P E T R 1 7 3 0 B
Journal Name Manuscript No.
Dispatch: 9.5.12 Journal: PETR CE: Suganya
Author Received: No. of pages: 13 PE: Vasanth

Pediatr Transplantation 2012

Pediatric Transplantation
DOI: 10.1111/j.1399-3046.2012.01730.x

Cardiovascular risk assessment in children following kidney transplantation

Dégi A, Kerti A, Kis É, Cseprekál O, Tory K, Szabó AJ, Reusz GS. Cardiovascular risk assessment in children following kidney transplantation.

Abstract: CV diseases are the leading cause of death among patients with ESRD. RTX decreases the CV risk; however, it still remains definitely higher than that of the general population. Large multicenter and longitudinal studies are difficult to perform and hard end-points of CV events are usually missing among pediatric population. Thus, appropriate estimation of CV risk is of crucial importance to define the potential hazards and to evaluate the effect of treatments aimed to reduce the risk. A number of validated non-invasive methods are available to assess the extent of CV damage in adults, such as calcification scores, cIMT, aPWV, 24-h ABPM, AASI, and HRV; however, they need adaptation, standardization, and validation in pediatric studies. cIMT and PWV are the most promising methods, as pediatric normative values are already present. The up-to-date treatment of ESRD aims not only to save life, but to offer the patient a life expectancy approaching that of the healthy population and to ensure a reasonable quality of life.

Arianna Dégi*, Andrea Kerti*, Éva Kis, Orsolya Cseprekál, Kálmán Tory, Attila J. Szabó and George S. Reusz
First Department of Pediatrics, Semmelweis University, Budapest, Hungary

Key words: renal transplantation – end-stage renal disease – heart – risk factors – children

George S. Reusz, First Department of Pediatrics, Semmelweis University, Bókay János u. 53-54., H-1083 Budapest, Hungary
Tel.: +36 30 9869545
Fax: +36 1 3138212
E-mail: reusz.gyorgy@med.semmelweis-univ.hu
*Arianna Dégi and Andrea Kerti contributed equally to this manuscript.

Accepted for publication 24 April 2012

The up-to-date treatment of ESRD aims not only to save life, but to offer the patient a life expectancy approaching that of the healthy population and to ensure a reasonable quality of life. Patients with ESRD are at high risk of CV disease. RTX decreases that risk; however, it still remains definitely higher than that of the general population. Thus, appropriate estimation of CV risk is of crucial importance to define the potential hazards and to evaluate the effect of treatments aimed to reduce the risk (1, 2).

Abbreviations: AASI, ambulatory arterial stiffness index; ABPM, ambulatory blood pressure monitoring; BP, blood pressure; CAC, coronary artery calcification; CaxP, calcium phosphorus product; cIMT, carotid intima–media thickness; CKD, chronic kidney disease; CV, cardiovascular; CVD, cardiovascular disease; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HRV, heart rate variability; HT, hypertension/hypertensive; LVMI, left ventricular mass index; LVH, left ventricular hypertrophy; MAP, mean arterial pressure; MH, masked hypertension; MRI, magnetic resonance imaging; aPWV, aortic pulse wave velocity; RTX, renal transplant/transplantation; SDS, standard deviation score; VC, vascular calcification; WCH, white coat hypertension.

CV risk in adult and pediatric ESRD

Even the earliest stages of CKD are associated with excess risk of subsequent coronary heart disease in people without manifest vascular disease (1, 3). The CV burden is even more severe in adult transplant recipients. The risk of cardiac death in ESRD has been shown to be two to three magnitudes higher than that in the general population. This is due in great part to the presence of traditional CV risk factors such as hypertension, hyperlipidemia, diabetes, physical inactivity, smoking, and older age, and it is aggravated by several non-traditional risk factors related to poor kidney function, such as anemia, volume overload, altered lipid and calcium phosphate metabolism, hyperparathyroidism, homocysteinemia, microalbuminuria, and chronic inflammation (4, 5).

Kidney transplantation significantly prolongs patient life by improving renal function and as a consequence slows down the progression of CV disease (6). Adult RTX recipients have significantly reduced rate of cardiac death compared with dialysis patients (7). However, even after successful transplantation, renal function still remains lower than that of the general popula-

tion; thus, a number of the above-mentioned risk factors are still present and RTX recipients still have up to 10 times the rate of cardiac death and 50 times the annual rate of fatal or non-fatal CV events as the general population. Furthermore, immunosuppressive treatment can impair the benefits of the functioning graft, as the standard immunosuppressive regimens are using non-selective drugs affecting various signaling pathways with nephrotoxic, metabolic, and CV side effects (1, 6; Fig. 1).

CVD is also present in pediatric transplant recipients. In fact, CVD has been found to be responsible for deaths in 36% of pediatric patients with ESRD – up to 34% of pediatric dialysis patients and 11% following pediatric transplantation (8). Unfortunately, the prevalence of chronic graft dysfunction following pediatric RTX is underestimated, and diagnosis is often made late (9, 10).

CV risk assessment

Appropriate assessment of CV risk in the general population is an important subject of medical research as it presents a major public health issue. Typical tools for CV risk assessment are the widely used and validated scoring systems. As a result, not only the actuarial CV risk, but also the possible effects of medical treatment can be predicted based on data of large longitudinal interventional studies (11, 12).

However, patients living with a functioning kidney graft present as a very special subpopulation. Beyond the risk factors due the burden of the pretransplant dialysis vintage, they are enduring the effect of slowly declining kidney function with all of its consequences, the side effects of immunosuppressive treatment, and consequent metabolic changes.

The traditional risk assessment – as the Framingham risk score – is underestimating the CV threat to this population in adults (13).

As childhood ESRD and transplantation is a rare condition compared to the adult population, large multicenter and longitudinal studies are difficult to perform, and hard end-points of CV events are usually missing, it is even more difficult to establish and predict the real CV risk in this population. Thus, there is a definite need for surrogate markers that are representative for subclinical damage, and the value has been established in large scale clinical studies (14).

As the impact of CKD and CV risk factors on long-term outcomes and recommended management strategies has been recently reviewed in this journal (15), we will focus herein on the theory and practice of non-invasive assessment of CV risk in patients on renal replacement therapy and especially in transplanted children.

Functional and morphological changes of the vasculature during growth. Basis for non-invasive CV monitoring

Arterial properties are physiologically influenced by aging and – in the growing child – by changes in the dimensions of the body and the arterial tree. The effect of the pathological processes – that means any alteration occurring beyond the physiological variations – can only be assessed if the age-related changes are documented and normalized.

Evidence that the dimensions and elastic properties of the arterial tree are intimately related to height was initially provided by morphological studies. Van Meurs et al. (16) demonstrated that higher body height goes hand in hand with increasing of internal diameter of the aorta. A fourfold increase in aortic diameter with

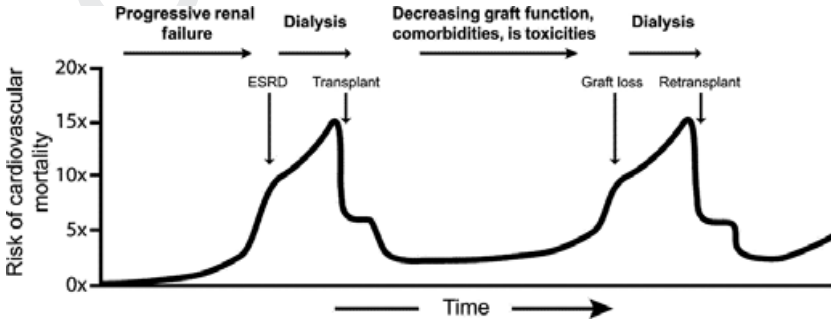

Fig. 1. Risk of CV mortality over time among adults with renal dysfunction, compared with the general population. The annual death rate from CV disease drops after RTX. It remains elevated in the immediate post-transplant period (0–3 months), but thereafter decreases to a level approximately twice that of the general population. Graft loss with a return to dialysis is associated with a significant increase in CV mortality, to the same degree as that observed in patients with ESRD. Retransplantation may reduce CV risk yet again. Adapted from (1).

body length means a twofold increase in the thickness of the tunica media (17). Ichida et al. (18) studied 173 children aged from 12 h to 15 yr and found a highly significant correlation between body surface area and aortic root dimension measured by echocardiography.

