Pediatric implications of heterozygous familial hypercholesterolemia

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Chapter 5

The measurement of arterial wall thickness as a surrogate marker for atherosclerosis

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Summary

Atherosclerosis is a protracted disease process of the arterial wall with onset decades prior to its clinical manifestations. To understand the determinants of the process and develop therapeutic approaches requires a lifelong follow-up if clinical endpoint data are used. This approach needs extensive time and resources. Therefore validated surrogate markers for atherosclerosis that can efficiently identify and describe populations at cardiovascular risk and investigate therapeutic regimens have drawn increasing attention. In this paper the preconditions for validated surrogate markers of atherosclerosis and why B-mode ultrasound intima-media thickness measurements meet these requirements are identified. In that context epidemiological studies and atherosclerosis regression trials are described. Moreover, an ultrasound imaging and image analysis protocol is presented to accentuate the need for standardization within and throughout studies if intima-media thickness measurements are used for modelling of arterial wall changes.
Surrogate markers of atherosclerosis: impact of statins

Introduction
Atherosclerosis is a generalized process of the arterial wall, which may progress or regress, depending on a plethora of factors.\(^1\)\(^6\) This condition, therefore, is a dynamic process and as such characterized by arterial wall remodelling, that may go unnoticed for a lifetime, but may also present as acute vascular disease and become clinically manifest.\(^2\) Since atherosclerosis progression requires decades epidemiological studies and intervention trials with clinical endpoints require long time follow-up, participation of large populations, or both. These requirements have to be met in order to provide data from which valid conclusions about the determinants of disease or the efficacy of a therapeutic intervention can be drawn.\(^7\) As a consequence, such studies consume precious time and financial resources.\(^8\) To overcome these challenges surrogate markers are currently the focus of intense attention.\(^9\) Such markers might be used to investigate determinants of atherosclerosis at an early stage of the process and can, subsequently, assess disease modifiers of atherosclerosis progression, such as life-style and pharmacological interventions. Criteria for the validity of such markers as a substitute for clinical endpoints have been proposed by Boissel and co-workers.\(^9\) These colleagues stipulated three conditions for the ascertainment of validity. First, a surrogate marker should be more sensitive and more readily available (sensitivity and availability) than the clinical endpoint. Also, the surrogate marker should be easy to evaluate (convenient), preferably by non-invasive means. Second, the causal relationship between the surrogate marker and the clinical endpoint (proximity) should be established on the basis of both epidemiological, pathophysiological and clinical studies. This entails that it is a prerequisite that patients with and without vascular disease exhibit differences in surrogate marker measurements (specificity). Third and last, in intervention studies, anticipated clinical benefits (assessment of benefit) should be deducible from the observed changes in the surrogate marker. The latter argument implies that it is not just cost and time that favours the development of surrogate markers. Validated surrogate markers enable the assessment of promising new drugs in a relatively short period of time at relatively low cost compared to clinical outcome measures and thus obviate the need to await the outcome of trials driven by clinical events.\(^10\) Moreover, the strength of a surrogate marker is enhanced by the fact that it may yield pathophysiological information at an early stage of the disease process. Surrogate markers, therefore, have an inherent value of their own.\(^9\) Early surrogate markers have originated from techniques available for the clinical assessment of patients with vascular disease, such as angiography and Doppler ultrasound. These techniques have significant clinical relevance but they do not provide useful information on the early stages of arterial wall thickening prior to lesion formation. Doppler ultrasound can only identify a stenosis in case of a 40-50% lumen area reduction,\(^11\) and angiography can only visualize luminal changes in the very late stages of the
disease process. Furthermore, both techniques are inadequate in light of the Glagov effect of initial arterial wall remodeling in the course of atherosclerosis progression.\textsuperscript{12,13}

In contrast, B-mode ultrasound imaging technology has evolved to such an extent that the walls of superficial arteries can be imaged non-invasively, in real-time and at high resolution. Unlike angiography, or ‘luminology’, ultrasound imaging can visualize the arterial wall itself in every stage of atherosclerosis, from ‘normal’ to complete arterial occlusion. Arterial wall thickness can therefore be measured as a continuous variable from childhood into old age, in patients as well as in healthy controls.\textsuperscript{14}

