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DOTTORATO IN IPERTENSIONE E PREVENZIONE DEL RISCHIO CARDIOVASCOLARE

PhD Thesis

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PULSE WAVE FORM ANALYSIS: COMPARATION OF DATA OBTAINED WITH TWO DIFFERENT METHODS.

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Introduction

Interest in the role that arterial stiffening plays in the pathogenesis of cardiovascular disease (CVD) has increased dramatically in the past decade, in large part because of studies that have used pulse pressure as a measure of stiffness. In general terms, if the wall of the aorta stiffens, pulse pressure increases, although there are exceptions to this generality that will be discussed later in this review.

Thus, pulse pressure is a widely accessible, if imperfect, indicator of arterial stiffness. Numerous studies performed over the past decade have shown that higher pulse pressures are associated with a moderate increase in the risk for major CVD events, such as myocardial infarction, heart failure, arrhythmia and stroke. (1),(2),(3),(4),(5),(6). In addition, excessive pressure pulsatility is associated with evidence of microvascular damage and dysfunction,(7) which may explain associations between increased pulse pressure and a number of conditions common in older people that are thought to involve a microvascular insult, such as cognitive impairment, macular disease and chronic kidney disease.

The potential importance of the disease burden attributable to increased arterial stiffness is underscored by the change in pulse pressure with advancing age. Pulse pressure increases rapidly after 50 years of age at a time when incidence and prevalence of hypertension and CVD also increase markedly.

Survey (NHANES) has shown that increased systolic pressure is nearly universal in hypertensives after 50 years of age. In this age range, more than 80% of cases have isolated systolic hypertension (ISH), which represents an isolated or predominant abnormality in pulse pressure (7). Data from the Framingham study has shown that contrary to prior beliefs, ISH arises de novo on a background of normal or high normal blood pressure and is not the terminal phase of longstanding essential hypertension. (8). On a population basis, pulse pressure increases by 10 mmHg per decade starting from about 50 mmHg at 50 years of age. Thus, average, but not optimal, pulse pressure in Western societies is roughly equal to age in middle-aged and older people. This level of pulse pressure is not optimal because even after adjusting for age and other potential confounders, each 10 mmHg increase in pulse pressure is associated with a 10-40% increase in risk for various major clinical events. Thus, a pulse pressure greater than 50 mmHg is reason for concern at all ages and should not be ignored in older people just because of the known increase in pulse pressure with advancing age.

In this way central aortic blood pressure has recently received significant interest as a more accurate predictor of outcome than brachial artery blood pressure (9). Peripheral blood pressure is higher than central blood pressure due to pressure amplification from the aorta to the brachial artery (10),(11).

However there is considerable variation in central blood pressures even between individuals with similar brachial blood pressure (12). Moreover, pharmacological intervention may produce a different decrease in central pressure as compared to peripheral pressure, (13),(14),(15). Although central blood pressure cannot be reliably inferred from a cuff measurement of brachial systolic and diastolic blood pressure, a number of techniques exist to assess the aortic pressure. Aortic pressure can be measured directly by cardiac catheterisation. However, this method is invasive and, therefore, not appropriate for large scale trials. A commercial device (SphygmoCor) records the radial pulse waveform using applanation tonometry, and then applies a transfer function to obtain the central aortic pressure waveform and a number of other indices, including central systolic blood pressure (cSBP). This estimated cSBP correlates strongly with data from direct invasive measurement of central blood pressure (11),(16). Central systolic blood pressure can also be estimated from the late systolic shoulder of the radial pulse waveform (pSBP2), without the use of a transfer function. Close correlation between cSBP2 and cSBP has been demonstrated (13) ,(14), (17) with similar absolute values. (13),(17). A new device, the Omron HEM-9000AI utilizes this technique to estimate central systolic blood pressure.

1. History

From antiquity the arterial pulse was associated with life, and its absence with death. The pulse was considered to carry vital information on the state of health, and was a central part of the physical examination in Chinese, Greek and Arabian medicine. Untold numbers of paintings show the physician feeling the radial artery pulse – though rarely conducting any other sort of examination. The Roman physician Galen (AD? 130–? 200), had a strong influence on this field for over fifteen hundred years. He wrote many papers on the arterial pulse, which had huge influence in subsequent years – though not at the time of his life. It appears that he was alone, and not one of influence among contemporary colleagues. He had no pupils, acolytes or disciples. His characterisation of pulses was incomprehensible. But it survived unquestioned until the time of William Harvey.

Harvey

Modern scientific medicine began with William Harvey, whose great work was done in London while in practice at St. Bartholomew's Hospital, and as an anatomist to the recently formed Royal College of Physicians. The College still has as its crest an illustration of a physician's fingers palpating the arterial pulse. Harvey had been educated in the traditions of Galileo through studies in Padua.

His brilliant monograph *de Motu Cordis et Sanguinis in Animalibus* remains the most important application of the scientific method in all medical history. Such being the case, it is surprising how few modern physicians or cardiovascular researchers have read any translation of the original Latin text. Fewer still have read or even been aware of the criticism that Harvey faced, and the way that he defended his views – most notably in his open letters to Jean Riolan of Paris (18).

The view that Harvey proposed is now universally accepted, but it was highly controversial at the time.

Harvey's views were based on human anatomy, namely the size of the heart in systole and diastole, the function of veins, on comparative physiology and quantitative assessment. He proposed that oxygenated blood was propelled by left ventricular contraction into the arterial tree, then was "continuously, evenly and uninterruptedly driven by the beat of the arteries into every member and part", then through veins, back to the heart, then from the right heart through similar small blood vessels in the lungs back to the left atrium and ventricle. He challenged the existing notion that arteries contain air, and that they expanded and contracted themselves independently of the heart. He stressed that they acted passively to accept blood from the left ventricle, and that left ventricular systole (contraction) caused arterial diastole (expansion), and that arterial

systole (relaxation) occurred during cardiac diastole when the two systems were separated by the closed aortic valve.

Harvey was a tolerant man and knew he was asking for a huge leap for others to accept his arguments. He praised his predecessors and where possible pointed out where other persons' views led on to his own. He even quoted Galen (whose basic views were utterly at odds) in relation to an experiment where a reed was used to occlude an artery with resulting strong reflection upstream – and in unambiguous terms "*unde et fluxus inhibitor et impetus refringitor eo quod supra ligaturiam reverberatur*".

Harvey's tolerance was limited, and so it was in 1649, 21 years after publication of *de Motu Cordis* that he wrote his second open letter (18) to Jean Riolan, first urging others against extremes in uncritical acceptance or malicious dismissal of any new technique, then attacking the latter in words that would now be considered improper, even defamatory:

> "There are, moreover, those who cry out that I have striven after the empty glory of vivisections, and they disparage and ridicule with childish levity the frogs, snakes, flies, and other lower animals which I have brought on to my stage. Nor do they abstain from scurrilous language. To return scurrility with scurrility, however, I judge unworthy in a philosopher and searcher after truth. I think it will be better and wiser to tone down these many indications of bad manners by the light of true and trustworthy indications. It is unavoidable that dogs bark and vomit their surfeit, or cynics are numbered among the assembled philosophers, but one must take care that they do not bite, or kill with their savage madness, or gnaw with a canine tooth the very bones and foundations of truth. While I resolved with myself that censurers, mummers, and stain-defiled writers of disapprobations should never be read (as being men from whom nothing sound or remarkable except scurrility was to be expected), I judged them even less worthy of answer. Let them enjoy their evil nature: I think they will scarcely ever have well-disposed readers: and the most good God does not give to the wicked that which is most outstanding and most to be desired, namely, wisdom. Let them continue with their scurrility until it irks if it does not shame them, and finally tires them out.".

Hales & Poiseuille

Harvey's work was of as much interest to natural philosophers (including members of England's Royal Society) as it was to physicians, who in any case had little way to apply it in the seventeenth and eighteenth century.

One such philosopher was the clergyman Stephen Hales, who conducted studies in vegetable statics (including movement of sap in trees) and on haemostatics, and was the first to measure arterial pressure (from the height the column of blood rose in a glass tube connected to a horse's crural artery). This was a measure of mean arterial pressure since inertia of the system prevented pulsations being accurately recorded. The value was measured at around 4–5 feet of blood, corresponding to around 100 mmHg. Hales showed the initial fall, then compensation in consequence of blood loss. Hales is credited with the concept of the Windkessel model of the arterial tree (distensibility of central arteries which cushioned flow pulsations and confined pulsations to these), and the concept of vascular resistance residing in small peripheral blood vessels (19). The French medical scientist J.L.M. Poiseuille confirmed the latter, having shown that pressure fall is trivial in the arterial vessels leading up to the microcirculation; Poiseuille's later work on viscosity and resistance was deliberately conducted in capillary tubes because of their physiological relevance to vascular resistance. Poiseuille also showed how arterial pressure could be measured more easily in mmHg and arranged a system where this could be recorded graphically.

Poiseuille's work in the nineteenth century was accompanied by other studies of arterial elasticity and pulse wave velocity by Thomas Young and on the physical principles of pulsatile blood flow in rigid and distensible tubes by many others. All this was summarised in the first and subsequent editions of *McDonald's Blood Flow in Arteries*. Nothing quite as contentious as Harvey's correspondence arose from this basic research.

London and Guy's Hospital

On the clinical scene, progress in the nineteenth century was associated with appreciation of the problems of high arterial pressure. Richard Bright (20) at Guy's Hospital in London had associated "hardness of the pulse" (denoting high blood pressure) with albuminuria, contraction of the kidneys, left ventricular hypertrophy, cardiac failure and stroke. Many of his cases had glomerulonephritis, and this was considered the cause of high blood pressure. After Bright's death, this work was continued at the same hospital by Gull and Sutton who suspected that the condition described by Bright was due to generalised arteriolar

constriction causing high peripheral resistance and arterial pressure, with secondary kidney damage. (20) Such views were presented gently to a receptive audience, and were supported by introduction of a quantitative sphygmogram by Frederick Mahomed, initially while a medical student at Guy's. (20) Mahomed showed high arterial pressure could be assessed from the form of the radial artery pulse. Using this method he subsequently described the natural history of what we now call essential hypertension, and confirmed that high arterial pressure could result from or lead to disease of the kidneys.(20).

Mahomed's instrument was an advance on that originally described by Etienne Marey in Paris during 1861. Marey was more interested in movement than in medicine, though he had a medical training. He was the first to describe the effects of age on contour of the radial artery pressure pulse – as subsequently confirmed by Freis, Kelly and others. In the nineteenth century, French and English sphygmograms were difficult to use, but they did give information on arterial pressure, and on cardiac rhythm, that was not available by any other method. In 1879 the first description of glyceryl trinitrate as an anti-anginal agent was accompanied by an illustration of the radial artery pulse which is virtually identical to that reported by Kelly et al. over a century later. Books and journals were liberally illustrated with sphygmograms. By the turn of the century Sir James Mackenzie had emerged as the most prominent physician in England on the basis of his books on the heart and the pulse, and on his use of sphygmograms in clinical practice. Mackenzie founded the journal "Heart" and is generally regarded as the first cardiologist in the English-speaking world. His view on heart failure was that this is attributable to myocardial exhaustion, as a consequence of arterial stiffening. This fits well with current views on the paramount importance of left ventricular load and left ventricular systolic pressure in development of heart failure (21). The main controversy about this time appears to have been proprietarily, between two English enthusiasts who claimed credit for initial clinical application, and whose feuding led to a Lancet editorial on plaguarism. The Dudgeon sphygmograph was a beautiful little device favoured by Mackenzie that sold around the world including to a Sydney suburban general practice. Dudgeon wished to improve the art of pulse interpretation which he described as follows:

"The physician of old made his diagnosis chiefly by
observation of the pulse and tongue. But, as the tongue
could be rapidly inspected, and anyone could judge of its

foulness or cleanness as well as himself, he concentrated his attention mainly on the pulse, in the feeling of which there was always scope for affecting the possession of peculiar skill and insight. To the uninitiated who regarded the doctor as the depositary of occult knowledge, and who received his dicta as though they were oracles, there was something very imposing in his method of pulse-palpation. The fingers of the right hand daintily grasping the patient's wrist, while the doctor's eyes were riveted on the loudticking gold chronometer he held in his left hand, his head gravely nodding the while synchronously with the arterial pulsations – all this formed a picture calculated to inspire beholders with reference and awe".

Riva Rocci

Introduction of cuff sphygmomanometric methods for blood pressure recording, first by Riva-Rocci, then by Korotkov led to the demise of the sphygmograph for blood pressure recording, while development of electrocardiography by Einthoven and clinical application by Lewis and others found another method for identifying cardiac arrhythmias. By 1917, the value of systolic blood pressure measurement for life insurance risk had been confirmed. Cuff sphygmomanometry then came to dominate clinical practice though it was not until the end of the twentieth century that views on diastolic pressure and pulse pressure were challenged. This was not the initial response. Korotkov had a hard time gaining recognition for his auscultatory method for determining systolic and diastolic pressure.

Cardiovascular physiologists

Through the early and mid twentieth century, methods were improved for direct measurement of arterial pressure and flow, notably in the USA whence scientists had fled from war-ravaged Europe. This too is summarised in the McDonald books. Most prominent were Wiggers in Cleveland, Hamilton and others in

Augusta, Georgia, and with clinical applications pressed by Paul Wood at the Mayo Clinic. A turning point was introduction of pulse waveform study in the frequency domain by Donald Macdonald and his colleagues John Womersley and Michael Taylor in London's St. Bartholomew's Hospital (where Harvey had practised). Basic to this was an assumption that the arterial system behaved in a linear fashion with respect to pressure and flow. This clearly was not the case, though Womersley's theoretic studies suggested that nonlinearities were sufficiently small to be neglected to a first approximation. Taylor's subsequent experimental work on vascular impedance and pressure/pressure transfer functions showed that nonlinearities were indeed far smaller than he and others had originally thought. This work moved on to explain pressure and flow in the arterial system in terms of wave travel and reflections. Details are summarised in the McDonald third to fifth editions. Original reaction to McDonald's work was profoundly sceptical. Data to support this was slow to emerge, and McDonald was a colourful and undiplomatic character. He was uncompromising in his view that pressure and flow waves could only be analysed properly in the frequency domain, and declined to consider how wave reflection may influence pressure and flow displayed by waveforms as a function of time (ie. in the time domain). This was the only way that contemporary physiologists operated. Consequently McDonald's views were systematically ignored by the American Physiological Society and its handbooks and reviews, with the first such not appearing until 1980. But others, including Taylor and his colleagues in Sydney, Milnor in Baltimore, Murgo in San Antonio and Westerhof in Amsterdam linked both approaches. By 1998, and in the fourth edition of McDonald's book, and 25 years after his death, there was general acceptance of his views on frequency analysis, but there was agreement that these could be considered with traditional analyses – and with all showing evidence of wave travel and strong wave reflection in the arterial tree.

2.2 Human application

Human application was advanced by introduction of a catheter-mounted electromagnetic flow meter by Mills from London in 1966, and a micromanometer by Millar and Murgo shortly afterwards. Use of these instruments at cardiac catheterisation extended the studies on experimental animals, but showed that humans were different, with far higher aortic impedance and pulse wave velocity than seen in experimental animals. This accounted for the characteristically different waveforms in older humans, and the evidence of deterioration in vascular/ventricular interaction that appeared to be the norm in humans by the age they came to cardiac catheterisation.

A series of studies on ascending aortic impedance at cardiac catheterisation were done in different countries notably in the USA, France and Japan from 1975 to 1995 and these established aging changes, effects of disease and of drugs. These however lapsed, and for a number of reasons. The catheters were very expensive, and not always approved for multiple uses. The flow waveforms were subject to distortion as a consequence of disturbed flow in the ascending aorta close to the heart. General agreement had been reached on the effects of age, hypertension, cardiac failure and of drugs. Non-invasive methods were improving and were far easier to apply.

Echocardiographic techniques are now widely used for measurement of ascending aortic flow velocity in routine assessment. These have been used with non-invasive carotid pressure (as a surrogate of ascending aortic pressure) for determination of ascending aortic impedance since 1988 (22). Mitchell and colleagues (23) and others have extended this work in recent times and have confirmed and advanced the invasive studies.

2.3 Tonometry supplements the brachial cuff

An important advance for non-invasive measurement of the arterial pressure waves was Millar's development of the applanation tonometer. Similar devices are now made by other manufacturers. All use the same principle as used in ocular tonometry, flattening of a small segment of the anterior wall of the artery. When this is achieved throughout a cardiac cycle, the pressure wave is identical to that recorded within the artery (24). These instruments are far more accurate and far easier to use than the mechanical sphygmogram, and more accurate than the microphonic systems as used for systolic time intervals in the 1970s.

Extensive studies of carotid tonometry have now been published. Carotid tonometry is presently being implemented as part of the Framingham study. The carotid pressure wave can be calibrated against upper limb pressure by assuming that mean pressure and diastolic pressure are the same in the carotid artery as in the brachial or radial artery; systolic pressure can then be calculated from the digitized waveform by extrapolation (22). This technique has acceptable accuracy (assuming cuff pressures are accurate) and can also be applied to the carotid diameter waveform (25),(26). Studies of carotid tonometry have to date provided conflicting results. Use with measured or assumed cardiac output for measuring systemic arterial compliance has not shown the clear-cut aging changes demonstrated through measurement of aortic pulse wave velocity. Calculation of central systolic pressure has not always been shown superior to brachial cuff

pressures or to measurement of pulse wave velocity in outcome studies. These problems could be technical. We have found the carotid pressure waveform difficult to measure accurately and reproducibly, and uncomfortable for the patient (27). We have also been concerned about dislodging plaque, especially when an operator has difficulty measuring the wave reproducibly through the full cardiac cycle, and seeks to persevere. In consequence we have preferred to generate aortic pressure from the radial pressure waveform.

Measurement of the radial pressure wave is hallowed by history, and was the technique used by Mahomed, Mackenzie and others over a five decade span. Records can be made easily, reproducibly and with no discomfort to the patient, and without the risks posed for the carotid. Calibration can reasonably be made directly with the brachial pressure cuff values. Central systolic and pulse pressure can be measured directly from the radial pressure wave through identification of the beginning of a reflected wave in later systole, but is generally undertaken through use of transfer functions which can be used to synthesise the whole aortic systolic pressure wave. This is the technique provided by the SphygmoCor device (and with whose manufacturer, AtCor Medical, M. O'Rourke is associated). Use of a generalised transfer function to generate central aortic from radial pressure waveform has been approved by the US FDA following a host of validation studies (11),(28). It does involve the same technique as introduced by McDonald, and extended by Taylor and colleagues. Challenges still appear in the literature to this method (29) but even these most ardent detractors have published data which is similar to that presented by others and used in the SphygmoCor system. The need for some form of transfer function to allow for distortion of the pressure pulse in the upper limb is apparent from all published transfer functions (28); all show substantial deviation from unity at frequencies between 2 and 7 Hz.

2.4 Marley-Whilst Franks' Model

Shortly after Marley (1863) made the first non-invasive measurements of the arterial pulse waveform it was noticed that the shape of the arterial pressure waveform changed markedly with ageing and disease. It was Frank, one of the most acclaimed physiologists of the last century, who proposed a model to explain these features, termed the arterial Windkessel. Whilst Frank's model could accurately describe the shape of the pressure waveform during diastole, it could not account for the sharp upslope of the pressure waveform during systole, or the shoulder later in systole. As a result, this technique lost popularity in favour of the newer techniques based on wave theory described by Womersely and McDonald and further developed by

Westerhof. This approach has gained wide acceptance and separates pressure into forward- and backward-travelling (or reflected) components.

One major limitation of these 'wave-only' techniques is that they are based on one-dimensional theory and consequently neglect the accumulation of blood due to radial expansion of the elastic arteries in systole: the aortic 'cushioning' or 'reservoir' effect. Womersely and McDonald were aware of this potential difficulty and cautioned that their theories were based on ''two very drastic assumptions'' namely that the aorta ''be regarded as a rigid tube'' and ''arterial expansion be neglected''.Clearly neither is true in the human aorta, however, as there was no other technique to explain all the features of the pressure waveform these assumptions are usually overlooked.

2.5. Modern blood pressure studies

Modern views on the arterial system are different to what they were twenty years ago, or even five years ago. Twenty years back, diastolic blood pressure levels were regarded as all important, and were required for a diagnosis of hypertension to be made. The landmark SHEP trial transformed this. Now, diastolic pressure is largely ignored in treatment of patients over age 50 and systolic (or pulse) pressure is considered all important. Elevated systolic pressure is now considered as the principal cause of the epidemic of heart failure in the elderly. Inaccuracy of casual clinical blood pressure recordings is increasingly recognised and recourse is now taken more frequently to measurement on multiple occasions at home. Lower levels of pressure are now targeted for treatment than before, especially in the presence of diabetes mellitus, renal insufficiency and cardiac failure.

2.6 Pulsations enter the microvasculature

Attention is now being focused on arterial stiffness as a cause of cardiovascular events especially in older persons, as well as in persons with diabetes mellitus, renal failure and cardiac failure. While explanations have been readily given as to how arterial stiffening increases left ventricular and aortic systolic pressure and so predisposes to left ventricular hypertrophy, cardiac failure and angina, attention is now shifting to the microvasculature (30). Harvey had referred to the microvasculature as being perfused "continuously, evenly and uninterruptedly" throughout the cardiac cycle as a consequence of the elastic properties of arteries. With large artery stiffening, pulsations cannot be cushioned in the large arteries, and extend further down into the

microvasculature, particularly that in the highly perfused organs (brain and kidneys) (31). High flow pulsations have been confirmed in cerebral arteries, and have even been shown extending through into the venous sinuses (32). Such high pulsation in the microvasculature appears to be responsible for partial rupture, with haemorrhage from these vessels resulting in fibrinoid necrosis and amyloid deposits and for endothelial damage and thrombosis with resultant micro-infarcts (31). Evidence is emerging that this is substantial in older persons, and is exaggerated when the smaller vessels are unusually fragile, as in diabetes mellitus, and/or when control of vasomotion is impaired (33). Evidence is emerging that the major problems of modern society (dementia and renal failure) are due to such later micro-vascular damage (34) as well as to the earlier aortic damage consequent on elastin fibre fracture and arterial stiffening.

3. - Basic principles of arterial stiffness

An understanding of the basic principles of haemodynamics is mandatory to appreciate fully the advantages and limitations of the various methodologies and indices used to assess arterial stiffness, and their potential clinical applications. Earlier physicists such as Young (1808), Poiseuille (1840), Moens (1878), and Korteweg (1878) established hydraulic and elastic theory. Physiologists/physicians, such as Marey (1860), Mahomed (1872), and Mackenzie (1902), developed various types of sphygmographs and made important contributions to the analysis of the pressure wave. Later, it appeared that the mechanical behaviour of large arteries was extremely complex and provided serious difficulties, both on the theoretical and technical aspects. Indeed, arteries have marked anisotropy, exhibit non-linear visco-elastic properties, and have powerful adaptive mechanisms. Moreover, no single arterial segment has identical viscoelastic properties, and it is impossible to extrapolate segmental arterial properties to the whole arterial tree.

Despite these obstacles, simple parameters derived either from the Windkessel model or based on arterial wave propagation have been developed. In several year has been, extensively contributiong to the clinical applications of these concepts, which proved useful not only in representing basic mechanical behaviour of the arterial system but also in predicting outcome and refining therapy.

3.1 Waves in arteries

Recently, waves in arteries have received increased attention as a possible independent mediator of cardiovascular risk (35),(36),(37),(38),(39) and a potential therapeutic target (40),(41),(42).

A wave is a disturbance that is propagated through a medium with an exchange between kinetic and potential energy. Implicit in this definition, though sometimes not appreciated, is that waves in the arterial circulation necessarily involve simultaneous changes in pressure and flow. It is also worth emphasizing that a wave involves the transmission of energy through displacement of particles or medium (blood) without implying a bulk movement of the medium. Hence waves can, and do, travel at speeds considerably faster than the bulk flow velocity of blood in the circulation. Often when people think of waves they think of transverse waves (e.g. waves in a violin string or a 'Mexican' wave at a sports stadium) where the displacement of the particle or medium is perpendicular to the direction of wave propagation; however, in the case of waves in arteries, the waves are predominantly longitudinal waves (i.e. the displacement of the medium is parallel to the direction of wave propagation), akin to acoustic waves or longitudinal waves in 'slinkies' The major difference between wave propagation in a rigid fluid-filled tube and an elastic fluid-filled tube (e.g. a blood vessel) is that the effective compressibility is the sum of the true compressibility of the fluid and the distensibility of the tube. This accounts for the much lower wave speeds seen in arteries than in rigid tubes.

From a fluid or gas dynamics perspective, longitudinal waves can be characterized as compression waves or expansion waves. These types of waves are characterized by the rate of change of pressure ($d\rho/dt$) they cause; compression wavefronts have $d\rho/dt > 0$; for expansion wave fronts $d\rho/dt < 0$. One apparently anomalous consequence of this definition is that compression wave fronts in arteries are associated with an expansion in arterial diameter, while expansion wave fronts are associated with a reduction in arterial diameter. However, less confusingly, compression of the blood stored in the ventricle during systolic contraction and ejection results in a forward travelling compression wave front, while a reduction in the rate of ventricular contraction in proto-diastole is associated with generation of an expansion wave front slowing aortic blood flow and assisting aortic valve closure (43).

Traditionally analysis of waves in the circulation has employed impedance analysis, a technique based on the Fourier transform. This approach considers waves as superimpositions of sinusoidal wave trains (harmonics) with different magnitudes, frequencies and phase. Impedance analysis is a powerful tool that has provided extensive insights into circulatory physiology, but it assumes linearity and periodicity of waves. In other words, waves are assumed to add together and the circulation is assumed to be in a state of steady-

state oscillation. This latter assumption implies that a given pulse can be influenced by the reflection of waves that originated in preceding cycles. This view is in conflict with observations made following ectopic or missed beats, where there is no evidence of a residual oscillation in pressure (44). In practice, however, heart rate is relatively stable in the majority of situations subjected to impedance analysis and the assumption of periodicity in these settings seems reasonable.

3.2 Wave Intensity Analysis

As mentioned above, a characteristic property of waves is that they allow the transport of energy without the need for net transport of material. By analogy with acoustic theory, the 'wave energy' is the part of a fluid's total energy, that is associated with a wave; while the 'wave intensity' is the rate of transport of wave energy, i.e. the instantaneous power carried by the wave per unit cross sectional area. The work done by the wave involves both kinetic energy associated with the velocity of flow and potential energy associated with expansion of the arterial wall (since blood is assumed to be effectively incompressible).

Wave energy and wave intensity are commonly expressed as J m^{-2} or W m^{-2}, respectively, although it has been proposed that wave intensity should be normalized with respect to the product of the derivatives of pressure ($d\rho/dt$) and velocity (dU/dt) with respect to time.

It is therefore plausible that the power and energy invested in waves reveals important information regarding ventricular performance and the state of the circulation, particularly with regard to wave reflection.

Wave intensity and wave energy are calculated by wave intensity analysis. This is a one-dimensional analytical technique that employs the 'method of characteristics' to assess wave travel at a specific location in the circulation. The approach is based on Riemann's invariant theory and similar approaches are widely employed for the analysis of non-linear effects in wave travel in gases and fluids, including shock waves. To perform wave intensity analysis only pressure (or arterial diameter changes) and flow velocity data from the same measurement site are required. These data are relatively easy to obtain using invasive or non-invasive techniques. Ideally these data should be acquired simultaneously, although sequentially acquired data has been successfully used (45),(46).

In itself, wave intensity analysis only provides information regarding the direction of net wave travel. However if the system is assumed to be linear (i.e. waves in different directions interact additively) then wave separation can be performed on the basis of the 'Water Hammer' equation, assuming that the local wave speed(c) is known (47). This technique of wave separation gives results that are very similar, if not identical,

to impedance based approaches, if the product of c x ρ (density of blood) is accepted as an estimate of characteristic impedance (Zc) per unit area (48).

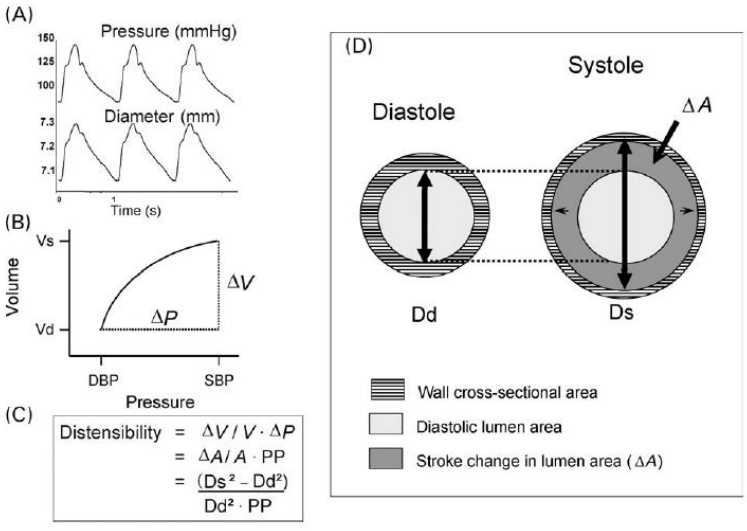
3.3 Waves in elastic tubes-Important Physical Properties

Waves travels is governed by factors determining 1) The displacement of blood along the axis of the vessel, with inertia being the most important effect in large arteries; and 2) The transverse displacement of vessel wall, i.e the stiffness of the vessels. The former is called the "longitudinal impedance" (ZL) and is approximated as ρ/A with ρ the density of the blood (about 1050 Kg/m3) and A the cross-sectional area of the vessel. The later is called "transverse impedance" (ZT).

The greater the longitudinal impedance (inertia), the slower the wave will travel along the vessel segment. The greater the transverse impedance (the stiffer the vessel), the less time will be lost in radial (transverse) displacement of the wall, and the faster the wave will travel in the tube.

3.4 Wave intensity analysis in the aorta and systemic arteries

Since its original description, a number of investigators have used wave intensity analysis to aid the understanding of physiological events in the arterial system. In addition to studies in the aorta (47),(49),(50),(51), coronaries (52),(53), (54) and other systemic arteries, (45),(55),(56), (57) wave intensity analysis has also been applied to the pulmonary circulation, (58) the venous circulation, (59) the chambers of the heart (60), (61),(62),(63),(64),(65) and cardiac assist devices (66).



Local arterial distensibility. (A) Simultaneous recording of stroke changes in BP and diameter. (B) Pressure–diameter curve. (C) Calculation of distensibility. (D) Schematic representation of the stroke change (DA) in lumen cross-sectional area.