The physiological basis of the influence of body size on arterial wall properties was demonstrated by Senzaki et al., who established reference ranges for age-associated changes in arterial pulsatile properties in 112 pediatric patients after cardiac catheterization. Their results indicated a progressive increase in arterial compliance despite a decrease in arterial wall elasticity, leading to the conclusion that the increase in arterial size accompanying increased body size outweighs the effects of age on intrinsic elastic properties of arterial walls (19). Such influence of body dimensions on the elastic properties of arteries was also demonstrated in a more recent study by Jourdan et al. (20), in which both intima–media thickness and arterial stiffness were shown to change with age and body size. Accordingly, the authors concluded that morphological and functional measurements of large arteries should be normalized to take into account changes during adolescence.

The relation between age, height, and arterial properties could be distorted if the growth of the child is impaired, as seen in children with CKD. In such specific cases, the effect of the disease on the arteries and the influence of the smaller arterial dimensions should be assessed in parallel. The use of controls matched by height is well established in pediatrics. BP, cIMT, and PWV are examples where reference to height is necessary rather than the automated use of age-matched controls (20–24).

Vascular calcification

VC is one of the major causes of arterial stiffening in CKD. London et al. (25) demonstrated that the degree and type of calcification are predictors of subsequent vascular mortality. VC may be classified by its location and association with atherosclerotic plaque formation. Atherosclerotic calcification located in the intimal layer is associated with atherosclerosis, and it involves cellular necrosis, inflammation, and lipid deposition. The other form is the Monckeberg type media sclerosis, in which amorphous mineral is deposited circumferentially along ~~or~~ the elastic lamellae of the medial layer. This apparition is more prevalent in children with CKD (26).

The contribution of traditional risk factors (as hypertension, aging, smoking, diabetes, and dyslipidemia) does not fully explain the high frequency of VC in CKD; thus, some other distinct pathogenesis may be involved.

VC in CKD was thought for many years to be a passive process resulting from elevated serum phosphate levels and an increase in the calcium phosphate product, resulting in oversaturated plasma. In dialysis patients, VC is associated with hypercalcemia, hyperphosphatemia, an elevated CaxP~~,~~ and ingested oral calcium. In addition, CAC occurs much earlier in pediatric patients undergoing dialysis than in the general population, and its progression positively correlates with serum phosphate levels, the CaxP~~,~~ and daily calcium intake (27–30).

Clinical studies also demonstrated a decreased mortality in dialysis patients ingesting the non-calcium-containing phosphate binder sevelamer; other studies showed that dialysis patients treated with sevelamer had little or no progression of VC when compared with those treated with calcium-containing phosphate binders (31).

However, recent studies revealed that many key regulators of bone formation and bone structural proteins are expressed in both calcified medial arterial layers and atherosclerotic plaques, suggesting that VC is rather an active process triggered by the disturbed calcium and phosphate metabolism, inducing osteoblastic transdifferentiation of vascular smooth muscle cells, the osteogenic lineage allocation and differentiation of multipotent vascular progenitors such as pericytes and calcifying vascular cells, and the loss of inhibitors of VC [recently reviewed by (32) and (33)].

Active vitamin D compounds are commonly used for the treatment of secondary hyperparathyroidism. Both low and increased levels of serum calcitriol are related to increased risk of VC, suggesting a bimodal association (34). As vitamin D receptor activation is beneficial in patients with CKD not only for the suppression of serum parathyroid hormone levels but also for improved survival, optimal vascular protective strategies in dialysis patients may require careful monitoring of both the vitamin D dose, as well as serum calcitriol levels. Continuous efforts have been made to develop new vitamin D analogs with lower calcemic and phosphatemic activities (30).

VC in children differs substantially from adults where it presents dominantly as atherosclerosis and affects medium and large vessels~~,~~ and intimal lesions are the most prominent. In contrast in pediatric ESRD, the occurrence of calcifications is very frequent, and the lesions are found mainly in the media (35–38). Hyperparathyroidism, an

increased serum calcium phosphate product, microinflammation, and hyperhomocystinemia are the main factors predisposing to vascular damage at young age. The effects of the pre-transplant uremic burden and the influence of these risk factors persist in part even after successful renal grafting (22, 29, 39). Consequently, clinical presentation in adult patients is manifested by symptoms of ischemic heart disease (e.g., angina, myocardial infarction, stroke), whereas subjects with childhood onset ESRD who later develop CVD usually are asymptomatic until death because of arrhythmia or other CV events (15).

Calcification scores

Calcium deposition is frequently seen on plain X-rays in the large arteries of dialysis patients. It is recognized as a direct sign of ectopic calcification and arteriosclerosis. Several non-invasive imaging technologies are suitable to detect VC and identify atherosclerosis before symptoms appear or major vascular events occur. These include X-ray and ultrasound techniques and computed tomography.

Few studies have used plain X-rays to assess VC. Aortic arch calcification detected by chest radiography is frequent in hemodialysis patients and is associated with CV disease, increasing age, and duration of hemodialysis (40, 41).

Arterial calcifications may be evaluated ultrasonographically in extra coronary arteries as described by Guérin et al. (28). Blacher et al. (27) defined a calcification score, which is a strong predictor of CV and all-cause mortality in patients with ESRD.

Calcium scores detected by electron beam tomography and helical or spiral CT imaging may be used to determine and quantify CAC. The method was introduced by Agatston et al. (42).

CAC has high prevalence in the aging general population and it is more often seen in males than in females. A significant relationship was found between CAC and traditional CV risk factors, such as hypertension, hypercholesterolemia, diabetes, smoking, sedentary lifestyle, and obesity. Increased CAC has prognostic value for future coronary events and shows relationship with increased mortality (43, 44).

Low GFR is related to increased CAC. It is frequent in predialysis patients and correlates with traditional and non-traditional risk factors for CVD. A high prevalence of CAC is present in adult patients with ESRD. In the study of Tomiyama et al. (45), the prevalence of coronary calcification (defined as a CAC score >0 Agatston units) in patients with CKD was 64% before dialysis. In a large study that included 1908 patients with CKD, a strong and graded relationship between CAC and CKD has been described. Lower kidney function was associated with increased severity of Agatston scores. CAC becomes most significant for kidney function with eGFR <30 mL/min/1.73 m^2. Odds ratios increased from 1.68 (95% CI, 1.23–2.31) for eGFR of 50–59 mL/min/1.73 m^2 to 2.82 (95% CI, 2.06–3.85) for eGFR <30 mL/min/1.73 m^2 (46).

Young adults receiving dialysis, especially when dialysis was initiated in childhood, are in particular susceptible to CAC (29, 47). In the study of Oh et al. (29), median calcium scores exceeded the age-specific 95th normal percentiles on average 10-fold in male and 17-fold in female patients.

The progression of coronary calcification is common in adult renal recipients and predicts CV events and mortality. Recipients with CAC score <100 had a better cumulative survival rate compared to the recipients with CAC score >100 (95.1% vs. 82.3%, $p = 0.03$) (48). Similarly, pediatric kidney transplant patients may also be at increased risk for CAC before they reach middle age (29, 35, 47, 49).

In conclusion, early selection of patients at risk for cardiac events is crucial (50), but in contrast to adults, no validated calcification scores exist for use in the pediatric population. Because of the risk of radiation exposure from CAC measurements, this method should be limited to very high-risk children and adolescents.

Pulse wave velocity

Functional and morphological arterial alterations are reflected by changes of the propagation of the pulse wave along the arterial tree. According to the Moens–Koerteweg formula ($PWV = Eh/2r\rho$, where E is the elastic modulus, h is the thickness of the arterial wall, 2r is the internal diameter of the arterial wall, ρ is the blood density), the higher the internal diameter of an elastic artery, the lower the PWV value, which means a higher elasticity of the arterial wall (51).

Central (aortic) PWV is a convenient integrated index of vascular pathology over a person's life course. It indicates the general burden inflicted upon the arteries over time by aging, arterio-atherosclerosis, hypertension, lipids, glycaemia, kidney function, disturbed calcium and phosphate metabolism, vitamin D, smoking, etc. EHS guidelines recommend its use

as an optional measure to characterize arterial function in adults (52, 53). In contrast, PWV measured in the peripheral, muscular arteries (brachial, femoral) had no prognostic value in ESRD. The morbidity associated with peripheral arteries is more influenced by caliber reduction and the presence of stenotic lesions. In the presence of hemodynamically relevant lower limb artery stenosis, the measure of PWV loses its significance as a measure of stiffness (53).

Aortic PWV may be measured by different methods, detecting the pulse wave at well-defined sampling sites (carotid artery and femoral artery). Devices based on applanation tonometry, oscillometry, ultrasound, and even MRI have been developed. The most popular devices used in children are based on applanation tonometry or oscillometry. Aortic PWV is measured by simultaneous or sequential ECG-gated recordings of the arterial pressure wave at the carotid and femoral arteries and defined as the distance of the sampling sites divided by the time difference between the rise delay of the distal and proximal pulse wave (54).

PWV is a sensitive marker of arterial stiffness and consequently a surrogate marker for CV events in adult hypertension and ESRD (55, 56). After RTX increased, PWV is attributable to incomplete restoration of GFR, the presence of subclinical inflammation (57), new-onset diabetes (58). One year after successful RTX, improvement in CV risk factors was associated with improvement in indices of arterial stiffness (59).

Data on arterial stiffness (PWV) in uremic and RTX children are sparse (14, 22, 60–62).

The difficulty of using PWV in children is its dependence on age and body dimensions (22).

To evaluate the data obtained in different pediatric patient groups, the practice was even recently to use controls matched for age. The problem of growth deficit is not solved by this approach (37, 60). Age, height, and MAP were found to be the major determinants of PWV, emphasizing the need to consider height in patients with growth deficit (62).

The reference data of PWV provided in a study involving more than 1000 healthy children and teenagers enable the calculation of appropriate age- and height-specific SDS values of PWV in the pediatric population (23; Fig. 2).

aPWV was increased both in children on dialysis (62, 63), and following RTX (22), compared to age- and height-matched healthy controls. The major determinants of aPWV in children with ESRD are the elevated phosphate level and CaxP product, the decreased fetuin-A level, the elevated CaxP/fetuin-A ratio, and the cumulative dose of calcitriol administered (61–63). After transplantation, the uremic burden including the calcitriol dose administered prior to transplantation and the graft function after

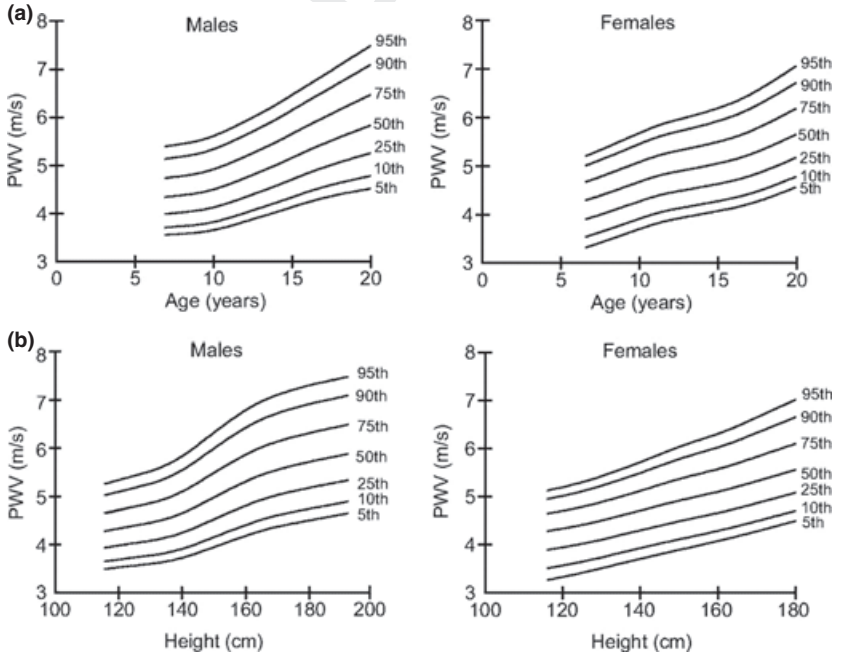

Fig. 2. Pulse wave velocity (PWV) percentile curves according to age (a) and height (b). PWV of the 1008 subjects showing a non-normal distribution. Percentile boundaries were calculated and plotted according to sex, age, and height. Adapted from (23).

transplantation are the major factors influencing PWV (22).

Reference values of PWV in children present a suitable tool for use in longitudinal interventional studies, as suggested by the statement of the American Heart Association (14). However, as there are different devices in use based on different techniques of pulse wave detection and distance measurement, it is of crucial importance to compare and validate the devices according to a standardized protocol, to ensure the interchangeability of the data. Recent studies have demonstrated a good concordance between the most frequently used instruments in pediatrics (64, 65). Assessing the comparability of PWV measurement by oscillometry (Vicorder, Skidmore Medical, UK) with applanation tonometry (PulsePen, DiaTecne, Italy; and Sphygmocor, AtCor, Australia), we found that following path length correction of the Vicorder, all three devices provided comparable results (Fig. 3).

This should allow extrapolating data between previously established normal PWV values for children and forthcoming studies using these instruments to assess children at long-term risk of CVD (66).

Intima–media thickness

The cIMT is a commonly used, validated parameter of structural vessel change. It is measured by high-resolution ultrasound at the common carotid artery (20, 67).

Increased common cIMT is considered an early phase of atherosclerosis and has been associated with CV risk and risk of coronary events. In addition to atherosclerosis, intima–media thickening also occurs in hypertension, aging, diabetes, and hyperlipidemia. Damage of the large arteries, characterized by increased IMT and arteriosclerosis, is a contributing factor of mortality in patients with ESRD (68, 69).

Influence of body dimensions on the morphologic and elastic properties of arteries has been shown by Jourdan et al. (20). Both IMT and arterial stiffness were found to change with age and body size. The authors concluded that morphological and functional measurements of large arteries should be normalized to take into account changes during adolescence, and tables of pediatric reference values have been provided (20; Fig. 4).

A relative increase in cIMT could be shown already in the first decade of life (63) as well as in predialysis CKD stages 2–4 (70, 71), compared to the healthy control values. However, although structural vascular changes are found in predialysis patients, the vessel may retain its normal compliance and distensibility (70). This could be explained by the fact that an increase in the vessel wall thickness or cIMT is coupled with remodeling of the vessel, so that an increase in the carotid artery lumen occurs, possibly to counter the stiffness or loss of compliance of the vessel (71). This compensatory remodeling in the early stages of CKD and the more plastic vessels of

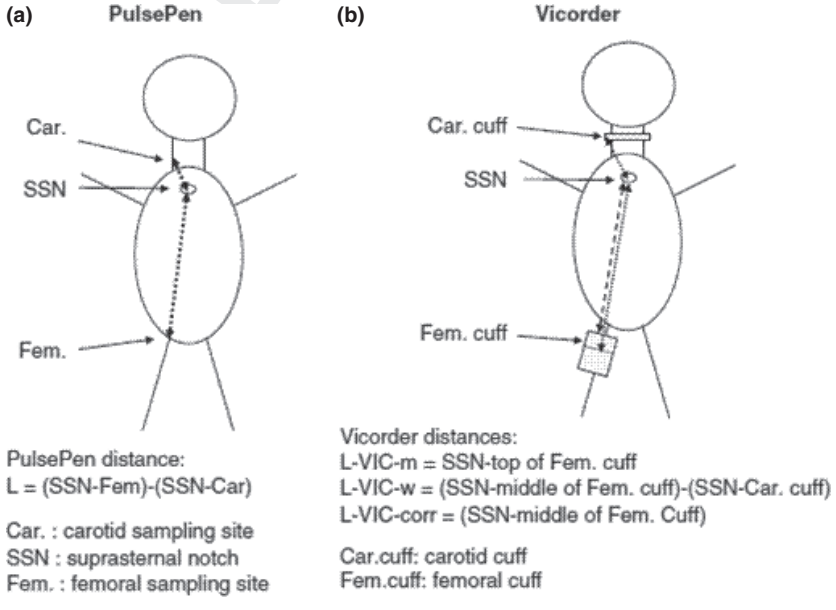

Fig. 3. Distance measurement (L) for the determination of pulse wave velocity by applanation tonometry (PulsePen device) (a) and oscillometry (Vicorder device) (b). Adapted from (64).

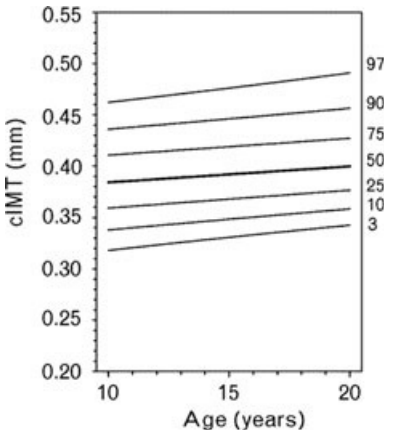

Fig. 4. Percentiles of carotid intima–media thickness (cIMT) distribution for age in healthy adolescents. Adapted from (20).

children may protect them against the deleterious consequences of vascular damage. However, with ongoing CKD and progressive severity of the uremic damage as found in dialysis patients, a further deterioration in cIMT coupled with increased vascular stiffness occurs. In addition, an increased cIMT is associated with an increased LVMI and higher systolic and diastolic BPs (70, 71).

In cross-sectional studies, there are correlations between cIMT- and uremia-associated risk factors. There is linear relationship between cIMT and the time spent on dialysis, Ca, P, and parathyroid hormone levels, as well as medication dosages of calcium-based phosphate binders and vitamin D compounds, suggesting that dysregulated calcium phosphate metabolism is crucial to the uremic vasculopathy (63, 70).

Pediatric studies have shown that cIMT correlated with a higher calcitriol dosage (34, 63, 70), and there is a bimodal association of vitamin D levels with cIMT (34). These effects may be determined by the influence of vitamin D on Ca-P homeostasis, as well as its pro-inflammatory action (34).

Post-transplantation vascular disease may be driven by hypertension, obesity, and related risk factors and possibly by immunosuppressive agents as suggested by studies in adults (72). Data in children are variable. In one study, there was no increase in cIMT following RTX with strict BP control (73), as cIMT progressively increases with age, a non-increase in cIMT in the course of a longitudinal study may be interpreted as a regression (74). This again points to the need to use the age- and height-specific normative values in pediatric follow-up studies (20). A prospective observational study among 56 children and adolescents with CKD provided evidence that successful RTX may induce partial regression of the large-vessel arteriopathy associated with uremia within one yr of follow-up. In contrast, continued renal failure was associated with measurable progression of vascular lesions. Within a period of on average 12 months, IMT increased significantly, by about 0.7 SD, in patients who remained in CKD or on dialysis. In turn, in dialysis patients with elevated IMT who received a renal allograft, IMT regressed by an average of 0.6 SD within one yr of grafting. IMT decreased significantly mainly in those subjects who had abnormally elevated IMT at baseline, indicating a trend toward improvement in a pathological condition and arguing against non-specific effects of factors related to transplantation, such as changes in intravascular hydration or a general anti-inflammatory effect of the immunosuppressive medication (75).

24-hour ABPM and AASI

ABPM

Hypertension is a risk factor of CV injury in the general population as well as in ESRD. Twenty-four-hour ABPM is performed to provide data on the normal daytime and nighttime BP. It is used to diagnose and optimize treatment of HT and to identify WCH and MH. ABPM predicts CV morbidity, mortality, and end organ damage in a hypertensive population (76, 77). Blunted diurnal BP variation is a strong predictor of CV events and death (78). High BP variability contributes to the increased CV risk related to MH and WCH (79). Ambulatory pulse pressure estimated with ABPM is a good predictor for long-term outcomes in hypertensive patients (80). The rate of rise of systolic BP is associated with increased cIMT (81).

The prevalence of HT increased dramatically in the past decade because of the epidemic of obesity in the general population. Children with ESRD are especially prone to HT. Although the efficacy of ABPM is established in children, and normative values according to age and height are available and used in the everyday practice (24, 82), we should consider that the definition of the daytime and nighttime periods influences the results. Thus, significant errors may occur in the evaluation (83, 84). HT leads to end organ damage (85), which increases the risk of CV morbidity and mortality. HT and pre-HT in children and adolescents were associated with elevated LVMI values. Patients with any stage of CKD and with even mildly elevated BP are at risk of developing renal damage (86, 87).

HT is an important risk factor in patients with CKD. Ambulatory systolic BP predicts all-cause mortality in this population. MH and WCH have prognostic significance (88). Flynn et al. (89) found a high prevalence of HT in pediatric patients with CKD: 54% from 432 subjects had BP levels ≥95th percentile. CKD children have increased cardiac output and LVMI and increased mortality. The prevalence of LVH in children on peritoneal dialysis was 70%. A systolic BP load ≥15% had a high sensitivity, specificity, and predictive value for LVH in this population (90, 91).

In the study of Mistnefes et al. (92), the prevalence of LVH was 75% in children with ESRD and 37.1% after kidney transplantation. RTX children have increased cIMT and decreased distensibility in association with HT (93). RTX children with HT have an increased risk for graft loss. One year after RTX, lower GFR is related to abnormal circadian BP pattern (94). BP may also be influenced by the choice of immunosuppressive therapy. Lower 24-hour systolic BP was found in a sirolimus-treated group in contrast to patients treated with calcineurin inhibitors (95). Assessing BP values in children is of primary importance to reduce the CV risk. ABPM is helpful to optimize treatment strategies and reduce CV events and chronic graft dysfunction among patients with CKD or post-RTX. Regular use of ABPM in transplant patients enables detection of masked and hidden HT (96).

AASI

Among the easy to generate measures of arterial wall properties, AASI has been proposed as a surrogate measure of arterial stiffness (97, 98) and as a marker of CV morbidity and mortality. The main limitation of the method is the lack of standardization in adults as well as in children.

The AASI is defined as one minus the regression slope of diastolic BP over systolic BP obtained by ABPM (97; Fig. 5). The mean AASI was found between 0.31 and 0.56 (97–100), and normal values of AASI are estimated as <0.50 and 0.70 in young and older subjects, respectively (98).

In primary HT patients, increased AASI implies a higher probability of target organ damage (101). AASI showed a positive correlation with triglycerides and urinary albumin excretion and was negatively related to estimated creatinine clearance, GFR, and renal volume-to-the resistive index ratio (102, 103). Patients with microalbuminuria, carotid abnormalities, or left ventricular hypertension showed increased AASI as compared to those without it (102).

AASI showed a positive correlation with IMT in hypertensive patients (104).

Only limited evidence on the value of AASI in children is available. Simonetti et al. studied 114 hypertensive and 71 normotensive, healthy children aged 5–16 yr. They showed that AASI is elevated in hypertensive children and correlates with the duration and the origin of HT in childhood. The cutoff of AASI distinguishing HT from normotensive children was set at 0.301 with an odds ratio of 8.2, a sensitivity of 81%, and a specificity of 65% (105). In the study of Stergiou et al. (106), among 82 children and adolescents (mean age 13.1 ± 2.9 yr), AASI correlated with weight, height, systolic BP, and LVMI; however, they could not confirm the

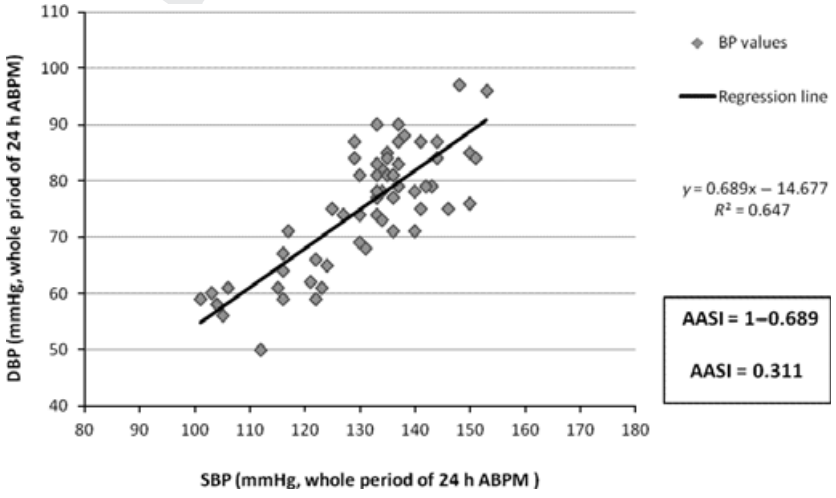

Fig. 5. The definition of ambulatory arterial stiffness index (AASI) from 24-h ABPM. DBP, diastolic blood pressure; SBP, systolic blood pressure.

difference between the AASI values of normotensives and patients with HT.

In conclusion, AASI is a predictor of target organ damage in adults. It is easy to measure non-invasively, quick, and simple. However, standardization of the method and establishment of a large pediatric database of normal values is needed to evaluate the real applicability of the method in children with CV risk.

HRV and autonomic dysfunction

CV mortality of patients with ESRD has also been shown to be related to the uremic autonomic dysfunction (107). Autonomic dysfunction, assessed by HRV, was found to be an independent risk factor of CV mortality in ESRD (108). HRV refers to the beat-to-beat variation of heart rate. As such, it represents the physiological sinus arrhythmia that depends on the vagal and the sympathetic innervation of the sinus node (109). Roughly, higher the parasympathetic activity, higher the HRV, whereas higher the sympathetic activity, lower the HRV (110).