Studies that evaluated the origin of the lumen-intima and the media-adventitia ultrasound interfaces in relation to carotid and femoral far wall arterial histology, demonstrated that the distance between these interfaces reflects the intima-media complex. Consequently, this distance is referred to as intima-media thickness or IMT.\textsuperscript{15,16}

Since B-mode ultrasound is non-invasive, these IMT measurements can be used in observational studies in healthy populations as well as in atherosclerosis regression trials, to assess medication efficacy.\textsuperscript{16} Here we present a short overview on these observational studies\textsuperscript{16,22} and intervention studies\textsuperscript{23,27} as well as the B-mode ultrasound imaging protocol as it is presently validated and standardized at our center. The need for standardization of IMT measurements in imaging trials is illustrated with recent data of patients with familial hypercholesterolemia and unaffected controls.

**Observational studies and intervention trials involving IMT measurements**

*IMT measurements in observational studies*

As mentioned before, B-mode intima-media thickness measurements allow for the investigation of determinants of atherosclerotic disease in the general population.

Two examples of such large follow-up studies are the Rotterdam Study\textsuperscript{17,19} and the Atherosclerosis Risk in Communities Study (ARIC).\textsuperscript{20,23} The Rotterdam Study is a single-center prospective follow-up study of a cohort of 8000 individuals over the age of 55, living in a suburb of Rotterdam.\textsuperscript{17,19} The objective of this study was to identify the determinants of the progression of atherosclerosis of the carotid arterial wall. In the Rotterdam project these ultrasound studies provided solid evidence that IMT measurements may indeed be used as an indicator of generalized atherosclerosis.\textsuperscript{17} Study results provided associations between carotid IMT and stroke, angina pectoris, myocardial infarction, intermittent claudication and essential hypertension.\textsuperscript{18,19} In the Atherosclerosis Risk in Communities Study (ARIC),\textsuperscript{20,23} a study in 15,800 American adults, high resolution B-mode ultrasound was shown to be able to assess all stages of atherosclerosis. In ARIC, the procedure showed a high level of reproducibility, was inexpensive and was established as a noninvasive independent predictor of coronary artery disease. Specifically, a seemingly small increase of 0.2mm in mean carotid IMT
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was shown to increase the relative risk for myocardial infarction and stroke with 33 and 28%, respectively.

**IMT measurements in clinical trials**

B-mode intima-media thickness measurements are also used to demonstrate the efficacy of lipid and blood pressure lowering drugs. The 4-year Cholesterol Lowering Atherosclerosis Study (CLAS)\(^{24}\) assessed the effects of colestipol-niacin therapy in men with previous coronary by-pass surgery and showed statistically significant treatment effects after 2 and 4 years of therapy (p-value <0.0001). The Asymptomatic Carotid Artery Progression Study (ACAPS)\(^{25}\) was a 3-year trial in which a daily dosage of 20 to 40 mg of lovastatin was investigated in asymptomatic men and women between 40 and 79 years of age with early carotid atherosclerosis. In this study lovastatin, compared to placebo, modified the combined IMT of 12 carotid artery wall segments (p<0.001). The Kuopio Atherosclerosis Prevention Study (KAPS)\(^{26}\) investigated the 3-year efficacy of pravastatin in hypercholesterolemic men between 44 and 65 years of age. In this trial, the primary outcome measure (combined IMT of 4 carotid arterial wall segments) showed near significance (p=0.06) and a highly significant effect on combined (right and left) common carotid IMT (p=0.002). In the Regression Growth Evaluation Statin Study (REGRESS)\(^{27,28}\) 40mg pravastatin was assessed in men with angiographically proven coronary artery disease and a total cholesterol between 4 and 8 mmol/L. In this 2-year atherosclerosis regression trial the efficacy of pravastatin was demonstrated by coronary angiography\(^{27}\) and by B-mode ultrasound of the peripheral arteries.\(^{28}\) Interestingly, the ultrasound component of REGRESS was highly significant (p<0.0001) in only 255 patients. A significance at this level was not obtained in any of the coronary angiographic parameters of 885 patients of the REGRESS cohort. These findings underline the usefulness of non-invasive ultrasound as a research tool in intervention trials; a field that was until recently dominated by coronary artery lumen measurements. In the 2-year ASAP trial\(^{29}\) the effects of atorvastatin 80mg and 40mg simvastatin QD were investigated in 325 patients with familial hypercholesterolemia (FH). In this trial aggressive cholesterol lowering with statins was more effective than conventional statin treatment. Specifically, the study showed an actual decrease in carotid IMT in the most aggressively treated group (± 51% LDL-cholesterol lowering) whereas the less aggressive treatment (± 41% LDL-C lowering) only showed inhibition of atherosclerosis progression. The outcome of the recent 1-year ARBTER study\(^{10}\) in 161 patients with cardiovascular disease is in line with our ASAP findings. To investigate whether lowering LDL-C below the National Cholesterol Education Program (NCEP) II criterion for secondary prevention of 100mg/dL would further reduce the burden of atherosclerotic disease, the effects of atorvastatin 80mg and pravastatin 40mg on carotid IMT were compared.
Atorvastatin reduced LDL-c by 49% to 76(±23)mg/dL; pravastatin with 27% to 110(±30)mg/dL. In the pravastatin group IMT stabilized; in the atorvastatin group the IMT decreased and showed atherosclerosis regression (p=0.03). Possibly, further LDL-c lowering, even beyond present guidelines, has a favourable effect on arterial walls and consequently the occurrence of future cardiovascular disease.

**IMT as a validated endpoint for atherosclerotic vascular disease**

Considering all of the above, intima-media thickness (IMT) measurements acquired by means of B-mode ultrasound imaging of carotid and femoral arterial walls meet all validity criteria of a surrogate marker. Moreover, B-mode ultrasound can provide data on peripheral arterial wall thickness in all stages of atherosclerosis. As was shown in prospective epidemiological studies already a modest increase of IMT substantially increases the relative risk for myocardial infarction and stroke. IMT measurements have also shown the benefit of cholesterol lowering and anti-hypertensive compounds. In particular, in one of these studies (the REGRESS trial) it was observed that statins have an impact on coronary lumen, IMT of carotid and femoral arteries and clinical cardiovascular events in a similar direction. Therefore, IMT has now been accepted by most as a validated surrogate marker for atherosclerotic vascular disease.

![Figure 1](image_url)

**Figure 1.** A 2x2cm B-mode ultrasound digital still image of the common carotid artery and its adjacent structures as depicted by a 5-10MHz linear array transducer. The white triangle on top is the sternocleidomastoid muscle; the black triangle over the common carotid is the jugular vein. The common carotid near and far arterial walls are clearly shown. The common carotid segment is defined as the arterial wall proximal (to the right) of the carotid dilatation (small white arrow). The lumen-intima and the media-adventitia interfaces.
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A standardized B-mode ultrasound imaging and image analysis protocol
To fully exploit the potential of ultrasound imaging in atherosclerosis research, ideally, standardized and strictly implemented imaging protocols should be used both in observational studies and in applied clinical research. The ultrasound protocol summarized below has been successfully applied in such studies and in atherosclerosis regression trials. The dynamic changes in the process of atherosclerosis, whether due to genetic or environmental factors or due to lipid lowering by drug treatment, were entered into a model. In our view, standardization of image acquisition provides complementary observational and trial data. In observational studies and lipid lowering intervention trials hundreds of subjects may be included, often in multi-center settings. Consequently, large numbers of images are generated. This necessitates a protocol that has been designed for efficiency of image acquisition and image analysis. In this B-mode ultrasound protocol, the common carotid, the carotid bulb, the internal carotid, the common femoral and the superficial femoral arterial far wall segments are scanned bilaterally. Acuson 128XP ultrasound instruments (Acuson, Moutainview, CA, USA) equipped with 5-10MHz linear array broadband L7 transducers and Extended Frequency (EF) software are used. A standard view of 2 by 2 centimeters is imaged. Images are saved as 4:1 compressed JPEG files. These image files are approximately 150k each; a file size that can be easily transferred over the internet (Figure 1). Image

