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.

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).

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).](image-url)
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 lipid deposition. The other form is the Monckeberg type media sclerosis, in which amorphous lipid deposition.

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 hyperparathyroidism, 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; 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 hyperhomocysteinemia are the main factors predisposing to vascular damage at young age. The effects of the pretransplant 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 m². Odds ratios increased from 1.68 (95% CI, 1.23–2.31) for eGFR of 50–59 mL/min/1.73 m² to 2.82 (95% CI, 2.06–3.85) for eGFR < 30 mL/min/1.73 m² (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 = \( Eh/2r \)), where \( E \) is the elastic modulus, \( h \) is the thickness of the arterial wall, \( 2r \) is the internal diameter of the arterial wall, \( \rho \) 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).

\[ \text{aPWV} \]

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) and oscilometry (Vicorder device). Adapted from (64).](image-url)
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.

**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.](image-url)
difference between the AASI values of normoten- 
sives 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 establish-
ment 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 auto-
nomic dysfunction (107). Autonomic dysfunc-

tion, assessed by HRV, was found to be an 

dependent risk factor of CV mortality in ESRD 

(108). HRV refers to the beat-to-beat variation 

of heart rate. As such, it represents the physi-

ological sinus arrhythmia that depends on the 

vagal and the sympathetic innervation of the 

sinus node (109). Roughly, higher the parasym-

athetic activity, higher the HRV, whereas higher 

the sympathetic activity, lower the HRV (110).

The reduction in HRV is a well-known conse-

quence 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 sympa-

thetic 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 sympa-

thetic activity (114). Taken together, the rela-

tionship 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 avail-
able (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, standard-

ization, and validation in pediatric studies 

(Table 1). In contrast to adults, hard end-points 

of CV events are sparse in the pediatric popula-

tion; thus, we urgently need data to establish the 

thresholds for the different non-invasive mea-

sures. 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

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

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References

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USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 8.0 or above). (Note that this document uses screenshots from Adobe Reader X)
The latest version of Acrobat Reader can be downloaded for free at: [http://get.adobe.com/reader/]

Once you have Acrobat Reader open on your computer, click on the Comment tab at the right of the toolbar:

This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the Annotations section, pictured opposite. We’ve picked out some of these tools below:

1. **Replace (Ins) Tool** – for replacing text.
   - Strikes a line through text and opens up a text box where replacement text can be entered.
   - How to use it:
     - Highlight a word or sentence.
     - Click on the Replace (Ins) icon in the Annotations section.
     - Type the replacement text into the blue box that appears.

2. **Strikethrough (Del) Tool** – for deleting text.
   - Strikes a red line through text that is to be deleted.
   - How to use it:
     - Highlight a word or sentence.
     - Click on the Strikethrough (Del) icon in the Annotations section.

3. **Add note to text Tool** – for highlighting a section to be changed to bold or italic.
   - Highlights text in yellow and opens up a text box where comments can be entered.
   - How to use it:
     - 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.
5. Attach File Tool – for inserting large amounts of text or replacement figures.

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.

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.

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: