

Determinants of premature atherosclerosis in children with end-stage renal disease

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Short title:

Atherosclerosis in children with renal failure

Summary

Cardiovascular disease is a major cause of morbidity and mortality in young adults with end-stage renal disease (ESRD), but its basis is still not well understood. We therefore evaluated the determinants of atherosclerosis in children with ESRD.

A total of 37 children with ESRD (with 31 who had undergone transplantation) were examined and compared to a control group comprising 22 healthy children. The common carotid intima-media thickness (CIMT) was measured by ultrasound as a marker of preclinical atherosclerosis. The association of CIMT with anthropometrical data, blood pressure, plasma lipid levels, and other biochemical parameters potentially related to cardiovascular disease was evaluated.

Children with ESRD had significantly higher CIMT, blood pressure, and levels of lipoprotein (a), urea, creatinine, ferritin, homocysteine, and serum uric acid as well as significantly lower values of apolipoprotein A. The atherogenic index of plasma ($\log(\text{triglycerides}/\text{HDL cholesterol})$) was also higher in patients with ESRD; however, this difference reached only borderline significance. In addition, a negative correlation was found between CIMT and serum albumin and bilirubin in the ESRD group, and this correlation was independent of age and body mass index. In the control group, a significant positive correlation was observed between CIMT and ferritin levels.

Factors other than traditional cardiovascular properties, such as the anti-oxidative capacity of circulating blood, may be of importance during the early stages of atherosclerosis in children with end-stage renal disease.

Keywords

Atherosclerosis, Children, Carotid intima-media thickness, End-stage renal disease

INTRODUCTION

Despite an improvement in the survival of children with severe chronic kidney disease, morbidity and mortality rates in these patients are still relatively high. The risk of death due to cardiovascular disease (CVD) in children with end-stage renal disease (ESRD) is 1,000-fold higher than in the general paediatric population (Kavey *et al.* 2006). After successful renal transplantation, the risk of CVD decreases considerably in children; nevertheless, it remains at least two-fold higher compared to the age-matched general population (Sarnak *et al.* 2003, Mitsnefes 2008).

Complications due to atherosclerosis, the main cause of death in adult ESRD populations, are rather rare in paediatric patients. A total of 1,380 deaths (for patients aged 0-30 years) recorded by the United States Renal Data System between 1990 and 1996 were analysed in a recent study (Parekh *et al.* 2002), and the authors found that the main causes of cardiovascular deaths in this study were sudden cardiac arrest, arrhythmia and cardiomyopathy. However, CVD of atherosclerotic origin has been shown to be the main

cause of morbidity and mortality in young adults with ESRD (Berenson 2002, Oh *et al.* 2002, Oren *et al.* 2003, MacDonald *et al.* 2004). Based on these observations, numerous studies have focused on the potential determinants of the extreme acceleration of atherosclerosis in patients with renal impairment. There is no doubt that renal replacement therapy contributes to specific proatherogenic factors such as chronic inflammation (due to catheters, dialysate leakage, chronic or recurrent infections during immunosuppression, raised cytokine production and reduced renal clearance) and hypertension or aggressive pharmaceutical treatment (steroids, immunosuppressives) (Lilien *et al.* 2003). The availability of high-resolution ultrasonographic methods has enabled non-invasive investigation of functional and morphological atherosclerotic changes in childhood (Sass *et al.* 1998, Yang *et al.* 2007, Jehlicka *et al.* 2009). The common carotid intima-media thickness (CIMT), as measured by ultrasound, is the most frequently used marker of preclinical atherosclerosis. Normative values of CIMT have already been proposed based on the examination of 247 healthy children (Jourdan *et al.* 2005). However, only a few studies have been published on preclinical atherosclerosis in paediatric ESRD patients with respect to the broad range of metabolic risk factors. These studies have revealed unequivocal findings of increased preclinical atherosclerosis in peripheral arteries (Mitsnefes *et al.* 2004, Litwin *et al.* 2005, Jourdan *et al.* 2005, Poyrazoglu *et al.* 2006, Bilginer *et al.* 2007, Civilibal *et al.* 2007).

The Czech Republic ranks among the countries with the highest incidence of CVD, caused by a high prevalence of cardiovascular risk factors including children (Urbanová *et al.* 1998). Therefore, the aim of our study was to detect determinants of atherosclerosis in children with ESRD from the high-risk Czech population. The particular aims of this study were as follows: to evaluate the presence of preclinical atherosclerosis in children with ESRD and to detect conventional and newly discussed determinants of preclinical atherosclerosis in this population.

METHODS

Thirty-seven children (20 boys and 17 girls, aged 14.5 ± 3.3 years) with ESRD who were undergoing renal replacement therapy (RRT) were examined. The study group consisted of 31 children that had undergone successful renal transplantation and 6 that had undergone peritoneal dialysis (PD). The renal diseases diagnosed in the study group were as follows: hereditary nephropathies (n=11), renal hypo- or dysplasia (n=6), glomerulonephritis (n=3), tubulointerstitial nephritis (n=3), obstructive nephropathy (n=2), reflux nephropathy (n=2), haemolytic-uremic syndrome (n=1), and unknown causes (n=2). All dialysed children were treated with continuous cycling peritoneal dialysis. None of the children suffered from diabetes mellitus, and no child was treated with any lipid-reducing medication. Thirty-four children (92 %) in the study group were treated for high blood pressure (BP). In the transplant group, only four children were treated with vitamin D analogues, and none were treated with phosphate binders. All children from the PD group were treated with phosphate binders, and four members of the PD group were given vitamin D analogues as well. The mean time between RRT and ultrasound examination in the patient group was 4.4 ± 2.7 years, and the mean time between dialysis and transplantation was 12.5 ± 11 months.

The height and weight of each patient were measured using a standard protocol, and the body mass index (BMI) was calculated as $(\text{weight in kg})/(\text{height in m})^2$. The office systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an Omron M5-1 automatic blood pressure monitor (Omron Corporation, Kyoto, Japan) with an appropriately sized cuff on the right arm of the seated patient after 5 minutes of rest. The mean arterial pressure was calculated as the diastolic pressure + $1/3(\text{systolic} - \text{diastolic blood pressure})$. All anthropometrical and biochemical measurements, including BP, were obtained on the same day as the ultrasonic examination for preclinical atherosclerosis in the carotid arteries.

As a control group, 22 healthy, gender-matched children (10 boys and 12 girls, aged 11.0 ± 4.4 years) were included in the study and examined using the same protocols. The control group comprised healthy children of the hospital staff and patients from the allergology and gastroenterology outpatient clinics who were not undergoing systemic steroid therapy nor had been diagnosed with lipid metabolism disorders. The study protocol was approved by the local ethics committee of the 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, and informed consent was obtained from the parents of all children involved in the investigation.

Measurement of CIMT

As a marker of atherosclerosis, CIMT was measured using a high-resolution ultrasonic device (SonoSite Titan, USA) with a 7.5-MHz linear transducer. This approach was a slight modification of a previously described method (Pitha *et al.* 1999). In brief, all measurements were performed on the far (deeper) wall of the common carotid artery, where the intima-media layer is defined as a space between the two visible leading edges (lumen-intima, media-adventitia) (Fig. 1), as was described and validated by Pignoli and others (Pignoli *et al.* 1986, Wendelhag *et al.* 1991, Raitakari 1999). In each patient, the CIMT of both left and right common carotid arteries was recorded, and the proper measurements were performed

offline using computer software (ImagePro Plus, USA). The optimal image of each left and right common carotid artery was frozen, and 10-mm-long segments proximal to the carotid bifurcation were marked (Fig. 2). The mean IMT in the entire 10-mm-long segment was measured, and the final value was calculated as the arithmetical mean of the two measurements of the left and right carotid arteries. The coefficient of variation in previous studies was 4.9 %.

Laboratory measurements

A venous blood sample was drawn from each patient after an overnight fasting (12 hours). Total cholesterol, triglycerides, and cholesterol in all fractions were determined with a Hoffman-LaRoche COBAS MIRA autoanalyser using enzymatic Boehringer Mannheim kits. Serum levels of urea, creatinine, albumin, total bilirubin, uric acid, and glucose were measured using enzymatic colorimetric reactions. Apolipoprotein A1 (ApoA1) and B (ApoB) were measured by an immunoturbidimetric method, and hs-C-reactive protein (CRP) was measured by a direct turbidimetric method with an Advia 1650 analyser (Bayer, USA). Lipoprotein (a) (Lp(a)) was determined by immunonephelometric method on an Image 8000 analyser (Beckmann Coulter, USA), homocysteine was measured using a fluorescence polarisation immunoassay with an IMx analyser (Abbott, USA) and ferritin was measured by a chemiluminescence immunoassay on an Advia Kentaur analyser (Bayer, USA). The atherogenic index of plasma (AIP) was calculated as the $\log(\text{triglycerides}/\text{HDL cholesterol})$ according to Dobiasova (Dobiasova and Frohlich 1991). Patients with values of hs-CRP protein above 10 mg/L were excluded from analysis (4 children in the ESRD group). The glomerular filtration rate was calculated by the Schwartz formula (Schwartz *et al.* 1976).

Statistical analysis

Continuous variables are given as means with their standard deviations, and the standardisation for age and BMI are presented as means with their standard errors. Discrete variables are given as absolute and relative frequencies. T-tests were applied for between-group comparisons of continuous variables, and the χ^2 test was applied for discrete variables (e.g., boys/girls). To evaluate the differences between correlation coefficients in different groups, the test of equality of correlation coefficients was applied. All tests were two-sided, and $p < 0.05$ was considered statistically significant. Because the children in the study group were significantly older than those in the control group, all significant differences were also adjusted for age.

RESULTS

The main characteristics of the study and control groups are shown in Table 1, with separate analysis for transplant children alone. Because children with ESRD were older than those in the control group, the data were standardised for age when appropriate. Children with ESRD had a mean CIMT that was significantly higher than that of the control group. Regarding other variables studied, blood pressure, AIP, and levels of Lp(a), serum creatinine, ferritin, homocysteine, and serum uric acid were significantly higher in children with ESRD than in the control group. On the other hand, ApoA1 and, as expected, the glomerular filtration rate were significantly lower in children with ESRD than in the control group. No difference between the ESRD and control groups was found in the case of BMI or levels of serum total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, ApoB, hs-C-reactive protein, albumin, total bilirubin, or glucose. When the analysis was restricted to only children who had undergone transplantation, the atherogenic index of plasma and Lp(a) were no longer significantly different between the study and control groups, whereas the other results remained the same.

We subsequently analysed the association between CIMT and the other variables studied in the group of transplanted children and in the control group (Table 2). All correlations were standardised for age and BMI. In the group of transplanted children, a significant negative correlation was found for serum albumin; in addition, if dialysed children were also included (data not shown), a significant negative correlation with total serum bilirubin was found ($r=-0.351$, $p<0.05$). In the control group, a significant positive correlation between CIMT and serum ferritin was found. No other significant correlation between CIMT and the factors under study was observed, including correlations between CIMT and indicators of calcium or phosphate metabolism (data not shown). Significantly different trends were found for the correlation of CIMT with diastolic blood pressure and mean arterial pressure in children with ESRD and those in the control group: the trend in the ESRD group was negative, whereas the trend was positive in the control group.

DISCUSSION

Presence of preclinical atherosclerosis

In our study, we demonstrated increased preclinical atherosclerosis in children with end-stage renal disease compared to healthy control children from the high-risk Czech population. Our findings confirm the previously described presence of advanced preclinical atherosclerosis in children with ESRD. The CIMT values observed in the study and control groups were highly similar to those found in previous studies (Mitsnefes *et al.* 2004, Litwin *et al.* 2005, Jourdan *et al.* 2005, Poyrazoglu *et al.* 2006, Bilginer *et al.* 2007, Civilibal *et al.* 2007). In contrast, impaired distensibility of carotid arteries, but not increased CIMT, was observed in patients with ESRD in a study by Groothoff *et al.* (Groothoff *et al.* 2002). However, older patients were included, and no metabolic factors were taken into account; therefore, this discrepancy could be explained by the different populations studied, including the control group, which also included relatives of the patients. This explanation is also supported by the higher carotid intima-media thickness in this population as compared to the values reported in other studies, including the control group in this report.

Conventional and nonconventional cardiovascular risk factors

Regarding conventional cardiovascular risk factors, blood pressure and markers of reverse cholesterol transport were less favourable in our study group than in children with normal renal function. Regarding nonconventional cardiovascular risk factors, higher levels of homocysteine and ferritin were found in the children with ESRD than in the control children. However, in the case of homocysteine, these differences were almost assuredly due to decreased renal function, whereas the higher ferritin levels were due to prior substitution or treatment.

Higher values of blood pressure in children with ESRD, despite frequent antihypertensive treatments, have already been described in several studies (Mitsnefes *et al.* 2004, Seeman 2004 *et al.*, Poyrazoglu *et al.* 2006), and it is likely that this finding is primarily due to renal disease and immunosuppressive drugs (steroids).

Regarding dyslipidemia, we only found less favourable values for markers of reverse cholesterol transport, in particular, lower values for ApoA1 and the atherogenic index of plasma. The atherogenic index of plasma was of borderline significance when only children with transplant kidney were included; therefore, the generalisability of this finding is unclear. Many different changes in the spectrum, composition, and metabolism of lipoproteins have been described in patients with chronic kidney disease; these changes vary not only between different types of renal replacement therapy but also between various published studies. The most frequent pathological findings regarding the lipid spectrum in patients with renal impairment are hypertriglyceridemia, low HDL cholesterol, and a reduction in the size of LDL particles. The majority of studies have shown that neither the synthesis nor secretion of lipoproteins is significantly affected by renal insufficiency (Querfeld 1993). Some studies have explained changes in lipoprotein levels (uremic dyslipidemia) by decreased catabolism of lipoproteins containing apoB (Querfeld 1993, Kronenberg *et al.* 1996, Atman *et al.* 1997). In addition, the correlation between cardiovascular-related death and hyperlipidemia in patients with chronic renal insufficiency has not been demonstrated, although the lipid profile in these patients has been found to be markedly atherogenic. One of the most frequently discussed confounding factors in patients with renal failure is malnutrition associated with low cholesterol levels (Ritz 1996).

Other differences between the study and control groups were obviously related to lower glomerular filtration rate (serum urea, homocysteine, and uric acid) and/or treatment for the disease (ferritin).

Determinants of preclinical atherosclerosis

The main determinants of preclinical atherosclerosis in children with ESRD were lower levels of albumin and bilirubin. In contrast, only ferritin strongly and positively correlated with preclinical atherosclerosis in children with normal renal function. No significant association between preclinical atherosclerosis and conventional cardiovascular risk factors was found in any group.

Our finding regarding the inverse relationship between CIMT and albumin has been previously described (Litwin *et al.* 2005) and explained by the nutritional status of the patients in the study. Recent studies of adults on chronic haemodialysis have shown that mild hypertension, hypercholesterolemia, and

being overweight seem to be protective and are associated with improved survival (Nurmohamed and Nube 2005). This phenomenon has been named “reverse epidemiology” and is thought to be caused by a malnutrition-inflammation-atherosclerosis complex.

To our knowledge, this is the first report of an inverse correlation between preclinical atherosclerosis and bilirubin in children with ESRD. Recent experimental and human studies have indicated that bilirubin plays an important role in cytoprotection as a physiological protector against oxidative stress damage (Sedlak and Snyder 2004). Oxidative stress is believed to play an important role in the development of the atherosclerotic process. Bilirubin in the serum may directly antagonise the oxidative stimuli within the blood stream and diminish their untoward effect on LDL particles (Neuzil and Stocker 1994). Thus, the negative correlation between serum bilirubin and preclinical atherosclerosis may indicate its important role as a physiological antioxidant in patients with ESRD.

We did not observe a correlation between preclinical atherosclerosis and any conventional cardiovascular risk factor under study, either in children with ESRD or in those in the control group. This finding could be caused by the different characteristics of the study populations, as a strong association was found in children of similar age but dialysed (Poyrazoglu *et al.* 2006, Civilibal *et al.* 2007) or in older children after renal transplantation (Bilginer *et al.* 2007). Therefore, the reason for the conflicting findings in our study could be due to the different durations of exposure to elevated blood pressure.

Limitations and strengths of the study

Some limitations of the study include its cross-sectional design, the relatively low number of participants, and their heterogeneity due to different causes of renal failure and frequent pharmacological treatment. These factors may contribute to several marginal results and some differences between our work and previous studies. However, compared to other similar studies (Litwin *et al.* 2005, Bilginer *et al.* 2007), our study population consisted of almost the same number of patients with a less variable duration of transplantation. An additional strength of this study is the evaluation of a wide spectrum of conventional and nonconventional cardiovascular risk factors, including bilirubin levels and the atherogenic index of plasma.

Conclusion

In summary, based on our results, anti-oxidative capacity, as represented by serum albumin and bilirubin levels, may be important in the early development of atherosclerosis in children with end-stage renal disease, even those treated with transplantation and antihypertensive drugs. Therefore, management focused exclusively on conventional cardiovascular risk factors should not be the only method for lowering cardiovascular risk in this high-risk group of children.

Based on our data, further prospective clinical trials and studies focused on the management of cardiovascular disease in young patients with end-stage renal disease should also take into account the role of nutritional status and antioxidants.

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Table 1

Anthropometrical, clinical, biochemical and ultrasonic characteristics in children with end-stage renal disease and control children. Data are given as mean \pm standard deviation or standard error.

	ESRD n=37	Transplant patients (Tx) n=31	Controls n=22	p ESRD vs. control	p Tx vs. control
Age (years)	14.6 \pm 3.3	14.8 \pm 3.3	11.0 \pm 4.4	0.003	0.002
Boys/girls (n)	20/17	16/15	10/12	NS	NS
Carotid intima-media thickness (mm)	0.45 \pm 0.04	0.44 \pm 0.03	0.38 \pm 0.03	<0.001	<0.001
Body mass index (kg/m ²)*	20.0 \pm 0.7	20.6 \pm 0.7	19.4 \pm 0.9	NS	NS
Systolic blood pressure (mmHg)*	120 \pm 1.70	120 \pm 1.90	113 \pm 2.20	0.027	0.047
Diastolic blood pressure (mmHg)*	79 \pm 11.30	78 \pm 10.60	70 \pm 9.80	0.003	0.006
Mean arterial pressure (mmHg)*	93 \pm 1.60	92 \pm 1.70	85 \pm 2.10	0.006	0.018
Serum total cholesterol (mmol/l)	4.80 \pm 1.3	4.60 \pm 0.9	4.40 \pm 0.7	NS	NS
Serum triglycerides (mmol/l)*	1.70 \pm 0.10	1.70 \pm 0.10	1.40 \pm 0.20	NS	NS
Serum HDL cholesterol (mmol/l)	1.21 \pm 0.31	1.21 \pm 0.31	1.32 \pm 0.30	NS	NS
Serum LDL cholesterol (mmol/l)	2.62 \pm 0.86	2.56 \pm 0.65	2.44 \pm 0.76	NS	NS
Atherogenic index of plasma (log(triglycerides/HDL cholesterol))*	0.11 \pm 0.05	0.11 \pm 0.05	-0.06 \pm 0.06	0.036	0.051
Serum ApoB (g/l)	0.81 \pm 0.25	0.78 \pm 0.16	0.7 \pm 0.18	NS	NS
Serum ApoA1 (g/l)	1.23 \pm 0.21	1.23 \pm 0.21	1.34 \pm 0.18	0.041	0.042
Lipoprotein (a) (mg/l)	482 \pm 652	449 \pm 688	213 \pm 251	0.031	NS
Serum C-reactive protein (mg/l)**	1.13 \pm 1.75	1.05 \pm 1.69	0.63 \pm 0.42	NS	NS
Serum urea (mmol/l)	10.6 \pm 5.3	9.8 \pm 5.1	3.8 \pm 0.8	<0.001	<0.001
Serum creatinine (mmol/l)	223 \pm 203	175 \pm 169	67 \pm 15	<0.001	<0.001
Glomerular filtration rate (ml/s/1.73 m ²)	0.92 \pm 0.38	1.16 \pm 0.27	1.69 \pm 0.2	<0.001	<0.001
Serum albumin (g/l)	45.6 \pm 4.6	46.6 \pm 3.1	46.3 \pm 3.7	NS	NS
Serum ferritin (μ g/l)	225 \pm 217	188 \pm 196	47 \pm 49	<0.001	<0.001
Serum homocysteine (μ mol/l) *	12.2 \pm 0.8	12.4 \pm 0.8	8.0 \pm 1.5	0.024	0.022
Serum total bilirubin (mg/dl)*	9.6 \pm 1.3	9.9 \pm 1.5	13.1 \pm 1.8	NS	NS
Serum uric acid (mg/dl)*	363 \pm 16	370 \pm 18	295 \pm 25	0.024	0.022
Serum glucose (mmol/l)	5.1 \pm 0.5	5.1 \pm 0.5	5.2 \pm 1.0	NS	NS

ESRD: end-stage renal disease; Tx: transplant subgroup of ESRD patients

*Adjusted for age

**Four children from the ESRD/Tx group with C-reactive protein above 10 mg/l were excluded from the analysis

Table 2

Partial correlation of intima-media thickness with cardiovascular risk factors and other selected parameters in children with end-stage renal disease after transplantation and in the control group adjusted for age and body mass index.

	Transplant patients n=31	Controls n=22	Equality of correlation coefficients p
Systolic blood pressure	-0.025	0.301	0.270
Diastolic blood pressure	-0.308	0.285	0.044
Mean arterial pressure	-0.239	0.340	0.049
Total cholesterol	-0.131	0.139	0.372
Triglycerides	-0.100	0.283	0.202
HDL cholesterol	-0.071	-0.291	0.460
LDL cholesterol	-0.089	0.249	0.267
Atherogenic index of plasma (log(triglycerides/HDL cholesterol))	-0.176	0.348	0.083
Apolipoprotein B	-0.030	0.400	0.150
Apolipoprotein A1	-0.176	0.087	0.370
Lp (a)	-0.171	-0.093	0.801
C-reactive protein	0.070	-0.094	0.657
Fasting serum glucose	-0.022	0.263	0.494
Serum creatinine	0.027	-0.100	0.676
Serum albumin	-0.422*	-0.250	0.239
Serum ferritin	0.049	0.609*	0.080
Serum homocysteine	0.242	-0.091	0.401
Serum bilirubin	-0.300**	-0.017	0.345
Serum uric acid	0.087	-0.461	0.075

*p<0.05

**p<0.05 if children on dialysis were also included (total number=37)

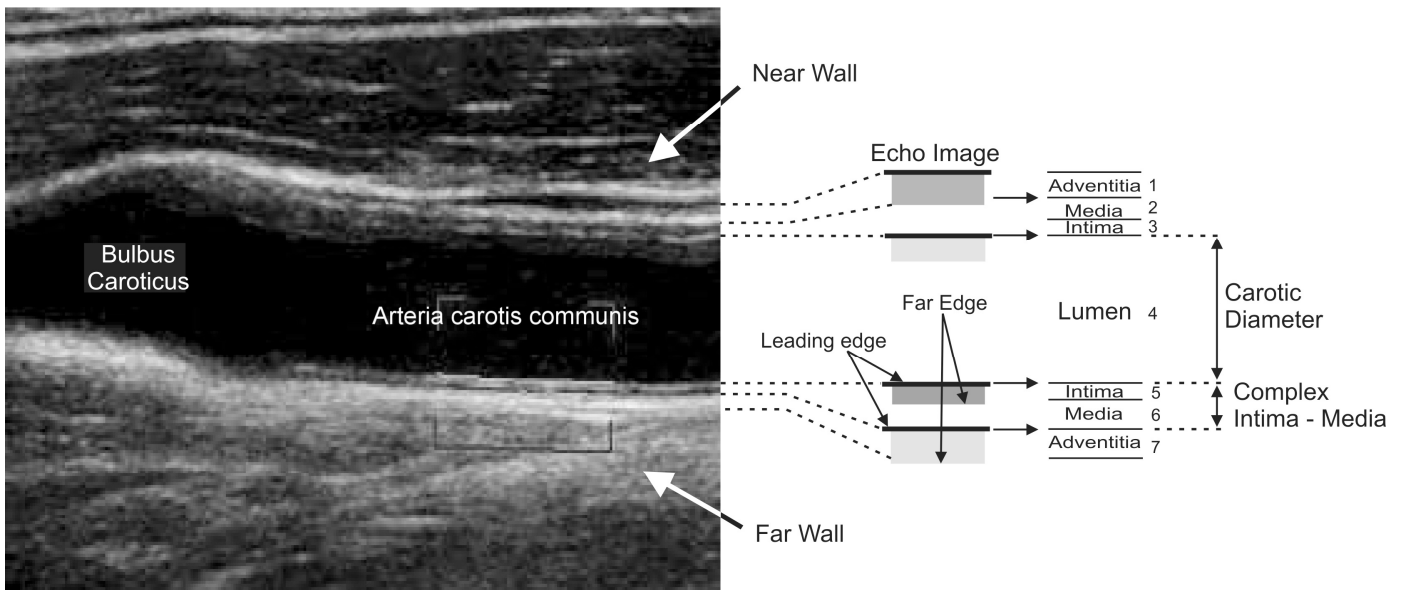


Fig. 1 Bifurcation of the common carotid artery (7 echo zones); measurements were performed between the leading edges of the far arterial wall (zones 5+6)

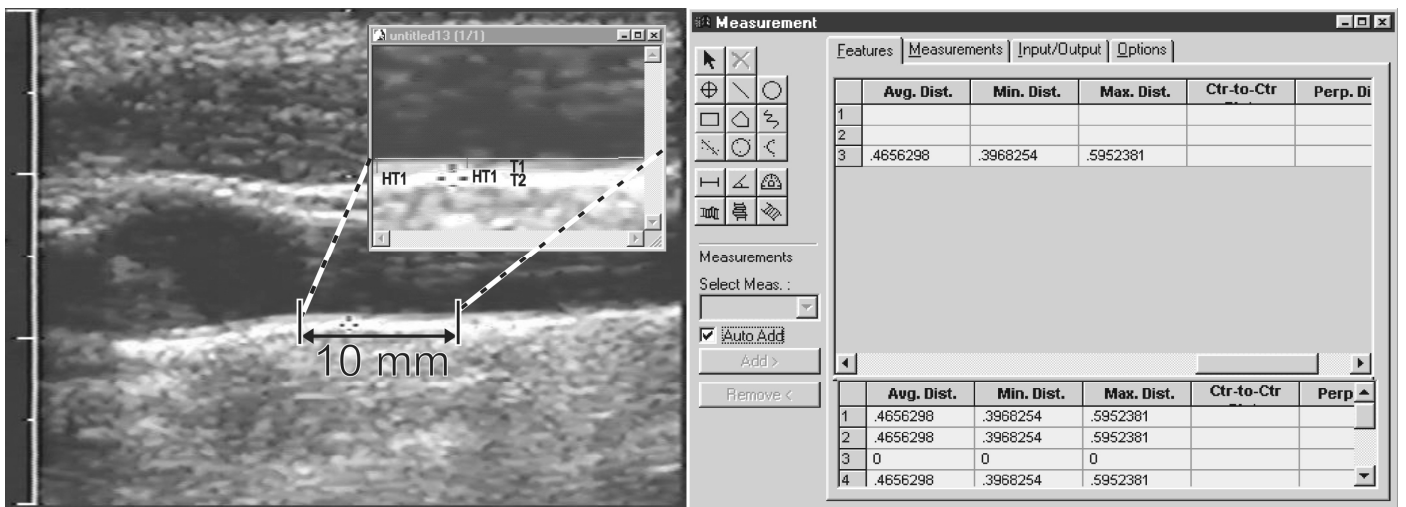


Fig. 2 Offline measurement of CIMT in a 10-mm-long segment of the common carotid artery