A comprehensive study of cardiovascular risk factors, cardiac function and vascular disease in children with chronic renal failure

Choni Rinat¹, Rachel Becker-Cohen¹,⁵, Amiram Nir²,⁵, Sofia Feinstein¹,⁵, David Shemesh³, Nurit Algur⁴, Efrat Ben Shalom¹,⁵, Benjamin Farber² and Yaacov Frishberg¹,⁵

¹Pediatric Nephrology, Shaare Zedek Medical Center, Jerusalem, Israel, ²Pediatric Cardiology, Shaare Zedek Medical Center, Jerusalem, Israel, ³Vascular Laboratory and Surgery, Shaare Zedek Medical Center, Jerusalem, Israel, ⁴Clinical Laboratory, Shaare Zedek Medical Center, Jerusalem, Israel and ⁵Hadassah-Hebrew University School of Medicine, Jerusalem, Israel

Correspondence and offprint requests to: Choni Rinat; E-mail: chrinat@szmc.org.il

Abstract

Background. Cardiovascular disease causes major morbidity and is an important determinant of premature death in the paediatric chronic kidney disease (CKD) population. It is composed of three separate, although interrelated, disease processes: atherosclerosis, arteriosclerosis (i.e. medial vascular calcifications) and myocardial disease. Myocardial consequences of atherosclerosis barely exist in children, thus providing a good opportunity to investigate the role that kidney disease plays in the development of cardiovascular disease.

Methods. We assessed 70 patients, aged 4 months to 18 years, with chronic kidney disease stages 3–5, for known risk factors of cardiovascular disease and for additional laboratory and clinical variables which may have an impact on this disease process. Carotid artery ultrasound was used to evaluate vascular structure and function, whereas myocardial disease was assessed by echocardiography.

Results. Traditional risk factors, although present in this cohort, did not accumulate with progression of chronic kidney disease. Non-traditional risk factors increased in number and severity in correlation with the stage of CKD. The main myocardial abnormalities were left ventricular hypertrophy and diastolic dysfunction. Vascular function tests correlated with calcium–phosphate metabolism variables, homocysteine and time-averaged serum uric acid.

Conclusions. This study shows that children with CKD are exposed to risk factors and demonstrate signs of cardiovascular disease already at a young age. The possible role of uric acid and homocysteine in the evolution of cardiovascular disease is discussed. Further studies looking at possible interventions to prevent cardiovascular morbidity and mortality in this high risk population are needed.

Keywords: arterial stiffening; CVS risk factors; myocardial dysfunction; renal failure; uric acid

Introduction

Improvement in therapeutic modalities in children with chronic renal failure (CRF) and the consequent extension of their life expectancy have drawn more attention towards secondary complications [1,2]. Cardiovascular (CVS) disease is the leading cause of death in adults with chronic kidney disease (CKD) [3,4] and has also been shown to be a late complication in children with renal failure [5,6].

Early appearance of vascular disease in patients with CKD is attributed to the combination of two parallel processes: atherosclerosis and arteriosclerosis. High prevalence of traditional cardiovascular risk factors, such as hypertension and dyslipidemia, are responsible for the formation of the intimal atherosclerotic plaque. On the other hand, there are newly emerging risk factors for the development of medial arteriosclerosis, which are all related to calcium phosphorous metabolism. Importantly, it has been shown that the presence of CVS risk factors already in childhood may lead to atherosclerosis in adulthood [7–9]. Very long-standing haemodialysis starting in childhood results in a very high prevalence (87.5%) of coronary calcifications [10]. Furthermore, the majority of children with...
CKD will need one or more renal transplantsations during their lifetime, which carries its own risk for CVS disease [11]. Altogether, this particular population is at a very high risk for vascular disease. This statement was recently supported by the American Heart Association, stratifying these patients in the same high risk group together with those having homozygous familial hypercholesterolaemia, type 1 diabetes mellitus and post-orthostatic heart transplantation [12].

It is therefore obvious that early recognition, identification and treatment of any risk factor is prudent.

A different aspect of cardiovascular disease in patients with CKD is the myocardial damage secondary to hypertension, volume overload [13,14] or the state of uraemia per se.

In this comprehensive cross-sectional study, we evaluated the prevalence of various traditional and non-traditional risk factors in a cohort of children with CKD stage 3–5. Detailed echocardiography studies were performed in order to detect cardiac pathologic processes and define their possible association with a number of clinical and laboratory variables. Long-term clinical endpoints of vascular disease are not practical in children. We used, therefore, carotid ultrasound, an accepted surrogate method for evaluating various vascularopathies. It included measuring carotid intimal–medial thickness (cIMT) and various measurements of the arterial biomechanical properties [15–17], and their correlation with the clinical and laboratory variables.

Methods

Patients

Seventy patients, aged 4 months to 18 years (27 <3 years, 21 age 3 to <10, 22 age 10s to <18), from a single center, with estimated glomerular filtration rate (eGFR) of <60 ml/min/1.73 m², were studied. Twenty three had end-stage renal disease (ESRD) treated with renal replacement therapy—four with peritoneal dialysis and 19 with haemodialysis. Twenty six children were Jewish and 44 were Arabs. Patients’ evaluation included the history of renal disease: age of onset, diagnosis, medications, except for water and regular medications. The following tests were performed: serum electrolytes, glucose, lipid profile, homocysteine, fibriogen, C-reactive protein (CRP) and urine total protein (TP)/creatinine ratio. For several parameters [calcium (Ca), phosphorous (Phos), uric acid, parathyroid hormone (PTH) levels and haemoglobin], a time-averaged level from the last 3 years was calculated to assess the time-integrated burden instead of a single random value. The average for every quartile was initially recorded, followed by calculating the average of all the time periods together.

Parameters such as serum haemoglobin and phosphate, in which the normal range vary with age and/or gender, were expressed as the number of standard deviations from the adjusted mean (Z score). This avoids the inaccuracy of comparing raw data.

Estimated GFR (eGFR) was calculated using the Schwartz equation and compared to normal values for age and gender. The same process of time-averaged eGFR was conducted.

Echocardiography

Echocardiography was performed by a senior paediatric cardiologist using HP Sonos 4500 and Vivid GE 7 machines. In patients undergoing haemodialysis, the study was performed immediately following treatment once they reached their dry weight. Left ventricular mass (LVM) was calculated using measurements obtained by two-dimensional echocardiography and the LVM index (LVMI) calculated to correct for patients’ height: LVMI = LV mass/height1.7 [18]. LVMI in infants and toddlers whose height is <1 m was compared to the normal range of this age group [19]. This was intended to avoid false-positive values in the very young, as LVMI is expressed per height in metres to the power of 2.7, which could erroneously result in very high values. Left ventricular end diastolic diameter (LVEDD) was indexed to the 90th percentile for the patients’ age. Shortening fraction was measured using M-mode echocardiography to assess systolic function [20], and diastolic function was assessed by Doppler measuring the mitral inflow e/a is the ratio between early (e, passive ventricular filling) and late (a, active atrial contraction) diastolic flow velocities. A control group consisted of 27 children and young adults, aged 3–27 years, who underwent echocardiography for evaluation of an innocent murmur, with no abnormal structural findings detected on the echocardiogram.

Carotid duplex examination

Carotid duplex examination was performed by a registered vascular technologist using Doppler ultrasound (Acuson Sequoia 512, Mountain View, CA, USA) with a 6L3 multifrequency linear array transducer. The measured variables were intimal–medial thickness and carotid diastolic and systolic diameter in a fixed point of the right common carotid artery, as previously described [21] with concomitant blood pressure measurement. Calculations of these measurements and how they are influenced by changes in blood pressure determined the following parameters: distensibility (DC) = 2(ΔD/ΔD(DSBP–DBP), where D denotes diastolic diameter and ΔD the change in artery diameter during systole. Stiffness (β) is defined as: (lnSBP/DBP)/(ΔD/D). The incremental modulus of elasticity (Einc) is a marker of the intrinsic properties of the arterial wall material, independent of the arterial geometry [14]. It is calculated using the ratio of the lumen cross-sectional area (LCSA) = π(D/2)² and the wall cross-sectional area (WCSA)/DC [22].

Statistical analysis

Statistical analysis was performed using SPSS version 14 (SPSS Inc., Chicago IL). Parameters were normalized for age/gender/height as need- ed. Association between two continuous variables was assessed by cal- culating the Pearson correlation coefficient or Spearman correlation test for parametric and non-parametric values, respectively. Levene test was used to define distribution width. For comparison of continuous vari- ables, the independent t-test was used when two groups were compared, and analysis of variance was employed when more than two groups were compared. For abnormal, asymmetric distribution of non-parametric vari- ables, the Kruskal–Wallis test was used. Chi-square and Mann–Whitney tests were employed to compare groups of categorical parameters in large and small groups, respectively.

Multiple stepwise linear regression analysis was performed to assess potential predictors of left ventricular hypertrophy (LVH), diastolic dysfunc- tion and arterial stiffening parameters.

Results

Patient characteristics and laboratory results

Patient characteristics are summarized in Table 1. There were no significant differences between groups of CKD stages.
It can almost exclusively be noted that wherever time-averaged and current laboratory variables were tested, either only the time-averaged variables had significant correlations or they had better correlations than the current results. Only the time-averaged results and its statistical analysis are shown.

Time-averaged haemoglobin levels and their age- and gender-adjusted Z scores were significantly lower and anaemia (Z score < −2) was more prevalent among children with higher CKD stage (Table 2). Serum Phos levels with their respective Z scores, Ca×P product and the parathyroid hormone levels were all higher and more prevalent in advanced CKD stages. Uric acid concentrations were significantly increased in patients with CKD 5. Homocysteine, inflammatory state (defined as either CRP >0.5mg/dl or fibrinogen >400mg/dl) and proteinuria [urine TP/creatinine >0.2 (mg/dl)/(mg/dl)] did not change significantly with stage of CKD.