The first and dominant peak intensity is a forward travelling compression wave (a pushing wave), which occurs when pressure and flow velocity rise simultaneously in the aorta. This occurs as a result of ventricular ejection and accelerates aortic blood flow. This is closely followed by a smaller backward-travelling compression wave or 'reflected wave' which originates from the distal circulation

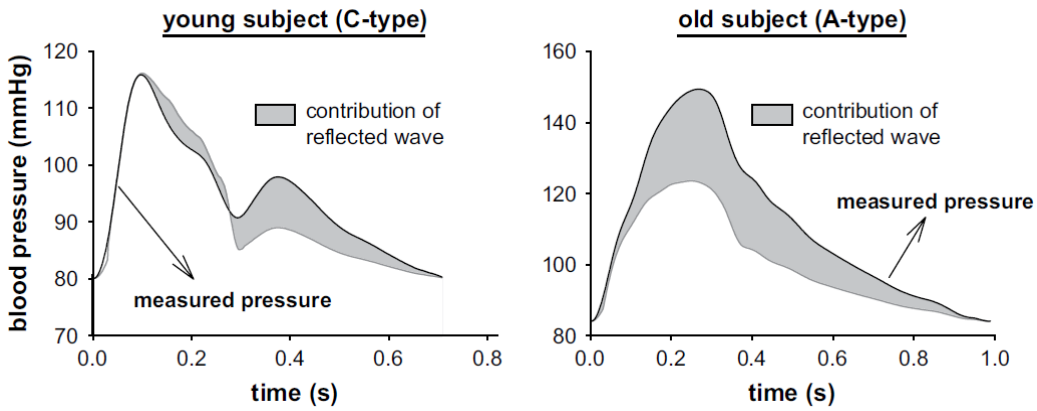
This wave is thought to arise when the forward-travelling compression wave is reflected back towards the proximal aorta from distal sites of impedance mismatch.

This wave increases total pressure, but decelerates blood flow. Towards the end of systole (protodiastole of Wiggers) there is a forward-travelling expansion (suction) wave that results from deceleration of ventricular contraction before closure of the aortic valve. The effect of this wave is to decelerate blood in the aorta (43),(67) and contribute to aortic valve closure. Relatively similar patterns of wave intensity have also been seen in carotid artery, (45),(68) brachial artery, (45) radial artery, (45) although the magnitude of the wave intensities differ between sites and in some cases additional waves, possibly arising as a result of re-reflection of these principal waves, are also evident.

3.5 Contribution of the wave reflection in central pressure

Despite of the complexity of wave reflection in the arterial trees, the global result can be schematized in a relatively simple way, where measured pressure is the result of forward and backward waveform (reflected) component.

For the central pressure wave form, this reflection appears to arise from an effective reflection site, a distance away from the heart. Age and disease affect arterial stiffness. PWV and the amplitude of the reflected wave generally increase, the reflected wave shifts towards the heart and the reflected wave arrives in early systole, rather in late systole to diastole as in young healthy subjects.



Contribution of the reflected pressure wave in young and old subjects, obtained using the principles of wave separation analysis. As well known, the reflected wave arrives in late systole and diastole in young (and tall) subjects, generating the typical ''C-type'' wave with late systolic inflection and adding little to the systolic blood pressure. In older subjects, increased pulse wave velocity causes the (more important) reflection to arrive at the central aorta in early systole, generating an early systolic inflection point (A-type wave) and boosting systolic blood pressure.

The consequence is not only a change in the visual appearance of the wave (**see figure above [4]**), but also a boost of the systolic pressure and a widening of the systolic-diastolic pressure difference (the pulse pressure), increasing the load of the heart (69). Although the major determinant of the pulse pressure is, by definition, the forward pressure wave (the reflected wave can never be higher than the forward wave), it's found in a normal middle age population, wave reflection does already explain about the 26% in pulse pressure (70).

3.7 Waves Reflection

When modelling the arterial tree has been also suggested that because the tube's end has a high level of resistance, waves are reflected and retrograde waves are generated. This would account for the secondary fluctuations of the pressure waveform in diastole and differences in the amplitude of the pressure wave between central and peripheral arteries and fits well with pathophysiological observations. In particular, it explains why an increase in the arterial stiffness increases central PP, with an associated increased systolic BP.

In the human body, wave reflections originate in various locations, including peripheral bifurcations of conducting arteries and smaller muscular arteries. The geometry, number of arterioles, and the architecture of the microvascular network play an important role in wave reflection. Indeed, arterial and arteriolar constriction results in reflection points closer to the heart, leading to earlier aortic wave reflections. In addition, with increased arterial stiffness, as observed, for example, in older subjects or hypertensive

patients, the reflected wave travels more rapidly along the arterial tree. Thus, both small and large arteries contribute to early reflected waves which arrive in early systole, superimpose on the forward wave, and boost the systolic pressure further, whereas blood pressure falls sharply in diastole with reduced diastolic fluctuations.

5. Molecular determinants of arterial stiffness

Aging and blood pressure are the 2 major determinants of arterial stiffness. Arterial stiffness is also increased in metabolic syndrome, type-1 and type-2 diabetes, chronic renal insufficiency and inflammatory diseases, which alter a large number of arterial wall components to various extents. The normal media layer is composed of smooth muscle cells (SMC) and extra-cellular matrix (ECM). The 2 major scaffolding proteins, collagen and elastin, provide structural integrity and elasticity. Any alteration in collagen and elastin production and molecular repair mechanisms can theoretically change arterial elasticity (71). The relative content of collagen and elastin is controlled by a dynamic process of production and degradation. Degradation occurs through collagenases and elastases, produced by inflammatory cells such as macrophages and polymorphonuclear neutrophils, and gelatinase activation (MMP-2 and MMP-9).

With age, in the load-bearing media of elastic arteries, there is a loss of the orderly arrangement of elastic fibres and laminae, which display thinning, splitting, fraying and fragmentation (72). Degeneration of elastic fibres is associated with an increase in collagenous material and in ground substance and often with the deposition of calcium in this and in degenerate elastic fibres. In several pathological conditions, these changes are associated with an infiltration of vascular SMC, macrophages and mononuclear cells, and an increased content of matrix metalloproteinases and cytokines (73). Inflammation, either acute such as in systemic vasculitis or chronic during rheumatoid arthritis, may stiffen the large arteries through various mechanisms(74) including endothelial dysfunction, leading to ‘‘functional’’ stiffening of the large arteries (reduction of nitric oxide bioavailability and increased activity of opposing mediators such as endothelin-1), cell release of a number of inducible matrix metalloproteinases (including MMP-9), medial calcifications, changes in proteoglycan composition and state of hydration, and cellular infiltration around the vasa vasorum leading to vessel ischemia. Abnormal, stiffer collagen molecules can result from non enzymatic glycation cross-linking, for instance during impaired glucose tolerance or diabetes. These irreversible cross-links form advanced glycation end products (AGEs) (72). Not only in diabetes, but also in chronic renal diseases, does the activation of systemic and local renin-angiotensin system promote SMC proliferation, low-grade

inflammation, and increase collagen content and AGE formation. Arterial stiffening in renal disease is also due to calcifications in the arterial media. Shanahan et al. (75) have suggested a sequence of molecular events in vascular calcification beginning with the loss of expression by VSMCs, of constitutive inhibitory proteins, and ending with expression by VSMCs and macrophages of chondrocytic-, osteoblastic-, and osteoclastic associated proteins that orchestrate the calcification process.

Thus, with aging, hypertension, diabetes, chronic renal disease and inflammation, the arterial wall can stiffen in response to a combination of several mechanisms. However, the respective role of each process is difficult to determine.

4.1 Endothelin

Three endothelins (ETs) have been recognized in mammals (ET-1, ET-2, and ET-3). These 21-amino acid peptides were originally identified as products of vascular endothelial cells but are now known to be produced in different organs. They are important regulators of cardiovascular function, including smooth muscle tone, but also affect digestive tract function, endocrine glands, the renal and genitourinary system, and the nervous system.

The main ET secreted by endothelium is ET-1, which is released in response to stimulation by high pressure and low shear stress; low pressure or high shear inhibits ET-1 production. ET release is also promoted by angiotensin II (Ang II), vasopressin, catecholamines, and transforming growth factor β among other factors. The multiple biologic actions of ET are mediated by at least two receptor subtypes, ET-A and ET-B. The ET-A receptor is widely expressed and is the principal receptor for the ET system in vascular smooth muscle. The ET-A receptor has higher affinity for ET-1 than for ET-3. ET-A receptor activation results in vasoconstriction via activation of phospholipase C and an increase in intracellular calcium. The ET-B receptor was initially thought to be expressed only in vascular endothelial cells and to release vasodilating substances such as Nitric Oxide (NO) and prostacyclin, as well as the newly identified vasodilating peptide adrenomedullin.

ET activation causes constriction and hypertrophy of blood vessels and promotes vascular fibrosis. The vascular endothelium is an important source of vasodilating and vasoconstricting factors. One of these vasoconstricting factors is ET-1, the most-potent human vasoconstrictor identified so far, which opposes, for example, the vasodilating actions of nitric oxide (NO) and the C-type (vascular) natriuretic peptide CNP.

ETs have positive chronotropic and inotropic effects on cardiac muscle and may induce cell hypertrophy. In the coronary circulation, the small number of endothelial ETB receptors suggests that ET acts mainly as a coronary vasoconstrictor.

The role of ETs in the pathophysiology of hypertension is still unclear, summarizes the current view of the potential implication of ET-1 in BP elevation and vascular hypertrophy, and the mechanisms whereby ET-1 contributes to vascular injury and the progression of atherosclerosis in stage 2 hypertension.

4.2 Nitric oxide (NO)

Originally described as endothelium derived relaxing factor (EDRF)—is released from endothelial cells in response to shear stress produced by blood flow, and in response to activation of a variety of receptors (76),(77). NO is a free radical gas with an in vivo half-life of a few seconds, which is readily able to cross biologic membranes (76),(78),(79). After diffusion from endothelial to vascular smooth muscle cells, NO increases intracellular cyclic guanosine monophosphate (cGMP) concentrations by activation of the enzyme guanylate leading to relaxation of the smooth muscle cells (80).

There are two major types of NOS in the vasculature: a constitutive and an inducible isoform. The former, which is present in endothelial cells, is called endothelial NOS (eNOS), the latter is an important inflammatory mediator released by macrophages in response to immunologic stimuli (81). NO has also antithrombogenic, antiproliferative, leukocyte-adhesion inhibiting effects, and influences myocardial contractility. (82),(83). The hemodynamic effects of pharmacologic NO inhibition include an increase in systemic and pulmonary arterial blood pressure, and a decrease in cardiac output.

The renin-angiotensin system plays a major role in hypertension (84). Apart from direct vasoconstrictor effects of angiotensin II (Ang II), there are important interactions between Ang II, oxygen radicals, and NO.

4.3 Matrix metalloproteinase types 2 and 9

Matrix metalloproteinase MMP-2 and MMP-9 are members of the zinc-containing endopeptidase family. They degrade native and denatured collagen and elastin, promote matrix protein degradation in vascular disease remodeling, and facilitate VSMC migration. Aortic MMP-2 activation in situ is progressively enhanced with aging, mainly localized to the intima, and co-localized with EC and VSMC in rats9,14 and monkeys.11 A recent study shows that in humans, MMP-2 and MMP-9 activity also is enhanced in situ in the grossly normal aortic segments with aging (85).

5. Inflammation and arterial stiffness

Arterial function is a crucial determinant of the overall cardiovascular (CV) performance. Several studies indicate that arterial stiffness, wave reflections and endothelial function are associated with the presence and extent of CV disease, and moreover, that they are independent predictors of outcomes in several populations. (86),(87),(88),(35), (39).

Atherosclerosis is currently recognized as an inflammatory disorder. Indeed, low-grade inflammation contributes to all stages of atherosclerosis, from the initial phase of increased endothelial permeability up to the formation of the mature atherosclerotic plaque and plaque rapture (89). Furthermore, accumulating data suggest that there is also an important link of clinically manifested inflammation, as in chronic inflammatory diseases or acute infectious disorders, with CV disease. Considering that arterial dysfunction indicates the presence and mediates the progression of CV disease and also predicts outcomes, current evidence with regard to the association of the different types of inflammation with arterial performance is of great interest, both in pathophysiological and clinical grounds.

It is established that subclinical low-grade inflammatory activation, as expressed by high circulating levels of inflammatory markers/mediators, is a common denominator of most CV risk factors. In diabetic patients, levels of C-reactive protein (CRP) and serum Amyloid-A, and white blood cell count are related to increased arterial stiffness (90). Notably, a relationship between abdominal adiposity and low carotid distensibility has been observed in diabetic patients, and this is fully accounted for by the increased levels of CRP and interleukin-6 (IL-6) (91). Similarly, in subjects with metabolic syndrome, there is a close association of arterial stiffness with the amount of subcutaneous fat, a tissue that is largely involved in the synthesis of proinflammatory cytokines (92). Likewise, carotid-femoral pulse wave velocity (PWV), a measure of aortic stiffness, is related to the level of CRP in hypercholesterolaemic patients (93).

The relation of arterial elastic properties with subclinical inflammation has also been studied in hypertensive populations. Our group and other investigators have observed that stiffness of large, elastic-type arteries deteriorates in parallel with increasing levels of CRP in hypertensive patients (94),(95). Moreover, we also observed that CRP is associated with stiffness of medium, muscular-type arteries, as expressed by the carotid-radial PWV (94). Concerning augmentation index (AIx), which is a composite index of wave reflections integrating arterial stiffness, peripheral vascular tone and characteristics and cardiac contractility, data regarding associations with CRP are not consistent (94),(95),(96).The relation of inflammation with

stiffness in hypertension has been further highlighted by a recent study showing that patients with low level of adiponectin, a cytokine with anti-inflammatory and atheroprotective properties, have high aortic stiffness (95). Furthermore, we have observed that the plasma level of fibrinogen, which is a pro-coagulant substance that also reflects the degree of inflammatory activation, is an independent determinant of large elastic artery stiffness in untreated hypertensives.

Associations between inflammatory compounds and arterial function have been observed even in the general population and in apparently healthy subjects, who are supposed to have the lowest degree of low-grade inflammatory activation. In such populations, CRP level has been related to pulse pressure, stiffness of elastic and muscular arteries and central wave reflections (97),(98). Furthermore, circulating level of matrix metalloproteinase-2 (MMP-2) is inversely associated with carotid-femoral PWV in healthy persons (99). In contrast to subjects with CV risk factors (100), it seems that in healthy, low-risk populations without the activation of low-grade inflammatory mechanisms, a higher MMP-2 level probably reflects a higher availability of nitric oxide (NO, a molecule with anti-inflammatory properties) (101) and more physiological extracellular matrix turnover, and thus it is associated with a lower aortic stiffness. Finally, has been observed that in healthy individuals, a readily available test such as white blood cell count may independently explain a greater amount of the variation of AIx than CRP (102).

Occult persistent infections represent another type of subclinical inflammation that may also coexist with impaired arterial function. In particular, Chlamydia pneumoniae seropositivity has been related to augmented arterial stiffness and aortic wall thickness even in asymptomatic children, indicating an adverse impact on vascular structure and function. Although recent studies showed that antibiotic therapy targeting these microorganisms does not reduce the incidence of CV disease, the debate on the significance of such infections for the initiation of atherosclerosis still continuous.

Cardiovascular disease is one of the leading causes of morbidity and mortality in patients with chronic inflammatory disorders. Recent studies have highlighted the close association between the level of inflammatory activation and impaired arterial performance in such patients. In rheumatoid arthritis, chronic inflammation affects adversely arterial stiffness, aortic haemodynamics and the performance of small arteries(103),(104),(105).Interestingly, as the majority of deaths in patients with rheumatoid arthritis are related to CV events, these data suggest that impaired arterial function may mediate a part of the increased CV risk of rheumatoid arthritis. In addition, arterial elastic properties are impaired in patients with systemic lupus erythematosus (103) or autoimmune systemic vasculitis(106). In children with Kawasaki syndrome, arterial stiffness and wave reflections are increased (107), presumably because the vasculitis is not confined

to coronary arteries but also affects other arterial beds. Furthermore, vascular function is compromised when inflammatory activation caused by chronic infections is present(108).

Apart from the link between chronic inflammation and atherosclerosis, accumulating data suggest also an association of acute inflammatory responses, as during an acute respiratory or urinary tract infection, with a short-term increased risk for a cardiovascular event (109). Regarding the arterial effects of acute inflammatory stimuli, earlier studies had found that infusion of proinflammatory cytokines in forearm arterial bed induces a transient and reversible endothelial dysfunction. Recent observational studies that employed more ‘‘physiological’’ inflammatory stimuli, showed that an acute respiratory infection may impair endothelial function for at least 2 weeks (110).

5.1 The role of C-reactive protein

Among several inflammatory markers, CRP is the most thoroughly investigated compound and the one that carries the highest amount of independent prognostic information regarding future CV events. Until recently, CRP was thought as an inert bystander of vascular inflammation, which indicates but does not directly contribute to arterial dysfunction. However, given that CRP may act through several mechanisms (complement activation, stimulation of synthesis of adhesion molecules and chemo attractant chemokines, modification of ionic channels), it was hypothesized that it may have a direct proatherosclerotic effect.

Concerning arterial function, it has been shown that exogeneously administered CRP may decrease NO production in vitro (111). However, these results were opposed by newer data showing that contaminating bacterial products found in the recombinant CRP preparations may actually account for the deleterious vascular effects attributed to CRP (112). Proceeding further, this latter study showed that CRP per se may actually benefit the NO availability (113). These findings suggest that CRP does not account for the arterial dysfunction observed in acute inflammatory conditions, and indicate the complexity of this issue. Animal experiments have recently shown that CRP may directly increase the extent of necrosis in the setting of acute myocardial infarction (114). However, regarding the involvement of CRP in the chronic process of atherosclerosis (and the accompanying arterial dysfunction) in humans, existing evidence favours the notion that CRP is a marker and not a mediator. Studies using the currently available specific CRP blockers (114) will demonstrate whether CRP has any definitive etiological role on arterial dysfunction and the atherosclerotic process in general.

5.2 Biochemical markers of endothelial dysfunction

A widely popular definition of endothelial dysfunction (ED) and one that we endorse entails an imbalance between vasodilating and vasoconstricting substances produced by (or acting on) endothelial cells (ECs). ED may participate in the elevation of blood pressure and can play a role in hypertension-related vascular damage. Indeed, the latter follows the acquisition of pro-inflammatory properties by the vascular endothelium. Current concepts of atherogenesis include involvement of the immune system and chronic inflammation. The up-regulation of the expression of adhesion molecules in the vascular endothelium is the initiating event in this process and allows leucocyte and monocyte adhesion to the EC surface. After adhesion, up-regulation of endothelial adhesins induces leucocyte and monocyte penetration into the sub-endothelial environment, where tumor necrosis factor (TNF)-Æ, interleukin (IL)-6 and other cytokines are released, resulting in recruitment of additional circulating cells. The membrane- spanning protein CD40 is also up-regulated in activated ECs and leucocytes and, after engagement with its natural ligand CD40L, which is expressed in monocytes and T lymphocytes, amplifies atherogenesis by further promoting cytokine release and adhesion of circulating cells to the endothelium. In a similar way, C-reactive protein (CRP), this is released by the liver in response to IL-6, acts both as a marker and a contributor to the vascular inflammatory process. A key determinant of inflammation is the increased generation of reactive oxygen species (ROS). Oxidized, but not native low-density lipoprotein (LDL), up-regulates endothelial adhesins expression in vitro and promotes endothelial–leukocyte interactions both directly and by modulating EC response to cytokines. In fact, antioxidants prevent adhesion molecule expression in ECs both in vitro and in vivo. The mechanism responsible for ROS-mediated endothelial up-regulation of adhesions and cytokines involves nuclear factor (NF)-kB, a pleiotropic modulator of inflammatory gene expression.

ROS also reduce nitric oxide (NO) synthase (NOS) activity and increase NO breakdown and thereby reduce the bioactivity of NO, a powerful inhibitor of NF-KkB activation under physiologic conditions. In addition, the ROS superoxide anion ($O^{2\bullet}$) and NO react to form peroxynitrite, which can be implicated in NO-mediated EC injury. ROS also lower the availability of tetrahydrobiopterin. If the latter occurs, the oxygenase function of NOS is replaced by its reductase function and ROS are produced instead of NO. Finally, since NO and endothelin (ET)-1 are reciprocally regulated an impaired NO availability could lead to increased ET-1 production, which affects various EC functions, and induce ED. The latter is the triggering event in atherosclerosis and is also fundamental in maintaining vascular inflammation. Thus, the integrity of the endothelial monolayer plays a pivotal role in counteracting such inflammatory events. Damaged ECs are

promptly removed and replaced by proliferating and migrating vascular ECs. Bone-marrow derived circulating EC progenitors can also migrate into a damaged endothelium to restore the endothelial monolayer. However, the continuous endothelial damage and repair, along with CV risk factors, may detrimentally impact on the endothelial progenitor cell population. Thus, endothelial injury in the absence of sufficient circulating progenitor EC availability may favour the progression of vascular disease EC progenitor cells could therefore represent a novel in vivo indicator of pre-clinical endothelial damage and a novel target for vascular protection. Although in-vitro methods allow investigation of these inflammatory processes, endothelial pro-inflammatory activation is difficult to evaluate in vivo. After adhesion, due either to enzymatic shedding or to alternative mRNA splicing, each endothelial adhesin releases its soluble component into the bloodstream. Thus, soluble adhesins are useful for assessing ED in vivo. Their circulating levels are higher in patients with coronary artery disease and their concentration in plasma has been related to the risk of future CV events. Increased circulating levels of soluble adhesion molecules have been described in association with CV risk factors, such as impaired glucose tolerance and hypercholesterolemia, where they correlate inversely with plasma nitrite + nitrate concentrations, which represent an accepted index of NO availability in vivo, at least under controlled dietary nitrate intake. Interestingly, patients with atherosclerosis [8] and at increased CV risk, also have increased circulating levels of ET-1.

As for CRP, the development of a highly sensitive (hs) assay has made possible the detection of mild elevations of hs-CRP which has prognostic value in acute coronary syndrome and represents an independent CV risk factor. Similarly, circulating levels of CD40 ligand are elevated in patients with stable and unstable angina and are associated with increased CV risk in healthy women. With regard to evaluation of oxidative stress, 8-iso-prostaglandin (8-iso-PG) F2Æ, an index of lipid peroxidation in vivo endowed with vasoconstrictive and platelet-activating properties, represents a surrogate for increased ROS production.

6. - Pathophysiology of cardiovascular complications

A generally accepted mechanistic view is that an increase in arterial stiffness causes a premature return of reflected waves in late systole, increasing central pulse pressure, thus systolic BP. SBP increases the load on the left ventricle, increasing myocardial oxygen demand. In addition, arterial stiffness is associated with left ventricular hypertrophy, a known risk factor for coronary events, in normotensive and hypertensive patients. The increase in central PP and the decrease in diastolic BP may directly cause subendocardial ischemia. The measurement of aortic stiffness, which integrates the alterations of the arterial wall, may also

reflect parallel lesions present at the site of the coronary arteries. Indeed, aortic stiffening accompanying age and cardiovascular risk factors is caused by various phenomena, including breaks in elastin fibers, accumulation of collagen, fibrosis, inflammation, medial smooth muscle necrosis, calcifications, and diffusion of macromolecules within the arterial wall. All these phenomena are known to occur in parallel at the site of the coronary circulation. An increased arterial stiffness can increase the risk of stroke through several mechanisms, including an increase in central PP, influencing arterial remodeling both at the site of the extracranial and intracranial arteries, increasing carotid wall thickness and the development of stenosis and plaques, the likelihood of plaque rupture, and the prevalence and severity of cerebral white matter lesion. The measurement of aortic stiffness, which integrates the alterations of the arterial wall, may also reflect parallel lesions present at the site of cerebral vasculature. Another explanation is given by the differential input impedance in the brain compared with other systemic vascular beds, leading to high-pressure pulsatility in brain and kidney. Finally, coronary heart disease and heart failure, which are favoured by high PP and arterial stiffness, are also risk factors for stroke.

7. - Pathophysiological conditions associated with increased stiffness and wave reflection

Arterial stiffness and wave reflections are widely used in observational studies to analyse the determinants of hemodynamic changes observed in various clinical conditions, and to understand the pathogenesis of their CV complications. A large number of publications and several reviews reported the various pathophysiological conditions associated with increased arterial stiffness and wave reflections.

Apart from the dominant effect of aging, they include (a) physiological conditions, such as sympathetic nervous system activity, low birth weight, menopausal status, lack of physical activity; (b) the genetic background such as a parental history of hypertension, diabetes or myocardial infarction, and genetic polymorphisms; (c) CV risk factors such as obesity, smoking, hypertension, hypercholesterolemia, impaired glucose tolerance, metabolic syndrome, type 1 and 2 diabetes, hyperhomocysteinemia, high CRP level, and acute inflammation; (d) CV diseases such as coronary heart disease, congestive heart failure, and fatal stroke; and (e) primarily non-CV diseases, such as end-stage renal disease (ESRD), moderate chronic kidney disease, rheumatoid arthritis, systemic vasculitis and systemic lupus erythematosus.

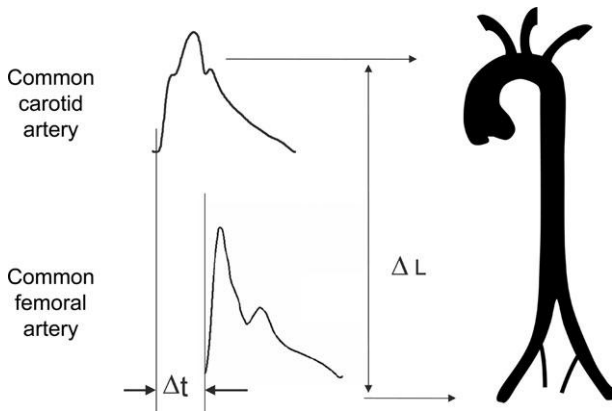
The contribution of these different factors to arterial stiffness and wave reflections has been studied in multivariate analyses: the major parameters to be taken into account, when evaluating the degree of arterial stiffness, are age and blood pressure, and to a lower extent, gender and classical CV risk factors.

8.-Measurement of arterial stiffness and central pulse-wave analysis

The indirect measure of arterial stiffness most commonly employed and shown to have prognostic value is derived from the assessment of carotid-femoral pulse wave velocity (Laurent Eur Heart J 2010).
In this work, however, we will focus on indices of arterial stiffness derived from arterial waveform analysis.

8.1 Pulse wave velocity

PWV is a measure of regional arterial stiffness of the arterial territory between the two measurement sites. This parameter is related not only to the elastic modulus (E) of the arterial wall (which represents the intrinsic stiffness of the wall), but also to the arterial geometry (thickness: h and radius: r) and blood density (ρ). During the end of the 19th century, Moens and Korteweg formulated this relationship as: $PWV^2=Eh/2r\rho$. Later on, Bramwell and Hill applied the Moense Korteweg equation to arterial physiology and described the relationship in terms of relative change in volume ($\Delta V/V$) and pressure ($\Delta\rho$) during ex vivo experiments: $PWV^2=\Delta\rho V/\Delta V\rho$. It is to be noticed that PWV is a direct measurement of arterial stiffness since it is the square value of 1/distensibility. In this aspect, it differentiates itself from indirect methods based on the models of circulation. The assessment of PWV involves measurement of two quantities: transit time of the arterial pulse along the analyzed arterial segment and distance on the skin between both recording sites.



Measurement of carotid-femoral PWV with the foot to foot method

8.2 Augmentation Index (Aix)

The augmentation index, AIx, is commonly defined as the ratio of the augmented pressure (AP), attributed to wave reflection, and the amplitude of the pulse (the pulse pressure; PP), often expressed as a percentage:

Aix= APXPP 100

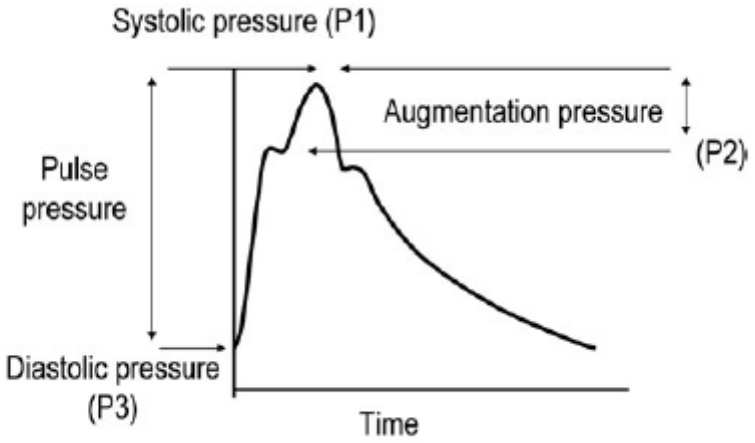
Since AIx is a relative measure, it can be derived from noncalibrated pressure tracings, as long as the morphology of the pressure wave is valid. The identification of the ‘‘characteristic point’’, where the reflected wave adds to the forward wave, is a critical step in the computation of AIx. For C-type waveforms (Fig), left panel), the reflected wave arrives late in systole, and there is an inflection point that occurs after the pressure has reached systolic pressure (69).In these cases, the augmented pressure is negative, and so is AIx. For A-type waveforms, the inflection point occurs prior to systolic pressure, and the reflected wave augments systolic blood pressure. AP is then positive, and so is AIx. Waveforms intermediate between type A and C can occur as well and the inflection point is then very close to or buried by the systolic peak. In early literature, the inflection point was visually identified on the waveform (69). Visual identification is however susceptible to subjective interpretation, which is why automated procedures have been developed, the most well known described by Takazawa et al. based on 4th order derivatives (115).The exact procedures embedded in commercial systems as the Sphygmocor (Atcor, Sydney, Australia) are not disclosed, but it is likely that they are equally based on higher order derivatives. We recently studied different automated algorithms and compared the estimated time of arrival of the reflected wave with the outcome of wave separation analysis (116). We found substantial differences between the different methods, and none corresponded with what we considered as the reference value of arrival of the reflected wave. In that same study we also explained a recent finding of Mitchell et al. who claimed that reflection sites shift away from the heart with age because of better matching of central

and peripheral impedance due to progressive stiffening of the central aorta (117) .We demonstrated that this is probably due to the poor definition of the timing of wave reflection using visual landmarks on the waveform(118). In the same work, we also had a closer look to the systematic finding that AIx is higher in women than in men, even after adjusting for physiological parameters such as height and heart rate. We speculated that this is also attributable to the poor definition of the time of arrival of the reflected wave (116), although more research is needed to further unravel this finding.

The drawback of AIx is that it is a composite measure which is sensitive to the magnitude of wave reflection, but also by many other factors affecting the timing of the reflected wave, such as the height of the subject, the heart rate (duration of systole), and the stiffness of the vessels(119). It has been suggested that, in order

to eliminate the heart rate effect, one could calculate AIx at a given heart rate, e.g. at 75 beats/min (also termed AIx@75). A linear regression equation has been derived within a population of 22 subjects with an implanted pacemaker, where it was shown that AIx (calculated from the Sphygmocor device) decreases by 3.9% for an increase in HR by 10 beats/min.24 An AIx of 32% for a heart rate of 62 bpm would translate into an AIx@75 of 32 - 3.9 (75 - 62) /10 = 26.9%. It is, however, always risky to apply a regression equation from one particular study to data measured in subjects who might be quite different from that population, with a different operator, potentially different equipment, etc. It is therefore probably safer to account for confounding factors by statistical means, i.e., by incorporating them into the statistical models as covariates.

At the same time, however, these confounding factors could also be seen as advantageous, because the net result of the reflected wave is largely determined by these timing factors and AIx quantifies the net result of wave reflection. The major problem with AIx was probably reported by McEneiry et al., who demonstrated that AIx tends to reach a plateau value of 50% around the age of 60, after which it does not further increase (120). As the amplitude of the reflected wave can never become more important than the amplitude of the forward wave, there is some logic in the levelling-off of AIx, but it is a bad feature of an index intended for clinical purposes that it loses its sensitivity beyond a certain age. Use of augmented pressure instead of augmentation index might resolve this problem. Finally, it can be mentioned that AIx can be derived from pressure curves measured at any location in the arterial tree. In recent years, people have shown the feasibility of assessing AIx from radial pressure tracings. Moreover, acceptable correlations have been shown between AIx measured at the radial and at the carotid artery. AIx measured on the radial artery and on synthesized central aortic pressure waveforms also show good comparison. (121), (122). These results are, to some extent, trivial since the aortic pressure waveforms were calculated from the radial pressure waves by means of a generalized transfer function (as with the Sphygmocor system). As such, correlations reach extremely high values (r = 0.94 - 0.96) and, as also stressed by Millasseau et al., it can be doubted whether using a transfer function to calculate central pressure waveforms has any added value at all (with respect to assessment of AIx) (121). Perhaps even more important is that changes in AIx induced by nitroglycerine and norepinephrine, also induced parallel changes in radial and central AIx (121).



Carotid pressure waveform is recorded by applanation tonometry.The height of the late systolic peak (P1) above the inflection (P2) defines the augmentation pressure, and the ratio of augmentation pressure to PP defines the AIx (in percent).

8.3 Central Blood Pressure

Due to the variable superimposition of incoming and reflected pressure waves along the arterial tree, aortic systolic and pulse pressure (i.e. the pressure exerted at the level of the heart, brain and kidney) may be different from the conventionally measured brachial pressure.

Furthermore, the claim has long been made that peripheral and central systolic and pulse pressures may be differently affected by antihypertensive drugs.

Furthermore, the results obtained in a large sub-study performed within a randomized trial have shown that central pulse pressure as assessed from the ‘‘augmentation index’’ is significantly related to cardiovascular events. However, the prognostic role of central as opposed to peripheral blood pressure needs to be further confirmed in more large-scale observational and interventional studies.

PULSE WAVE FORM ANALYSIS: COMPARATION OF DATA OBTAINED WITH TWO DIFFERENT METHODS.

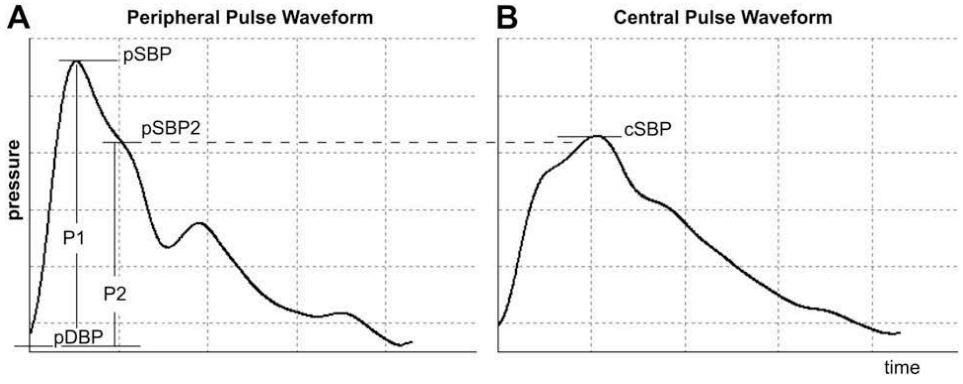
9. - AIMS

Introduction

Central aortic blood pressure has received in recent years more interest as a more accurate predictor of outcome than brachial artery blood pressure. However there is considerable variation in central blood pressures even between individuals with similar brachial blood pressure. Number of techniques exists to assess the aortic pressure. Aortic pressure can be measured directly by cardiac catheterisation but this method is invasive.

A commercially available device (SphygmoCor) records the radial pulse waveform using applanation tonometry, and then applies a transfer function to obtain the central aortic pressure waveform and a number of other indices, including central systolic blood pressure (cSBP). This estimated cSBP correlates strongly with data from direct invasive measurement of central blood pressure (11)

Central systolic blood pressure can also be estimated from the late systolic shoulder of the radial pulse waveform (pSBP2), without the use of a transfer function. Close correlation between pSBP2 and cSBP has been demonstrated (13),(14). A new device, the Omron HEM-9000AI utilizes this technique to estimate central systolic blood pressure with one important difference. pSBP2 is identified in the radial pressure waveform but a commercial algorithm is then applied to estimate the cSBP (14). The aim of this thesis is to compare the values of central systolic pressure and peripheral augmentation index calculated by the Omron and the SphygmoCor systems.



Relationship between peripheral pulse waveform and central pulse waveform. A, showing peripheral (radial) pulse waveform with peripheral systolic pressure (pSBP), peripheral late systolic shoulder ($pSBP_2$) and definitions of P1 and P2 as used to calculate peripheral augmentation index (pAIx). B, showing central (aortic) waveform with central systolic blood pressure (cSBP), corresponding in amplitude to $pSBP_2$.

Primary aim

- To compare the values of central systolic pressure and peripheral augmentation index in hypertensive patients calculated by the Omron and the SphygmoCor systems.

Inclusion criteria

- Aged ≥18 and
- Hypertension
- Antihypertensive Treatment

Exclusion Criteria

- Unstable angina before a period of stabilization,
- CHF
- Advanced A-V Blockade
- Arrhythmias
- Severe Hypertension.
- Known Arrhythmia
- Acute cases of Low Blood Pressure
- Insufficient peripheral circulation
- Diabetes
- Patients whose artery cannot be found by palpation

10. - Methods

Eighty-four (84) hypertensive subjects (40 males and 44 females), mean aged 58 ± 12 years were examined between February 2010 and June 2010 at the Hypertension Unit at San Luca Hospital (Istituto Auxologico Italiano) Milan Italia. All 84 subjects were treated with antihypertensive therapy. They had no history of

cardiovascular events, stroke, other serious disease or diabetes, and were in antihypertensive therapy. Ca Antagonist (42), ACEI (44), ARA II inhibitors (25), Diuretics (61), some of these patients were taken a convinated therapy. 16 Subjects were under statin therapy; 41 had history of familiar cardiovascular disease. 62 no smokers, 13 currently smoke, and 9 ex-smokers.

The two devices compared were the SphygmoCor® (AtCor Medical, Sydney, Australia) and the Omron® HEM-9000AI (Omron® Healthcare Co., Ltd., Kyoto, Japan). The late systolic shoulder (pSBP2; Fig. (above) represents the influence of the reflected wave, and is provided by both systems' software. The Omron system then estimates central systolic blood pressure (cSBPomr) from the pSBP2 value, using linear regression. The SphygmoCor® estimates central systolic pressure (cSBPsphy) from the aortic pressure wave, which is derived from the radial pressure wave using a generalized transfer function. Both devices use inbuilt software to derive peripheral augmentation index (pAIx) from the radial pulse waveform, and the same equation is used by both.

pAIx=(P2/P1)x100 where, P1=pSBP- pDBP (PP), P2=pSBP2-pDBP (AP)

Pulse Wave Analysis with SphygmoCor®

Radial artery wave forms at the right wrist were performed in the 84 subjects. Subjects were rested for at least 5 minutes prior to measurement, and were advised of not talk. All readings were made by one trained operator.

The subjects were examined on one visit. During the visit, two blood pressure measurements with consecutive radial pulse wave registrations were performed; the pulse wave was always registered immediately after the blood pressure measurement and the time elapsed was usually up to 30 s. Blood pressure was measured in accordance with the international recommendations, an oscillometric sphygmomanometer Omron® 705 IT, (Omron Corp., Matsusaka, Japan) was used.

Pulse wave was recorded on radial artery by the Sphygmocor® system (PWV Medical Ltd, Sydney, Australia) with the pencil-like Millar pressure tonometer. Using this method, pulse waves recorded during approximately a 10 s period are evaluated. From each peripheral pulse wave, a central wave is derived using a generalized transfer function. The pulse waveforms are the means of individual cycles. The quality of registration is determined according to homogeneity and height of the waves. The operator aimed to obtain recordings of optimal quality as defined by the manufacturer. The main quality control parameter is the Operator Index. The Operator Index is a number that is calculated from a weighting equation using the four qualities. The Operator Index range is 0 -100. As a general guide, if the Operator Index is 80 it is considered

acceptable. If the Operator Index is between 75-79 it is of borderline quality, and analysis of the Quality Control Indices should be undertaken to determine if the recording is acceptable. If the Operator Index was under 74 scale the recording was unacceptable and was repeated.

Pulse Wave Analysis Omron® HEM-9000AI

Immediately after of the pulse wave analysis with Sphygmocor® device, the pulse wave analysis with Omron HEM-9000AI device was performed in all the 84 subjects.

AI measurement with Omron HEM-9000AI device is intended only for adults with a wrist circumference of 10-19 cm around the radius protrusion. We adjusted the height of the chair to align the height of the wrist with the height of the heart. By sitting back and keeping the back straight, the arm will naturally be straight. The AI pulse wave measurement unit was placed on a flat, horizontal (non-sloping) surface. The elbow was placement board of the AI pulse wave measurement unit according to the length of the arm of the patient. The elbow placement board was extended approximately 10 cm the patient was instructed to relax and not to move during measurement.

The wrist rest has a slope of approximately 30 degrees. When putting one's hand along the slope of the wrist rest, the wrist goes up by a slope of approximately 30 degrees.

We selected the blood pressure cuff according to the circumference of the patient's arm. The medium size adult arm cuff (22 - 32cm) was used for all the subjects because all was in this size range.

The measurement was performed in about a minute, Aix, central aortic pressure and brachial blood pressure was calculated at the same time, brachial blood pressure was used to calibrate and calculated the AiX and the central aortic pressure.

The non-invasive radial arterial pulse wave measurement of Omron® HEM-9000AI, system consists of a tonometry sensor unit, radial pulse measurement unit, and a Main Unit (display unit). The sensor unit has a pressure sensor consisting of an array of 40 micro transducer elements. As one of these 40 sensor elements is automatically selected to obtain optimal radial arterial pressure waveforms, this method is thought to be a more objective approach. Signals of the ascending aortic and radial arterial pulse waves were low pass-filtered at a cut-off frequency of about 25 Hz and 105 Hz, respectively. Inflection points or peaks that corresponded to early and late systolic blood pressure were obtained by multidimensional derivatives of the original pressure pulse waveforms. The measurement was repeated if the AiX Standard deviation was less of 6.0.

As an index of wave reflection, AI was defined as the ratio of the height (P2/P1) of the late systolic shoulder/peak (P2) to that of the early systolic shoulder/peak (P1) the maximal systolic blood pressure (r-SBP) and diastolic pressure in the radial artery were corrected to the brachial systolic blood pressure and brachial diastolic blood pressure, respectively. The late systolic blood pressure in the radial artery (r-SBP2) was calculated by the following equation: r-SBP2 [mmHg] = (P2/PPh) X (SBP [mmHg] −DBP [mmHg]) + DBP [mmHg] where PPh, wave height corresponding to radial arterial pulse pressure; SBP and DBP, brachial systolic and diastolic blood pressures, respectively.

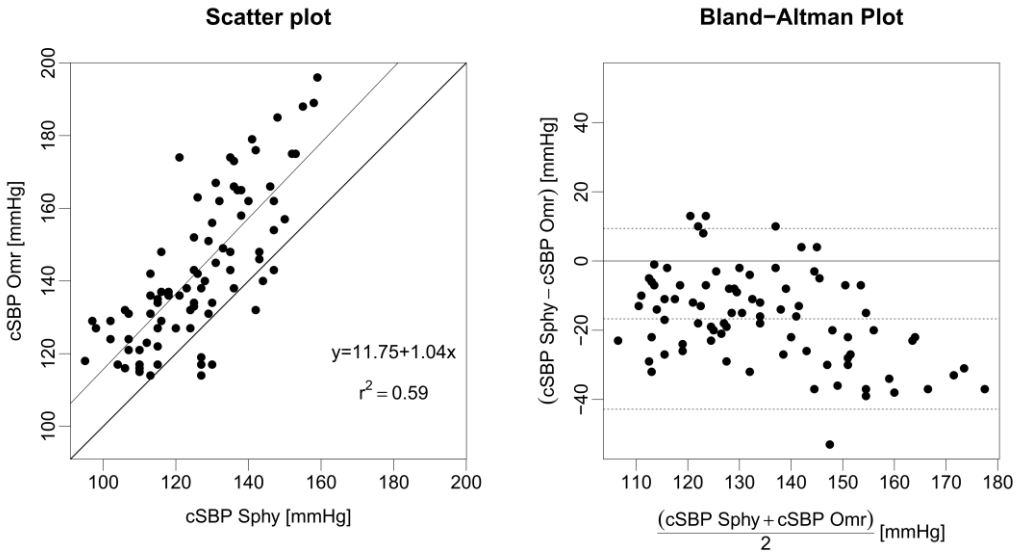
11. - Results

Statistics

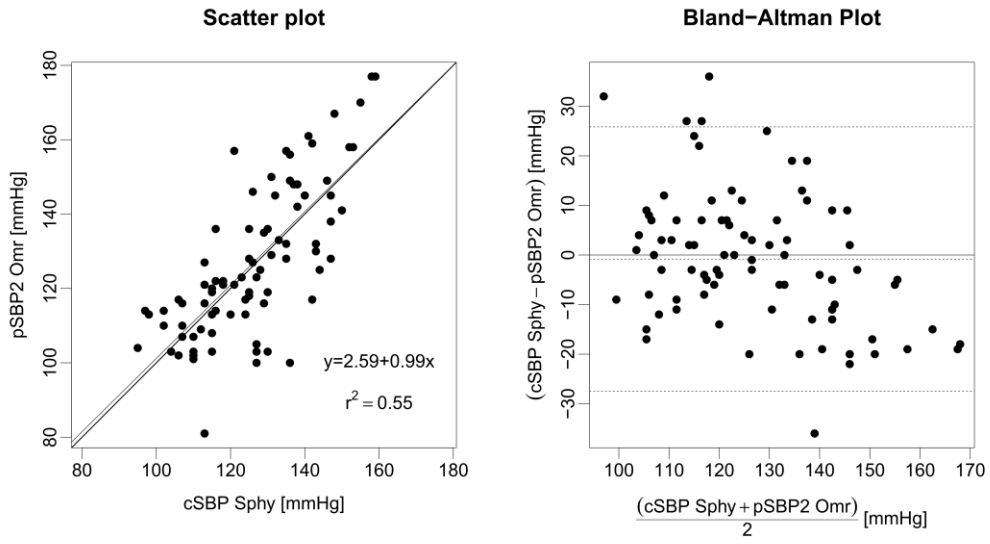
Data were analyzed using Statistica software version 9.0 (StatSoft Tulsa, Oklahoma. Inc). Pearson product–moment correlation coefficient (r) was used to determine associations between variables. Bland–Altman plots were used to assess agreement between methods. Dependent t tests were used to compare means.

1) Comparison between central pressure values provided by Sphygmocor and by Omron.

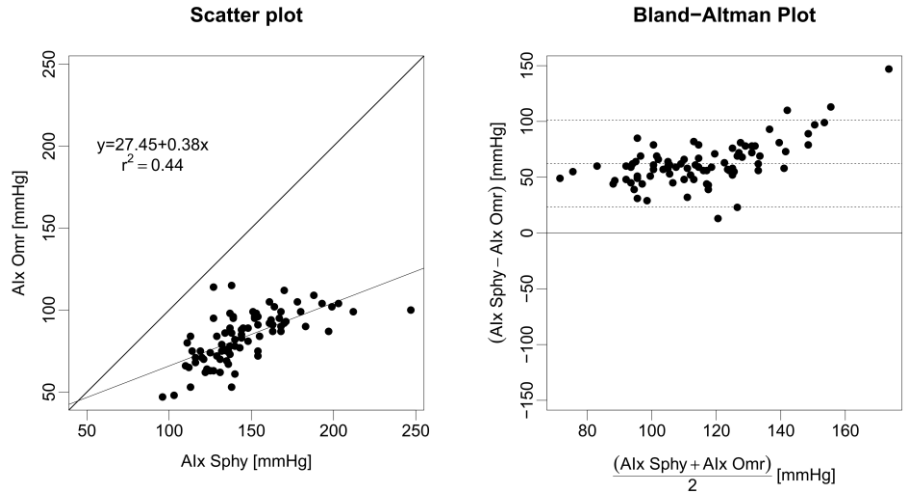
There was a good correlation between cSBPsphy and cSBPomr (r=0.76; r^2=58, P=< 0.001), but with a mean difference of -16 ± 13 mmHg, indicating a systematic underestimation by Omron device



2) Comparison between cSBPsphy and pSBP2omr values showed also a good correlation, r= 0.74; r²=55, P <0.001, with a mean difference of only -0.8 ± 13 mmHg, indicating a good mean agreement



3) Peripheral augmentation index measured by both devices showed close correlation (r=0.66; r²= 43 P < 0.001) but a mean difference Sphy-Omr of 62 ± 19, indicating important overestimation by the Sphygmocor.



12. - Discussion

Many papers have recently reported, aortic systolic blood pressure holds important predictive value for calculating future cardiovascular events, independently of brachial pressure (123). McEniery et al. (12) demonstrated that for a given level of brachial systolic pressure, aortic systolic pressure was higher in those

individuals with cardiovascular risk factors or disease compared with healthy subjects. If measurement of central pressure is to become routine, it is important to be able to perform measurements quickly and non-invasively in large populations. The Omron device is semi-automated, and performs applanation tonometry through an array of 40 sensors applied to the radial pulse in the wrist. This measures the radial pulse waveform and calculates cSBP and pAIx. The findings suggest that the late systolic shoulder of the radial pressure wave (pSBP2) may provide a better estimate of aortic systolic pressure than the estimated cSBP value provided by the Omron device.

Use of pSBP2 as a direct estimate of aortic systolic pressure has been validated against invasive measurements. (13),(14).

The mean difference between pSBP2 and aortic systolic pressure was 1.8 ± 4 mmHg in the study by Munir et al.,6 and 1 ± 2 mmHg in the study by Pauca et al. (17). However, using the Omron device, Takazawa et al. (14) observed that although pSBP2 and aortic systolic pressure were highly correlated, the mean difference between them was 11.7±7.1 mmHg. The Omron system extrapolates the pSBP2 to provide an estimate of cSBP, using linear regression. When comparing the values of the late systolic shoulder of the radial pulse wave identified by both devices (pSBP2sphy and pSBP2omr).

When comparing the original pSBP2omr values with the cSBP calculated by SphygmoCor, the mean difference was only -0.8 ± 13 mmHg. As we did not make invasive measurements of aortic pressure, it is impossible to know whether cSBPomr or cSBPsphy is closer to the true aortic SBP. However, the SphygmoCor's system of estimating cSBP has been validated against invasive measurements (17),(16) and it is therefore likely to be reliable. Clearly this is not correct as peripheral pressure amplification increases brachial pressure values above those in the aorta, as has been confirmed by invasive data (17). As pSBP2omr is very closer to cSBPsphy than the estimation of cSBPomr, use of the conversion factor to obtain cSBPomr seems unnecessary. Further comparison with direct invasive measurements of aortic pressure may yield a more appropriate algorithm to convert pSBP2omr to cSBPomr. In this way to estimate central arterial pressure accurately using the Omron device, investigators should use pSBP2 rather than the estimated value of cSBP.

Peripheral augmentation index is calculated by the same formula in both devices, there was a good correlation between the values of pAIx calculated by each device ($r=0.66$; $r^2= 0.43$, $P=< 0.001$), but a very poor agreement.

In summary estimated cSBP provided by the Omron system good correlation and limited agreement with values obtained from the SphygmoCor system.

Conversely cSBP with Sphygmocor showed good agreement with pSBP2 measured by OMRON.

Further investigations should compare estimates of pSBP2 by the Omron system to direct measurements of aortic pressure by cardiac catheterisation. The results suggest that the Omron system has potential for use in large scale trials, giving accurate, of pSBP2 which show strong correlations with those of the SphygmoCor device

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