Figure 2. The ultrasound digital still images are imported as 4:1 compressed JPEG files into a dedicated software program (etrack). In the image a region of interest is identified by the reader. The IMT of this particular arterial far wall is 0.621 ± 0.065 mm (Courtesy Dr W.J. Stok, MD and Dr J.M. Karemaker MD PhD, Departments of Vascular Medicine and Physiology, Academic Medical Centre, Amsterdam, The Netherlands).
acquisition and image analyses have been digitized according to DICOM standards.\(^{33}\) This approach allows for high quality ultrasound image capture,\(^{34}\) the ability to control measurements from image acquisition to manuscript writing, and conform with and anticipate on regulatory guidelines for drug trials using image based measurements as endpoints. In clinical research, image analyses are performed in the controlled environment of an ultrasound core laboratory, where validated hardware and software should be used. The associated off-line measurement is illustrated in Figure 2. Output of the image analysis consists of a textfile with demographic, quality assessment and measurement data as well as an associated 7k sized control JPEG image file which shows how the measurement was done (Figure 3). Standardized image acquisition is a key issue in arterial wall imaging studies. The use of digital imaging techniques and the internet allows for a more user friendly study environment. This can be illustrated by data comparing patient populations known to be at a high risk for cardiovascular disease and unaffected healthy people at low risk.

Standardized IMT measurements in those at high cardiovascular risk

The ultrasound protocol described above was used to investigate carotid and femoral IMT in patients with FH and unaffected siblings. FH is a common autosomal dominant disorder of lipoprotein metabolism, affecting approximately 1 in 400 individuals in the Netherlands.\(^{35}\) Patients have elevated levels of low-density lipoprotein cholesterol (LDL-C) due to mutations in the LDL-receptor gene. As a result of the excessively high LDL-C levels FH-patients are at very high risk for premature cardiovascular disease (CVD).\(^{36}\) Presently, CVD risk due to FH can only be reduced by lipid-lowering agents. This need for lipid lowering therapy and the age that treatment should be started is an important question for parents with an FH child.

Table 1. Clinical and Biochemical Characteristics of Unaffected Controls and FH subjects

<table>
<thead>
<tr>
<th>Population samples</th>
<th>Adolescents Controls</th>
<th>Mid-Aged Controls</th>
<th>Seniors, Controls</th>
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<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>26</td>
<td>48</td>
</tr>
<tr>
<td>Gender, Male/Female</td>
<td>22/22</td>
<td>11/15</td>
<td>24/24</td>
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<tr>
<td>Age (SD), years</td>
<td>14.9(2.8)</td>
<td>34.5(9.6)</td>
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<td>Total Cholesterol, mmol/L</td>
<td>4.2(0.7)</td>
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<td>5.9(1.1)</td>
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<td>LDL-C, mmol/L</td>
<td>2.5(0.6)</td>
<td>2.7(0.9)</td>
<td>3.9(1.1)</td>
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<tr>
<td>HDL-C, mmol/L</td>
<td>1.4(0.3)</td>
<td>1.5(0.4)</td>
<td>1.3(0.4)</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>0.8(0.3)</td>
<td>1.3(0.4)</td>
<td>1.7(0.24)</td>
</tr>
<tr>
<td>IMT(SD), mm</td>
<td>0.53(0.03)</td>
<td>0.59(0.07)</td>
<td>0.77(0.12)</td>
</tr>
</tbody>
</table>

Major demographic and lipid characteristics of three unaffected groups and three groups with familial hypercholesterolemia (FH). IMT's were calculated as the population means of the per subject combined and averaged carotid and femoral IMT measurements. IMT=Intima-Media Thickness, LDL=low-density lipoprotein, HDL=high-density lipoprotein, C=cholesterol, SD=Standard Deviation.
Figure 3. The software program provides IMT data as well as an associated control JPEG image of 7K with every arterial wall thickness measurement. These images allow for sonography and image analysis quality control and quality assurance issues.

To estimate atherosclerosis progression from childhood into seniority in controls and in FH subjects, we used cross-sectional standardized IMT measurements in six unaffected and affected age groups. Since measurements were standardized we could extrapolate atherosclerosis progression estimates from these cross-sectional data for each of the groups and, consecutively, for the combined groups as a whole. This approach does not obviate the need for longitudinal studies and clinical endpoint data, but it does circumvent the need to perform lifelong studies to describe vascular wall changes and

