

Thesis for doctoral degree (Ph.D.)  
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# ROLE OF AMBULATORY BLOOD PRESSURE MONITORING AFTER PEDIATRIC RENAL TRANSPLANTATION

Rafael Tomás Krmar

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### List of original publications

This thesis is based in the following papers, which will be referred to in the text by their Roman numerals:

- I. Ferraris JR, Ghezzi L, Waisman G, **Krmar RT**. Potential cardiovascular risk factors in pediatric renal transplant recipients. *Pediatric Nephrology* 2006; 21(1): 119-125.
- II. **Krmar RT**, Berg UB. Long-term reproducibility of routine ambulatory blood pressure monitoring in stable pediatric renal transplant recipients. *American Journal of Hypertension* 2005; 18(11): 1408-1414.
- III. Ferraris JR, Ghezzi L, Waisman G, **Krmar RT**. ABPM vs. office blood pressure to define blood pressure control in treated hypertensive pediatric renal transplant recipients. *Pediatric Transplantation* 2007; 11 (1): 24-30.
- IV. **Krmar RT**, Balzano R, Jogestrand T, Cedazo-Minguez A, Englund MA, Berg UB. Prospective analysis of carotid arterial wall structure in pediatric renal transplants with ambulatory normotension and in treated hypertensive recipients. *Pediatric Transplantation* 2008; 12: 412-419.
- V. **Krmar RT**, Berg UB. Blood pressure control in hypertensive pediatric renal transplants: Role of repeated ABPM following transplantation. *American Journal of Hypertension* 2008; 21 (10): 1093-1099.

## Summary

**Background** Office blood pressure (BP) readings, *i.e.*, BP obtained in a clinical setting, are the basis of the present knowledge concerning the risks associated with hypertension. However, adult studies indicate that ambulatory BP measurements obtained over a 24-h period (ABPM) provides a much better representation of the patient's BP pattern than office readings, and that ABPM is a much better predictor of adverse outcome than office BP. Therefore, there has been a lot of focus on ABPM as a clinical tool to improve the estimate of true BP in hypertensive children. Since hypertension is a common post renal-transplant complication we carried out a series of studies in an attempt to determine the actual role of ABPM in our pediatric renal transplant recipients. **Methods** In all, 96 recipients from Argentina (72% male) were included in papers I and III and 136 recipients from Sweden (55% male) were included in papers II, IV, and V. Office BP was measured either by mercury manometer (paper I and III) or by an automated device (paper II). ABPM was applied in papers II, III, IV, and V. **Results** In paper I we observed that 76% of our study population was on antihypertensive treatment at recipients' last follow-up. We noted that a rather low number of recipients (prevalence 19%) were diagnosed as having non-controlled hypertension, *i.e.*, office BP within the hypertensive range while on antihypertensive treatment. A limitation in the data interpretation is that BP was in fact not evaluated by means of ABPM and therefore we may have derived a misleading conclusion. In paper III, and in line with this hypothesis, we found that almost one-third of the treated hypertensive recipients, in whom BP appeared to be controlled according to office BP, had in fact non-controlled hypertension by ABPM criteria. We also observed that the two methods of BP measurement did not agree closely. In a previous study (paper II), we demonstrated that long-term reproducibility of ABPM is superior to that for office BP. In paper IV we aimed to analyze the impact of BP on arterial wall structure, which is regarded as a potential surrogate marker of hypertensive vascular damage, as evaluated by repeated vascular ultrasound examinations. Baseline carotid artery intima-media thickness (cIMT) was found to be significantly higher in renal transplants compared with healthy controls. After a 4-year follow-up, and regardless of recipients' ambulatory BP status, follow-up cIMT was not significantly different compared with baseline cIMT. In paper V we analyzed the role of repeated ABPM following transplantation as a reference method to better characterize recipients' BP pattern. At the recipients' last follow-up visit we observed that more than three quarters of our treated hypertensive recipients displayed controlled BP. This figure was significantly higher compared to our historical control group, in whom ABPM was applied for the first time during antihypertensive treatment after transplantation while therapeutic decisions were driven by office BP measurements (difference between proportions 48.6% (80.6% – 32%); 95% CI for difference, 36% to 60%,  $P = 0.001$ ). **Conclusions** In a population at high risk for hypertension we observed that the reproducibility of ABPM was superior to that for office BP measurements and that the two methods of BP measurement do not closely agree. We infer, therefore, that office BP and ABPM should not be used interchangeably. In our study population, cIMT was not found to be a reliable surrogate marker of hypertensive organ damage. Finally, we demonstrated that the routine use of repeated ABPM following transplantation has significantly improved our method of identification and management of hypertensive renal transplant recipients.

**List of abbreviations**

ABPM	Ambulatory blood pressure monitoring
BP	Blood pressure
CI	Confidence interval
cIMT	Common carotid artery intima-media thickness
SD	Standard deviation
SDD	Standard deviation of the mean difference
SDS	Standard deviation score

## **Preface**

On approaching the task of writing this preface for my doctoral thesis the first thing I am conscious of is that I should start with *a caveat*; the aim of this thesis is not to provide an in-depth review of the epidemiology, pathophysiology, evaluation, and treatment of hypertension in childhood diseases, a clinical field that is continuously expanding.

I would rather already from the Introduction approach the main aim of my thesis by concentrating on the clinical background that has both awakened my interest in the field of pediatric hypertension after renal transplantation and justify the clinical research that I will present in my dissertation, as stated in the last paragraph of the Introduction. Likewise, the reader will also notice that I have preferred to describe the methods and the discussion of the results focusing only on the main aim of each paper. For this purpose, and in order to make my thesis more readable, I relied upon a more comprehensive statistical approach to present and discuss the data which otherwise are identical to the original publications.

Finally, I will end this thesis with a clinical view that is important and still unresolved.

*R.T.K*

*Stockholm, November 2008*

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## 1 Introduction

### 1.1 A brief historical overview of the importance of BP measurements in children

For many years the natural history of hypertension in childhood was poorly documented and very little was known about the health consequences of hypertension in the young.<sup>1</sup>

In the early 1960s, the erroneous belief in considering hypertension as a rarity in children was weakened by two retrospective longitudinal studies from the same Center, reporting on 20 years experience of investigating hypertensive children.<sup>2, 3</sup> These reports clearly showed that children with severe office hypertension, *i.e.*, blood pressure (BP) measurements obtained in a clinical setting, resulted in adverse outcomes if left untreated. They also demonstrated that severe hypertension can occur at any age and showed that no sustained rise in BP in a child can necessarily be regarded as safe and non-progressive.<sup>2, 3</sup>

At the time, a contemporary researcher reported on the office BP patterns in 1473 children aged 4-15 years.<sup>4</sup> An engrossing observation presented in this study was that, by means of repeated office BP measurements, 35 apparently healthy children (prevalence 2.3%; 95% CI, 1.6% to 3.2%) were identified as having systolic and/or diastolic BP values persistently greater than the 95<sup>th</sup> percentile for their age, *i.e.*, their office BP readings were unusually high relative to the reference study population.

Eight years later, a review article summarized the data available at that time on hypertension in children, adolescents, and young adults, which showed that the prevalence of essential hypertension, *i.e.*, no specific medical cause could be found to explain the patient's condition, ranged from 1% to 11%.<sup>5</sup> The author however pointed out some drawbacks on interpreting the pooled data. Firstly, the body position in which office BP was measured differed considerably from study to study, making it difficult to compare the results. Secondly, and most importantly, was the fact that the definition of hypertension was based on cut-off office BP values derived from adult epidemiological studies and therefore any possible relationship of BP to age was not considered. Indeed, there were pediatric data suggesting that office BP values were lower in children compared to adults.<sup>4, 6</sup> If this was the case, the author argued that the cut-off points to define high BP in childhood would certainly be lower than the adult criteria.<sup>5</sup> Still, despite all these limitations, the aforementioned review contributed to refute the belief that hypertension was a disorder limited only to the adult population.

Another pioneering pediatric study observed that a child's office BP level persists over time, suggesting that high BP in an adult might in fact be recognizable by repeated BP measurements already in childhood.<sup>7</sup> The relevance of this observation and therefore the ability to identify children at risk for developing hypertension in early adult life was confirmed many years later in longitudinal epidemiological studies.<sup>8, 9</sup> Previous to the latter studies being published, there was yet no normative data for interpretation of office BP values in children. Therefore there was an urgent need for more knowledge regarding the normal distribution and history of BP across the pediatric ages that would allow for better criteria in defining high BP and consequently enable early identification and prevention of hypertension in children and adolescents. As a consequence, substantial efforts devoted to the investigation of BP in healthy children and adolescents were conducted, and resulted in the publication of The First Task Force Report on Blood Pressure Control in Children in North America

published in 1977.<sup>10</sup> This report was the first in a series of new and more extensive epidemiological data.<sup>11</sup> After the publication of the 1977 Report, measurement of office BP became a routine procedure in the pediatric population and from a historic standpoint was perhaps the beginning of a more rational approach to study BP levels in the first two decades of life.

## **1.2 Usual considerations in the measurement of office BP**

Non-invasive measurement of BP is a safe and painless procedure that provides reliable information when performed accurately. Virtually all epidemiological adult and pediatric BP data have been obtained by means of mercury manometer and the auscultatory technique,<sup>11-14</sup> *i.e.*, the Riva-Rocci/Korotkoff technique.<sup>15, 16</sup> The mercury manometer has always been regarded as the definitive method for clinical measurement of BP, and is considered the device to which all other devices for BP measurement should be compared.<sup>17</sup> However, the routine use of this method is likely to change, not because of any technological advance, but because of the progressive prohibition of the medical use of mercury. This prohibition is based on environmental concerns about mercury contamination from broken manometers.<sup>18-20</sup>

Regardless of the method used to measure office BP, it must be recognized that BP is highly variable and is influenced by several factors (see below), not least being the circumstances of the measurement itself. Understanding this concept is important, as the observer has to consider the large variability that may occur in BP from moment to moment with respiration, emotion, exercise, temperature, pain, and eating. Thus, if variability in BP is ignored,<sup>21, 22</sup> hypertension will be erroneously diagnosed. As a consequence, the diagnosis of hypertension should be based on multiple BP measurements taken on separate occasions.

In addition, the subject's posture also affects BP and therefore BP measurements are usually standardized in the sitting position after the subject has been sitting calmly and comfortably with his/her feet flat on the floor for at least 5 minutes.<sup>23</sup> There are also additional issues that are important in obtaining accurate office BP readings. In short, the available devices for BP measurement must be maintained, calibrated and functional. A cuff containing a bladder of inappropriate dimensions has also been shown to be a serious source of error.<sup>24, 25</sup> To avoid overestimation of BP (bladder too small) or underestimation of BP (bladder too large), it is recommended that the inflatable bladder has a width which is at least 40% of the arm circumference and a length that encircles 80% to 100 % of the upper arm, between the olecranon and acromion processes.<sup>11</sup>

## **1.3 Methods of BP measurement in a clinical setting**

This section concentrates on the description of two BP instruments, namely the standard mercury manometer and automated oscillometric devices.

In an adult population, mercury readings obtained by trained health care personnel have been the standard for epidemiological studies for several decades, and the basis for most prognostic data in hypertension.<sup>26, 27</sup> This technique was subsequently adopted for examining BP in children and adolescents.<sup>10, 14</sup>

In any circumstance where BP is measured, the goal is to characterize the subject's BP as accurately as possible. Consequently, it is crucial to minimize human observer error, which is by far the most fallible component in achieving accurate and reliable BP readings when the mercury manometer/auscultatory technique is used.<sup>28</sup> It is well known that by using the mercury manometer/auscultatory technique, the observer may be prone to a systematic error. Such an error cannot be removed by repeating measurements or averaging large numbers of readings. One of the most important factors that leads to intra-observer error is the failure to interpret precisely the Korotkoff sounds. The digit preference, *i.e.*, the observer rounds off the pressure reading to a digit of his or her preference, is another source of error. Finally, there is a serious bias of simply adjusting the BP to meet observer's preconceived notion of what the BP should be.<sup>28</sup> Since the accuracy of BP measurement is of paramount importance in the clinical setting as well as in the field of clinical research, it is expected that the observer has been trained to the highest possible level.<sup>29</sup>

In children, controversy exists over whether muffling (Korotkoff phase 4) or the disappearance of sound (Korotkoff phase 5) should be used for measurement of diastolic BP. There is a general consensus, applicable to both adults and children, that by releasing the air from the cuff so that the mercury falls at the rate of 2 mm Hg per second, the onset of Korotkoff phase 1, *i.e.*, the appearance of clear tapping sounds, corresponds to systolic BP.<sup>11-14</sup> In adults, diastolic BP is determined by Korotkoff phase 5. The same definition has been adopted in children,<sup>11</sup> with the exception that if very low Korotkoff phase 5 persists, Korotkoff phase 4 should be recorded as diastolic BP.<sup>11</sup> A recent pediatric study has questioned this approach based on the observation that Korotkoff phase 4 predicts a stronger diastolic BP tracking into adulthood.<sup>30</sup> The auscultation technique, however, may be difficult to use in neonates, infants, and younger children because the movements of the arm can jeopardize accurate monitoring of the Korotkoff sounds.<sup>31</sup>

Several automated devices for measuring BP have replaced the mercury manometer in a large number of medical Centers, where there has been concern regarding environmental contamination with mercury.<sup>17</sup> The first commercial oscillometric device for BP measurement was manufactured under the name Dinamap, an acronym for "device for indirect non-invasive mean arterial pressure".<sup>32</sup> The fundamental concept underlying these devices is the same as that of other cuff-based BP measuring devices. However, the Dinamap device uses the oscillometric principle of measuring BP which is not based on the same physiological observation as auscultation. Indeed, it measures BP by first inflating the cuff rapidly above systolic BP and then deflating the cuff in a stepwise fashion in 5-10 mm Hg decrements. When blood starts flowing through the artery, oscillations are detected by the surrounding cuff. The point of maximal oscillations corresponds to mean arterial BP. Systolic and diastolic BP are then calculated using a proprietary algorithm of the slope of rise and fall of the oscillations, as functions of a mean, and calibrated to be equivalent to corresponding intra-aortic pressures.<sup>32</sup> The digital monitor displays values for the mean, systolic, and diastolic BP. The device is easy to use and requires little training, involving attention to factors such as environment, application of the appropriate cuff, and correct use of machine controls. It is relatively easy to use on infants and young children because there is no need for auscultation.<sup>33</sup> Compared with mercury manometers, the main advantages of oscillometric devices is the minimization of observer bias or digit preference and

no risk for mercury contamination.<sup>24, 34</sup> An additional strength of oscillometric devices is the strong correlation with intra-arterial readings,<sup>31</sup> which obviates the auscultatory dilemma of whether Korotkoff phase 4 or Korotkoff phase 5 is the more accurate reflection of diastolic BP.<sup>30</sup>

The use of Dinamap has nevertheless also potential weaknesses. The Dinamap device is an expensive piece of equipment that requires periodic calibration to ensure optimal function. Different models of oscillometric devices have different measurement algorithms and therefore measure different quantities. Thus, one needs validation data for each model to be used in routine clinical practice.<sup>34</sup> The rapid rate of inflation of the cuff by the machine may cause anxiety in children leading to erroneously high readings, known as a first-reading effect, *i.e.*, the first of several readings is higher than subsequent readings.

The oscillometric device is still dependent on a standard mercury manometer since the consistency and accuracy of measurements generated by an algorithmic interpretation of BP have to be compared in order to be validated for clinical use.<sup>35</sup> Briefly, the recommendations state that for these devices to be acceptable, no more than 25% of measurements should have an error of more than 10 mm Hg and no more than 10% at 15 mm Hg.<sup>17</sup>

A recent study compared the Dinamap model 8100 with BP readings obtained by mercury manometer in prepubertal children (aged 8 to 13 years).<sup>36</sup> According to the British Hypertension Society protocol, which validates the accuracy of oscillometric devices from A to D (grades A and B are acceptable while C and D are unacceptable),<sup>17</sup> the Dinamap model 8100 reached a satisfactory B grading for both systolic and diastolic measurements. The device also satisfied the Association for the Advancement of Medical Instrumentation criteria for both systolic and diastolic BP.<sup>37</sup> Another large pediatric single center study compared also the Dinamap model 8100 with BP readings obtained by mercury manometer.<sup>38</sup> This study showed however that the mean Dinamap readings were higher for both systolic and diastolic BP. The authors concluded that the two methods are not interchangeable and recommended the use of Dinamap-specific normative BP standards rather than the normative tables produced by and for the auscultatory method (see below) when BP is obtained by the Dinamap model 8100.<sup>39</sup> Similarly, recent adult studies have also raised the question as to whether oscillometric devices can replace mercury manometers.<sup>40-42</sup>

#### **1.4 Particular considerations when measuring office BP in children**

In contrast to an adult population, healthy children undergo an age- and height-related rise in BP as they mature, making it therefore necessary to redefine the threshold for BP normality throughout childhood.<sup>11</sup> Thus, tables are available that provide the systolic and diastolic BP level at the 95<sup>th</sup> percentile according to age, sex, and height.<sup>11, 14</sup> These tables should always be consulted to determine whether a child's office BP measurements are normal or elevated. Similar to the past three decades, the current standard for defining hypertension in children is based on BP values that exceed the 95<sup>th</sup> percentile derived from population percentiles of the normal BP distribution within a specific age, gender, and height-percentile combination.<sup>11, 14</sup> Asymptomatic elevated BP measurements must be confirmed on repeated visits since raised BP tends to fall as a result of an accommodation effect

(e.g., reduction of anxiety) and a regression to the mean, a non-biological phenomenon that derives from mathematical considerations.<sup>43</sup>

In an adult population, there is essentially a linear relationship between the height of office BP and the likelihood of cardiovascular complications even at BP values equal to or below 139/89 mm Hg, which is accepted as the upper normal limit for defining office normotension.<sup>44</sup> Treatment of hypertension, defined as office systolic and/or diastolic BP values  $\geq$  140/90 mm Hg, has been associated with cardiovascular protection.<sup>44, 45</sup> Thus, to label an adult subject as hypertensive presupposes an operational definition derived from findings of trials showing that lowering office BP has an unequivocal clinical benefit.

In children, there is no such data supporting the validity of current pediatric norms for defining hypertension in predicting the risk of adverse cardiovascular events. It seems however unlikely that this type of information would ever be available since the occurrence of cardiovascular events, as a consequence of hypertension, is fortunately extremely rare during childhood. Also, so far the current definition of hypertension has not been systematically correlated with end organ damage, *i.e.*, the identification of hypertensive-related functional or structural organ abnormalities. In other words, the significance of high BP in the young, unless extremely elevated or symptomatic,<sup>2, 3</sup> has not been related to disease outcomes. Consequently, the upper limit of office normotension, *i.e.*, BP readings  $\leq$  95<sup>th</sup> age-matched, sex-matched, and height-matched percentile of the reference standard,<sup>11, 14</sup> is an arbitrary statistical definition rather than a functional one. Hence hypertension defined on descriptive cut-off points derived from epidemiological data may not have a biological meaning.<sup>46</sup> To date, there is no direct evidence to disprove this notion, as no longitudinal studies in children have investigated whether normalization of office BP to  $<$  95<sup>th</sup> percentile would prevent end organ damage.

Although the exact management of BP thresholds seems to be debated, the current definition of hypertension based upon office BP measurement has gained momentum, and no child with persistent elevated BP, as defined above, will be left without appropriate investigation, close follow-up, and treatment considerations.

Finally, recent investigations have reported that atherogenesis appears to begin early in life, in association with traditional risk factors for coronary disease, including office systolic and diastolic BP,<sup>47</sup> therefore raising the question as to whether adequate BP control in childhood will result in a reduced risk for cardiovascular events in adult life.

### **1.5 Ambulatory BP monitoring technique: A review of the data available in an adult population**

Having briefly examined the methods of office BP measurement and their inherent limitations, it should be noted that these methods are not infallible, and there are important concerns related to the interpretation of office BP. As a result, investigations have been conducted to develop a more reliable and accurate non-invasive method of BP measurement.<sup>48</sup> More than 40 years ago the ambulatory BP monitoring (ABPM) technique was first described in an attempt to improve the estimate of a subject's true BP.<sup>49</sup> It is now acknowledged that this non-invasive technique substantially enhances the clinician's understanding of BP behavior in hypertensive patients and aids in diagnosis and therapeutic decision making.<sup>50</sup>

Over the past 20 years, non-invasive automatic devices have been developed for clinical research and hypertension management. Ambulatory BP monitors have become much more practical to use. The devices are quite small (less than 0.5 kg in most instances), precise, and are simple to apply in the clinical setting. The currently available ambulatory monitors are fully automatic and can record BP for 24 hours or longer while patients go about their normal daily activities. Most monitors use the oscillometric technique. As previously described, oscillometric measurement depends on the detection of pulsatile oscillations from the brachial artery into the cuff. The amplitude of the oscillations is related to a standard form of BP, *i.e.*, mercury manometer/auscultatory technique, and an algorithm is then developed. The oscillometric methodology is fairly accurate in routine clinical use in patients who hold their arm still during cuff inflation and deflation.<sup>51</sup> The monitor is connected to a manometer cuff on the upper arm and is usually programmed to take readings every 15 to 30 minutes throughout the day and night. Standard protocols are used to evaluate the accuracy of the monitors and approved devices are usually accurate to within 5 mm Hg compared to readings taken with a mercury manometer.<sup>17</sup> An up-to-date list of validated monitors is available via a not-for-profit web site: <http://www.dableducational.org>, accessibility verified as of November 2008.<sup>52</sup> All ABPM devices are sold with individual software packages, which basically present data as average day- and night-time BP values as well as a visual plot of the profile of BP behavior under the 24-h period.