The reduction in HRV is a well-known consequence of ESRD, both in adults and childhood. Similarly to adults, HRV reduction is reversible after transplantation (111). Previously, reduction of HRV in ESRD was suggested to be the consequence of autonomic neuropathy (112). However, as HRV was similarly reduced in children with HT but normal GFR to that of children with ESRD (111), the role of sympathetic overactivity was suggested. In a double-blind, placebo-controlled, randomized, crossover study, improvement in HRV with propranolol proved that sympathetic overactivity is at least partly responsible for HRV reduction in uremia (113).

Similar results have been found in adults with chronic renal failure. The effect of enalapril on the baroreflex response to changes in arterial pressure expressed either by muscle sympathetic nerve activity or heart rate was investigated. Enalapril normalized the sympathetic nerve activity and the heart rate by reducing sympathetic activity (114). Taken together, the relationship between autonomic dysfunction and high CV mortality in ESRD probably reflects the deleterious effect of sympathetic overactivity on CV mortality that is well known in patients with congestive heart failure or post-myocardial infarction. Sympathetic activation plays an important role in HT and target organ damage associated with chronic renal failure (115).

HRV is a bedside, non-invasive, low-cost, and simple to perform method, requiring standard hospital equipment and a dedicated software. Normal ranges for pediatric patients are available (116, 117). As it primarily reflects the autonomic innervation of the sinus node, whose assessment is easier by the simple measurement of the heart rate, its role in the daily clinical practice is still to be established.

Conclusion

Functional and structural arterial damage are already present in children with ESRD, but it may be improved by kidney transplantation. A number of validated non-invasive methods are available to assess the extent of CV damage in adults; however, they need adaptation, standardization, and validation in pediatric studies (Table 1). In contrast to adults, hard end-points of CV events are sparse in the pediatric population; thus, we urgently need data to establish the thresholds for the different non-invasive measures. A reasonable approach is to use the 95 percentile values as the upper limits for normal and to use these specific percentiles in the follow-up studies, to check the tracking of the parameter studied. The picture is complicated by the growth deficit of children with ESRD, where height rather than age-specific percentiles should be used.

cIMT and PWV are the most promising methods, as pediatric normative values are already present. However, it is to note that although these markers have been extensively

Table 1. Methods of non-invasive assessment of early vascular changes in pediatric patients

Parameter	Methodology	Use in pediatrics
cIMT	B-mode ultrasound, operator dependent	Established normal values in children (20)
Coronary calcification	Electron beam computed tomography, high radiation exposure	Studies available, but no established normal values in children (29, 35, 47, 49)
aPWV and pulse waveform analysis	Various techniques (applanation tonometry and oscillometry), in part operator dependent	Established normal values in children for aPWV (23, 64, 65)
AASI	24-h ABPM, operator indepedent, easy to measure, quick, and simple	No established normal values in children
HRV	Operator independent, easy to perform, needs specific equipment	Studies available but no established normal values in children (111, 113, 116, 117)

used in many studies of vascular outcome, there is recent evidence to show that very early vascular damage may be missed even by these methods. It must be remembered that despite significant vascular damage and Ca accumulation on vessel biopsies, the corresponding vascular scans have remained normal (118). Thus, even a normal or negative result must be interpreted with caution, until the results of longitudinal follow-up and interventional studies are available.

Acknowledgements

The work was supported by the following Hungarian National Grants: OTKA 100909, PD83431; TAMOP 4.2.3/08/1/KMR-2008-0003, 4.2.2. B-10/1.2010-2013; LP 2001-008/2011 and by the Hungarian Society of Nephrology.

References

1. Liefeldt L, Budde K. Risk factors for cardiovascular disease in renal transplant recipients and strategies to minimize risk. Transpl Int 2010: 23: 1191–1204.
2. Stenvinkel P. Chronic kidney disease: A public health priority and harbinger of premature cardiovascular disease. J Intern Med 2010: 268: 456–467.
3. Di Angelantonio E, Chowdhury R, Sarwar N, Aspelund T, Danesh J, Gudnason V. Chronic kidney disease and risk of major cardiovascular disease and non-vascular mortality: Prospective population based cohort study. BMJ 2010: 341: c4986.
4. Lentine KL, Hurst FP, Jindal RM, et al. Cardiovascular risk assessment among potential kidney transplant candidates: Approaches and controversies. Am J Kidney Dis 2010: 55: 152–167.
5. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol 1999: 10: 1606–1615.
6. Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A, Kaplan B. Kidney transplantation halts cardiovascular disease progression in patients with end-stage renal disease. Am J Transplant 2004: 4: 1662–1668.
7. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998: 32: S112–S119.
8. Parekh RS, Carroll CE, Wolfe RA, Port FK. Cardiovascular mortality in children and young adults with end-stage kidney disease. J Pediatr 2002: 141: 191–197.
9. Chavers BM, Li S, Collins AJ, Herzog CA. Cardiovascular disease in pediatric chronic dialysis patients. Kidney Int 2002: 62: 648–653.
10. Feber J, Wong H, Geier P, Chaudry B, Filler G. Complications of chronic kidney disease in children post-renal transplantation - a single center experience. Pediatr Transplant 2008: 12: 80–84.
11. Hippisley-Cox J, Coupland C, Vinogradova Y, et al. Predicting cardiovascular risk in England and Wales: Prospective derivation and validation of QRISK2. BMJ 2008: 336: 1475–1482.
12. Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. Lancet 1999: 353: 89–92.
13. Silver SA, Huang M, Nash MM, Prasad GV. Framingham risk score and novel cardiovascular risk factors underpredict major adverse cardiac events in kidney transplant recipients. Transplantation 2011: 92: 183–189.
14. Urbina EM, Williams RV, Alpert BS, et al. Noninvasive assessment of subclinical atherosclerosis in children and adolescents: Recommendations for standard assessment for clinical research: A scientific statement from the American Heart Association. Hypertension 2009: 54: 919–950.
15. Filler G. Challenges in pediatric transplantation: The impact of chronic kidney disease and cardiovascular risk factors on long-term outcomes and recommended management strategies. Pediatr Transplant 2011: 15: 25–31.
16. Van Meurs-Van Woezik H, Krediet P. Measurements of the descending aorta in infants and children: Comparison with other aortic dimensions. J Anat 1982: 135: 273–279.
17. Van Meurs-Van Woezik H, Klein HW, Markus-Silvis L, Krediet P. Comparison of the growth of the tunica media of the ascending aorta, aortic isthmus and descending aorta in infants and children. J Anat 1983: 136: 273–281.
18. Ichida F, Aubert A, Denef B, Dumoulin M, Van Der Hauwaert LG. Cross sectional echocardiographic assessment of great artery diameters in infants and children. Br Heart J 1987: 58: 627–634.
19. Senzaki H, Akagi M, Hishi T, et al. Age-associated changes in arterial elastic properties in children. Eur J Pediatr 2002: 161: 547–551.
20. Jourdan C, Wuhl E, Litwin M, et al. Normative values for intima-media thickness and distensibility of large arteries in healthy adolescents. J Hypertens 2005: 23: 1707–1715.
21. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004: 114: 555–576.
22. Csepreka l O, Kis E, Schaffer P, et al. Pulse wave velocity in children following renal transplantation. Nephrol Dial Transplant 2009: 24: 309–315.
23. Reusz GS, Csepreka l O, Temmar M, et al. Reference values of pulse wave velocity in healthy children and teenagers. Hypertension 2010: 56: 217–224.
24. Wuhl E, Witte K, Soergel M, Mehls O, Schaefer F. Distribution of 24-h ambulatory blood pressure in children: Normalized reference values and role of body dimensions. J Hypertens 2002: 20: 1995–2007.
25. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. Nephrol Dial Transplant 2003: 18: 1731–1740.
26. Shroff RC, Shanahan CM. The vascular biology of calcification. Semin Dial 2007: 20: 103–109.
27. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. Hypertension 2001: 38: 938–942.
28. Guerin AP, London GM, Marchais SJ, Metivier F. Arterial stiffening and vascular calcifications in end-stage renal disease. Nephrol Dial Transplant 2000: 15: 1014–1021.
29. Oh J, Wunsch R, Turzer M, et al. Advanced coronary and carotid arteriopathy in young adults with childhood-onset chronic renal failure. Circulation 2002: 106: 100–105.
30. Shroff R. Dysregulated mineral metabolism in children with chronic kidney disease. Curr Opin Nephrol Hypertens 2011: 20: 233–240.
31. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. Kidney Int 2007: 71: 438–441.

32. Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: The killer of patients with chronic kidney disease. J Am Soc Nephrol 2009: 20: 1453–1464.
33. Shroff R, Knott C, Rees L. The virtues of vitamin D – but how much is too much? Pediatri Nephrol 2010: 25: 1607–1620.
34. Shroff R, Egerton M, Bridel M, et al. A bimodal association of vitamin D levels and vascular disease in children on dialysis. J Am Soc Nephrol 2008: 19: 1239–1246.
35. Sheth RD, Perez MD, Goldstein SL. Cardiovascular calcifications in pediatric patients receiving maintenance dialysis. Pediatri Nephrol 2003: 18: 810–813.
36. Parekh RS, Gidding SS. Cardiovascular complications in pediatric end-stage renal disease. Pediatri Nephrol 2005: 20: 125–131.
37. Querfeld U. The clinical significance of vascular calcification in young patients with end-stage renal disease. Pediatri Nephrol 2004: 19: 478–484.
38. Shroff R, Weaver DJ Jr, Mitsnefes MM. Cardiovascular complications in children with chronic kidney disease. Nat Rev Nephrol 2011: 7: 642–649.
39. Briese S, Claus M, Querfeld U. Arterial stiffness in children after renal transplantation. Pediatri Nephrol 2008: 23: 2241–2245.
40. Coll B, Betriu A, Martinez-Alonso M, et al. Large artery calcification on dialysis patients is located in the intima and related to atherosclerosis. Clin J Am Soc Nephrol 2011: 6: 303–310.
41. Goldsmith DJ, Covic A, Sambrook PA, Ackrill P. Vascular calcification in long-term haemodialysis patients in a single unit: A retrospective analysis. Nephron 1997: 77: 37–43.
42. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990: 15: 827–832.
43. Wong ND, Kouwabunpat D, Vo AN, et al. Coronary calcium and atherosclerosis by ultrafast computed tomography in asymptomatic men and women: Relation to age and risk factors. Am Heart J 1994: 127: 422–430.
44. Wang L, Jerosch-Herold M, Jacobs DR Jr, Shahar E, Detrano R, Folsom AR. Coronary artery calcification and myocardial perfusion in asymptomatic adults: The MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol 2006: 48: 1018–1026.
45. Tomiyama C, Higa A, Dalboni MA, et al. The impact of traditional and non-traditional risk factors on coronary calcification in pre-dialysis patients. Nephrol Dial Transplant 2006: 21: 2464–2471.
46. Budoff MJ, Rader DJ, Reilly MP, et al. Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) Study. Am J Kidney Dis 2011: 58: 519–526.
47. Goodman WG, Goldin J, Kuizon BD, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 2000: 342: 1478–1483.
48. Roe P, Wolfe M, Joffe M, Rosas SE. Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. Atherosclerosis 2010: 212: 589–594.
49. Ishitani MB, Milliner DS, Kim DY, et al. Early subclinical coronary artery calcification in young adults who were pediatric kidney transplant recipients. Am J Transplant 2005: 5: 1689–1693.
50. Weiner DE, Tighiouart H, Amin MG, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: A pooled analysis of community-based studies. J Am Soc Nephrol 2004: 15: 1307–1315.
51. Mackenzie IS, Wilkinson IB, Cockcroft JR. Assessment of arterial stiffness in clinical practice. QJM 2002: 95: 67–74.
52. Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: Methodological issues and clinical applications. Eur Heart J 2006: 27: 2588–2605.
53. Pannier B, Guerin AP, Marchais SJ, Safar ME, London GM. Stiffness of capacitive and conduit arteries: Prognostic significance for end-stage renal disease patients. Hypertension 2005: 45: 592–596.
54. Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new non-invasive portable tonometer for determining arterial pressure wave and pulse wave velocity: The PulsePen device. J Hypertens 2004: 22: 2285–2293.
55. Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001: 37: 1236–1241.
56. London GM, Marchais SJ, Guerin AP. Arterial stiffness and function in end-stage renal disease. Adv Chronic Kidney Dis 2004: 11: 202–209.
57. Verbeke F, Van Biesen W, Peeters P, Van Bortel LM, Vanholder RC. Arterial stiffness and wave reflections in renal transplant recipients. Nephrol Dial Transplant 2007: 22: 3021–3027.
58. Opazo Saez A, Kos M, Witzke O, Kribben A, Nurnberger J. Effect of new-onset diabetes mellitus on arterial stiffness in renal transplantation. Transpl Int 2008: 21: 930–935.
59. Zoungas S, Kerr PG, Chadban S, et al. Arterial function after successful renal transplantation. Kidney Int 2004: 65: 1882–1889.
60. Covic A, Mardare N, Gusbeth-Tatomir P, et al. Increased arterial stiffness in children on haemodialysis. Nephrol Dial Transplant 2006: 21: 729–735.
61. Kis E, Cseprekal O, Biro E, et al. Effects of bone and mineral metabolism on arterial elasticity in chronic renal failure. Pediatr Nephrol 2009: 24: 2413–2420.
62. Kis E, Cseprekal O, Horvath Z, et al. Pulse wave velocity in end-stage renal disease: Influence of age and body dimensions. Pediatr Res 2008: 63: 95–98.
63. Shroff RC, Donald AE, Hiorns MP, et al. Mineral metabolism and vascular damage in children on dialysis. J Am Soc Nephrol 2007: 18: 2996–3003.
64. Kis E, Cseprekal O, Kerti A, et al. Measurement of pulse wave velocity in children and young adults: A comparative study using three different devices. Hypertens Res 2011: 34: 1197–1202.
65. Kracht D, Shroff R, Baig S, et al. Validating a new oscillometric device for aortic pulse wave velocity measurements in children and adolescents. Am J Hypertens 2011: 24: 1294–1299.
66. Querfeld U, Anarat A, Bayazit AK, et al. The Cardiovascular Comorbidity in Children with Chronic Kidney Disease (4C) study: Objectives, design, and methodology. Clin J Am Soc Nephrol 2010: 5: 1642–1648.
67. Litwin M, Niemirska A. Intima-media thickness measurements in children with cardiovascular risk factors. Pediatri Nephrol 2009: 24: 707–719.
68. Benedetto FA, Mallamaci F, Tripepi G, Zoccali C. Prognostic value of ultrasonographic measurement of carotid intima media thickness in dialysis patients. J Am Soc Nephrol 2001: 12: 2458–2464.
69. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. Circulation 2007: 115: 459–467.
70. Mitsnefes MM, Kimball TR, Kartal J, et al. Cardiac and vascular adaptation in pediatric patients with chronic kidney disease: Role of calcium-phosphorus metabolism. J Am Soc Nephrol 2005: 16: 2796–2803.

71. Litwin M, Wuhl E, Jourdan C, et al. Altered morphologic properties of large arteries in children with chronic renal failure and after renal transplantation. J Am Soc Nephrol 2005: 16: 1494–1500.
72. De Lima JJ, Vieira ML, Viviani LF, et al. Long-term impact of renal transplantation on carotid artery properties and on ventricular hypertrophy in end-stage renal failure patients. Nephrol Dial Transplant 2002: 17: 645–651.
73. Krmar RT, Balzano R, Jogestrand T, Cedazo-Minguez A, Englund MS, Berg UB. Prospective analysis of carotid arterial wall structure in pediatric renal transplants with ambulatory normotension and in treated hypertensive recipients. Pediatr Transplant 2008: 12: 412–419.
74. Mitsnefes MM. Understanding carotid artery intima-media thickness in childhood: Lessons from studies in children with renal transplants. Pediatr Transplant 2008: 12: 377–380.
75. Litwin M, Wuhl E, Jourdan C, et al. Evolution of large-vessel arteriopathy in paediatric patients with chronic kidney disease. Nephrol Dial Transplant 2008: 23: 2552–2557.
76. De La Sierra A, Segura J, Banegas JR, et al. Clinical features of 8295 patients with resistant hypertension classified on the basis of ambulatory blood pressure monitoring. Hypertension 2011: 57: 898–902.
77. O'brien E, Cox JP, O'malley K. Ambulatory blood pressure measurement in the evaluation of blood pressure lowering drugs. J Hypertens 1989: 7: 243–247.
78. Brotman DJ, Davidson MB, Boumitri M, Vidt DG. Impaired diurnal blood pressure variation and all-cause mortality. Am J Hypertens 2008: 21: 92–97.
79. Ogedegbe G, Agyemang C, Ravenell JE. Masked hypertension: Evidence of the need to treat. Curr Hypertens Rep 2010: 12: 349–355.
80. Kao YT, Huang CC, Leu HB, et al. Ambulatory pulse pressure as a novel predictor for long-term prognosis in essential hypertensive patients. J Hum Hypertens 2011: 25: 444–450.
81. Stamatelopoulos KS, Manios E, Barlas G, et al. Time rate of blood pressure variation is superior to central hemodynamics as an associate of carotid intima-media thickness. J Hypertens 2010: 28: 51–58.
82. Reusz GS, Hobor M, Tulassay T, Sallay P, Miltenyi M. 24 hour blood pressure monitoring in healthy and hypertensive children. Arch Dis Child 1994: 70: 90–94.
83. Jones HE, Sinha MD. The definition of daytime and nighttime influences the interpretation of ABPM in children. Pediatr Nephrol 2011: 26: 775–781.
84. Soergel M, Kirschstein M, Busch C, et al. Oscillometric twenty-four-hour ambulatory blood pressure values in healthy children and adolescents: A multicenter trial including 1141 subjects. J Pediatr 1997: 130: 178–184.
85. Feber J, Ahmed M. Hypertension in children: New trends and challenges. Clin Sci (Lond) 2010: 119: 151–161.
86. Lubrano R, Travasso E, Raggi C, Guido G, Masciangelo R, Elli M. Blood pressure load, proteinuria and renal function in pre-hypertensive children. Pediatr Nephrol 2009: 24: 823–831.
87. Stabouli S, Kotsis V, Rizos Z, et al. Left ventricular mass in normotensive, prehypertensive and hypertensive children and adolescents. Pediatr Nephrol 2009: 24: 1545–1551.
88. Agarwal R, Sinha AD, Light RP. Toward a definition of masked hypertension and white-coat hypertension among hemodialysis patients. Clin J Am Soc Nephrol 2011: 6: 2003–2008.
89. Flynn JT, Mitsnefes M, Pierce C, et al. Blood pressure in children with chronic kidney disease: A report from the Chronic Kidney Disease in Children study. Hypertension 2008: 52: 631–637.
90. Bircan Z, Duzova A, Cakar N, et al. Predictors of left ventricular hypertrophy in children on chronic peritoneal dialysis. Pediatr Nephrol 2010: 25: 1311–1318.
91. Weaver DJ Jr, Kimball TR, Koury PR, Mitsnefes MM. Cardiac output and associated left ventricular hypertrophy in pediatric chronic kidney disease. Pediatr Nephrol 2009: 24: 565–570.
92. Mitsnefes MM, Daniels SR, Schwartz SM, Meyer RA, Khoury P, Strife CF. Severe left ventricular hypertrophy in pediatric dialysis: Prevalence and predictors. Pediatri Nephrol 2000: 14: 898–902.
93. Mitsnefes MM, Kimball TR, Witt SA, Glascock BJ, Khoury PR, Daniels SR. Abnormal carotid artery structure and function in children and adolescents with successful renal transplantation. Circulation 2004: 110: 97–101.
94. Wadei HM, Amer H, Griffin MD, Taler SJ, Stegall MD, Textor SC. Abnormal circadian blood pressure pattern 1-year after kidney transplantation is associated with subsequent lower glomerular filtration rate in recipients without rejection. J Am Soc Hypertens 2011: 5: 39–47.
95. Steigerwalt SP, Brar N, Dhungel A, et al. Improved 24-hour blood pressure control with sirolimus versus calcineurin inhibitor based immunosuppression in renal transplant recipients. Transplant Proc 2009: 41: 4184–4187.
96. Paripovic D, Kostic M, Spasojevic B, Kruscic D, Peco-Antic A. Masked hypertension and hidden uncontrolled hypertension after renal transplantation. Pediatr Nephrol 2010: 25: 1719–1724.
97. Dolan E, Thijs L, Li Y, et al. Ambulatory arterial stiffness index as a predictor of cardiovascular mortality in the Dublin Outcome Study. Hypertension 2006: 47: 365–370.
98. Li Y, Dolan E, Wang JG, et al. Ambulatory arterial stiffness index: Determinants and outcome. Blood Press Monit 2006: 11: 107–110.
99. Dechering DG, Adiyaman A, Van Der Steen M, Thien T. Interstudy variability in the ambulatory arterial stiffness index. Hypertension 2007: 50: e65; author reply e66.
100. Hansen TW, Staessen JA, Torp-Pedersen C, et al. Ambulatory arterial stiffness index predicts stroke in a general population. J Hypertens 2006: 24: 2247–2253.
101. Ratto E, Leoncini G, Viazzi F, et al. Ambulatory arterial stiffness index and renal abnormalities in primary hypertension. J Hypertens 2006: 24: 2033–2038.
102. Leoncini G, Ratto E, Viazzi F, et al. Increased ambulatory arterial stiffness index is associated with target organ damage in primary hypertension. Hypertension 2006: 48: 397–403.
103. Mule G, Cottone S, Cusimano P, et al. Inverse relationship between ambulatory arterial stiffness index and glomerular filtration rate in arterial hypertension. Am J Hypertens 2008: 21: 35–40.
104. Garcia-Garcia A, Gomez-Marcos MA, Recio-Rodriguez JI, et al. Relationship between ambulatory arterial stiffness index and subclinical target organ damage in hypertensive patients. Hypertens Res 2011: 34: 180–186.
105. Simonetti GD, Von Vigier RO, Wuhl E, Mohaupt MG. Ambulatory arterial stiffness index is increased in hypertensive childhood disease. Pediatr Res 2008: 64: 303–307.
106. Stergiou GS, Kollias A, Giovas PP, Papagiannis J, Roussias LG. Ambulatory arterial stiffness index, pulse pressure and pulse wave velocity in children and adolescents. Hypertens Res 2010: 33: 1272–1277.
107. Robinson TG, Carr SJ. Cardiovascular autonomic dysfunction in uremia. Kidney Int 2002: 62: 1921–1932.
108. Ranpuria R, Hall M, Chan CT, Unruh M. Heart rate variability (HRV) in kidney failure: Measurement and consequences of reduced HRV. Nephrol Dial Transplant 2008: 23: 444–449.

109. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996: 93: 1043–1065.
110. Fukuta H, Hayano J, Ishihara S, et al. Prognostic value of heart rate variability in patients with end-stage renal disease on chronic haemodialysis. Nephrol Dial Transplant 2003: 18: 318–325.
111. Tory K, Suveges Z, Horvath E, et al. Autonomic dysfunction in uremia assessed by heart rate variability. Pediatr Nephrol 2003: 18: 1167–1171.
112. Kurata C, Uehara A, Sugi T, et al. Cardiac autonomic neuropathy in patients with chronic renal failure on hemodialysis. Nephron 2000: 84: 312–319.
113. Tory K, Horvath E, Suveges Z, et al. Effect of propranolol on heart rate variability in patients with end-stage renal disease: A double-blind, placebo-controlled, randomized crossover pilot trial. Clin Nephrol 2004: 61: 316–323.
114. Ligtenberg G, Blankestijn PJ, Oey PL, et al. Reduction of sympathetic hyperactivity by enalapril in patients with chronic renal failure. N Engl J Med 1999: 340: 1321–1328.
115. Schlaich MP, Socratous F, Hennebry S, et al. Sympathetic activation in chronic renal failure. J Am Soc Nephrol 2009: 20: 933–939.
116. Bar-Haim Y, Marshall PJ, Fox NA. Developmental changes in heart period and high-frequency heart period variability from 4 months to 4 years of age. Dev Psychobiol 2000: 37: 44–56.
117. Massin M, Von Bernuth G. Normal ranges of heart rate variability during infancy and childhood. Pediatr Cardiol 1997: 18: 297–302.
118. Shroff RC, McNair R, Figg N, et al. Dialysis accelerates medial vascular calcification in part by triggering smooth muscle cell apoptosis. Circulation 2008: 118: 1748–1757.

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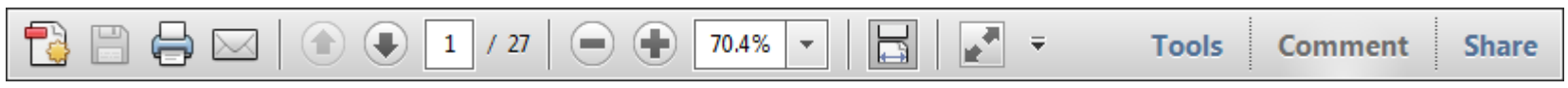

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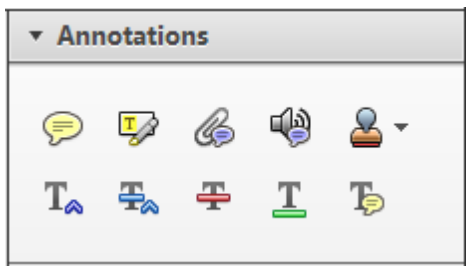

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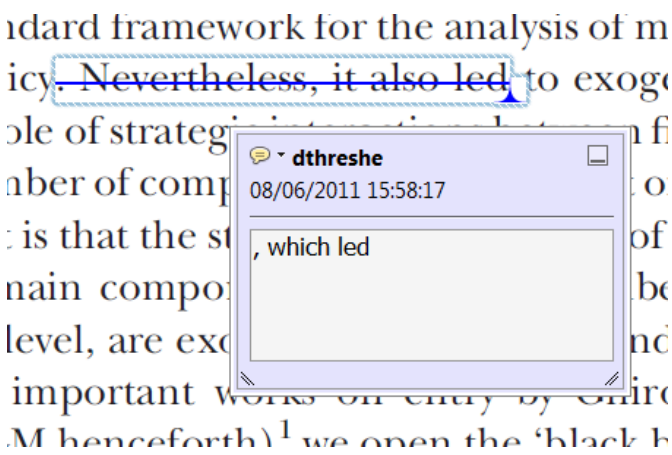

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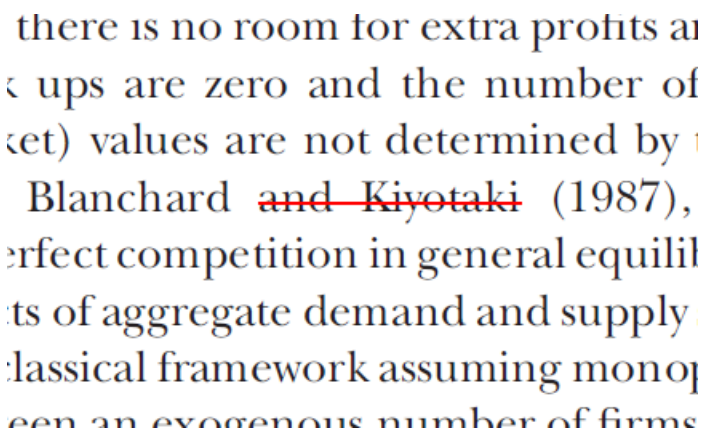

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Highlights text in yellow and opens up a text box where comments can be entered.

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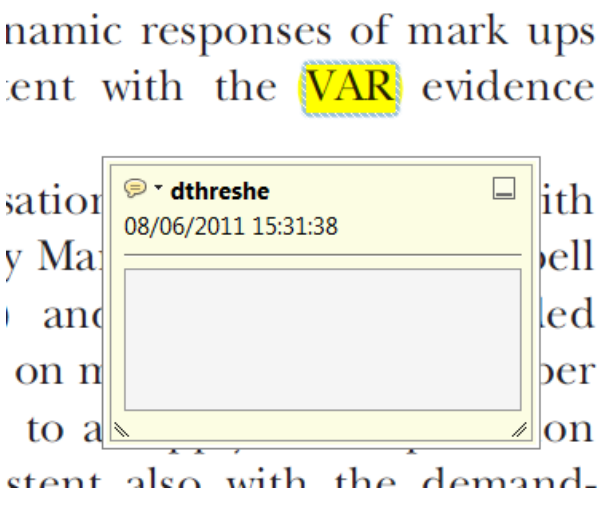
- Highlight the relevant section of text.
- Click on the Add note to text icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

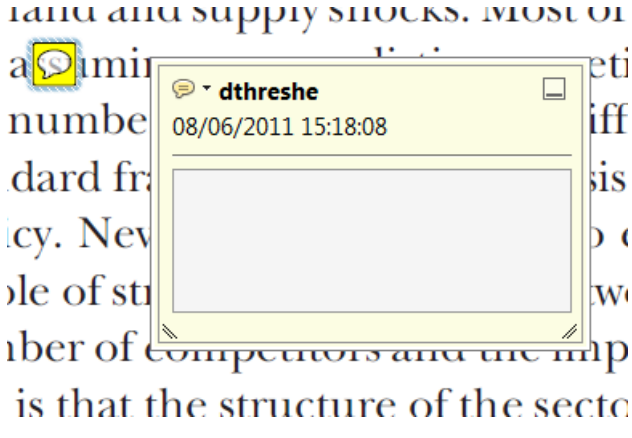


4. Add sticky note Tool – for making notes at specific points in the text.

Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the Add sticky note icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



WILEY-BLACKWELL

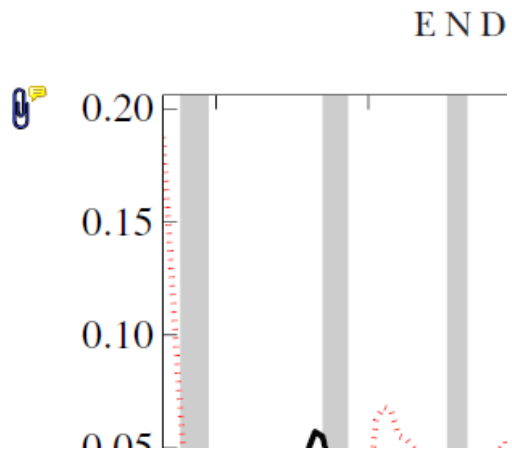
USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

5. Attach File Tool – for inserting large amounts of text or replacement figures.

Inserts an icon linking to the attached file in the appropriate pace in the text.

How to use it

- Click on the Attach File icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

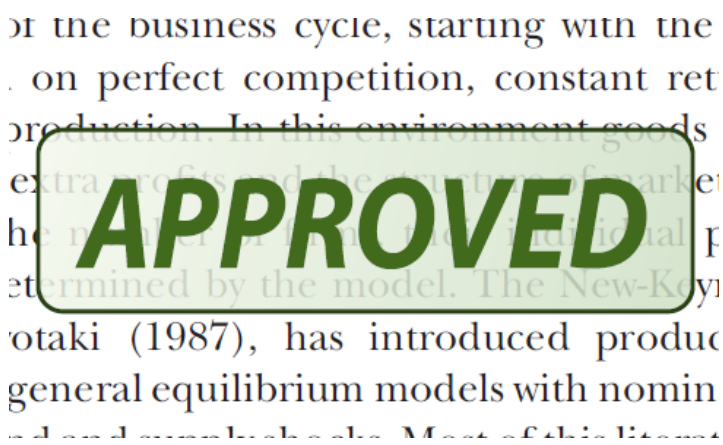


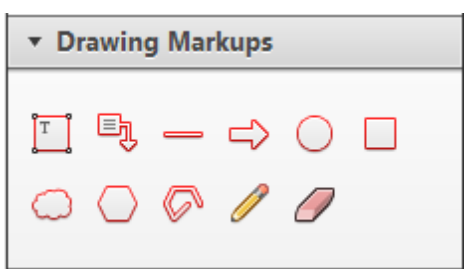
6. Add stamp Tool – for approving a proof if no corrections are required.

Inserts a selected stamp onto an appropriate place in the proof.

How to use it

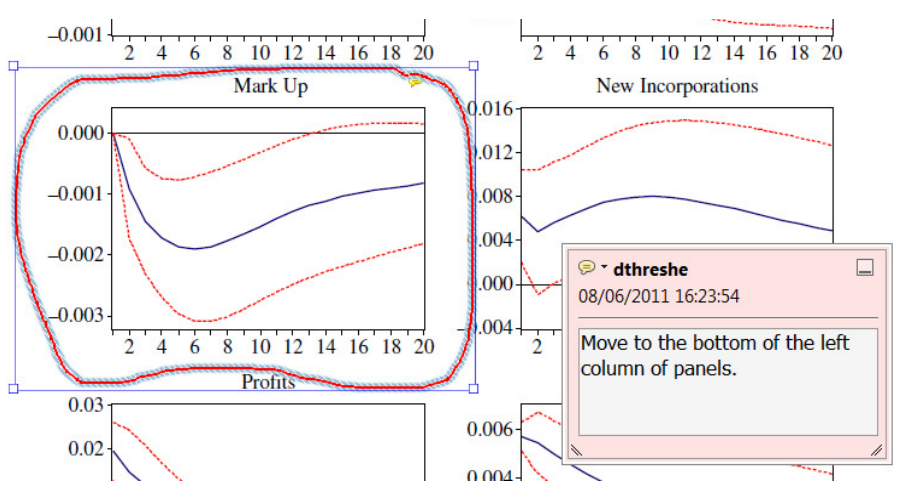
- Click on the Add stamp icon in the Annotations section.
- Select the stamp you want to use. (The Approved stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).





7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.

For further information on how to annotate proofs, click on the Help menu to reveal a list of further options: