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THE IMPACT OF ARTERIOVENOUS FISTULAS ON AORTIC STIFFNESS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Résumé

Contexte. La création d'une fistule artérioveineuses (FAV) chez les patients atteints d'insuffisance rénale chronique (IRC) a des effets néfastes sur le profil central de l'onde de pouls, suggérant l'augmentation de la rigidité artérielle. Le but de la présente étude est d'évaluer de manière prospective l'effet de la création d'une FAV sur la rigidité artérielle.

Méthode. Trente et un patients atteints d'IRC stade 5 ont subi une évaluation hémodynamique avant et 3 mois après la création d'une FAV. La pression artérielle (PA), l'analyse centrale et carotidienne du profil de l'onde de pouls et la vitesse de l'onde de pouls (VOP) carotido-fémorale et carotido-radiale ont été étudiées. Le test-t de Student et le test de Wilcoxon ont été utilisés pour comparer les paramètres hémodynamiques pré-FAV et post-FAV , le cas échéant. Pour déterminer l'association entre les variables, des corrélations de Pearson ainsi que des régressions linéaires simples et multiples ont été utilisées.

Résultats. Après la création de la FAV, la PA périphérique et la PA centrale ont diminué, sans changements significatifs de la fréquence cardiaque (FC) ou de la pression pulsée. La VOP carotido-fémorale (VOPc-f) a diminué de 13,2 ± 4,1 à 11,7 ± 3,1 m/s ($P < 0,001$). L'indice d'augmentation centrale a monté de 20,8 % ± 11,5 à 23,7 % ± 11,6, à la limite de la signifiance statistique ($P = 0,08$). Le ratio de viabilité sous-endocardique a diminué de façon significative (153 % ± 34 versus 143 % ± 32, $P < 0,05$), principalement comme conséquence de la diminution de l'indice de temps de la pression diastolique (ITPD), sans modification significative de la durée diastolique. La réduction de la VOPc-f s'explique par les changements de la PA moyenne et de la FC ($R^2 = 0,29$). La réduction de l'ITPD était liée à des changements de la PA diastolique centrale et de la PA fin systolique centrale ($R^2 = 0,87$). L'amélioration significative de la rigidité aortique est principalement le résultat de la réduction relative de la VOPc-f dans le sous-groupe de patients ayant une valeur basale de la VOPc-f supérieure à la valeur médiane de 13 m/s.

Conclusion. La création de la FAV est associée à une amélioration passive de la rigidité aortique, en particulier chez les patients avec artères plus rigides. Cette amélioration de la rigidité artérielle pourrait être bénéfique pour le système cardiovasculaire.

Abstract

Background. The creation of arteriovenous fistulas (AVF) in patients with advanced chronic kidney disease (CKD) has been shown to have adverse effects on their central pulse wave profile suggesting a likely increase in arterial stiffness. The aim of the present study was to directly evaluate the effect of AVF on arterial stiffness.

Method. Thirty-one stage-5 CKD patients underwent haemodynamic assessment prior to and 3 months after creation of AVF. Haemodynamic assessment included measurement of blood pressure (BP), central and carotidal pulse wave profile analysis, and carotido-femoral and carotidoradial pulse wave velocities (PWV). Pre-AVF and post- AVF haemodynamic parameters were compared using the Wilcoxon signed-rank test or the paired Student t-test as appropriate. Pearson correlations, single and multiple linear regressions were used to determine the association between variables.

Results. After creation of AVF, peripheral and central BPs decreased without significant change in heart rate (HR) or pulse pressure. Carotido-femoral PWV (c-fPWV) fell from 13.2 ± 4.1 to 11.7 ± 3.1 m/s ($P < 0.001$). There was an increase in the central augmentation index (20.8% ± 11.5 versus 23.7% ± 11.6, $P = 0.08$) of borderline significance, and a significant reduction in the subendocardial viability ratio (153% ± 34 versus 143% ± 32, $P < 0.05$), which was mainly the result of a decrease in the diastolic pressure time index (DPTI) without any significant change in the diastolic duration. The reduction of c-fPWV was explained by changes in mean BP and HR ($R^2 = 0.29$). The reduction in DPTI was related to changes in central diastolic BP and changes in end-systolic BP (adjusted $R^2 = 0.87$). The significant improvement in aortic stiffness was mostly the result of the relative reduction of c-fPWV in the subgroup of patients with baseline c-fPWV above the median value of 13 m/s.

Conclusion. The creation of AVF is associated with a passive improvement of aortic stiffness especially in patients with stiffer arteries. This improvement in arterial stiffness could potentially be beneficial to the cardiovascular system

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You cannot walk straight when the road bends...
- old gipsy proverb -

I will always be grateful to my mentor,
for helping me to walk straight when my road bent.

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CHAPTER 2

Abbreviations

AGEs - advanced glycation end products

AIx - augmentation index

Ap - augmented pressure

APD - automated peritoneal dialysis

AVF - arteriovenous fistulas

AVG - arteriovenous synthetic grafts

BP - blood pressure

CAD - coronary artery disease

CAPD - continuous ambulatory peritoneal dialysis

caPWP - carotid artery pulse wave profile

c-fPWV - carotido-femoral pulse wave velocity

CKD - chronic kidney disease

CKD-MBD) - Chronic kidney disease - mineral and bone disorder

cPWP - central pulse wave profile

c-rPWV - carotido-radial pulse wave velocity

CVC - central venous catheters

DD - diastolic duration

DPTI - diastolic pressure time index

ECM - extra cellular matrix

ED - ejection duration

EPO – erythropoietin

ESRD - End Stage Renal Disease

GFR - Glomerular Filtration Rate

HD - hemodialysis

HR - heart rate

Kf - glomerular capillary filtration coefficient

MBP - mean blood pressure

MW - molecular weight

NO - nitric oxide

pPWP - peripheral pulse wave profile

PTH - parathyroid hormone

PWA - pulse wave analysis

PWV - pulse wave velocity

RAGE - AGEs specific receptors

RBC - red blood cell

SEVR - subendocardial viability ratio

SPTI - systolic pressure time index

Tr - time of wave reflection

Introduction

The end-stage renal disease population is growing worldwide. In Canada, there are almost 37,000 patients that need renal replacement therapy in order to survive and each year, approximately 5,000 new patients become a part of this population. The best treatment that can be offered, regarding the survival and the quality of life, is renal transplantation. Unfortunately, as the organ supply for transplantation is limited, dialysis remains the main modality of renal replacement therapy.

Although dialysis improves the quality of life and essentially keeps these patients alive, the 5-year survival rate is approximately 40%. The extremely increased mortality rate is mostly related to cardiovascular disease that cannot be solely explained by the traditional cardiovascular risk factors. An increased aortic stiffness associated with altered central hemodynamics parameters was proved to be a predictor of all-cause and cardiovascular mortality in dialysis population. Consequently, the aortic stiffness determinants in end stage renal disease represents a matter of interest as the reversal of this process may positively influence the survival of these patients. The invasive process of hemodialysis (HD) session, the metabolic disturbances like uremia and altered mineral metabolism are some of the obvious processes that could play a role in the aortic stiffening. However, in the present research project we take into account that a large part of the HD population has another important particularity: the presence of an arteriovenouse fistula (AVF) used for vascular access. The AVF are considered to be the vascular access of choice in HD, as there is evidence of decreased cardiovascular mortality in patients utilizing an AVF when compared to other types of long-term vascular access[1].

The National Kidney Fundation (USA) recommends that at least 50% of incident HD patients should have an AVF at the beginning of therapy and at least 40% of prevalent patients undergoing hemodialysis should use a fistula for vascular access. In the USA, a prevalence of almost 55% was reached at the beginning of 2010. In Canada, the Canadian

Society of Nephrology Clinical Practice Guidelines (1999) state that more than 60% of prevalent HD patients should be hemodialysed by an AVF fistula. As approximately 50% of end stage renal disease population is treated by HD, it is obvious that there is a large number of patients with a functional AVF and that this number will continuously grow.

The national and international guidelines that strongly encourage the use of an AVF as vascular access in HD are based on solid evidence of the improved morbidity and mortality in patients being hemodialysed by an AVF when compared with other types of vascular access. However, several studies and numerous case reports showed that AVF are associated with altered central cardiovascular parameters, left ventricular hypertrophy, high-output cardiac failure, exacerbation of coronary ischemia and decreased subendocardial perfusion. Such findings raised questions about the hemodynamic influences of an AVF in a population with 10 to 30 fold increase in cardiovascular mortality when compared to general population.

Previous studies suggested, by indirect evidence, an increased aortic stiffness after AVF creation. Hence, it was of concern that this procedure could have a negative influence on a hemodynamic parameter that was proved to be an all-cause and cardiovascular mortality predictor. The aim of our study was to investigate the influence of an AVF creation on aortic stiffness and central hemodynamic parameters in a longitudinal study. In fact, it was for the first time that the 'gold standard' for measuring aortic stiffness (which is carotido-femoral pulse wave velocity) was studied before and after AVF creation in chronic kidney disease patients.

CHAPTER 1

THEORETICAL NOTIONS

1. Anatomy and Physiology of the kidneys

The major role of the kidneys in human body is to excrete metabolic waste products, to regulate the body concentration of water and salt, to ensure the acid-base equilibrium and to serve as an endocrine organ by secreting hormones. The decline in kidney function can be classified depending on the time interval within which this failure occurred. A sudden decline in function leading to the accumulation of waste products and disturbances in volume, electrolytes and acid-base balance has been traditionally described as acute renal failure; recently, this term was proposed to be replaced in the literature[2] with **acute kidney injury**, diagnosed on the basis of clinical history and laboratory findings[3]. A gradual, progressive loss of renal function is known as **chronic kidney disease (CKD)** characterized by disturbances of all renal functions, including full-blown metabolic disturbances as a consequence of endocrine function loss.

In this subchapter, a very short review of renal anatomy and physiology introduces the definition, classification, epidemiology and causes of CKD. By understanding the metabolic consequences of renal function loss and the renal replacement therapies, the reader will become familiar with the conditions of the patients participating in our study. As we will see later, the increased cardiovascular morbidity and mortality in this population completely justify the need for a better comprehension of the central hemodynamics and the factors that could influence it, including arteriovenouse fistulas.

1.1. Anatomy and structure of the kidney

The kidneys are paired organs situated behind the peritoneum (retroperitoneal) on each side of the vertebral column. Each kidney weights approximately 150g and is about 12cm in length. On the medial side of the bean shaped parenchyma there is the renal hilus containing the renal artery and vein, the ureter, lymphatics and nerve supply. A tough, external fibrous capsule protects the inner structure of the kidneys.

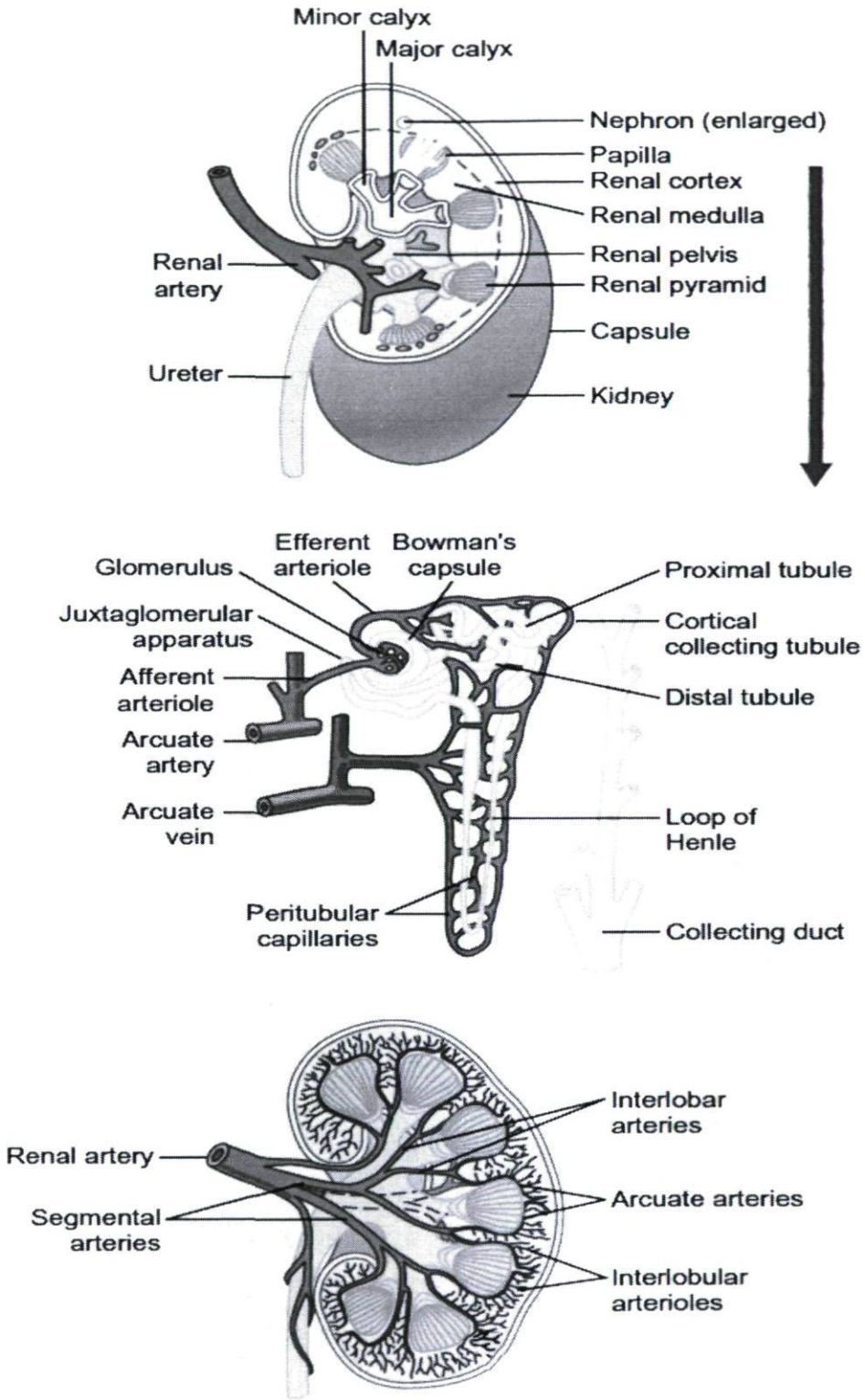


Fig.1.1. Schematic section of the human kidney, nephron representation and renal arterial supply showing the major vessels and the microcirculation of each nephron. ***Image adapted from Guyton[4].***

Macroscopically (Fig. 1.1), on a transversal section of the kidney, we can visualize two regions: an outer, darker, 1.2-1.5 cm in thickness region called **cortex** and an inner, paler region called **medulla**; renal medulla is divided into multiple pyramidal structures representing the **renal pyramids**; each pyramid originates near the cortex and terminates in a **renal papilla** which collects urine in the **minor calyces** (about 12 in each kidney) that will form open-ended pouches called **major calyces**. The major calyces organize in a funnel-shaped structure, the **renal pelvis** that continues with the **ureter** (a tubular structure responsible for transporting urine to the bladder).

Microscopically (Fig.1), each renal parenchyma contains approximately 1 million morphofunctional units called **nephrons** that are present from birth and cannot be regenerated. With normal aging or kidney injury, there is a gradual decrease in nephron number (after age 40, their number physiologically decreases by 10% every 10 years). The nephron contains a **glomerulus** formed by a tuft of capillaries surrounded by an epithelial capsule (Bowman's capsule) and a long epithelial **tubule** (proximal, loop of Henle and distal tubule), which joins the tubules of other nephrons in **collecting ducts**. The glomeruli (found in the cortex) function as a blood filter and the tubules (found in the cortex and medulla) transform the glomerular filtrate in urine.

The main **renal artery** (Fig. 1.1) is divided into anterior and posterior segments; from these, **interlobar arteries** form the **arcuate arteries** that give rise to **interlobular arteries**. From interlobular arteries, **afferent arterioles** emerge and form the glomerular tuft (20-40 capillary loops) that merges and exits the glomerulus as **efferent arterioles**. Efferent arterioles from superficial nephrons (cortex) form a capillary network that encircles cortical tubules (**peritubular vascular network**); the efferent arterioles of juxtamedullary nephrons (20-30% of the nephrons) will form the **vasa recta**, which supplies the outer and deeper medulla. Thus, the renal vasculature forms a unique, specialized type of capillary network characterized by the sequence arteriole-capillary-arteriole, known as the **arterial port system.**

Approximately two thirds of the renal blood flow is received by the cortex and one third by the medulla; the high cortex flow assures the glomerular filtration and the medullar flow maintains the reabsorption of water and electrolytes from the tubules.

1.2. Renal blood flow and hemodynamics

Contrary to most organs of the human body where the main purpose of the blood supply is to ensure oxygenation, in the kidney, the main purpose of the blood flow is to maintain the hydroelectrolytic balance and to ensure the expel of metabolic wastes. The completion of this task requires the largest distribution of cardiac output to an organ: 20 to 25% of the cardiac output[5] or 1000ml/min for an average 70 kg man. This considerable demand in blood flow is explicable by a perfusion rate of approximately 4000ml/kg*min, ten to five times higher than in the coronary territory or exercising muscle, perfusion rate surpassed only by the thyroid tissue[6].

In the human body, the organs compete for blood flow and the result of this competition depends upon their respective flow resistances. As the kidney has a very low resistance / gram of tissue, it succeeds to ``attract`` a significant percentage of the cardiac output. From a hemodynamic point of view, it represents a low resistance, high flow system[7]. The explanation of the kidney's low vascular resistance resides in the parallel arrangement (Fig.1.2) of a tremendous number of glomeruli within the renal arterial tree. Contrary to a series arrangement of resistance (Rt=R1+R2+...+Rn), in parallel arrangement (1/Rt=1/R1+1/R2+...+1/Rn), the total resistance of the system lowers with the addition of components.

In the kidney, 1 million glomeruli arranged in parallel assure a very low resistance. The loss of glomeruli from any causes (aging or kidney injury) leads to increased resistance of the renal vasculature with repercussions on total peripheral resistance.

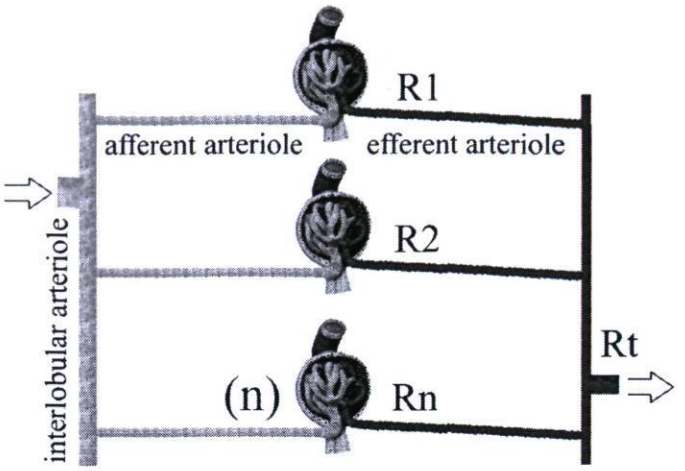


Fig.1.2. Schematic representation of the parallel arrangement of glomeruli in the renal circulation. Total resistance of the system (Rt) and resistance of one glomerulus (R1,2,n). ***Original figure.***

The afferent and efferent arterioles control (by vasoconstriction / vasodilatation) the glomerular flow. Under physiologic conditions, the mechanism of renal autoregulation changes the arteriolar resistance so that blood flow is constantly preserved within a wide variation of perfusion pressure (80–180 mmHg).

1.3. Urine formation and Glomerular Filtration Rate (GFR)

The urine formation begins with the process of glomerular filtration, continues with tubular reabsorption and secretion and ends with the accumulation in the bladder, ready to be discarded by micturition. As the purpose of this text is not to give an extensive review of the renal physiology but rather to explain the pathogenesis and the classification of CKD, we will pay particular attention to the filtration process, which has a central role in the classification of CKD.

The glomerular filtration represents the first step of the renal excretory function and can be quantitatively expressed as Glomerular Filtration Rate (GFR) in milliliters/minute. In normal conditions, of the approximately 1500L of blood received daily (more than half of this volume representing plasma, depending of hematocrit), 180L of acellular fluid (20% of

the plasmatic, free volume passing through the Bowman's capsule) are filtered by the glomeruli and delivered to proximal tubules. The quantification of renal filtration is a measure of volume in time and is expressed as: GFR = volume of filtered plasma by the glomerular capillaries / time. Another way to express GFR is by multiplying the glomerular capillary filtration coefficient (Kf) with the pressure (hydrostatic and osmotic) difference between the capillary lumen and Bowman's capsule (Δp). Kf can be defined as the quantity of liquid able to be filtered in one minute by the sum of glomerular capillaries when applying a pressure difference of 1mmHg. It becomes clear that GFR is dependent of Δp, the number of glomerular capillaries and the permeability of the capillary wall. **In normal condition** the kidney is able to maintain Δp constant at approximately 10mmHg. In such conditions, **GFR is a measure of the number of healthy perfused glomeruli**.

The most simple and utilized method in the clinical practice for measuring the GFR is the estimation based on serum creatinine concentration. Creatinine is an endogenous substance produced by muscle and eliminated by the kidneys. The renal creatinine excretion is composed of filtration through the glomerulus (80%) and tubular secretion (20%), without any tubular reabsorption.

Equations for Predicting GFR in Adults Based on Serum Creatinine Concentration

Abbreviated MDRD study equation:

$$GFR\ (mL\ per\ minute\ per\ 1.73\ m^2) = 186 \times (S_{Cr})^{1.154} \times (Age)^{0.203} \times (0.742\ if\ female) \times (1.210\ if\ black)$$

Cockcroft-Gault equation:

$$C_{Cr}\ (mL\ per\ minute) = \frac{(140 - age) \times weight}{72 \times S_{Cr}} \times (0.85,\ if\ female)$$

Table 1.1. Equations for predicting GFR in adults based on serum creatinine concentration. Glomerular filtration rate (GFR); Modification of Diet in Renal Disease (MDRD); serum creatinine concentration (Scr); creatinine clearance (Ccr). For each equation, SCr is in milligrams per deciliter, age is in years, and weight is in kilograms. ***Table adapted from Johnson, C.A., et al.[8].***

Therefore, serum creatinine concentration is a marker of kidney function and is used to estimate GFR by the abbreviated MDRD formula and/or Cockcroft-Gault equation with the condition that creatinine serum concentration is stable (Table 1.1). The normal values are around 120ml/min/1.73m^2.

1.4. Regulation of water, electrolyte balance and blood pressure

The renal function assures the homeostasis of water and electrolytes in the organism. Homeostasis is a term referring to the capacity of a system to maintain constant certain biological parameters. For completing this task, the kidneys must match the intake (drinking and eating habits) with the urinary excretion. Renal function loss or alteration leads to accumulation or depletion of water and electrolytes with serious consequences on the cardiovascular, nervous and muscular systems.

The kidneys play the most important role in the regulation of blood pressure. **The long-term control** is accomplished by excreting variable amounts of water and sodium, establishing the intravascular and extravascular volume. When the body contains too much fluid, the blood volume and pressure will raise and, in normal conditions, the kidney will excrete this excess (pressure diuresis). Conversely, when blood pressure falls below the needs of the organism, the kidneys conserve the body water until a new equilibrium is reached. The kidneys also contribute to the **short-term regulation** of blood pressure by secreting vasoactive substances acting on the entire peripheral circulation. As mentioned above, the variation of renal vascular resistance (acute, in response to physiologically stimuli or chronic by glomerular loss) influences the total peripheral resistance with repercussions on blood pressure.

1.5. Metabolic functions of the kidney

Regulation of acid-base equilibrium – The control of blood pH within the range of 7.35-7.45 is realized by the regulation of hydrogen ion (H^+, acid) concentration which is dictated by the ratio of CO_2 partial pressure (PCO_2) and the plasmatic bicarbonate concentration (HCO_3^-, base). The lung controls PCO_2 in a range of 40mmHg and the

kidney is responsible for maintaining the HCO_3^- concentration within a range of 24-26 mEq/L. Each day, about 1mEq/kg of nonvolatile acid is produced by the protein metabolism, acid that will be fixed and buffered by plasmatic HCO_3^- (which is "lost" from the organism). In order to be able to maintain a constant plasmatic HCO_3^-, the kidney must reabsorb from the tubules all of the filtered HCO_3^- and replace all the loses by producing HCO_3^-. New HCO_3^- formation in the kidney is dependent of its H^+ secreting capacity: for each H^+ lost in the urine a new molecule of HCO_3^- is gained. The kidney secrets H^+ by two means: by utilizing an H^+ATPase pump and by hydrolyzing glutamine (which will generate ammonia, NH_4^+, excreted in the urine). The NH_4^+ formation is quantitatively more important than the active secretion of H^+ by the H^+ATPase pump and also represents the main renal response to acidosis.

Erythrocyte production – Erythrocytes or red blood cell (RBC) synthesis is hormonally controlled by erythropoietin (EPO). The kidney accounts for EPO secretion and controls the production of RBC. In physiological conditions, hypoxia represents an important stimulus for EPO secretion. In the context of renal function loss, the lack of EPO production leads to anemia.

Vitamin D production – The kidneys produce the active form of vitamin D, 1.25-dihidroxycholecalciferol under the stimulation of parathyroid hormone (PTH) and under the control of plasmatic calcium levels. Vitamin D is one of the key players in the homeostasis of mineral metabolism: it promotes intestinal calcium and phosphate absorption and favors (in small, physiologically doses) bone calcification. Its principal actions target the maintaining of a normal, healthy bone. On the other hand, PTH is a hormone whose principal actions target the maintaining of a normal calcemia: it increases calcium and phosphate mobilization from the bone, decreases calcium excretion and increases renal phosphate excretion. In the context of renal function loss, there is a reduced production of 1.25- dihydroxycholecalciferol leading to hypocalcemia.

2. Chronic Kidney Disease and End Stage Renal Failure

2.1 Definition and Classification

Chronic kidney disease is defined by the National Kidney Fundation[9] as GFR less than 60 mL/minute/1.73m2 for more three months, with or without kidney damage or kidney damage for more than three months with or without decreased in GFR. Kidney damage is defined as a structural or functional abnormality of the kidney, manifested by pathologic abnormalities or markers of kidney damage, including abnormalities in the composition of the blood or urine or abnormalities in imaging tests.

A GFR less than 15ml/min/1.73m^2 is known as End Stage Renal Disease (ESRD) or stage 5 CKD. In this situation, the organism is incapable to maintain its homeostasis; these patients need renal replacement therapies in order to survive.

Classification of Chronic Kidney Disease

Stage	*Description†*	*GFR (mL per minute per 1.73 m^2)*
1	Kidney damage with normal or elevated GFR	≥ 90
2	Kidney damage with mildly decreased GFR	60 to 89
3	Moderately decreased GFR	30 to 59
4	Severely decreased GFR	15 to 29
5	Kidney failure or End Stage Renal Disease (ESRD)	< 15 (or dialysis)

Table 2.1. National Kidney Foundation classification of Chronic Kidney Disease. ***Table adapted from Johnson, C.A., et al.[10].***

2.2 Renal replacement therapies

As kidneys fail, the excess of water and sodium, the acidosis and the accumulation of a myriad of uremic toxins lead to uremia. At this stage, patients start to lose appetite, have fluid overload, heart failure, encephalopathy, coagulopathy and other organ dysfunctions. If left untreated, patients will inevitably die of renal failure. Renal replacement therapy can be achieved by kidney transplantation, peritoneal dialysis or hemodialysis.

Kidney transplantation gives the best chance of survival and a better quality of life. Unfortunately, the lack of kidneys availability for transplantation somehow forces patients to receive dialysis.

Dialysis treatment is based on uremic toxins diffusion across a semi-permeable membrane. **Peritoneal dialysis** requires the insertion of a catheter into the peritoneal cavity. The peritoneum serves as the dialysis membrane for uremic toxins clearance. The patients usually require between 10-15 L of fluid per day in order to achieve an adequate blood clearance.

Hemodialysis is an extracorporeal method of uremic toxin epuration. The patient's blood passes through a semi-permeable synthetic filter, while a dialysis fluid passes through the dialysate compartment. The uremic toxins leave the patients blood by diffusion across the membrane into the dialysate compartment, which is then eliminated. Usually, the standard hemodialysis treatment is performed three times per week and each session lasts about 4 hours.

2.3 Epidemiology of CKD and ESRD

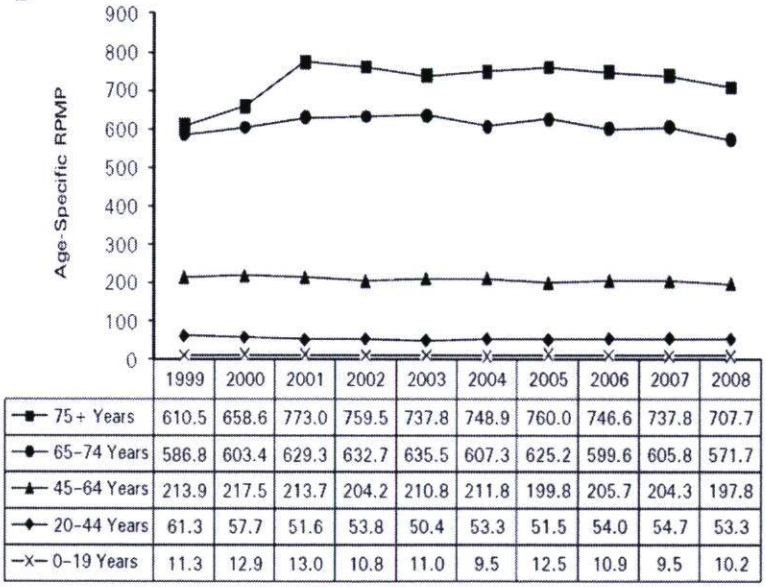
Chronic kidney disease has become a major public health problem worldwide[11]; its incidence and prevalence have constantly raised in the developed countries principally due

to the aging of the general population and the emergence of diabetes and hypertension as the principals causes of CKD[12, 13]. In a review[14] of 26 population-based studies originating from North America, Australia, Europe and Asia, the prevalence of CKD was 7.2% in the group of 30 years of age or older and varied from 23.4% to 35.8% in the group of 64 years of age or older. In the following text, we will discuss the incidence and prevalence of ESRD in Canada[15] as it is relevant to our study.

A

Age Group		1999	2008
0–19 Years	N	90	80
	%	2.0	1.5
20–44 Years	N	717	622
	%	15.8	11.5
45–64 Years	N	1,483	1,827
	%	32.6	33.6
65–74 Years	N	1,253	1,376
	%	27.5	25.3
75+ Years	N	1,008	1,526
	%	22.2	28.1
Total	**N**	**4,551**	**5,431**

B



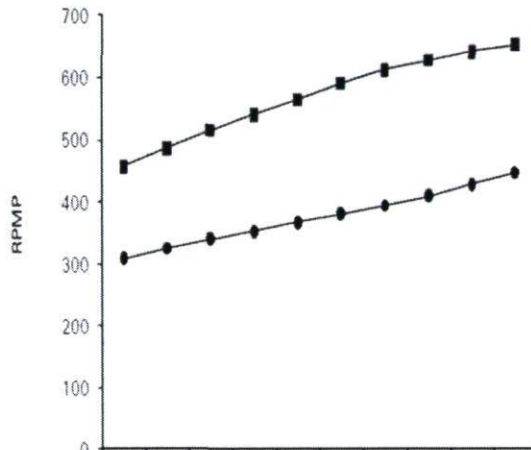
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
75+ Years	610.5	658.6	773.0	759.5	737.8	748.9	760.0	746.6	737.8	707.7
65–74 Years	586.8	603.4	629.3	632.7	635.5	607.3	625.2	599.6	605.8	571.7
45–64 Years	213.9	217.5	213.7	204.2	210.8	211.8	199.8	205.7	204.3	197.8
20–44 Years	61.3	57.7	51.6	53.8	50.4	53.3	51.5	54.0	54.7	53.3
0–19 Years	11.3	12.9	13.0	10.8	11.0	9.5	12.5	10.9	9.5	10.2

Fig.2.1 (A) Distribution of Incident End-Stage Renal Disease Patients by Age Group, Canada, 1999 and 2008 (Number, Percent of Total); (B) Incident ESRD patients, age-specific rate per million population, Canada, 1999 to 2008. Rate per million population (RPMP). Sources: Canadian Organ Replacement Register, 2009, Canadian Institute for Health Information and Statistics Canada.

Between 1999 and 2008, the **incidence** of ESRD increased by 19%, from 4,551 to 5,431 patients. The highest number of new patients belonged to the group aged 45 to 64 years (33.6% in 2008, Fig. 2.1A). The highest age-specific incidence rate throughout this period was observed in the 75 years of age and older group. As showed in Fig. 2.1B, it reached a maximum in 2001 and then, in 2005, started to slowly decline. In the 20 to 44 years of age group, the incidence rate of ESRD declined by 13% (Fig. 2.1A).

The **prevalence** of ESRD in Canada steadily increased since 1999 (Fig. 2.2). Between 1999 and 2008, there was a 43% increase in the prevalent rate for dialysis and a 45% increase in the rate of kidney transplant patients. In 2008, 36,638 patients were treated for ESRD: 48% receiving hemodialysis, 41% with a kidney transplant and 3,989 treated with peritoneal dialysis.

A



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Dialysis	457.0	486.1	516.1	540.9	565.4	591.2	613.5	629.3	642.3	653.0
Functioning Transplant	308.7	325.5	340.4	353.4	367.6	380.9	394.9	410.3	429.0	446.8

B

Type of Treatment*		Que.	Canada
HD Home	N	83	**721**
	%	1.1	**2.0**
HD Institutional	N	3,712	**17,044**
	%	48.4	**46.5**
CAPD	N	349	**1,622**
	%	4.6	**4.4**
APD	N	253	**2,367**
	%	3.3	**6.5**
Transplant	N	3,265	**14,884**
	%	42.6	**41.0**
Total	**N**	**7,662**	**36,638**

Fig.2.2. (A) Prevalence rate for patients on dialysis or with functioning transplant in Canada, 1999 to 2008. Rate per million population (RPMP). (B) Prevalent End-Stage Renal Disease patients, by type of treatment in Québec and Canada, 2008 (Number, Percent). Hemodialysis (HD); continuous ambulatory peritoneal dialysis (CAPD); automated peritoneal dialysis (APD). Sources: Canadian Organ Replacement Register, 2009, Canadian Institute for Health Information and Statistics Canada.

2.4 Causes of ESRD

In the last decades, the etiology of CKD changed: glomerulonephritis and infectious etiologies were outnumbered as primary etiologies by diabetes and hypertension. In Canada, the leading cause of ESRD is diabetic nephropathy followed by renal vascular disease (Table 2.2).

		Type of Treatment*			Total
		HD	PD	Tx	
Diagnosis					
Glomerulonephritis	N	2,474	732	4,479	**7,685**
	%	13.9	18.4	30.1	**21.0**
Diabetes	N	5,973	1,200	2,269	**9,442**
	%	33.6	30.1	15.2	**25.8**
Renal Vascular Disease	N	3,157	681	917	**4,755**
	%	17.8	17.1	6.2	**13.0**
Polycystic Kidney Disease	N	798	231	1,669	**2,698**
	%	4.5	5.8	11.2	**7.4**
Drug Induced	N	296	62	194	**552**
	%	1.7	1.6	1.3	**1.5**
Pyelonephritis	N	866	156	1,195	**2,217**
	%	4.9	3.9	8.2	**6.1**
Other	N	1,754	397	1,998	**4,149**
	%	9.9	10.0	13.4	**11.3**
Unknown	N	2,447	530	2,163	**5,140**
	%	13.8	13.3	14.5	**14.0**

Table 2.2. Prevalent End-Stage Renal Disease patients by treatment and primary diagnosis, Canada, December 31, 2008 (Number, Percent). Hemodialysis (HD); peritoneal dialysis (PD); transplant (Tx). Source: Canadian Organ Replacement Register, 2009, Canadian Institute for Health Information

2.5 Consequences of ESRD

2.5.1 Hemodynamic consequences of reduced renal blood flow

The organs of the human body are arranged in parallel circulation along the cardiovascular system (Fig. 2.3). Consequently, as discussed in section 1.2, this arrangement results in a total vascular resistance that decreases when a local resistance is added and increases when a local resistance is removed ($1/Rt=1/R1+1/R2+\ldots+1/Rn$). The same is true when we consider the renal circulation as a component of the cardiovascular system. In ESRD, because of the glomerular loss, the kidney blood flow is impaired and a low resistance, high flow component of the circulatory system is lost. As a consequence, the total peripheral resistance increases, a phenomenon that can be experimentally demonstrated in animals[16]. This fact should not be confused with the capacity of the kidney to hormonally

regulate the blood pressure[17] and the peripheral arteriolar tonus. Indeed, injured and hypoperfused kidneys in an ESRD patient can activate the rennin-angiotensin system and produce a number of vasoactive substances that directly increase peripheral resistance leading to vasoconstriction and increased blood pressure. In rare circumstances, even unfunctioning kidneys can lead to malignant hypertension that can only be treated by surgical nephrectomy[18].

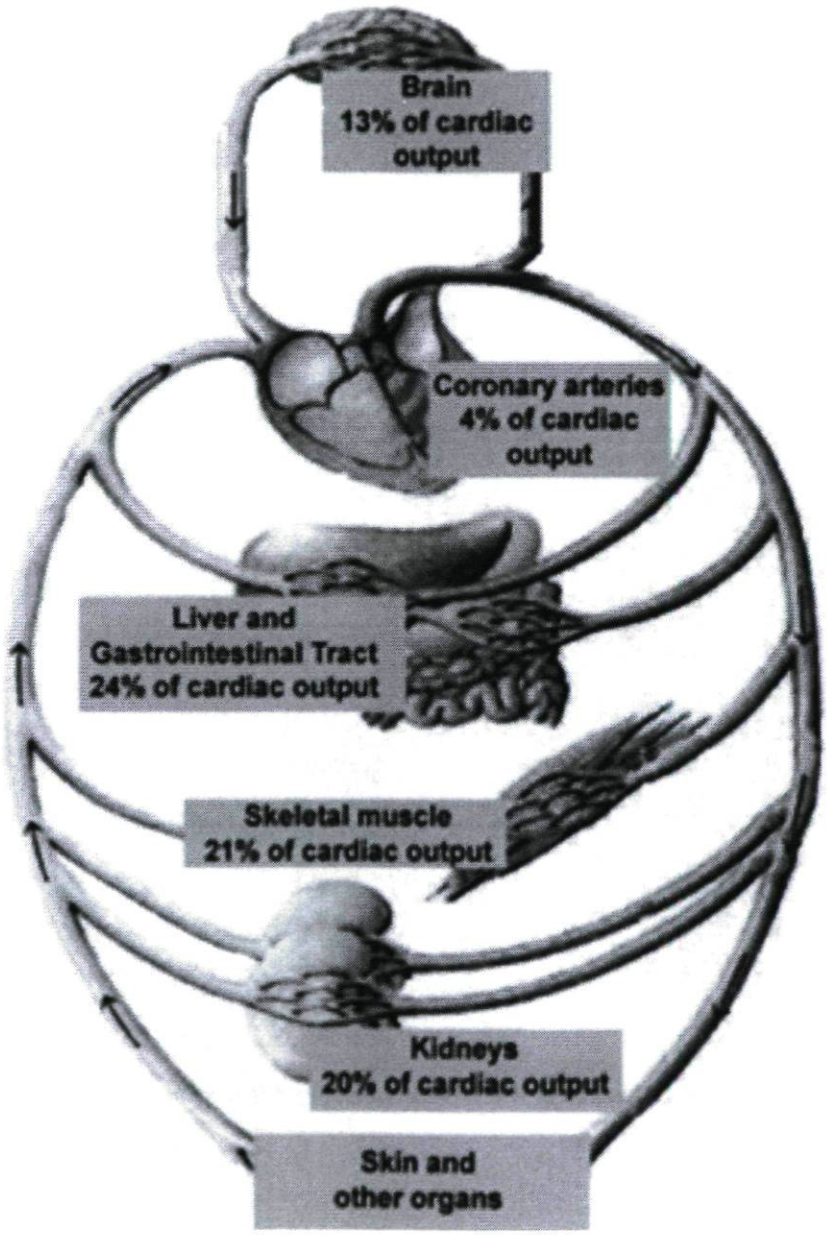


Fig.2.3. Schematic representation of parallel arrangement of the peripheral organs in the systemic circulation. ***Image adapted from Despopoulos [19].***

2.5.2 Hypertension

Hypertension is a common complication of ESRD, especially in patients with glomerular, tubulointerstitial and adult polycystic kidney disease. The mechanisms of hypertension in ESRD are numerous and complex[17, 20] including extracellular volume expansion[21], altered response of the renin-angiotensin system[22, 23], overactivity of the sympathetic system[24], dysregulation of endothelin system and endothelial dysfunction[25]. In ESRD patients, volume expansion can be controlled to a degree by dialysis. However, a large percentage of these patients remain hypertensive and require additional pharmacological intervention.

2.5.3 Disorders of metabolic functions

Abnormal mineral and bone metabolism begin early in CKD and are associated with increased mortality and morbidity[26-28]. ESRD patients have the tendency to hypocalcemia as a result of 1.25- Dihidroxycholecalciferol deficiency. In order to maintain plasma calcium concentration, PTH secretion will increase and calcium, along with phosphate, is mobilized from the bone. Because of decreased or lost renal function, the excretion of phosphate (normally promoted by PTH) is impaired and it starts to accumulate in the organism resulting in an increased calcium-phosphate product that favors the calcification of soft tissues and blood vessels[29]. However, it was demonstrated that vascular calcification is not just a passive precipitation process due to calcium and phosphate levels exceeding their solubility product in the extracellular space, but an active cell-mediated process associated with deposition of bone matrix proteins in the arterial media[30, 31]. ***Chronic kidney disease - mineral and bone disorder (CKD-MBD***[32] is defined as the abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism along with the resulting abnormalities in the bone turnover, mineralization, volume, linear growth, or strength and the calcification of vascular or other soft-tissue. ***Renal osteodystrophy***[32] represents the skeletal component of the CKD-MBD that is quantifiable by bone biopsy and includes osteitis fibrosa, osteomalacia and adynamic bone disease[33, 34]. These conditions are clinically characterized by bone pain and increased incidence of fractures and deformities.

Anemia was clearly shown to correlate with the magnitude of GFR decline and is present in the majority of ESRD patients[35]. Decreased hemoglobin (Hb) levels were shown to be associated to left ventricular hypertrophy[36, 37] and congestive heart failure[38] that were demonstrated to regress after treatment with recombinant human erythropoietin[39]. However, the present guidelines recommend a target Hb between 11g/dL and 12g/dL that should not exceed 13g/dL, as there is evidence of increased cardiovascular morbidity and mortality above this value in ESRD patients[40]. It is also notable that ESRD patients show an increased blood pressure in response to erythropoietin treatment[41, 42], response that could partially explain the relation between the improvement in Hb values and cardiovascular risk. In a study by Yang *and all[43],* Hb variability was shown to be associated with increased mortality, fact explained by the authors as being the result of an increased physiological stress.

Uremic solute retention in ESRD results in a gradual increase in plasma concentration of organic compounds. The kidney is able to clear and metabolize organic molecules with a molecular weight (MW) up to 58kDa. Although low MW molecules (MW up to 300Da) are efficiently removed by hemodialyse, the high MW molecules are unable to pass through the dialysis filter and therefore they accumulate in the body. There is increasing evidence that these molecules play a significant role in the clinical, metabolic and biochemical disturbances in ESRD. It has been demonstrated that patients on peritoneal dialysis, where the peritoneal membrane permits the epuration of higher MW compounds, had improved toxic symptoms as compared with the patients dialysed with older hemodialyse filters[44] (modern filters or high-flux membranes have the capacity to remove organic compounds up to a MW of 12kDa). Examples of such organic compounds that could have a role in the pathophysiology of ESRD complications include β2-microbulin[45], advanced glycation end products (AGEs)[46] or endothelin-1[47, 48].

Chronic inflammation in ESRD has been proposed to play an important role in the burden of cardiovascular disease[49]. Patients receiving dialysis have elevated plasma levels of acute phase proteins and other inflammatory biomarkers that have been shown to be

associated with increased cardiovascular mortality[50]. Several factors may contribute to chronic inflammation in ESRD; among them, there are the contact of the blood with hemodialyse filter (which represents a foreign body for the immune system), the decreased clearance of pro-inflammatory cytokines and the increased oxidative stress[51, 52].

Protein and energetic metabolism in ESRD is characterized by protein malnutrition in 18% to 70% of dialysed patients and it is one of the strongest predictors of morbidity and mortality in this population[53]. The causes of protein malnutrition are numerous and include: inadequate food intake secondary to anorexia; a catabolic response to superimposed illnesses and to chronic inflammation; nutrients removal by dialysis; blood loss; endocrine disorders of uremia (e.g. insulin resistance, hyperglucagonemia, hyperparathyroidism); accumulation of endogenously formed uremic toxins or the ingestion of exogenous toxins.

2.6 Cardiovascular risk in CKD

2.6.1 Cardiovascular morbidity and mortality is increased in CKD

In 1974, Linder published the first study that demonstrated a high cardiovascular morbidity and mortality in patients receiving maintenance hemodialysis[54]. He observed a higher incidence of myocardial infarction, angina pectoris and strokes when compared with age-matched normal or hypertensive patients without renal disease. At a time when long time survival on hemodialysis became a reality for ESRD patients, this study provided the first evidence of a clinical problem that could increase the morbidity and mortality of uremic patients on chronic hemodialysis. These findings were confirmed by an analysis of over 50,000 European patients which showed annual death rates from coronary heart disease to be much higher among uremic patients on dialysis, particularly for the younger age group[55]. In studies where cardiovascular disease is clearly defined and sudden death is included among cardiac deaths, Foley *at all* and recent data from *U.S. Renal Data System* [56, 57]showed a tremendous increase of cardiovascular mortality in dialysis patients.

Cardiovascular disease accounts for approximately half the deaths among ESRD adults undergoing regular dialysis and is 10–20 times higher than in the general population, even after stratification by age, gender, race, and presence of diabetes.

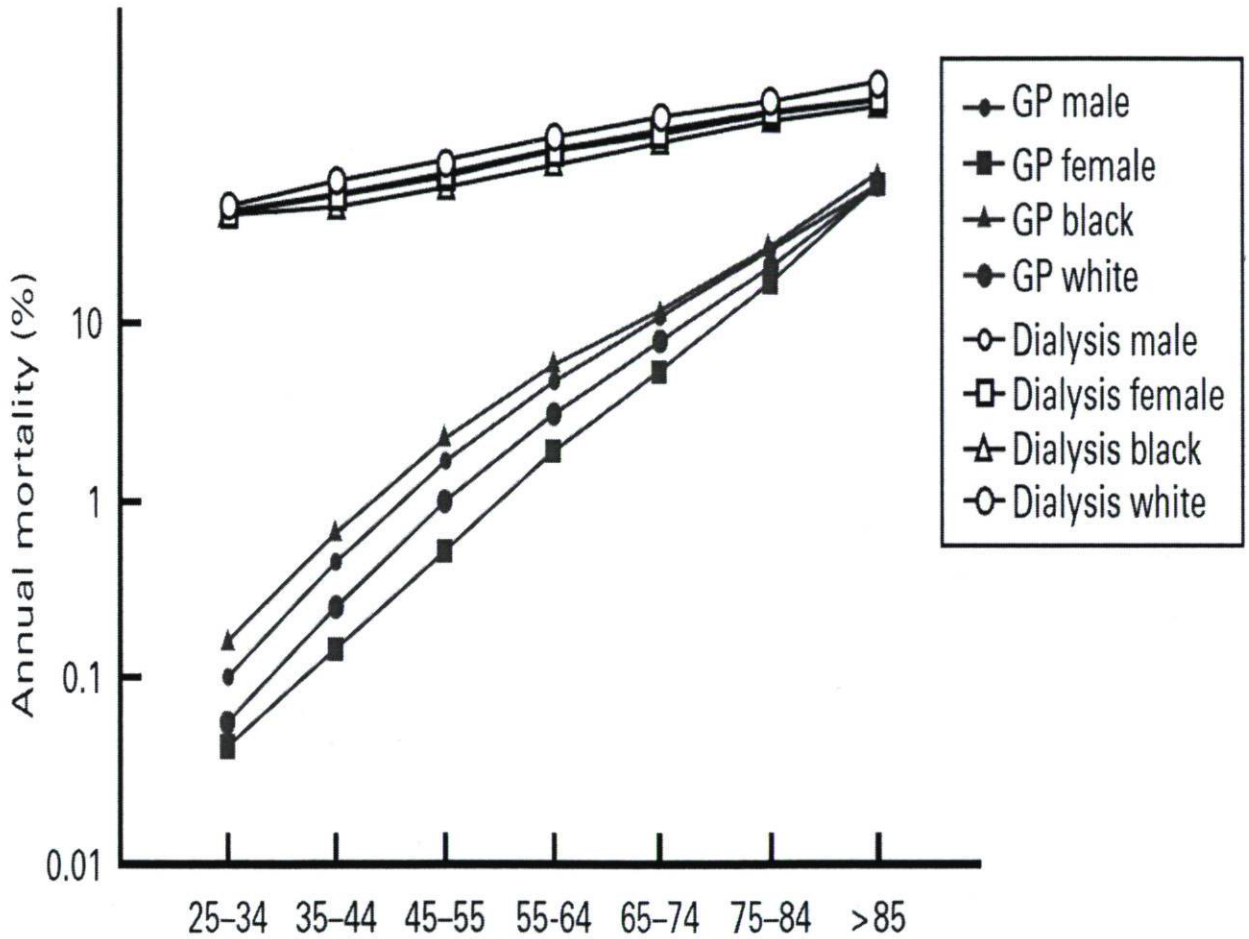


Fig.2.4. Cardiovascular mortality rates in patients on renal replacement therapy compared with normal background population. Data from the US Renal Disease Service (USRDS) 2006.

As it was unclear if the pre-existing cardiovascular disease or the uremic state leads to the high cardiovascular morbidity and mortality in ESRD, Beddhu et *al*[58] demonstrated that even moderate renal failure increases the risk of myocardial infarction and death independent of clinical variables, baseline angiographic evidence of coronary disease and

treatment. It is now well recognized that CKD is an independent cardiovascular risk factor[59-61].

In patients aged between 25 and 35 years, cardiovascular mortality is hundreds of time more elevated than in the general population so that a young adult receiving dialysis has a annual cardiovascular risk of an elderly without renal disease (Fig. 2.4). Children and young adults with ESRD die primarily of cardiovascular disease, having a cardiovascular mortality risk higher than the mortality risk from infection or malignancy[62, 63].

The decrease in cardiovascular mortality after restoration of renal function by kidney transplant in ESRD[64] strongly supports the evidence that uremia is an independent cardiovascular risk factor.

2.6.2 Conventional cardiovascular factors and reverse epidemiology in ESRD

The increased cardiovascular mortality in ESRD is not completely supported by the traditional (Framingham) cardiovascular risk factors[65].

In the general population, cardiovascular mortality increases sharply with advancing age. In contrast, the effect of aging on cardiovascular mortality is much less obvious in ESRD[56]. More than that, traditional risk factors valid in the general population such as body mass index, serum cholesterol and blood pressure were found to inversely relate to mortality in hemodialysis patients (Fig. 2.5). ***The protective role of obesity, hypercholesterolemia, increased blood pressure and increased plasmatic creatinine in hemodialysis is referred to as reverse epidemiology***[65].