Ambulatory BP monitoring is usually well tolerated by patients. However, a few problems do exist. A minority of patients does not sleep well with the recorders. Cuffs can rotate and different arm positions can change BP significantly. Rarely, patients may develop erythema, or echymoses in the area distal to cuff placement. These soft tissue injuries are typically mild and self limited. Finally, the oscillometric technique cannot measure BP in patients with arrhythmias, such as rapid atrial fibrillation.

Given the great variability in BP,<sup>21, 22, 53</sup> the reproducibility of a single method for measuring BP (which refers to whether a technique used to measure BP produces the same results in identical circumstances) is essential for accurate diagnosis of BP abnormalities. When two methods are compared and one of them has poor reproducibility, a poor agreement between the two methods is expected.<sup>54</sup> Several studies conducted both in normotensive and hypertensive adult subjects have demonstrated that ABPM has a higher short- and long-term reproducibility than office BP measurement.<sup>55-58</sup>

Ambulatory BP monitoring outside of the medical care environment can provide information that is of potential value in the clinical field: (i) an estimate of the true, or mean BP level, (ii) the diurnal rhythm of BP, and (iii) BP variability.<sup>48</sup>

Three main studies form the background of the current adult definition of ambulatory normotension.<sup>59-</sup>

<sup>61</sup> It is worth to mention that even though the approach to define “normality” varies from study to study, from statistically derived values<sup>59, 61</sup> to outcome-based analysis of ambulatory BP,<sup>60</sup> the aforementioned studies reached similar conclusions about “normality”. Mean ambulatory BP value of < 135/85 mm Hg is considered as the upper normal limit for average daytime BP, which corresponds to an office BP of < 140/90 mm Hg.<sup>62</sup> This is the threshold above which cardiovascular risk appears to increase markedly.<sup>63</sup> As a result, the American and European guidelines for evaluation and treatment of arterial hypertension in an adult population have incorporated ambulatory BP threshold values for

diagnosing hypertension by ABPM.<sup>13, 64</sup> A recent review based on a meta-analysis pointed out that the optimal daytime BP values were found to be about 120/80 mm Hg.<sup>65</sup>

Ambulatory BP monitoring is the only non-invasive method that allows the evaluation of BP behavior over a 24-h period. A pioneering study published in the early 1980s showed that in normotensive subjects, the onset of sleep was associated with a significant systolic and diastolic BP decrease of approximately 10% - 20%.<sup>66</sup> A similar pattern was observed in hypertensive patients, but the diurnal profile of BP was set to a higher level.<sup>66</sup>

In some subjects, regardless of their BP status, the nocturnal decline in BP is diminished (< 10%), and this is referred to as a non-dipping pattern, in contrast to the normal dipping pattern.<sup>67</sup> Embedded within the 24-h diurnal variation are ultradian components, *i.e.*, rhythmic components similar to sleep cycles with a period of less than 24-h (typically 6, 8, or 12-h).<sup>68</sup> Ultradian rhythms have been observed to be reduced in non-dipper patients.<sup>69</sup>

By means of ABPM it is also possible to explore the amplitude and frequency of BP fluctuations occurring during the entire 24-h period.<sup>70</sup> This physiological parameter is known as BP variability and increased BP variability is a well-established feature in hypertensive patients.<sup>71</sup> Blood pressure variability is easily assessed by calculating the standard deviation (SD) of BP during a defined time period. However, there are certain limitations due to the fact that ABPM yields BP values every 15 to 30 minutes instead of beat-to-beat BP changes.

So far, the most relevant operational aspects concerning the use of ABPM have been summarized. Given that ABPM yields multiple BP readings during patient's activities throughout the whole day, including sleep-time, and that ambulatory BP measurements have high reproducibility, ABPM provides less biased and more accurate measurement, and therefore a much better representation of patient's true BP pattern than office BP measurements. In addition, ABPM is not subject to digit preference, and avoids the transient rise of patient's BP in response to the observer, referred to as the "white-coat effect".<sup>72</sup> There are, however, as described in the next section, additional aspects of the utmost importance from the clinical perspective.

## **1.6 Clinical implications of ABPM in an adult population**

Over the past 10 years, studies have been conducted to investigate whether ABPM is clinically superior to office BP in predicting target organ damage.<sup>73-75</sup> Two studies prospectively analyzed the relationship between treatment-induced regression of hypertensive target-organ damage, namely left ventricular mass, with change in office and 24-h ambulatory BPs.<sup>73, 74</sup> Both studies reached the same conclusion that regression of left ventricular mass was more closely related to changes in ABPM than to office BP changes. They also demonstrated that changes in daytime BP were no more or no less important than the changes in night-time BP in terms of treatment-induced regression of left ventricular mass. Another study, a large cross-sectional one, investigated the adverse vascular consequences of hypertension, assessed by means of ultrasound examinations of carotid artery intima-media thickness (cIMT),<sup>75</sup> which is linked to coronary artery and cerebrovascular disease.<sup>76, 77</sup> This study showed that the best determinants of cIMT were the average of systolic 24-h BP and the magnitude of systolic BP variation, *i.e.* how much BP oscillated during the 24-h period. In

addition, both daytime and night-time BP contributed similarly to cIMT. Likewise, a recent investigation showed that the relationship between ABPM and cIMT remains significant even after adjusting for office BP, suggesting therefore that ABPM provides an independent contribution to risk stratification.<sup>78</sup> In the all the aforementioned investigations<sup>73-75</sup> except the latter,<sup>78</sup> in which office BP was measured by nurses, office BP readings were made by doctors. It could be speculated that a transient rise of patient's office BP in response to the observer might have played a role in the poor predictor of office BP in target organ damage. With regard to whether office BP recordings performed by trained nurses would give equally good estimates of hypertensive load on the heart and the blood vessels, a cross-sectional study examined the relationship of office BP and 24-h BP profile to left ventricular mass and cIMT in non-treated hypertensive subjects.<sup>79</sup> This study showed that office BP measured by nurses correlated equally well with left ventricular mass and with cIMT, as did ABPM. This observation is somewhat in line with a previous validation study which showed that office BP readings made by a doctor, compared with office BP measurements obtained by a trained nurse, were significantly higher than ABPM, pointing out that the "white-coat effect" associated with measurement by doctors is not an artefact of research studies.<sup>80</sup> The authors conclude therefore that if ABPM is not possible, repeated measurements by a nurse is a better estimate of a subject's true BP.

Several long-term prospective follow-up studies of mortality outcome compared the ability of ABPM versus office BP in predicting cardiovascular risk.<sup>81-85</sup> One study, which included only treated hypertensive patients, observed that after adjustment for classic risk factors including office BP measurements, 24-h ambulatory systolic and diastolic BP provided additional prognostic information concerning cardiovascular events (myocardial infarction or sudden death, stroke, new episodes of angina pectoris, congestive heart failure, and peripheral vascular disease).<sup>81</sup> Because of the study design, this investigation could not address the predictive value of pre-treatment versus in-treatment ABPM. In another study, which also included hypertensive patients, ABPM was applied as a research tool before and in-treatment, while therapeutic decisions were driven by office BP measurement.<sup>86</sup> After an average of 3.7 years of follow-up, ambulatory BP control (defined as daytime BP within the normotensive range < 135/85 mm Hg), was found to be superior to office BP control (< 140/90 mm Hg) for prediction of individual cardiovascular risk in treated hypertensive subjects. In other surveys, the study populations were mainly composed of patients who were not receiving antihypertensive therapy at the initial ABPM and were treated according to their office BP during the follow-up.<sup>82-84, 87</sup> Overall, the main finding was that ABPM was a stronger predictor of cardiovascular events than office BP, confirming therefore the observations derived from a previous seminal study.<sup>88</sup>

According to the analysis of day-to-night variability of BP, it has been observed that loss of nocturnal decline in BP, so-called non-dipping status, was a predictor of cardiovascular events among hypertensive patients.<sup>67</sup> There is some evidence suggesting that normotensive subjects with a diminished nocturnal decline in BP showed a greater risk of cardiovascular mortality compared with those who displayed dipping status, *i.e.*, a nocturnal BP decline of at least 10%.<sup>89</sup> It was also observed, from the same community-based study, that elevated daytime BP was associated with increased intra-cerebral haemorrhage mortality and elevated night-time BP was associated with

increased cerebral infarction mortality and heart disease.<sup>90</sup> Recently, a prospective study showed that an increase in erratic BP variability was accompanied by an increased cardiovascular risk.<sup>91</sup>

When used in conjunction with office BP readings, ABPM also has the potential value to identify patients with white coat hypertension. This is a phenomenon which refers to persistently elevated office BP readings in non-treated subjects while actual BP, as evaluated by means of ABPM, is within the normal range. It is a well known condition requiring the use of out-of-office BP measurements for both an accurate diagnosis and prognostic evaluation.<sup>92</sup> This condition has a profound clinical relevance since decisions relying only on office BP measurements will result in an inappropriate diagnosis and treatment.

Early studies, using different thresholds to diagnose white coat hypertension, showed a prevalence of this condition as high as 30%.<sup>92, 93</sup> Today, most experts define white coat hypertension as elevated office BP  $\geq$  140/90 mm Hg and daytime ambulatory BP  $<$  135/85 mm Hg.<sup>94</sup> By applying this definition, a recent study conducted in untreated subjects attended in a primary care setting, showed a prevalence of white coat hypertension of 39.4% (95% CI, 34% to 44.7%).<sup>95</sup>

The clinical prognosis of this condition in terms of cardiovascular events (specifically stroke) seems to be comparable to that of subjects with normal office BP values.<sup>96</sup> Interventional trials showed that treating white coat hypertension has no significant benefit on morbid events.<sup>97</sup> On the other hand, there is some evidence derived from a small study, suggesting that some subjects with white coat hypertension can actually develop ambulatory hypertension.<sup>98</sup> In a more recent multiethnic study it was observed that the incidence of stroke in subjects with white coat hypertension tends to increase in the long-term (after 6 years of follow-up).<sup>99</sup> Taken together, these data raise the question of whether white coat hypertension should be considered an "innocent" condition. As a consequence, repeated ABPM seems to be a critical test for proper assessment and long-term management of subjects with white coat hypertension.<sup>50</sup>

A subset of hypertensive diagnoses has also been elucidated as a result of applying ABPM in the clinical practice, *e.g.*, labile hypertension,<sup>100</sup> evaluation of refractory hypertension<sup>101</sup> and masked hypertension, a condition that is the reverse of white coat hypertension.<sup>102</sup>

Masked hypertension, in contrast to white coat hypertension, has been found to have a prognosis similar to sustained hypertension in terms of left ventricular hypertrophy and cardiovascular events.<sup>103-105</sup> This phenomenon, if left unrecognized, can therefore lead to under-treatment of hypertension and increase in morbidity and mortality. Compared to white-coat hypertension, the prevalence of masked hypertension, defined as office BP  $<$  140/90 mm Hg and daytime ambulatory BP  $\geq$  135/85 mmHg, in presumed healthy subjects fortunately seems to be lower (prevalence 22.8%; 95% CI, 18.3% to 27.8%);<sup>106</sup> but contrary to white coat hypertension, masked hypertension needs to be looked for and there are few clinical hints to its presence. This condition appears to occur more frequently in smokers and in treated hypertensive patients.<sup>105, 107</sup>

The use of ABPM also provides additional insights into responses to antihypertensive therapy. Ambulatory BP measurements were shown to be associated with a more sustained BP control.<sup>108</sup> A subset analysis of an intervention trial showed that office BP readings were poor predictors of ABPM response to treatment.<sup>109</sup> Results from a recent analysis based on predictable therapy cost, indicate

that the use of ABPM at the initial confirmatory diagnosis of hypertension may result in substantial cost saving.<sup>110</sup>

Currently, it can be ascertained that ABPM provides more precise BP averages than office BP readings with multiple measurements recorded during usually daily activities. According to the data discussed above as well as results derived from a growing number of other surveys, ABPM has been shown to be superior to office BP for cardiovascular risk stratification in both untreated and treated hypertensive patients. Thus, information derived from ABPM recordings can explain many of the benefits of antihypertensive treatment. Although the routine use of ABPM is not universally recommended, the International guidelines for evaluation and treatment of hypertension have emphasized the use of ABPM as a valuable adjunct method to office BP measurements.<sup>13, 50, 64</sup>

### **1.7 Ambulatory BP monitoring in hypertension in childhood**

Essential hypertension or primary hypertension is a heterogeneous disorder with polygenic manifestations, which accounts for most adult human hypertension.<sup>111</sup> In children, essential hypertension accounts for fewer than 25% of cases of hypertension, being more likely diagnosed in children older than 10 years of age and associated with obesity and increased body mass index.<sup>8, 112</sup> The importance of increased BP in the context of the childhood obesity epidemic has been firmly established in numerous studies.<sup>113, 114</sup>

In contrast, secondary hypertension, which is not a disease entity but results from defects in identifiable systems that amplify physiological processes to raise BP, accounts for most cases of hypertension in the young.<sup>115-117</sup> Before adolescence, renal disorders are the most common underlying condition identified in children with hypertension.<sup>117</sup>

Regardless of the underlying cause of hypertension, a number of pediatric reviews, published since the use of ABPM was first described in children,<sup>118</sup> argue that ABPM offers more than office BP for both diagnosis and monitoring of hypertension, advocating therefore its use in children and adolescents referred for evaluation of hypertension.<sup>119-132</sup> In the aforementioned reviews, the clinical usefulness of office BP measurements is not underestimated but, based on the adult literature and on available pediatric data; the advantages of ABPM are stressed.

In children, the ability to obtain high quality ambulatory BP recordings correlates positively with age (younger patients appear to have more difficulty complying with instructions to hold their arm still during BP measurements).<sup>133</sup> Other factors that may also limit the accuracy of ABPM are obesity, weak pulse, and male gender (boys more often manually cancel the automatic measurement compared to girls).<sup>134, 135</sup> However, several pediatric studies, including children as young as 2 months of age, have provided evidence on the reliability of ABPM.<sup>136-138</sup>

Among the few oscillometric ambulatory BP devices that have been evaluated in children, the Space Labs 90207 and the later model 90217 monitors are the most commonly used ambulatory BP monitors in pediatric populations,<sup>139, 140</sup> including very young children.<sup>138</sup> Like any other test, the validity of the use of ABPM in children is influenced by its reproducibility. Similar to an adult population, studies conducted in normotensive and hypertensive children showed a high reproducibility in mean ambulatory BP values.<sup>141, 142</sup> In addition, a more recent study demonstrated adequate reproducibility

for longitudinal interpretation.<sup>143</sup> On the other hand, the reproducibility of circadian BP patterns seems to be lower compared to mean BP values.<sup>141</sup>

The availability of normative data against which ABPM results can be compared is critical to the interpretation of ABPM. To date, the largest and most widely used ABPM normative data were obtained in healthy central European children and adolescents. This database shows, as seen with office BP, that normal values for ABPM rise with age and therefore BP values must be adjusted for body size, as a surrogate for maturational age.<sup>140</sup> As the authors found that in the same sample cohort, the distribution of ambulatory BP values tended to be non-Gaussian across the entire pediatric age range, the data were reanalyzed in a later study in an attempt to normalize them to gender and height.<sup>144</sup> For this purpose, they applied a statistical technique (LMS method, based on age- and gender-specific estimates of the distribution median [M], coefficient of variation [S], and degree of skewness [L]) to account for the skewed distribution of BP.<sup>145</sup> In this study it is also reported that across ages mean daytime systolic and diastolic BP values are higher and mean night-time systolic and diastolic BP are lower than the published reference values for office BP measurements.<sup>11, 14</sup> The importance of this observation is that ABPM values should not be compared to BP measurements obtained in a clinical setting.

The concept of BP load (defined as the percentage of ABPM recordings higher than pre-established cut-off ambulatory BP values) emerged as a potential diagnostic tool in adult patients with untreated essential hypertension.<sup>146</sup> In children, combined mean ambulatory BP and BP load have been proposed to define the severity of hypertension.<sup>124, 147, 148</sup> However, there is as yet no consensus as to what is the acceptable limit of BP load above which BP causes harm.<sup>62</sup>

Due to the fact that overt morbid cardiovascular events are rare in pediatric hypertensive patients, investigations have relied upon surrogate markers of hypertensive target organ damage in an attempt to better identify hypertensive children at risk for cardiovascular complication later in early adult life.<sup>124</sup> These include left ventricular hypertrophy, as a marker of heart disease, and cIMT, as an indicator of large vessel disease. In cross-sectional studies conducted in children and adolescents with untreated essential hypertension, defined by means of office readings,<sup>149</sup> or by ABPM measurements,<sup>150</sup> the prevalence of left ventricular hypertrophy, defined according to adult criteria, *i.e.*, left ventricular mass above 51 g/m<sup>2.7</sup>, which is associated with 4-fold higher risk for the development of cardiovascular endpoints,<sup>151</sup> was 8.4% (95% CI, 4.3% to 14.6%) and 27% (95% CI, 13.7% to 44.1%), respectively. Several studies have shown a correlation between the severity of ambulatory BP elevation and the likelihood of developing left ventricular hypertrophy.<sup>150, 152</sup> On the other hand, a recent multi-center study found that abnormal ambulatory BP results were not predictive of left ventricular hypertrophy in a cohort of 184 children aged 3 to 20 years with newly diagnosed primary hypertension.<sup>153</sup> Increased cIMT has also gained acceptance as a potential marker of hypertensive vascular damage in pediatric patients with essential hypertension diagnosed by means of office<sup>154, 155</sup> and ambulatory BP recordings.<sup>156, 157</sup> Altogether, these studies showed that vascular end-organ damage can already be identified in children and adolescents with primary hypertension. Their results emphasize therefore the importance of the early recognition and treatment of hypertension across the pediatric ages as well as the need to

investigate the presence of hypertensive target-organ damage. These investigations also suggest that the use of ABPM in children and adolescents might offer a more precise prediction of target injury related to BP than office BP readings. On the other hand, these investigations have in common that they were designed as cross-sectional studies. This is a limitation when interpreting associations with left ventricular hypertrophy or increased cIMT and hypertension, as a possible risk factor. This is due to the fact that the study-populations were investigated on only one occasion, and therefore both sets of information were obtained at the same time. Hence, the aforementioned investigations can only suggest possible causal links. Prospective interventional studies are necessary to better address whether there is indeed a causal link and its clinical significance.<sup>158</sup>

The latest Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents,<sup>11</sup> like adult guidelines, acknowledges the use of ABPM for the diagnosis of white coat hypertension. In untreated children and adolescents referred to a pediatric hypertension clinic for persistently elevated office BP, the prevalence of this condition, defined as the average of three systolic and/or diastolic office BP greater than or equal to age, gender, and height-specific Task Force 95<sup>th</sup> percentile<sup>11</sup> and daytime systolic and diastolic BP < 95<sup>th</sup> percentile by gender and height from normative pediatric ambulatory BP data,<sup>140</sup> was 30.9% (95% CI, 20.5% to 43%), appearing to be more common in children with mild elevations of office BP.<sup>159</sup> As it seems the likelihood of white coat hypertension decreases as office BP increases,<sup>159, 160</sup> children and adolescents with borderline and mild sustained office hypertension will benefit from ABPM to confirm or exclude true hypertension.<sup>159</sup> Studies designed to evaluate whether this condition carries an increased risk for target organ damage did not detect significant differences between children with white coat hypertension and normotensive controls in terms of left ventricular mass<sup>152, 161</sup> and cIMT.<sup>161</sup> However, it still remains to be clarified whether children with white coat hypertension, as suggested in adult studies,<sup>99</sup> may actually progress to a true hypertensive condition. Therefore, close follow-up with repeated ABPM seems to be warranted until more data becomes available.

Masked hypertension has only recently been described in children.<sup>161, 162</sup> According to these studies, the prevalence of this condition appears to be lower than that observed in adults (prevalence 7.9%; 95% CI, 5.9% to 10.2%). In contrast to children diagnosed as having white coat hypertension, children with masked hypertension were observed as having greater left ventricular mass compared to normotensive controls.<sup>161</sup> In one study, children with masked hypertension were followed-up with repeated ABPM for an average of 34 months.<sup>162</sup> At follow-up examination, children with persistent masked hypertension had higher left ventricular mass than normotensive controls. In this investigation, children with masked hypertension were more obese than controls and twice as likely to have parents with a history of hypertension.<sup>162</sup> Altogether, this condition warrants follow-up with repeated ABPM. However, no clinical criteria are so far available to properly identify this risk population, hence making it rather difficult to determine pediatric patients who will benefit from undergoing screening with ABPM.

Loss of nocturnal decline in BP is often observed in hypertensive children with a known cause of hypertension such as renal or endocrine disorders, than in children with essential hypertension.<sup>163</sup> There is indeed increasing information about dipping status in children with disorders such as diabetes,<sup>164, 165</sup> liver transplant recipients<sup>166</sup> sleep disorders,<sup>167</sup> and particularly in children with renal

parenchymal reduction,<sup>168</sup> chronic renal failure<sup>169</sup> as well as in children undergoing dialysis,<sup>170</sup> and after renal transplantation.<sup>171</sup> However, the biological significance of loss of nocturnal decline in BP in hypertensive pediatric patients still remains to be clarified.

Finally, although ambulatory hypertension, and hypertension defined on office BP readings, has neither been systematically correlated to end organ damage nor associated to cardiovascular events, the current evidence, based on the observed advantages of ABPM over office BP readings, makes ABPM a stronger diagnostic tool. When used in conjunction with office BP readings, ABPM has the potential value to identify children with white coat hypertension and masked hypertension, confirming therefore normotension or hypertension, respectively. In addition, there has been much interest in the potential role of ABPM in medical decision making of hypertensive children.<sup>136, 172</sup>

### **1.8 Post-renal transplant hypertension: On the importance of the diagnosis of hypertension and adequate BP control of hypertensive recipients**

Kidney transplantation is the renal replacement therapy of choice across ages for most patients with end-stage renal disease, not only improving quality of life but also offering extended life expectancy compared with dialysis.<sup>173-175</sup>

Compared with the general population adult renal transplant recipients are at high risk for morbidity and mortality, largely as a result of cardiovascular disease including disorders of cardiovascular perfusion (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) and disorders of cardiac function (congestive heart failure and left ventricular hypertrophy).<sup>176</sup> Adult patients undergoing renal transplantation are usually burdened by a high risk of cardiovascular disease.<sup>177</sup> Even though cardiovascular mortality decreases significantly after renal transplantation when compared to cardiovascular death rates of dialysis patients on waiting list,<sup>178</sup> the annual risk of cardiovascular disease in adult transplant recipients is still considerably higher than in the general population.<sup>179, 180</sup> Traditional cardiovascular risk factors (systemic hypertension, diabetes mellitus, hypercholesterolemia, obesity, tobacco use, left ventricular hypertrophy, anemia),<sup>181-183</sup> allograft dysfunction,<sup>184</sup> and novel cardiovascular risk factors, which are still under intense study (markers of inflammation such as C-reactive protein, hyperhomocysteinemia, advanced glycation end products),<sup>181-183</sup> have been implicated in the pathogenesis of cardiovascular disease in adult renal transplant recipients.

Among traditional risk factors, hypertension, defined as office BP  $\geq$  140/90 mm Hg,<sup>13, 64</sup> is the most prevalent cardiovascular risk factor reported in adult renal transplants, affecting 75% to 90% of recipients.<sup>181, 185</sup> In a large cohort of adult renal transplants with functioning graft the prevalence of hypertension, defined as the use of antihypertensive medication, was 96.5% (95% CI, 95.3% to 97.4%).<sup>186</sup> Risk factors for hypertension after renal transplantation include calcineurin inhibitor use, corticosteroid use, quality of the donor, delayed graft function, acute graft rejection, chronic graft rejection, recurrent or *de novo* glomerulonephritis, transplant artery stenosis, high renin output from diseased native kidneys, and obesity.<sup>187</sup>

Recent observational adult studies suggest a 1% to 2% increased risk of fatal and non-fatal cardiovascular disease events for every 1 mm Hg increase in systolic BP.<sup>179, 185, 186</sup> In addition, a large

study with a long follow-up period found that graft survival rate was significantly related to both systolic and diastolic BP.<sup>188</sup>

In an adult general population, isolated treatment of hypertension is no longer sufficient to reduce total cardiovascular risk, as described in recent treatment guidelines which incorporate the concept of global cardiovascular risk management.<sup>13, 64</sup> However, it is well established that reduction in BP is essential to improve long-term cardiovascular health in the general population.<sup>189, 190</sup> Therefore, hypertensive renal transplant recipients should also be vigorously treated as recently pointed out in a large adult study where the number of treated hypertensive recipients with adequate office BP control was shown to be rather low at 1 year after transplantation (prevalence 46.8%; 95% CI, 44% to 49.6%).<sup>186</sup>

Earlier investigations conducted in pediatric renal transplant recipients found that hypertension is also a common post-transplant complication.<sup>191, 192</sup> A large pediatric study reported 63% (95% CI, 61.2% to 64.6%) prevalence of hypertension, defined as the use of antihypertensive medication, at 2 years after transplantation.<sup>193</sup> While this study demonstrated a high prevalence of post-transplant hypertension, no data were reported on the level of controlled BP. In a recent pediatric study it was observed that at 1-year following transplantation less than half of treated hypertensive recipients had controlled office BP, defined as office systolic and diastolic BP lower than age, gender, and height-specific Task Force 95<sup>th</sup> percentile while on antihypertensive medication (prevalence 36.4%; 95% CI, 28.7% to 44.7%).<sup>194</sup>

As kidney transplantation has become the treatment of choice for children with chronic renal failure, much concern arose about the long-term cardiovascular outcomes of patients who received a renal transplantation in childhood and had reached adulthood. In one single center study the main cause of mortality was cardiovascular complications.<sup>195</sup> In another series, a questionnaire sent to recipients who underwent renal transplantation during childhood and reached adulthood, reported a 2% incidence of myocardial infarction.<sup>196</sup> In line with adult results, recent investigations have identified cardiovascular mortality as the most common cause of death among children, adolescents, and young adult renal transplant recipients.<sup>197, 198</sup> In addition, hypertension has been identified as one of the most prevalent potential cardiovascular risk factors among pediatric renal transplant recipients.<sup>191, 199, 200</sup> Moreover, pediatric recipients exposed to a long duration of hypertension are more likely to die in early adult life than recipients exposed to a relatively short period of hypertension.<sup>197</sup>

It should be stressed that the data for post-transplant hypertension summarized above were obtained from office BP measurements, which, as previously described, are poorly reproducible by ABPM. Thus, ABPM would be the preferred method of choice to increase diagnostic accuracy of hypertension as well as response to antihypertensive therapy and to better predict outcomes. Indeed, during the past years, the utility of ABPM after kidney transplantation has been the subject of several adult<sup>201-203</sup> and pediatric<sup>129, 162, 195-205</sup> cross-sectional studies. These investigations largely contributed to the acceptance of ABPM as a potential useful modality for the evaluation of BP in renal transplant recipients in both hypertension research and in the clinical setting. On the other hand, despite the fact that in an adult general population ABPM was shown to be superior to office BP readings in predicting cardiovascular events,<sup>81-86</sup> there is no prospective outcome-based trial of transplant and

cardiovascular endpoints comparing BP treatment decisions on the basis of office or ambulatory BP values to properly define the role of ABPM in post-renal transplant BP management.

Finally, while all the pediatric studies cited thus far agree that office BP is not a reasonable surrogate for ambulatory BP in renal transplant recipients, these investigations suggest, but do not prove, the potential clinical implications of ABPM in this population at high risk for hypertension.

## **2 Aims**

The aim of this thesis was to test the hypothesis whether (i) the routine use of repeated ABPM after renal transplantation improves the identification of true hypertensive recipients and (ii) facilitates the management of hypertension. We conducted therefore a series of studies in an attempt to determine the actual role of ABPM in our pediatric renal transplant population.

## **3 Materials and Methods**

### **3.1 Patients**

A detailed description of study populations' inclusion and exclusion criteria as well as recipient's and healthy control's demographic characteristics are fully depicted in the original articles.

Previous investigations reported that tacrolimus-based immunosuppressive therapy is associated with less hypertension compared with cyclosporin-treated renal transplant recipients. Since in our renal transplant population tacrolimus has replaced cyclosporin, we retrospectively investigated office BP in 56 children and adolescents and 14 young adults ( $\geq 18 \leq 22$  years) from a total of 100 recipients who underwent consecutive kidney transplantation (paper **I**). Recipients were non-randomly allocated, before December 2000, to cyclosporin ( $n = 38$ , 29 males; median age 13.7, range 4.1 to 22 years), and, after December 2000, to tacrolimus ( $n = 32$ , 23 males; median age 13.8, range 3.5 to 22 years) given with mycophenolate mofetil and corticosteroids. Recipients' immunosuppressive protocol was not modified throughout the study period and the study population was followed at monthly intervals on an outpatient basis. All recipients were followed 2 years after transplantation.

Adult studies have clearly shown that ABPM is a more repeatable method of BP measurement than office BP readings. No pediatric study has however evaluated the reproducibility of ambulatory BP readings in renal transplants. Therefore the aim of paper **II** was to evaluate the long-term reproducibility of average office and ambulatory BP readings in 18 stable pediatric renal transplant recipients (8 males) in three visits (annual controls), 12 months apart, over a 2-year period. Recipients' immunosuppressive treatment as well as concomitant medication remained unchanged throughout the study period. At the time of annual control 1, the median time of follow-up after transplantation was 7 years (range, 2 to 11 years) and the median age was 10.4 years (range, 5.7 to 14.6 years).

In paper **III** we aimed to analyze, in a cross-sectional study, whether the sole use of office BP is reliable for the diagnosis of BP control. Twenty-six treated hypertensive recipients (18 males; median age 14.2, range 6.8 to 18 years) underwent ABPM at the median interval after transplantation of 2 years (range, 0.4 to 9.5 years).

The aforementioned study was followed by a prospective and longitudinal study designed to investigate whether ambulatory BP contributes to the morphological characteristics of carotid artery walls in normotensive and hypertensive pediatric renal transplant recipients (paper **IV**). This study was conducted in 31 recipients (16 males) with a median age of 10.5 years (range, 3.8 to 16.8 years) who underwent their first carotid artery ultrasound examination (baseline) at the median interval after transplantation of 5 years (range, 1 to 13.3 years). The median time from the baseline to the subsequent carotid artery ultrasound (follow-up examination) was 4 years (range, 2 to 6 years). Diagnosis of hypertension and treatment was based on ABPM criteria. Twenty-one healthy children (9 males) with a median age of 10.8 years (range, 3.3 to 18.1 years) were recruited for B-mode ultrasound assessment of the carotid artery and were regarded as the control group to our study population.

Finally, in paper **V** we investigated the role of repeated ABPM, performed at yearly intervals following transplantation, in the assessment and decision-making processes of post-transplant hypertension.

In 1998, ABPM was introduced as a diagnostic tool at the recipients' annual controls and became the reference method to identify true hypertensive recipients, to evaluate drug resistance, and to facilitate long-term hypertension management. Since then, ABPM has been performed on a regular basis at yearly intervals, regardless of recipient's BP status. In this study, we analyzed ambulatory BP results in 37 recipients (23 males; median age at time of transplantation of 10.6, range 1 to 16 years) who were transplanted since the systematic introduction of ABPM (study group). The median time between their first ABPM, which was performed at 1 year following transplantation (baseline examination), and their most recent annual ABPM examination (last follow-up) was 3 years (range, 1 to 8 years).

We also reviewed ambulatory BP status in 50 additional recipients (28 males; median age at time of transplantation of 5.1, range 0.4 to 15.7 years), who underwent their first ABPM examination at their annual control in 1998 (median time between transplant surgery and first ABPM of 6 years, range 2 to 15 years). These recipients were regarded as our historical control group and their ABPM results, obtained in 1998, were compared to the study group ambulatory BP status at baseline and at last follow-up, respectively.

### **3.1.1 Ethical considerations**

All studies were approved by the Ethics Committee either at Karolinska University Hospital, Huddinge, Sweden (papers **II**, **IV**, and **V**) or at Hospital Italiano, Buenos Aires, Argentina (paper **I** and **III**). Oral informed consent was obtained from both the children and the parents.

## **3.2 Methods**

### **3.2.1 Office BP measurement**

In paper **I** and **III**, office BP was measured by doctors and determined with a mercury manometer on the right arm with cuffs of adequate size. The first and the last Korotkoff sounds (K1 and K5) were taken as systolic BP and diastolic BP, respectively. The mean of three consecutive BP readings, taken approximately 1 min apart with a patient in a sitting position after  $\geq 5$  min of rest, was used to ascertain the recipient's office systolic BP and diastolic BP. Seated office BP was always measured in the morning and shortly after the recipients had taken their medication(s), including antihypertensive drugs.

For patients under 18 years (paper **I** and **III**), office BP within the hypertensive range was defined as the mean of three systolic BP and/or diastolic BP readings exceeding the 95<sup>th</sup> age-, sex-, and height-matched percentile of the adapted reference standard on at least three different outpatient visits.<sup>11</sup> For patients aged 18 years or older (paper **I**), BP values  $\geq 140$  mmHg (systolic BP) and/or  $\geq 90$  mmHg (diastolic BP), also based on the average of three readings measured on three office visits, were considered office hypertension.<sup>204</sup> In most cases, anti-hypertensive treatment was introduced if non-pharmacological therapy failed to lower BP within a period of several months.

In paper **I**, for analysis purposes, anti-hypertensive treatment was regarded as an indicator of office hypertension.

In paper **III**, controlled office BP, *i.e.*, BP within the normotensive range while the recipient was on antihypertensive therapy, was defined as the average of three replicate seated systolic and diastolic office BP recordings less than or equal to the 95<sup>th</sup> age-, sex- and height-matched percentile of the adapted reference standard on the three outpatient visits closest to ABPM.<sup>11</sup>

In paper **II**, office BP readings were recorded on all visits twice, at least 1 min apart after  $\geq 5$  min of rest, from the right arm with cuffs of the adequate size and with the recipient in the sitting position and using the same oscillometric device (Dinamap Compac S Monitor, Critikon, Newport, UK). Seated office BP was always measured between one and two hours after taking morning medication(s) including antihypertensive medication. For each recipient, seated office BP was measured on two occasions, one day apart in the morning and by the same nurse. In paper **II**, the average of these two measurements was regarded as recipient's office BP at each annual control. Office BP within the hypertensive range was defined as systolic and/or diastolic BP average exceeding the 95<sup>th</sup> percentile sex- and height-matched of the adapted reference standard.<sup>14</sup>

### **3.2.2 Ambulatory BP monitoring**

Ambulatory BP monitoring was recorded on the non-dominant arm using an appropriate arm cuff and was carried out using the oscillometric monitor, Model 90207, Space Labs, Redmond, WA (paper **II**, **III**, **IV**, and **V**). This automatic monitor is widely used and its validation has been previously confirmed in children.<sup>205</sup> The device was programmed for cuff insufflations every 20 min from 7 am to 10 pm and every 30 min from 10 pm to 7 am (paper **II**, **IV**, and **V**) and every 10 min from 6 am to 10 pm and every 20 min from 10 pm to 6 am in paper **III**. The cuff was attached to the non-dominant upper-arm and a series of calibration readings were taken with a mercury manometer (paper **III**) or

Dinamap Compac S Monitor, Critikon, Newport, UK (paper **II**, **IV**, and **V**), to ensure that the device was giving accurate readings (within 5 mm Hg of the manual readings). To improve the quality of BP recordings, recipients were given tailored instructions on the procedure and encouraged to maintain their usual activities. They were asked to complete a diary of events during the 24-h period, including their awake and asleep times. Blood pressure readings of systolic BP > 240 or < 70 mm Hg, diastolic BP > 150 or < 40 mm Hg, and pulse pressure > 150 or < 20 were automatically discarded (paper **II**, **III**, **IV**, and **V**). Night-time was defined according to the period of night-time sleep based on recipients' diaries.

The definition of arterial hypertension was based on mean daytime ABPM values. This arbitrary definition relied upon two premises. First, at the time the ABPM procedure was introduced both in Argentina and in Sweden there were no pediatric data to support targeting BP at specific periods of the 24-h cycle. Second, office BP reference values did not report on night-time BP measurements.<sup>11, 14</sup> Since 1998, we have used the pediatric normative data for ABPM published by Soergel *et al* (paper **IV**, and **V**),<sup>140</sup> and since 2002 we used their later revised version as ABPM reference values (paper **II**, **III**, **IV**, and **V**).<sup>144</sup>

Thus, four different BP status were defined according to recipients' ambulatory BP patterns as follows: (i) normotension, mean systolic and diastolic daytime ambulatory BP values  $\leq$  95<sup>th</sup> distribution adjusted height- and sex- related percentile for daytime described in the published pediatric normative data for ABPM, (ii) controlled BP, mean systolic and diastolic daytime BP readings within the normotensive range while the recipient was on antihypertensive therapy, (iii) hypertension, mean systolic and/or diastolic daytime ambulatory BP value(s) above the 95<sup>th</sup> distribution adjusted height- and sex- related percentile for daytime, and (iv) non-controlled hypertension, mean systolic and/or diastolic daytime ambulatory BP readings within the hypertensive range while the recipient was on antihypertensive therapy. In recipients whose height was < 120 cm their mean daytime BP values were compared to the 120 cm reference values.

Systolic and diastolic 24-h BP load were calculated as the sum of day- and night-time recordings above the 95<sup>th</sup> distribution adjusted height- and sex-related percentile for day- and night-time normative data for ABPM,<sup>140, 144</sup> divided by the total number of recordings during the 24-h period multiplied by 100 (paper **II** and **IV**).

Prescription and starting dose of antihypertensive drug therapy as well as further dose titration were decided on an individual basis by the treating physician according to recipient's ABPM results (paper **II**, **IV**, and **V**).

In the original articles, ABPM results are presented either as raw data (paper **II**, **III**, **IV**, and **V**) or as normalized to standard deviation scores (SDS) by use of the least mean square method (LMS-method), taken from the formula by Cole and Green as adapted by Wühl *et al* (paper **IV** and **V**).<sup>144</sup> This analysis accounts for the non-Gaussian distribution of the original pediatric normative ABPM data published by Soergel *et al*.<sup>140</sup> This linear transformation represents the distance in units of SD between a given individual's mean ambulatory BP values and the mean reference ambulatory BP values for a given sex and height provided in the revised version of the original pediatric normative

ABPM data.<sup>144</sup> Thus, the calculation of SDS values permits a more precise evaluation of pooled ambulatory BP data over time independent of sex and height.

### 3.2.3 Carotid B-mode ultrasonography

Carotid arteries were examined bilaterally with a duplex scanner (Aspen; Acuson, Mountain View, CA, USA) using a 7-MHz linear array transducer following a predetermined and standardized scanning protocol (paper **IV**), as previously described.<sup>206</sup> All recordings were performed by two experienced vascular sonographers, with the subjects in supine position, and the head slightly turned away from the sonographer. The scans were videotaped and analyzed by a computer system<sup>207</sup> with automated tracing of echo interfaces and in some cases corrected manually. The analyses were performed blinded to the clinical background at the end of the follow-up by one investigator (Rita Balzano). Measurements of distances between the wall echoes within a 10-mm-long section of the common right and left carotid artery were made in late diastole defined by a simultaneous electrocardiographic recording. The far wall of the common carotid artery, 0.5 to 1.0 cm proximal to the delimitation of the carotid bulb, was used for measurements of the cIMT and common carotid artery lumen diameter (ALD). The cIMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo. The ALD was defined as the distance between the leading edge of the intima-lumen echo of the near wall and the leading edge of the lumen-intima echo of the far wall. The measured values of the cIMT and ALD within each 10-mm-long section from the right and left artery were averaged, and the mean values were used for analysis. The cross-sectional intima-media area, a value derived from the cIMT and the lumen dimension, was calculated to compensate for the BP-dependent change in the wall thickness by using the formula:  $3.14 [(ALD / 2 + IMT)^2 - (ALD / 2)^2]$ .<sup>208</sup> This approach also allowed a better estimate of the cIMT among recipients with different BP, and between the two ultrasound examinations in the same subject. To assess intraobserver agreement, the measurements of 40 randomly chosen carotid segments were repeated. The differences between repeated measurements of cIMT and ALD, *i.e.*, the variability of the within-subject differences, were 7% and 1%, respectively, with a cIMT of 0.3 to 0.6 mm and an ALD of 4.9 to 7.0 mm.

### 3.2.4 Statistical analysis

Ninety-five percent confidence intervals (95% CI) for proportions and point estimate (point prevalence) were calculated using the adjusted Wald method in order to present our sample statistics as estimates, if the total population was studied.<sup>209</sup> Thus, 95% CI is the range of value which one can be confident includes the true value for the examined variable. An important consideration when using this descriptive method is that there is a small chance that the 95% CI from a single sample will not include the true population's prevalence of the variable being analyzed whatever the sample size. However, the width of the 95% CI depends on the sample size. Indeed, a wide 95% CI suggests a poor precision, which will be improved by a larger sample size. The upper and lower limits therefore provide a means of assessing whether the results are clinically important. Proportions between groups were compared with  $\chi^2$  test.

Validation of ABPM for application to clinical practice in pediatric renal transplant recipients requires comparison with the established office BP measurement technique or with the assessment of outcomes. The latter, as previously described, would be difficult to undertake in a hypertensive pediatric population due to the fact that the occurrence of cardiovascular events is extremely rare at this age. Thus, the primary aim of comparison would be to determine whether the two methods to monitor BP, *i.e.*, office BP readings and ABPM, agree sufficiently to be used interchangeably, or, in other words, how much one method is likely to differ from the other when the two methods are applied on the same subject.

Bland and Altman proposed a method for assessing agreement between two methods of measurement, based on quantifying the variation in between-differences for individual patients (paper **III**).<sup>54</sup> Their analysis first calculates the difference in measurements obtained by the two methods on the same subject, which is computed as the value determined by one method minus the value determined by the other method. The mean of such difference in a sample of subjects is the mean of the difference or the estimated bias. The standard deviation of the differences (SDD) measures random fluctuations around this mean. If the differences are normally distributed it would be expected that 95% of the differences between the two methods of measurement lie between the mean of the difference – 1.96SD and the mean of the difference + 1.96SD, or  $\pm 2SD$ . These calculated levels are called 95% “limits of agreement”. The calculation of the 95% limits of agreement is based on the assumption that the differences are normally distributed. Such differences are indeed very likely to follow a normal distribution because subjects are left with the measurement error, which is expected to be normal anyway.

An essential feature of the analysis is the graphical representation of the data with the difference between the measurements obtained by the two methods (*y* axis) plotted against the mean of the two measurements (*x* axis). Concerning BP, the topic being discussed in this thesis, intra-arterial BP is regarded as the “gold standard” against which to judge new methods, in particular non-invasive methods such as office and ambulatory BP measurements. Intra-arterial BP measurement is however an invasive method which is not practical in the clinical setting. Therefore, the mean of the BP measurements obtained by office and ambulatory BP measurements (*x* axis) would be the best estimate of BP since the true BP is unknown.

Bland-Altman plots are generally interpreted without further analysis. However, several questions arise when, as in the present example, pairs of BP measurements are plotted as described above. A clinical question, not a statistical one, is whether the mean difference between measurements obtained by the two methods mentioned above is large enough to be relevant in clinical decision making. If the provided differences are not clinically important then the two methods could be used interchangeably. There will also be a variation about this mean, *i.e.*, the measurement errors. These estimates will be meaningful if it is assumed that the measurement errors are uniform throughout the range of measurements.

Repeatability is relevant to the study of method comparison because poor repeatability, *i.e.*, considerable variation in repeated measurements on the same subject, precludes the assessment of the amount of agreement which is possible. If one method has poor repeatability, the agreement

between the two methods is bound to be poor. Therefore, repeatability of each method separately should be examined before agreement between methods can be estimated (paper II).

The examination of repeatability can be approached in the same way as the assessment of agreement. Thus, the mean of the difference, the SDD within subjects, and the mean of duplicate measurements are calculated using the same method as before. Then the SDD can be compared to see which method is more repeatable. Each SDD can be also used to calculate the limits within which the differences between two measurement obtained by the same method would be expected to lie. Here the mean of the difference is expected to be approximately zero since the same method is used. It should be noted that if the mean difference between duplicate measurements is significantly different from zero, as assessed by the paired *t*-test or one-way analysis of variance, when more than two repeated measurements are analyzed, it can then be inferred that there is a systematic difference between the pairs of results, and, as a consequence, the data should not be assessed for repeatability.

Finally, 95% of the differences, *i.e.*, the maximum difference which is likely to occur between two measurements, are expected to be less than 2SD. This is also known as repeatability coefficient, which is expressed, assuming a normal distribution of differences, as the mean of the observed differences  $\pm 1.96SD$ .

#### 4 Results and Discussion

In paper I, the use of antihypertensive treatment at 2-years after transplantation was regarded as an indicator of hypertension. At this time point, we observed that 33 of 38 cyclosporin-treated recipients (prevalence 86.8%; 95% CI, 71.9% to 95.5%) and 20 of 32 tacrolimus-treated recipients (prevalence 62.5%; 95% CI, 43.6% to 78.9%) were regarded as hypertensive recipients. The observed prevalence of recipients on antihypertensive treatment was significantly lower in the tacrolimus- than in the cyclosporin-treated group (difference between proportions 24.3% (86.8% – 62.5%); 95% CI for difference, 4% to 43%,  $P = 0.01$ ). This observation is in line with a large retrospective cohort study using the North American Renal Transplant Cooperative Study (NAPRTCS) database where recipients were treated, like in our study, either with cyclosporin ( $n = 391$ ) or tacrolimus ( $n = 77$ ) associated to mycophenolate mofetil and corticosteroids.<sup>210</sup> After adjusting for donor source, use of induction therapy, year of transplantation, presence of delayed graft function and recipients gender, age and race, tacrolimus-treated recipients were significantly less likely to require antihypertensive medication at 2-years after transplantation; Cox proportional hazard regression analysis, relative risk 0.67 (95% CI, 0.56% to 0.79%). These data indicate that tacrolimus-treated recipients had a reduction of ~33% in the need for antihypertensive medication. A recent retrospective study, using the US Renal Data System database, investigated the history of hypertension in adult recipients with functioning grafts who were receiving either cyclosporin ( $n = 17108$ ) or tacrolimus ( $n = 7225$ ) associated with mycophenolate mofetil and corticosteroids, and who were followed at least one year after renal transplantation.<sup>211</sup> Our results are also in accordance with this large adult study, which shows that history of hypertension was significantly lower in the tacrolimus- than in the cyclosporin-treated group (difference between proportions 2.9% (72% – 69.1%); 95% CI for difference, 1.8% to 4%,  $P < 0.0001$ ).

We also looked at the number of recipients with non-controlled hypertension based on the recipient's average of three office BP readings obtained at last follow-up. The prevalence of non-controlled hypertension in the cyclosporin-treated group was similar to the tacrolimus-treated group (difference between proportions 1.2% (20% – 18.8%); 95% CI for difference, –18% to 22%,  $P = 0.8$ ). The overall prevalence of non-controlled hypertension observed in our entire study population was 18.8% (95% CI, 9.4% to 31.9%). This observation is however in contrast to a previous pediatric retrospective study including recipients with different immunosuppressive protocols.<sup>194</sup> Of 148 recipients receiving antihypertensive medications, 94 displayed non-controlled hypertension at 1 year after transplantation (prevalence 63.5%; 95% CI, 55.2% to 71.2%), this figure being higher compared to our study group. One important methodological difference that makes the latter study superior to ours in terms of study design and consequently in data interpretation, is that the analysis of controlled BP and non-controlled hypertension was based on the mean value for systolic and diastolic office BP for each recipient's clinical visit within the first year after transplantation and not on the last clinical visit as in our study. Still, the authors recognize, as we did in our own study, that ABPM would be a much more superior method for improving the estimate of recipients' true BP status.

Overall, 75.7% of our study population (95% CI, 63.9% to 85.1%) was regarded as hypertensive recipients at last follow-up. A much larger retrospective pediatric study from NAPRTCS, aimed to determine the prevalence of post-transplant hypertension, defined also as the use of antihypertensive medications, found that 1822 of 2889 recipients were on antihypertensive therapy 2 years after transplantation (prevalence 63%; 95% CI, 61.2% to 64.8%).<sup>193</sup> Although in this investigation, similar to our study, a large proportion of recipients were regarded as hypertensives, the observed prevalence of treated hypertensive recipients was significantly lower in the aforementioned investigation compared to our study (difference between proportions 12.7% (75.7% – 63%); 95% CI for difference, 3% to 21%,  $P = 0.03$ ). The most likely explanation for this inconsistency might be due to the fact that all our recipients were on calcineurin inhibitors whereas in the aforementioned study the analysis included recipients with and without calcineurin inhibitors. This is in line with earlier studies showing that the introduction of calcineurin inhibitors has increased the prevalence of hypertension in solid organ transplants.<sup>212, 213</sup>

In order to examine the degree of reproducibility of repeated office and ambulatory BP measurements we first tested whether pairs of results were significantly different. As presented in paper **II**, mean office and ambulatory BP differences between the measurements obtained at annual controls 1, 2, and 3 were not significantly different (one way analysis of variance). Table 1 summarizes the descriptive statistics used for analyzing the degree of reproducibility, expressed as the SDD, between annual controls for both office and ambulatory BP recordings.

**Table 1| Descriptive statistics of reproducibility of office and ambulatory blood pressure recordings over a period of 2 years in 18 pediatric renal transplant recipients**

Variables	Annual control 2 - control 1		Annual control 3 – control 2		Annual control 3 – control 1	
	Mean difference	SDD	Mean difference	SDD	Mean difference	SDD
Office BP (mmHg)						
Systolic	0.5	13.4	1.1	12.4	1.6	13.7
Diastolic	1.9	6.3	2.1	8.0	4.0	9.5
24-h BP (mmHg)						
Systolic	1.1	6.2	-0.1	7.0	0.9	7.3
Diastolic	0.3	5.5	0.6	5.1	0.9	5.6
Daytime BP (mmHg)						
Systolic	0.7	6.9	-0.2	8.6	0.4	7.1
Diastolic	0.0	6.9	0.7	5.7	0.7	5.5
Night-time BP (mmHg)						
Systolic	1.3	4.8	-0.7	7.0	0.6	8.9
Diastolic	0.8	4.7	0.1	5.3	0.9	6.5

SDD = + 1 standard deviation of the mean BP differences between visits; BP, blood pressure.

Respective SDDs for office and ambulatory BP recordings display similar values between annual controls over the 2-year period, suggesting therefore a good repeatability of each individual method. However, SDDs for office BP were consistently above SDDs for all ambulatory BP recordings except for daytime diastolic BP between annual control 1 and 2.

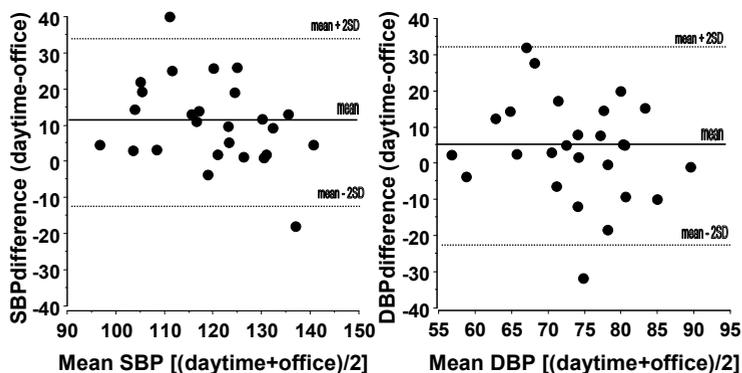
Unfortunately so far there is no published study conducted in pediatric renal transplant recipients that applies the same approach to evaluate repeatability of each of the methods of BP measuring separately, against which we could compare our results. However, our observation extends previous results derived from adult studies conducted in normotensive and hypertensive subjects showing that SDDs, used also as the reciprocal of BP reproducibility, were lower for ABPM than for office BP readings.<sup>55, 56, 58, 214</sup> Altogether, these data show a better reproducibility for ambulatory BP recordings compared to office BP readings.

In paper **III**, we retrospectively analyzed 26 treated hypertensive pediatric renal transplant recipients who underwent ABPM procedure at one time-point after renal transplantation. We found that eight recipients were categorized as having controlled BP by both office and ABPM criteria and two recipients as having non-controlled hypertension by the two methods of BP measurement. In eight recipients, BP was controlled when measured by office but not by ABPM criteria, with the opposite observed in three recipients. In five recipients, BP status according to BP measurements could not be categorized, since their office readings fluctuated in one or two of the three outpatient visits closest in time to ABPM. If it is assumed (as a probability) that in the latter five

recipients their BP status would have agreed using office BP and ABPM criteria, the overall (and *a priori* the lowest estimate) prevalence of recipients with controlled office BP, who in fact had non-controlled hypertension by ABPM criteria, was 30.7% (95% CI, 14.3% to 51.7%). By keeping the same assumption, in 11 out of 26 recipients, their office BP status were not consistent with their respective ABPM results (prevalence 42.3%; 95% CI, 23.3% to 63%).

Overall, 12 recipients from the entire study population had non-controlled hypertension according to ABPM criteria (prevalence 46.4%; 95% CI, 28.7% to 64.5%). Our observations extend the results derived from previous pediatric cross-sectional studies reported worldwide showing that office BP readings are not a reasonable surrogate for ambulatory BP in identifying true responders to antihypertensive therapy after renal transplantation.<sup>215-223</sup>

Agreement between the two methods of measurement according to the Bland-Altman method is shown in Figure 1.



**Figure 1| Comparison of systolic and diastolic daytime ambulatory and office blood pressure according to the method of Bland and Altman (SBP, systolic blood pressure; DBP, diastolic blood pressure)**

For systolic and diastolic BP the mean difference was 10.7 and 3.9 mm Hg, respectively, and the 95% limits of agreement of the difference were -12.6 to 34.1 mm Hg for systolic and -23.9 to 31.7 mm Hg for diastolic BP. From this analysis it can be inferred that in our study population, office BP measurement might give systolic BP values between 13 mm Hg above daytime readings and 34 mm Hg below it. From both these data and the above described observation, we conclude that the mean difference between measurements obtained by office BP readings and ABPM in our study population was large enough to have important clinical implications.

Over the past decade, the study of structural changes in the arterial wall related to BP has attracted major interest, as blood vessels represent an important target organ for hypertension. Carotid ultrasonography has been shown to be a valid technique that allows a safe, accurate and non-invasive reproducible quantification of asymptomatic structural vascular change.<sup>224, 225</sup> Although this method does not allow for differentiation between pre-atherosclerotic intimal lesion and hypertension-induced tunica media hypertrophy,<sup>226</sup> it reflects disease in other vascular beds, and has gained acceptance as a potential surrogate marker of hypertensive vascular damage. The aim in paper **IV** was to determine, by means of repeated high-resolution B-mode vascular ultrasound, whether BP contributes to the morphological characteristics of the carotid artery wall. Table 2 displays BP status throughout the study period.

**Table 2| Blood pressure status at baseline and at last follow-up in 31 renal transplant recipients**

<b>BP status</b>	<b>Baseline<sup>a</sup></b> (n = 31)	<b>Last follow-up<sup>b</sup></b> (n = 31)
Normotensive	16	9
New-onset hypertension	3	3
Controlled BP	5	19
Non-controlled hypertension	7	–

<sup>a</sup> Baseline denotes the first carotid artery ultrasound examination which was performed at the median time of 5 years (range, 1 to 13.3 years) after transplantation. <sup>b</sup> Last follow-up denotes the second carotid artery ultrasound examination performed at the median time of 4 years (range, 2 to 6 years) after the baseline ultrasound examination. BP, blood pressure.

As shown in Table 2, none of the treated hypertensive recipient had non-controlled hypertension at last follow-up.

Baseline cIMT and calculated common carotid artery cross-sectional intima media area (cIMa), both in recipients with strict normotension, (*i.e.*, ambulatory normotension without antihypertensive therapy), at baseline and throughout the study period, and in recipients with treated hypertension and newly diagnosed hypertension, were significantly higher than in healthy controls (Table 3).

**Table 3| Carotid ultrasound variables of healthy controls and the study group**

Variable	Control children (n = 21)	Ambulatory normotension		Ambulatory hypertension	
		Baseline (n = 9)	Follow-up (n = 9)	Baseline (n = 22)	Follow-up (n = 22)
cIMT (mm)	0.43 ± 0.03	0.48 ± 0.05‡	0.48 ± 0.05	0.47 ± 0.07‡	0.48 ± 0.05
cIMa (mm <sup>2</sup> )	7.57 ± 1.00	8.61 ± 1.3‡	8.79 ± 1.4	8.6 ± 1.5‡	8.54 ± 1.3

Data are presented as mean ± 1 standard deviation. cIMT, common carotid artery intima-media thickness; cIMa, calculated common carotid artery cross-sectional intima-media area. Comparison between control, normotensive, and hypertensive groups at baseline carotid artery ultrasound examination was carried out using analysis of variance (ANOVA) followed by Bonferroni test to allow for multiple pair-wise comparisons, ‡  $P = 0.02$  compared with control children.

On the other hand, we did not observe any significant difference in baseline and follow-up cIMT and cIMa between recipients with ambulatory normotension and ambulatory hypertension. Similarly, pairwise comparisons showed that baseline and follow-up cIMT and cIMa within each subgroup of recipients were not significantly different.

Associations between cIMT and hemodynamic variables in the entire study group and separately in recipients with ambulatory normotension and hypertension, both at baseline and at follow-up carotid ultrasound examination, were analyzed by calculating Pearson correlation coefficients. Regardless of the time-point, we did not observe any significant association between systolic and diastolic 24-h BP, daytime BP, night-time BP, ABPM-SDSs, BP loads and cIMT. According to these results we conclude that in our study population ambulatory BP was not a contributing factor to increased cIMT. Thus, increased cIMT seems not to reflect an adaptive response to intraluminal pressure as suggested in a previous adult study.<sup>227</sup> However, we cannot overlook the possibility that the contribution of BP to the development of increased cIMT, in a study population with rather good BP control, was too weak to be detected, or that BP operates in conjunction with other factor(s) in the pathogenesis of increased cIMT.

In healthy children, different results in regard to the influence of BP on cIMT have been reported.<sup>228, 229</sup> In one study it was observed that cIMT was not affected by age and body habitus up to 18 years of age and that office BP seems to have little influence on cIMT.<sup>228</sup> In another study, cIMT was noted to increase in the second decade of life and office BP was found to be a strong determinant of cIMT.<sup>229</sup>

Contrary to our study, a previous pediatric investigation including a similar number of recipients as in the current study ( $n = 31$ ), showed a positive association between BP and cIMT.<sup>230</sup> However, a significant positive correlation was only observed between office BP and cIMT, while ambulatory BP recordings were not significantly associated with cIMT. The aforementioned investigation was designed as a cross-sectional study instead of a longitudinal and prospective study like the present one, precluding therefore the examination of recipients' cIMT pattern over time. Still, both studies agree on one relevant point: pediatric renal transplant recipients have increased cIMT when compared

with healthy controls. In line with this observation, a recently published pediatric longitudinal study including 19 renal transplant recipients, found that at the time of transplant surgery (baseline) as well as 1 year after transplantation, a large number of study recipients displayed cIMT measurements above the upper normal reference values derived from healthy children and adolescents (13 out of 19 recipients at baseline and 11 out of 19 recipients at 1 year after transplantation displayed higher cIMT than healthy controls).<sup>231</sup> This study also showed that successful transplantation was accompanied by a significant decrease in cIMT at 1 year after transplantation compared to baseline values. This relevant observation, if confirmed in larger studies, gives support to the authors' and to other investigators' hypotheses<sup>230, 232, 233</sup> that successful pediatric renal transplantation might exert a beneficial effect on asymptomatic uremic arteriopathy.

So far, our data do not prove that the routine use of ABPM following transplantation would improve our ability to identify true hypertensive recipients and true responders to antihypertensive therapy. Consequently, we analyzed the individual data of our pediatric recipients who underwent renal transplantation from the systematic introduction of ABPM onward (paper **V**).

Table 4 displays BP status of the study group both at baseline and at last follow-up as well as BP status of the historical control group. Throughout the follow-up period, antihypertensive therapy was either commenced or increased in 23 recipients. At last follow-up none of the recipients were categorized as having new-onset hypertension, whereas 24 out of 29 treated hypertensive recipients were classed as having controlled BP (prevalence 80.6%; 95% CI, 64.9% to 92.8%) (Table 4). The prevalence of recipients with controlled BP in the historical control group was 32% (95% CI, 15.4% to 51%).

**Table 4| Blood pressure status at baseline and at last follow-up in 37 renal transplant recipients and in the historical control group**

BP status	Study group		Historical control group (n = 50)
	Baseline <sup>a</sup> (n = 37)	Follow-up <sup>b</sup> (n = 37)	
Normotensive	15	8	22
New-onset hypertension	6	–	5
Controlled BP	6	24	7
Non-controlled hypertension	10	5	16

<sup>a</sup> Baseline denotes annual examination performed 1 year after transplantation. <sup>b</sup> Last follow-up is regarded as the most recent ABPM annual examination

The observed prevalence of recipients with new-onset hypertension in the study group at baseline was not statistically different when compared to the historical control group (difference between proportions 11.8% (30.4% – 20.6%); 95% CI for difference, –14% to 33.8%, *P* = 0.4). The prevalence of controlled

BP in the study group at baseline was also similar to the control group (difference between proportions 6.8% (38.8% – 32%); 95% CI for difference, –22% to 35%,  $P = 0.6$ ). However, the observed prevalence of controlled BP in the study group at last follow-up examination was significantly higher than in the control group (difference between proportions 48.6% (80.6% – 32%); 95% CI for difference, 36% to 60%,  $P = 0.001$ ).

In a *post-hoc* analysis, the above mentioned results were compared to the observed prevalence of controlled BP in the study population reported in our paper **III**, in whom BP status was defined according to the same ABPM criteria as in the present investigation. It was noted that the prevalence of controlled BP both in the study group at baseline and in the historical control group were similar to that observed in paper **III** (difference between proportions 19.6% (46.4% – 38.8%); 95% CI for difference, –20% to 35%,  $P = 0.3$  and difference between proportions 14.4% (46.4% – 32%); 95% CI for difference, –11% to 40%,  $P = 0.09$ , respectively). However, the observed prevalence of controlled BP in the study group at last follow-up examination was significantly higher than in recipients described in paper **III**, in whom, similar to the historical control group in paper **V**, ABPM was applied for the first time during antihypertensive treatment while therapeutic decisions were driven by office BP measurements (difference between proportions 34.2% (80.6% – 46.4%); 95% CI for difference, 18% to 49.9%,  $P = 0.02$ ). The result of this analysis gives further support to our initial finding that the routine use of repeated ABPM following renal transplantation may clearly improve BP control in a population at high risk for hypertension.

## 5 Conclusions and future perspectives

I have tried to focus on our most relevant results, and I conclude from my thesis, as far as statistics permit, that (i) hypertension is a common post-transplant complication, (ii) ABPM shows a better reproducibility compared to office BP readings, (iii) the two methods of BP measurement do not closely agree, and (iv) office BP measurements fail to detect a substantial number of recipients who are hypertensive by ABPM criteria. Therefore, on clinical grounds, the two methods of BP measurement should not be used interchangeably. In addition, in our study population, cIMT was not found to be a reliable surrogate marker of hypertensive organ damage. Not least, the routine use of repeated ABPM performed at yearly intervals has clearly improved the method of identification and management of hypertension in our study population. Our results, however, cannot yet be generalized to all pediatric renal transplant recipients until larger studies confirm our findings.

Finally, to date there is no data on the clinical impact of controlled ambulatory BP on aging in this selected group of patients. In addition, contrary to recent outcome-based published evidence derived from adult studies,<sup>234, 235</sup> no conclusive evidence is available so far as to whether both the daytime and night-time period of the 24-h BP cycle should be used to define ambulatory hypertension and consequently which could be the treatment target.

Therefore, it is necessary to follow up pediatric recipients until young adulthood to determine the clinical impact of controlled ambulatory BP, assessed as the occurrence of cardiovascular events or, hopefully, by means of reliable surrogate marker(s) of cardiovascular health. Unfortunately this is not a

simple task, as it requires patience and close follow-up, and once again this goal could only be fulfilled by a large prospective longitudinal survey.



## 6 Acknowledgements

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