf-PWV prove that different factors, and mainly blood pressure levels, are the most influencing determinants of arterial stiffness.

Conversely, the relation between cf-PWV and MAP in our population was stronger than that reported in previous studies [80]. This relation is linear when analyzed in the whole population, but major differences exist between different age categories. The strongest relation was in the age range from 30 to 39 years (R^2=0.35), while in the subsequent years the R^2 becomes lower. Therefore, the relation between blood pressure and cf-PWV becomes weaker in the older age, in the hypertensive population. This is an important result that was not sufficiently underlined in previous studies. Although we do not have data about the change of cf-PWV with MAP for each single patient, we can affirm that a possible explanation of this difference in the relation between MAP, cf-PWV and age resides in the vascular pathophysiology of vascular ageing. Data from experimental and modeling studies concluded that the relationship of BP and arterial stiffness is different between younger and older arterial vascular system and is determined by different viscoelastic properties. In younger age, a strong direct relation exists between cf-PWV and MAP, while in older age, arterial stiffness becomes less BP-dependent [81]. Our results could be considered as a confirmation of this experimental hypothesis in a population level. A clinical implication for this result may be that it is more difficult to reduce arterial stiffness, measured as cf-PWV, in older individuals than in younger ones by reducing blood pressure levels. Nevertheless, such conclusion should be confirmed in intervention studies.

To further investigate these issues, we analyzed data from the intervention study conducted in a subgroup of 105 hypertensive patients older than 60 years. These patients were enrolled and randomized into three groups with different systolic blood

pressure goals. Although results of this study are only preliminary, at 6-month follow-up analysis of data, a significant result was achieved regarding the reduction of cf-PWV. The group with a more intensive treatment for blood pressure reduction (SBP < 130 mmHg) achieved a significantly greater reduction rate of cf-PWV. This is an important evidence considering the recent controversies about blood pressure targets, as in the SPRINT trial [82]. The SPRINT trial confirmed that lower blood pressure targets translate into a clinical benefit, i.e. a reduction of cardiovascular events, in high risk patients. Although many uncertainties exist regarding the blood pressure measurement in the SPRINT trial (because the automated office blood pressure measurement was used as a reference in this study), our preliminary results are going in the same direction. A larger reduction in blood pressure levels in the group of the more intensive SBP target (<130 mmHg) leads to a significant reduction of cf-PWV. In this reduction of arterial stiffness, a major role is played by the passive reduction of arterial stiffness given by the reduction of MAP, given the intrinsic blood pressure dependency of arterial stiffness. We cannot exclude that an additional benefit of BP reducing therapies is involved in the improvement of structural properties of large vessels, although it is not possible to specifically assess the specific contribution of antihypertensive drug therapy in the changes in arterial stiffness. Whether a reduction in cf-PWV in the high intensity treatment group of our trial would translate in a reduction of major cardiovascular end-points, it will be possible to conclude only after an adequate follow-up period.

Another important finding of our study regards the relationship between cf-PWV and ba-PWV. Unlike previous studies in this topic, that analyzed only lower number of subjects [83,84], our study found only a weak relation between cf-PWV and ba-PWV. The poor relation between cf-PWV and ba-PWV is surprising considered that these two measures are often both considered as appropriate markers of central aortic stiffness [85],

and both showed in previous studies a significant and independent predictive value for cardiovascular events. If we take cf-PWV, which is considered the gold standard measure of aortic stiffness [15], as the reference measure, we can say that probably ba-PWV, as measure of central arterial stiffness, is flawed by the inclusion of arterial segments different from the aorta. Brachial and lower extremities arteries are considered peripheral muscular arteries [86], and stiffness in these arteries may be the expression of peripheral rather than central arterial stiffness. Stiffness in peripheral arteries do not change significantly with age and may be affected by different risk factors than aortic stiffness [1]. Unlike previous studies, that analyzed healthy and younger subjects [85], our study focused in a hypertensive population that was significantly elder. Moreover, the Chinese rural population presents a high prevalence of smoking habit (which was nearly 30% in the studied population), a vascular risk factor affecting prevalently peripheral arteries and causing peculiarly arterial disease in femoral and tibial arteries, which are included in the evaluation of ba-PWV. The differences between cf-PWV and ba-PWV cause a weak correlation between the two variables and may therefore be explained by the older age and by the burden of cardiovascular risk factors in the considered population. If confirmed by further analyses, ba-PWV should not be considered an appropriate measure of aortic stiffness, in patients as those considered in our study.

The poor agreement between the augmentation index evaluated from the Vascular Profiler-1000 device (CRAI) and the carotid tonometry-derived augmentation index (Aix) is less surprising. The central waveform profile derived from the carotid artery could be considered as fully superimposable to the central (aortic) waveform, as demonstrated by invasive validation [78]. This is not the case of CRAI, which is elaborated from the signal of the brachial oscillometric arterial pressure, and is therefore a surrogate measure. A computer calculates the CRAI from the brachial signal with an undisclosed algorithm, but

this methodology, to our knowledge, has not undergone an independent validation procedure. CRAI cannot therefore be considered as an adequate surrogate of AIx, and further studies are warranted to introduce improvements in the evaluation of central augmentation index by the analysis of peripheral arterial waveform.

Another original contribution given by our study to the current knowledge is the relationship that we have explored between cf-PWV and homocysteine levels. An interesting finding is that cf-PWV significantly rose with increasing homocysteine levels. This relation was proven to be independent by the logistic regression analysis, that was adjusted for many possible confounding factors (Age, Sex, SBP, DBP, BMI, FPG, TC, TG, Smoke). As evidenced by the spline curve, it seems that cf-PWV significantly increase in the range of borderline homocysteine levels (10-15 μmol/l), and was steadily high in the pathological range (>15 μmol/l).

Abnormally high levels of homocysteine in the serum, above 15 μmol/L, are a medical condition called hyper-homocysteinemia, which has been claimed to be a significant risk factor for the development of a wide range of diseases, including thrombosis. It is also found to be associated with the risk of future cardiovascular disease [87]. A previous study explored this association, in a smaller cohort [88], but our study has the advantage to include a higher number of patients with a wide range of cardiovascular risk factors. The association between hyper-homocysteinemia and cf-PWV is confirmed, but the independent association observed in our study suggests that there are possible biological mechanisms accounting for the effect of plasma homocysteine at the vascular level. Homocysteine may induce endothelial dysfunction, which could be a precocious damage leading to arterial stiffness, and could stimulate vascular smooth muscle cell proliferation [89], linking homocysteine levels to arterial stiffening. Nevertheless, this finding should be confirmed in different populations and

possibly considered in future experimental settings.

Conclusions

In conclusion, by the analyses of arterial stiffness variables in a wide cohort of patients from a Chinese rural area, our study found several important results:

- Age and mean BP are the main factors affecting arterial stiffness levels, evaluated as cf-PWV.
- The relationship between cf-PWV and age is weaker in this population than in other population studies conducted in the Europe and in the US. Conversely, the relation of cf-PWV with mean BP levels is stronger, in every age range.
- In an intervention study in a subpopulation, a tighter blood pressure control was associated with a greater reduction in cf-PWV.
- A poor agreement was found between reference methodologies of pulse wave velocity and augmentation index evaluation (cf-PWV, Aix) and surrogate measures (ba-PWV, CRAI), which include peripheral arterial segments.
- Homocysteine levels were significantly and independently associated with cf-PWV in this population.

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