Only non-traditional risk factors accumulate with CKD stage. Risk factors for vascular disease were divided into traditional and non-traditional. The former (Table 3) included positive family history, diabetes mellitus, smoking (either passive or active), hypertension and dyslipidaemia (defined as high total serum cholesterol, increased LDL cholesterol or decreased HDL cholesterol; specific values

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>CKD 3 (N = 30)</th>
<th>CKD 4 (N = 17)</th>
<th>CKD 5 (N = 23)</th>
<th>Overall (N = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD (range)</td>
<td>7.1 ± 5.7 (0.5–16.5)</td>
<td>9.5 ± 5.6 (0.3–17.5)</td>
<td>5.1 ± 4.3 (0.3–15)</td>
<td>7.0 ± 5.4 (0.3–17.5)</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>16 (53.3%)</td>
<td>11 (64.7%)</td>
<td>17 (73.9%)</td>
<td>44 (62.9%)</td>
</tr>
<tr>
<td>Ethnic origin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arab</td>
<td>18 (60%)</td>
<td>12 (70.6%)</td>
<td>14 (60.9%)</td>
<td>44 (62.9%)</td>
</tr>
<tr>
<td>Jew</td>
<td>12 (40%)</td>
<td>5 (29.4%)</td>
<td>9 (39.1%)</td>
<td>26 (37.1%)</td>
</tr>
<tr>
<td>Primary disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysplastic/obstructive</td>
<td>15 (45.7%)</td>
<td>5 (27.8%)</td>
<td>7 (30.4%)</td>
<td>27 (38.6%)</td>
</tr>
<tr>
<td>Familial</td>
<td>7 (22.9%)</td>
<td>9 (50.0%)</td>
<td>2 (8.7%)</td>
<td>18 (25.7%)</td>
</tr>
<tr>
<td>SRNSa</td>
<td>4 (14.3%)</td>
<td>0 (0%)</td>
<td>6 (26.1%)</td>
<td>10 (14.3%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (17.1%)</td>
<td>3 (22.2%)</td>
<td>8 (34.8%)</td>
<td>15 (21.4%)</td>
</tr>
<tr>
<td>CRF years</td>
<td>3.42 ± 3.91</td>
<td>5.33 ± 3.57</td>
<td>2.16 ± 2.21</td>
<td>3.44 ± 3.51</td>
</tr>
<tr>
<td>Dialysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>19 (27.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>4 (5.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dialysis vintage (years; mean±SD)</td>
<td>0.70 ± 2.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>0.70 ± 2.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>0.95 ± 2.45</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*SRNS—steroid-resistant nephrotic syndrome. HD—haemodialysis, PD—peritoneal dialysis.

### Table 2. Laboratory data according to CKD stages

<table>
<thead>
<tr>
<th>Laboratory variable</th>
<th>CKD 3 (N = 30)</th>
<th>CKD 4 (N = 17)</th>
<th>CKD 5 (N = 23)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Overall (N = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (time averaged, g/dl; mean±SD)</td>
<td>12.2 ± 1.5</td>
<td>11.6 ± 1.5</td>
<td>10.9 ± 0.9</td>
<td>NS</td>
<td>0.003</td>
<td>11.6 ± 1.3</td>
</tr>
<tr>
<td>Z score (mean±SD)</td>
<td>−1.37 ± 1.61</td>
<td>−2.14 ± 3.42</td>
<td>−2.90 ± 1.99</td>
<td>NS</td>
<td>−2.06 ± 2.35</td>
<td></td>
</tr>
<tr>
<td>Anemia: number of patients with Z score &lt; −2; n (%)</td>
<td>11 (36.7%)</td>
<td>12 (70.6%)</td>
<td>15 (65.2%)</td>
<td>NS</td>
<td>0.03</td>
<td>38 (54.3%)</td>
</tr>
<tr>
<td>PTH (time averaged, pg/ml; mean±SD)</td>
<td>148 ± 189</td>
<td>402 ± 314</td>
<td>467 ± 357</td>
<td>NS</td>
<td>&lt; 0.001</td>
<td>315 ± 317</td>
</tr>
<tr>
<td>Number of patients with PTH &gt;88pg/ml; n (%)</td>
<td>20 (66.7%)</td>
<td>17 (100%)</td>
<td>21 (91.3%)</td>
<td>0.006</td>
<td>NS</td>
<td>58 (82.8%)</td>
</tr>
<tr>
<td>Phosphorous (time averaged, mg/dl; mean±SD)</td>
<td>5.15 ± 0.56</td>
<td>5.01 ± 0.85</td>
<td>5.78 ± 1.22</td>
<td>0.02</td>
<td>NS</td>
<td>5.32 ± 0.94</td>
</tr>
<tr>
<td>Z score (mean±SD)</td>
<td>1.02 ± 1.85</td>
<td>1.31 ± 2.49</td>
<td>2.81 ± 4.29</td>
<td>NS</td>
<td>1.68 ± 3.07</td>
<td></td>
</tr>
<tr>
<td>Number of patients with Z score &gt; 2; n (%)</td>
<td>8 (26.7%)</td>
<td>6 (35.3%)</td>
<td>10 (43.5%)</td>
<td>NS</td>
<td>NS</td>
<td>24 (34.3%)</td>
</tr>
<tr>
<td>Ca×P (time averaged, mg ²/dl²; mean±SD)</td>
<td>47.5 ± 6.53 3</td>
<td>46.3 ± 9.48 3</td>
<td>53.5 ± 13.4</td>
<td>0.005</td>
<td>NS</td>
<td>49.2 ± 10.3</td>
</tr>
<tr>
<td>Number of patients with Ca×Phos &gt; 55; n (%)</td>
<td>3 (10.0%)</td>
<td>3 (17.6%)</td>
<td>8 (34.8%)</td>
<td>0.08</td>
<td>NS</td>
<td>14 (20.0%)</td>
</tr>
<tr>
<td>Homocysteine μmol/l (mean±SD)</td>
<td>11.4 ± 16.2</td>
<td>10.3 ± 6.2</td>
<td>11.5 ± 8.9</td>
<td>NS</td>
<td>NS</td>
<td>11.2 ± 12.0</td>
</tr>
<tr>
<td>Number of patients with Hcy &gt;15μmol/l [n (%)]</td>
<td>16 (7.7%)</td>
<td>2 (11.7%)</td>
<td>5 (21.7%)</td>
<td>NS</td>
<td>NS</td>
<td>12 (17.1%)</td>
</tr>
<tr>
<td>Uric acid (time averaged, mg/dl; mean±SD)</td>
<td>5.48 ± 1.38</td>
<td>6.52 ± 1.62</td>
<td>6.56 ± 1.21</td>
<td>0.01</td>
<td>6.09 ± 1.47</td>
<td></td>
</tr>
<tr>
<td>Inflammatory state n (%)</td>
<td>12 (40.0%)</td>
<td>9 (52.9%)</td>
<td>14 (60.1%)</td>
<td>NS</td>
<td>NS</td>
<td>35 (50.0%)</td>
</tr>
<tr>
<td>Proteinuria n (%)</td>
<td>26 (86.7%)</td>
<td>14 (82.3%)</td>
<td>15 (65.2%)</td>
<td>NS</td>
<td>NS</td>
<td>55 (78.6%)</td>
</tr>
<tr>
<td>Average number of non-traditional risk factors</td>
<td>2.83 ± 1.42</td>
<td>3.70 ± 1.26</td>
<td>3.83 ± 1.34</td>
<td>0.02</td>
<td>3.37 ± 1.42</td>
<td></td>
</tr>
</tbody>
</table>

Selected laboratory values in the different CKD stages; comparison between absolute levels, Z scores, where relevant, and percentage of patients with abnormal results. Time averaged, see text. NS—not significant. Ca×P—calcium phosphorous product. Hcy—homocysteine.

<sup>a</sup> Between CKD 3 and either 4 or 5.

<sup>b</sup> Between CKD 3 and either 4 or 5.
vary with age [23]). Hypertension (controlled or uncontrolled) was much more prevalent among CKD 5 patients. All other risk factors, including dyslipidaemia, were equally distributed among the three CKD stage groups. Similarly, there was no significant difference in the mean number of risk factors per patient between these groups (1.67 ± 1.15 risk factors in CKD stage 3, 1.76 ± 1.03 in CKD stage 4 and 2.00 ± 0.74 in CKD stage 5 patients; \( P = 0.5 \)).

Non-traditional risk factors included inflammatory state, hyperphosphataemia, Ca×P product >55mg²/dl², hyperparathyroidism (iPTH >88pg/ml), hyperhomocysteinaemia (>15μmol/l) and the presence of proteinuria or anaemia (Table 2). Overall, there was a significant increase in the mean number of these risk factors per patient with higher CKD stages (2.83 ± 1.42 risk factors in CKD stage 3, 3.70 ± 1.26 in CKD stage 4 and 3.83 ± 1.34 in CKD stage 5 patients; \( P = 0.02 \)).

Cardiac status. The two main findings identified were LVH and diastolic dysfunction (15.7% and 18.6% of the patients, respectively; Table 4). Decreased systolic function was detected in 7.1% of the patients.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>CKD 3 (N = 30)</th>
<th>CKD 4 (N = 17)</th>
<th>CKD 5 (N = 23)</th>
<th>P-value</th>
<th>Overall (N = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of CVD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passive n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncontrolled n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBPI (mean ± SD)</td>
<td>0.97 ± 0.18</td>
<td>0.91 ± 0.11</td>
<td>1.16 ± 0.23</td>
<td>&lt;0.001*</td>
<td>1.01 ± 0.21</td>
</tr>
<tr>
<td>DBPI (mean ± SD)</td>
<td>0.86 ± 0.20</td>
<td>0.77 ± 0.17</td>
<td>1.12 ± 0.30</td>
<td>&lt;0.001*</td>
<td>0.93 ± 0.27</td>
</tr>
<tr>
<td>DBPI &gt;1 [μg/2/dl²]</td>
<td>6 (20.0%)</td>
<td>2 (11.8%)</td>
<td>15 (65.2%)</td>
<td>&lt;0.001*</td>
<td>23 (32.9%)</td>
</tr>
<tr>
<td>Controlled hypertension</td>
<td>2 (6.7%)</td>
<td>4 (23.5%)</td>
<td>1 (4.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>0 (0%)</td>
<td>1 (5.5%)</td>
<td>0 (0%)</td>
<td></td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>21 (70.0%)</td>
<td>11 (64.7%)</td>
<td>19 (82.6%)</td>
<td>0.4</td>
<td>51 (72.9%)</td>
</tr>
<tr>
<td>Cholesterol high</td>
<td>14 (46.7%)</td>
<td>6 (35.3%)</td>
<td>14 (60.9%)</td>
<td>0.3</td>
<td>34 (48.6%)</td>
</tr>
<tr>
<td>LDL high</td>
<td>10 (35.7%)</td>
<td>5 (31.3%)</td>
<td>8 (38.1%)</td>
<td>0.8</td>
<td>23 (33.5%)</td>
</tr>
<tr>
<td>LDL low</td>
<td>10 (33.3%)</td>
<td>7 (41.2%)</td>
<td>10 (45.5%)</td>
<td>0.7</td>
<td>27 (38.6%)</td>
</tr>
<tr>
<td>Average number of risk factors</td>
<td>1.67 ± 1.15</td>
<td>1.76 ± 1.03</td>
<td>2.00 ± 0.74</td>
<td>0.5</td>
<td>1.80 ± 1.00</td>
</tr>
</tbody>
</table>

LDL—low density lipoprotein cholesterol, HDL—high density lipoprotein cholesterol, NS—not significant.

\( \text{a} \) Between CKD 5 and either CKD 3 or 4.

Table 3. Traditional risk factors for cardiovascular disease in pediatric CKD patients

Table 4. Echocardiographic and carotid duplex data
statistically significant differences between those with or without LVH were noted with respect to serum PTH levels, Ca×P product, age or CKD stage. Logistic regression analysis showed that only systolic BPI correlated significantly with LVH ($P = 0.05$).

**Diastolic dysfunction is associated with SBPI, the presence of time-integrated anaemia and inflammatory state.**

Diastolic dysfunction (DD) increased in prevalence with progression of kidney disease. Whereas none of the children in CKD 3 group had DD, it was detected in 17.6% of CKD 4 patients and in 43.4% of the CKD 5 group ($P < 0.0001$; Figure 1A). Patients with diastolic dysfunction had on average higher BPIs than those with normal diastolic function (SBPI $1.20 \pm 0.30$ vs $0.98 \pm 0.16$, $P < 0.05$; DBPI $1.15 \pm 0.40$ vs $0.87 \pm 0.20$, $P < 0.05$; Figure 1B). Lower time-averaged haemoglobin levels (Figure 1C) and $Z$ scores ($10.5 \pm 0.86$ vs $11.8 \pm 1.30$g/dl, $P < 0.005$ and $-3.52 \pm 2.02$ vs. $-1.72 \pm 2.30$, $P = 0.01$, respectively) were detected among children with diastolic dysfunction. An inflammatory state was also more common among patients with DD (92% vs 40.4%; $P < 0.001$). In addition, time-averaged PTH levels were higher in patients with DD ($532 \pm 382$pg/ml vs $265 \pm 261$pg/ml; $P < 0.05$; Figure 1D). Average patients’ age was similar in these two groups. Backward stepwise logistic regression showed that the following parameters significantly correlated with diastolic dysfunction: systolic BPI, inflammatory state and time-averaged haemoglobin $Z$ score ($P < 0.05$ for all).

**Systolic dysfunction is associated with SBPI, DBPI and time-averaged anaemia.** Systolic dysfunction was identified in only five patients (7.1%; Table 4). Time-averaged haemoglobin levels and $Z$ scores were significantly lower in patients with systolic dysfunction ($10.2 \pm 1.13$ vs $11.7 \pm 1.29$g/dl, $P < 0.05$ and $-4.40 \pm 1.91$ vs $-1.88 \pm 2.29$, $P < 0.05$, respectively). They also had higher SBPI and DBPI ($1.37 \pm 0.33$ vs $0.99 \pm 0.17$, $P < 0.005$ and $1.30 \pm 0.45$ vs $0.90 \pm 0.23$, $P < 0.005$, respectively).

**Vascular structure and function**

cIMT is not correlated with traditional risk factors but is associated with Ca–P-related parameters. In this cohort of patients, cIMT was overall found to be within normal range for age (97th percentile: 0.46mm at age 10 years, 0.49mm at 20 years; [24]). It did correlate, however, with the number of years in ESRD ($r = 0.316$; $P = 0.02$) but no association was seen with any of the traditional atherosclerosis or non-traditional risk factors, nor with echocardiographic findings. We found a tendency for thicker cIMT in CKD 5 patients ($0.43mm \pm 0.05mm$) compared to patients in CKD 4 ($0.40mm \pm 0.06mm$) and 3 ($0.39 \pm 0.05mm$).
Dependent variable Variable $\beta$ $P$-value

$E_{\text{inc}}$: $\tau^2 = 0.352$
- Homocysteine 0.247 0.05
- Uric acid 0.259 0.05
- Phos Z score 0.261 0.05

Distensibility: $\tau^2 = 0.353$
- Homocysteine $-0.252$ 0.05
- Uric acid $-0.250$ 0.05
- PTH $-0.296$ 0.05

**Arterial stiffening parameters correlate with some Ca–P metabolism-related variables.** Various parameters describing arterial stiffening were analyzed. Time-averaged serum PTH concentrations correlated with decreased arterial distensibility and with increased stiffness ($r = -0.353$, $P = 0.01$ and $r = 0.290$, $P = 0.03$, respectively). Time-averaged phosphorus Z score correlated with $E_{\text{inc}}$ ($r = 0.297$, $P = 0.04$). Other Ca–P-related parameters were not significantly correlated.

**Uric acid, homocysteine levels and the presence of AV fistula are correlated to arterial stiffening.** Significant correlations were found between arterial stiffness and time-averaged uric acid levels (distensibility: $r = -0.378$, $P = 0.005$; $E_{\text{inc}}$: $r = 0.353$, $P = 0.01$; Figure 2A–B) and serum homocysteine concentrations (distensibility: $r = -0.267$, $P = 0.05$; $E_{\text{inc}}$, 0.350, $P = 0.01$).

Parameters of vascular function did not correlate with any of the following: age, anaemia, blood pressure variables (SBPI, DBPI or pulse pressure), inflammatory state, eGFR, duration of CRF or ESRD, total, HDL or LDL cholesterol.

Similarly, no significant correlation was found between vascular function and echocardiographic structural (e.g. LVH) or functional (e.g. diastolic dysfunction) parameters.

**Analysis of covariance pointed at serum homocysteine levels, time-averaged uric acid and PTH levels as significant independent variables which affect vascular distensibility (all $P < 0.05$; $\tau^2 = 0.353$; Table 5).** Only time-averaged PTH levels were independently and significantly correlated with stiffness, whereas homocysteine levels, time-averaged uric acid and time-averaged phosphorous Z score were determined as independent factors affecting $E_{\text{inc}}$ ($P < 0.05$ for all; $\tau^2 = 0.352$; Table 5).

**Discussion**

This is the first study on various CVS manifestations in CKD involving very young patients and, to the best of our knowledge, the first time the role of uric acid as a risk factor was investigated. In this study, we show that only non-traditional risk factors for vascular disease increase in number and severity with progression of kidney disease. High SBPI was significantly associated with worsening of all echocardiographic parameters. Low time-averaged haemoglobin levels were associated with both diastolic and systolic cardiac dysfunction, whereas inflammatory state is associated with diastolic dysfunction only. cIMT was only associated with the duration in ESRD.

Several parameters describing arterial stiffness were assessed with respect to potential risk factors. Hyperhomocysteinaemia, time-averaged PTH and uric acid levels significantly correlated with distensibility. Time-averaged PTH was also associated with arterial stiffness. Serum homocysteine concentrations, time-averaged uric acid and phosphorous Z scores were found to correlate significantly with $E_{\text{inc}}$.

Vascular disease should be divided into two separate disease processes: intimal atherosclerosis and medial arteriosclerosis. These two clinical entities differ with respect to risk factors, pathophysiology, histological findings and clinical manifestations. Classical atherosclerosis is caused by intimal plaque formation, secondary to traditional vascular disease risk factors. The clinical manifestations are due to alterations in the conduit function of the arteries. On the other hand, medial arteriosclerosis is caused by diffuse mineral deposition in the tunica media of the arterial wall, as a result of high calcium–phosphate product, suppression of natural crystallization inhibitors [including matrix Gla protein (MGP), osteoprotegerin (OPG) and fetuin A] and vascular smooth muscle cell phenotypic changes leading to osteoblastic differentiation [26,27]. Together they create a permissive environment for nucleation of Ca–P crystals. The known risk factors, in addition to the Ca–P-related parameters, are cumulative doses of Ca containing Phos binders and vitamin D analogs. Clinically, arteriosclerosis causes vascular stiffness and secondarily results in hypertension, increased pulse pressure and LVH [26]
The major factors leading to myocardial disease include volume overload, anaemia and the uremic milieu per se. Important components of the latter are the endogenous corticosteroids, digitalis-like substances whose secretion is augmented in CRF patients as an adaptation to the uraemic electrolyte–water imbalance [28–30]. Their action results in LVH, myocardial fibrosis and diastolic dysfunction.

In children with CKD, vascular disease rarely reaches clinical manifestations. On the other hand, myocardial disease can cause symptomatic diastolic and/or systolic dysfunction. When cardiac death occurs, it is rarely caused by atherosclerosis or arteriosclerosis but rather by congenital cardiac anomalies or secondary to electrolyte imbalance, hypertensive crisis or fluid overload. A comprehensive study [6] pointed to cardiovascular-related diseases as the main cause of death (overall 41%) in a cohort of patients with an early onset of ESRD (0–14 years of age). The list of aetiologies included cerebrovascular events, congestive heart failure, cardiac arrest and dissection of the aorta—all of them, at least in part, are consequences of hypertension, electrolyte anomalies and volume overload rather than secondary to vascular or myocardial disease per se. Nevertheless, three of the patients died of myocardial infarction at a very young age (24–36 years old). The high death rate declined by 50% from the decade of 1972–1981 to 1982–1992, and the CVS mortality declined even further by 61%.

Overall, the cIMT was within the normal range in the present study, although the average cIMT in CKD stage 5 (0.43 ± 0.05 mm) was higher than the published normal mean values (0.39 ± 0.05 mm and 0.40 ± 0.05 mm at age 10 and 20 years, respectively [24]). Previous studies [31–33] demonstrated increased cIMT in paediatric CKD patients, especially in those receiving renal replacement therapy. The difference between our results and those described in previous studies may stem from the fact that we included children in CKD 3–5 rather than only those with ESRD or from dissimilarities in age groups. Our patients were significantly younger (on average 7.0 years compared with ~15 years), and since cIMT increases with age [24], this probably impacts on the mean values for the entire cohort.

Lack of published normal values for cIMT during the first decade of life precluded Z scoring in this part of the study (as was done by Litwin et al. [31]), and may have compromised the interpretation of the results.

Of note, since cIMT measures both intima and media, it reflects not only intimal atherosclerosis but also medial arteriosclerosis or other causes of medial thickening. In paediatric CKD patients, when arteriosclerosis is in its very early stages, cIMT seems to be more a marker of arteriosclerosis.

The main cardiac abnormalities detected in our study were LVH and diastolic dysfunction, with prevalence comparable to that of previous publications. LVH was found to correlate with systolic and diastolic hypertension and with the presence of an AV fistula. Other studies have pointed at hypertension, anaemia and parameters associated with Ca-P metabolism as affecting LVH in patients with CKD. In the paediatric population, Mitsnefes et al. found LVH to correlate with factors related to Ca-P metabolism and with haemoglobin levels and hypertension [34,35]. Matteucci et al. found anaemia, low GFR, high BMI and young age as independent correlates with LVM [36]. It can be argued that, in young children <1 m in height, indexing the LVM by dividing it by the height to the power of 2.7 may cause artifactually high values. This was corrected in our study by using age-adjusted LVMI normal values [19].

We found homocysteine levels to correlate with vascular distensibility and $E_{inc}$ but not with cIMT. Serum homocysteine levels were previously found to correlate with arterial stiffening in both the general population [37] and in haemodialysis adult patients [38], and with coronary calcifications in young adults with childhood onset CRF [39]. It remains controversial whether there is a direct influence on cardiovascular disease. Several trials failed to show that using homocysteine-lowering agents reduces the risk for clinical CVS disease, even in CKD patients [40]. Other studies were aimed at testing the hypothesis that lowering homocysteine levels would improve endothelial dysfunction in the general population and in CKD patients (both adults and pediatric), but the results were not consistent [41–43].

Since classical atherosclerosis and arteriosclerosis are different disease processes, one can argue that the effect of homocysteine is more on the arterial media, contributing to arteriosclerosis (and secondarily to arterial stiffness) and not to intimal atherosclerosis. This might solve the paradox between the consistent finding of positive correlation between arterial stiffness and hyperhomocysteinaemia and the absence of a favorable effect of lowering homocysteine levels on ischaemic heart disease or stroke. The absence of obvious improvement in parameters of endothelial function following homocysteine lowering could potentially reflect irreversible damage or long-term effect of hyperhomocysteinaemia to the endothelium. If this is the case, treatment can be still justified to avoid further damage.

We have demonstrated good and significant correlations between time-averaged serum uric acid levels and both decreased distensibility and increased $E_{inc}$. Hyperuricaemia has many emerging deleterious effects; in children, it was found to be related to primary hypertension [44] and was suggested as having a role in its pathogenesis. In addition, since hyperuricaemia is strongly associated with pre-eclampsia, it was assumed that it is not merely an association by chance but rather uric acid has a pathogenic role in the development of hypertension and kidney disease in this context [45]. A large epidemiological study concluded that elevated uric acid levels increases the risk for new onset kidney disease, independent of eGFR, components of the metabolic syndrome, gender, age and antihypertensive drugs [46]. Also, our findings are consistent with previous studies pointing to the deleterious effect of uric acid on endothelial function and arterial stiffness, even when uric acid levels are within the normal range [47,48]. Mechanistically, experimental animal models have suggested that high uric acid levels may induce primary hypertension, probably by causing renal afferent arteriolopathy [49]. Another possible mechanism for vascular damage is through increased production of reactive oxygen species and a decrease in the potent vasodilator nitric oxide [50]. Treatment of patients with allopurinol improved endothelial dysfunction in both congestive heart failure and in type 2 diabetes mellitus [51,52]. These data point to possible direct deleterious effects of hyperuricaemia on arteries and may provide explanation to our findings.
Current indications for lowering uric acid with allopurinol are limited. It is widely accepted that patients with uricosuria, regardless of the blood uric acid levels, will benefit from such treatment to prevent nephrolithiasis or nephrocalcinosis. On the other hand, asymptomatic patients with isolated hyperuricaemia are often not treated as they are at a very low risk for gout or for renal disease. The risk of hyperuricaemia for CVS disease is not well established, and frequently it is only regarded as a component of the metabolic syndrome [53]. Since arterial stiffening is strongly associated with cardiovascular morbidity and mortality [54], any factor directly causing arterial stiffness should be considered as a potential target for treatment.

Parameters related to calcium–phosphorous metabolism have been shown to have an impact on arterial stiffening which is confirmatory of previous observations [34,35,39]. Phosphor and calcium reference values are higher in younger ages [23]. Having Ca×P product above the recommended level (>55 mg²/dl²) often occurs with normal Ca/P concentration range for age. The physiology of this phenomenon is not known. Theoretically, higher concentrations of the crystallization inhibitors at these ages could have been an adequate explanation, but apparently young children have lower concentrations of fetuin A and OPG (but not MGP; [55]). Better understanding is needed so appropriate guidelines can be established for this age group.

In this study, we used several means to ensure gender, age and height corrections for variables, including Z scoring of laboratory and echocardiographic results, correction of L VMI for children <1 m in height and calculating time-averaged values. This proved to be effective since stratifying the patients by age did not significantly change the data analysis results. Although the use of time-averaged values for various laboratory variables was previously implemented in a few studies [15,31], it is not yet a common practice. We believe this approach improves the accuracy of the comparisons and provides better understanding of the pathological processes.

In the very young group, we encounter specific problems in achieving recommended therapeutic goals: hypertension is more difficult to control—it was found in 42.8% of the entire group, but was much more prevalent in the age group of 0.4–3 years (66.7%) compared to 30.2% in the age group of 3–18 years. This might stem from lower BP limits in younger age, dependence on liquid feeding formulation, which puts the patient at risk of chronic volume overload, and the extreme burden of the treatment in general on the patients’ parents that may result in inadequate compliance. In addition, statin use is limited to children >8 years of age, thus there are no effective tools to combat hypercholesterolaemia at lower age, although lowering cholesterol levels was not shown to be effective in reducing CVS events or mortality in adult haemodialysis patients [56,57].

Limitations of this study include the following: this is a cross-sectional and single-centered study, and some of the patients had short follow-up. For all vascular measurement variables, there are no published normal values, so adjustment for age was impossible. In addition, only 87 of 70 (75.7%) patients had carotid artery duplex examination.

In summary, CVS disease in CKD patients is a combination of three interrelated disease processes—arteriosclerosis, atherosclerosis and myocardial disease—with different risk factors, which together have an impact on morbidity and mortality. Studies should be undertaken with this concept in mind to avoid misinterpretation of their results. These disease processes start already in the first decade of life, from CKD stages 3 and on. Therapeutic measures directed at risk factor reduction have to be intensively taken. In this regard, controlling blood pressure and avoidance of anaemia are obvious, but other measures must be considered. Guidelines concerning the optimal Ca–P level in children with CKD should be defined and consideration should be given to the dilemma whether hyperuricaemia and hyperhomocysteinaemia should be treated. Further studies are needed to explore the long-term effects of these agents on arterial stiffness.

Acknowledgements. This study was supported by the Mirsky Fund, Shaare Zedek Medical Center.

Conflict of interest statement. None declared.

References

12. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation 2006; 114: 2710–2738
Cardiovascular risk factors, cardiac function and vascular disease in children with CRF

42. Title LM, Cummings PM, Giddens K et al. Effect of folic acid and antioxidant vitamins on endothelial dysfunction in patients with coronary artery disease. J Am Coll Cardiol 2000; 36: 758–765

Received for publication: 1.3.09; Accepted in revised form: 30.9.09