<table>
<thead>
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<th>Adolescents</th>
<th>Young Adult</th>
<th>Older Adult</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FH</td>
<td>FH</td>
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<tr>
<td></td>
<td>44</td>
<td>23</td>
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<td></td>
<td>23/21</td>
<td>12/11</td>
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<td>14.8(2.8)</td>
<td>28.5(8.8)</td>
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<td></td>
<td>7.8(1.9)</td>
<td>7.5(1.8)</td>
<td>9.6 (1.9)</td>
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<td>6.1(1.8)</td>
<td>5.5(1.7)</td>
<td>7.6(1.9)</td>
</tr>
<tr>
<td></td>
<td>1.2(0.3)</td>
<td>1.3(0.4)</td>
<td>1.2(0.3)</td>
</tr>
<tr>
<td></td>
<td>1.0(0.3)</td>
<td>1.6(0.6)</td>
<td>1.9(0.9)</td>
</tr>
<tr>
<td></td>
<td>0.55(0.05)</td>
<td>0.65(0.08)</td>
<td>0.86(0.18)</td>
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</table>
substantiates the need for preventive measures in those at high cardiovascular risk. The clinical and demographic characteristics of the FH subjects and controls are given in Table 1. Carotid and femoral IMT was measured in all subjects and combined to a per subject average. First, for each dataset characteristics of arterial walls with age and vascular risk were investigated. In children at the age of 10 mean combined carotid and femoral IMT of FH and controls was similar (both 0.53(SD)±0.03mm: p>0.15). Then, the IMT measurements were extended till the age of 76 to estimate atherosclerosis progression from childhood into old age. For the scatter of each dataset Lowess splines were used (Figure 4, black dotted lines). These splines consequently indicated a similar linear IMT increase with age in all of the six populations. Second, the data of FH (0.79 ± 0.20 mm, range 0.45-1.53 mm) and unaffected populations (0.63 ± 0.14 mm, 0.48 -1.14 mm) were pooled into two datasets. The FH group consisted of 315 subjects (age range 11-67 years; LDL-C 7.2 ±1.8 mmol/L); the control group consisted of 118 unaffected subjects (age range 11-76 years; LDL-C 3.1 ± 0.8

Figure 4. Arterial wall thickness progression estimates in individuals heterozygous for FH (LDL-C 7.2 ± 2.0 mmol/L, 0.79 ± 0.20, range 0.45-1.53 mm) and healthy controls (LDL-C 3.4 ± 0.8 mmol/L; 0.63 ± 0.14, 0.48-1.14 mm, blue dots). For the pooled FH and the pooled control data (blue line) IMT increase was estimated by linear regression (with respective 95% CI’s in grey lines). On average, healthy controls reach an IMT of approximately 0.8mm at age 80, where FH-subjects reach this value (and, if untreated, often their first cardiovascular symptoms) around the age of 40.
mmol/L). We then applied an overall linear regression analysis to estimate IMT increase with age between groups and found that IMT increase with age was at least twice as large in FH as in controls (0.009 and 0.004 mm/year). The mean differential IMT change between FH and controls was 0.005 mm/year (p<0.001)(Figure 4).

**Discussion**

These observations led us to estimate that on average an healthy individual reaches an IMT of 0.78 mm at the age of 76 years. In FH individuals this IMT is already reached at the age of 37 years. Moreover, as can be observed from the scatterplots of Figure 4, although statistically correct, linear regression is a poor representation of the chaos (and the accompanying symptoms of disease) of arterial walls that emerges once an arterial wall thickness of approximately 0.8 mm is reached. In our opinion, it is precisely that stage of arterial wall chaos that should be. Our analyses indicate rapid atherosclerosis progression in FH individuals and are in line with the fact that CAD will manifest before the age of 50 in many FH individuals. This IMT graph therefore not only illustrates the opportunity to study effective new drugs in populations such as FH, but also shows the need for primary prevention in young individuals with the disorder. The short time slot available for disease prevention in FH individuals emphasizes the need to modify atherosclerosis progression at a very early time point in life.

Intima-media thickness (IMT) measurements can accurately describe the process of arterial wall changes due to atherosclerosis from coherence to chaos as a continuous *in-vivo* variable. IMT measurements can provide information on apparently healthy and at risk populations. Also, IMT measurements can provide data on efficacy of novel lipid modifying medications. It may therefore be concluded that IMT measurements are a validated surrogate endpoint for atherosclerosis and vascular disease risk. Lastly, if IMT outcome is to be used as an argument in discussions on whether or not to apply preventive measures in presumed at risk populations, and, whether the results of therapeutic response of drugs in populations are valid, the strength of the argument is better supported if the IMT measurements are performed in a standardized environment.

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