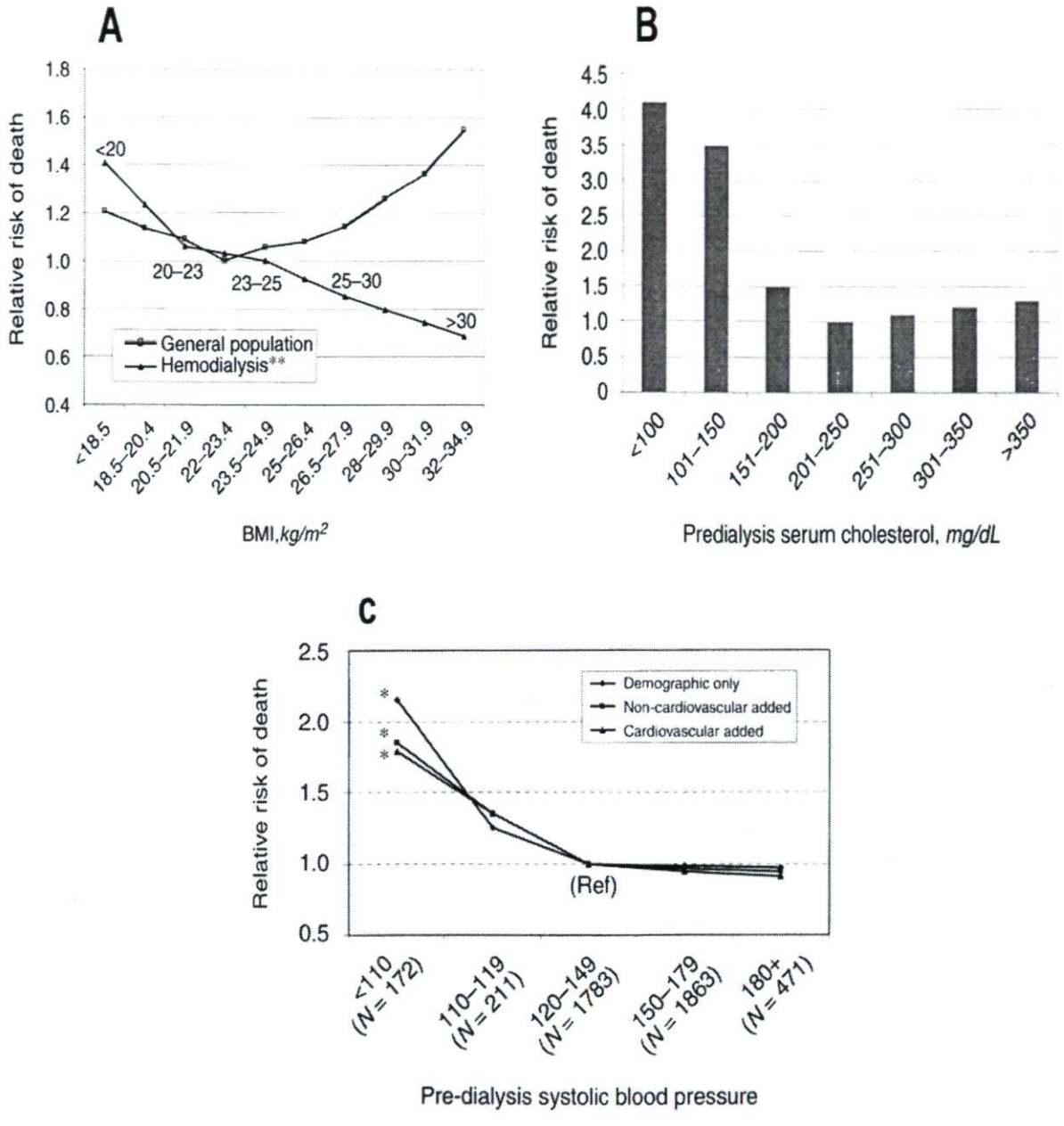


Fig.2.5. (A) Comparison between the impact of body mass index (BMI) on all-cause mortality in the general versus hemodialysis population. ***Figure adapted from Kalantar-Zadeh, K., et al.[65].*** **(B) Relative risk of death in hemodialysis patients according to serum cholesterol concentration compared to the reference group (cholesterol 200 to 250 mg/dL).** ***Figure from Lowrie, E.G. and N.L. Lew [66].*** **(C) Predialysis blood pressure and mortality risk in hemodialysis patients.** ***Figure from Port, F.K., et al.[67].***

2.6.3 The survival bias in ESRD

One of the possible explanations for reverse epidemiology is the survival bias. This term refers to the process of survival selection undergone by ESRD patients. Statistical data show that in the USA, ESRD population represents less than 5% of the CKD patients[68]. This finding suggests that ***the majority of CKD patients die before reaching ESRD***. The patients that survive to all CKD stages and begin renal replacement therapies might not be representative for the general population regarding the morbidity and mortality risk factors.

2.6.4 Non conventional cardiovascular risk factors in ESRD

The unexplained cardiovascular mortality in ESRD determined an increased recognition of the non-traditional risk factors. Examples include excessive calcium and phosphorus load, hyperparathyroidism[69], anemia[70], inflammation[71-73], increased asymmetric dimethylarginine[74], hyperhomocysteinemia[75], increased oxidative stress[76] and aortic stiffness. The influence of an increased aortic stiffness on cardiovascular mortality in ESRD patients is discussed in section 4.3.

3. Arterial Stiffness

Arteries deliver blood to peripheral tissues and transform the pulsatile output of the left ventricle in a continuous, smooth blood flow. To fulfill this mission, arteries have strong vascular walls, capable to sustain high pressures and velocities while being also distensible. Distensibility, one of the most important properties of arterial system, refers to the capacity of an artery to increase its diameter in response to increased pressure. The alteration of this function leads to increased arterial stiffness, a reverse measure of distensibility.

For a better understanding of arterial stiffness pathophysiology, the following text will shortly review the composition, the structure and the mechanical properties of the arterial wall; a brief discussion regarding the consequences of increased arterial wall stiffness on arterial functions will introduce the central hemodynamic effects and the clinical methods of measuring the arterial stiffness. Finally, the adverse effects on central blood pressure, ventricular loading, subendocardial perfusion, cardiovascular morbidity and mortality will also be reviewed.

3.1. Arterial wall

The arterial wall is a well-organized structure composed of endothelial cells, smooth muscle cells and extra cellular matrix (ECM) formed by collagen and elastic fibers along with proteoglycans (glycosoaminoglycans). Within the cardiovascular system, the general architecture of arteries is similar. The variation in the proportion of constituents rather than their type determines the physiological mechanical properties of the arterial wall, reflecting the functional requirements at different levels in the arterial tree. Alteration in wall composition, constituent's proportion and structure may lead to increased arterial stiffness with strong negative repercussions on the cardiovascular system.

3.1.1 Building blocks and structure of the arterial wall.

Endothelial cells – Endothelial cells constitute the simple squamous (single cell thick) lining of the circulatory system. Vascular endothelium is a multifunctional tissue responsible for maintenance of the vessel wall homeostasis. It has synthetic and metabolic properties, participates in the blood-tissue interaction and ***controls the transfer of small and large molecules across the vascular wall***. One of their most important roles is to modulate the blood flow and ***vascular reactivity*** by synthesizing vasoconstrictors like endothelin I or vasodilators like NO in response to physiologically stimuli. Failure of the endothelial tissue to assure a normal vasoreactivity defines the endothelial dysfunction, which is, in part, responsible for thrombus formation, atherosclerosis and vascular remodeling.

Vascular smooth muscle cells – Vascular smooth muscle cells are the predominant cellular element of the arterial wall. Their role in the vascular tissues includes but doesn't resume to: ***vasoconstriction / vasodilatation***; synthesis of collagen, elastin and proteoglycans; elaboration of growth factors and cytokines. The smooth muscle cells are also important elements in the ***vascular repair, remodelling*** and atherosclerotic process as they are able to migrate to the intima and proliferate following vascular injury. The regulation of their functions is complex and includes angiotensin II, catecolamines, NO, endothelin-1, platelet-derived factor, fibroblast growth factor, transforming growth factor-beta and interleukins.

Collagen fibers – Collagen fibers are responsible for the ***strength and integrity*** of the connective tissues and organs. Currently, 27 types of collagen are known; however collagen I and III are found in the arterial wall, being the most abundant load-bearing and reinforcing element. The strength of collagen fibers transposed to our macroscopic world approaches the strength of steel; this property is the result of a highly organized molecular and microscopic structure. Basically, all of the collagens molecules consist of a triple helix formed by three α polypeptide chains; slightly different amino acid compositions of these

chains are responsible for the different types of collagen. In the process of fiber formation, the triple helix of the collagen molecule lines up and begins to form fibrils. This step is called ***crosslink formation*** and is promoted by a specialized enzyme, lysyl oxidase. The reaction places stable crosslinks within (intramolecular crosslinks) and between the molecules (intermolecular crosslinks) and is the critical step that gives the collagen fibers a tremendous strength. The collagen fibrils organize in bundles forming collagen fibers, which are loose and wavy in the arterial wall; they begin to straighten and exercise their reinforcing properties when the vascular wall begins to distend.

Elastic fibers – Elastic fibers confers ***resilience and elastic recoil*** to the tissues. They are extracellular matrix biopolymers assembled from different proteins or glycoproteins and are composed of two distinct morphological entities: elastin and microfibrills. Elastin is an amorphous material containing highly cross-linked, hydrophobic proteins. The microfibrillar component of elastic fibers is formed by several glycoproteins, of which the best known are fibrillin-1 and fibrillin-2. Elastic microfibrils provide a three-dimensional scaffold for the assembly of elastin during the formation of elastic fibers. The resilience and elastic recoil of elastic fibers are conferred by elastin. Elastogenesis occurs in most elastic tissues during late prenatal and neonatal development. Once synthesized in early development, elastic fibers undergo very little turnover in most normal adult tissues. For example, the elastic fibers deposited in the aorta during childhood are usually the same elastic fibers that the person will die with. However, in a variety of elastic-tissue diseases, new synthesis in adult tissue results in aberrant accumulation of dysfunctional elastic fibers. Examples of such common disorders include hypertension and aortic aneurysms.

Proteoglycans – Proteoglycans are large macromolecules consisting of a core protein that is covalently attached to approximately 100 glycosaminoglycan molecules (long polysaccharide chains composed of repeating disaccharide units) and a link protein, which binds hialuronic acid. Proteoglycans are very large, highly negative charged macromolecules that attract water into the extracellular matrix giving it a ***gel-like consistency***. This highly hydrated gel is ***able to resist compressive forces while allowing diffusion of O_2 and nutrients between the blood and tissue cells***. In the arterial wall they

form important structural links between fibrous (collagen and elastic fibers) and cellular components, influencing the structural integrity of the vascular wall.

Extracellular matrix (collagen, elastic fibers and proteoglycans) plays a crucial role in determining the mechanical properties of the vascular wall. **Elastic fiber to collagen ratio determines the stiffness of the arterial wall**. One of the important things to remember is that in the aortic wall and most vessels, ***collagen is able to be synthesized in response to injury; contrary, elastic fibers have a turn over covering the entire life and are not believed to be synthesized after childhood.***

The architecture of an artery follows the same pattern along the arterial tree and consists of three concentric layers (Fig. 3.1):

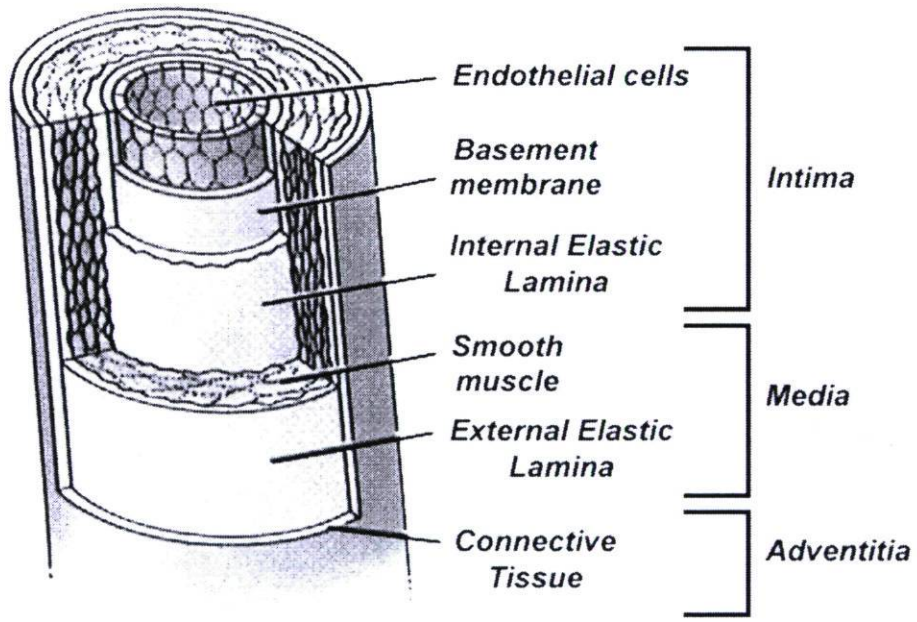


Fig.3.1. Schematic representation of arterial wall layers.

Intima – Intima is a single layer of ***endothelial cells*** lined on a basement membrane with minimal underlying connective tissue. It is separated from the media by a dense elastic membrane called ***internal elastic lamina***, which forms its chief thickness. The intima of

large arteries is substantially thicker than that seen in medium and small arteries; in aorta it accounts for about one quarter of the thickness of the whole wall and many fenestrated elastic laminae and fibers are present in the so-called ***subendothelial layer*** between the endothelium and the internal elastic lamina

Media – Media is a complex three-dimensional network formed by ***bundles of collagen fibers***, ***elastic fibers*** and ***smooth muscle cells***. The outer limit of the media in most arteries is a well-defined ***external elastic lamina***. In the large arteries like aorta, the collagen, the elastin and the smooth muscle cells are found to be organized in a varying number of ***medial lamellar units*** that are circumferentially aligned and are responsible for the high strength and resilience of this layer. For example, in the human abdominal aortic media, there is an average of 40 lamellar units[77] and each of them is about 10μm in thickness[78]. This pattern looses its organization toward the periphery, so that the laminated structure of the media is hardly present in the medium size or small arteries.

Adventitia – consists chiefly of ***elastic and collagen fibers*** along with ***nerve fibers*** and ***vasavasorum*** (small arterioles arising from outside the vessel coursing in to the outer 1/2 – 2/3 of the media).

3.1.2 Mechanical properties of arterial wall

The most important property of the arterial wall resides in the ability of an arterial segment to change its length in response to a tensile force (force that is pulling along the length of a segment) and to return to its original length when the force ceases to exert action. This mechanical property, defined as the elasticity of the arterial wall, is a consequence of numerous elastic fibers present in the arterial media.

Elastic fibers are highly extensible and, even at large deformations, can be characterized by a linear relation between stress and tensile strain (Fig. 3.2). Stress (σ) is defined as the intensity of force (F) acting across an area (A): $\sigma=F/A$. Tensile strain (ε) is defined as a

measure of length change increase (Δl) divided by the initial length (l) of the material: $\varepsilon = \Delta l/l$. The slope of the graphic represents the **Young modulus of elasticity** (E): $E = \sigma/\varepsilon$.

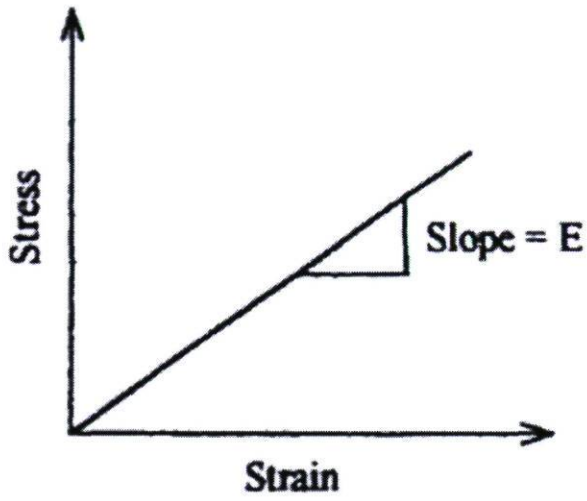


Fig.3.2 Linear relation between stress and strain. Young modulus of elasticity (E).

The Young modulus is a material property and is a measure of the stiffness and not of the elasticity; in other words, as the modulus increases, the tensile force necessary to produce the same variation in length also increases. A high modulus value implies increased stiffness and reduced elasticity. For example, the Young modulus of collagen fibers which are relatively inextensible is almost 1000 times higher than that of elastic fibers[79].

The **elastic modulus of elastic fibers** should not be confounded with the **elastic modulus of the arterial wall**. As discussed above, the arterial wall components accounting for the majority of its mechanical properties are collagen, elastic fibers and smooth muscular cells. In the arterial media, the elastin is organized into a three-dimensional, interconnecting lamellar network designed to transfer stress throughout the vessel wall; between the lamellar layers, there are smooth muscles cells and bundles of wavy collagen that show no definite overall arrangement at low pressure but become circumferentially aligned as pressure increases. This multiple-phase composition of the vessel wall provides nonlinear

stress-strain relationships. In a theoretical model, the organization of the arterial wall components is shown in Figure 3.3.

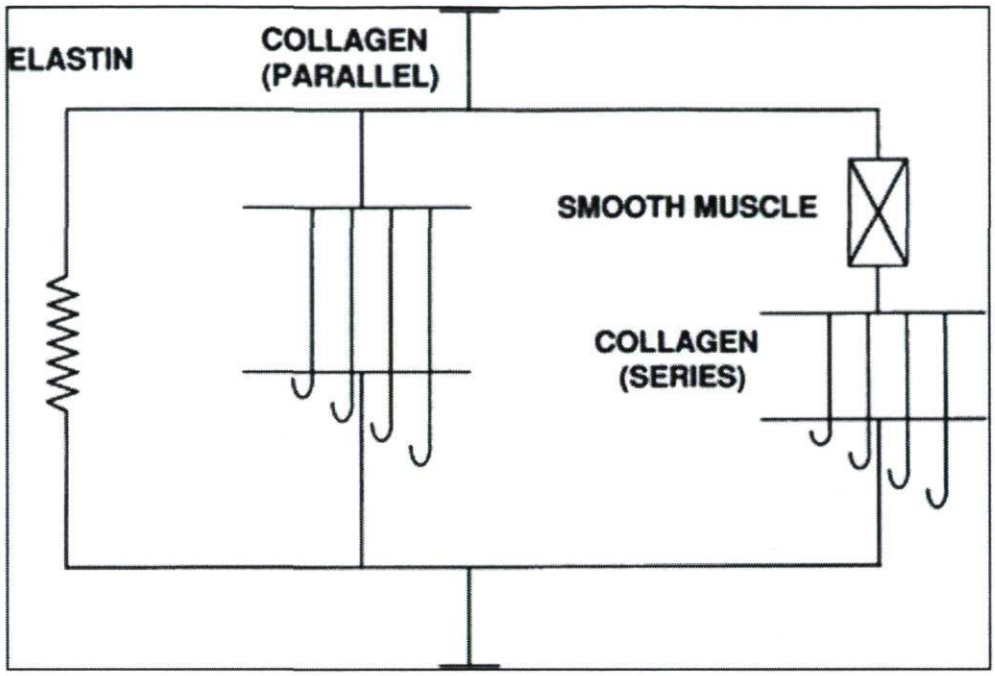


Fig.3.3. Schematic representation of elastin, collagen and smooth muscle organization in arterial media. Collagen is represented by stiff springs that are recruited as the arterial wall extends (parallel collagen) or as the smooth muscle contracts (series collagen). ***Figure from Bank, A.J., et al.[80].***

In the resting state (no tensile strain), the wavy collagen fibers bear no load. At low strains, because the elastic modulus of elastin dominates the mechanical behavior, the wall is relatively extensible. At high strains, as the collagen fibers unfold, the elastic modulus of collagen dominates and the wall is relatively inextensible. Previous studies suggest that in the aortic wall, approximately 10% of collagen fibers are engaged at physiological strain[81] whereas at higher strains, the wall becomes progressively less extensible as collagen fibers are recruited to support wall tension and restrict aortic distension. In normal conditions, these properties determine an optimal behavior for the expansion and contraction of the blood vessel during the cardiac cycle. It limits over distension and damage when exposed to extreme pressures.

As the arterial wall exhibits a curved stress-strain relationship, it cannot be characterized by a single Young modulus but rather by an **incremental modulus**, defined as the slope of the stress-strain relation. For the biological tissue, the incremental elastic modulus increases with strain. In the blood vessel, the arterial wall becomes stiffer with increasing blood pressure, which represents the stress. Figure 3.4 shows the incremental elastic modulus of an artery and the relation with the arterial diameter (dependent of blood pressure).

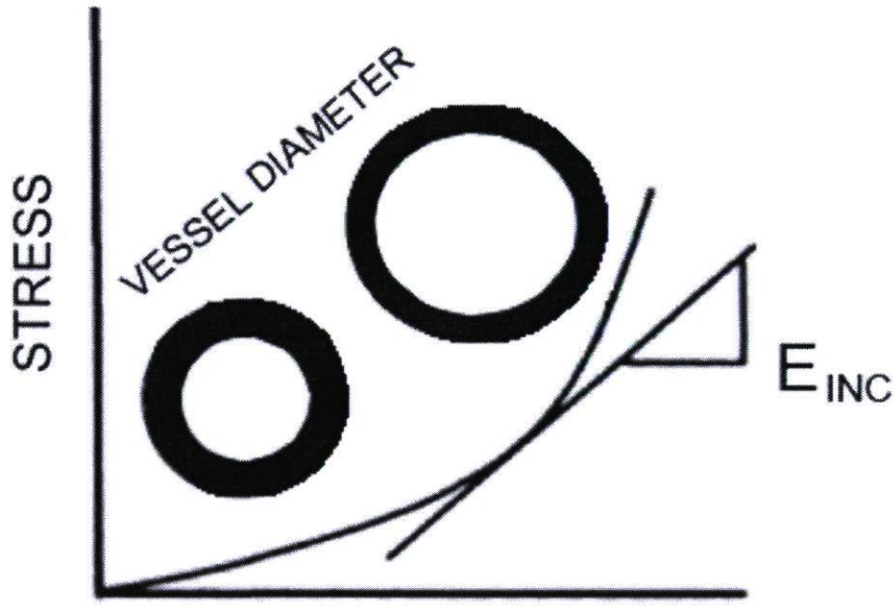


Fig.3.4. Nonlinear (incremental) relation between stress and strain of the arterial wall. Incremental elastic modulus (E_{inc}).

The individual mechanical role of elastin and collagen was demonstrated by selectively digesting elastin and collagen from a human external iliac artery[82] (Fig. 3.5). The initial stiffness of the normal (control) arterial wall at low pressure is approximately equal to the slope of the tension–radius curve of the "collagen-digested" artery representing the contribution of the elastin. The final slope of the tension–radius curve at high pressure equals the slope of the tension–radius curve of the "elastin-digested" artery representing the contribution of tensed collagen fibers. The control (untreated) artery shows the typical J-shaped mechanical response, characterized by the incremental elastic modulus.

The net effect of the nonlinear relation between stress and strain has repercussions on arterial stiffness: for the same artery, the Young modulus at a given point is different depending on the blood pressure. An increase in mean blood pressure determines an increase in arterial stiffness, without any structural changes in the arterial wall. This may lead to confusion and questioning about the morphologic changes in the vascular wall. An example is in hypertension: when elastic properties are derived at the mean pressure of the individual patient, the vessels of hypertensive patients are found to have a higher incremental elastic modulus as when compared with normal subjects. The arterial stiffening could be only apparent, the result of the higher means blood pressure[83], but could also represent pathologic modification in the structure of the vessel wall. In order to conclude if mechanical properties have changed or not either a stress-strain graph should be made or the incremental elastic modulus should be compared at similar strains or stresses.

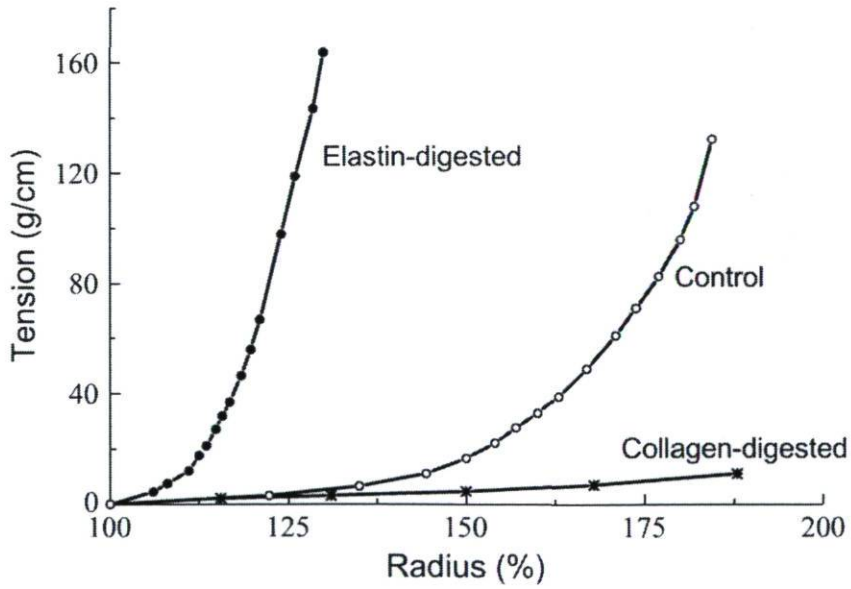


Fig.3.5. Tension–radius responses of human iliac arteries: control (untreated artery), collagen digested (collagen is removed from the artery by formic acid), elastin-digested (elastin is removed by trypsin). ***Figure from Roach, M.R. and A.C. Burton [82].***

Another important aspect regarding the elastic properties of the arterial wall is the different behavior of the wall layers under mechanical stress[84] (Fig. 3.6). The intima is very thin in normal, healthy arteries and makes an insignificant contribution to mechanical properties of

the arterial wall. From a mechanical perspective, the media is the most significant layer in a healthy artery. As a consequence of its rich elastin content, media expresses the highest elasticity and the pathologic processes affecting this layer have the greatest impact on arterial stiffness. The adventitia contributes significantly to the stability and strength of the arterial wall. In no distended and no stressed adventitial tissue, the collagen fibers are embedded in a wavy form in the extracellular matrix, which causes this layer to be less stiff than the media. However, at significant levels of strain the collagen fibers reach their straightened lengths and the mechanical response of the adventitia changes to that of a stiff "tube" that prevents the smooth muscle from acute over distension.

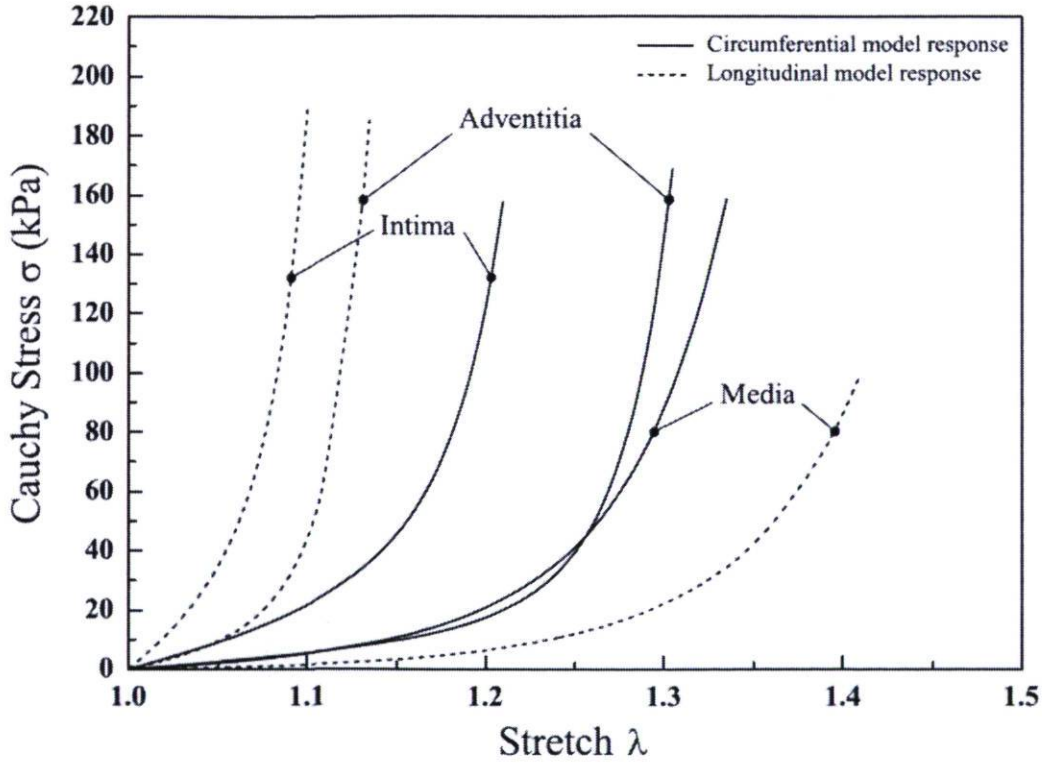


Fig.3.6. Stress–stretch model response representing mean mechanical data of the three arterial layers in circumferential and longitudinal directions obtained from 13 non-stenotic human left anterior descending coronary arteries. ***Figure from Holzapfel, G.A., et al.[84].***

The arterial wall, like any other biomaterial has a high water content, approximately 70% of it's weight[85, 86]. The water is attracted by proteoglycans and is giving to extracellular matrix a gel-like consistency, which deeply influences the mechanical properties. Vascular

walls have not only elastic, but also viscous properties. Visco-elastic properties refer to the initial, larger force necessary to change the length of a material. As the force continues to stress, the viscous contribution decreases, a phenomenon known as stress relaxation. When there is a sudden increase in stress, the strain of a visco-elastic material is delayed and will lag behind, a phenomenon called **hysteresis**. Those properties are the determinants of the **complex elastic modulus**, which depends on the frequency of the stress oscillations (the heartbeats). This text is not meant to explain these phenomenons but rather to remind the complexity of the biomaterials and cardiovascular hemodynamics.

3.1.3 Pathophysiology of arterial wall stiffness

As shown in Figure 3.7, the mechanisms of arterial stiffness are numerous, complex and not yet completely elucidated. The following text does not mean to explore these pathways but rather to briefly review the morphopathologic changes of the arterial wall and their associations with normal physiology and disease.

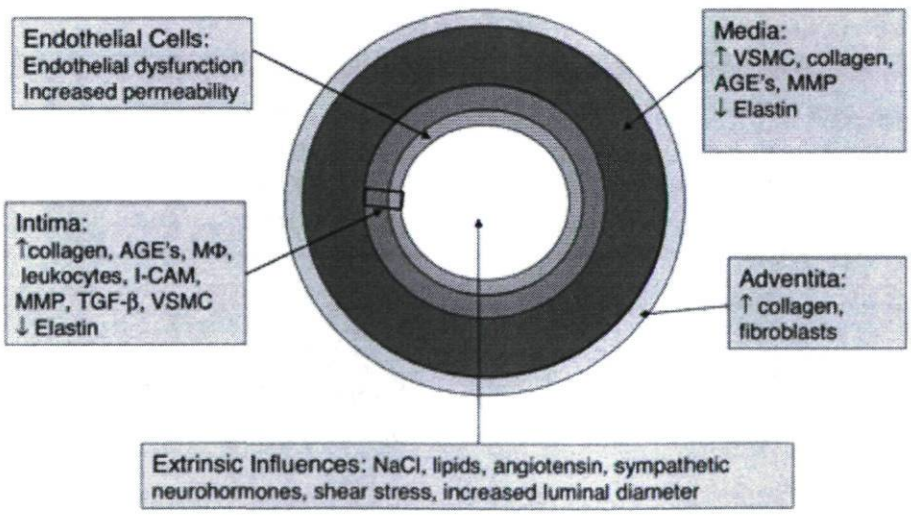


Fig.3.7. Summary of arterial stiffness pathophysiology. Advance end glycation products (AGE's); intracellular cell adhesion molecules (I-CAM); matrix metalloproteases (MMPs); transforming growth factor beta (TGF-β); vascular smooth muscle cell (VSMC). ***Figure from Zieman, S.J.[87].***

Arterial stiffening is referred as **arteriosclerosis**, a generic term describing the thickening and loss of elasticity of the arterial wall[88] (from the Greek Arterio, meaning artery,

and sclerosis, meaning hardening). The major pathological processes responsible for arteriosclerosis include arterial remodeling, degenerative changes of collagen and elastin fibers, medial calcification (Mönckebrg sclerosis) and atherosclerosis. Atherosclerosis should not be confused with arteriosclerosis. While atherosclerotic process is responsible especially for the loss of conductance arterial function by occlusive lesions (section 3.2.2), arteriosclerosis is responsible for dampening or capacitance function loss by decreasing arterial distensibility.

Atherosclerosis represents a chronic endothelial cell injury allowing monocytes and lymphocytes to adhere and migrate into the intima. This event leads to plaque formation by intracellular and extracellular accumulation of oxidize low-density lipoproteins and muscle cells migration into the intima where they convert their normal contractile role in a secretory one, producing extracellular matrix. Atherosclerotic plaques are associated with the ***calcification of the arterial intima***, thus increasing the stiffness of the affected arterial wall. However, usually, these lesions are not continuous but rather ***patchy and localized***. The arteries are most severely affected at ostia and at bifurcations, where laminar flow is disrupted. In general, there is a descending order of the vascular involvement severity: abdominal aorta, coronary arteries, popliteal arteries, etc. However, patients with severe coronary artery atherosclerosis can have a relatively disease-free aorta[89].

The term of **vascular remodeling** refers to the reorganization of the existing components of the vascular wall and/or smooth muscle cell proliferation followed by the synthesis of new extracellular matrix constituents such as collagen. This process results in a different composition of the tissue with an increased collagen to elastin ratio. In normal conditions, the vascular remodeling acts as a physiologic response to vascular endothelial injury or increased vascular wall stress. ***Intimal remodeling*** and thickening represent the healing response of the organism after vascular injury and include the formation of a neointima[88]. Studies using balloon catheter denudation of the common carotid artery as a model of endothelial injury found that smooth muscle cells within the media proliferate and then migrate across the internal elastic lamina into the intima. There they continue to proliferate and synthesize a new matrix, resulting in a dramatically thickened

neointima[90]. Contrary to a normal, healthy artery, where the intima is represented by a thin membrane, in a vessel that suffered intimal remodeling, the thickened, hyperplastic neointima significantly contributes to the mechanical properties of the arterial wall. ***Medial remodeling*** represents an adaptative response of the vascular wall to increased pressure. In order to normalize the circumferential stress seen for example in hypertension, wall thickness increases by deposition of extracellular matrix (especially collagen), hyperplasia and hypertrophy of smooth muscle cells in the medial layer[91, 92]. It was also shown that the collagen density increases in the medial layer with aging while the amount of elastin remains stable or declines[93].

Degenerative changes of the vascular wall are normally associated with the aging process and refer to the quantitative and qualitative changes affecting the components of the extracellular matrix. Aortic medial elastic fibers were found to be disorganized, thinner, and more fragmented in old animals and humans when compared with those of younger age. These findings may be partially explained by ***increased elastase activity*** in old individuals[93-95]. Age related arterial stiffening may also be related to the accumulation and generation of advanced glycation end products (AGEs) and to the ***nonenzymatic glycation of the extracellular matrix proteins***. AGEs formation begins with a nonenzymatic reaction between glucose and proteins (Maillard reaction) which forms reversible early glycation products (Schiff base). These products transform over a period of days or weeks into more stable products (Amadori products) that tend to accumulate on proteins such as collagen and undergo further reactions to form AGEs[96]. The net result is the formation of irreversible cross-links between collagen molecules and fibers[97]. AGE-linked collagen is stiffer and less susceptible to hydrolytic turnover resulting in the accumulation of structurally inadequate collagen fibers[98]. The nonenzymatic glycation of extracellular matrix proteins represents the receptor-independent action of AGEs. Another pathway is represented by the receptor-dependent action: AGEs specific receptors (RAGE) along with other binding proteins are responsible for signal transduction that activates multiple intracellular signaling pathways. The RAGE receptor is currently viewed as most biologically significant in the setting of atherosclerosis[99]. Aging is also associated with the ***deposition of chondroitin sulfate, heparin sulfate, proteoglycans, and***

fibronectin that can also thicken and stiffen the extracellular matrix of the vessel's walls[100].

Medial calcification (Mönckebrg's sclerosis or medial calcinosis) is characterized by calcification of the tunica media. Contrary to atherosclerosis, where the mineral deposits are localized in the intima, at the level of atherosclerotic plaque, in medial calcinosis, there are ***diffuse mineral deposits within the arterial media***. In healthy individuals, medial calcification is associated with advanced age. It was shown that in elderly individuals there is an increased mineralization of medial elastic fibers because of the increased affinity of elastin for calcium[101].

3.2 Artery types

Based on their diameter, their structural features and the proportion of constituents, arteries are divided in three types:

- large or elastic – aorta and its large branches, innominate, subclavian, common carotid and iliac arteries;
- medium-sized or muscular – comprising other branches of aorta like coronary and renal arteries;
- small arteries and arterioles – found in the tissues.

Figure 3.8 shows the difference of diameter, wall thickness and relative proportion of constituents between different types of arteries. Elastin is the major component of aortic wall as smooth muscular cells are the major component of medium-sized arteries vascular wall.

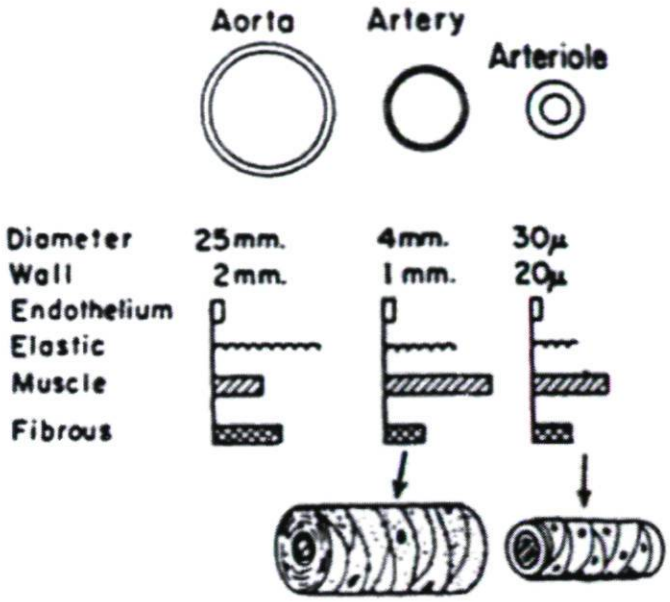


Fig.3.8 Diameter and relative contents of endothelium, elastic and fibrous tissues and smooth muscle in different type of arteries. ***Figure adapted from Rushmer, R.F.[102].***

The major regulator of the arterial wall stiffness in the resting state of a healthy artery is the proportion of elastin to collagen within the extracelular matrix. As we mentioned above, in an artery, this proportion varies from type to type but also within the same vessel in the case of aorta. Figure 3.9 shows the ratio of elastin and collagen in the arterial tree depending of topography. Thoracic aorta is the vessel with the highest content and proportion of elastin, being the most elastic artery in the circulatory system[103].

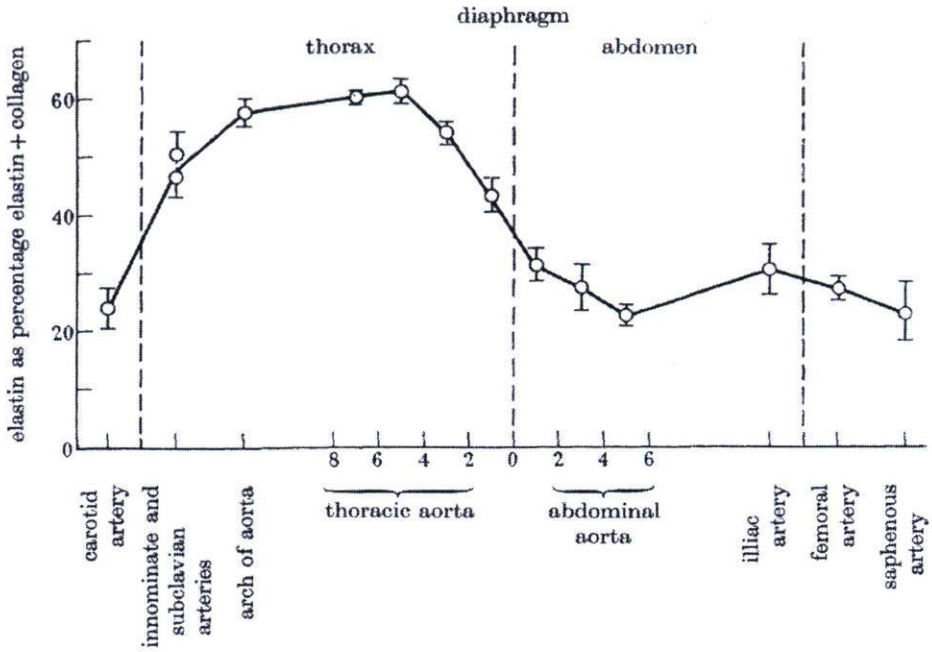


Fig.3.9. Ratio of elastin to elastin+collagen in the arteries of dogs. Figures along the abscissa represent the distance from the diaphragm in centimetres. ***Figure from Harkness, M.L.R et al.[85].***

3.3 Arterial hemodynamics

3.3.1 Conduit, capacitance functions and windkessel model

Arterial wall stiffness is a regulator and a marker of the two important and interconnected functions of the large and medium-sized arteries: dampening (cushioning) and conduit (distributing) functions.

Dampening (cushioning) function is responsible for transforming the intermittent ventricular blood flow in a continuous one and for buffering the oscillations of the blood pressure. This function is dependent of the **visco-elastic properties** of the arterial wall, the main determinant of arterial **distensibility**. Arterial distensibility reduces the vascular impedance by augmenting the vessel radius and allowing the artery to accommodate the pulsatile output of the left ventricle. It stores energy and averages out the blood pressure

pulsations. From a hemodynamic point of view it is important that the total quantity of blood that can be stored for each unit of pressure rises. This measure is called **compliance or capacitance**. Large, elastic arteries exhibit the highest distensibility and compliance within the arterial tree and are known as **capacitance arteries**. Stiffening of capacitance arteries (especially thoracic aorta) interferes with dampening function and is mainly the result of pathologically changes in the **medial layer** of the arterial wall.

$$\text{Vascular distensibility} = \frac{\text{Increase in volume}}{\text{Increase in pressure} \times \text{Original volume}}$$

$$\text{Vascular compliance} = \frac{\text{Increase in volume}}{\text{Increase in pressure}}$$

Conduit (distributing) function, is responsible for transferring the blood from the central circulatory system to different organs. The arterial wall of the arteries supplying the tissues with blood regulates the flow by **acute changes in the arterial diameter** in response to physiologically stimuli. The capacity to vasoconstrict/vasodilate characterizes the **muscular**, **medium size arteries** also known as **conductance (distributing) arteries**. The alteration in the function of these arteries is the result of the narrowing or occlusion of their lumen, mainly as a consequence of atherosclerosis. Although arterial stiffness is not the cause of atherosclerosis, plaque formation will stiffen the arterial wall.

Dampening and conduit functions are interrelated. Capacitance arteries are the main determinant of the blood pressure dampening, but it must be remembered that conductance arteries are also distensible and contribute to this function.

There is some confusion in the literature regarding the "conduit" term. Some authors use "conduit" referring to the large, elastic arteries, and they refer to medium-size, muscular arteries as "distributing". In this text, **by conduit arteries we refer to the medium-size muscular arteries** and **by capacitance arteries we refer to the large, elastic vessels**.

Windkessel vascular model combines the conduit and dampening functions; it refers to the concept that arteries act as storage elastic vessels and transform the intermittent ventricular ejection into a continuous and smooth flow at arteriolar and capillary level (Fig. 3.10). The origin of the name is associated with the early fire engines, which utilized a compressed air chamber (windkessel in German) to transform the intermittent flow of the water pump in a continuous one. In the arterial system, the high systolic pressures generated during the ventricular ejection stretch the arteriolar walls: thus, the arteries will distend and store blood. After the aortic valve closes, the elastic recoil of the vessel walls maintains blood flow during diastole.

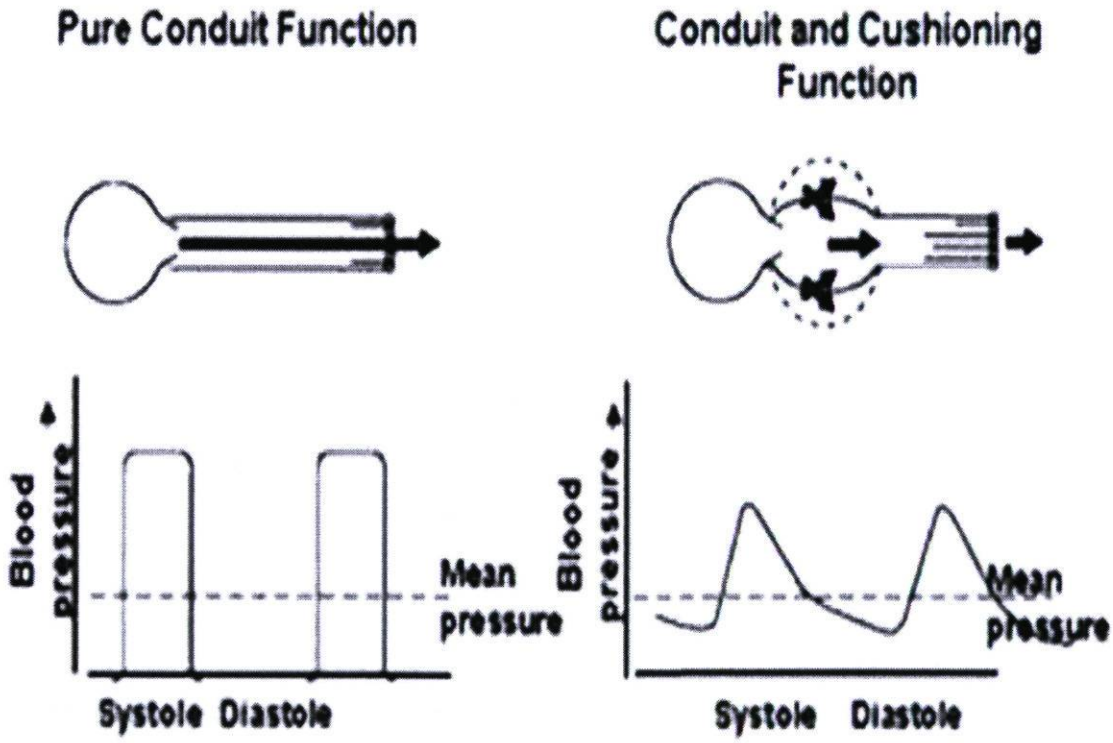


Fig.3.10. Schematic representation of conduit and dampening (cushioning) arterial function.

Although windkessel explains the cushioning function of large arteries, it is a simple model assuming that the whole arterial tree is distending at the same time, following the same linear elastic modulus. In reality, these assumptions are not true. The aorta is a tubular

structure and the distension of the aortic root is followed by the distal segments at a speed (velocity) that can be measured. At the same time, the complex elastic modulus of the arteries increases with the distance from the heart to periphery.

3.3.2 Pulse wave and pulse wave reflection

A more realistic model of arterial hemodynamics includes the propagation of the pulse wave (generated by the left ventricle) through the visco-elastic arterial tree and the reflection of this wave when reaching the increased peripheral resistance. These notions can be imagined by thinking to a simple experiment: a long spring with one free end and the other end being fixed against a hard, inextensible surface (peripheral resistance); on giving a sudden flip (ventricular ejection) to the free end of the spring, we'll create a compressional force passing down its length like a wave (**pulse wave**). The speed of this wave (**wave velocity**) is dependent of the spring rings elasticity. As the wave approaches the fixed end, it will compress the last few rings, which will bounce back (**wave reflection**).

In 1878, Moens and Korteweg derived an expression known as the Moens-Korteweg equation, which describes the relationship between the arterial stiffness and pulse wave velocity (PWV):

$$v = \sqrt{(Ec/2\rho y)}$$

were v is the PWV, **E** is the Young incremental elastic modulus, c is the arterial wall thickness, ρ is the density of blood and y is the arterial diameter in diastole. According to this equation, the PWV is directly related to arterial stiffness (modulus of elasticity): an increased arterial stiffness determines a high PWV.

The propagation and the velocity of the pulse wave was also described by Frank, Bramwell and Hill[104] in the early 1920's. As in clinical setting it was impossible to determine the incremental elastic modulus, they modified the Moens-Korteweg equation and assumed that ρ is constant and equal to 1.055. The resulting equation is the following:

$$v = 0.357\sqrt{(\mathrm{V}/[d\mathrm{V}/dp])}$$

were ***v*** is the PWV, **V** is the arterial volume per unit of length, **dV** is the change in arterial volume per unit of length, when a difference of pressure is applied (**dp**). As dV/dp*V represents arterial distensibility, it become clear that PWV is inversely related with the capacity of an artery to distend.

The continuous change in the arterial elastic properties causes a progressive increase in PWV from aorta to periphery. The increased peripheral resistance and the variation in arterial caliber result in wave reflection[105]. O'Rourke proposed a largely accepted model[105, 106] in which the arterial tree is viewed as a distensible tube with numerous ramifications represented by peripheral resistances. The initial incident pulse wave generated by the left ventricle travels through aorta. The wave is reflected when it reaches the arterial bifurcations, the discontinuities of the vasculature and especially the increased peripheral resistances. This reflection is generating a retrograde wave that travels backwards to the ascending aorta. In this model, the final aortic waveform is represented by the summation of incident and reflected pulse waves (Fig. 3.11). An early reflection during the cardiac cycle augments the systolic pressure stressing the ventricle; a delayed reflection augments the diastolic pressure and helps maintaining a continuous blood flow through diastole.

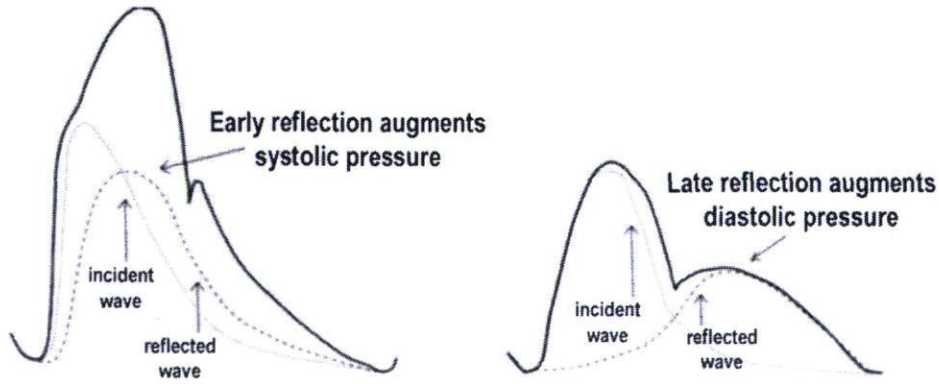


Fig.3.11. Hemodynamic consequences of early and late wave reflection in ascending aorta.

The PWV and the location of the reflection sites determine the timing of the reflected pulse wave in the proximal aorta[105].

A high PWV (increased arterial stiffness) determines an early reflection, as the time needed for the incident and for the reflected waves to travel from the proximal aorta to the reflection sites and back is short. A low PWV implies a delayed wave reflection, as the traveling time of the incident and the reflected waves is longer. Figure 3.12 schematically illustrates the pulse wave propagation correlated with the aortic pressure form during a cardiac cycle at high and low wave velocities.

A short distance from the heart to the wave reflection sites determines an early reflection; if this distance increases, the reflection is delayed. The hypothetical reflection site in humans can be found in the descending aorta, about 40 to 55 cm from the heart[105]. The arterial branching points, the presence of arterial segments with altered distensibility and the arteriolar vascular tonus influence its location[105]. In response to arteriolar vasoconstriction, the reflection site is brought closer to the heart; contrary, arteriolar vasodilatation is driving it away. When the pulse wave velocity is known, the reflection site distance can be derived[107] :

$$L_p = PWV\ \Delta t_p/2$$

were Lp is the distance from the heart to the reflection site, PWV is the pulse wave velocity and Δt_p is the time from the initial upstroke of the pressure wave to the inflection point of the reflected wave.

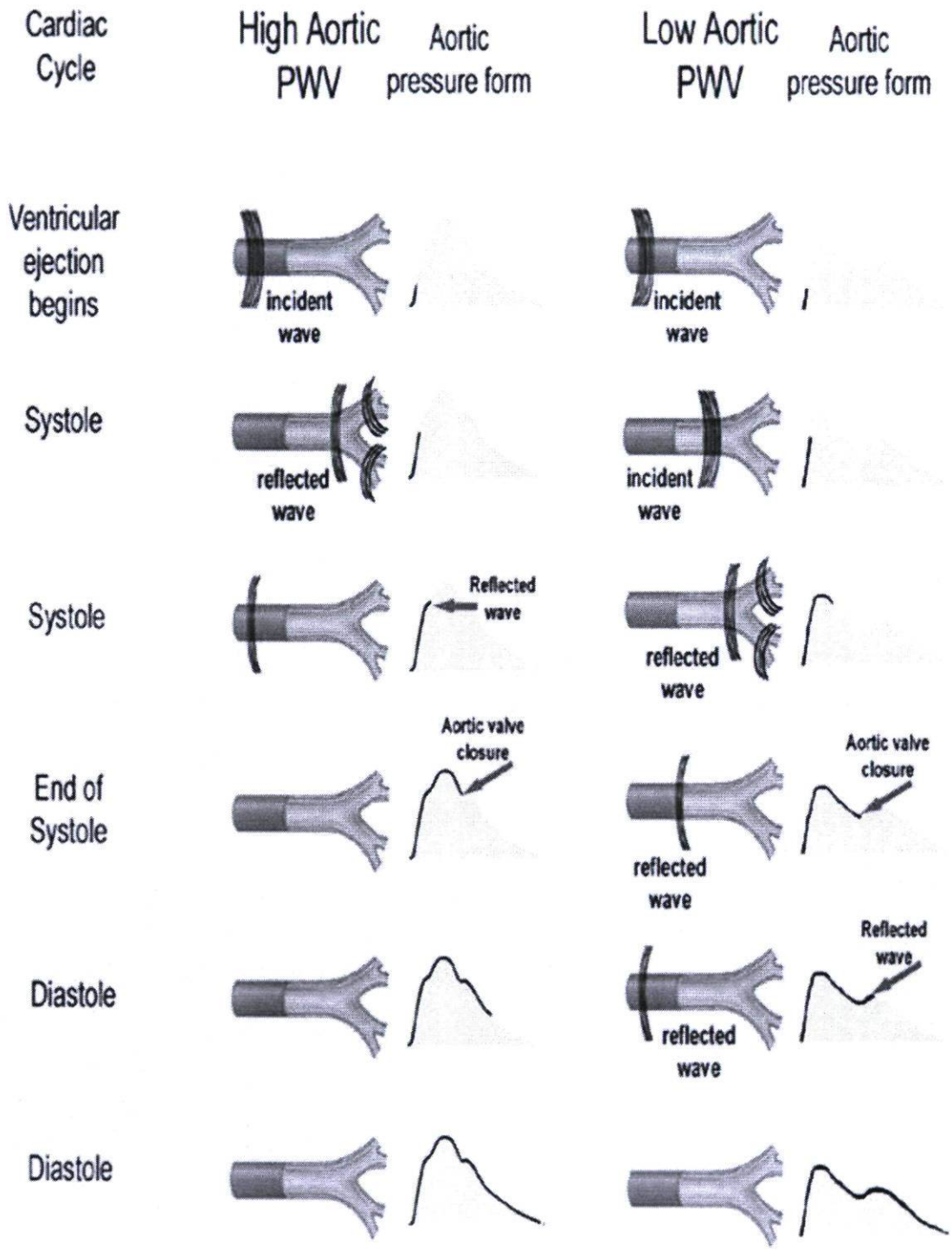


Fig.3.12. Schematic presentation of aortic pulse wave and pressure waveform, during a cardiac cycle at high and low pulse wave velocity. At high velocity, the reflected wave arrives early, during systole. At low velocity, the reflected wave arrives later in the cardiac cycle. *Original figure.*

3.4 Arterial stiffness and blood pressure

3.4.1 The influence of arterial stiffness on systolic, pulse and diastolic pressure – windkessel function alteration

An increased arterial stiffness implies a decreased arterial distensibility, which alters the arterial dampening function. In a stiff artery, the amplitude of the pulsatile flow is not decreased by the windkessel effect, resulting in an ***increased systolic and pulse pressure***, along with a ***decreased diastolic pressure***.

3.4.2 Central versus peripheral blood pressure and the pulse pressure amplification phenomenon – wave reflection

The variation in arterial distensibility (elasticity) between central and peripheral vessels changes the shape of arterial pulse wave as it travels along the arterial tree. The pulse wave reflection is more pronounced in the periphery as the arterial stiffness is greater and the distance to the reflection sites is shorter. The net result is that the amplitude of the arterial pulse pressure increases from ascending aorta to middle-size, muscular (brachial, femoral) arteries. In other words, ***the pulse pressure increases from the central to peripheral locations*** and the cuffed measured brachial pressure does not corresponds to central blood pressure[108]. These findings were experimentally demonstrated[109] and the importance of central versus peripheral (brachial) blood pressure is largely and clinically accepted[110, 111].

Normally, in humans, central systolic and pulse pressure are lower (with approximately 14mmHg) than peripheral systolic and pulse pressure, whereas the diastolic and mean pressures are almost identical[112, 113]. The amplification of pulse pressure from aorta to periphery is known as ***"pulse pressure amplification"***. For a certain individual, the pulse pressure amplification varies and is dependent of aortic stiffness, reflection sites and reflection coefficient[105]. As shown in Figure 3.13, for a given peripheral pulse pressure,

the central pulse pressure markedly decreases in the case of an elastic, distensible aorta; as aortic stiffness increases, the central values approach the peripheral pulse pressure.

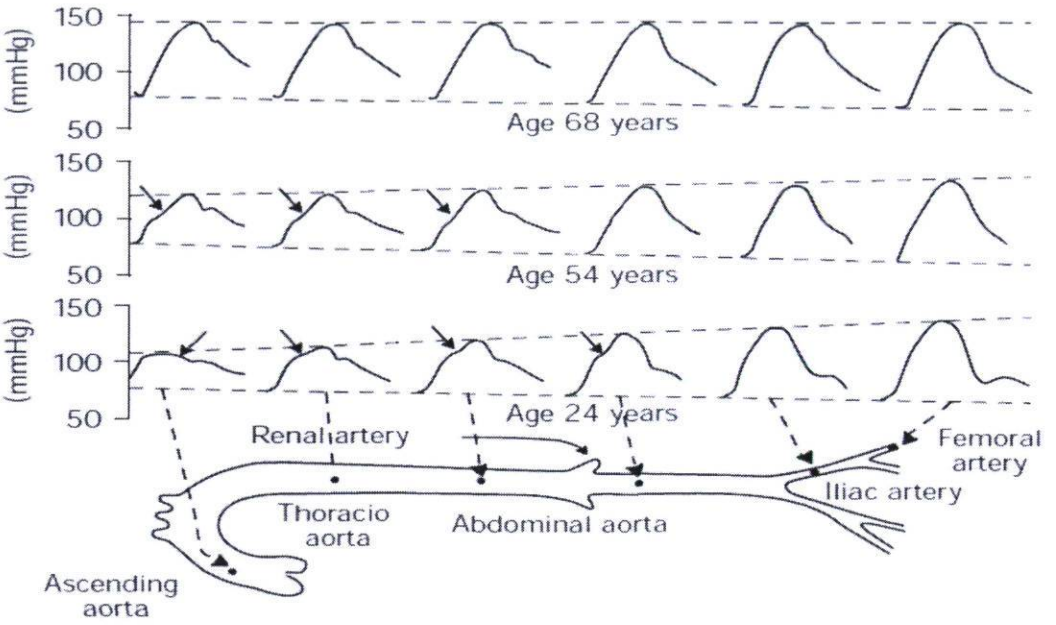


Fig.3.13. Schematic presentation of changes in pressure wave contour along the aortic trunk in adult human subjects aged 24, 54 and 68 years. Aortic stiffness increases with age. The arrow on the waveforms indicates the first systolic inflection corresponding to the beginning of the reflected wave at different positions in the arterial tree. Note that this point tends to occur earlier in systole with advancing age. ***Figure from Wilmer W. Nichols and M.F. O'Rourke [105].***

The theoretical concept is confirmed by epidemiological studies, as shown in Figure 3.14.

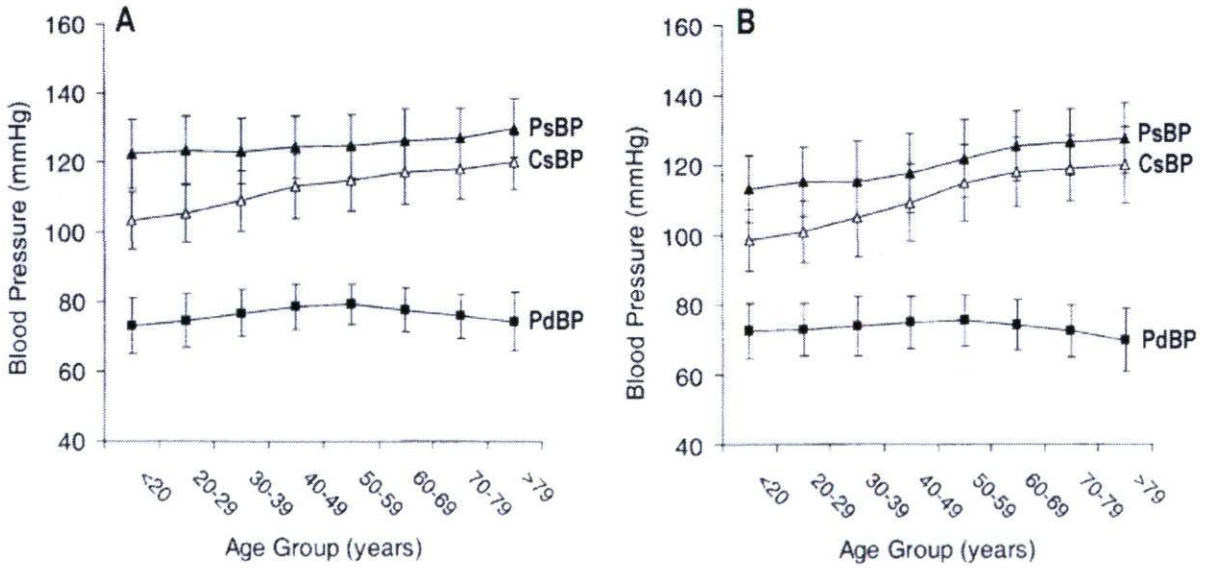


Fig.3.14. Blood pressure averaged for deciles of age for males (A) and females (B). Peripheral systolic pressure (PsBP); central systolic blood pressure (CsBP); peripheral diastolic blood pressure (PdBP). ***Figure adapted from McEniery, C.M., et al.[114].***

Conventional measurement of cuff blood pressure in the brachial artery does not take into account the aortic stiffness. A clinical approach based only on peripheral pressure measurements risks to ignore important and relevant informations. For example, in a study of 10,613 men and women aged from 18 to 101 years old, more than 70% of individuals with high-normal brachial pressure had similar aortic pressures as those with stage 1 hypertension. The data demonstrated that the assessment of central pressure may improve the identification and management of patients with elevated cardiovascular risk[108].

Because of the pulse pressure amplification phenomenon, an expert consensus was reached regarding the brachial and central pulse pressure measurements: it is inaccurate to use brachial pulse pressure as a surrogate for aortic or carotid pulse pressure, particularly in young subjects[115]. Central pulse pressure can be measured invasively, directly from the aorta or noninvasive by analyzing the peripheral wave pressure form as detailed later in this text.

Arterial stiffening increases systolic and pulse pressure as a consequence of decreased compliance and alteration in dampening (windkessel) function. Aortic stiffening increases peripheral systolic and pulse pressure as a consequence of decreased compliance and alteration in dampening (windkessel) function ***and*** **increases the central (bringing it closer to the peripheral values) systolic and pulse pressure as a consequence of increased arterial wave reflections.**

In summary, aortic stiffness increases the systolic pressure by two means: decreased distensibility and increased arterial wave reflections.

3.5 Non-invasive clinical measurements of arterial stiffness and wave reflections

Regional and local arterial stiffness can be measured non-invasively by different means[115]. Local arterial stiffness can be determined using ultrasound devices (carotid arteries) or cine magnetic resonance imaging (aorta). Regional stiffness can be measured at various sites along the arterial tree by pulse wave velocity (PWV). As discussed above, arterial and especially aortic stiffness have an important influence on central hemodynamics parameters and pulse wave reflections that can be assessed by pulse wave analysis (PWA).

3.5.1 Carotido-femoral pulse wave velocity ($_{c\text{-}f}$PWV) and Complior® system.

Carotido-femoral pulse wave velocity ($_{c\text{-}f}$PWV) is considered to be the gold standard measurement of arterial stiffness. It is a simple, non-invasive, and reproducible method that measures the PWV along the arterial pathway (thoracic and aorto-femoral territory) with the greatest influence on central hemodynamics[115]. At the base of this concept is the Moens-Korteweg equation (found in section 3.2.4) which states that the arterial wall stiffness (incremental elastic modulus) of an artery is directly proportional with the speed of the pulse wave traveling along its length. An increased arterial stiffness determines a high $_{c\text{-}f}$PWV.

$_{C\text{-}f}$PWV is usually measured using the foot-to-foot velocity method from arterial waveforms, which are usually obtained transcutaneously at the common carotid and femoral artery. The arterial waveform can be acquired from different types of captors including echotracking[116, 117] and Doppler[118] probes.

In the study presented here, we used a Complior® system[119] which uses a mechanotransducer to capture the waveform. The software is mathematically derivating the waveform and identifies the foot of the wave (the beginning of the up-stroke inflection),

then the time interval (Δt) between the two wave feet. The c-fPWV is calculated in meters/second by dividing the distance between the two arterial sites by Δt (Fig. 3.15)

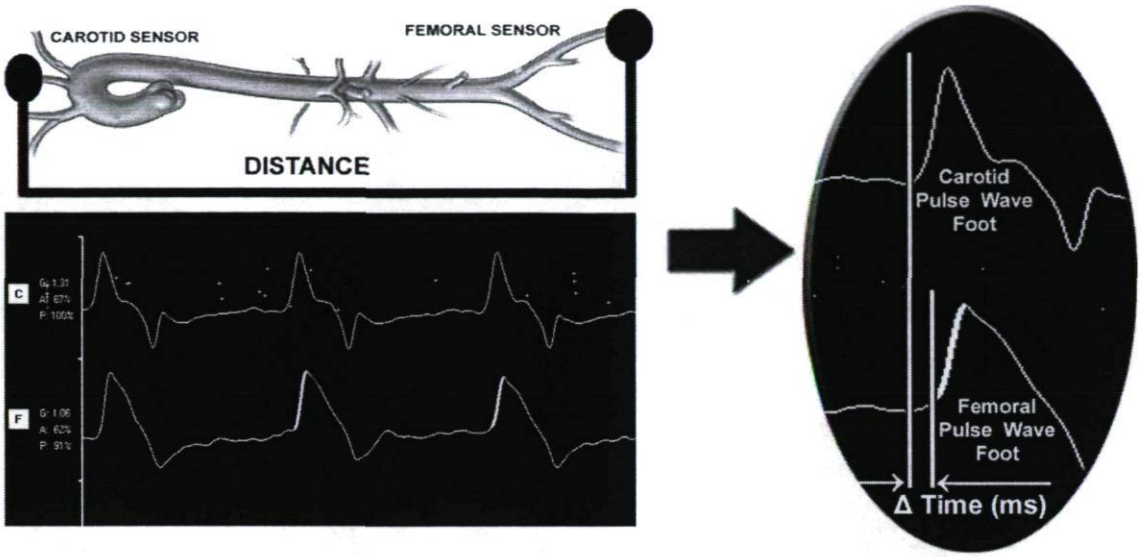


Fig.3.15. Complior® carotidal and femoral pulse wave tracing along with schematic representation of the time spacing (Δ Time) between the carotidal and femoral foot wave. ***Original Figure.***

The distance between the carotidal and femoral site should be measured precisely because small inaccuracies may influence the absolute value of PWV[120]. Abdominal obesity, particularly in men, and large bust size in women can make distance measurements inaccurate[121]. In our study we tried our best to measure the distance from the carotidal to femoral site in a straight line with the patient in a supine position ignoring the external shape of the body.

The distance that should be used when measuring the $_{c\text{-}f}$PWV by the foot-to-foot method is controversial and the majority of the investigators recommend:

1. using the total distance between the carotid and femoral sites of measurement (like in our study) **or**
2. subtracting the distance from the carotid location to the suprasternal notch from the total distance **or**
3. subtracting the distance from the carotid location to the suprasternal notch from the distance between the suprasternal notch and the femoral site of measurement.

All three methods are approximations and absolute differences are unimportant in intervention studies with repeated measurements when investigators use the same method. However, when comparing two populations, differences in the methods used to assess the path length will be critically important. Recent expert opinions[122, 123] underlined that the method used to calculate the distance for measuring aortic PWV should be by **subtracting the distance from the carotid location to the suprasternal notch from the distance between the suprasternal notch and the femoral site.** This method would lead to the standardization of PWV values and allow the comparability between different noninvasive devices[124].

By measuring the Δt between the carotid and femoral pulse wave foot and using as distance the method from 3), the aortic PWV can be computed. Aortic PWV can be also measured by applanation tonometry using electrocardiography as the "time reference".

As mentioned earlier, in normal physiologic conditions, the main determinants of aortic stiffness are blood pressure and age. This was confirmed in a study[125] of 77 healthy young individuals were the authors found that after multiple regression analysis, c-fPWV was positively correlated with diastolic blood pressure and age. In another study[126] in which 296 normal individuals were followed for a period of six years, multivariate analysis showed that determinants of baseline c-fPWV were age, body mass index and mean arterial pressure. The only significant determinant of the c-fPWV progression in normal, healthy individuals was age: there is an increased progression of c-fPWV with aging. Monnier *et all*[127] also showed that PWV progressively increases with age, more rapidly after age 45.

3.5.2 Pulse waveform analysis (PWA) and Sphygmocor® system.

The graphic recordings and analysis of the pulse waveform date from last century when Mahomed (1872) and Marey (1860) introduced the sphygmograph as a clinical tool in medical examination. With the arrival of the sphygmomanometer (blood cuff), the simplicity and the accuracy of this technique overcome, at that time, the pulse wave analysis.

In the modern era, the observation of early pioneers that the peripheral pulse waveform is different from the central one was explained by McDonald[128] as being the result of the pulse wave reflections. The "pulse pressure amplification" and the epidemiological evidence demonstrating the importance of central hemodynamics parameters in evaluating the cardiovascular risk[129] raised the interest in the central pulse waveform analysis (PWA).

The central pulse waveform can be directly obtained by invasive catheterization of proximal aorta. With the introduction of high fidelity tonometers, the peripheral (e.g. radial) pulse waveform can be noninvasively and easily obtained and analyzed. The central (aortic) pulse waveform can be computed from periphery by using a "transfer function". Once this function was validated[130], the central hemodynamic parameters can be non-invasively estimated.

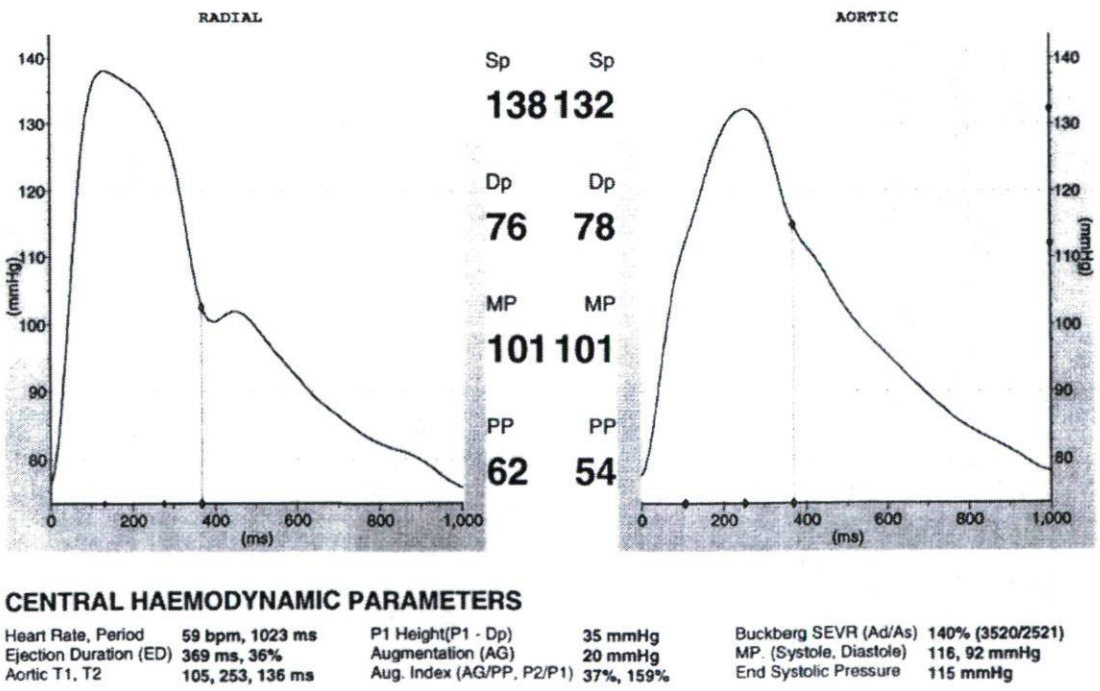


Fig.3.16. Sphygmocor® interface. Systolic pressure (Sp); diastolic pressure (Dp), mean pressure (MP), pulse pressure (PP). ***Figure from O'Rourke, M.F.[131].***

The Sphygmocor® system acquires the pulse waveform from the radial artery and than computes the central pulse waveform[131]. Figure 3.17 shows the interface of the system. On the left side of the image is the radial pulse waveform recorded by a high fidelity sensor using applanation tonometry. The waveform is calibrated using systolic and diastolic pressure values from conventional cuff measurement. On the right side of the image, there is the computed aortic pulse waveform. An average waveform is calculated from the ensemble average of a series of contiguous pulses.

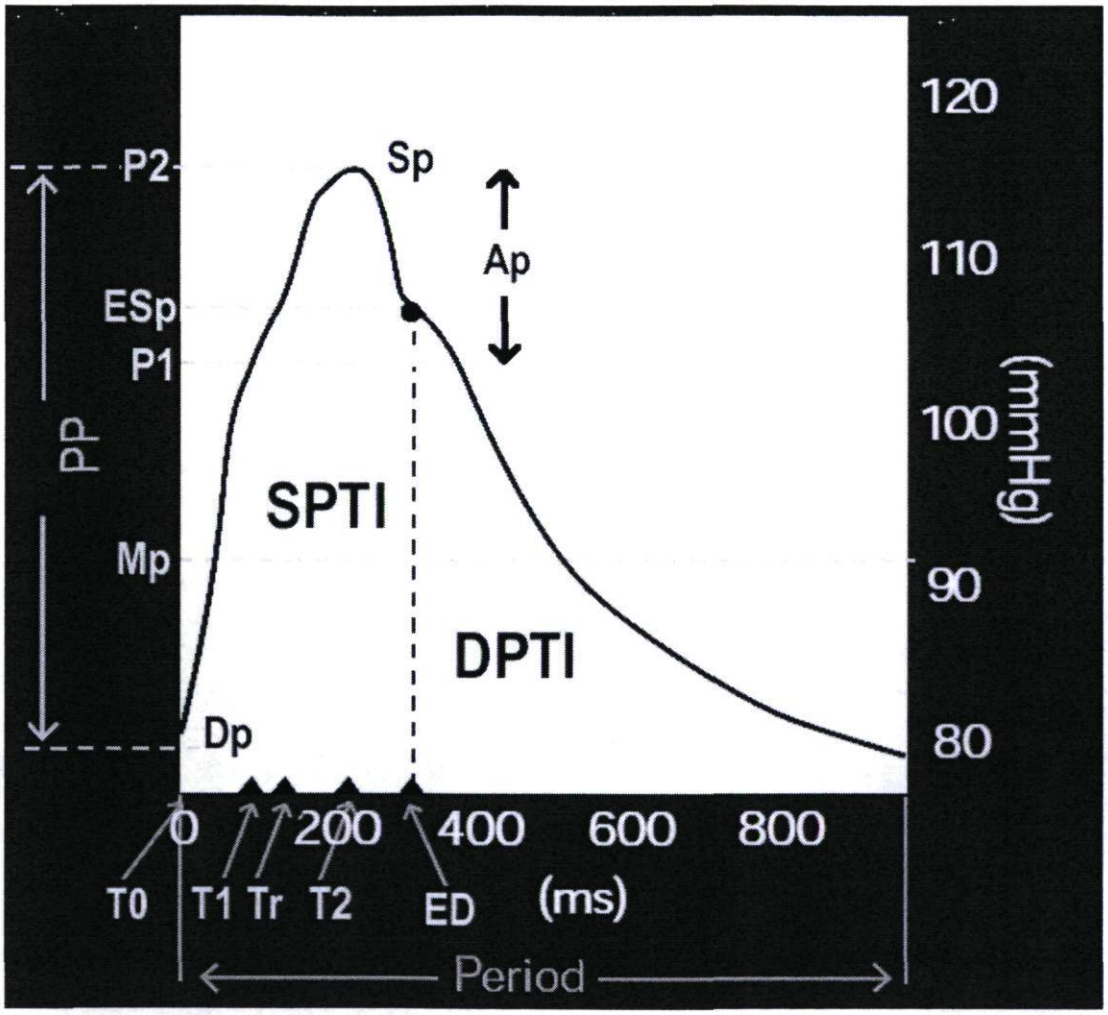


Fig.3.17. Aortic pressure waveform. Systolic pressure (Sp); diastolic pressure (Dp); pulse pressure (PP); mean pressure (Mp) calculated as the area under the curve of radial waveform; end systolic pressur (ESp); first systolic shoulder (P1) or peak flow; systolic peak pressure (P2); beginning of ventricular ejection (T0); time at P1 (T1); time to return of the reflection wave (Tr); time at P2 (T2); ejection duration (ED); augmentation pressure (Ap); systolic pressure time index (SPTI); diastolic pressure time index (DPTI). ***Figure adapted from[132].***

The artic pressure waveform starts with the ejection of the left ventricle at T0. In a very elastic, compliant aorta, peak pressure coincides with peak flow. On the contrary, in a stiffer aorta (Fig. 3.17), peak flow occurs before peak pressure (hysteresis of the arterial wall, section 3.1.2) and can be noticed on the aortic waveform as a systolic shoulder appearing at P1. In other words, after the time corresponding to peak flow (T1), the aortic waveform changes its slope into a "flatter one" as a result of a decrease in flow. The reflected wave arrives at Tr (time of the reflected wave) and augments the pressure at systolic peak (P2). The difference between P2 and P1 (P2-P1) represents the augmented pressure (Ap). Augmentation index (AIx) represents the ratio Ap/PP*100.

AIx depends on the magnitude of the wave reflection and the time of wave reflection (Tr). The magnitude of the wave reflection (the amount of reflection) is determined by the reflection coefficient[133] and will not be discussed here. An early reflection (short Tr) determines an increased AIx, which among others is influenced by:

- **pulse wave velocity** - An increased aortic stiffness and PWV determines an increased Aix[134]. Unpublished data from our laboratory shows that in 172 chronic hemodialysis patients, in a multiple regression analysis using as covariates age, mean blood pressure and c-fPWV, the AIx corrected for a heart rate of 75bpm is positively associated with c-fPWV ($r=0.409$, $p<0.001$).
- **reflection sites location** - AIx increases as the distance from the ascending aorta to the reflection sites decreases. Examples include peripheral vasoconstriction or short body height, which brings the reflection sites closer to the aorta. On the contrary, vasodilatation and increased body height decrease the Aix[135-138].
- **heart rate** - An increased heart rate shortens the ventricular ejection duration. As the time needed for the pulse wave to travel remains the same, the Tr will appear later in the systole because of the shortened ejection duration. The net result is a decreased in AIx of approximately 4% for 10bpm increase in heart rate[139]. In our study, we used the corrected (by Sphygmocor® software) value of AIx for a heart rate of 75bpm.

In a study[140] of 330 healthy young males (mean age of 28 year), the authors found that after multiple regression analysis, the AIx was positively associated with age, aortic PWV and mean arterial pressure and inversely associated with body height and heart rate.

The area under the systolic curve of the aortic pulse waveform is referred to as ***systolic pressure time index (SPTI)*** and is directly related to cardiac work and oxygen consumption. The area under the diastolic curve is referred to as diastolic ***pressure time index (DPTI)*** and is directly related to pressure and time of coronary perfusion. The ratio DPTI / SPTI*100 express the relation between cardiac supply and demand, also known as ***subendocardial viability ratio (SEVR) or Buckberg Index***. In normal conditions, SEVR has a value between 130% and 200%. When it is below 100%, the subendocardium layers were found to be underperfused[141] and may represent an aggravating factor in patients with coronary artery disease[142]. SEVR was found to decrease with high heart rates (by decreasing the diastolic / systolic time ratio)[143] and increased aortic stiffness[144]. Some authors also suggest that in patients with increased aortic stiffness, SEVR obtained by non-invasive means could be underestimated[145].

Previous studies have shown differences between intra-arterial and cuff blood pressure measurements[146, 147]. One of the Sphygmocor® system limitations is that noninvasive estimation of aortic pressure relates to calibration from the sphygmomanometer cuff. However, the aortic pressure waveform generated from the radial waveform calibrated with the sphygmomanometer cuff improves the hemodynamic information in comparison with that provided by the peripheral blood pressure alone.

3.5.3 $_{C\text{-}f}$PWV and PWA as a measure of aortic stiffness.

AIx and PWV (aortic or c-f) cannot be used interchangeably as an index of aortic stiffness. AIx may not be a true indicator of aortic stiffness, but an index of wave reflection and PWV[148, 149]. The findings of some authors demonstrating that AIx can change independently of aortic PWV after short[135] and long[150] term pharmaceutical interventions, support these views.

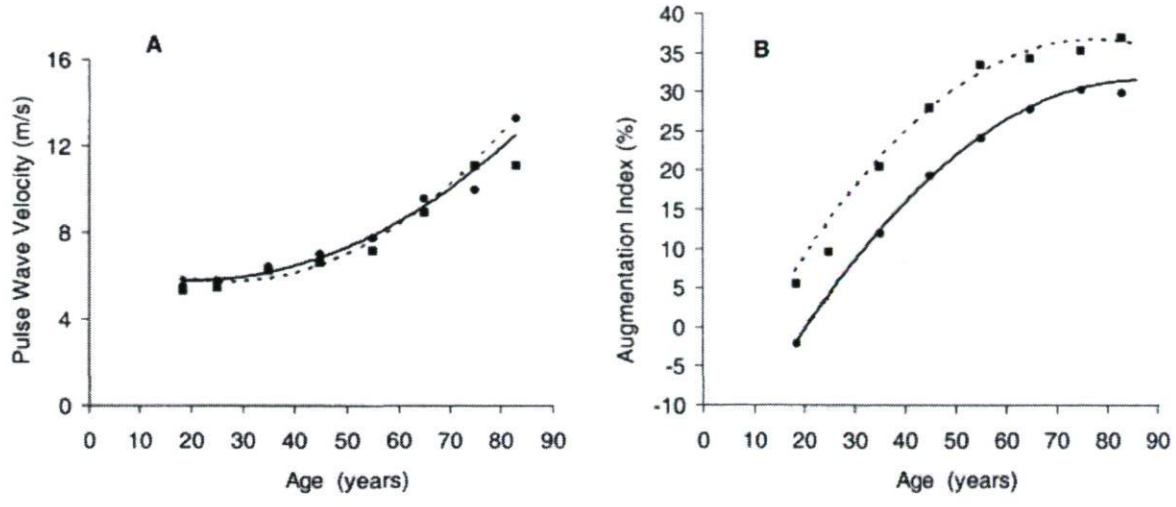


Fig.3.18. Regression curves representing the effect of age on aortic PWV (A) and augmentation index (B) in males (circles, solid lines) and females (squares, dashed lines). ***Figure adapted from McEniery, C.M., et al.[114].***

The different pattern of age-related changes (Fig. 3.18) is another evidence that PWV and AIx are markers of vascular aging while representing a spectrum of somehow different pathophysiological mechanisms. AIx increases more in younger individuals whereas aortic PWV increases more in older individuals. Overall, these data suggest that AIx might be a more sensitive marker of arterial aging in younger individuals, and aortic PWV is more sensitive in those over 50 years of age.

3.6 Pathophysiologic consequences of arterial stiffening

3.6.1 Effects on macro and microvasculature

The increased blood and pulse pressure associated with aortic stiffening leads to chronic alterations of mechanical forces acting on the arterial wall. As a consequence, the large and medium-sized vessels suffer a remodeling process demonstrated by changes in the composition and geometry of the artery along with a progressive increase in diameter[151].

An increased pulse pressure was also found to have deleterious effects on microcirculation by inducing small artery and arteriolar remodeling. In an animal model using arteriovenous fistula for raising the pulse pressure, the authors reported the hypertrophy of cerebral arterioles[152]. Others demonstrated that experimental stiffening of the large arteries by a medial elastocalcinosis model[153] resulted also in hypertrophic remodeling of middle cerebral and mesenteric arteries. These findings confirm the hypothesis that large artery stiffness has a negative influence on small arteries and arterioles by raising the pulse pressure and augmenting the arteriolar wall stress.

3.6.2 Ventricular hypertrophy

As we mentioned above, in normal conditions, in a young healthy adult, the PWV, the peripheral resistance and the ventricular contraction are physiologically coupled, so that the arrival of the reflected wave in proximal aorta occurs in the diastolic phase when the aortic valve is closed. When there is an increased arterial stiffness as measured by carotido-femoral pulse wave velocity **and / or** the reflection sites change their location more proximally (vasoconstriction, increased peripheral resistance), the reflected wave arrives while the aortic valve is still open, thus increasing the pressure load[107]. The causal relation between an increased aortic augmentation and ventricular hypertrophy is schematically proposed in Figure 3.20: the arrival of the reflected wave during systole (opened aortic valve) gives a "ventricular kick". In this situation, the stress on the ventricular wall is dependent on the timing of the reflected wave. An early arrival as measured by an increased aortic AIx increases the ventricular pressure load and "kicks" the ventricle early in systole when the ventricular radius is higher and the wall thinner. Thus, the reflecting wave is applying a greater wall stress (according to Laplace law – Fig. 3.19) and it determines ventricular remodeling and hypertrophy (conform to Frank-Starling mechanism[4]).

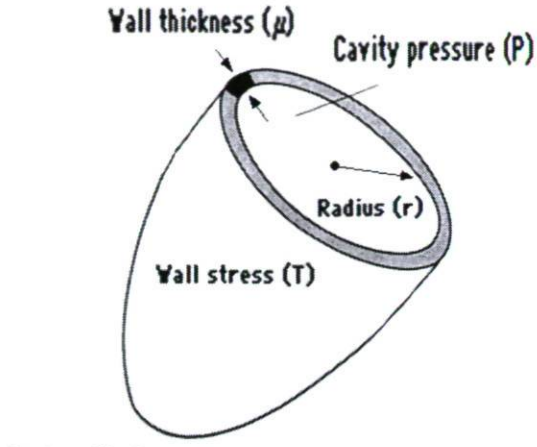


Fig.3.19. Laplace law: the wall stress is directly related to radius and inversely related to wall thickness.

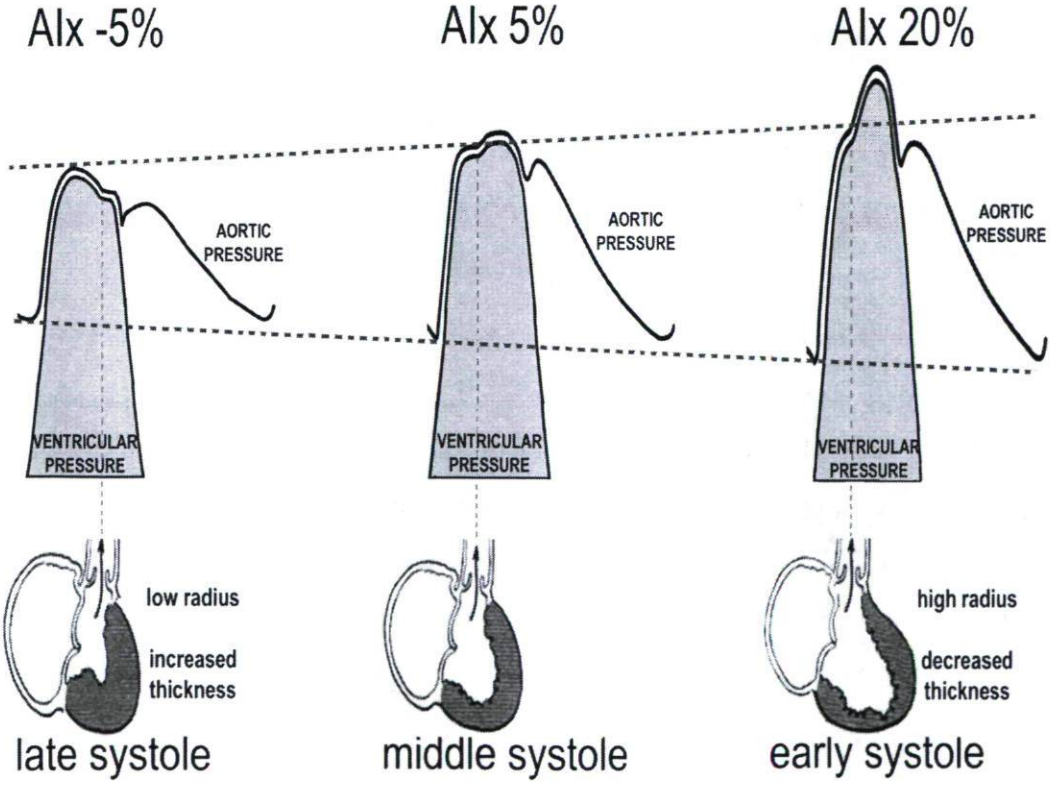


Fig.3.20. Aortic augmentation index and ventricular coupling. A late arterial reflection wave stresses the ventricle at a time of low radius and increased thickness. An early reflection stresses the ventricle at a time of high radius and decreased thickness. Augmentation index (AIx). *Original figure.*

As the AIx approaches values closer to 0, the additional pressure load and the "ventricular kick" arrive at a low radius and increased thickness, thus applying a lower wall stress.

The association of increased aortic stiffness (measured by carotido-femoral, aortic or brachial-ankle pulse wave velocity) with ventricular hypertrophy was demonstrated in young healthy subjects[154], hypertensive[155-159] and ESRD patients[160]. In a study of 256 normotensive subjects and hypertensive patients, pressure-dependent methods for measuring arterial stiffness showed an association with left ventricular hypertrophy whereas pressure-independent methods were associated with left ventricular concentric remodeling[161] . The AIx was found to be an independent predictor of left ventricular mass in normotensive subjects[162], hypertensive[159, 163, 164] and ESRD patients[165]. It was also shown that the regression of left ventricular hypertrophy during antihypertensive treatment positively correlated with the degree of AIx reduction[166] and delayed wave reflection[167]. Experimental models in animals, designed to explore the relation between arterial stiffness and ventricular function, found an association between PWV, AIx and ventricular hypertrophy[168]. It was also demonstrated that the loss of arterial distensibility negatively influence the myocardial energetic metabolism[169].

3.6.3 Decreased subendocardial perfusion

As we mentioned in section 3.4, an increased aortic stiffness determines decreased diastolic pressure and increased systolic and pulse pressure. The net result is a decreased DPTI / SPTI ratio (decreased Buckberg Index). As the heart is mainly perfused during diastole, it can be concluded that a stiff aorta could have a negative influence on coronary perfusion[170].

Experimental studies using hydraulic models suggested that low aortic compliance is associated with a reduction in coronary blood flow[171]. Animal studies have shown that a reduction in aortic distensibility resulted in decreased coronary flow, especially subendocardial[172, 173]. However, these findings are controversial, as others have found

that an increased in pulse pressure stimulates and augments the coronary blood flow. In one of this studies, the authors took into account only the coronary pulse pressure and waveform (the coronary blood flow was driven by a computerized servopump system)[174]. Others showed that, during acute experimentally induced decrease in aortic compliance, the coronary blood flow increased due to a higher coronary systolic flow, which was sustained by an increased heart rate [175].

Studies in humans confirmed that aortic stiffness is related to decreased coronary flow velocity and reserve in healthy individuals[176], diabetics with a negative coronary angiogram[177], CAD patients[178] and in patients with stable angina after percutaneous coronary intervention[179, 180].

3.7 Aortic stiffness as a predictor of cardiovascular morbidity and mortality

There is strong evidence that aortic stiffness as measured by PWV is an independent predictor of cardiovascular risk[181, 182]. In 2007, The Europeans Society of Hypertension guidelines for the management of arterial hypertension[183] listed a carotido-femoral pulse wave velocity higher than 12m/s as evidence of subclinical organ damage. Increased aortic stiffness, as evaluated by central hemodynamic parameters (central pulse pressure and AIx), was also found to correlate with cardiovascular risk.

Carotido-femoral and aortic pulse wave velocities were found to predict cardiovascular morbidity and mortality in a wide range of individuals: general[184-187] and elderly population[188], diabetics[189], hypertensive[190-194] and ESRD patients[195-197]. The influence of blood pressure independent carotido-femoral pulse wave velocity on cardiovascular mortality was demonstrated in ESRD patients: the individuals who did not respond by a regression of aortic stiffness following reduction in blood pressure showed an increased cardiovascular mortality[198]. Based on these prospective studies, it was suggested that 12m/s represents the threshold for an abnormally elevated carotido-femoral pulse wave velocity[183].

In a review[129, 199] analyzing the results of 11 longitudinal studies including general[200] and elderly population[201], hypertensive[202], coronary artery disease[203-207] and ESRD patients[208-210], the authors concluded that central hemodynamic parameters are independent predictors of future cardiovascular events and all-cause mortality. AIx was found to predict the cardiovascular events independently of peripheral pressures. The authors also concluded that central pulse pressure values result in a better prediction of cardiovascular events when compared with peripheral pulse pressure values (marginally significantly). In a very recently published study[211] including 520 patients undergoing coronary angiography, a 10% increase in the AIx (derived from noninvasive pulse waveform analysis) was associated with 31.4% increased risk of all cause mortality, myocardial infarction, stroke or revascularization.

4. Aortic stiffness in ESRD

4.1 Aortic stiffness is increased in CKD patients

Renal function is an important determinant of aortic stiffness. It has been found that even mild, within normal values, deterioration in renal function is associated with increased aortic PWV independently of blood pressure[212, 213]. Studies designed to reveal the determinants of aortic stiffness progression in healthy and hypertensive individuals found that serum creatinine levels are a major determinant of accelerated aortic stiffness progression[126]. In already established CKD, an advanced stage and decrease glomerular filtration rate is associated with an increase aortic PWV[214, 215].

In ESRD patients it was demonstrated that aortic stiffness, as determined by functional (increased systolic and pulse pressure), hemodynamic (increased aortic, c-fPWV and Aix) and structural (increased intima to media ratio) parameters[216-219] is increased when compared to general population. Some authors[220] found that aortic PWV is higher in hemodialysis patients even when compared with age and blood pressure matched controls. These altered hemodynamic parameters are accompanied by cardiovascular morphologic changes like increased left ventricular mass and increased arterial diameter[218, 221-223]. Table 4.1 is an example of such findings in hemodialysis patients without previous history of cardiovascular disease when compared with age-matched healthy controls. Although the increased aortic stiffness could be a marker of numerous cardiovascular comorbidities and risk factors found in this patients, it has been hypothesized that the alteration of arterial architecture and function could play a primary and direct role in the development of cardiac complications in ESRD patients[224, 225].

The origin of the increased aortic stiffness in hemodialysis is not completely elucidated. Although it is obvious that the exposure to CKD is partially responsible for this phenomenon, the influence of chronic hemodialysis on aortic stiffness is a matter of debate. Some authors[226] concluded that hemodialysis has no adverse effect on aortic stiffness

that is present before starting the renal substitution therapy. However, the results of this cross-sectional study might be influenced by the survival bias (section 2.6.3) and the volume status of the patients at the time of evaluation. The long term influence of hemodialysis on aortic stiffness could be elucidated by further prospective studies, a work in progress in our laboratory[227-229].

	Normal controls ($n=260$)	ESRD patients ($n=257$)	p
Systolic BP (mmHg)	118.9±16.1	133.4±29.8	**<0.0001**
Diastolic BP (mmHg)	68.9±11.1	76.0±17.1	**<0.0001**
Pulse pressure (mmHg)	50.0±10.6	57.4±18.9	**<0.0001**
AI (%)	15.2±19.0	22.5±18.6	**<0.0001**
PWV (m/s)	8.3±3.0	10.7±4.6	**<0.0001**
CCA IMT (×100) (mm)	7.2±1.6	8.2±1.8	**<0.0001**
LVM (g)	144±44	207±68	**<0.0001**
Aorta inner diameter (mm)	30±4	31±4	**<0.0001**
CCA inner diameter (mm)	5.5±0.6	6.3±1.0	**<0.0001**

Table 4.1. End-stage renal disease (ESRD); blood pressure (BP); augmentation index (AI); aortic pulse wave velocity (PWV); common carotid artery (CCA); intima media thickness (IMT); left ventricular mass (LVM) in controls and ESRD patients. ***Table adapted from Hsu, P.F., et al.[218].***

4.2 Pathogenesis of aortic stiffness in CKD patients

The underlying mechanisms for an increased aortic stiffness in CKD are not well defined. Extensive literature reviews of this subject[230, 231] suggest a list of potential pathophysiological processes associated with arterial stiffening in CKD patients: hypertension, chronic fluid overload, arterial calcifications and disturbed phosphocalcic

metabolism, inflammation, malnutrition, vitamin deficiency, sympathetic nervous system over-activity, activation of the renin–angiotensin system, oxidative stress and increased lipid oxidation, endothelial dysfunction and abnormalities in NO metabolism. It has also been proposed that aortic stiffness is the result of the accumulation of advanced glycation end products with subsequent crosslinking of the elastin and collagen fibers, and enhanced accumulation of extracellular matrix in the arterial wall[232-235].

Among the morpho-pathologic lesions of the arterial wall that could be responsible for arterial stiffening, **medial arteriosclerosis and calcification** was proved to be more prevalent in hemodialysis patients than in the general population, especially at younger age[29, 236]. These lesions, which can be quantified by noninvasive means like electron-beam and multi-slice computed tomography, ultrasonography and plain radiographs, were found to be correlated with aortic PWV[237, 238]. Experimental studies in animals demonstrated that induced uremia is also associated with **vascular smooth muscle cells hyperplasia and hypertrophy, increase in aortic extracellular matrix**, increased cross-sectional area of the aortic media and increased aortic wall thickness[239, 240].

It was also shown that in the aortas of uremic patients there is an enhanced AGE-related modification of proteins [241]. The hypothesis that **AGE accumulation in the vascular tissue increases protein cross-linking** is supported by the findings of a marked decrease in aortic collagen solubility of the diabetic rats[242]. The implication of advanced glycation end products in the pathophysiology of aortic stiffness in hemodialysis patients is a matter of interest and future research in our laboratory[243-249].

4.3 Aortic stiffness and mortality in ESRD

As showed in Figure 4.1 and 4.2, aortic stiffness, measured by aortic PWV[197] and AIx[208], was found to be an independent survival predictor in ESRD patients, although some authors expressed reserves regarding the AIx[210]. It is well established that kidney transplant improves the survival of ESRD patients[250]; however, there is little and scarce data about the reversal of aortic stiffness in this population. Some authors found that after

successful kidney transplant, there is a reduction in aortic stiffness, without tacking into account the changes in blood pressure[251, 252]. Others found that the improvement in aortic stiffness is blood pressure dependent[253], while others found no improvement[254].

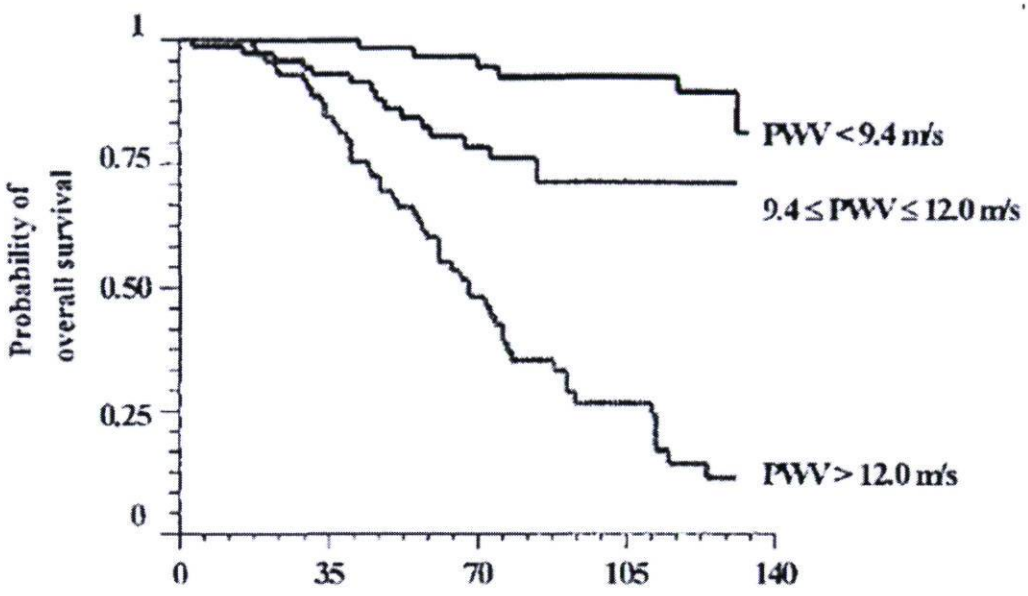


Fig.4.1. Aortic PWV and overall mortality in ESRD. ***Figure from Blacher, J., et al[197].***

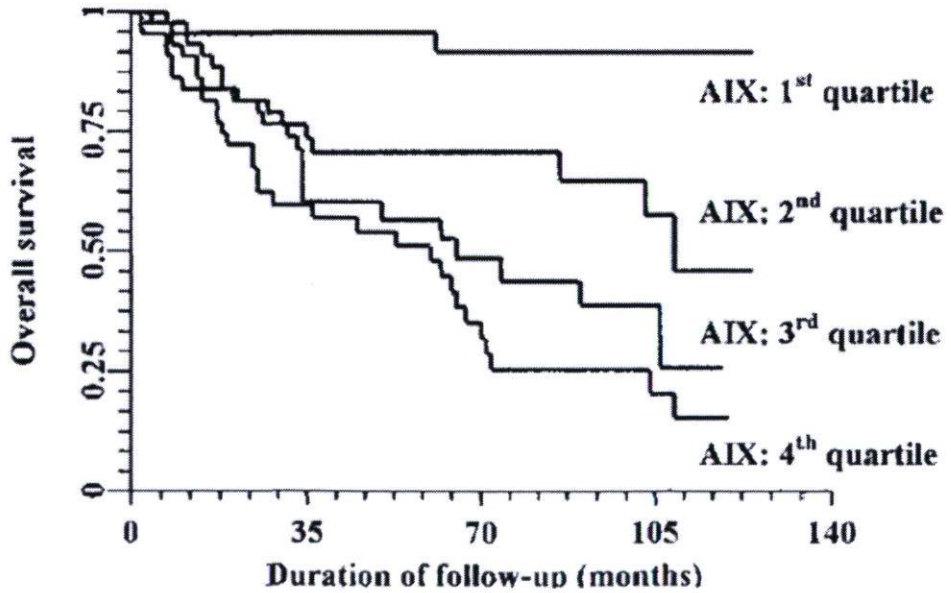


Fig.4.2. Augmentation Index (AIX) and overall mortality in ESRD. ***Figure from London, G.M., et al.[208].***

Recent data from our laboratory shows that there is a decrease in the AIx after kidney transplantation along with a blood pressure independent improvement in c-fPWV. The improvement in c-fPWV seams to be age-dependent and more important in patients of older age[255-257]. These findings further support the hypothesis that aortic stiffness is not only an independent mortality risk factor but also a modifiable one in ESRD.

5. Arteriovenouse fistulas in hemodialysis

5.1 Definition and utility of an arteriovenous fistula in hemodialysis

Arteriovenous fistulas describe an abnormal communication between an artery and a vein that can be congenital or acquired. **Congenital fistulas** can be divided in tumors (high turnover of endothelium) and malformations (dysmorphogenesis with no evidence of abnormal endothelial turnover). **Acquired fistulas** are secondary to trauma, tumors or as a result of surgery. **Arteriovenous fistula** is a term describing a singular communication between an artery and a vein that usually has an acquired etiology. It can be located anywhere in the human body, in the systemic or pulmonary circulation. In the following text we will refer to the **arteriovenous fistulas located in an extremity** (arm or leg) as it is relevant to our study.

In hemodialysis, an arteriovenouse fistula plays the role of the **vascular access**, which is necessary to connect the patient's circulatory system to the hemodialyse machine: blood flows from the patient; it is dialysed and it returns to the patient. To obtain a sufficient clearance of uremic toxins, 60 to 80 L of blood are treated during the four hours of a hemodialysis session. Therefore, the blood flow through the dialysis filter should reach to a level as high as 350-400 mL/min. Such a significant flow could be obtained by accessing a large vein or a middle size artery. As hemodialysis sessions are as frequent as three to four times a week, repetitive puncture or cut down of a native vessel leads to inflammation, scarring, fibrosis and thrombosis with the loss of the lumen. The problem of a vascular access that can be used repetitively was solved by **placing a catheter** into a large vein or by **surgically creating a connection between an artery and vein**. The surgically created arteriovenouse fistula fulfills the role of a vascular access that has a high blood flow and sustains repetitive punctures for a long period of time.

5.2 Types of vascular access for hemodialysis

There are three principal forms of vascular access for hemodialysis (Fig. 5.1): central venous catheters (CVC), native arteriovenous fistulas (AVF) and arteriovenous synthetic grafts (AVG).

The **CVC** (Quinton or Quinton/Hickman catheters) are placed in the femoral, jugular or subclavian vein and contain two separate lumens: one carries blood from the patient and the other returns it to the circulation. The **Quinton catheter** is placed percutaneously and represents a temporary access necessary if dialysis is required in emergent situations, before a permanent device has been placed or is ready for use. The **Quinton/Hickman catheter**s are referred to as permanent catheters and have a subcutaneous tunnel before entering the central veins. Because these catheters are less likely to become infected they can be used for longer periods of time and even for long-term access in patients with no other potential form of access, in patients with contraindication of conventional vascular access or as a bridge in patients awaiting living-related kidney donor transplantation.

The surgically created arteriovenous connection is positioned just beneath the skin so it can provide a quick and easy access. It is used for long-term vascular accesses and represents the conventional vascular access in hemodialysis. It also requires the lapse of a certain period of time after the surgery in order to be ready for use. The circulatory system of the patient can be then connected to the hemodialysis machine by placing two needles: one needle provides arterial blood to the machine and the other returns it to the venous side. The construction of a side-to-side-anastomosis between the radial artery and the cephalic vein at the wrist (Cimino fistula) was described in 1966[258]. It was a milestone in the treatment of hemodialysis patients, representing the birth of the AFV. Presently, an **AVF** is created by joining a large vein directly to an artery in the forearm or higher in the arm. The main techniques includes: **simple direct fistulas** (the artery and vein are connected in their natural position in a side-to-side or a side-artery-to-vein-end anastomosis), **transposed vein fistulas** (a vein is moved and tunneled through the tissue to connect to an artery in end-to-side fashion) and **translocated vein fistulas** (a vein is removed from its anatomical

location and is connected to an artery and a vein). If a patient's vasculature is unsuitable for the creation of an AVF, an **AVG** can be used. Most of these grafts are made from polytetrafluorethylene (PTFE). When used for hemodialysis access, the PTFE graft is typically a 6-millimeter diameter tube, which is tunneled in a loop under the skin and creates a connection between an artery and a vein. These grafts are similar in function to AFV.

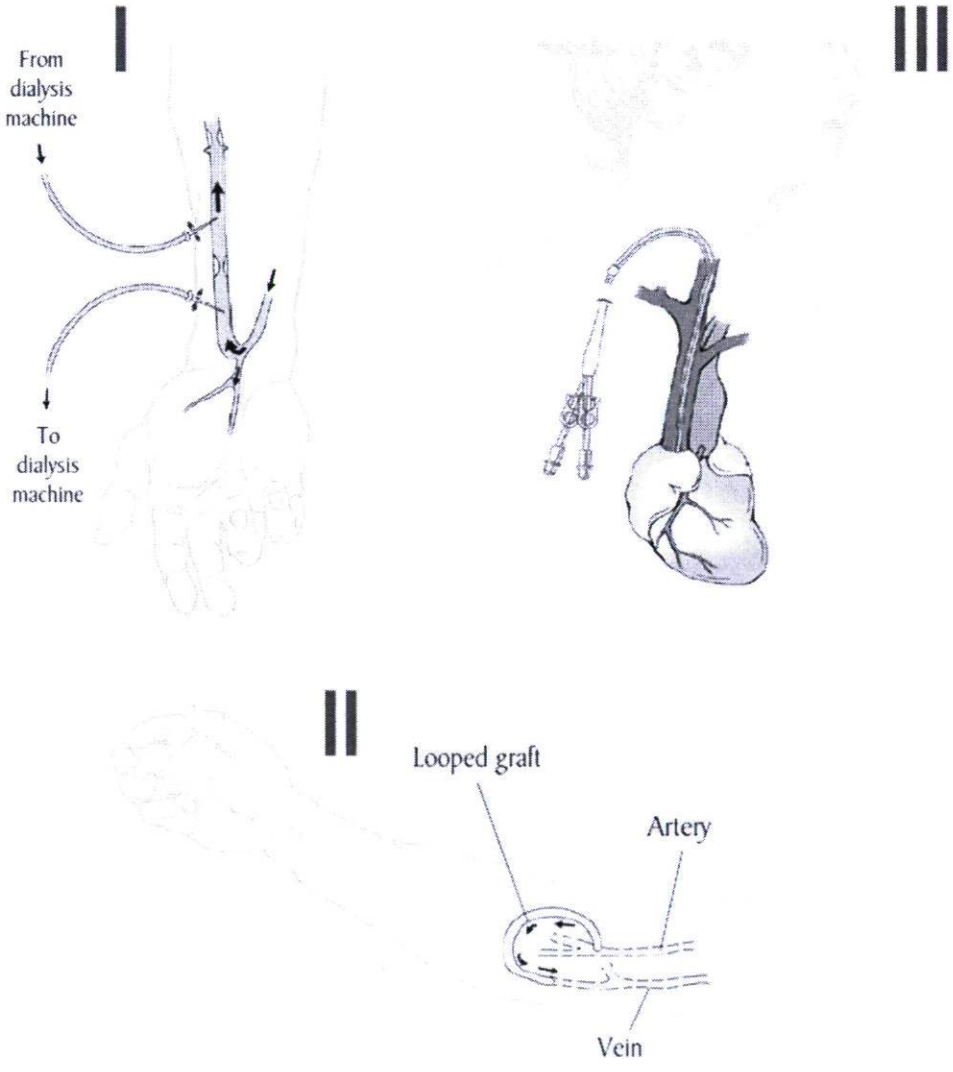


Fig.5.1. Schematic representation of vascular access for hemodialysis. AVF (I), AVG (II) and CVC (III). ***Image adapted from[259].***

5.3 AVF is associated with decreased morbidity and mortality in hemodialysis

The AVF are considered to be the best long-term vascular access in hemodialysis because of the numerous advantages over the AVG and particularly over the CVC. When compared with AVG, the AVF have the best permeability rate, the lowest rate of thrombosis, require the fewest interventions[260-263] and provide the longest access survival rate[264-267]. It was also shown that AVF are associated with lower rates of infections than AVG[268], differences that are markedly accentuated when compared with CVC[269]. As a result, the use on an AVF as long-term vascular access for hemodialysis offers the lowest hospitalization rates[270]. AVF are also the most cost-effective[271].

There is also evidence of a better survival rate in patients being hemodialysed by an AVF then in patients using a CVC as a long-term vascular access. It may be argued that such differences may be the result of patients' selection (an AVF is created in patients with good prognosis while CVC are chosen for patients with a more reserved outcome). Some authors found that the AVF are associated with a decreased in all-cause mortality even after adjustment for comorbidities[270, 272] while others found that the differences in mortality disappeared after correction for comorbidity, suggesting that CVC use per se is not associated with increased mortality risks with respect to AVF[273].

5.4 Prevalence, creation and timing of an AVF in hemodialysis

Based on the evidence of decreased morbidity and mortality, the present clinical practice guidelines suggest a goal of 65 % of prevalent hemodialysis patients having an AVF[274]. In the United States, the "FistulaFirst" website[275] (an organization dedicated to the promotion of the AVF as the first choice of vascular access) reports that the national AVF rate reached 54.9 % in February 2010. It also has been noticed that fewer AVF are reported in female than in male and in African-American versus non-African-American hemodialysis patients[276-280], differences that are not clearly explained.

While fistula creation is a procedure with low morbidity and often performed under local anesthesia, fistulas need time for maturation in order to provide adequate blood flow and sustain repetitive puncture. Both AVF and AVG require several weeks to mature before they can be used for hemodialysis. In an AVF, the vein needs to become larger and thicker and in an AVG, the tissue around the graft needs to heal and incorporate the graft. Clinical data indicate that AVF should be assessed for maturation between four to six weeks after its creation. Initial access may need to be combined with lower initial blood flow and smaller dialysis needles; flow can then be increased and needles advanced to regular size.

Creating the AVF before it is required for hemodialysis allows for maturation to take place and avoids the use of CVC. The patient should be referred and evaluated by a surgeon well before the time of anticipated need. Fistula creation at least four months before starting chronic hemodialysis is associated with the lowest risk of sepsis and death, as the use of catheters is reduced[281]. The clinical practice guidelines[274] recommend that, in order to give the patient a better chance of having a functional permanent access at the initiation of hemodialysis, a fistula should be placed at least six months before the anticipated start of the therapy.

5.5 Hemodynamic pathophysiology of AVF

The creation of a free arteriovenous connection in the systemic circulation causes shunting of blood from the high-pressure arterial system to the low-pressure veins. This creates an abnormal low-resistance circuit with hemodynamic local and central consequences.

5.5.1 Local hemodynamic pathophysiology

The blood flow follows the path of the least resistance. If the resistance in an AVF is sufficiently low, the fistulous tract steals blood from the distal arterial supply[282] (in extreme cases the flow can be even reversed), phenomenon known as parasitic circulation. As the blood pressure decreases in the distal arterial segment, it can cause ischemia or even gangrene. In general, when creating the AVF, an anastomosis more proximal in the arterial

tree should be smaller to limit maximal fistula flow and prevent the steal syndrome. Also, end-to-end anastomosis imposes a high risk for peripheral ischemia since there is a complete disruption of the artery.

The increased efferent AVF flow determines a high peripheral venous pressure that can lead to swelling, varicosities and ulcers of the limb. For example, a large anastomosis in the side-to-side technique, without ligating the distal venous limb, may result in venous hypertension[283].

The increased pressure and flow in an AVF causes diameter enlargement, thickening of the media and fibrosis of the vein wall[284], changes known as "arterialization" of the vein. This phenomenon is in fact beneficial and enables the vein to provide the high flows needed for hemodialysis and to withstand repeated needle punctures. It typically takes 4 to 8 weeks before a newly created native AVF fistula goes through these morphological changes (the process we referred earlier as the "maturation" of an AVF) and is ready for use. Unfortunately, with time, the AVF increases in size and can lead to cosmetic objections, aneurysm formation and increase in blood flow that can exacerbate the central hemodynamic effects.

5.5.2 Central hemodynamic pathophysiology

In a **healthy individual, in acute settings**, a high-pressure arterial flow directly deviated to a low-resistance, high capacitance vein, creates a high flow shunt. This shunt is not fulfilling normal physiological functions and transmits ambiguous signals to regulatory mechanisms:

- as a result of a decreased "functional" arterial volume, there is a decrease in blood pressure[285-287], particularly diastolic[288-290], which can be interpreted as a "false" intravascular volume loss with the subsequent activation of sympathetic mechanisms[291] and peripheral vasoconstriction[285].
- the increased efferent fistulous flow determines an increase in central venous blood flow[285] and a rise in right atrial preload which can be interpreted as a "false"

increase in the intravascular volume with subsequent release of atrial natriuretic peptide[286].

Figure 5.2 shows the acute hemodynamic changes following the closure and reopening of a femoral AVF[285]. It was demonstrated that the kidneys also respond to these changes: they attempt to control the body fluid balance by modifying the sodium excretion while the glomerular filtration rate remained constant. Acute compression of an AVF determined an augmentation of urinary sodium while compression of the AVF resulted in a fall of urinary sodium bellow the control values[290]. The overall response of the circulatory system following the acute opening of an arteriovenous shunt is an attempt to maintain and stabilize the blood pressure by increasing the heart rate and cardiac output[285, 286]. Inversely, compression of an existent AVF is followed by a reflex decrease in heart rate: the resultant increase in blood pressure distends the aortic arch activating the vagal baroreceptors[292]. This reflex is also known as Nicoladoni-Israel-Branham sign. Finally, it has been demonstrated that creation of a high flow AVF (2 L/min) is associated with an acute decrease in coronary perfusion and a subendocardial ischemia[141].

The opportunity to study the **long term hemodynamic effects of an AVF in healthy individuals** was provided by patients with trauma-induced AVF, especially war-related trauma patients. It was recognized that an AVF decreases blood pressure, increases the venous return and augments the cardiac output. These hemodynamic changes lead to left ventricular hypertrophy and high-output heart failure that can result over time in congestive heart failure[288, 289]. The increased cardiac output is modulated by several mechanisms, including enhanced sympathetic activity, neurohormonal changes, and increased blood volume. The kidneys play a significant role in these hemodynamic changes by controlling the fluid balance and adapting the circulating volume to the physiological needs.

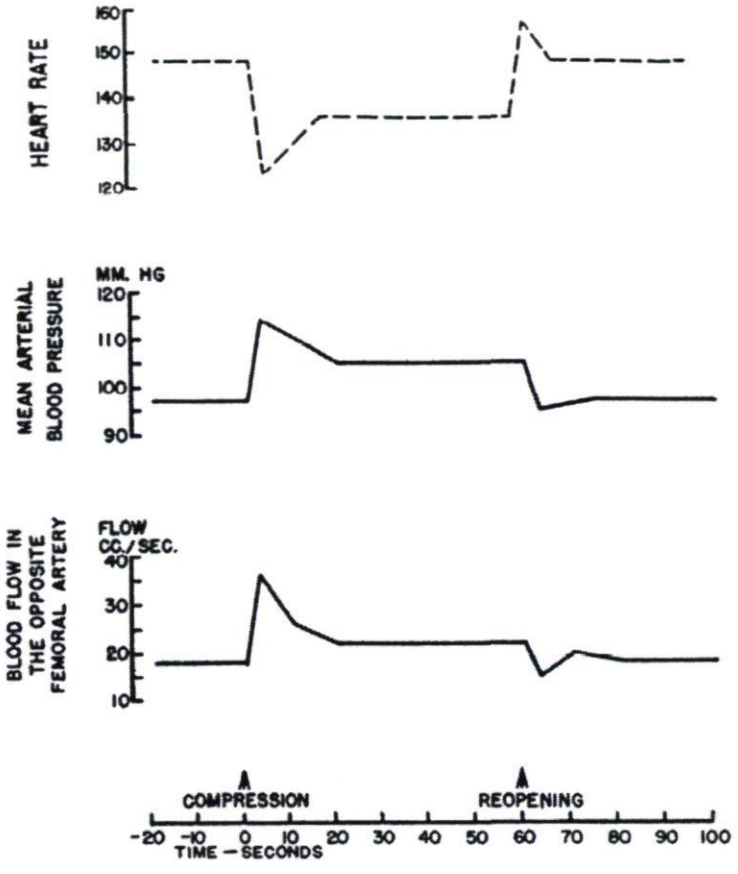


Fig.5.2. Acute changes in heart rate, mean blood pressure and blood flow in the opposite femoral artery following compression and reopening of a femoral AVF in dogs. ***Figure adapted from Van Loo, A. and E.C. Heringman [285].***

The long term hemodynamic effects of an AVF in hemodialysis patients were studied in the quest of a possible explanation for the increased cardiovascular mortality in these patients[293, 294]. Before briefly reviewing the evidences found in the literature, it should be noted that there are several differences between the circulatory system of a healthy individual and the circulatory system of a typical hemodialyse patient:

- with the loss of renal function, these patients lost their ability to self-regulate the fluid balance and to adapt the circulating volume to physiological needs. Stimuli triggered by osmotic, baro and chemoreceptors determine a cardiovascular response regulated by the sympathetic or parasympathetic nervous system (vasoconstriction / dilatation) without the intervention of renal mechanisms.

- cardiac function may be already impaired in this patients as CKD represents a cardiovascular risk factor; the compensatory rise in the cardiac output may be impaired.
- it has already be shown that CKD patients have an increased aortic stiffness; loss of the large arteries capacitance function may represent an impediment in maintaining the diastolic pressure when the mean blood pressure is decreasing as a result of the arteriovenous shunt.

Increased aortic augmentation index – A cross-sectional study in renal transplant patients found that a functional AVF is associated with an increased aortic AIx[295]. A small longitudinal study in CKD patients showed that, after the creation of an AVF, there was an increase in the aortic AIx that failed to reach statistical significance [296]. An AVF could lead in several ways to an early wave reflection and an increased AIx. To name a few, they may be the result of increased aortic stiffness, increased cardiac output or a more proximal location of the aortic pulse wave reflection sites. The increased peripheral sympathetic activity and vasoconstriction after AVF creation[291] could bring the pulse wave reflection sites closer to the ascending aorta. In addition, it must be emphasis that changes in the arterial impedance of a limb alone could influence the location of the aortic reflection site[297].

Left Ventricular Hypertrophy – AVF is associated with significant changes in the structure and function of the heart. Prospective studies showed that the prevalence of left ventricular hypertrophy increased after fistula creation[298]. The regression of the left ventricular hypertrophy in renal transplant patients, after AVF closure, supports this evidence in several studies[299-302].

High-Output Cardiac Failure - There are numerous case reports of hemodialysis patients with AVF and congestive heart failure that resolved after the fistulae's flow was reduced or obliterated[303-312]. Although some authors suggest that cardiac decompensation is likely to occur only in patients with underlying cardiac disease[313], and there was no clear evidence of increased heart failure risk in patients with AVF when compared to other types

of vascular access[314], others[294] recommends that patients with high-flow fistulas (flow greater than 2 L/min) should have a flow reduction procedure since they are at risk for developing heart failure.

Exacerbation of Coronary Ischemia and decreased subendocardial perfusion - Savage et al.[296] used pulse wave analysis to determine the subendocardial viability ratio (SEVR) in a group of CKD patients before and after AVF creation. In this study, SEVR immediately decreased after AVF creation and remained low throughout a 6-month period, demonstrating the negative impact of an AVF on subendocardial perfusion. In another study[315], the SEVR was obtained in 10 kidney transplant patients with a functioning AVF. After acute AVF compression, SEVR increased along with systolic and diastolic pressure. There was also a decrease in heart rate and cardiac output that could explain the decrease in oxygen demand of the myocardium. Some authors[294] recommend that patients with preexisting severe ischemic heart disease should avoid AVF placement if the underlying ischemia cannot be treated and that patients with an AVF who develop worsening ischemic heart disease despite maximum medical and surgical cardiac intervention should have AVF ligation.

5.6 AVF and cardiac morbidity and mortality in hemodialysis

As mentioned above, an AVF creation in CKD patients was found to have negative hemodynamic consequences. However, there is a lack of evidence of AVF' negative effects on cardiovascular morbidity and mortality[314]. More than that, patients utilizing an AVF as long-term vascular access demonstrated a lower cardiovascular mortality when compared to patients using other types of vascular access. In a study of 4,854 hemodialysis patients[1], the use of an AVF 90 days after dialysis initiation remained significantly associated with lower cardiovascular mortality (hazard ratio 0.69) when compared with catheter use. These findings suggest that vascular access type influences mortality beyond the effects of increased infection in patient using CVCs and strongly support the existing guidelines recommending the use of an AVF early in the course of hemodialysis therapy[274].

CHAPTER 2

THE IMPACT OF ARTERIOVENOUS FISTULAS ON AORTIC STIFFNESS IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Keywords: arterial stiffness; arteriovenous fistula; augmentation index; chronic kidney disease; pulse wave velocity

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The impact of arteriovenous fistulas on aortic stiffness in patients with chronic kidney disease

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Abstract

Background. The creation of arteriovenous fistulas (AVF) in patients with advanced chronic kidney disease (CKD) has been shown to have adverse effects on their central pulse wave profile suggesting a likely increase in arterial stiffness. The aim of the present study was to directly evaluate the effect of AVF on arterial stiffness.

Method. Thirty-one stage-5 CKD patients underwent haemodynamic assessment prior to and 3 months after creation of AVF. Haemodynamic assessment included measurement of blood pressure (BP), central and carotidal pulse wave profile analysis, and carotido-femoral and carotido-radial pulse wave velocities (PWV). Pre-AVF and post-AVF haemodynamic parameters were compared using the Wilcoxon signed-rank test or the paired Student *t*-test as appropriate. Pearson correlations, single and multiple linear regressions, were used to determine the association between variables.

Results. After creation of AVF, peripheral and central BPs decreased without significant change in heart rate (HR) or pulse pressure. Carotido-femoral PWV ($_{c\text{-}f}$PWV) fell from 13.2 ± 4.1 to 11.7 ± 3.1 m/s ($P < 0.001$). There was an increase in the central augmentation index (20.8% ± 11.5 versus 23.7% ± 11.6, $P = 0.07$) of borderline significance, and a significant reduction in the subendocardial viability ratio (153% ± 34 versus 143% ± 32, $P < 0.05$), which was mainly the result of a decrease in the diastolic pressure time index (DPTI) without any significant change in the diastolic duration. The reduction of $_{c\text{-}f}$PWV was explained by changes in mean BP and HR ($R^2 = 0.29$). The reduction in DPTI was related to changes in central diastolic BP and changes in end-systolic BP (adjusted $R^2 = 0.87$). The significant improvement in aortic stiffness was mostly the result of the relative reduction of $_{c\text{-}f}$PWV in the subgroup of patients with baseline $_{c\text{-}f}$PWV above the median value of 13 m/s.

Conclusion. The creation of AVF is associated with a passive improvement of aortic stiffness especially in patients with stiffer arteries. This improvement in arterial stiffness could potentially be beneficial to the cardiovascular system despite an associated deterioration in the aortic pulse wave profile.

Keywords: arterial stiffness; arteriovenous fistula; augmentation index; chronic kidney disease; pulse wave velocity

Introduction

Cardiovascular complications are the main cause of morbidity and mortality in patients with advanced chronic kidney disease (CKD). The increased cardiovascular morbidity in this population cannot be fully explained by classical risk factors, suggesting that other factors may also play a role. Among these, increased aortic stiffness, as measured by carotido-femoral pulse wave velocity ($_{c\text{-}f}$PWV), and enhanced and early central pulse wave reflection, as measured by the aortic augmentation index (AIx), have been shown to be predictors of cardiovascular mortality in chronic haemodialysis patients [1–6]. Despite the fact that an AVF is still considered to be the vascular access of choice, it does create a high flow and low resistance vascular system that increases cardiac output and may lead to left ventricular hypertrophy [7–10]. In addition, AVF can also alter the central pulse profile leading to decreased cardiac oxygen supply that could be deleterious to cardiovascular health in this population [11–14].

A longitudinal study by Savage *et al.* [13] showed a reduction in the diastolic pressure time index (DPTI) 3 months after the AVF was created without any significant change in the systolic pressure time index (SPTI). These changes resulted in a significant reduction in the subendocardial viability ratio (SEVR). However, while AIx increased after AVF creation, it failed to reach statistical significance in this study that included only nine patients [13]. In addition, Ferro *et al.* [12] described an association between the increased AIx and the presence of a functioning AVF in a cross-sectional study involving renal transplant recipients.

It is thought that increased central pulse pressure, early wave reflection, increased cardiac workload and reduced myocardial perfusion are some of the physiological consequences of aortic stiffness that may be clinically relevant to cardiovascular disease. Therefore, an increase in aortic stiffness is expected to reduce the travel time of the reflecting wave thereby increasing the AIx and decreasing the SEVR. Based on increased AIx and reductions in the SEVR, results from previous studies suggest that arterial stiffness may be either stable or increased in patients with AVF. Other factors apart from arterial stiffness, such as ventricular systolic duration and peripheral vascular tone, however, are important determinants of AIx [15–19].

In light of these observations, we hypothesized that a reduction in DPTI and a potential increase in AIx after AVF creation should be accompanied by an increase in aortic stiffness as measured by assessment of $_{c\text{-}f}$PWV. To our knowledge, the effect of AVF on aortic stiffness has never been studied directly. The aim of the present study was to assess the impact of AVF on aortic stiffness and central pulse wave profile (PWP) prior to and 3 months after creation of AVF.

Methods

Study design and patient population

$_{c\text{-}f}$PWV and carotido-radial pulse wave velocity ($_{c\text{-}r}$PWV) and PWP were performed within a month of AVF creation (pre-AVF) and 3 months after surgery (post-AVF), as described below. This longitudinal study was conducted at the Centre Hospitalier Universitaire de Québec, L'Hôtel-Dieu de Québec Hospital between October 2004 and 2007. All stage-5 CKD patients who were scheduled for their first AVF creation were invited to participate. Exclusion criteria were no functional AVF 3 months following the surgical intervention, extreme blood pressure (BP) values (systolic pressure >190 mmHg or <80 mmHg), severe congestive heart failure and patients having had a previous AVF. In addition, patients with acute heart failure, stroke or acute coronary syndrome in the preceding 3 months and during the study period were excluded. Demographics, medical history, medication and laboratory parameters were assessed from the patient's chart. All patients gave informed consent to participate in this study, which was also approved by the ethic committee of the institution.

During the study period, 48 patients were enrolled. Seventeen patients were excluded for the following reasons: non-functional AVF ($n = 7$), transfer to another institution ($n = 7$), severe cardiac failure ($n = 1$), death ($n = 1$) and extremely low BP ($n = 1$). Among the 31 patients who completed the study, 16 were already on haemodialysis (median of 6 months) and 15 were being followed up in the predialysis clinic at the time of the baseline examination. At the time of follow-up examination, however, 6 of these 15 subjects had started on haemodialysis after a median of 58 days post-AVF creation. The aetiologies of CKD were diabetic nephropathy ($n = 12$), IgA nephropathy ($n = 5$), chronic glomerulonephritis ($n = 4$), obstructive nephropathy ($n = 4$), renal dysplasia ($n = 2$), nephroangiosclerosis ($n = 1$), medullary cystic disease ($n = 1$), reflux nephropathy ($n = 1$) and unknown ($n = 1$).

Of the 22 patients who were treated by haemodialysis, the access blood flow was measured, at the time of the follow-up visit, by the ultrasound dilution method with HD02 Hemodialysis Monitor System (Transonic Systems, Inc., Ithaca, NY, USA) [20]. Haemodialysis was performed three times weekly with a filter of 2.1 m^2 surface area, dialysis duration of 3–4 h/session and a blood flow of 350–400 mL/min. A bicarbonate-based buffer dialysis solution was used with sodium concentrations of 138–142 mmol/L, a potassium concentration of 1–3 mmol/L, a calcium concentration of 1.25 mmol/L and a dialysate flow rate of 500–750 mL/min.

Haemodynamic measurements

After 10 min of rest in the supine position, the brachial artery BP was recorded six times consecutively, with a 2-min interval between each measurement, using an automatic sphygmomanometer BPM-100 (BP-Tru, Coquitlam, Canada). The average of the last five measurements was used as brachial systolic and diastolic BPs [21,22]. All measurements (BP, PWP and PWV) were done on the opposite side of the expected or existing AVF. In haemodialysis patients, all measurements were done just prior to the second haemodialysis session of the week.

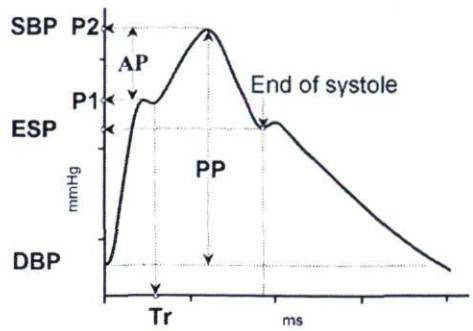

Fig. 1. Central pulse wave profile. The graph shows the first peak of pressure (P1), second peak of pressure (P2), time of return of the reflection wave (Tr), end-systolic pressure (ESP), diastolic blood pressure (DBP), systolic blood pressure (SBP), pulse pressure (PP) and augmented pressure (AP).

The $_{c\text{-}f}$PWV was determined according to the foot-to-foot method, using the Complior® device (Artech Medical, Pantin, France) as previously validated [23]. Briefly, two transducers were placed, one at the base of the neck over the common carotid artery and one over the femoral artery. The software automatically determines the transit time between the carotid and the femoral pulse waves by using the second derivative algorithm to identify the foot of the wave. The distance was assessed by direct measurement of the superficial distance between the two probes. Each measurement of PWV (m/s) was expressed as the mean of 8–10 consecutive cardiac cycles. The average of three separate measurements was used for analysis. The $_{c\text{-}r}$PWV was determined using the same technique by positioning the second sensor on the radial artery at the level of the wrist. In our laboratory, the intrasession and intersession coefficients of variation were 2.9% and 8.9%, respectively.

PWP was assessed by arterial tonometry using the Sphygmocor® Px Pulse Wave Analysis System (AtCor Medical Pty Ltd, West Ryde, Australia). Briefly, a Millar tonometer was placed over the radial artery to obtain a peripheral PWP (pPWP). The mean BP (MBP) was then derived using radial artery pulse wave analysis and brachial systolic and diastolic BPs. Thereafter, the central PWP (cPWP) was estimated by using the generalized transfer function [24]. However, in order to bypass the prerequisite validity of generalized transfer function in the advanced CKD patient, with or without AVF, we also used the non-processed arterial wave profile of the common carotid artery (caPWP) as a surrogate for cPWP ($n = 29$). The pulse wave analysis was performed three times, and the average of the three measurements was used for analysis. Then, cPWP and caPWP were analysed using the same system to determine the following parameters: central mean pressure of systole, central mean pressure of diastole, augmented pressure (AP), heart rate adjusted AIx, ejection duration (ED) and diastolic duration (DD) as shown in Figures 1 and 2. In addition, the SERV was calculated by the dividing DPTI by SPTI (DPTI/SPTI) as shown in Figure 2. In our laboratory, the intrasession and intersession coefficients of variation for AIx were 2.6% and 6%, respectively.

Data analysis

Data analysis was performed using the SPSS software (version 10.0 for Windows, SPSS Inc., Chicago, IL, USA). Data were expressed as means ± SD unless otherwise specified. Pre-AVF and post-AVF haemodynamic parameters were compared using the Wilcoxon signed-rank test or the paired Student *t*-test as appropriate. The Mann–Whitney test was used to compare changes between groups. The McNemar test was used to assess any changes in the class of medication. Pearson correlations, single and multiple linear regressions, were used to determine the association between variables. A two-sided *P*-value of <0.05 was considered to be statistically significant. Using a two-sided one-sample *t*-test, a sample size

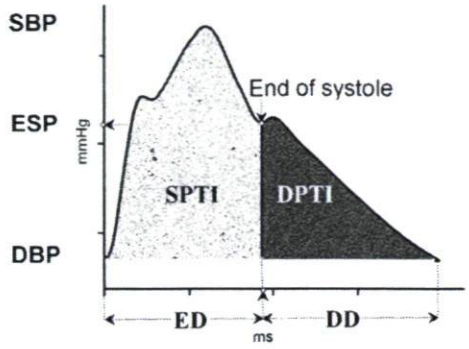

Fig. 2. Central pulse wave profile and subendocardial viability ratio (SEVR), a marker of subendocardial perfusion expressed as the ratio of the diastolic pressure time index (DPTI) and systolic pressure time index (SPTI). The other parameters in the graphic are ejection duration (ED), diastolic duration (DD) and end-systolic pressure (ESP).

Table 1. Baseline characteristics

Parameter	$n = 31$
Male	20 (65%)
Age (years)	58 ± 15
Weight (kg)	78 ± 13.6
Body mass index (kg/m^2)	28.8 ± 5.6
Smoking (active or past history)	15 (48%)
Hypertension	26 (84%)
Coronary artery disease	9 (29%)
History of myocardial infarction	3 (10%)
Peripheral artery disease	5 (16%)
Diabetes	12 (39%)
Haemodialysis at the beginning of the study	16 (52%)
Haemodialysis at the end of the study	22 (71%)

Values are expressed as number (percentage) or mean ± SD.

of 30 subjects would provide 91% power to detect a difference of 1.5 m/s in $_{c\text{-}f}$PWV between the two periods, assuming a standard deviation of 2.5 with a significance level (alpha) of 0.05.

Results

Twenty men and 11 women, with a mean age of 58 ± 15 years, were studied. The baseline characteristics are listed in Table 1. The peripheral, central and carotidal haemodynamic parameters and the PWV measurements prior to and 3 months following the AVF creation are summarized in Table 2.

There was a significant reduction in the peripheral systolic and diastolic BPs by 7.3 ± 18.5 and 6.8 ± 10.8 mmHg, respectively ($P < 0.05$). The central systolic BP decreased from 119.8 ± 17.8 to 113.9 ± 22.9 mmHg ($P = 0.07$), and the central diastolic BP decreased from 78.6 ± 11.3 to 72.0 ± 12.3 mmHg ($P < 0.005$); however, there were no significant changes in heart rate (HR) or in peripheral, central and carotidal pulse pressures. The $_{c\text{-}f}$PWV fell from 13.2 ± 4.1 to 11.7 ± 3.1 m/s (<0.001), but the $_{c\text{-}r}$PWV showed only a slightly non-significant reduction (9.3 ± 2.2 versus 8.9 ± 1.6 m/s, $P = 0.16$). In contrast, despite the reduction in MBP and $_{c\text{-}f}$PWV, there was a non-significant increase in the central AIx (20.8% ± 11.5 versus 23.7% ± 11.6, $P = 0.08$) and the carotidal AIx (12.1% ± 11.8 versus 14.1% ± 15.8, $P = 0.48$). There was no significant change in the augmentation pressure and the timing of wave reflection. There was, however, a slight increase in the central systolic duration that failed to reach statistical significance (305 ± 42 versus 318 ± 30 ms, $P = 0.08$). The DPTI decreased significantly and SPTI remained stable. As a result, there was a significant reduction in both central and carotidal SEVRs (153% ± 34 versus 143% ± 32, $P < 0.05$ and 159% ± 33 versus 146% ± 26, $P < 0.005$, respectively). These results were consistent among the various groups of patients: younger or older than

Table 2. Pre- and Post-AVF haemodynamic parameters

	Pre-AVF	Post-AVF	*P*-value
Peripheral Haemodynamic parameters			
Systolic BP (mmHg)	131.7 ± 17.4	124.4 ± 22.7	<0.05
Diastolic BP (mmHg)	77.7 ± 11.1	70.8 ± 11.9	<0.001
Mean BP (mmHg)	95.6 ± 11.8	89.3 ± 15.4	<0.01
Pulse pressure (mmHg)	54.0 ± 16.8	53.6 ± 17.6	0.87
Heart rate (bpm)	71 ± 14	70 ± 14	0.66
Central haemodynamic parameters			
Systolic BP (mmHg)	119.8 ± 17.8	113.9 ± 22.9	0.07
Diastolic BP (mmHg)	78.6 ± 11.3	72.0 ± 12.3	<0.005
Pulse pressure (mmHg)	41.1 ± 17.9	41.9 ± 18.3	0.77
End-systolic BP (mmHg)	106.7 ± 14.9	100.3 ± 18.0	<0.05
Tr (ms)	136.4 ± 10.6	137.4 ± 11.5	0.54
AP (mmHg)	9.1 ± 7.6	10.2 ± 8.7	0.21
P1 (mmHg)	109 ± 12	101 ± 16	0.004
AIx (%)	20.8 ± 11.5	23.7 ± 11.6	0.08
Ejection duration (ms)	305 ± 42	318 ± 30	0.08
Diastolic duration (ms)	569 ± 153	570 ± 164	0.94
DPTI (ms × mmHg)	3428 ± 515	3091 ± 504	<0.001
SPTI (ms × mmHg)	2309 ± 425	2266 ± 595	0.52
DPTI/SPTI (SEVR) (%)	153 ± 34	143 ± 32	<0.05
Carotid haemodynamic parameters			
Systolic BP (mmHg)	119.1 ± 15.8	114.3 ± 21.8	0.19
Diastolic BP (mmHg)	79.3 ± 11.1	71.9 ± 12.3	<0.01
Pulse pressure (mmHg)	39.7 ± 12.5	42.3 ± 15.6	0.36
End-systolic BP (mmHg)	101.8 ± 13.4	94.4 ± 17.1	<0.05
Tr (ms)	145 ± 23	140 ± 30	0.39
AP (mmHg)	4.8 ± 4.9	5.8 ± 6.8	0.38
P1(mmHg)	111 ± 14	106 ± 17	0.05
AIx (%)	12.1 ± 11.8	14.1 ± 15.8	0.48
Ejection duration (ms)	301 ± 47	313 ± 31	0.18
Diastolic duration (ms)	598 ± 156	594 ± 150	0.87
DPTI (ms × mmHg)	3451 ± 512	3118 ± 507	<0.001
SPTI (ms × mmHg)	2236 ± 421	2190 ± 530	0.53
DPTI/SPTI (SEVR) (%)	159 ± 33	146 ± 26	<0.005
Pulse wave velocity			
$_{c\text{-}r}$PWV (m/s)	9.3 ± 2.2	8.9 ± 1.6	0.16
$_{c\text{-}f}$PWV (m/s)	13.2 ± 4.1	11.7 ± 3.1	<0.001
Adjusted $_{c\text{-}r}$PWV (m/s)	9.3 ± 2.2	9.2 ± 2.4	0.25
Adjusted $_{c\text{-}f}$PWV (m/s)	13.2 ± 4.1	12.9 ± 4.3	0.16

Values are expressed as mean ± SD.
Tr, time of return of the reflected wave; AP, augmented pressure adjusted for the heart rate of 75 beats per minute; AIx, augmentation index adjusted for the heart rate of 75 beats per minute; DPTI, diastolic pressure time index; SPTI, systolic pressure time index; c-r, carotido-radial; c-f, carotido-femoral; PWV, pulse wave velocity; adjusted, heart rate and mean blood pressure post-AVF adjusted $_{c\text{-}r}$PWV and $_{c\text{-}f}$PWV.

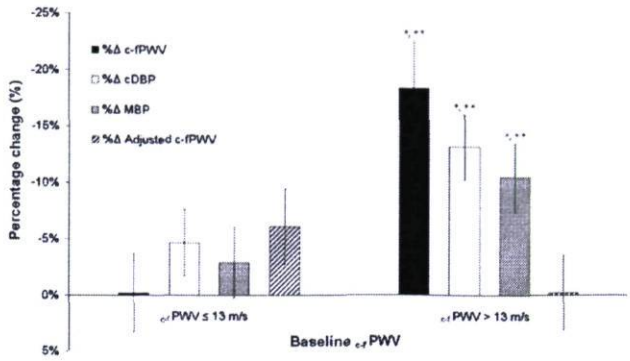

Fig. 3. Relative variation of BP and $_{c\text{-}f}$PWV according to baseline $_{c\text{-}f}$PWV. The graph shows the percentage (%) variation (Δ) of carotido-femoral pulse wave velocity ($_{c\text{-}f}$PWV), central diastolic blood pressure (cDBP) and mean blood pressure (MBP) and adjusted $_{c\text{-}f}$PWV. $^{*}P < 0.05$, for between group changes in each parameter. $^{**}P < 0.01$, for relative variation within the group of patients with baseline $_{c\text{-}f}$PWV >13 m/s.

Table 3. Pre and post-AVF laboratory parameters

	Pre-AVF	Post-AVF	*P*-value
Urea (mmol/L)	22.5 ± 8.6	20.3 ± 6.2	0.12
Creatinine (μmol/L)	590 ± 239	595 ± 235	0.84
Calcium (mmol/L)	2.20 ± 0.17	2.25 ± 0.16	0.19
Phosphate (mmol/L)	1.78 ± 0.78	1.67 ± 0.33	0.47
Albumin (g/L)	38.3 ± 4.1	40.4 ± 5.2	<0.01
PTH (ng/L)	315 ± 272	324 ± 253	0.80
Haemoglobin (g/L)	108 ± 15	118 ± 10	0.22

Values are expressed as mean ± SD.

50 years of age and with or without previous cardiovascular disease, diabetes or haemodialysis.

Using linear regression, the reduction in end-systolic BP was the most important determinant of change in DPTI (adjusted $R^2 = 0.80$). In multivariate analysis, both end-systolic and diastolic BPs remained the most significant determinants of change in DPTI (adjusted $R^2 = 0.87$). The change in the central diastolic BP was the single most important determinant of the change in $_{c\text{-}f}$PWV ($R^2 = 0.29$). However, physiologically it is more appropriate to adjust PWV for MBP and HR ($R^2 = 0.29$). After adjustment for changes in MBP and HR, there were no significant differences in either $_{c\text{-}r}$PWV or $_{c\text{-}f}$PWV.

Figure 3 shows the relative changes in $_{c\text{-}f}$PWV, central diastolic and MBPs according to the baseline $_{c\text{-}f}$PWV below or above the median value of 13 m/s. The figure clearly demonstrates that patients with higher initial $_{c\text{-}f}$PWV were the most likely to experience a relative reduction of $_{c\text{-}f}$PWV after creation of AVF.

After 3 months of follow-up, the mean access blood flow rate was 1050 ± 410 mL/min. There was no relationship, however, between the access blood flow rate and the change in BP, AIx, timing of wave reflection, DPTI or $_{c\text{-}f}$PWV. The medication and laboratory parameters before and after AVF creation are listed in Tables 3 and 4. The number and class of antihypertensive drugs were similar throughout the study period. During the same period, there was no significant change in patient weight (78.2 ± 13.6 versus 78.0 ± 13.4 kg, $P = 0.73$); however, there was a slight but significant increase in the serum albumin level (38.3 ± 4.1 versus 40.4 ± 5.2 g/L, $P < 0.01$). No correlation was observed between the degree of change in the albumin level and the degree of change in systolic, diastolic or MBPs.

Table 4. Pre and post-AVF medication

Medication	Pre-AVF *n* (%)	Post-AVF *n* (%)	*P*-value
Antihypertensive drugs			
ACEI or/and ARA	20 (65%)	17 (55%)	0.25
β-blockers	11 (35%)	14 (45%)	0.45
Ca channel blockers	18 (58%)	14 (45%)	0.12
Diuretics	23 (74%)	19 (62%)	0.21
Mean number of antihypertensive drugs	2.5 ± 1.5	2.2 ± 1.4	0.10
Other drugs			
Darbepoetin alfa	24 (77%)	29 (94%)	0.06
Vitamin D	11 (35%)	14 (45%)	0.25
Sevelamer HCl	5 (16%)	7 (23%)	0.50
Calcium carbonate	22 (71%)	27 (87%)	0.06
Statins	20 (65%)	17 (55%)	0.25
Aspirin	11 (35%)	14 (45%)	0.45

Values are expressed as number (percentage) or mean ± SD
ACEI, angiotensin-converting enzyme inhibitor; ARA, angiotensin II receptor antagonist.

Discussion

The results of the present study show that the creation of an AVF in stage-5 CKD patients was associated with a reduction in both peripheral and central BPs and, for the first time, a reduction in aortic stiffness. The relative reduction in aortic stiffness was most significant in patients with stiffer arteries; however, this improvement in aortic stiffness was not associated with the expected improvement of arterial PWP. In fact, there was no change in the timing of wave reflection in addition to a non-significant increase in the central AIx and a significant reduction in the SEVR.

Aortic stiffness has been shown to have a negative impact on cardiovascular morbidity and mortality in patients with hypertension and in patients on haemodialysis [1,25]. As a structural change in arterial wall composition is highly unlikely during this short period of follow-up, it is believed that reduction of $_{c\text{-}f}$PWV is due to BP-related change in arterial stiffness. This reduction in BP may relieve the tension from the collagen fibres and put the aorta in a more favourable pressure–diameter relationship, resulting in a passive improvement in aortic stiffness [16]. Indeed, the significant decrease in $_{c\text{-}f}$PWV is best predicted by the reduction in central diastolic BP ($R^2 = 0.29$), or by changes in MBP and HR ($R^2 = 0.29$).

Normally, an improvement in aortic stiffness is expected to increase the travel time of the reflecting wave, reduce the AIx and increase the SEVR. However, this expected response is not supported by our findings. Our results show a statistically non-significant increase in the central AIx ($P = 0.08$) after AVF creation. This is in keeping with the

results published by Ferro *et al.* [12] in which they found a clear association between increased AIx and the presence of a functioning AVF in renal transplant recipients. A high AIx increases left ventricular after load and may potentially be a contributing factor in AVF-induced left ventricular hypertrophy [10,26,27]. However, in addition to PWV, other factors such as ventricular systolic duration and peripheral vascular tone are important determinants of AIx [15–19]. It is therefore hypothesized that other vascular compensatory mechanisms may play a role in outweighing the effects of the decrease in $_{c\text{-}f}$PWV on central PWP. Indeed, previous studies have demonstrated that AIx can change independently of aortic PWV during administration of nitroglycerin or angiotensin II, which primarily has peripheral effects [28,29]. In this regard, AIx is no longer considered to be a reliable marker of arterial stiffness [29,30]. The absence of reduction in AIx after the creation of AVF in the present study could be accounted for by an increase in regional vascular tone, possibly splanchnic, that would proximalize the arterial reflection sites [19]. This contention is further supported by the association of AVF with reflex vasomotor change in blood vessels and increased sympathetic activity, which could lead to peripheral vasoconstriction and proximal displacement of the reflection sites in the aorta [31,32]. In addition, the constant timing of wave reflection despite a decrease in PWV is consistent with this hypothesis. In the study by Savage *et al.* [13], despite a rise in BP, there was a non-significant increase in AIx after creation of AVF in nine stage-5 CKD patients who were followed up prospectively for up to 6 months. Interestingly, the levels of baseline and follow-up AIx are similar in our present study as compared to the study by Savage *et al.* [13]. These findings suggest a potentially clinically relevant increase in AIx that does not reach statistical significance due to a small sample size.

In agreement with previous studies, our results also confirmed a reduction in the SEVR (DPTI/SPTI), which is a reflection of deteriorating endocardial/epicardial blood flow ratios [33]. Indeed, it was shown by Bos *et al.* [11] that acute compression of AVF is associated with an increase in the SEVR. In this study, since there was a reflex reduction in HR, it could be argued that the HR modification might interfere with the proper interpretation of the findings [11]. However, these results were also validated in the study by Savage *et al.* [13], who reported a reduction in the SEVR immediately after AVF creation that persisted throughout the 6 months of follow-up. As observed in previous studies, the reduction in the SEVR is mainly the result of a decrease in DPTI, as there was no significant change in the SPTI after 3 months. The change in DPTI is strongly related to changes in central or carotidal end-systolic pressure (Figure 2), with a R^2 of 0.80. The addition of the diastolic BP value into the equation, although significant, increased the adjusted R^2 by only 0.07.

Taken together, there are presently no data supporting the impact of the reduced SEVR on mortality. In addition, contrary to the study by London *et al.* [4], the study by Covic *et al.* [34] failed to show a statistically significant impact of AIx on mortality in a group of middle-aged patients on haemodialysis. Therefore, negative clinical impact of AVF on central PWP should be interpreted with caution, especially in light of beneficial effect of AVF on aortic stiffness. Although there are no data to directly support that AVF-induced reduction in aortic stiffness is beneficial in terms of survival, data from Guérin *et al.* [35] suggest that a BP-dependent reduction in $_{c\text{-}f}$PWV is associated with a better survival in a group of haemodialysis patients. It may therefore be argued that the cardiovascular survival benefit of AVF could be due, at least partly, to an improvement in aortic stiffness, especially in the group of patients at higher risk of mortality [36]. However, in the study by Guérin *et al.* [35], the reduction of $_{c\text{-}f}$PWV was a result of pharmacological BP reduction, and therefore, the extension of these findings into BP reduction by any other means should be interpreted with caution.

There are also a number of other confounding factors in our study that need to be discussed. First, there was a lack of strict control on the anti-hypertensive drug therapies. However, BP medication did remain relatively constant and it is unlikely that they played a significant role in the main findings of the study. Second, although patient weight remained stable during the study period, there was an increase in serum albumin level that might indicate a better nutritional status or a better control of fluid overload. However, we found no association between the degree of decrease in the BP and the degree of increase in the albumin level that might suggest a significantly better control of fluid overload. Third, we acknowledge that six patients started HD and that HD might have had a significant influence on vascular function. In order to address this bias, we pooled data from patients who remained in the same treatment category and found similar findings. Fourth, although this is the largest longitudinal study, it was not designed to detect a small BP-independent (or structural) change in arterial stiffness because of the relatively short duration of the study that does not take into account the chronic adaptation of the cardiovascular system to an AVF. The strength of a longer duration of follow-up, however, might be hampered by the loss of AVF, loss of patients, change in medications and intercurrent illness. Finally, an evaluation of cardiac output and aortic diameter before and after creation of AVF could have shed more light on the comprehensive interpretation of these findings.

In summary, our findings show, for the first time, that AVF creation is associated with a BP-related reduction in aortic stiffness without the expected positive effect on central haemodynamic parameters. Future studies are required to re-evaluate cardiovascular risk assessment by aortic stiffness and central PWP analysis with regard to the type of vascular access.

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Conflict of interest statement. None declared.

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CHAPTER 3

GENERAL DISCUSSION AND CONCLUSION

The ESRD population is rapidly growing worldwide and the best treatment that can be offered to these patients is renal transplantation. Unfortunately, the limited kidney transplant availability forces a large part of this population to receive dialysis in order to survive. In theory, renal replacement therapy by peritoneal dialysis or hemodialysis can be received indefinitely. In practice, the associated cardiovascular complications are a great source of morbidity and mortality. Consequently, in hemodialysed patients, the cardiovascular mortality and morbidity are several times to hundred of times higher than in general population especially at younger ages. Surprisingly, the high cardiovascular mortality cannot be explained only by traditional risk factors. In this context, aortic stiffness emerged as a non-traditional risk factor associated with cardiovascular morbidity and mortality in dialysis patients.

The guidelines regarding the vascular access in hemodialysis strongly encourages the use of AVF as it was proved that AVF are associated with a better survival when compared to other types of vascular access. However, previous studies showed that an AVF creation has negative effects on cardiovascular hemodynamics and suggested an increase in aortic stiffness by indirect means. The absence of a prospective study that measures the aortic stiffness by the gold standard (PWV) before and after an AVF creation questioned the influence of an AVF on a parameter that is proved to be a cardiovascular risk factor.

The aim of our study was to determine in a prospective manner the influence of an AVF creation on aortic stiffness in an ESRD population. This section has no intent to repeat the discussions from Chapter 2, but rather to summarize our main findings and to add some topics that were not addressed in the main article.

In the present study, the results obtained after AVF creation in CKD patients confirmed the previous findings of a negative impact on the cardiac oxygen supply/demand ratio (Buckberg Index) as measured by pulse wave profile. In addition, we found an increase in central AIx of borderline statistical significance ($p=0.08$). However, contrary to previous studies, our results demonstrate for the first time that AVF creation is associated with a

blood pressure dependent decrease in c-fPWV, especially in patients with higher c-fPWV baseline values.

The decrease in Buckberg Index represents a non-desirable effect of AVF creation. However, there is no evidence of the negative influence of this parameter on cardiovascular mortality in ESRD patients. Moreover, the majority of our patients showed a pre and post-AVF Buckberg Index (mean of 159 and 146% respectively) within normal values, which vary from 130 to 200%. As subendocardial ischemia was shown to appear at values below 100%, the clinical significance of our findings remains questionable in the absence of cardiac workload and coronary perfusion measures.

Although the increase in the central AIx was of borderline significance, this finding is in keeping with the results published by Ferro *at all.* As mentioned in the discussion of the main article, the increased AIx could be explained by vascular compensatory mechanisms. The increased regional (possibly splanchnic) vascular tone may play a role in outweighing the hemodynamic effects of aortic stiffness improvement. However, another hypothesis not discussed yet implies the effects of an upper extremity AVF on arterial wave reflections. The addition of a high-flow, low-resistance circuit in the vascular branches originating near the ascending aorta could lead to a proximal displacement of the reflection sites[297], increasing the AIx. If this is the case, it logically implies that the creation of a lower extremity AVF could result in the distal displacement of the aortic reflection sites and a decrease in AIx coupled with an improvement in aortic stiffness. Although completely hypothetical, we consider it should be mentioned at least as a further prospect for future research. Currently, the creation of a leg (thigh) AVF or graft is reserved for patients who have multiple failure of upper-arm vascular access and demonstrated a comparable survival time[316, 317].

It should also be noted that the increased AIx, after the creation of an AVF, occurred in the setting of decreased blood pressure and aortic stiffness. Recent data from our laboratory[227, 228] suggest that in hemodialysis patients, c-fPWV, adjusted for the changes in blood pressure, is increasing at a rate of 0.5m/s/year, which is far superior than

the rate observed in general population[126]. The accelerated aortic stiffening is also accompanied by an increasing mean and systolic blood pressure. Under these circumstances, one can question whether the negative effect of an AVF on AIx may be further accentuated if, in the long term, the c-fPWV and the blood pressure return to or surpass the pre-AVF values due to hemodialysis.

Despite the negative effects on subendocardial perfusion and AIx, the AVF is associated with decreased cardiovascular mortality in hemodialysis patients[1]. The present study shows a blood pressure dependent reduction of aortic stiffness that could explain these findings. The introduction of the high-flow, low-resistance component (represented by the AVF) in the circulatory system should be also viewed in the context of our patients' hemodynamics. As discussed in section 2.5.1, ESRD represents not only a loss in renal function, but, in the majority of cases, a loss in renal blood flow. Consequently, at the time of AVF creation, the circulatory system of these patients has already lost a high-flow, low-resistance component that normally receives 20% of the cardiac output. It might be hypothesised that, in this context, the creation of the AVF represents a 'return' to more physiologically conditions, which were present before the loss of the renal blood flow.

In summary, we demonstrated for the first time that AVF creation is associated with a blood pressure related reduction in aortic stiffness without the expected positive effect on central haemodynamic parameters. Future studies are required to re-evaluate the relation between cardiovascular risk, assessed by aortic stiffness and central PWP analysis, and the type of vascular access in ESRD.

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