Carotid intima-media thickness

Studies into methodological aspects

Soner Doğan

Carotid Intima-media thickness: studies into methodological aspects.

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Carotid intima-media thickness: studies into methodological aspects

Intima-media dikte van de arteria carotis: studies naar methodologische aspecten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
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Promotor:	Prof.dr. D.E. Grobbee
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A person who knows all the answers, has an opinion on everything, has a certainty backed up by rational argument, has very little possibility of further progress. Such a person is unlikely to walk away from a discussion with anything more than a reaffirmation of how right he or she has been all along.

Creativity involves breaking out of established patterns in order to look at things in a different way.

Edward de Bono

Voor mijn ouders

Manuscripts based on the studies presented in this thesis

Chapter 2
S Dogan, Y Plantinga, JM Dijk, Y van der Graaf, DE Grobbee, ML Bots, on behalf of the SMART study group*. Online common carotid intima-media thickness measurements: a direct comparison of risk factor relations and associations with future events. *In review: Ultrasound in Medicine and Biology*

Chapter 3
S Dogan, DE Grobbee, JJP Kastelein, ML Bots. Mean common or mean maximum carotid intima-media thickness as primary outcome in lipid lowering trials. *Submitted*

Chapter 4
S Dogan, R Duivenvoorden, DE Grobbee, JJP Kastelein, CL Shear, WT Duggan, GW Evans, FLJ Visseren, ML Bots, on behalf of the RADIANCE 1 & 2 study group*. Completeness of carotid intima media thickness measurements depends on body composition: findings in the RADIANCE 1 and 2 study. *Submitted*

Chapter 5.1
S Dogan, Y Plantinga, GW Evans, R Meijer, W Riley, DE Grobbee, ML Bots, on behalf of the OPAL investigators*. Ultrasound protocols to measure carotid intima-media thickness; a comparison of reproducibility and rate of progression in healthy post-menopausal women, the OPAL study. *In review: Curr.Med.Res. Opin.*

Chapter 5.2
S Dogan, Y Plantinga, JR Crouse, GW Evans, JS Raichlen, DH O'Leary, MK Palmer, DE Grobbee, ML Bots, on behalf of the METEOR Study Group*. Ultrasound protocols to measure carotid intima-media thickness; a comparison of reproducibility, rate of progression and treatment effect in asymptomatic subjects with mild to moderate subclinical atherosclerosis, the METEOR study. *Submitted*

Chapter 5.3
S Dogan, R Duivenvoorden, DE Grobbee, JJP Kastelein, CL Shear, WT Duggan, GW Evans, FLJ Visseren, ML Bots, on behalf of the RADIANCE 1 & 2 study group*. Ultrasound protocols to measure carotid intima-media thickness; a comparison of reproducibility, rate of progression and treatment effect in subjects with familial hypercholesterolemia and subjects with mixed dyslipidemia, the RADIANCE 1 and RADIANCE 2 study. *Submitted*

Chapter 6
S Dogan, J Durga, GW Evans, FJ Kok, P Verhoef, DE Grobbee, ML Bots. Effect of batch reading on carotid intima-media thickness progression in randomized controlled trials. *Submitted*

Chapter 7
ML Bots, MK Palmer, S Dogan, Y Plantinga, JS Raichlen, GW Evans, DH O'Leary, DE Grobbee, JR Crouse, on behalf of the METEOR Study Group*. Intensive lipid lowering reduces progression of carotid atherosclerosis within 12 months of treatment: The METEOR study. *Submitted*

* members of the studygroups are listed in the appendix

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Chapter 1 :

General introduction

General introduction

Carotid intima-media thickness (CIMT) is a non-invasive alternative marker of atherosclerotic disease that has been used extensively since 1987 [1]. CIMT is defined as the distance between the lumen-intima interface, which corresponds to the inner and outer echogenic lines seen on the B-mode ultrasound image. Increased CIMT has consistently been shown to predict future vascular events [2,3]. In addition, change in CIMT over time is currently used in randomized controlled trials (RCTs) as an alternative (surrogate) end point for cardiovascular events to evaluate the efficacy of interventions [4-14] because of its advantage of considerable reductions in sample size and duration of follow-up in comparison to traditional morbidity-mortality event trials. Despite of the long history and its frequent use there is little uniformity in the measurement of CIMT. There are no accepted standardized CIMT measurement protocols that are based on published evidence. Choice of ultrasound protocols and measurement approaches are based generally on expert opinion and personal experience rather than on evidence. This is caused mostly by the absence of published information on methodological issues concerning CIMT measurements that could provide guidance. This diversity of ways to measure CIMT may have substantial effects on the published results of studies and on the interpretation thereof.

There are many different approaches to obtain CIMT data and a CIMT value per individual. First of all approaches vary in the definition and selection of primary and/or secondary outcome measure(s) which define the ultrasound protocol. Furthermore there is variability in the CIMT measurement procedure, which can roughly be divided in two different stages: acquisition of ultrasound CIMT data and the actual quantification of CIMT. Variability in the acquisition stage exists in choice of the ultrasound protocol (which segments, walls and angles of the carotid artery does one want to measure). The actual quantification can be performed in many ways. CIMT measurements can be performed at the same time as the ultrasound scan is performed (online measurement) or after performing the ultrasound scan (off line measurement). Online measurements can be done manually by the sonographer or through an automated edge detection program. Off line measurements can be done over a long time period (non-batch reading approach) or in a short time span (batch reading approach).

When a study, either an intervention or an observational study, with CIMT as a primary outcome measure is designed, these features all may have effects on the conduct, the size of the study, the precision of the CIMT measure, and the ability to show relations of interest.

Rationale of this thesis

The aim of this thesis is to provide more insight on different methodological aspects of CIMT measurements to facilitate an evidence-based decision making with respect to CIMT measurements when designing a study that makes use of it as an outcome measure.

Outline of this thesis

In **chapter 2,** we compare two techniques, an automated RF approach and a manual B-mode approach to measure CIMT. The comparison is performed on reproducibility, risk factor relationship and association with future cardiovascular events. In **chapter 3** the pros and cons of two different CIMT assessments as primary end points in trials on lipid lowering drugs, i.e., the mean common CIMT and the mean maximum CIMT, are considered. In **chapter 4** we describe the completeness of CIMT data that can be achieved with an ultrasound protocol in a population with familial hypercholesterolemia and in a population with mixed dyslipidemia and try to answer the question to what extent the amount of missing measurements are related to angles, segments and walls of several ultrasound protocols.

The relation between the extensiveness of an ultrasound protocol (number of walls, segments and angles measured) and the reproducibility, progression rates of CIMT and treatment effects is examined in **chapter 5**. Firstly, in a population of healthy postmenopausal women (**chapter 5.1**, the OPAL study), secondly in a low-risk population with subclinical atherosclerosis (**chapter 5.2,** the METEOR study) and finally in a population of subjects with familial hypercholesterolemia and a population of subjects with mixed dyslipidemia (**chapter 5.3**, the RADIANCE 1 and RADIANCE 2 study). In **chapter 6**, the implications of batch-reading and non-batch reading of CIMT images on progression rates and treatment effect are discussed. The time period that is needed in a lipid lowering trial to observe treatment effect is studied in **chapter 7**. Finally in **chapter 8** we summarize the results of the studies that are performed in this thesis and put them in perspective.

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Chapter 2

Online common carotid intima-media thickness measurements:
manual B-mode versus automated Radio-Frequency measurements

Abstract

Background

Carotid intima-media thickness (CIMT) serves as an indicator of atherosclerosis and cardiovascular risk in observational and intervention studies. Manual measurements of B-mode ultrasound images with manual tracing or automated-edge-detection programs are the most applied methods. Automated measurements with radio-frequency (RF) are suggested as an alternative. We compared these methods in terms of risk-factor relations and associations with future events.

Methods

Data from participants of the SMART-study was used. Far wall common CIMT was measured with manual B-mode and automated RF. Detailed risk-factor information was obtained. All participants were followed for occurrence of vascular events (mean follow-up 2.1 years). CIMT was related to risk factors with linear-regression models and to future events with Cox-Proportional-Hazards models.

Results

Data were available for 2146 participants. Agreement between the two methods was modest (Intraclass correlation coefficient = 0.34). Risk factor relations with age and systolic blood pressure were stronger for B-mode than for RF. Association with future events (Hazard Ratios) was better for B-mode than for RF: vascular death (1.27 vs. 1.00) and ischemic stroke (1.45 vs. 1.03). After selecting participants with thinner CIMT (< 0.9 mm) RF showed a stronger association with future events than B-mode: all events (1.59 vs. 1.09), vascular death (1.72 vs. 0.93) and coronary ischemic events (1.65 vs. 1.05).

Conclusion

The choice for either B-mode or RF measurements in research is partly driven by type of study-population, expected presence of local atherosclerotic abnormalities, and main aim of the study (risk-factors or events). Given our findings, in higher-risk populations a B-mode approach should be preferred, whereas in relatively more healthy populations, with thinner common CIMT, the automated RF approach is the method of choice.

Introduction

Carotid intima-media thickness (CIMT) is widely used in observational and intervention studies to study determinants of atherosclerosis and its consequences for cardiovascular events [1-4]. In addition, change in CIMT over time is currently used as an alternative for cardiovascular events as primary outcome in intervention studies [5-15]. The approaches to measure CIMT can be classified generally in two major categories: manual versus automated measurements. Manual measurement can be subdivided in off-line and online measurements. In off-line measurements B-mode ultrasound images are stored on videotape or a digital medium. The CIMT measurements are performed later, with either manual tracing or an automated edge detection program [16]. In routine clinical practice this approach may seem elaborate and time-consuming. Alternatively, in clinical practice CIMT can be measured manually and online with calipers of the ultrasound machine which provides a direct estimate of CIMT [17]. Disadvantages of manual measurements are that it can be relatively time-consuming. Furthermore reader subjectivity due to personal interpretation of the lumen-intima and media-adventitia borders on the B-mode image, reflectivity of the structures, gain setting and compression characteristics of the ultrasound system are other disadvantages. A technically different approach is the M-mode ultrasonography, an on-line automated measurement approach which uses radiofrequency (RF) signals [18]. In this approach, the diameter and relative increase in cross-sectional area are obtained with a wall track system which processes the raw RF signals that are received along a single line of observation (M-line processing). The RF measurements are performed in the distal common carotid artery 2 cm proximal to the origin of the carotid bulb [17,19,20]. Automated RF is more time efficient and involves a minimum of user interaction. RF calculates mean IMT locally over a cardiac cycle instead of mean IMT over a 10-mm segment at end-diastole. With RF simultaneous assessment of arterial wall properties such as distensibility can be obtained at the same location, facilitating detailed study of the intrinsic wall characteristics [21]. Variability in CIMT due to sonographers in the RF approach remains but the reproducibility of the automated RF approach has been shown to be good, as reported in previous studies [18,22,23].

Manual B-mode and automated RF imaging provide simple methods for measuring common CIMT. Both techniques are widely available, cheap, easy to operate and therefore suitable for the use in trials and observational studies. Furthermore both approaches can be performed on-line on one ultrasound machine. Despite differences in technique and location of measurement, the end result of the two techniques (common CIMT) is a surrogate marker which can be used to rank subjects in categories of vascular risk in the same way as other measures like intra vascular ultrasound (IVUS), coronary calcification scores, ankle brachial index or even more basic parameters as blood pressure and cholesterol levels. Up until now it is unclear which approach ranks subjects best in high and low CIMT order and provides the most adequate CIMT information, i.e. a CIMT-value that relates strongest to risk factors and to future risk of vascular events. As far as we know, no direct comparison for these relationships has yet been performed between B-mode and RF measured CIMT. So we set out to study these aspects using CIMT data collected in routine clinical practice in high-risk patients with two different commercially available ultrasound systems that use either the manual or the automated approach.

Materials and methods

Study population

Data were used of participants of the SMART study (Second Manifestations of ARTerial disease), an ongoing prospective single-center cohort study in subjects with manifest arterial disease and / or with cardiovascular risk factors. Persons aged 18 – 80 years that were newly referred to the University Medical Center Utrecht (UMC Utrecht) for treatment of clinical manifestations of atherosclerosis (transient ischemic attack, ischemic stroke, peripheral arterial disease (PAD), abdominal aortic aneurysm (AAA), coronary heart disease (CHD)) or who were otherwise at high risk of developing symptomatic arterial disease (diabetes mellitus, hypertension, hyperlipidemia) filled out a questionnaire and underwent vascular screening, blood chemistry and ultrasonography. Written informed consent was obtained from all participants. The study was approved by the medical ethics committee of the UMC Utrecht. The rationale and design of the SMART study have been described in detail elsewhere [17]. For the current study we used data of the first consecutive 2545 patients with manifest arterial disease that were enrolled in the SMART study up until March 1 2003. After that date no measurements with the RF were performed. Of 156 participants, B-mode values were missing due to equipment failure or logistic reasons. Of 243 participants RF values were missing. Finally, data of 2146 participants with both B-mode as RF values were available for analyses. We performed analyses on the total population and on subpopulations with different threshold values for B-mode CIMT. Results for the subpopulation with CIMT < 0.9 mm are presented. Threshold values were based on B-mode measured CIMT values as this method has no upper limit for measuring CIMT.

Vascular risk screening

Vascular risk screening was conducted on a single day at the UMC Utrecht. Blood samples were collected after an overnight fast. Glucose, total cholesterol, triglycerides and HDL-cholesterol were measured. LDL-cholesterol was calculated by use of Friedewald's formula [24]. Height and weight were measured without shoes and heavy clothing, hip to waist ratio and body mass index (BMI) were calculated. Carotid arterial stiffness was measured with a Wall Track System (Scanner 200, Pie Medical, Maastricht, The Netherlands) equipped with a 7.5 MHz linear array transducer and vessel wall moving detector system [25]. Blood pressure was measured in supine position at the right brachial artery every 4 minutes during the arterial stiffness measurement with a semi-automatic oscillometric device (Omega 1400, Invivo Research Laboratories Inc., Broken Arrow, OK, USA). Medical history, use of current medication and current and past cigarette smoking behavior were derived from a questionnaire as described in detail elsewhere [17,19,20].

Follow-up

Participants were biannually asked to complete a questionnaire on hospitalizations and outpatient clinic visits. The endpoints of interest were ischemic stroke, coronary ischemic events, vascular death and the composite of all vascular events. Definitions of the events have been described previously [17,19,20]. If participants or family recorded such an event, hospital discharge letters and results of relevant laboratory and radiology examinations were retrieved. On the basis of this information all events were

audited by three members of the Outcome Event Committee (OEC). In case of disagreement, the opinion of other members of the OEC was sought and final adjudication was based on majority of classifications obtained.

Ultrasound, online B-mode

The left and right common carotid arteries were examined in anterolateral, posterolateral and mediolateral direction, with an ATL Ultramark 9 (Advanced Technology Laboratories, Bethel, WA, USA), equipped with a 10 MHz linear array transducer. Measurements were performed on the far wall 1 cm proximal to the beginning of the dilation of the carotid bulb [17]. In short, the right carotid artery was scanned in three angles. The sonographer traced the leading edges corresponding to the transition zones between lumen-intima and media-adventitia over a length of 1 cm proximal to the carotid bulb. Total intima-media surface of this selected area was calculated automatically. The same was done for the left side. The mean CIMT of the six measurements in each participant was calculated. An interobserver variability study on manual CIMT measurements among 25 participants performed in this cohort showed a coefficient of variation of 11.7% and an intra-observer variability of 7.7%[26].

Ultrasound, on-line automated RF

Automated RF measurements were performed with the Wall Track System, Pie Medical Scanner 200, a commercially available ultrasound system. CIMT measurements were retrieved from measurements performed to assess arterial stiffness. The measurements were performed in the far wall of the distal common carotid artery 2 cm proximal to the origin of the carotid bulb [17,19,20]. In short, at the right carotid artery 5 measurements were performed of each 4 seconds (equal to several cardiac cycles, depending on the heart rate of the subject in resting state). The vessel movement detector system registered for each subsequent cardiac cycle the change in arterial diameter and end-diastolic diameter and the common CIMT of the far wall. First the CIMT within a single measurement was averaged. Next the results of the 5 assessments were averaged. The same procedure was used for the left carotid artery. In the same reproducibility study among 25 participants performed in this cohort the inter-observer variability showed a coefficient of variation of 19.1% and an intra-observer variability of 14.3% for the arterial stiffness measurements [27].

Data analysis

Means and standard deviations (SD) of the B-mode and automated RF values were computed and reported for descriptive variables. Univariate correlation coefficients were determined (Intraclass correlation coefficients (ICC)) to describe the relation between the two methods. We constructed cross tables to assess agreement and ranking of both methods after division in tertiles and next calculated kappa's (measure of agreement). Linear regression analysis models were used to estimate beta coefficients and 95% confidence intervals for the relation of common CIMT (both B-mode and automated RF) as independent variable with vascular risk factors as dependent variable. The model used was:

”risk factor” = alpha + beta1*CIMT + beta2*age + beta3*sex

In etiologic analyses CIMT is usually used as a dependent variable and ‘risk factors’ as independent variables. In this study we used ‘risk factor’ as dependent variable and ‘CIMT’ as independent variable. The reason is that betas of a regression model heavily depend on the distribution of the dependent variable. If the distributions of dependent variables (B-mode and RF) differ too much, beta coefficients will differ due to differences in distribution and a direct comparison cannot be made. The distributions of the two methods were considerably different. Attempts to obtain more equal distributions by several transformations (logarithmic rescaling, rescaling into z-scores) were not successful. Hence we studied the relation per standard deviation increase of CIMT. R squared (R^2) was calculated assessing the proportion of variation in dependent variable (risk factor) explained by the independent parameters (CIMT, age and sex). Cox proportional hazards models were used to estimate hazards ratios (HR) and 95% confidence intervals for the occurrence of cardiovascular events during follow-up associated with an increase of one standard deviation of B-mode CIMT and of one standard deviation of RF CIMT. The follow-up (FU) period used for the survival analyses was the period 1996 – march 2003. If participants had multiple events, the first event was used in analyses. Additional analyses, based on different CIMT ranges were performed. If the beta or the HR of one method was not present in the 95% CI of the beta or the HR of the other method, we concluded significant difference in strength of association. Statistical analyses were performed with the statistical package SPSS (version 12.0.1 for Windows).

Results

Baseline characteristics of the study population are given in table 1. The total number of participants was 2146. Mean age was 55.2 years (SD 12.7), 67% of the participants were aged 50 years and over, 70% was male and 25% had diabetes. B-mode CIMT ranged from 0.38 to 4.52 mm, with a mean of 0.88 mm (SD 0.30). RF CIMT ranged from 0.34 to 1.32 mm, with a mean of 0.64 mm (SD 0.12).

Table 1: Baseline characteristics of the study-population (n=2146)

Men (%)	70
Age (years)	55.2 (12.7)
Systolic blood pressure (mm. Hg.)	138 (19)
Diastolic blood pressure (mm. Hg.)	80 (10)
Triglycerides (mmol/l.)	2.01 (1.44)
Cholesterol (mmol/l.)	5.51 (1.22)
HDL cholesterol (mmol/l.)	1.21 (0.38)
LDL cholesterol (mmol/l.)	3.43 (1.11)
Hemoglobin (mmol/l.)	8.9 (0.8)
Diabetes mellitus* (%)	25
Body mass index (kg./m.²)	26.5 (4.2)
Hip to Waist-ratio	0.91 (0.08)
Current smoking (%)	31
Ever smoking (%)	73
B-mode measured common CIMT (mm.)	0.88 (0.30)
RF measured common CIMT (mm.)	0.64 (0.12)

Data are mean (standard deviation) or %, CIMT = Carotid Intima Media Thickness;
* glucose lowering medication, fasting glucose ≥ 7.0 mmol/l or non-fasting glucose ≥ 11.1 mmol/l

The correlation between RF and B-mode measured CIMT was poor for the total population (ICC = 0.34), but improved slightly for subjects with thinner CIMT values. ICC for subjects with CIMT < 0.9 mm was 0.50. Agreement in ranking into high versus low CIMT by both methods was poor with a kappa of 0.15. Selection of a population with thinner CIMT did not improve the agreement.

Stronger relations were found for age and systolic blood pressure with B-mode CIMT (beta 6.05 and 2.80 respectively) as compared to the RF (beta 3.52 and 1.93 respectively) (table 2). For pack years of smoking and body mass index statistically significant associations were found with B-mode CIMT that were not seen with RF (betas 2.55 and 0.20 versus 1.52 and -0.12, respectively). Betas of these variables for B-mode were not present in the 95% confidence interval (95% CI) of the RF, suggesting significant differences in strength of relations. Total explained variance (R^2) was 23 % for age in B-mode CIMT and 10 % for RF. For all other variables the R^2 of B-mode CIMT and automated RF approach was more or less equal.

During a mean follow up of 2.1 years (range: 0.0 to 5.0 years) 98 participants experienced a new vascular event (table 3), of whom 46 died from a vascular cause. In regression models adjusted for age and sex (model I) increase in B-mode CIMT per standard deviation of 0.30 mm was significantly related to increased risk of any vascular event, vascular death and ischemic stroke. Increase in B-mode CIMT was also related to increased risk of coronary ischemic events, but this relation did not reach the level of statistical significance. RF measured CIMT (per standard deviation of 0.12 mm) showed no statistically significant relations with all events, ischemic stroke or coronary ischemic events. After additional adjustment for vascular risk factors known to be associated with an increased CIMT and with cardiovascular risk (model II: age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, ever smoking and use of antihypertensive medication at baseline), the strength of the relations attenuated. HR for vascular death and for ischemic stroke of RF CIMT were not present in the 95% CI of the B-mode, so B-mode was stronger related with vascular death and ischemic stroke than RF measured CIMT.

After selection of participants with B-mode CIMT < 0.90 mm, the remaining number of subjects was 1374. The magnitude of the relations for age and systolic blood pressure with B-mode CIMT did not change substantially (beta 6.47 and 2.55, respectively) but became weaker with the RF approach (beta 2.81 and 0.90 respectively) (table 2). For LDL-cholesterol, a stronger relation was found with the RF CIMT approach (beta 1.38 versus B-mode 0.58).

38 Participants experienced a new vascular event, of which 15 died from a vascular cause (table 3). In regression models adjusted for age and sex increase in B-mode CIMT was related to increased risk of all vascular events, ischemic stroke and coronary ischemic events. Increase in RF CIMT was related to increased risk of all events, vascular death, ischemic stroke and coronary ischemic events. These relations were all stronger for RF than for B-mode except for ischemic stroke. For ischemic stroke B-mode was stronger associated than RF (HR 3.16 and HR 1.40 respectively) however the HR of RF was present in the 95%CI of the HR of the B-mode. RF CIMT was stronger related to all events, vascular death and coronary ischemic events than B-mode CIMT, with absence of HR of B-mode in the 95% CI of RF.

Table 2: Risk factor relations of B-mode and RF measured CIMT for the total population (n = 2146) and for subjects with a CIMT < 0.9 mm (n=1374)

Total population

	B-mode measured CIMT				*Automated RF measured CIMT*			
	Regression coefficient	95% CI	R2	p-value	Regression coefficient	95% CI	R2	p-value
Age	6.12	(5.64;6.59)	0.23	< 0.01	3.53	(3.01;4.05)	0.08	< 0.01
Age (corrected for sex)	6.05	(5.58;6.53)	0.23	< 0.01	3.52	(3.01;4.04)	0.09	< 0.01
Corrected for age and sex								
Systolic blood pressure (mm. Hg)	2.80	(1.93;3.67)	0.12	< 0.01	1.93	(1.14;2.72)	0.11	< 0.01
Diastolic blood pressure (mm. Hg)	0.02	(-0.47;0.50)	0.02	0.95	0.46	(0.02;0.90)	0.02	0.04
Cholesterol (mmol/dl.)	0.40	(-0.19;0.99)	0.02	0.18	0.89	(0.36;1.43)	0.03	< 0.01
Triglycerides (mmol/dl.)	-0.35	(-1.05;0.34)	0.01	0.32	-0.45	(-1.08;0.19)	0.01	0.17
HDL cholesterol (mmol/dl.)	-0.13	(-0.30;0.04)	0.11	0.14	-0.09	(-0.24;0.07)	0.10	0.29
LDL cholesterol (mmol/dl.)	0.60	(0.06;1.14)	0.01	0.03	1.03	(0.53;1.53)	0.02	< 0.01
Pack years smoking	2.55	(1.57;3.54)	0.10	< 0.01	1.52	(0.64;2.40)	0.09	< 0.01
Body Mass Index (kg/m²)	0.20	(0.00;0.40)	0.01	0.05	-0.12	(-0.31;0.06)	0.00	0.20
Hip to Waist-ratio*	0.06	(0.02;0.09)	0.29	< 0.01	0.03	(-0.00;0.06)	0.29	0.07

Subjects with CIMT < 0.9 mm

	B-mode measured CIMT				*Automated RF measured CIMT*			
	Regression coefficient	95% CI	R^2	p-value	Regression coefficient	95% CI	R^2	p-value
Age	6.47	(5..90; 7.03)	0.27	< 0.01	2.81	(2.17;3.45)	0.05	< 0.01
Age (corrected for sex)	6.42	(5.85;6.98)	0.27	< 0.01	2.81	(2.17;3.44)	0.06	< 0.01
Corrected for age and sex								
Systolic blood pressure (mm. Hg)	2.55	(1.51;3.58)	0.09	< 0.01	0.90	(-0.02;1.81)	0.07	0.06
Diastolic blood pressure (mm. Hg)	0.69	(0.08;1.30)	0.03	0.03	0.18	(-0.35;0.72)	0.03	0.50
Cholesterol (mmol/dl.)	0.75	(-0.04;1.53)	0.02	0.06	1.08	(0.40;1.76)	0.03	< 0.01
Triglycerides (mmol/dl.)	0.28	(-0.71;1.26)	0.01	0.58	-0.66	(-1.51;0.20)	0.01	0.13
HDL cholesterol (mmol/dl.)	-0.11	(-0.34;0.11)	0.14	0.32	-0.18	(-0.37;0.02)	0.14	0.08
LDL cholesterol (mmol/dl.)	0.58	(-0.17;1.33)	0.01	0.13	1.38	(0.74;2.02)	0.02	< 0.01
Pack years smoking	1.76	(0.63;2.89)	0.09	< 0.01	1.75	(0.78;2.72)	0.09	< 0.01
Body Mass Index (kg/m²)	0.42	(0.14;0.69)	0.01	< 0.01	-0.24	(-0.48;0.00)	0.00	0.05
Hip to Waist-ratio*	0.05	(-0.00;0.09)	0.28	0.07	0.05	(0.00;0.09)	0.28	0.04

Regression coefficients per standard deviation (SD) increase in CIMT (SD total population: B-mode 0.30 mm, RF 0.12 mm; SD subjects with CIMT < 0.9 mm : B-mode 0.11 mm, RF 0.11 mm).

* Hip to Waist-ratio multiplied by 10, performed for more easy interpretable results. CIMT as independent variable i.e. “risk factor” = constant + CIMT*beta1 + age*beta2 + sex*beta3

Table 3: Association of B-mode and RF measured CIMT with future events for the total population (n=2146) and for subjects with a CIMT < 0.9 mm (n=1374)

Total population

			B-mode measured CIMT			RF measured CIMT		
	Model	# events	HR	(95% CI)	p-value	HR	(95% CI)	p-value
All events	I	98	1.20	(1.03;1.39)	0.02	1.14	(0.95;1.37)	0.16
	II		1.10	(0.91;1.33)	0.31	1.09	(0.90;1.32)	0.39
Vascular death	I	46	1.27	(1.05;1.54)	0.01	1.00	(0.77;1.31)	0.98
	II		1.16	(0.90;1.49)	0.24	0.97	(0.74;1.29)	0.86
Ischemic Stroke	I	25	1.45	(1.24;1.69)	< 0.01	1.03	(0.70;1.51)	0.87
	II		1.34	(1.04;1.71)	0.02	0.86	(0.57;1.30)	0.48
Coronary ischemic events	I	60	1.09	(0.86;1.37)	0.47	1.24	(0.98;1.56)	0.07
	II		1.02	(0.78;1.33)	0.89	1.21	(0.95;1.53)	0.12

Subjects with CIMT < 0.9 mm

			B-mode measured CIMT			RF measured CIMT		
	Model	# events	HR	(95% CI)	p-value	HR	(95% CI)	p-value
All events	I	38	1.09	(0.73;1.64)	0.67	1.59	(1.19;2.11)	< 0.01
	II		0.97	(0.63;1.50)	0.90	1.57	(1.15;2.14)	< 0.01
Vascular death	I	15	0.93	(0.47;1.81)	0.82	1.72	(1.10;2.67)	0.02
	II		0.79	(0.39;1.63)	0.52	1.77	(1.09;2.90)	0.02
Ischemic Stroke	I	4	3.16	(0.66;15.21)	0.15	1.40	(0.56;3.49)	0.47
	II		3.01	(0.58;15.75)	0.19	1.19	(0.44;3.24)	0.73
Coronary ischemic events	I	26	1.05	(0.65;1.69)	0.86	1.65	(1.17;2.31)	< 0.01
	II		0.95	(0.57;1.59)	0.85	1.65	(1.14;2.38)	0.01

HR: Hazards ratio per standard deviation (SD) increase in CIMT (SD total population: B-mode 0.30 mm, RF 0.12 mm; SD subjects with CIMT < 0.9 mm : B-mode 0.11 mm, RF 0.11 mm).
Model I with adjustment for age and sex
Model II with adjustment for age, sex, systolic blood pressure, diastolic blood pressure, diabetes mellitus, ever smoking and use of antihypertensive medication at baseline

Discussion

Our study showed that common CIMT measured with B-mode provided stronger relations with the well-established risk factors age and systolic blood pressure in a population that is at high risk. These relations continued to exist in participants with thinner CIMT. B-mode measured CIMT showed stronger associations for any vascular event, vascular death and ischemic stroke in the total population. However, in a population with common CIMT (< 0.9 mm), the associations of RF measured CIMT became stronger for the occurrence of any vascular event, vascular death and coronary ischemic events. B-mode measured CIMT still provided the strongest relation for ischemic stroke.

Before discussing these findings into more detail, some methodological aspects need to be addressed. It is important to emphasize the difference in techniques. In manual B-mode measurements raw RF-waves are processed and displayed on the monitor of the ultrasound machine, next the sonographer traces the lumen-intima and media-adventitia borders in the common carotid artery segment that is located starting 1 cm distal from the tip of the flow divider in the carotid bifurcation and extending 1 cm towards the heart. Finally a computer program determines the maximum thickness over this 1 cm segment. In automated RF measurements unprocessed raw radio frequency signals are used and measurements are performed at a single spot (M-line) that is located 2 cm proximal to the origin of the carotid bulb. Measurements at this location are recommended by the manufacturer, to ensure high reproducibility.

In the total population there were besides increased common CIMT also carotid plaques present. This is clearly illustrated by the range of B-mode CIMT in table 1 (0.38 – 4.52 mm). The difference in association with future events may partly be explained by the ability of the B-mode approach to measure plaques. In this line of reasoning, relations between CIMT estimates and risk factors and between CIMT estimates and events may be different due to the fact that plaques show different relations with age, blood pressure and events as compared to 'non-plaque' CIMT. [28]. After selection of subjects with a CIMT < 0.9 mm we obtained a population within the operational range of the RF equipment. By this selection carotid plaques were excluded and the association with future events attenuated for B-mode CIMT and improved for RF CIMT. The HR for all but future ischemic stroke were more predictive for RF CIMT than for B-mode CIMT.

The ultrasound machines that were used in this study are not actual any longer. In currently available automated RF equipment the range for IMT measurements has been extended to 1500 μm. Whether this will lead to an improvement of risk prediction and risk factor-relationship remains to be determined since a direct comparison between older and more recent automated RF methods, as far as we know, has not been conducted. There is no published data on comparative studies with newer equipments similar to our analyses (survival analyses) or comparative studies between older and newer equipments. However in our opinion it is not likely that newer ultrasound machines have improved so much, that ranking into high versus low CIMT order would result in significantly different ranking with older machines.

The correlation between the two approaches in our study was poor. The ICC was only 0.34. Previous comparative studies all reported correlations above 0.80 [22,23]. The correlation improved to 0.50 in

population with CIMT < 0.9mm. This difference in agreement between both approaches may be explained by a number of differences between our study and the previous studies: i.e. the study setting, study population, blinding of investigators and a different way of ranking. The previous studies were performed in a research setting with clear emphasis on reproducibility of CIMT values. Our study was performed in a routine fashion, with sonographers who were unaware of the comparison between the two methods that was conducted several years later. Secondly, differences across studies in study populations may explain part of the findings. In the earlier studies, the study populations consisted of healthy and younger subjects. At younger ages the CIMT is more similar along a section of the carotid artery than it is in older populations. Consequently measurements in the area of 1-3 cm from the bifurcation may tend to be more similar and thus better agreement is found. The present study was performed in older patients with manifest vascular disease.

The low kappa's for the total population and different subpopulations (results not shown) seem to indicate that the two techniques classify and rank participants in different strata of thin, medium and thick CIMT. Reason for this difference in stratifying could be due to different disease processes that each technique measures. The presence of carotid plaques represents a more advanced stage of atherosclerosis, which is correlated with increased risk of stroke. CIMT measured closer to the bifurcation (as with B-mode) is more likely to contain plaques. CIMT measured more proximal to the heart (as with the RF) correlates to general atherosclerosis and therefore, to coronary ischemic processes. This corresponded well with the difference in associations for future outcome categories of events we found for both techniques.

In conclusion, the implication of our findings may be that the choice for either B-mode CIMT or RF CIMT measurements in research is partly driven by the type of study population, the expected presence of local atherosclerotic abnormalities, and of course the main aim of the study (risk factors or events). Common CIMT measurements by the manual B-mode approach are preferred in higher-risk populations, in which thicker common CIMT measurements are expected. In relatively more healthy populations, with thinner common CIMT, the automated RF approach is the method of choice.

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Chapter 3

Mean common or mean maximum carotid intima-media thickness as primary outcome in lipid lowering intervention studies

Abstract

Background

Carotid intima-media thickness (CIMT) measurements are increasingly used as a disease outcome in randomized controlled trials that assess the effects of lipid modifying treatment on atherosclerosis progression. There is an ongoing debate on whethermean common CIMT or mean maximum CIMT should be used as a primary outcome in such studies. The decision to use a specific parameter is often based on personal preference and expert opinion. We set out to evaluate the published data in order to facilitate a more evidence based decision.

Methods

We reviewed the literature (PUBMED search up to March 2008). Fifteen trials with lipid modifying treatment were identified that provided information on both mean common CIMT and mean maximum CIMT progression. Common CIMT and mean maximum CIMT were compared on reproducibility, strength of relation with LDL and HDL cholesterol, segment specific progression rates and on treatment effects and congruency of their results (harm/neutral/beneficial) with data from event trials.

Results

The reported reproducibility was high for both measures, but a direct comparison was not possible. The relations of in trial achieved LDL-C and HDL-C levels with CIMT progression were modest and not different in magnitude between both measures. CIMT progression rates differed across carotid segments as did segment specific treatment effects without a clear preference pattern. In eight trials results were in similar directions for both CIMT measures. In four trials expected effects were found for the mean maximum CIMT and not for the mean common CIMT, whereas in three trials the effect was found for the mean common CIMT and not for the mean maximum CIMT. The results from CIMT trials appeared to follow results from the clinical event trials on lipid modifying treatment. Thus had one relied on mean maximum CIMT only, one would have encountered three negative trial results (no effect on CIMT, clear effect on event rate), whereas had one relied on mean common CIMT only, four false negative studies would have been encountered

Conclusion

Based on the literature, reproducibility and lipid relations appear similar for both the mean maximum and mean common CIMT measurement, but, the mean maximum CIMT results more often are congruent with event findings. Therefore, the mean maximum CIMT as primary outcome is preferred. An additional advantage is that information on mean common CIMT can also be obtained in protocols assessing the mean maximum CIMT, but not the other way around.

Introduction

In studies that assess the effect of interventions that modify atherosclerotic vascular disease, notably by lipid modifying drugs, the change in carotid intima-media thickness (CIMT) over time is increasingly used as an alternative outcome measure for cardiovascular events. In measuring CIMT, there are several options to choose from with regard to the primary outcome parameter. In some trials, the primary outcome is the change in common CIMT progression, whereas in others the change in mean maximum CIMT progression is chosen. Mean common CIMT is based on CIMT measurements obtained from only the far or both the far and near wall of the common carotid artery segment at the left and right side, 1 to 3 cm proximal to the carotid bifurcation. Suitable ultrasound images are usually stored once or multiple times. CIMT is generally measured off line from these stored images where the measurement is performed over a 10 mm artery segment for each image, and expressed as a mean common CIMT in mm. The mean maximum CIMT is a summary measure that is computed as the mean of the single maximum CIMT measurements measured in 6 to 12 standard carotid artery walls (far or near wall of the 3 distinct carotid segments: the common carotid segment (CCA), the carotid bifurcation (BIF) and the internal carotid artery (ICA) segment) at both the left or right side. A mean maximum CIMT ultrasound assessment also enables the reader to acquire the mean common CIMT measure.

The choice for one or the other CIMT measure as primary outcome is generally based on personal preference and/or expert opinion. An evaluation of the published data to support the use of either measure is lacking, but its availability may facilitate an evidence based decision. Arguments in favor of a common CIMT measurement over a mean maximum CIMT measurement generally include higher reproducibility [1-3], more complete measurement assessment, an equally strong relation with future events, a stronger relation between progression rates and lipid levels, a higher susceptibility for lipid lowering treatment, and a more rapid ultrasound protocol. Support for mean maximum CIMT measurement above common CIMT measurement includes the view that reproducibility, completeness, risk prediction, and lipid level relations are similar to that of the common CIMT, but that a mean maximum CIMT provides a more complete coverage of the extent of carotid atherosclerosis. Furthermore, CIMT progresses differently over the carotid segments and it appears unpredictable at which segment lipid modifying treatment might have its effect. In addition, when mean maximum CIMT measurement is chosen, also information on the mean common CIMT is collected so one can use both the mean maximum CIMT and the mean common CIMT as outcome measurement.

We summarized the available data on the performance of either measure by reviewing the published literature and we will present our results to facilitate a more evidence based choice.

Methods

Identification of articles

The library database PUB-MED (www.ncbi.nlm.nih.gov) was used to identify all published lipid modifying randomized controlled trials that used CIMT as an outcome parameter (table 1). The search was performed up to the end of March 2008. Two authors (SD, MLB) reviewed all articles. In addition, references in the retrieved articles were checked and added when the initial search did not include

these trials. Studies were excluded when they did not present progression rates for both mean common CIMT measurement and mean maximum CIMT measurements. Information retrieved from the articles included reproducibility measures; LDL and HDL cholesterol levels at baseline and at end of study per treatment arm; segment specific CIMT progression rates; mean common CIMT progression rates; mean maximum CIMT progression rates; treatment effect and numbers of subjects in the control and intervention arms. In addition, we performed a search to identify trials with clinical events as an outcome, for the lipid lowering drugs that were used in the CIMT trials.

Table 1: Headings that were used to identify articles

Database	Domain	Determinant	Outcome
Pubmed	Randomized	Drug	Carotid
1966- March 2008	Controlled	Therapy	Intima
	Trial	Treatment	Media
	RCT	Lipid	Thickness
	Etiology	Lowering	CIMT
	Intervention	Statin	IMT

Data analyses

Results on reproducibility and differences in segment specific CIMT progression rates are presented in a descriptive manner. The relation between achieved end of study HDL- and LDL- cholesterol levels and CIMT progression was examined using a weighted linear regression model. Progression rates of each treatment arm in a trial were used. Studies were weighted by number of subjects in the treatment arm. The relation between mean common and mean maximum CIMT progression was assessed using a Spearman correlation coefficient. Finally to assess congruency we presented similarities and differences in study outcome of the mean common CIMT and the mean maximum CIMT with the outcome of the event trials. As CIMT is a surrogate measure for atherosclerotic cardiovascular disease, results of CIMT trials should mirror event trials.

Results

Search strategy

The literature search gave 461 hits. Scanning through title, abstract and when needed the main paper we identified 27 CIMT trials in which the effect of lipid lowering on CIMT progression was reported[4-30]. Of these, 14 provided information on both mean common CIMT and mean maximum CIMT. Through the reference check one additional trial was identified [31]. Ultimately, this resulted in 15 CIMT trials for analyses [5,7-10,12,13,15,16,18,19,21,22,30,31].

For each drug used in the CIMT trials, the corresponding event trials were searched resulting in 15 clinical event trials for lovastatin, pravastatin, fluvastatin, simvastatin, atorvastatin and torcetrapib [32-46]. For ezetimibe and rosuvastatin no event trials had yet been performed up to March 2008.

Reproducibility

For three trials [8,13,30] information on reproducibility could not be retrieved from the publication (table 2). Reproducibility was expressed in various manners and for different processes (re-reading / repeated visits). In none of the papers reproducibility information for both the mean common CIMT and mean maximum CIMT was reported. While results generally showed excellent reproducibility for either measure, a direct comparison of reproducibility between mean common CIMT and mean maximum CIMT measurements could not be made.

Table 2: Reproducibility of randomized controlled trials on lipid modifying drugs with mean maximum and mean common CIMT as outcome measure

	Reproducibility						
	Not reported	**ICC**	**CoV**	**MAD**	**MD (SD)**	**Pearson R**	**Corr.Coef**
PLAC-II [8]	-						
BCAPS [13]	-						
PHYLLIS [30]	-						
ACAPS [12]		0.75*					
RADIANCE 1 [15]		0.90*					
RADIANCE 2 [7]		0.92*					
ENHANCE [16]		0.92					
REGRESS [10]		0.88					
METEOR [9]		0.93*		0,056			
ASAP [22]			<5%				
INDIA [21]			<1%				
HYRIM [31]			<6%				
CAIUS [18]				0,07	0,007 (0.095)		0.85
CERDIA [5]					0,004 (0,054)		
KAPS [19]						CCA : 0.89	
						BIF : 0.79	

ICC : intra class correlation coefficient at baseline, CoV : coefficient of variance, MAD : mean absolute difference (mm), MD(SD mm: mean difference (standard deviation)in millimeters, Pearson R : Pearson correlation coefficient, Corr.Coef : correlation coefficient, CCA : common carotid artery, BIF : bifurcation of carotid artery. * : ICC based on repeated visits

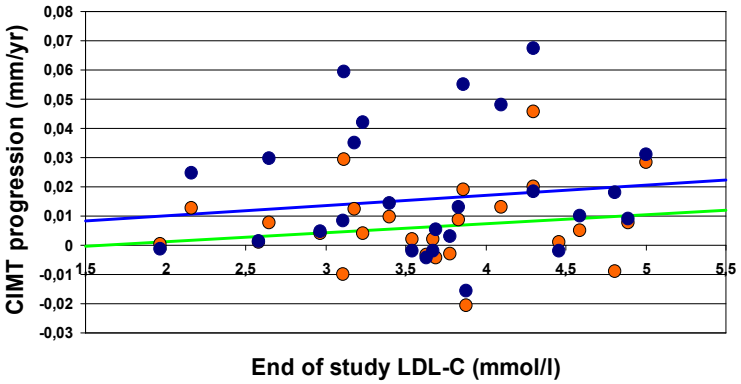
Achieved HDL and LDL levels and CIMT progression

The strength of the relation between achieved (end of trial) LDL cholesterol level and CIMT progression was modest (figure 1). The correlation coefficient was 0.18 for the mean maximum CIMT and 0.23 for the mean common CIMT progression. There was no difference in strength of the association between mean maximum and mean common CIMT progression, with overlapping 95% confidence intervals.

The correlation with achieved HDL cholesterol level was -0.27 for the mean maximum CIMT and

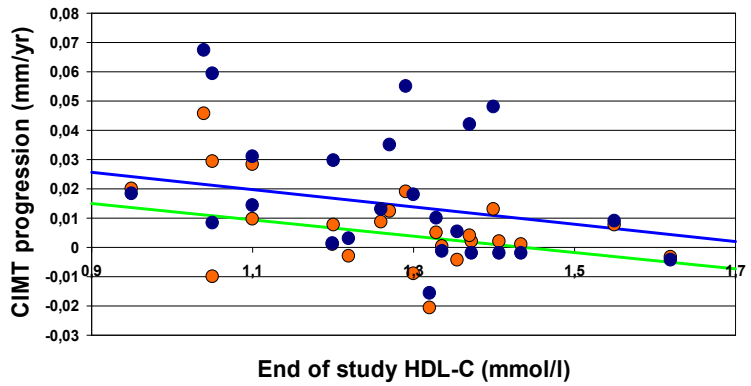
-0.038 for the mean common CIMT progression (figure 2). There was no difference in strength of the association between mean maximum and mean common CIMT progression. Figure 3 shows a strong relation between mean common CIMT progression and mean maximum CIMT progression (Spearman correlation of 0.71).

Figure 1. Relation of achieved LDL cholesterol with common CIMT progression (green line, orange dots) and with mean maximum CIMT progression (blue line, blue dots). Based on the references (5,7-10,12,13,15,18,19).



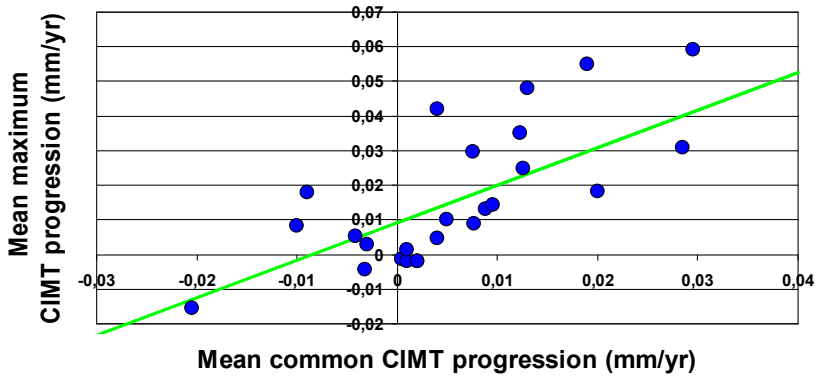
Weighted regression, for mean common CIMT (light dots) regression slope : 0.23 (p=0.25), for mean maximum CIMT (dark dots) regression slope : 0.18 (p=0.39)

Figure 2: Relation of achieved HDL cholesterol with common CIMT progression (green line, orange dots) and with mean maximum CIMT progression (blue line, blue dots). Based on the references (5,7-10,12,13,15,18,19).



Weighted regression, for mean common CIMT (light dots) regression slope : -0.38 (p-value = 0.07), for mean maximum CIMT (black dots) regression slope : -0.27 (p-value = 0.21). The estimates of the treatment arm with torcetrapib in the RADIANCE studies have not been included because of the extreme achieved HDL levels.

Figure 3. Relation of common CIMT progression with mean maximum CIMT progression



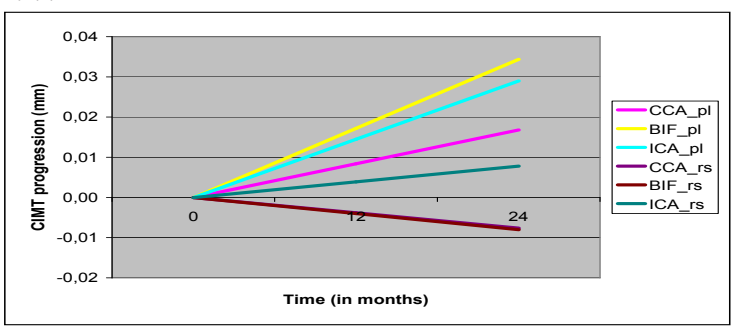
Weighted regression slope = 0.71 (p-value < 0.01).Dots represent CIMT progression of the intervention and the placebo group in the studies.

Segment specific CIMT progression and treatment effects

CIMT progresses differently over time in the common carotid segment, the carotid bifurcation and the internal carotid artery [47]. This is further exemplified by the results from the placebo arms in the randomized controlled clinical trials (figure 4 and table 3). In the placebo arms of the trials, absolute CIMT progression was higher for the carotid bifurcation as compared to the internal carotid and common carotid segments (table 3).

In all eight placebo controlled trials the carotid bifurcation CIMT progression was lower in the intervention arm as compared in the placebo arm. For the common CIMT progression this was for seven out of eight studies and for the internal CIMT progression in four out of five. The magnitude of segment specific CIMT progression differed across trials ranging from -0.021 to 0.046 mm / year in the common, from -0.022 to 0.090 mm / year in the bifurcation and from -0.035 to 0.044 mm / year in the internal carotid artery.

Figure 4. Segment specific CIMT progression rates as observed in the treatment and placebo arms of the METEOR study (9)



CCA: common carotid artery; BIF = carotid bifurcation; ICA = internal carotid artery; _pl= placebo arm; _rs = rosuvastatin arm

Table 3. Carotid intima-media thickness progression (mm/year) per carotid segment

		Change from baseline in CIMT per segment (mm. / year)		
Study name	**Intervention**	**CCA**	**BIF**	**ICA**
KAPS [19]	Pravastatin 40 mg	0.010‡	0.028	***NR***
	Placebo	0.029‡	0.040	***NR***
PLAC-II [8]	Pravastatin 40 mg	0.030‡	0.090	0.044
	Placebo	0.046‡	0.104	0.043
CAIUS [18]	Pravastatin 40 mg	-0.003‡	-0.013‡	***NR***
	Placebo	0.008‡	0.004‡	***NR***
REGRESS [10]	Pravastatin 40 mg	-0.005	0.030	-0.030
	Placebo	0.010	0.045	-0.035
ASAP [22]	Atorvastatin 80 mg	-0.021	-0.011‡	-0.016
	Simvastatin 40 mg	-0.009	0.031‡	0.044
BCAPS [13]	Fluvastatin 40 mg	0.004‡	0.057	***NR***
	Placebo	0.012‡	0.079	***NR***
CERDIA [5]	Simvastatin 20 mg	0.002	-0.017	0.005
	Placebo	-0.006	-0.010	0.031
INDIA [21]	Atorvastatin 10 mg	-0.008‡	-0.022‡	-0.009‡
	Placebo	0.011‡	0.013‡	0.007‡
METEOR [9]	Rosuvastatin 40 mg	–0.0038‡	–0.0040‡	0.0039‡
	Placebo	0.0084‡	0.0172‡	0.0145‡
RADIANCE 1 [15]	Atorvastatin 56.5 mg* & torcetrapib 60 mg	0.0038	***NR***	***NR***
	Atorvastatin 56.5 mg*	-0.0014	***NR***	***NR***
RADIANCE 2 [7]	Atorvastatin 13.5 mg* & torcetrapib 60 mg	0.0126	0.0283	0.0252
	Atorvastatin 13.5 mg*	0.0076	0.033	0.0357
ENHANCE [16]	Simvastatin 80 mg & Ezetimibe 10 mg	0.0010	0.0072	0.0050
	Simvastatin 80 mg	0.0012	0.0031	-0.0004

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment;
NR=not reported in the paper or not assessed in the study;
* Mean titrated daily dose used in the trial. ‡ difference between interventions statistically significant ($p < 0.05$).
ACAPS[12], PHYLLIS[30] and HYRIM[31] did not report the progression rates separately per carotid segment.

Congruency between results for mean common and mean maximum CIMT from CIMT trials and results from event trials

The outcomes of the CIMT trials for mean common CIMT, for mean maximum CIMT, and the congruency with results from event trials are presented in table 4. Results of event trials indicated that treatment with lovastatin, pravastatin, fluvastatin, atorvastatin and simvastatin has beneficial effects on events [32,33,35-40,42-46] (table 4). For rosuvastatin only one event trial was performed that studied effect of rosuvastin treatment in older patients with systolic heart-failure[48]. This study showed no benefit of rosuvastatin use on mortality, but was performed in a population where in general a CIMT trial would not be performed. The results of the torcetrapib event trials were classified harmful (harm), as the event trial showed increased risk of mortality and morbidity [34]. For ezetimibe no results from event trials have been presented yet.

In eight CIMT trials results showed similar direction for both CIMT and event outcome measure, while in seven CIMT trials congruency with event trials depended on the choice of the primary outcome

Table 4. Congruency of outcome of CIMT trials with outcome of event trials

Study name	Event-trial	CIMT-trial outcome		Congruency CIMT trial and event trial		
		Mean common CIMT	Mean Maximum CIMT	No Difference	Favors† mean common CIMT	Favors† mean maximum CIMT
ACAPS [12]	Benefit [38]	***Neutral***	Benefit			X
REGRESS [10]	Benefit [32,45,46]	***Neutral***	Benefit			X
PHYLLIS [30]	Benefit [32,45,46]	***Neutral***	Benefit			X
ASAP [22]	Benefit [35-37,42,44]	***Neutral***	Benefit			X
CERDIA [5]	Benefit [36,42]	***Neutral***	***Neutral***	X		
PLAC-II [8]	Benefit [32,45,46]	Benefit	***Neutral***		X	
BCAPS [13]	Benefit [33,39,40,43]	Benefit	***Neutral***		X	
KAPS [19]	Benefit [32,45,46]	Benefit	Benefit	X		
CAIUS [18]	Benefit [32,45,46]	Benefit	Benefit	X		
HYRIM [31]	Benefit [33,39,40,43]	Benefit	Benefit	X		
INDIA [21]	Benefit [35,37,44]	Benefit	Benefit	X		
RADIANCE 1 [15]	**Harm** [34]	**Harm**	***Neutral***		X	
RADIANCE 2 [7]	**Harm** [34]	***Neutral***	***Neutral***	X		
METEOR [9]	* no event data yet	Benefit	Benefit	X		
ENHANCE [16]	*no event data yet	***Neutral***	***Neutral***	X		

Harm = significant increase in CIMT progression in the intervention group compared to the control group= reject new treatment; **Neutral** = no significant difference in CIMT progression between the intervention group compared to the control group = reject new treatment; **Benefit** = significant reduction in CIMT progression in the intervention group compared to the control group = accept new treatment

† favor = defined as that outcome measure that confirms the primary research question

measure. In four trials [10,12,22,30] expected effects were found for the mean maximum CIMT and not for the mean common CIMT, whereas in three trials [8,13,15] the effect was found for the mean common CIMT and not for the mean maximum CIMT. Thus when relied on the mean maximum CIMT only, three trials would have been falsely negative (no effect on CIMT, clear clinical events effect). When relied on common CIMT only, four studies would have been falsely negative.

Discussion

Our review of findings from 15 randomized intervention studies shows that the reported reproducibility was high for both measures, although a direct comparison was not possible. The relations of achieved LDL-C and HDL-C levels with CIMT progression were modest and showed no difference in magnitude between both CIMT measures. CIMT progression rates differed across carotid segments with highest progression rates observed in the bifurcation segment. Treatment effects also differed across carotid segments without a clear preference pattern. Furthermore, the trials using mean maximum CIMT progression more often (12 out of 15 studies) paralleled the findings of the event trials in contrast to the mean common CIMT (11 out of 15 studies). By the use of only one or the other outcome measure important results of lipid modifying trials on CIMT progression might have been missed. In four trials the effect on mean maximum CIMT progression was beneficial whereas the effect of the mean common CIMT was neutral. In three trials, effects for the common CIMT were different from the mean maximum CIMT.

Some issues of this review need to be considered. In the published reports information on reproducibility for mean common and mean maximum CIMT was not given separately. Furthermore, the parameters to reflect reproducibility presented in the reports varied so much that a direct formal comparison of reproducibility of both measures was not possible to perform. Most trials have reported excellent reproducibility for the primary outcome parameter. As far as we know, there are no published reports which compare reproducibility parameters for the outcome measures separately. Secondly, for the comparison between the two CIMT measures we restricted ourselves to trials with both CIMT measurements. Therefore several trials that measured common CIMT progression only were not mentioned here in detail. The results in four of these studies were congruent with results from events trials [6,14,24,25] and in one were not[17].

In this era in which imaging of atherosclerosis becomes increasingly important in studying the efficacy of new anti atherosclerotic treatments the choice of the endpoints becomes crucial. It is important for trials that use CIMT progression as a surrogate marker for vascular events, that the findings in the trial parallel the results of event trials. The studies included in this review were lipid modifying trials using a statin versus placebo or versus another less potent statin. Had the 15 trials used only the mean common CIMT measurement as primary outcome, then in four a different, and potentially 'wrong', conclusion would have been reached. In contrast, had the trials used mean maximum CIMT measurements as primary outcome three trials would have led to a different conclusion. However if the mean maximum CIMT is measured, information on mean common CIMT is also collected and would have been available as secondary outcome.

In a randomized controlled trial one has to pick one primary endpoint. Apart from data presented here, several other aspects should be taken into account in the choice between mean common and mean maximum CIMT. This includes information on a direct comparison of reproducibility between the two measurements and a comparison of the anticipated effect sizes with corresponding sample size considerations. Unfortunately, published information on these issues is lacking. Finally, also budgetary aspects may, to some extent, be important in the choice for the primary endpoint. The mean maximum CIMT measurement is associated with a a more laborious ultrasound protocol and with more time requirements for the off line CIMT reading afterwards compared to the mean common CIMT measurement. This makes the mean maximum approach more costly than the mean common approach. Data comparing the information in terms of expected benefit and costs up untill now is not available.

In conclusion, based on the literature, reproducibility and lipid relations appear similar for both the mean maximum and mean common CIMT measurement, but, the mean maximum CIMT results more often are congruent with event findings. Therefore, the mean maximum CIMT as primary outcome is preferred. An additional advantage is that information on mean common CIMT can also be obtained in protocols assessing the mean maximum CIMT, but not the other way around.

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Chapter 4

Completeness of Carotid Intima Media Thickness Measurements depends on body composition

Abstract

Background

Ultrasound protocols to measure carotid intima media thickness (CIMT) differ considerably with regard to the inclusion of the number of carotid segments and angles used. Detailed information on completeness of CIMT information is often lacking in the published reports, and at most overall percentages are presented. We studied completeness of CIMT measurements and its relation with vascular risk factors using data from two CIMT intervention studies: one among familial hypercholesterolemia (FH) patients, the Rating Atherosclerotic Disease change by Imaging with A New CETP Inhibitor (RADIANCE 1) and one among mixed dyslipidemia (MD) patients the Rating Atherosclerotic Disease change by Imaging with A New CETP Inhibitor (RADIANCE 2).

Methods

We used baseline ultrasound scans from the RADIANCE 1 (n=872) and RADIANCE 2 (n=752) study. CIMT images were recorded at 12 artery-wall combinations (the near and far wall of the left and right common carotid artery (CCA), bifurcation (BIF) and internal carotid artery (ICA) segments) at 4 set angles, resulting in 48 possible measurements per patient. Presence or absence of CIMT measurements was assessed per artery-wall combination and per angle. The relation between completeness and patient characteristics was evaluated with logistic regression analysis.

Results

In 89% of the FH patients information on CIMT could be obtained on all twelve carotid segments, and in 7.6% eleven segments provided CIMT information (nearly complete 96.6%). For the MD patients this was 74.6% and 17.9%, respectively (nearly complete: 92.5%). Increased body mass index and increased waist circumference were significantly ($p = 0.01$) related to less complete data in FH patients. For MD patients, relations were seen for increased waist circumference ($p<0.01$) and baseline mean maximum CIMT ($p=0.07$). Segment specific data indicated that in FH patients completeness was somewhat less for the near wall of the right internal carotid artery (94%) as compared to other segments (all >98%). In MD patients completeness was lower for the near wall of the right and left carotid artery: 86.0% and 90.8%, respectively as compared to other segments (all > 97%).

Conclusion

With the current ultrasound protocols it is possible to obtain a high level of completeness. Apart from the population studied, body mass index and waist circumference are important in achieving completeness of CIMT measurements.

Introduction

Carotid intima-media thickness (CIMT) measurements have been used widely to evaluate cardiovascular disease (CVD) risk factors and CVD morbidity and mortality [1-3]. Changes in CIMT over time are being used to measure efficacy of pharmacological interventions where CIMT is used as a surrogate marker for atherosclerotic vascular disease risk [4-17]. At present there is little agreement on ultrasound protocols to measure CIMT and they vary in measuring single or double walls (near and/or far wall), or single or multiple segments at set / pre specified angle(s) or unspecified free angle(s). Evidence to favor one approach over another appears to be lacking.

Completeness of CIMT information is often an argument to restrict protocols to imaging of the far wall of the common carotid artery only, or to imaging of the far wall of the other segments. One of the underlying assumptions is that 'missingness of CIMT information' may bias the observed rate of change in CIMT. Unfortunately, detailed published information on aspects of completeness of carotid segments and walls is not available. In published reports from CIMT trials, if anything, generally overall information on completeness is presented[9,10,16,18,19]. In addition, information on factors that reduce completeness of CIMT measurement assessment has not been widely addressed. From experience, it is well known that carotid ultrasound imaging is more difficult in subjects with a short and thick neck. In addition, the general view appears to be that adequate CIMT measurements of images from the near wall seem to be difficult to obtain and that characteristics of the patient population also may affect the completeness rates.

In view of the lack of published information on these issues, we set out to study this using a large body of data obtained in two recently performed randomized controlled trials.

Methods

General

We used data of the RADIANCE 1 [15,20] and RADIANCE 2 trial[20]. These studies have been described in detail before. In summary RADIANCE 1 was a double-blind randomized placebo-controlled multi-center study in which 850 patients with heterozygous familial hypercholesterolemia (FH) were randomly assigned to receive either atorvastatin monotherapy or atorvastatin combined with 60 mg of torcetrapib, a cholesteryl ester transfer protein(CETP) inhibitor, for 2 years to study the effect on CIMT progression. RADIANCE 2 was a comparable study, a double-blind, placebo-controlled multi-center study in which 752 participants with mixed dyslipidemia were randomly assigned to atorvastatin monotherapy or atorvastatin combined with 60 mg of torcetrapib for 2 years to assess the effect of torcetrapib on the progression of atherosclerosis.

Carotid Ultrasound Examinations

The ultrasound protocol for assessment of carotid intima-media thickness has been described in detail elsewhere[8,15,20,21]. In short, duplicate scans were made at baseline and at each patient's final visit and single scans at visits at 6, 12, and 18 months, to give a maximum of seven scans for each patient.
At each visit sonographers acquired and recorded CIMT images of the 12 artery-wall combinations of the

near and far walls of the right and left carotid artery for the common, bifurcation, and the internal carotid artery segments, at four predefined angles of 30º steps (90º to 180º on the right side and 270º to 180º on the left side) using the Meijers carotid arc. This resulted in 48 possible measurements per patient. All imaging centers used the same ultrasound protocol to acquire images and equipment (Sequoia 512 scanners equipped with 8L5 transducers; Siemens AG, Munich, Germany). Forty eight 5-seconds image sequences (video clips) were saved in DICOM format (Digital Imaging in Communications in Medicine, National Electrical Manufacturers Association, Rosslyn, VA, USA). Imaging data were transferred directly from the study sites to the two reading centers (Vascular Imaging Center, University Medical Center, Utrecht, The Netherlands, and Wake Forest University Medical Center, Ultrasound Reading Center, Winston-Salem, NC, USA), where standardized equipment and protocols were used to process stored images. From every image sequence, readers selected one frame in end diastole for measurement of carotid intima-media thickness. Maximum thickness (and also mean for the common carotid artery) was measured semi automatically with Artery Measurement System software (Chalmers University, Göthenburg, Sweden)[22]. Readers were unaware of the interventions assigned to patients, and of previous measurements. Quality assurance protocols have been described elsewhere[20].

Statistical Methods

Completeness, defined as the presence of a CIMT measurement, was addressed in various ways since information on CIMT availability was present on an angle level (4 angles), on wall level (near and far wall), on a carotid segment level (CCA, BIF, ICA), on a carotid side level (left, right). Furthermore, when in a study the interest lies in measurement of mean maximum CIMT, information of CIMT in the twelve segments should be available, and thus completeness for the (2 walls x 3 carotid segments x 2 sides =) 12 artery-wall combinations was studied separately.

First, completeness was presented by angle and carotid segment separately. Next, availability of one or more angle specific CIMT measurements for each carotid artery segment was presented separately. Next, data were presented per segment for each angle. Finally, the completeness of CIMT was presented by segments (complete was defined as CIMT available at all 12 artery-wall combinations).

The relation of completeness, i.e. a CIMT measurement available at all 12 segments, with several vascular risk factors was studied using a logistic regression model. The outcome 'complete' was defined as CIMT available at all 12 segments. The odds ratio (OR) and its corresponding 95% confidence interval (95%CI) was estimated for several risk factors. An increased OR (> 1) should be interpreted as an increased risk of obtaining incomplete data. Results are presented for the two RADIANCE studies separately. Data were analyzed using SPSS version 14.0.

Results

Baseline characteristics of the two study populations are given in table 1. Participants of the RADIANCE 1 study were younger, had higher levels of LDL cholesterol, lower levels of triglycerides, lower waist circumference values and lower body mass index than participants of the RADIANCE 2 study (table 1). There were more male participants and hypertension was less common in RADIANCE 1 compared

to RADIANCE 2. Furthermore, CIMT was considerably higher in MD patients as compared to the FH patients.

Table 1: Baseline characteristics of the RADIANCE 1 and RADIANCE 2 study

	RADIANCE 1	RADIANCE 2
Subjects, n	872	752
Age (years)	45 (12.5)	57(8.2)
Male, n (%)	431 (49.4)	482 (64)
Systolic blood pressure (mm. Hg)	116 (11)	120 (11)
Diastolic blood pressure (mm. Hg)	73 (7)	74 (7)
HDL cholesterol (mg/dl)	52(13)	47(11)
LDL cholesterol (mg/dl)	139 (37)	100 (20)
Triglycerides (mg/dl)	113(64)	183(80)
Waist circumference (cm)	89(12)	100(13)
BMI (kg/m²)	26.7(4.4)	30.1(4.4)
Hypertension*, n (%)	230(26.4)	390(52)
Mean maximum CIMT (mm)	1.14 (0.30)	1.31 (0.31)
Mean common CIMT (mm)	0.72 (0.15)	0.83 (0.15)

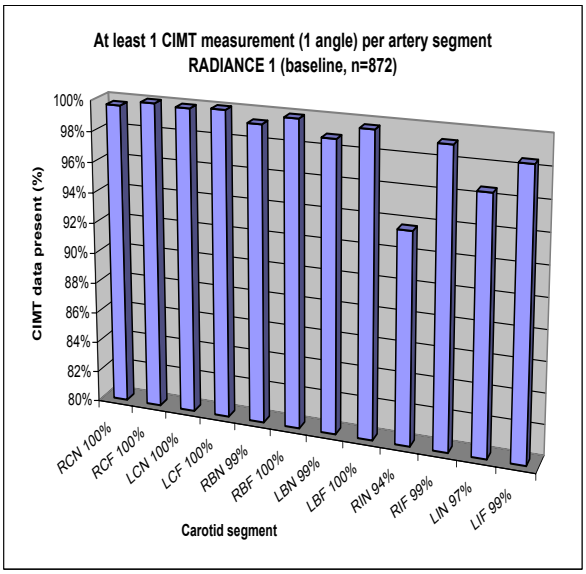
Values presented as mean (standard deviation) or number (percentage); BMI: body mass index.
* hypertension defined as: systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg or use of antihypertensive medication

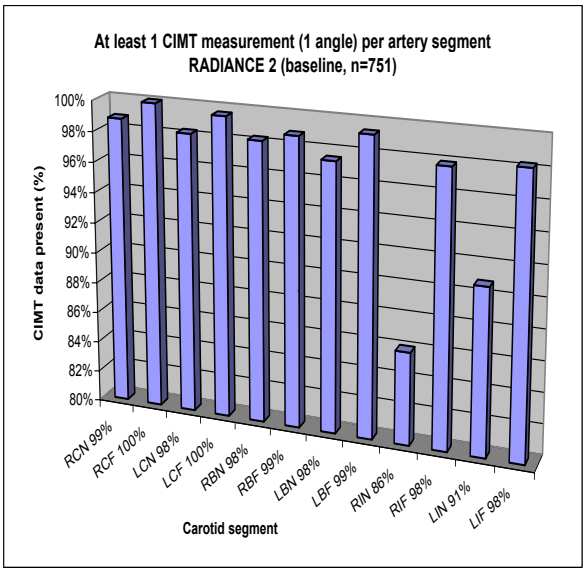
Completeness per segment

The availability of at least 1 CIMT measurement (out of the 4 (angles)) was 94% in the FH patients trial and 86% in the MD patients trial (figure 1). Segment specific data indicated that in FH patients completeness was somewhat less for the near wall of the right internal artery (94%) as compared to other segments (all >98%). In MD patients completeness was lower for the near wall of the right and left carotid artery: 86% and 91%, respectively as compared to other segments (all > 97%).

When completeness was defined as the availability of all four angle CIMT measurements, percentages were still considerably high in the FH patients (figure 2). Completeness percentages in general were higher for far wall measurements as compared to near wall measurements. Also, the completeness percentages declined when going from the common carotid segment, to the bifurcation segment to the internal carotid segment. A similar pattern was seen for the completeness rates among MD patients, although the rates were lower than those found for FH patients (figure 2). CIMT information from the near walls of the internal carotid segments was most difficult to obtain.

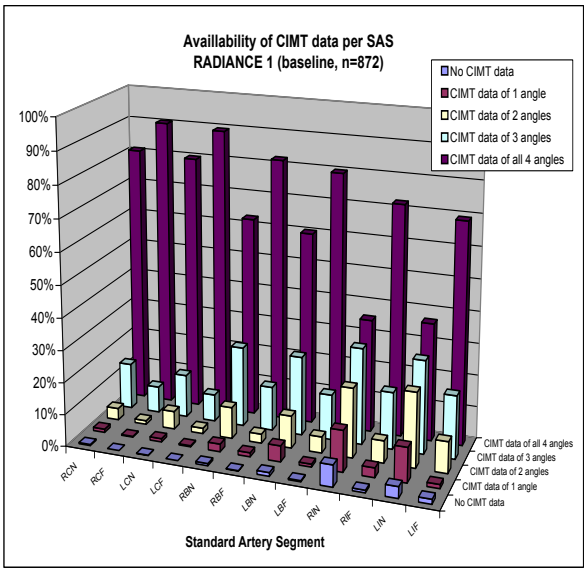
Figure 1: Availability of CIMT measurements per standard artery segment

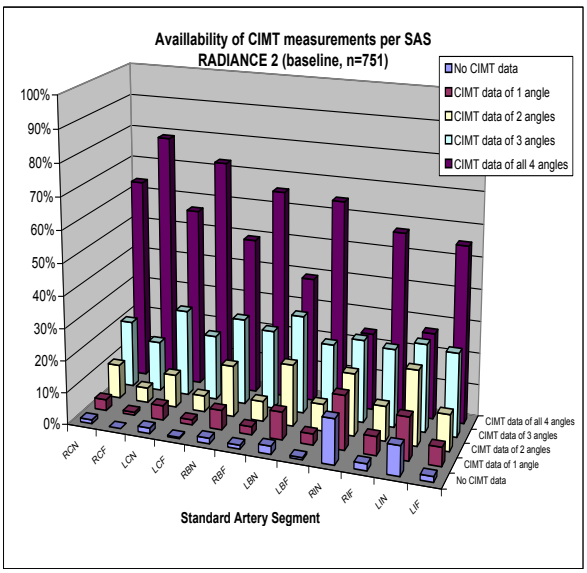




Availability is defined as, at least one CIMT measurement at any one of the four angles / measurements of each one of twelve standard artery segments. RCN : near wall of the right common carotid artery; RCF : far wall of the right common carotid artery; LCN : near wall of the left common carotid artery; LCF : far wall of the left common carotid artery; RBN : near wall of the right bifurcation; RBF ; far wall of the right bifurcation; LBN :near wall of the left bifurcation; LBF : far wall of the left bifurcation; RIN : near wall of the right internal carotid artery; RIF : far wall of the right internal carotid artery; LIN : near wall of the left internal carotid artery; LIF : far wall of the left internal carotid artery

Figure 2: Availability of CIMT measurements per standard artery segment.





CIMT measurements of 0 (= none) / 1 / 2 / 3 / 4 (all 4 angles) available. RCN : near wall of the right common carotid artery; RCF : far wall of the right common carotid artery; LCN : near wall of the left common carotid artery; LCF : far wall of the left common carotid artery; RBN : near wall of the right bifurcation; RBF ; far wall of the right bifurcation; LBN :near wall of the left bifurcation; LBF : far wall of the left bifurcation; RIN : near wall of the right internal carotid artery; RIF : far wall of the right internal carotid artery; LIN : near wall of the left internal carotid artery; LIF : far wall of the left internal carotid artery

Completeness of CIMT measurements per segment, wall, angle and side

Table 2 provides information on the percentage of participants with a CIMT measurements present per segment, wall, angle and side for both studies. Completeness was higher in FH patients than in MD patients for nearly all combinations. The near wall of the internal carotid artery of both the left and right side provided the lowest completeness in both studies. Measurements of the 120°/240° and 150°/210° angles were most complete for these segment-wall combinations.

In studies where the interest is on assessment of mean maximum CIMT for each participant, i.e., taking the average of the maximum CIMT measurement of each of the 12 segments, information on completeness (having at least one CIMT measurement per segment) is important. At least 89.0% of FH patients had one CIMT measurements at 12 segments. In addition, 96.6% had at least one CIMT measurement in 11 segments. For the MD patients these percentages were 74.6% and 92.7%, respectively (table 3).

Table 2: Percentage of CIMT measurements presented by wall, segment and angle in RADIANCE 1 (872 scans) and RADIANCE 2 (752 scans) at baseline.

	RADIANCE 1					RADIANCE 2			
	90°	**120°**	**150°**	**180°**		**90°**	**120°**	**150°**	**180°**
RCN	92,7%	96,0%	95,8%	89,4%	RCN	84,6%	90,9%	88,5%	78,4%
RCF	95,3%	98,2%	98,5%	96,8%	RCF	90,3%	94,9%	96,0%	90,9%
RBN	90,8%	90,6%	85,8%	78,4%	RBN	76,0%	89,3%	87,1%	79,4%
RBF	90,6%	95,8%	96,4%	93,5%	RBF	83,0%	94,1%	95,3%	90,7%
RIN	71,4%	75,0%	64,0%	59,4%	RIN	80,7%	83,9%	79,0%	72,2%
RIF	84,3%	92,2%	91,5%	88,4%	RIF	82,0%	90,0%	91,1%	87,4%
	270°	**240°**	**210°**	**180°**		**270°**	**240°**	**210°**	**180°**
LCN	92,1%	96,4%	94,4%	88,9%	LCN	69,8%	81,1%	75,8%	68,3%
LCF	94,0%	98,5%	98,2%	96,3%	LCF	77,9%	89,3%	91,3%	88,5%
LBN	84,1%	90,0%	82,7%	79,5%	LBN	62,6%	62,1%	56,5%	48,7%
LBF	89,0%	94,4%	95,4%	92,9%	LBF	75,2%	82,3%	87,9%	82,6%
LIN	80,4%	75,6%	65,0%	60,9%	LIN	66,2%	73,5%	58,6%	51,1%
LIF	80,8%	89,9%	92,1%	88,8%	LIF	71,0%	83,8%	87,6%	84,6%

The right carotid artery is scanned at 90°,120°,150° and 180°; the left artery scanned at the equivalent angles 270°, 240°, 210° and 180°. RCN : near wall of the right common carotid artery; RCF : far wall of the right common carotid artery; LCN : near wall of the left common carotid artery; LCF : far wall of the left common carotid artery; RBN : near wall of the right bifurcation; RBF ; far wall of the right bifurcation; LBN :near wall of the left bifurcation; LBF : far wall of the left bifurcation; RIN : near wall of the right internal carotid artery; RIF : far wall of the right internal carotid artery; LIN : near wall of the left internal carotid artery; LIF : far wall of the left internal carotid artery)

Table 3: Frequencies of complete artery segment – wall combination measurements per participants in the RADIANCE 1 and RADIANCE 2 study, at baseline.

	RADIANCE 1	RADIANCE 2
Number of artery-wall segments with a measurements	**% (n)**	**% (n)**
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	0.5% (4)
8	-	0.7% (5)
9	0.3 % (3)	1.6% (12)
10	3.1 % (27)	4.5% (34)
11	7.6 % (66)	17.9% (135)
12 (complete)	89.0 % (776)	74.6% (562)

The 12 artery segment walls comprise of the 2 (near and far) walls of the 3 (common carotid artery, bifurcation, internal carotid artery) segments of the 2 (left and right) common carotid arteries

Determinants of completeness

An increased waist circumference and increased body mass index in FH patients were related to a statistically significant increased risk of incompleteness (i.e., CIMT measurement in less than 12 segments): OR (95%CI) 1.28 (1.07; 1.54) and 1.92 (1.21; 3.05) respectively (table 4). Age, lipid levels, blood pressure and CIMT at baseline showed no relation with completeness. In MD population an increased body mass index was related to an increased risk of incompleteness with an OR (95%CI) 2.04(1.40; 2.98). Waist circumference showed the same trend, however, this did not reach statistical significance. An increase in mean maximum CIMT showed a borderline significant relation with completeness, OR (95%CI) 0.51(0.25; 1.05).

Change over time in completeness of CIMT measurements

To study whether there were changes in completeness of CIMT measurements over time, we performed similar analyses for both studies at the end of study (results not shown). The results were comparable with a slight improvement for completeness in all angles and artery-wall combination, most probably due to gain of experience of the sonographers who performed the ultrasound scans. Again, the near wall of the internal carotid artery and the extreme angle 180° were associated with the least complete measurements.

Table 4: Relation between vascular risk factors with incomplete CIMT measurements.

	RADIANCE 1			RADIANCE 2		
	OR	95% CI	p-value	OR	95% CI	p-value
Age (years)	1.08	(0.91;1.28)	0.38	0.97	(0.80;1.19)	0.79
Systolic blood pressure (mm. Hg)	0.97	(0.79;1.19)	0.75	1.08	(0.93;1.26)	0.31
Diastolic blood pressure (mm. Hg)	1.05	(0.76;1.44)	0.77	1.16	(0.91;1.48)	0.22
HDL-cholesterol (mg/dl)	0.94	(0.80;1.11)	0.46	0.87	(0.74;1.02)	0.09
LDL-cholesterol (mg/dl)	1.00	(0.95;1.06)	0.95	0.98	(0.90;1.06)	0.57
Triglycerides (mg/dl)	1.02	(0.99;1.05)	0.23	1.02	(0.94;1.11)	0.60
Waist circumference (cm)	1.28	(1.07;1.54)	0.01	1.12	(0.98;1.28)	0.09
Body mass index (kg/m²)	1.92	(1.21;3.05)	0.01	2.04	(1.40;2.98)	<0.01
mean common CIMT (mm)	1.34	(0.61;2.94)	0.47	0.61	(0.21;1.71)	0.34
mean maximum CIMT (mm)	0.94	(0.46;1.90)	0.86	0.51	(0.25;1.05)	0.07

OR: odds ratio; 95%CI: 95% confidence interval. Odds ratios represent the odds on incomplete measurements. Incomplete CIMT measurements were defined as having CIMT measurements in < 12 artery wall segments. For more easy interpretable results all riskfactors were multiplied by 10; except for the mean common and the mean maximum CIMT.

Discussion

The present study provides empirical data that completeness of CIMT measurements is related to the segments of the carotid artery, the walls and the angles in which they are examined. Furthermore, it shows that increased body mass index and waist circumference affect the ability to obtain CIMT measurements. Finally, characteristics of the study population appear to be of influence, too.

There are some methodological issues that need to be considered. In the current analyses we recorded presence of CIMT measurement without further studying the CIMT values on correctness of the CIMT values. It might be that some CIMT measurements were incorrect and thus should have been removed from the dataset and thus our completeness rates may have been overestimated. However, because of the high standard of the core laboratory in which the measurements were performed, with periodical quality assessments of the intra- and inter-reader reproducibility, we consider a material effect of wrong measurements on our findings unlikely.

Comparison of our findings with other studies is difficult, since previous publications in general only present overall completeness rates, which to some extent are unclear as to what determines the numerator and denominator. Overall completeness rates that have been reported included 88% in the ENHANCE-study[16] and 88.6% in the OPAL study[23]. Specified completeness measures were provided in the Muscatine study among young and middle aged adults [19] with percentages of complete CIMT measurements at the common carotid artery (near wall: 98.9%; far wall: 99.7%), the carotid bifurcation (near wall: 92.7%; far wall: 92.8%) and the internal carotid artery (near wall: 74.0%; far wall: 88.0%). Our overall estimates in FH and MD subjects compare favorably with these studies.

In the RADIANCE studies sonographers and readers were trained and certified before the start of the study. Furthermore, continuous quality control measures were taken. Our longitudinal findings

suggest that experience of sonographers and readers minimally affect improvement in measurements availability of segments and angles, and thus point towards patient characteristics as the main source of completeness.

Completeness was less in the mixed dyslipidemia population as compared to the familial hypercholesterolemia population. This may be attributed to several aspects. First, the MD population was clearly more overweight. Overweight is related to more incompleteness through either a physical limitation (short, thicker neck) which makes image acquisition more difficult, or through the fact that the ultrasound appearance of the CIMT in the near and far wall in these subjects is more difficult to distinguish because of surrounding tissues. Secondly, the MD population has a more extensive atherosclerosis burden, as indicated by thicker common and mean maximum CIMT. It is well appreciated that arterial walls are more difficult to visualize in the presence of acoustic shadowing, and thus a CIMT measurement is more difficult to perform. Finally, the way atherosclerosis develops in FH patients may be different to how it develops in MD patients. The former giving rise to more circular homogenous development, whereas the latter more heterogeneous and plaque like [24].

In contrast to what is generally thought, our findings indicate that high levels of completeness can be obtained at most of the artery-wall combinations of the carotid artery if one performs multiple measurements per artery-wall combination (multiple angles). The question that is not addressed yet, is whether 'missingness' affects the estimated CIMT progression rates and affects the direction and magnitude of the treatment effect. At present, several statistical models exists that can be applied to datasets with missing data, e.g. multilevel linear mixed-effects model [25-27]. In these statistical approaches regression lines are fitted using restricted maximum likelihood methods to site-specific CIMT values rather than to means over carotid sites to deal with missing data. The reason for this is that some carotid artery sites are consistently more difficult to visualize than others, giving rise to missing data, depending on the site. These models, using all available CIMT measurement points at all visits, provide progression estimates that are less biased by missingness. Another approach to deal with missing data is imputation [28-30]. To the best of our knowledge, the consequence of imputation on CIMT progression estimates and treatment effects has never been quantified yet. Finally, an approach that can be taken is to restrict the ultrasound protocol to segments and walls and angle that show high completeness levels. Studies into the effect of such choices on reproducibility, CIMT progression estimates and treatment effects have been published in abstract form at conferences [31,32]. Generally, these analyses indicate that multi angle protocols perform 'better' in terms of reproducibility (higher), progression rates (more precise) and treatment effect (larger and more precise). Where we chose in this paper to give a detailed description, a next step might be to quantify which segments, walls and angles contribute most to missingness of CIMT data using regression techniques.

In conclusion, with the advanced ultrasound technology available today it is possible to obtain a high level of complete CIMT measurements. Apart from the type of population studied, body mass index, waist circumference are important in achieving completeness of CIMT measurements.

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Chapter 5.1

Ultrasound protocols to measure carotid intima-media thickness; a comparison of reproducibility and rate of progression in healthy post-menopausal women

Abstract

Background

Current ultrasound protocols to measure carotid intima-media thickness (CIMT) differ in number of carotid segments, angles and walls measured. No published evidence is available to help in the deciding which approach is best. We performed a post-hoc analysis to compare different ultrasound protocols on reproducibility and progression estimates in the 'Osteoporosis Prevention and Arterial effects of tiboLone' (OPAL) study, a 3 year randomized controlled trial among healthy postmenopausal women,

Methods

CIMT information was collected from both carotid arteries (left and right) from 2 walls (the near and far wall) of 3 segments (common carotid, bifurcation and internal carotid artery) at 5 fixed angles. Based on combinations of these (2 x 2 x 3 x 5 =) 60 measurements, we built 66 different ultrasound protocols to estimate a CIMT for each individual (22 protocols for mean common CIMT and 44 protocols for mean maximum CIMT). Each protocol was compared on reproducibility at baseline (Intra class correlation (ICC) and standard deviation (SD) of the mean difference of duplicate scans) and on the ratio of CIMT rate of progression in the placebo group and its corresponding standard error (progression-precision ratio).

Results

Duplicate scans at baseline and end of study were available for 675 women (78% of 759 subjects). ICC ranged from 0.69 to 0.88. Mean difference in CIMT of duplicate scans and its standard deviation, ranged from 0.0010 mm to 0.0137 mm and from 0.0561 to 0.1770, respectively. CIMT rate of progression ranged from -0.0001 to 0.0113 mm/year, with the progression/precision rate ranging from 0.03 to 4.04. The protocols with highest reproducibility and highest CIMT progression-precision were mean common CIMT protocols measuring both near and far wall at ≥ 2 angles. Of the mean maximum protocol, those with three segments and ≥2 angles performed best, yet with lower estimates as for the common CIMT protocols.

Conclusion

In healthy middle-aged subjects mean common CIMT protocols that included measurements at both near and far walls at multiple (≥ 2) angles produced CIMT measurements with highest reproducibility and highest rate of progression with its associated precision and are to be recommended in this population.

Introduction

Carotid intima-media thickness (CIMT) is widely used in observational and intervention studies to study determinants of atherosclerosis and its consequences for cardiovascular events [1-4]. In addition, change in CIMT over time is currently used as an alternative for cardiovascular events as primary outcome in intervention studies [5-11]. At present there is little uniformity in ultrasound protocols to measure CIMT. Protocols vary in the selection of carotid segments, angles, and walls of the carotid artery measured. Measurements can be made of the near wall and/or the far wall along the common carotid artery (CCA), the carotid artery bifurcation (BIF) and the internal carotid artery (ICA) at different angles of insonation. Although CIMT measurements have been performed for years in several studies and settings, significant differences exist between ultrasound protocols. In the published literature [12-21], the most commonly used ultrasound protocols allow CIMT measurements to be taken from combinations of segments (CCA; CCA and BIF; CCA, BIF and ICA), walls (far wall; near and far wall) and angles (single angle or a combinations of several angles). With these measurements one can estimate two different CIMT measures: the mean common CIMT and the mean maximum CIMT. The mean common CIMT is estimated as the mean value of the CIMT measurements that are performed over a 10 mm part of the far wall or both the far and near wall of the common carotid artery segment. The mean maximum CIMT is a summary measure that is computed as the mean of the single maximum CIMT measurements that are measured in 4 to 12 standard carotid artery walls: the far wall or both the far and near walls of the 2 or 3 distinct carotid segments: CCA, BIF and ICA segment at both the left and right side.

The choice of an ultrasound protocol depends on the choice of outcome parameter. If the outcome parameter is chosen to be mean common CIMT, measurements of only the CCA segment are performed. If the outcome parameter is mean maximum CIMT, measurements can be performed at two segments (CCA and BIF) or at all three segments of the carotid artery (CCA, BIF and ICA).

To determine which ultrasound protocol (combination of segments, angles and walls measured) would be the most optimal, many issues are important of which the following matter most: 1 the reproducibility; 2 the ability of the method to assess change over time (CIMT progression rates); 3 ability to be related to risk factors or treatment; 4 ability to provide complete information on measurements; and 5 the expense and time requirements needed to accomplish the ultrasound measurements. The choice of one ultrasound protocol over another is partly based on some of the above mentioned aspects, and finally for a large part based on personal experience of the investigator(s). Up until now formal evaluations of differences in methodological aspects between ultrasound protocols and their results on above mentioned parameters have not yet been published. This is caused largely due to a lack of studies that included data from all segments, walls and angles to enable such a formal comparison. Yet, in view of the potential use of CIMT measurements in etiologic and intervention studies, published evidence on these issues is needed. Therefore we set out to study differences in reproducibility and rate of progression across various ultrasound protocols using CIMT information that were collected in the Osteoporosis Prevention and Arterial effects of tiboLone (OPAL) study, a randomized controlled trial among 866 healthy postmenopausal women.

Methods

General

The OPAL study was an international multicenter three-arm, randomized, placebo-controlled, double-blind trial to determine the effects of tibolone (2.5 mg tablet daily) and continuous combined conjugated equine estrogens (CEE) plus medroxyprogesterone acetate(MPA) (0.625 mg + 2.5 mg tablets daily) on the progression of carotid intima-media thickness among 866 healthy postmenopausal women[22]. Approval for conduct of the OPAL study was obtained from the institutional review boards of the participating clinics. Written informed consent was obtained from all study participants.

Carotid ultrasound and CIMT measurements

The ultrasound protocol used in the OPAL study has been described in detail elsewhere[22]. In short, duplicate carotid ultrasound scans were made at baseline and at each patient's final visit and single scans every 6 months. At each visit sonographers acquired CIMT images of 12 standard artery segments (SAS) of near and far walls of the right and left carotid artery for the common carotid artery (CCA), the carotid bifurcation (BIF) and the internal carotid artery (ICA), at five predefined angles of 30º steps (60º to 180º on the right side and 300º to 180º on the left side) using the Meijers carotid arc. This resulted in 60 possible measurements per patient. All 11 imaging centers used the same imaging acquisition protocol and equipment (Acuson Aspen, Mountain View, CA, USA) with identical pre-sets and 7.0 (10-5.0) MHz linear array transducer. The entire ultrasound examination was recorded on super VHS videotapes, which were collected at the two reading centers (Vascular Imaging Center, University Medical Center, Utrecht, Netherlands, and Wake Forest University Medical Center, Ultrasound Reading Center, Winston-Salem, NC, USA). All ultrasound scans were read with Image Pro® software. On each image, the visualized blood-intima and media-adventitia boundaries were manually marked with a computer mouse-controlled caliper within images of the 12 standard artery segments. Maximum thickness (and also mean for the common carotid artery) was measured semi-automatically with Artery Measurement System software (Chalmers University, Göthenburg, Sweden)[23], dedicated software added to ensure standardized settings across reading stations and continent. Readers were unaware of the interventions assigned to patients, and of previous measurements.

Ultrasound protocols

By selecting CIMT information from different combinations of segments, walls and angles of both carotid arteries we constructed 22 ultrasound protocols to estimate mean common CIMT and 44 ultrasound protocols to estimate mean maximum CIMT, resulting in a total of 66 different protocols.
The protocols that have been used in the analyses of the OPAL study were protocol 55 for the mean maximum CIMT and protocol 11 for the mean common CIMT (table 1).

Data analysis

We compared the CIMT estimates of each ultrasound protocol on two aspects: reproducibility and rate of CIMT progression over time in the placebo group with its corresponding precision.

Table 1: The 66 different ultrasound protocols in the OPAL study, with statement of walls, segments and angles measured

			Protocol	Walls		segments			Angles				
				Fw	Nw	CCA	BIF	ICA	60	90	120	150	180
									300	270	240	210	180
Mean common CIMT protocols													
Segment	**Wall**	**Angle**											
CCA	FW & NW	1	1	+	+	+				+			
			2	+	+	+					+		
			3	+	+	+						+	
			4	+	+	+							+
		2	5	+	+	+				+	+		
			6	+	+	+					+	+	
			7	+	+	+						+	+
		3	8	+	+	+				+	+	+	
			9	+	+	+					+	+	+
		4	10	+	+	+				+	+	+	+
		5	11	+	+	+			+	+	+	+	+
	FW	1	12	+		+				+			
			13	+		+					+		
			14	+		+						+	
			15	+		+							+
		2	16	+		+				+	+		
			17	+		+					+	+	
			18	+		+						+	+
		3	19	+		+				+	+	+	
			20	+		+					+	+	+
		4	21	+		+				+	+	+	+
		5	22	+		+			+	+	+	+	+
Mean maximum CIMT protocols													
CCA & BIF	FW & NW	1	23	+	+	+				+			
			24	+	+	+					+		
			25	+	+	+	+					+	
			26	+	+	+	+						+
		2	27	+	+	+	+			+	+		
			28	+	+	+	+				+	+	
			29	+	+	+	+					+	+
		3	30	+	+	+	+			+	+	+	
			31	+	+	+	+				+	+	+
		4	32	+	+	+	+			+	+	+	+
		5	33	+	+	+	+		+	+	+	+	+
	FW	1	34	+		+	+			+			
			35	+		+	+				+		
			36	+		+	+					+	
			37	+		+	+						+
		2	38	+		+	+			+	+		
			39	+		+	+				+	+	
			40	+		+	+					+	+
		3	41	+		+	+			+	+	+	
			42	+		+	+				+	+	+
		4	43	+		+	+			+	+	+	+
		5	44	+		+	+		+	+	+	+	+
CCA & BIF & ICA	FW & NW	1	45	+	+	+	+			+			
			46	+	+	+	+				+		
			47	+	+	+	+					+	
			48	+	+	+	+						+
		2	49	+	+	+	+	+		+	+		
			50	+	+	+	+	+			+	+	
			51	+	+	+	+	+				+	+
		3	52	+	+	+	+	+		+	+	+	
			53	+	+	+	+	+			+	+	+
		4	54	+	+	+	+	+		+	+	+	+
		5	55	+	+	+	+	+	+	+	+	+	+
	FW	1	56	+		+	+	+		+			
			57	+		+	+	+			+		
			58	+		+	+	+				+	
			59	+		+	+	+					+
		2	60	+		+	+	+		+	+		
			61	+		+	+	+			+	+	
			62	+		+	+	+				+	+
		3	63	+		+	+	+		+	+	+	
			64	+		+	+	+			+	+	+
		4	65	+		+	+	+		+	+	+	+
		5	66	+		+	+	+	+	+	+	+	+

CCA: common carotid artery segment, BIF: bifurcation segment, ICA: internal carotid artery segment; NW: near wall, FW: far wall

Reproducibility was assessed in two ways: Intraclass correlation coefficient (ICC) and mean difference of the duplicate CIMT scans at baseline with their corresponding standard deviations. We choose the SD of the mean difference as reproducibility parameter, as the ideal and best protocol would have a mean difference of 0 with a small variation around this mean difference. The parameters for reproducibility were estimated from the duplicate baseline scans.

CIMT progression for the placebo group was calculated as the difference between the average of the duplicate scans at end of study and the average of the duplicate scans at baseline. We considered only the placebo group since in this group there is no influence by the intervention and CIMT should theoretically increase over time. An absolute progression-precision (PP) ratio was calculated by dividing the progression over time by its standard error (SE). We used this ratio as this measure reflects both magnitude of rate of CIMT progression and its associated precision; e.g. an ultrasound protocol that provides a large progression rate with a high precision (small SE) will have a high ratio.

The protocols were ranked by the ICC (the higher, the better), the standard deviation (SD) of the mean difference (the lower, the better), and the progression-precision ratio (the higher, the better). Finally, a summary score for each protocol was calculated, based on summation of the ranks of high reproducibility and highest PP-ratio after categorization of these scores in sixtiles. The protocols were ranked by this summation score. Sixtiles were used as we anticipated that differences between the protocols were not large enough to justify a difference of 66 versus 1 between the best and the worst protocol, whereas a difference of 6 versus 1 between the best and the worst protocols was considered reasonable.

For the present study, we restricted our attention to the use of data of the duplicate scans at baseline and at the end of 36 months of the study. Only those participants that had data for all four scans were included in the analyses (675 out of 759 participants). Analyses were performed by the complete-case analysis principle. Imputation of missing data was not performed. Analyses were performed using SPSS statistical software (version 12.0).

Results

Data on examinations at baseline and end of study was available for 675 (78%) out of 759 subjects completing the trial. Mean age was 59 years and mean systolic blood pressure 129 mm Hg. The mean common CIMT at baseline in the total population (standard deviation) was 0.72 (0.11) mm and the mean maximum CIMT 1.10 (0.22) mm.

Reproducibility

The ICC ranged from 0.720 to 0.881 in the mean common CIMT protocols, from 0.689 to 0.840 in the mean maximum CIMT protocols. Looking upon reproducibility as expressed by the ICC, mean common CIMT protocols performed better than mean maximum CIMT protocols. Protocols with ≥ 3 angles had the highest ICC in both the single wall (only far wall) as the two wall protocols. These results applied both to the mean common CIMT as to the mean maximum CIMT protocols (table 2).

The mean difference in CIMT between the two baseline examinations was close to 0 for almost

Table 2: Reproducibility of CIMT measurements at baseline in the OPAL study (n= 675), estimated as Intraclass correlation coefficients (ICC) and the mean difference in CIMT (mm) between duplicate scans and ranking for all protocols.

				ICC	Ranking of ICC			Mean difference	SD	Ranking of SD		
Segment	Wall	Angle			Mean common CIMT	Mean maximum CIMT	Overall			Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,796	6		33	0,0023	0,0782	7		51
			2	0,824	10		48	0,0015	0,0715	8		52
			3	0,777	4		26	0,0020	0,0817	5		49
			4	0,720	1		4	0,0026	0,0909	1		45
		2	5	0,852	12		56	0,0015	0,0638	13		57
			6	0,861	13		57	0,0016	0,0614	14		58
			7	0,817	8		44	0,0040	0,0702	10		54
		3	8	0,874	17		61	0,0016	0,0577	18		62
			9	0,868	16		60	0,0022	0,0587	17		61
		4	10	0,876	18		62	0,0021	0,0562	21		65
		5	11	0,876	19		63	0,0024	0,0561	22		66
	FW	1	12	0,793	5		32	0,0052	0,0882	2		46
			13	0,811	7		40	0,0012	0,0801	6		50
			14	0,777	3		24	0,0027	0,0857	4		48
			15	0,734	2		12	0,0010	0,0861	3		47
		2	16	0,864	15		59	0,0031	0,0662	12		56
			17	0,851	11		55	0,0019	0,0671	11		55
			18	0,820	9		47	0,0022	0,0703	9		53
		3	19	0,878	20		64	0,0029	0,0601	16		60
			20	0,862	14		58	0,0017	0,0608	15		59
		4	21	0,881	22		66	0,0025	0,0565	20		64
		5	22	0,879	21		65	0,0021	0,0569	19		63
CCA & BIF	FW & NW	1	23	0,732		10	11	0,0066	0,1660		3	3
			24	0,741		11	13	0,0054	0,1560		11	11
			25	0,722		4	5	0,0056	0,1580		10	10
			26	0,703		2	2	0,0054	0,1640		6	6
		2	27	0,787		25	29	0,0066	0,1530		15	15
			28	0,790		26	30	0,0038	0,1470		23	23
			29	0,761		17	19	0,0073	0,1490		20	20
		3	30	0,813		34	41	0,0091	0,1490		21	21
			31	0,781		23	27	0,0044	0,1530		16	16
		4	32	0,809		31	37	0,0085	0,1510		18	18
		5	33	0,828		41	51	0,0059	0,1420		26	26
	FW	1	34	0,727		7	8	0,0108	0,1710		2	2
			35	0,689		1	1	0,0011	0,1770		1	1
			36	0,719		3	3	0,0064	0,1640		5	5
			37	0,726		6	7	0,0059	0,1610		8	8
		2	38	0,759		16	18	0,0078	0,1650		4	4
			39	0,756		13	15	0,0034	0,1630		7	7
			40	0,758		14	16	0,0068	0,1500		19	19
		3	41	0,797		28	34	0,0113	0,1560		12	12
			42	0,758		15	17	0,0040	0,1600		9	9
		4	43	0,791		27	31	0,0114	0,1530		17	17
		5	44	0,811		33	39	0,0065	0,1380		29	29
CCA & BIF & ICA	FW & NW	1	45	0,752		12	14	0,0091	0,1400		28	28
			46	0,761		18	20	0,0010	0,1320		37	37
			47	0,762		19	21	0,0063	0,1310		39	39
			48	0,723		5	6	0,0056	0,1450		25	25
		2	49	0,804		29	35	0,0059	0,1310		40	40
			50	0,814		36	43	0,0037	0,1230		44	44
			51	0,772		21	23	0,0062	0,1330		36	36
		3	52	0,827		40	50	0,0091	0,1290		41	41
			53	0,814		35	42	0,0052	0,1290		42	42
		4	54	0,819		38	46	0,0096	0,1370		33	33
		5	55	0,840		44	54	0,0094	0,1290		43	43
	FW	1	56	0,729		8	9	0,0111	0,1540		13	13
			57	0,730		9	10	0,0017	0,1540		14	14
			58	0,785		24	28	0,0060	0,1350		34	34
			59	0,769		20	22	0,0072	0,1470		24	24
		2	60	0,777		22	25	0,0068	0,1490		22	22
			61	0,809		32	38	0,0048	0,1370		32	32
			62	0,805		30	36	0,0084	0,1340		35	35
		3	63	0,828		42	52	0,0105	0,1380		30	30
			64	0,818		37	45	0,0077	0,1380		31	31
		4	65	0,825		39	49	0,0137	0,1410		27	27
		5	66	0,836		43	53	0,0110	0,1320		38	38

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; NW: near wall, FW: far wall. ICC: Intra class correlation; SD: standard deviation;

Protocols were ranked from 1 to 66 in an order of low to high reproducibility (increasing ICC and decreasing standard deviation of mean difference)

all ultrasound protocols, with a range of 0.0010 to 0.0137 (table 2). The standard deviation (SD) of the mean difference ranged from 0.0561 to 0.0909 for the mean common CIMT ultrasound protocols and from 0.1230 to 0.01770 for the mean maximum CIMT ultrasound protocols. Mean common CIMT protocols with measurements at ≥ 2 angles had the smallest SD. Mean maximum CIMT protocols with measurements at ≥ 3 angles had the smallest SD (table 2).

Rate of CIMT progression over time

The rate of CIMT progression over time in the placebo-group ranged from -0.0001 to 0.0113 mm/year with corresponding standard errors from 0.0014 to 0.0033 (table 3). The progression-precision ratio (PP-ratio) ranged from 1.21 to 4.04 for mean common CIMT protocols and from 0.03 to 3.97 for mean maximum CIMT protocols. The mean common CIMT protocols showed the highest ratios between rate of CIMT progression over time and its corresponding precision. The highest ratios were found in the mean common CIMT protocols that included measurements of both walls at ≥ 2 angles. Protocols with measurements of both walls at ≤ 3 angles had the highest ratios in the mean maximum CIMT protocols.

Overall ranking of ultrasound protocol based on reproducibility and rate of CIMT progression

The protocols with the highest overall summation scores (table 5: overall score ≥13) were mean common CIMT protocols. The summation score increased if measurements were performed at more angles. In 6 out of the best 9 protocols with overall score ≥17, the protocols included measurements of both near and far wall at ≥ 2 angles. When the interest lies in the mean maximum CIMT protocols, the highest ranking protocols were those with near and far wall measurements and ≥ 2 angles in three segments.

Discussion

In a population of healthy postmenopausal women the composition of the ultrasound protocol to measure CIMT affected reproducibility and the magnitude and precision of CIMT progression rates. The best balance between reproducibility and rate of progression with corresponding precision was found for mean common CIMT ultrasound protocols that used information from both the near and far wall. Mean maximum CIMT protocols that used measurements of both near and far wall at multiple angles were associated with the best values of reproducibility, rate of CIMT progression and its precision, yet these were lower than for the mean common CIMT protocols.

Some aspects of this study need to be addressed. Firstly, all CIMT data in our study was acquired by highly-trained sonographers that used an extensive ultrasound protocol. After collection scans were read by experienced readers in a core-lab under strict quality assurance conditions. This provided excellent high-quality CIMT data. The fixed angle approach guaranteed that consecutive scans would be performed at approximately the same angle. Such a standardized approach to CIMT measurements may have enhanced reproducibility and improved quality of CIMT data. Others studies have used

Table 3: Annual rate of progression of CIMT (mm), its precision (standard errors), ratio of progression-precision and ranking of the ratio for all 66 ultrasound protocols in the OPAL study for the placebo-group (n=216)

				Progression (mm / year)	SE	Absolute ratio	Ranking of absolute ratio		
Segment	Wall	Angle					mean common CIMT	mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,0038	0,0017	2,29	2		22
			2	0,0057	0,0016	3,57	13		53
			3	0,0063	0,0016	4,02	21		65
			4	0,0056	0,0017	3,25	7		46
		2	5	0,0046	0,0014	3,23	6		45
			6	0,0057	0,0014	4,04	22		66
			7	0,0054	0,0015	3,58	14		54
		3	8	0,0051	0,0014	3,78	16		58
			9	0,0053	0,0014	3,74	15		56
		4	10	0,0051	0,0014	3,78	17		59
		5	11	0,0046	0,0014	3,37	9		48
	FW	1	12	0,0023	0,0019	1,21	1		11
			13	0,0070	0,0018	3,89	18		61
			14	0,0057	0,0017	3,38	10		49
			15	0,0048	0,0018	2,68	3		31
		2	16	0,0047	0,0016	2,91	5		35
			17	0,0062	0,0016	3,98	20		64
			18	0,0052	0,0015	3,39	11		50
		3	19	0,0050	0,0015	3,34	8		47
			20	0,0057	0,0014	3,93	19		62
		4	21	0,0049	0,0014	3,46	12		52
		5	22	0,0041	0,0014	2,88	4		33
CCA & BIF	FW & NW	1	23	0,0093	0,0031	3,03		34	39
			24	0,0113	0,0029	3,83		43	60
			25	0,0102	0,0028	3,69		41	55
			26	0,0089	0,0029	3,03		35	40
		2	27	0,0080	0,0029	2,77		29	32
			28	0,0112	0,0028	3,97		44	63
			29	0,0087	0,0027	3,20		39	44
		3	30	0,0085	0,0029	2,91		31	36
			31	0,0102	0,0030	3,43		40	51
		4	32	0,0074	0,0030	2,48		24	26
		5	33	0,0067	0,0028	2,38		22	24
	FW	1	34	0,0039	0,0033	1,17		9	9
			35	0,0093	0,0032	2,90		30	34
			36	0,0061	0,0028	2,23		20	21
			37	0,0042	0,0030	1,42		12	13
		2	38	0,0023	0,0033	0,68		4	4
			39	0,0075	0,0031	2,38		21	23
			40	0,0033	0,0028	1,17		10	10
		3	41	0,0021	0,0033	0,62		3	3
			42	0,0046	0,0031	1,48		13	14
		4	43	-0,0001	0,0032	0,03		1	1
		5	44	0,0002	0,0028	0,05		2	2
CCA & BIF & ICA	FW & NW	1	45	0,0086	0,0028	3,09		37	42
			46	0,0091	0,0024	3,74		42	57
			47	0,0063	0,0025	2,48		25	27
			48	0,0059	0,0024	2,42		23	25
		2	49	0,0078	0,0025	3,09		38	43
			50	0,0073	0,0025	2,94		33	38
			51	0,0061	0,0024	2,50		26	28
		3	52	0,0067	0,0027	2,51		27	29
			53	0,0076	0,0025	3,07		36	41
		4	54	0,0066	0,0026	2,51		28	30
		5	55	0,0056	0,0026	2,16		19	20
	FW	1	56	0,0064	0,0031	2,04		18	19
			57	0,0085	0,0029	2,93		32	37
			58	0,0053	0,0029	1,84		16	17
			59	0,0023	0,0025	0,92		7	7
		2	60	0,0044	0,0029	1,52		14	15
			61	0,0060	0,0030	1,99		17	18
			62	0,0033	0,0027	1,22		11	12
		3	63	0,0035	0,0030	1,15		8	8
			64	0,0049	0,0028	1,73		15	16
		4	65	0,0022	0,0029	0,77		6	6
		5	66	0,0020	0,0027	0,73		5	5

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; SE: standard error; ratio = calculated as progression / SE, protocols were ranked in an order of lowest to highest absolute value of progression/SE ratio

Table 4: Ranking of the ultrasound protocols based on summation of the transformed rank scores on reproducibility and progression-precision ratio for the OPAL study

Segment	Wall	Angle		Mean common CIMT	Mean maximum CIMT	Overall score
CCA	FW & NW	1	1	5		10
			2	7		15
			3	7		14
			4	4		11
		2	5	8		17
			6	11		18
			7	7		14
		3	8	12		18
			9	11		18
		4	10	13		18
		5	11	11		17
	FW	1	12	3		9
			13	8		15
			14	4		13
			15	3		10
		2	16	7		16
			17	10		16
			18	7		15
		3	19	10		17
			20	10		18
		4	21	12		17
		5	22	10		15
CCA & BIF	FW & NW	1	23		7	6
			24		9	9
			25		8	7
			26		6	6
		2	27		9	8
			28		11	12
			29		10	8
		3	30		11	10
			31		10	10
		4	32		9	9
		5	33		11	11
	FW	1	34		3	3
			35		6	6
			36		5	4
			37		4	4
		2	38		4	4
			39		6	6
			40		7	5
		3	41		7	7
			42		5	5
		4	43		6	6
		5	44		9	8
CCA & BIF & ICA	FW & NW	1	45		11	9
			46		12	12
			47		11	9
			48		7	7
		2	49		14	12
			50		13	12
			51		10	10
		3	52		13	12
			53		13	12
		4	54		13	11
		5	55		13	11
	FW	1	56		5	5
			57		7	7
			58		9	9
			59		7	6
		2	60		8	7
			61		10	9
			62		10	10
		3	63		10	9
			64		11	10
		4	65		9	9
		5	66		11	10

CCA: common carotid artery segment, BIF: bifurcation segment, ICA: internal carotid artery segment

protocols that were dedicated in finding the previous image (on-sight / digital memory) or in going to the visually maximum measurements (independent of angles), or in using a single angle protocol that visually measured the best image from which CIMT should be measured, regardless of a set angle. However, there are no data available to indicate how protocols with absence of angle approaches would rank in our tables. Secondly, our study was performed in healthy postmenopausal women free from symptomatic vascular disease. It might be that ultrasound protocols rank differently in populations with a lesser or a heavier burden of carotid atherosclerosis. It is assumed that when more carotid plaques are found, reliable CIMT measurements tend to be more difficult to obtain. Therefore, the findings of the study are restricted to general populations of healthy middle-aged subjects. Thirdly, it is difficult and to some extent arbitrary to assign the parameters the precise amount of weight and rank the protocols based on these parameters. Therefore we assigned equal weight to reproducibility and CIMT progression-precision ratio. Finally, although important for trials, the differences in treatment effect between protocols in this comparative study of ultrasound protocols were not taking into account. The ability to show effect of treatment on CIMT-progression with a high precision is one of the most important parameters that outweighs the choice of an ultrasound protocol, since this is the main reason why randomized controlled trials are performed. The OPAL analyses showed that there was no evidence of benefit of treatment with tibolone and CEE/MPA on mean-max CIMT progression rates and there was even some indication of harm [22]. Therefore in that setting it was difficult to define what the effect of treatment should be. Due to absence of a clear treatment effect we did not include treatment effect in the present analyses.

We assessed reproducibility by means of ICC and the standard deviation of the difference between paired scans. The ICC is a commonly used and well known measure to evaluate reproducibility. It compares the variability of different measurements of the same subject to the total variation across all measurements and subjects. One characteristic of the ICC is that it depends on the magnitude of the variation between individuals. If the variation between individuals is large relatively to the measurement error, the ICC will become high. In contrast if the variation between individuals is small and the CIMT is measured with the same measurement error the ICC will become small. Multiple segment protocols could therefore provide higher ICC than single segment protocols based on larger variation between individuals rather than on larger reproducibility. To overcome this we ranked the protocols also on the standard deviation of the mean difference of the duplicate scan, since this measure is also a suitable test to assess reproducibility. However, the results showed that this did not affected reproducibility measures, as ICC was high in the mean common CIMT protocols. Based on the ICC, the mean common CIMT protocols were best reproducible. The results were confirmed by the SD of the mean difference. In both the mean common CIMT protocols and the mean maximum CIMT protocols, measurements at both near and far wall at multiple angles were associated with higher values of reproducibility. Ranking of the protocols on the mean absolute difference (MAD) provided similar results as ranking based on SD of the mean difference (results not shown).

For decades a lively discussion is ongoing regarding the desirability of performing near wall CIMT measurement.[24] Reluctance to do so is based on the physics of ultrasound indicating that the near wall

CIMT is at best an approximation of the true value, whereas the advocates indicate that the addition of the near wall measurements increases precision and hence increases precision in risk prediction and evaluation of drug effects. Our study indicated that the best protocols were all predominantly based on data collection of both the near and far wall. This referred to both mean common CIMT protocols as to mean maximum CIMT protocols

In every study that uses CIMT measurements, cohort study or randomized controlled trial, a choice needs to be made regarding the balance of benefit and harm. Benefit relates to high reproducibility, precise measurements, and when appropriate good balance between high rate of CIMT progression and its precision. For intervention studies, also the balance between treatment effect and its precision is of importance. From observational studies and from placebo control arms in trials it has been shown that CIMT progresses differently across the different segments of the carotid artery [10,11,15,25]. Furthermore, it is difficult to predict at which segment or combination of segments the treatment will have it main effects. Harm relates to time needed to acquire CIMT information (ultrasound scanning and reading) and the costs related to that process. Given these findings, the choice for an ultrasound protocol depends on a well-considered balance of the parameters that are of importance for the study that will be carried out. We feel that the present analyses provide sufficient information for the reader to make that balance based on evidence.

In summary, our findings support the notion that the number and specific combination of segments, angles and walls influence reproducibility, magnitude and accuracy of progression of CIMT over time. In healthy middle-aged subjects mean common CIMT protocols that included measurements at both near and far walls at multiple (≥ 2 angles) produced CIMT measurements with highest reproducibility and highest rate of progression with its associated precision.

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Chapter 5.2

Ultrasound protocols to measure carotid intima-media thickness: a comparison of reproducibility, rate of progression and treatment effect in asymptomatic subjects with mild to moderate subclinical atherosclerosis

Abstract

Background

Current ultrasound protocols to measure carotid intima-media thickness (CIMT) in trials differ considerably. The best CIMT protocol would be one that combines high reproducibility, a large and precise rate of CIMT progression and a large and precise treatment effect. We performed a post-hoc analysis to determine the best CIMT protocol using data from the Measuring Effects on intima-media Thickness: an Evaluation Of Rosuvastatin study, a randomized double-blind, placebo-controlled study among 984 low risk individuals on the effect of rosuvastatin on CIMT progression.

Methods

CIMT information was collected from 2 walls (near and far wall), 3 segments (common carotid, bifurcation and internal carotid artery), 5 different angles (for the right carotid artery– 60, 90, 120, 150, and 180 degrees on the Meijer's Carotid Arc; for the left– 300, 270, 240, 210, and 180 degrees) of 2 sides (left and right carotid artery), resulting in possibly (2 x 3 x 5 x 2 =) 60 measurements. Based on combinations of these measurements, we built 66 different ultrasound protocols to estimate a CIMT for each individual (22 protocols for mean common CIMT, 44 protocols for mean maximum CIMT). For each protocol we assessed reproducibility at baseline (Intra-class correlation (ICC), mean difference of duplicate scans), 2 year CIMT progression rate in the placebo group with its corresponding standard error and treatment effect and its corresponding standard error.

Results

Data of duplicate ultrasound examinations at baseline and end of study were available for 688 participants (70% of 984). The ICC ranged from 0.81 to 0.95. CIMT progression rates in the placebo group ranged from 0.0046 mm/yr to 0.0177 mm/yr, with SE ranging from 0.00134 to 0.00337. Treatment effects ranged from 0.0141 to 0.0388 mm/year. The protocols with highest reproducibility, highest CIMT progression/precision and highest treatment effect/precision were those measuring both near and far wall at ≥ 2 angles.

Conclusion

Ultrasound protocols that include CIMT measurements at multiple angles of both near and far wall give the best balance between reproducibility, rate of CIMT progression, treatment effect and their associated precision in a low-risk population with subclinical atherosclerosis. Multiple segment protocols that measure mean maximum CIMT performed slightly better than single segment protocols that measure mean common CIMT.

Introduction

Carotid intima-media thickness (CIMT) is a non-invasive alternative marker of atherosclerotic disease that has been used extensively since 1987. It is defined as the distance between the lumen-intima interface, which corresponds to the inner and outer echogenic lines seen on the B-mode ultrasound image that includes the intimal layer of the wall wherin atheroma develop [1]. CIMT is widely used in observational and intervention studies to study determinants of atherosclerosis and its consequences for cardiovascular events[2-5]. In addition, change in CIMT over time is currently used as a primary outcome in intervention studies to provide guidance for anticipated outcome in cardiovascular event trials [6-10]. CIMT can be measured in various ways. Measurements can be made of the near wall and/or the far wall along the common carotid artery (CCA), the carotid artery bifurcation (BIF) and the internal carotid artery (ICA) at different angles of insonation. Although CIMT measurements have been performed for years in several studies and settings, significant difference exists between ultrasound protocols[11,12]. In the published literature, the most commonly used ultrasound protocols allow CIMT measurements to be taken from combinations of segments (CCA; CCA and BIF; CCA, BIF and ICA), walls (far wall; near and far wall) and angles (single angle or a combinations of several angles). With these measurements one can estimate two different outcome measures: the mean common CIMT and the mean maximum CIMT. The mean common CIMT is estimated as the average value of the mean CIMT measurement that is performed over a 10 mm part of the far wall or both the far and near wall of the common carotid artery segment of one or both carotid arteries. The mean maximum CIMT is a summary measure that is computed as the mean of the single maximum CIMT measurements that are measured in 4 to 12 standard carotid artery walls: the far wall or both the far and near walls of the 2 or 3 distinct carotid segments: CCA, BIF and ICA segment at both the left and right side. The choice of an ultrasound protocol depends on the choice of outcome parameter. If the outcome measure of a trial is chosen to be the mean common CIMT, measurements of only the CCA segment are performed. If the mean maximum CIMT is chosen, measurements can be performed at two segments (CCA and BIF) or at all three segments of the carotid artery (CCA, BIF and ICA).

To determine which ultrasound protocol (combination of segments, angles and walls measured) would be the most optimal, many issues are important including: the reproducibility of the method; the ability of the method to progression over time; the ability of the method to show an effect of treatment; and the expense and time requirements needed to accomplish the ultrasound measurements. The choice of one ultrasound protocol over another is partly based on some of the above mentioned aspects and for a large part based on personal views and experience of the investigator(s). Formal evaluations of differences in methodological aspects between ultrasound protocols have not been published, since there have previously been no studies that included data from all segments, walls and angles to enable such a formal comparison. Yet, in view of the potential use of CIMT measurements in etiologic and intervention studies, published evidence on these issues is crucial and uniformity in protocols could lead to an optimal use of CIMT measurement.

Therefore we set out to study this using data from the Measuring Effects on intima-media Thickness: an Evaluation of Rosuvastatin (METEOR) study, a randomized double-blind, placebo-controlled study

among 984 low risk individuals on the effect of rosuvastatin on CIMT progression.

Methods

General

The rationale, design and main findings of the METEOR study have been described in detail elsewhere[13,14]. In short, METEOR was a 2-year, double-blind, placebo-controlled trial comparing rosuvastatin 40 mg with placebo treatment in middle-aged asymptomatic subjects with moderately elevated cholesterol and low risk of cardiovascular disease according to the National Cholesterol Educational Program (NCEP) Adult Treatment Panel (ATP) III guidelines criteria (0-1 risk factor or ≥2 risk factors with a 10-year coronary heart disease (CHD) risk <10%). The study was conducted in accordance with the ethical principles in the Declaration of Helsinki, the International Conference on Harmonization, Good Clinical Practice guidelines, and appropriate regulatory requirements. The study protocol was approved by the appropriate Institutional Review Board and/or Independent Ethics Committee at each clinical site. All participants provided written informed consent. Main inclusion criteria were: age 45 to 70 years (male) or 55 to 70 years (female); screening low density lipoprotein cholesterol (LDL-C) 120 to 190 mg/dL (3.1 to 4.9 mmol/L) for those with only age as a CHD risk factor, or 120 to 160 mg/dL (3.1 to 4.1 mmol/L) for individuals with 2 or more CHD risk factors and a 10-year risk of CHD events <10%; high density lipoprotein cholesterol (HDL-C) ≤60 mg/dL (1.6 mmol/L); triglycerides (TG) <500 mg/dL (5.7 mmol/L); and a maximum CIMT measurement of at least 1.2 but <3.5 mm on 2 separate ultrasound examinations. Eligible potential participants were randomized to either rosuvastatin or placebo in blocks of seven (5 rosuvastatin, 2 placebo) at each clinical site.

Carotid ultrasound and CIMT measurements

Carotid ultrasound examinations were performed 7 times: twice before randomization, once each at 6, 12 and 18 months after randomization, and then twice at the end of 24 months of study treatment. At each visit standardized longitudinal B-mode images of the near and far walls of the 3 segments of the carotid artery were obtained [13,14]. The common carotid artery was assessed in the segment extending from 10 to 20 mm proximal to the tip of the flow divider. The carotid bifurcation was assessed from the tip of the flow divider, extending 10 mm proximal to the tip of the flow divider. The internal carotid artery was assessed in the 10 mm distal to the tip of the flow divider. Meijer's Carotid Arc® was used to image the artery at pre-specified angles[15,16]. All ultrasound scans were read with Image Pro® software using a uniform reading protocol that ensured standardized settings across reading stations and core laboratories. The image boundaries were marked manually. For CIMT measurements, trailing edges were traced on the near wall boundaries and leading edges on the far wall boundaries. Measurements were performed on images from selected angles: for the right carotid artery – 60, 90, 120, 150, and 180 degrees on the Meijer's Carotid Arc; for the left carotid artery – 300, 270, 240, 210, and 180 degrees. For the near and far walls of the right and left carotid bifurcation and internal carotid artery, all of which were imaged separately, measurements were made only of the maximum CIMT at all selected angles. For the common carotid artery, measurements were made of both the mean and maximum CIMT of

each wall at all selected angles. All readers completed a uniform training program. After each individual had finished the study, all 7 scans were read in batch by a single reader blinded to the timing of each scan[13,14].

Assessment of various protocols to measure CIMT

By selecting the CIMT information from different combinations of segments, angles, walls and both carotid arteries we constructed 22 ultrasound protocols to estimate the mean common CIMT and 44 ultrasound protocols to estimate the mean maximum CIMT resulting in a total of 66 ultrasound protocols (table 1). The protocols that have been used in the analyses of the METEOR study were the most comprehensive protocols: protocol 55 for the mean maximum CIMT and protocol 11 for the mean common CIMT.

Data analysis

We compared the CIMT estimates of each ultrasound protocol on three aspects: reproducibility, CIMT-progression and treatment effect. Reproducibility was assessed in two ways. We estimated the Intraclass correlation coefficient (ICC) and the mean difference of the duplicate CIMT scans at baseline with their corresponding standard deviations for all protocols. Ultrasound protocols were ranked by the level of the ICC (the higher, the better) and by the standard deviation (SD) of the mean difference in CIMT (the lower, the better). The ideal and best protocol would have a mean difference of zero with a small variation around this mean difference.

CIMT progression was estimated as the difference between the average of the 2 duplicate scans at the end of the study and the average of the 2 duplicate scans at baseline for the placebo group. We considered only the placebo group since CIMT progression in this group is not influenced by the intervention. Precision of the different approaches for measuring CIMT progression was defined by their standard error (SE) to obtain an absolute progression/precision (PP) ratio. The choice for ranking by this PP-ratio is that ideally one would like to use an ultrasound protocol that shows the highest progression with the highest precision (i.e., smallest SE).

To study the treatment effect on CIMT change we estimated differences in CIMT progression and their standard errors with regression models in which CIMT progression over time was the dependent variable and treatment (yes or no) was the independent variable. Next a treatment/precision (TP) ratio was calculated by dividing the regression coefficient by its corresponding SE, and the ultrasound protocols were ranked by this TP ratio. This way the ultrasound protocols were ranked based on a combination of strongest relation with treatment and highest precision.

Finally, we ranked the protocols by all three aspects for the mean common CIMT protocols, the mean maximum protocols and for all 66 protocols. We calculated a summary score for each protocol that was based on summation of the ranks of all three parameter sets (highest reproducibility, highest TP-ratio and highest PP-ratio) after categorization of these scores in groups of five and six. We used groups of five for the 22 mean common CIMT protocols and 44 mean maximum CIMT protocols and six for the total ranking of the 66 protocols. This approach was applied rather than focusing on the absolute

Table 1: The 66 different ultrasound protocols in the METEOR study, with statement of segments, walls and angles measured

			Protocol	Walls		Segments							
				Fw	Nw	CCA	BIF	ICA	60	90	120	150	180
									300	270	240	210	180
Mean common CIMT													
Segment	Wall	Angle											
CCA	FW & NW	1	1	+	+	+				+			
			2	+	+	+					+		
			3	+	+	+						+	
			4	+	+	+							+
		2	5	+	+	+				+	+		
			6	+	+	+					+	+	
			7	+	+	+						+	+
		3	8	+	+	+				+	+	+	
			9	+	+	+					+	+	+
		4	10	+	+	+				+	+	+	+
		5	11	+	+	+			+	+	+	+	+
	FW	1	12	+		+				+			
			13	+		+					+		
			14	+		+						+	
			15	+		+							+
		2	16	+		+				+	+		
			17	+		+					+	+	
			18	+		+						+	+
		3	19	+		+				+	+	+	
			20	+		+					+	+	+
		4	21	+		+				+	+	+	+
		5	22	+		+			+	+	+	+	+
Mean maximum CIMT													
CCA & BIF	FW & NW	1	23	+	+	+				+			
			24	+	+	+					+		
			25	+	+	+	+					+	
			26	+	+	+	+						+
		2	27	+	+	+	+			+	+		
			28	+	+	+	+				+	+	
			29	+	+	+	+					+	+
		3	30	+	+	+	+			+	+	+	
			31	+	+	+	+				+	+	+
		4	32	+	+	+	+			+	+	+	+
		5	33	+	+	+	+		+	+	+	+	+
	FW	1	34	+		+	+			+			
			35	+		+	+				+		
			36	+		+	+					+	
			37	+		+	+						+
		2	38	+		+	+			+	+		
			39	+		+	+				+	+	
			40	+		+	+					+	+
		3	41	+		+	+			+	+	+	
			42	+		+	+				+	+	+
		4	43	+		+	+			+	+	+	+
		5	44	+		+	+		+	+	+	+	+
CCA & BIF & ICA	FW & NW	1	45	+	+	+	+			+			
			46	+	+	+	+				+		
			47	+	+	+	+					+	
			48	+	+	+	+						+
		2	49	+	+	+	+	+		+	+		
			50	+	+	+	+	+			+	+	
			51	+	+	+	+	+				+	+
		3	52	+	+	+	+	+		+	+	+	
			53	+	+	+	+	+			+	+	+
		4	54	+	+	+	+	+		+	+	+	+
		5	55	+	+	+	+	+	+	+	+	+	+
	FW	1	56	+		+	+	+		+			
			57	+		+	+	+			+		
			58	+		+	+	+				+	
			59	+		+	+	+					+
		2	60	+		+	+	+		+	+		
			61	+		+	+	+			+	+	
			62	+		+	+	+				+	+
		3	63	+		+	+	+		+	+	+	
			64	+		+	+	+			+	+	+
		4	65	+		+	+	+		+	+	+	+
		5	66	+		+	+	+	+	+	+	+	+

CCA : common carotid artery segment, BIF : bifurcation segment, ICA : internal carotid artery segment; NW : near wall, FW : far wall

ranks because we anticipated that differences between the protocols were not large enough to justify a difference of 66 versus 1 between the best and the worst protocols, whereas a difference of 6 versus 1 between the best and the worst protocols was reasonable.

For the present study, we restricted our attention to the use of data of the duplicate scans before randomization and the duplicate scans at the end of 24 months of study. Only those participants that had data for all four visits were included in the analyses (n= 688). Analyses were performed by the complete-case analysis principle. Imputation of missing data was not done. Analyses were performed using SPSS statistical software (version 12.0).

Results

Data on both baseline examinations and both end of study examinations was available for 688 out of 984 participants (70%). At baseline, the mean age was 56.7 years, mean HDL-cholesterol was 49.2 mg/dL (1.28 mmol/L) and the mean LDL-cholesterol was 153.5 mg/dL (3.99 mmol/L). The mean common CIMT at baseline [mean(standard deviation)] was 0.76(0.12) mm and the mean maximum CIMT was 1.16(0.20) mm.

Reproducibility

Reproducibility was high (table 2). ICC ranged from 0.829 to 0.945 for the mean common CIMT protocols, and from 0.805 to 0.924 for the mean maximum CIMT protocols. Looking upon reproducibility as expressed by the ICC, mean common CIMT protocols performed better than mean maximum CIMT protocols. Protocols with ≥ 3 angles showed the highest ICC in both the single wall (only far wall) and the two wall protocols for both the mean common CIMT protocols and the mean maximum CIMT protocols. Multiple segment protocols (all three segments) had higher ICC than two segment protocols.

The mean difference in CIMT between the two baseline examinations was close to 0 for almost all ultrasound protocols, with a range of -0.0060 to 0.0061 mm (table 2). The standard deviation (SD) of the mean difference ranged from 0.0887 to 0.0397 mm for the mean common CIMT ultrasound protocols and from 0.1308 to 0.0747 mm for the mean maximum CIMT ultrasound protocols. Mean common CIMT protocols with measurements of both the near and far wall at ≥ 3 angles had the smallest SD. This also applied to the mean maximum CIMT: protocols with measurements of both near and far wall at ≥ 3 angles had the smallest SD (table 2)

Rate of CIMT progression over time

The rate of CIMT progression over time in the placebo-group ranged from 0.0046 to 0.0177 mm/year and the standard errors ranged from 0.00134 to 0.00337 (table 3). The progression-precision ratio ranged from 1.63 to 6.39 for the mean common CIMT protocols and from 3.92 to 13.01 for the mean maximum CIMT protocols. The mean maximum CIMT protocols showed the highest ratios between rate of CIMT progression over time and its corresponding precision. The highest ratios were found in the mean maximum CIMT protocols that comprised measurements of both walls in all three carotid segments. The protocols with measurements of both walls at ≥ 2 angles had the highest ratios in the mean common CIMT protocols.

Table 2: Reproducibility of CIMT measurements at baseline in the METEOR study (n= 688), estimated as Intraclass correlation coefficients (ICC) and the mean difference in CIMT (mm) between duplicate scans and ranking for all protocols.

				ICC	Ranking of ICC			Mean difference	SD	Ranking of SD		
Segment	Wall	Angle			Mean common CIMT	Mean maximum CIMT	Overall			Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,845	4		15	-0,0021	0,0700	6		50
			2	0,866	8		27	0,0020	0,0673	7		51
			3	0,865	7		24	0,0057	0,0660	8		52
			4	0,829	1		6	-0,0009	0,0733	5		49
		2	5	0,911	13		52	0,0008	0,0520	15		59
			6	0,915	14		54	0,0042	0,0514	16		60
			7	0,904	9		44	0,0026	0,0535	13		57
		3	8	0,935	18		62	0,0023	0,0436	20		64
			9	0,930	17		61	0,0028	0,0451	19		63
		4	10	0,945	22		66	0,0018	0,0397	22		66
		5	11	0,944	21		65	0,0024	0,0398	21		65
	FW	1	12	0,834	2		9	-0,0036	0,0887	1		29
			13	0,858	6		19	0,0012	0,0845	2		35
			14	0,848	5		17	0,0061	0,0818	3		39
			15	0,839	3		13	0,0007	0,0805	4		42
		2	16	0,907	12		49	-0,0007	0,0645	9		53
			17	0,907	11		48	0,0041	0,0635	10		54
			18	0,904	10		45	0,0041	0,0611	11		55
		3	19	0,928	15		59	0,0018	0,0546	12		56
			20	0,929	16		60	0,0032	0,0529	14		58
		4	21	0,941	19		63	0,0017	0,0476	17		61
		5	22	0,943	20		64	0,0025	0,0462	18		62
CCA & BIF	FW & NW	1	23	0,819		4	4	-0,0008	0,0998		20	20
			24	0,825		5	5	0,0047	0,0985		22	22
			25	0,838		9	11	-0,0001	0,0956		24	24
			26	0,829		6	7	-0,0008	0,0955		25	25
		2	27	0,877		22	30	0,0031	0,0872		31	32
			28	0,876		21	29	0,0042	0,0874		30	31
			29	0,874		20	28	-0,0003	0,0862		32	33
		3	30	0,897		31	39	0,0032	0,0828		36	38
			31	0,891		28	36	0,0023	0,0843		34	36
		4	32	0,905		37	47	0,0017	0,0811		37	40
		5	33	0,909		38	50	0,0014	0,0810		38	41
	FW	1	34	0,805		1	1	-0,0030	0,1296		2	2
			35	0,813		3	3	0,0035	0,1308		1	1
			36	0,830		7	8	-0,0031	0,1235		3	3
			37	0,834		8	10	-0,0045	0,1208		4	4
		2	38	0,865		18	25	0,0029	0,1162		6	6
			39	0,865		19	26	0,0025	0,1171		5	5
			40	0,863		17	23	-0,0048	0,1146		9	9
		3	41	0,890		27	35	0,0018	0,1101		13	13
			42	0,881		23	31	-0,0024	0,1131		11	11
		4	43	0,899		33	41	-0,0027	0,1076		14	14
		5	44	0,903		35	43	-0,0022	0,1062		16	16
CCA & BIF & ICA	FW & NW	1	45	0,845		12	16	-0,0013	0,0882		29	30
			46	0,862		16	22	0,0010	0,0860		33	34
			47	0,861		15	21	-0,0020	0,0892		28	28
			48	0,839		10	12	-0,0025	0,0943		26	26
		2	49	0,896		30	38	0,0003	0,0791		40	44
			50	0,899		32	40	0,0006	0,0792		39	43
			51	0,883		24	32	-0,0028	0,0841		35	37
		3	52	0,917		42	56	0,0011	0,0747		44	48
			53	0,905		36	46	0,0000	0,0788		41	45
		4	54	0,921		43	57	0,0004	0,0750		42	46
		5	55	0,924		44	58	0,0000	0,0748		43	47
	FW	1	56	0,812		2	2	-0,0011	0,1158		7	7
			57	0,844		11	14	0,0012	0,1156		8	8
			58	0,859		14	20	-0,0018	0,1126		12	12
			59	0,858		13	18	-0,0060	0,1145		10	10
		2	60	0,883		25	33	-0,0003	0,1029		19	19
			61	0,895		29	37	0,0000	0,1030		18	18
			62	0,888		26	34	-0,0060	0,1062		15	15
		3	63	0,910		39	51	-0,0009	0,0981		23	23
			64	0,903		34	42	-0,0028	0,1033		17	17
		4	65	0,915		41	55	-0,0035	0,0987		21	21
		5	66	0,913		40	53	-0,0024	0,0919		27	27

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; NW: near wall, FW: far wall. ICC: Intra class correlation; SD: standard deviation; Protocols were ranked from 1 to 66 in an order of low to high reproducibility (increasing ICC and decreasing standard deviation of mean difference)

Table 3: Annual rate of progression of CIMT (mm), its precision (standard errors), ratio of progression-precision and ranking of the ratio in the METEOR study for the placebo-group (n=196)

				Progression (mm / year)	SE	Absolute ratio	Ranking of absolute ratio		
Segment	**Wall**	**Angle**					mean common CIMT	mean maximum CIMT	overall
CCA	**FW & NW**	**1**	**1**	0,0082	0,00214	3,84	12		**12**
			2	0,0100	0,00199	4,99	**16**		**18**
			3	0,0093	0,00196	4,75	14		**16**
			4	0,0088	0,00227	3,87	13		**13**
		2	**5**	0,0093	0,00171	5,41	**17**		**19**
			6	0,0099	0,00164	6,00	**19**		25
			7	0,0087	0,00180	4,81	**15**		**17**
		3	**8**	0,0094	0,00148	6,35	**21**		31
			9	0,0091	0,00163	5,61	**18**		**20**
		4	**10**	0,0090	0,00146	6,15	**20**		27
		5	**11**	0,0090	0,00141	6,39	**22**		32
	FW	**1**	**12**	0,0046	0,00282	1,63	**1**		**1**
			13	0,0065	0,00261	2,51	**3**		**3**
			14	0,0051	0,00248	2,06	**2**		**2**
			15	0,0087	0,00337	2,57	**4**		**4**
		2	**16**	0,0061	0,00222	2,73	**5**		**5**
			17	0,0062	0,00204	3,03	8		**8**
			18	0,0070	0,00252	2,76	**6**		**6**
		3	**19**	0,0056	0,00192	2,93	**7**		**7**
			20	0,0069	0,00221	3,13	11		**11**
		4	**21**	0,0063	0,00205	3,08	10		**10**
		5	**22**	0,0061	0,00202	3,04	9		**9**
CCA & BIF	**FW & NW**	**1**	**23**	0,0138	0,00166	8,32		25	**47**
			24	0,0157	0,00147	10,67		**37**	**59**
			25	0,0087	0,00153	5,66		**3**	**21**
			26	0,0125	0,00158	7,91		21	43
		2	**27**	0,0151	0,00154	9,80		**32**	**54**
			28	0,0143	0,00145	9,84		**33**	**55**
			29	0,0110	0,00149	7,37		19	41
		3	**30**	0,0148	0,00148	9,99		**35**	**57**
			31	0,0147	0,00149	9,87		**34**	**56**
		4	**32**	0,0157	0,00150	10,47		**36**	**58**
		5	**33**	0,0142	0,00149	9,53		**30**	**52**
	FW	**1**	**34**	0,0139	0,00226	6,15		**7**	26
			35	0,0150	0,00204	7,32		18	40
			36	0,0077	0,00198	3,92		**1**	**14**
			37	0,0146	0,00223	6,55		**11**	33
		2	**38**	0,0154	0,00218	7,09		16	38
			39	0,0125	0,00201	6,22		**8**	28
			40	0,0119	0,00202	5,88		**5**	23
		3	**41**	0,0123	0,00209	5,90		**6**	24
			42	0,0145	0,00205	7,08		15	37
		4	**43**	0,0142	0,00211	6,72		**12**	34
		5	**44**	0,0140	0,00208	6,73		**13**	35
CCA & BIF & ICA	**FW & NW**	**1**	**45**	0,0144	0,00148	9,71		**31**	**53**
			46	0,0177	0,00136	13,01		**44**	**66**
			47	0,0082	0,00140	5,85		**4**	**22**
			48	0,0095	0,00152	6,27		**9**	29
		2	**49**	0,0160	0,00134	11,97		**42**	**64**
			50	0,0153	0,00134	11,49		**39**	**61**
			51	0,0100	0,00140	7,15		17	39
		3	**52**	0,0157	0,00136	11,58		**41**	**63**
			53	0,0161	0,00139	11,52		**40**	**62**
		4	**54**	0,0171	0,00141	12,13		**43**	**65**
		5	**55**	0,0154	0,00139	11,10		**38**	**60**
	FW	**1**	**56**	0,0157	0,00198	7,93		22	44
			57	0,0172	0,00183	9,37		29	**51**
			58	0,0084	0,00182	4,61		**2**	**15**
			59	0,0137	0,00217	6,30		**10**	30
		2	**60**	0,0168	0,00184	9,16		27	**49**
			61	0,0144	0,00179	8,04		23	**45**
			62	0,0134	0,00192	6,98		**14**	36
		3	**63**	0,0143	0,00183	7,79		20	42
			64	0,0165	0,00192	8,58		26	**48**
		4	**65**	0,0160	0,00193	8,27		24	**46**
		5	**66**	0,0158	0,00170	9,32		28	**50**

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment; SE: standard error; ratio = calculated as progression / SE, protocols were ranked in an order of lowest to highest absolute value of progression/SE ratio

Treatment effect on CIMT change over time

The effect of treatment as compared to placebo on CIMT progression ranged from -0.0205 mm/yr to -0.0138 mm/yr for the mean common CIMT protocols and from -0.0388 mm/yr to -0.0141 mm/yr for the mean maximum CIMT protocols (table 4). The largest changes in CIMT due to treatment were found in both the two and three segment protocols. The smallest treatment effects were found in the single segment protocols or in single angle protocols.

The treatment-precision ratios ranged from 2.18 to 5.36 for the mean common CIMT protocols and from 1.75 to 6.50 for the mean maximum CIMT protocols. The highest ratios were found in both the mean common CIMT protocols and the mean maximum CIMT protocols. Protocols that combined measurements of both walls at multiple angles had the highest ratios between treatment effect and precision in all one, two and three segment protocols.

Overall ranking of ultrasound protocol based on reproducibility, rate of CIMT progression and treatment effect

The protocols with the highest overall summation scores (table 5: overall score ≥18) were all protocols that included measurements of both near and far wall. The summation score increased if measurements were performed at more angles. The protocols with the highest overall summation scores (overall score ≥22) were all found in the mean maximum CIMT protocols with measurements at both walls at 3 or more angles.

Discussion

Our findings indicate that the best balance between high reproducibility; large and precise estimate of CIMT progression rates and the large and precise estimates of treatment effect is achieved with mean maximum CIMT and mean common CIMT ultrasound protocols that include measurements of both walls at multiple angles. Mean maximum CIMT protocols overall performed slightly better than mean common CIMT protocols.

To the best of our knowledge this type of data has never been published before, most likely since no other study had such a wealth of detailed information on CIMT measurements as the METEOR study.

Some aspects of this study need to be addressed. All CIMT measurements in our study were fixed with regard to the angle af insonation through use of the Meijer's Carotid Arc. This guaranteed that consecutive scans would be performed at the same angle. Our results might differ from CIMT ultrasound protocols that are dedicated to finding the previous image (on-sight / digital memory) or those that interrogate the maximum measurements (independent of angles), or interrogate from a single angle that according to the sonographer is the best image from which CIMT should be measured. However, there are no data available to indicate how protocols with absence of angle approaches would rank in our tables.

It is difficult to precisely assign weight to the parameters we considered or to rank the protocols based on a weighted selection. A prerequisite for each test is a good reproducibility. If this condition is not met,

Table 4. Effect of treatment on CIMT rate of progression over time in METEOR study (n=688). Expressed as the decrease in CIMT per year due to treatment with rosuvastatin.

				Treatment effect (mm/year)	SE	Absolute ratio	Ranking of absolute ratio		
Segment	**Wall**	**Angle**					mean common CIMT	mean maximum CIMT	overall
CCA	**FW & NW**	**1**	**1**	-0,0190	0,0049	3,91	11		36
			2	-0,0184	0,0045	4,06	**15**		42
			3	-0,0164	0,0045	3,62	10		26
			4	-0,0149	0,0053	2,82	**3**		**9**
		2	**5**	-0,0190	0,0038	5,00	**21**		**51**
			6	-0,0185	0,0038	4,83	**18**		**48**
			7	-0,0138	0,0041	3,39	**7**		**19**
		3	**8**	-0,0185	0,0034	5,36	**22**		**61**
			9	-0,0159	0,0037	4,35	**17**		**47**
		4	**10**	-0,0168	0,0034	4,99	**20**		**50**
		5	**11**	-0,0162	0,0033	4,93	**19**		**49**
	FW	**1**	**12**	-0,0205	0,0062	3,29	**6**		**16**
			13	-0,0183	0,0060	3,04	**5**		**14**
			14	-0,0141	0,0057	2,50	**2**		**5**
			15	-0,0142	0,0065	2,18	**1**		**3**
		2	**16**	-0,0205	0,0050	4,07	**16**		43
			17	-0,0166	0,0049	3,42	8		**20**
			18	-0,0145	0,0050	2,91	**4**		**12**
		3	**19**	-0,0180	0,0045	4,02	13		38
			20	-0,0159	0,0045	3,51	9		**22**
		4	**21**	-0,0172	0,0043	4,02	14		39
		5	**22**	-0,0167	0,0042	4,00	12		37
CCA & BIF	**FW & NW**	**1**	**23**	-0,0292	0,00728	4,02		26	40
			24	-0,0359	0,00638	5,63		**41**	**63**
			25	-0,0202	0,00674	3,00		**9**	**13**
			26	-0,0217	0,00695	3,12		**10**	**15**
		2	**27**	-0,0343	0,00670	5,12		**33**	**54**
			28	-0,0332	0,00632	5,25		**36**	**57**
			29	-0,0243	0,00654	3,71		20	30
		3	**30**	-0,0336	0,00644	5,21		**35**	**56**
			31	-0,0337	0,00648	5,20		**34**	**55**
		4	**32**	-0,0348	0,00651	5,35		**38**	**59**
		5	**33**	-0,0331	0,00650	5,09		**32**	**53**
	FW	**1**	**34**	-0,0377	0,00994	3,80		23	33
			35	-0,0342	0,00897	3,81		24	34
			36	-0,0155	0,00874	1,77		**2**	**2**
			37	-0,0247	0,00984	2,51		**4**	**6**
		2	**38**	-0,0388	0,00954	4,07		28	44
			39	-0,0295	0,00884	3,34		**11**	**17**
			40	-0,0238	0,00890	2,67		**5**	**7**
		3	**41**	-0,0310	0,00919	3,37		**12**	**18**
			42	-0,0329	0,00900	3,66		18	28
		4	**43**	-0,0337	0,00926	3,64		17	27
		5	**44**	-0,0346	0,00915	3,78		21	31
CCA & BIF & ICA	**FW & NW**	**1**	**45**	-0,0238	0,00649	3,66		19	29
			46	-0,0381	0,00586	6,50		**44**	**66**
			47	-0,0169	0,00619	2,74		**6**	**8**
			48	-0,0190	0,00670	2,83		**7**	**10**
		2	**49**	-0,0312	0,00582	5,35		**39**	**60**
			50	-0,0333	0,00578	5,76		**43**	**65**
			51	-0,0217	0,00616	3,52		15	24
		3	**52**	-0,0312	0,00590	5,29		**37**	**58**
			53	-0,0346	0,00604	5,73		**42**	**64**
		4	**54**	-0,0335	0,00611	5,48		**40**	**62**
		5	**55**	-0,0306	0,00605	5,06		**31**	**52**
	FW	**1**	**56**	-0,0299	0,00870	3,44		**13**	**21**
			57	-0,0348	0,00801	4,34		30	**46**
			58	-0,0141	0,00804	1,75		**1**	**1**
			59	-0,0237	0,00960	2,47		**3**	**4**
		2	**60**	-0,0338	0,00805	4,20		29	**45**
			61	-0,0298	0,00787	3,78		22	32
			62	-0,0245	0,00846	2,90		**8**	**11**
		3	**63**	-0,0283	0,00805	3,51		**14**	23
			64	-0,0324	0,00841	3,85		25	35
		4	**65**	-0,0302	0,00849	3,55		16	25
		5	**66**	-0,0299	0,00744	4,02		27	41

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; SE: standard error; treatment effect: regression coefficient of CIMT; represent the amount of decrease in CIMT per year due to treatment with Rosuvastatin; ratio: calculated as the regression coefficient divided by the SE;
the regression model used: 'Progression Rate = a + beta (treatment)'

Table 5, Ranking of the ultrasound protocols based on summation of the transformed rank scores on reproducibility, progression-precision ratio and treatment-precision ratio

Segment	Wall	Angle		Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	9		13
			2	11		14
			3	9		13
			4	6		9
		2	5	15		18
			6	15		19
			7	10		14
		3	8	18		21
			9	16		19
		4	10	18		20
		5	11	19		20
	FW	1	12	5		7
			13	5		9
			14	4		8
			15	4		8
		2	16	10		15
			17	9		13
			18	8		13
		3	19	11		17
			20	12		15
		4	21	13		17
		5	22	13		17
CCA & BIF	FW & NW	1	23		10	12
			24		14	15
			25		6	8
			26		9	10
		2	27		15	16
			28		15	17
			29		13	13
		3	30		16	20
			31		16	19
		4	32		19	21
		5	33		18	19
	FW	1	34		6	8
			35		7	10
			36		4	5
			37		5	6
		2	38		9	12
			39		7	9
			40		5	8
		3	41		8	11
			42		9	11
		4	43		10	13
		5	44		11	13
CCA & BIF & DCA	FW & NW	1	45		13	13
			46		16	18
			47		8	8
			48		7	9
		2	49		19	20
			50		19	20
			51		11	14
		3	52		20	23
			53		19	22
		4	54		20	23
		5	55		19	22
	FW	1	56		7	8
			57		11	13
			58		6	7
			59		7	7
		2	60		13	15
			61		12	14
			62		8	11
		3	63		13	15
			64		12	15
		4	65		13	15
		5	66		15	17

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment

the ensuing parameters are of less importance. The primary outcome that researchers are interested in while conducting lipid-lowering trials is the ability and precision of the measurement to detect change in CIMT due to treatment. Since it would be arbitrary to assign one or another parameter a certain weight in the overall ranking and since there is no data to support any such weighings in the literature on this matter, we decided not to assign any weight to the parameters.

We assessed reproducibility by the ICC and the standard deviation of the mean difference. The ICC is a commonly used and well known measure to evaluate reproducibility. It assesses measurement reliability by comparing the variability of different measurements of the same subject to the total variation across all measurements and all subjects. One characteristic of the ICC is that it depends on the magnitude of the variation between individuals. If the variation between individuals is large relative to the measurement error, the ICC will become high. In contrast if the variation between individuals is small and CIMT is measured with the same measurement error the ICC will become small. Multiple segment protocols could therefore produce higher ICC than single segment protocols based only on this difference in between individual variation. To overcome this we ranked the protocols also on the standard deviation of the mean difference between the duplicate scans, since this measure is also a suitable test to assess reproducibility. One could also assess reproducibility by evaluating the mean absolute difference (MAD) of the duplicate scans. We calculated this MAD and found that ranking of the protocols on the MAD (results not shown) provided similar results as ranking based on SD of the mean difference. The correlation between these two parameters was close to 1, which indicated that these two parameters measured the same phenomenon.

The generalizability of our study is determined to a great deal by our study population. Our study population consisted of participants who were at low risk of coronary heart disease (CHD) by conventional clinical criteria, although their IMT criteria indicated the presence of subclinical atherosclerosis. Since reproducibility, progression with its precision, and treatment effect with its precision may be affected by the level of atherosclerosis, our findings are most applicable to populations with similar levels of CIMT across the segments.

For decades a lively discussion is ongoing whether or not to perform near wall CIMT measurements[12]. The reluctance is based on the physics of ultrasound indicating that the near wall CIMT is at best an approximation of the true value, whereas the advocates indicate that the addition of the near wall measurements increase precision and hence increase precision in risk prediction and evaluation of drug effects. Our study indicated that the best protocols with regard to reproducibility, PP-ratio and TP-ratio were all based on data collected from both the far wall and the near wall. This was found both for mean common CIMT protocols and for mean maximum CIMT protocols. We observed also that protocols that included more angles were associated with increase in scores on reproducibility, progression and its associated precision, and treatment effect and its associated precision. These results support the view that ultrasound protocols that measure both walls at multiple angles should be preferred above protocols that measure only the far wall.

In every study that uses CIMT measurements, cohort study or a randomized controlled trial, a choice needs to be made regarding the balance of benefit and cost. Benefit relates to high reproducibility,

precise measurements, a good balance between high CIMT progression and its precision, and a good balance between treatment effect and its precision. Cost relates to the time needed to acquire CIMT information (ultrasound examination and reading of the scans) and the expense related to that process. Given these findings, the choice of an ultrasound protocol should depend on a well-considered balance of the parameters that are of importance for the study that will be carried out. We feel that the present analyses provide sufficient information for each reader to make that balance in evidence based way.

In summary, our findings support the opinion that the number and specific combination of segments, angles and walls interrogated are associated with differences in reproducibility; magnitude and accuracy of progression of CIMT over time and treatment effect. The best balance between these parameters was found in mean maximum CIMT protocols followed by the mean common CIMT protocols, that icluded measurements of both walls at multiple angles. Protocols in which only the far wall was measured performed worse than protocols in which both the near and far wall were measured.

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Chapter 5.3

Ultrasound protocols to measure carotid intima-media thickness: a comparison of reproducibility, rate of progression and treatment effect in subjects with familial hypercholesterolemia and subjects with mixed dyslipidemia

Abstract

Background

Current ultrasound protocols to measure carotid intima-media thickness (CIMT) differ in a number of aspects. The ideal study protocol combines high reproducibility with high precision, includes patients with robust progression rates and is able to detect a change in progression as induced by the intervention. To assess these characteristics, a post-hoc analysis was performed using data from two randomized double-blind, placebo-controlled trials: one among 872 subjects with familial hypercholesterolemia (FH) and the other among 752 subjects with mixed dyslipidemia (MD), respectively. Both studies evaluated the effect of the addition of torcetrapib to atorvastatin mono-therapy on progression of CIMT.

Methods

CIMT information was collected from the left and right carotid artery from 2 walls (the near and far wall) of 3 segments (common carotid, bifurcation and internal carotid artery) at 4 different angles (right–90, 120, 150, and 180 degrees on Meijer's carotid arc; left–270, 240, 210, and 180 degrees, respectively). Based on combinations of these (2 x 2 x 3 x 4 =) 48 measurements, 60 theoretical protocols were constructed to estimate a CIMT measure per participant (20 protocols for mean common CIMT, 40 protocols for mean maximum CIMT). Each protocol was assessed on three parameters: 1. reproducibility at baseline (intra-class correlation (ICC), mean difference of duplicate scans); 2. CIMT progression rate in the atorvastatin mono-therapy group with its corresponding precision (standard error); 3. effect of torcetrapib and atorvastatin versus atorvastatin alone on CIMT progression rate with its corresponding precision (standard error). Based on these three parameters the protocols were ranked.

Results

Reproducibility: ICC ranged from 0.77 to 0.91 among FH patients and from 0.68 to 0.85 among MD patients. CIMT progression rates ranged from -0.0030 to 0.0020 mm/yr in the FH trial and from 0.00084 to 0.01057 mm/yr in the MD trial, with standard errors ranging from 0.00054 to 0.00162 and from 0.00083 to 0.00229, respectively. The difference in CIMT progression rate between treatment arms ranged from -0.00133 to 0.00400 mm/year in the FH trial and from -0.00231 to 0.00486 mm/year in the MD trial. In both the FH and MD trial the best overall outcome for all three parameters was achieved with mean common CIMT protocols with measurement of the near and far wall at multiple angles. When the interest is in mean maximum CIMT, protocols using multiple segments and angles provided the best balance.

Conclusion

Common CIMT assessed at both walls at multiple angles resulted in the best balance between high reproducibility, large rate of CIMT progression and large effect of treatment, all measured with high precision, in particularly in FH patients.

Introduction

Carotid intima-media thickness (CIMT) is a non-invasive marker of atherosclerotic vascular disease that has been studied extensively since 1987. It is defined as the distance between the lumen-intima interface, which corresponds to the inner and outer echogenic lines seen on the B-mode ultrasound image [1]. CIMT is widely used in observational and intervention studies to assess determinants of atherosclerosis and its consequences for cardiovascular events [2-5]. In addition, change in CIMT over time is currently used as a surrogate for cardiovascular events as primary outcome in intervention studies [6-10].

Measurements can be made of the near and/or the far wall along the common carotid artery (CCA), the carotid bifurcation (BIF) and the internal carotid artery (ICA) at different angles of insonation. At present there is considerable diversity in the choice of outcome measure and ultrasound protocols that are used to measure CIMT [11,12]. The most commonly used ultrasound protocols allow CIMT measurements to be taken from different combinations of segments (CCA; CCA and BIF; CCA, BIF and ICA), walls (only far wall; both far and near wall) and angles (single angle or a combination of angles). Moreover, with these parameters two different CIMT outcome measures can be estimated: the mean common CIMT and the mean maximum CIMT. The mean common CIMT is estimated as the mean value of the mean CIMT measurements that are performed over a 10 mm part of the far wall or of both the far and near wall of the common carotid artery segment. The mean maximum CIMT is a summary measure that is computed as the mean of the single maximum CIMT measurements that are measured in 6 to 12 standard carotid artery walls: the far wall or both the far and near wall of the 2 or 3 distinct carotid artery segments: the common carotid segment, the carotid bifurcation and the internal carotid artery segment at both the left and right side.

The ultrasound protocol depends on the outcome parameter. If the outcome measure of a trial is the mean common CIMT, measurements of only the CCA segment are performed. If the outcome measure is mean maximum CIMT, measurements can be performed at two segments (CCA and BIF) [13,14] or at all three segments of the carotid artery (CCA, BIF and ICA) [15-17].

To determine which ultrasound protocol (combination of segments, angles and walls measured) would be most optimal, many issues are important: the reproducibility of the method; the ability of the method to assess change over time (the CIMT rate of progression); the ability to be related to risk factors or treatment; the ability to provide complete information on measurements; and the expense and time requirements to accomplish the ultrasound measurements. The choice of one ultrasound protocol over another is at present partly based on some of the above mentioned aspects but to a large extent on personal preference. Up until now, formal evaluations of differences in methodological aspects between ultrasound protocols have not yet been published. This is largely due to a lack of studies that included data from all segments, as well as walls and angles to enable such a formal comparison.

We therefore compared ultrasound protocols in a post-hoc analysis in the RADIANCE 1 and RADIANCE 2 studies to determine the protocol that could provide the best balance between reproducibility, magnitude of CIMT change over time and its associated precision, and magnitude of effect of intervention on CIMT change over time and its associated precision.

Methods

General

We used data of the RADIANCE 1[17,18] and RADIANCE 2 study [15,18]. These studies have been described in detail before. In summary RADIANCE 1 was a double-blind randomized placebo-controlled multi-center trial in which 850 patients with heterozygous familial hypercholesterolemia were randomly assigned to receive either atorvastatin monotherapy or atorvastatin combined with 60 mg of torcetrapib for 2 years to study the effect on CIMT progression. RADIANCE 2 was a comparable study, a double-blind, placebo-controlled multi-center trial in which 752 participants with mixed dyslipidemia were randomly assigned to atorvastatin monotherapy or atorvastatin combined with 60 mg of torcetrapib for 2 years to assess the effect of torcetrapib, a CETP inhibitor, on the progression of atherosclerosis, by measuring the thickening of carotid intima-media.

Carotid Ultrasound Examinations

The ultrasound protocol for assessment of the carotid intima-media thickness has been described in detail elsewhere[15,17]. In short, duplicate scans were made at baseline and at each patient's final visit and single scans at visits at 6, 12, and 18 months, to give a maximum of seven scans for each patient. At each visit sonographers acquired and recorded CIMT images of 12 artery-wall segments of the near and far walls of the right and left carotid artery for the common, bifurcation, and the internal carotid artery segments, at four predefined angles of 30º steps (90º to 180º on the right side and 270º to 180º on the left side) using the Meijers carotid arc. This resulted in 48 possible measurements per patient. All imaging centers used the same imaging acquisition protocol and equipment (Sequoia 512 scanners equipped with 8L5 transducers; Siemens AG, Munich, Germany). A total of 48 image sequences (5 seconds lasting video clips) were saved in DICOM format (Digital Imaging in Communications in Medicine, National Electrical Manufacturers Association, Rosslyn, VA, USA). Imaging data were transferred directly from the study sites to the two reading centers (Vascular Imaging Center, University Medical Center, Utrecht, Netherlands, and Wake Forest University Medical Center, Ultrasound Reading Center, Winston-Salem, NC, USA), where standardized equipment and protocols were used to process stored images. From every image sequence, readers selected one frame in end-diastole for measurement of carotid intima-media thickness. Maximum thickness (and also mean for the common carotid artery) was measured semi automatically with Artery Measurement System software (Chalmers University, Göthenburg, Sweden)[19]. Readers were unaware of the interventions assigned to patients, and of previous measurements. Quality assurance protocols have been described elsewhere[20].

Assessment of Various Protocols to Measure CIMT

By selecting CIMT information of different combinations of segments, angles, walls and both carotid arteries 20 ultrasound protocols were constructed to estimate a mean common CIMT value and 40 ultrasound protocols to estimate a mean maximum CIMT value resulting in a total of 60 ultrasound protocols (table 1). The protocols that have been used in the analyses of the RADIANCE 1 and RADIANCE 2 study were protocol 10 for the mean common CIMT and protocol 50 for the mean

maximum CIMT.

Data Analysis

The CIMT estimates of each ultrasound protocol were compared on reproducibility, rate of CIMT progression and effect of intervention on change of CIMT over time with its associated precision.

Reproducibility was assessed in two ways. Intraclass Correlation Coefficients (ICC) and the mean difference of the duplicate CIMT scan at baseline with its corresponding standard deviation were calculated for all protocols and the protocols were ranked by the level of the ICC (the higher, the better) and the standard deviation (SD) of the mean difference (the lower, the better). The ideal and best protocol would have a mean difference of zero with a small variation around this mean difference.

Annual CIMT progression rate in the comparison group (atorvastatin only) was calculated by subtracting the mean CIMT value of the 2 duplicate scans at baseline from the mean CIMT value of the 2 duplicate scans at end of study and dividing it by 2. In this group the rate of CIMT progression was expected to be slowed down and pointing towards zero, as lipid lowering trials have shown statins to hold progression [21]. Precision of CIMT progression was defined by the standard error (SE). To obtain a parameter that weighs the magnitude of the progression rate and the precision of the measurement an absolute progression-precision (PP) ratio was constructed by dividing the mean progression rate by its SE and transforming this value into its absolute value. The absolute value was chosen as it is unknown, whether atorvastatin treatment would only slow down CIMT progression or would even cause regression (negative rate of progression). The protocols were ranked by this absolute PP-ratio from small to large, with the largest value defined as the protocol that achieved the best balance between CIMT progression rate and precision, as ideally one would like to use an ultrasound protocol that shows the highest progression with the highest precision (i.e., smallest SE).

To study the effect of the intervention on CIMT progression rates we estimated the differences in CIMT progression and their corresponding standard errors with regression models in which CIMT progression over time was the dependent variable and treatment (atorvastatin with torcetrapib versus atorvastatin alone) was the independent variable. Next an absolute treatment/precision (TP) ratio was calculated by dividing the regression coefficient (represents change in CIMT progression due to treatment with torcetrapib) by its corresponding standard error and transforming this into an absolute value. Next the ultrasound protocols were ranked by this absolute TP ratio from low to high, as ideally one would like to use an ultrasound protocol that shows the highest effect of treatment on CIMT progression (largest regression coefficient) with the highest precision (i.e., smallest SE).

Finally the protocols were ranked by all three parameters for the mean common CIMT protocols, the mean maximum CIMT protocols and all 60 protocols. A summary score was calculated for each protocol, based on summation of the overall ranks of reproducibility, progression-precision ratio and treatment-precision ratio after categorization of these scores in groups of five and six. We used groups of five for the 20 mean common CIMT protocols and 40 mean maximum CIMT protocols and six for the total ranking of the 60 protocols. This approach was applied rather than focusing on the absolute ranks because we anticipated that differences between the protocols were not large enough to justify a

Table 1: Ultrasound protocols to measure CIMT in the RADIANCE 1 and RADIANCE 2 study, with statement of walls, segments and angles measured

				Walls		Segments			Angles			
Segment	**Wall**	**Angle**		**Fw**	**Nw**	**CCA**	**BIF**	**ICA**	**90**	**120**	**150**	**180**
									270	**240**	**210**	**180**
Mean common CIMT protocols												
CCA	FW & NW	1	**1**	+	+	+			+			
			2	+	+	+				+		
			3	+	+	+					+	
			4	+	+	+						+
		2	**5**	+	+	+			+	+		
			6	+	+	+				+	+	
			7	+	+	+					+	+
		3	**8**	+	+	+			+	+	+	
			9	+	+	+				+	+	+
		4	**10**	+	+	+			+	+	+	+
	FW	1	**11**	+		+			+			
			12	+		+				+		
			13	+		+					+	
			14	+		+						+
		2	**15**	+		+			+	+		
			16	+		+				+	+	
			17	+		+					+	+
		3	**18**	+		+			+	+	+	
			19	+		+				+	+	+
		4	**20**	+		+			+	+	+	+
Mean maximum CIMT protocols												
CCA & BIF	FW & NW	1	**21**	+	+	+			+			
			22	+	+	+				+		
			23	+	+	+	+				+	
			24	+	+	+	+					+
		2	**25**	+	+	+	+		+	+		
			26	+	+	+	+			+	+	
			27	+	+	+	+				+	+
		3	**28**	+	+	+	+		+	+	+	
			29	+	+	+	+			+	+	+
		4	**30**	+	+	+	+		+	+	+	+
	FW	1	**31**	+		+	+		+			
			32	+		+	+			+		
			33	+		+	+				+	
			34	+		+	+					+
		2	**35**	+		+	+		+	+		
			36	+		+	+			+	+	
			37	+		+	+				+	+
		3	**38**	+		+	+		+	+	+	
			39	+		+	+			+	+	+
		4	**40**	+		+	+		+	+	+	+
CCA & BIF & ICA	FW & NW	1	**41**	+	+	+	+		+			
			42	+	+	+	+			+		
			43	+	+	+	+				+	
			44	+	+	+	+					+
		2	**45**	+	+	+	+	+	+	+		
			46	+	+	+	+	+		+	+	
			47	+	+	+	+	+			+	+
		3	**48**	+	+	+	+	+	+	+	+	
			49	+	+	+	+	+		+	+	+
		4	**50**	+	+	+	+	+	+	+	+	+
	FW	1	**51**	+		+	+	+	+			
			52	+		+	+	+		+		
			53	+		+	+	+			+	
			54	+		+	+	+				+
		2	**55**	+		+	+	+	+	+		
			56	+		+	+	+		+	+	
			57	+		+	+	+			+	+
		3	**58**	+		+	+	+	+	+	+	
			59	+		+	+	+		+	+	+
		4	**60**	+		+	+	+	+	+	+	+

CCA : common carotid artery segment, BIF : bifurcation segment, ICA : internal carotid artery segment; NW : near wall, FW : far wall

difference of 60 versus 1 between the best and the worst protocol, whereas a difference of 5 or 6 versus 1 between the best and the worst protocols was reasonable.

For the present study only data of the duplicate scans before randomization and the duplicate scans at the end of study were used. Participants that had data for all four visits were included in the analyses. Analyses were performed by the complete-case analysis principle. Imputation of missing data was not done. SPSS statistical software (version 12.0) was used for the analyses.

Results

The study populations of the RADIANCE 1 and RADIANCE 2 study differed on several baseline characteristics (table 2). Participants of the RADIANCE 1 study were younger, had higher levels of LDL cholesterol, lower levels of triglycerides, lower waist circumference values and lower body mass index than participants of the RADIANCE 2 study. There were more male participants and less hypertension in the RADIANCE 1 study as in the RADIANCE 2 study

The RADIANCE 1 study findings

Data on both pre-randomization examinations and both end of study examinations was available for 748 out of 850 participants (88%) for the RADIANCE 1 study.

Table 2: Baseline characteristics of the RADIANCE 1 and RADIANCE 2 study populations

	RADIANCE 1		RADIANCE 2
Subjects, n	872		752
Age (years)	45 (12.5)		57(8.2)
Male, n(%)	431 (49)		482 (64)
Mean maximum CIMT (mm)	1.14 (0.30)		1.31 (0.31)
Mean common CIMT (mm)	0.72 (0.15)		0.83 (0.15)
Systolic blood pressure (mm. Hg)	116 (11)		120 (11)
Diastolic blood pressure (mm. Hg)	73 (7)		74 (7)
HDL cholesterol (mg/dl)	52(13)		47(11)
LDL cholesterol (mg/dl)	139 (37)		100 (20)
Triglycerides (mg/dl)	113(64)		183(80)
Waist circumference (cm)	89(12)		100(13)
BMI (kg/m²)	26.7(4.4)		30.1(4.4)
Hypertension*, n(%)	230(26)		390(52)

Values presented as mean (standard deviation) or number (percentage); BMI * hypertension defined as: systolic blood pressure > 130 mm Hg or diastolic blood pressure > 85 mm Hg or use of blood pressure-lowering medication

Reproducibility

ICC ranged from 0.774 to 0.908 overall, from 0.803 to 0.908 in the mean common CIMT protocols, from 0.774 to 0.902 in the mean maximum CIMT protocols (table 3). The highest values of ICC were found for mean maximum CIMT and mean common CIMT protocols that included measurements at both walls at multiple angles (≥ 3 angles). The mean difference in CIMT between the two baseline examinations was close to 0 for almost all ultrasound protocols. The standard deviation (SD) of the mean difference ranged from 0.0777 to 0.0866 for the mean common CIMT ultrasound protocols and from 0.1215 to 0.2051 for the mean maximum CIMT ultrasound protocols. Mean common CIMT protocols had smaller SD than mean maximum CIMT protocols. Mean common CIMT protocols with measurements of both walls at ≥ 2 angles had the smallest SD. Mean maximum CIMT protocols with measurements of both walls of all three carotid segments had the smallest SD.

Rate of CIMT Progression over Time in the Atorvastatin Group.

The rate of CIMT progression in the atorvastatin alone group ranged from -0.00302 to 0.00029 mm/year for the mean common CIMT protocols (table 4). All but 1 protocol showed negative slopes. The CIMT progression for the mean maximum protocols showed positive and negative directions depending on the angles that were used. The absolute progression-precision ratio (PP-ratio) ranged from 0.44 to 3.73 for the mean common CIMT protocols and from 0.06 to 1.50 for the mean maximum CIMT protocols. The highest ratios were obtained with mean common CIMT protocols that included measurements of the near and far wall at multiple angles. In the mean maximum CIMT protocols the highest ratios were found in 2-segment protocols that included measurements of the far wall and in 3-segment protocols with measurements of the near and far wall.

Effect of Treatment Effect on CIMT Change over Time

The effect of co-therapy with torcetrapib as compared to placebo on CIMT progression ranged from 0.00087 to 0.000374 mm/year for the mean common CIMT protocols and from -0.00133 to 0.00400 mm/year for the mean maximum CIMT protocols (table 5). The smallest treatment effects were found in the mean maximum protocols, notably in protocols that included measurements of all 3 segments.
The absolute treatment-precision ratios (TP-ratios) ranged from 0.75 to 3.91 for the mean common CIMT protocols and from 0.03 to 2.63 for the mean maximum CIMT protocols. The highest TP-ratios were found predominantly in the mean common CIMT protocols. Protocols that included measurements of both walls at multiple angles had higher ratios than protocols that were based on measurements of only the far wall at one angle. When the interest was in mean maximum CIMT protocols, the highest TP-ratios were found in two-segment protocols with measurements of the near and far wall.

Overall Ranking based on Reproducibility, Rate of CIMT Progression and Effect of Treatment on CIMT Change over Time

Mean common CIMT protocols provided the best balance between high reproducibility, large and

Table 3: Reproducibility of CIMT measurements at baseline in the **RADIANCE 1** study (n= 748), estimated as Intraclass correlation coefficients (ICC) and the mean difference in CIMT (mm) between duplicate scans and ranking for all protocols.

				ICC	Ranking of ICC			Mean difference	SD	Ranking of SD		
Segment	Wall	Angle			Mean common CIMT	Mean maximum CIMT	Overall			Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,843	5		11	0,0032	0,0866	1		53
			2	0,868	9		24	0,0058	0,0852	14		54
			3	0,864	8		20	0,0000	0,0874	13		52
			4	0,811	3		4	0,0029	0,1000	7		46
		2	5	0,891	15		46	0,0075	0,0805	18		58
			6	0,904	18		58	0,0020	0,0777	20		60
			7	0,884	12		39	0,0014	0,0849	15		55
		3	8	0,908	20		60	0,0048	0,0784	19		59
			9	0,904	17		57	0,0026	0,0808	17		57
		4	10	0,906	19		59	0,0041	0,0814	16		56
	FW	1	11	0,803	1		2	0,0016	0,1162	2		41
			12	0,830	4		8	0,0058	0,1094	4		43
			13	0,846	6		14	-0,0004	0,1043	6		45
			14	0,809	2		3	0,0033	0,1129	3		42
		2	15	0,856	7		17	0,0070	0,1061	5		44
			16	0,879	11		33	0,0023	0,0984	10		49
			17	0,871	10		30	-0,0007	0,0993	8		47
		3	18	0,886	13		41	0,0036	0,0985	9		48
			19	0,886	14		42	0,0007	0,0983	11		50
		4	20	0,891	16		47	0,0016	0,0979	12		51
CCA & BIF	FW & NW	1	21	0,839		6	10	0,0023	0,1476		21	21
			22	0,863		12	19	-0,0061	0,1391		25	25
			23	0,869		17	26	-0,0025	0,1323		31	31
			24	0,844		7	12	0,0023	0,1363		28	28
		2	25	0,880		23	34	0,0000	0,1425		24	24
			26	0,890		31	45	-0,0081	0,1354		29	29
			27	0,886		28	40	-0,0034	0,1314		32	32
		3	28	0,893		32	48	-0,0032	0,1425		23	23
			29	0,896		34	50	-0,0074	0,1368		27	27
		4	30	0,899		36	52	-0,0032	0,1427		22	22
	FW	1	31	0,774		1	1	-0,0022	0,2051		1	1
			32	0,815		3	6	-0,0058	0,1932		3	3
			33	0,846		9	15	-0,0073	0,1790		8	8
			34	0,817		4	7	0,0008	0,1858		7	7
		2	35	0,836		5	9	-0,0023	0,1963		2	2
			36	0,869		18	27	-0,0116	0,1778		9	9
			37	0,868		15	23	-0,0115	0,1760		11	11
		3	38	0,868		16	25	-0,0059	0,1871		5	5
			39	0,870		19	28	-0,0140	0,1859		6	6
		4	40	0,876		21	31	-0,0086	0,1883		4	4
CCA & BIF & ICA	FW & NW	1	41	0,864		13	21	0,0006	0,1295		33	33
			42	0,883		26	37	-0,0104	0,1239		39	39
			43	0,879		22	32	0,0010	0,1215		40	40
			44	0,851		10	16	0,0037	0,1286		36	36
		2	45	0,893		33	49	-0,0045	0,1290		35	35
			46	0,900		37	53	-0,0081	0,1246		38	38
			47	0,888		29	43	0,0004	0,1253		37	37
		3	48	0,898		35	51	-0,0040	0,1342		30	30
			49	0,902		40	56	-0,0071	0,1293		34	34
		4	50	0,901		38	54	-0,0042	0,1373		26	26
	FW	1	51	0,902		39	55	-0,0056	0,1721		13	13
			52	0,844		8	13	-0,0111	0,1696		16	16
			53	0,861		11	18	-0,0024	0,1593		20	20
			54	0,812		2	5	0,0025	0,1777		10	10
		2	55	0,865		14	22	-0,0097	0,1697		15	15
			56	0,881		24	35	-0,0134	0,1616		19	19
			57	0,871		20	29	-0,0054	0,1661		18	18
		3	58	0,882		25	36	-0,0105	0,1691		17	17
			59	0,884		27	38	-0,0130	0,1699		14	14
		4	60	0,890		30	44	-0,0109	0,1723		12	12

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment; NW: near wall, FW: far wall. ICC : Intra class correlation; SD : standard deviation. Protocols were ranked from 1 to 66 in an order of low to high reproducibility (increasing ICC and decreasing standard deviation of mean difference)

Table 4: Annual rate of progression of CIMT (mm), its precision (standard errors), absolute ratio of progression-precision and ranking of the ratio for all 60 ultrasound protocols in the **RADIANCE 1** study for the atorvastatin only group (n=364)

				Progression (mm/year)	SE	Absolute ratio	Ranking of absolute ratio		
Segment	Wall	Angle					Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,00029	0,00066	0,44	**1**		25
			2	-0,00056	0,00054	1,05	**4**		38
			3	-0,00220	0,00063	3,52	**18**		**58**
			4	-0,00302	0,00081	3,73	**20**		**60**
		2	5	-0,00040	0,00059	0,67	**2**		32
			6	-0,00172	0,00055	3,15	**16**		**56**
			7	-0,00259	0,00071	3,65	**19**		**59**
		3	8	-0,00145	0,00061	2,36	8		**48**
			9	-0,00210	0,00065	3,25	**17**		**57**
		4	10	-0,00167	0,00067	2,48	9		**49**
	FW	1	11	-0,00092	0,00085	1,09	**5**		39
			12	-0,00069	0,00074	0,93	**3**		36
			13	-0,00220	0,00081	2,71	14		**54**
			14	-0,00193	0,00087	2,21	7		**47**
		2	15	-0,00150	0,00073	2,07	6		**46**
			16	-0,00186	0,00072	2,60	12		**52**
			17	-0,00212	0,00079	2,68	13		**53**
		3	18	-0,00215	0,00074	2,90	15		**55**
			19	-0,00188	0,00075	2,52	10		**50**
		4	20	-0,00197	0,00078	2,53	11		**51**
CCA & BIF	FW & NW	1	21	0,00133	0,00115	1,16		**36**	**41**
			22	0,00045	0,00096	0,47		25	26
			23	-0,00036	0,00091	0,40		22	22
			24	-0,00018	0,00113	0,16		**7**	**7**
		2	25	0,00069	0,00105	0,65		30	31
			26	-0,00037	0,00092	0,40		23	23
			27	-0,00020	0,00097	0,20		11	**11**
		3	28	0,00020	0,00098	0,20		12	**12**
			29	-0,00017	0,00097	0,18		**9**	**9**
		4	30	0,00037	0,00102	0,36		19	**19**
	FW	1	31	-0,00022	0,00157	0,14		**6**	**6**
			32	0,00022	0,00130	0,17		**8**	**8**
			33	-0,00084	0,00133	0,63		29	30
			34	0,00196	0,00162	1,21		**38**	**43**
		2	35	-0,00052	0,00136	0,38		21	21
			36	-0,00101	0,00127	0,80		**32**	34
			37	0,00031	0,00136	0,23		14	**14**
		3	38	-0,00099	0,00132	0,75		**31**	33
			39	-0,00008	0,00133	0,06		**1**	**1**
		4	40	-0,00012	0,00139	0,09		**4**	**4**
CCA & BIF & ICA	FW & NW	1	41	0,00163	0,00108	1,50		**40**	**45**
			42	0,00035	0,00086	0,41		24	24
			43	0,00090	0,00089	1,01		**34**	37
			44	0,00006	0,00110	0,06		**2**	**2**
		2	45	0,00109	0,00098	1,11		**35**	40
			46	0,00049	0,00090	0,54		26	27
			47	0,00054	0,00092	0,59		28	29
		3	48	0,00126	0,00096	1,31		**39**	**44**
			49	0,00053	0,00093	0,57		27	28
		4	50	0,00119	0,00099	1,19		**37**	**42**
	FW	1	51	0,00043	0,00140	0,31		17	**17**
			52	-0,00030	0,00111	0,27		15	**15**
			53	0,00032	0,00116	0,28		16	**16**
			54	0,00134	0,00149	0,90		**33**	35
		2	55	-0,00042	0,00116	0,36		20	**20**
			56	-0,00040	0,00113	0,35		18	**18**
			57	0,00026	0,00122	0,21		13	**13**
		3	58	-0,00012	0,00116	0,10		**5**	**5**
			59	-0,00023	0,00120	0,19		**10**	**10**
		4	60	-0,00010	0,00122	0,08		**3**	**3**

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment; SE: standard error; ratio = calculated as progression / SE, protocols were ranked in an order of lowest to highest ratio,

Table 5: Effect of treatment on CIMT rate of progression over time in **RADIANCE 1** (n=748).

				Treatment effect (mm./year)	SE	Absolute ratio	Ranking of absolute ratio		
Segment	Wall	Angle					Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,00107	0,00089	1,21	**2**		29
			2	0,00196	0,00078	2,50	7		**46**
			3	0,00320	0,00091	3,52	**17**		**57**
			4	0,00315	0,00104	3,02	11		**51**
		2	5	0,00156	0,00081	1,93	**5**		39
			6	0,00310	0,00083	3,74	**19**		**59**
			7	0,00366	0,00094	3,91	**20**		**60**
		3	8	0,00252	0,00086	2,92	9		**49**
			9	0,00334	0,00089	3,73	**18**		**58**
		4	10	0,00281	0,00091	2,73	8		**48**
	FW	1	11	0,00087	0,00116	0,75	**1**		**20**
			12	0,00192	0,00104	1,86	**4**		38
			13	0,00374	0,00112	3,35	**16**		**56**
			14	0,00180	0,00116	1,55	**3**		37
		2	15	0,00207	0,00100	2,07	6		**43**
			16	0,00328	0,00102	3,23	15		**55**
			17	0,00342	0,00108	3,16	14		**54**
		3	18	0,00313	0,00102	3,07	12		**52**
			19	0,00307	0,00104	2,96	10		**50**
		4	20	0,00287	0,00105	3,09	13		**53**
CCA & BIF	FW & NW	1	21	0,00305	0,00155	1,97		**36**	**41**
			22	0,00043	0,00129	0,34		**8**	**8**
			23	0,00343	0,00131	2,63		**40**	**47**
			24	0,00208	0,00150	1,39		**31**	33
		2	25	0,00159	0,00140	1,14		25	26
			26	0,00265	0,00129	2,06		**37**	**42**
			27	0,00328	0,00140	2,35		**39**	**45**
		3	28	0,00270	0,00138	1,96		**35**	40
			29	0,00208	0,00136	1,53		**34**	36
		4	30	0,00207	0,00142	1,46		**33**	35
	FW	1	31	0,00213	0,00209	1,02		23	24
			32	-0,00098	0,00174	0,56		15	**15**
			33	0,00400	0,00186	2,15		**38**	**44**
			34	-0,00048	0,00214	0,22		**5**	**5**
		2	35	0,00046	0,00182	0,25		**6**	**6**
			36	0,00243	0,00174	1,40		**32**	34
			37	0,00248	0,00196	1,27		28	30
		3	38	0,00233	0,00178	1,31		30	32
			39	0,00082	0,00186	0,44		13	**13**
		4	40	0,00074	0,00188	0,39		**10**	**10**
CCA & BIF & ICA	FW & NW	1	41	0,00173	0,00144	1,20		26	27
			42	-0,00022	0,00117	0,19		**4**	**4**
			43	0,00141	0,00126	1,12		24	25
			44	0,00124	0,00140	0,88		21	22
		2	45	0,00055	0,00130	0,42		11	**11**
			46	0,00077	0,00124	0,62		17	**17**
			47	0,00171	0,00131	1,30		29	31
		3	48	0,00095	0,00131	0,72		19	**19**
			49	0,00058	0,00129	0,45		14	**14**
		4	50	0,00058	0,00136	0,43		12	**12**
	FW	1	51	0,00126	0,00189	0,67		18	**18**
			52	-0,00133	0,00154	0,86		20	21
			53	0,00200	0,00167	1,20		27	28
			54	-0,00012	0,00192	0,06		**2**	**2**
		2	55	-0,00006	0,00162	0,03		**1**	**1**
			56	0,00057	0,00157	0,37		**9**	**9**
			57	0,00178	0,00180	0,99		22	23
		3	58	0,00094	0,00161	0,59		16	**16**
			59	0,00014	0,00171	0,08		**3**	**3**
		4	60	0,00043	0,00173	0,25		**7**	**7**

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; SE: standard error; treatment effect: regression coefficient of CIMT: represent the amount of increase in CIMT per year due to treatment with torcetrapib; ratio: calculated as absolute value of beta divided by the SE; the regression model used: 'Progression Rate = a + beta (treatment)'

Table 6: Ranking of the ultrasound protocols based on summation of the transformed rank scores on reproducibility, progression-precision ratio and treatement-precision ratio for the RADIANCE 1 study

Segments	Walls	Angle		RADIANCE 1		
				Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	**8**		14
			2	10		**18**
			3	**15**		**20**
			4	11		**18**
		2	5	12		**19**
			6	**19**		**24**
			7	**17**		**22**
		3	8	**15**		**22**
			9	**20**		**24**
		4	10	**14**		**23**
	FW	1	11	**5**		12
			12	**4**		14
			13	12		**19**
			14	**5**		15
		2	15	**7**		**17**
			16	13		**21**
			17	13		**20**
		3	18	13		**22**
			19	13		**20**
		4	20	**14**		**22**
CCA & BIF	FW & NW	1	21		14	14
			22		11	**9**
			23		15	15
			24		10	10
		2	25		14	14
			26		**16**	**16**
			27		15	15
		3	28		14	14
			29		**16**	13
		4	30		**16**	15
	FW	1	31		**6**	**6**
			32		**5**	**5**
			33		12	11
			34		**8**	**8**
		2	35		**6**	**6**
			36		13	12
			37		10	10
		3	38		11	12
			39		**7**	**7**
		4	40		**7**	**7**
CCA & BIF & ICA	FW & NW	1	41		**16**	15
			42		13	12
			43		**16**	15
			44		11	10
		2	45		**17**	15
			46		**17**	15
			47		**17**	**16**
		3	48		**17**	**16**
			49		**16**	15
		4	50		**16**	**16**
	FW	1	51		13	12
			52		**8**	**9**
			53		11	**9**
			54		**9**	**7**
		2	55		**8**	**8**
			56		11	**9**
			57		11	10
		3	58		10	**9**
			59		**9**	**8**
		4	60		**8**	**9**

CCA: common carotid artery segment, BIF: bifurcation segment, ICA: internal carotid artery segment

precise rates of CIMT progression and effect of treatment (table 6). Mean common CIMT protocols that measured both walls were ranked higher than single wall protocols. Mean maximum CIMT protocol, the protocols that included measurements of the near and far wall provided the highest total rank scores. Overall 3-segment mean maximum CIMT protocols that included measurements of the both walls at multiple angles had the highest total rank scores.

The RADIANCE 2 study findings

Data on both pre-randomization examinations and both end of studye examinations were available for 521 out of 752 participants (69%) in the RADIANCE 2 study.

Reproducibility

ICC ranged from 0.678 to 0.847 for the mean common CIMT protocols and from 0.684 to 0.861 for the mean maximum CIMT protocols (table 7). The highest values of ICC were found for mean common CIMT protocols that included measurements at multiple angles (≥ 2 angles) and for mean maximum CIMT protocols that included measurements of both near and far wall at ≥ 2 angles. The mean difference in CIMT between the two baseline examinations was close to 0 for almost all ultrasound protocols (table 7). The standard deviation (SD) of the mean difference ranged from 0.0974 to 0.1572 for the mean common CIMT ultrasound protocols and from 0.1584 to 0.2561 for the mean maximum CIMT ultrasound protocols. Mean common CIMT protocols had smaller SD than mean maximum CIMT protocols. Mean common CIMT protocols with measurements of both walls at ≥ 2 angles had the smallest SD. Of the mean maximum CIMT protocols, 3-segment protocols using measurements of both walls at multiple angles were ranked highest.

Rate of CIMT Progression over Time in the Atorvastatin Group.

The CIMT progression rates ranged from 0.00084 to 0.00539 mm/year for the mean common CIMT protocols and from 0.00398 to 0.01057 mm/year for the mean maximum CIMT protocols. The progression-precision ratio (PP-ratio) ranged from 0.84 to 5.61 for the mean common CIMT protocols and from 1.85 to 5.44 for the mean maximum CIMT protocols (table 8). Overall mean common CIMT protocols that included measurements of both walls were ranked highest. Mean maximum CIMT protocols that included measurements at all three segments of both walls at multiple angles showed the highest ratios between rate of CIMT progression over time and its corresponding precision.

Treatment Effect on CIMT Change over Time

The effect of co-therapy with torcetrapib as compared to placebo on CIMT progression ranged from -0.00101 to 0.00400 mm / year for the mean common CIMT protocols and from -0.00231 to 0.00486 mm/year for the mean maximum CIMT protocols (table 9). The absolute treatment-precision ratios (TP-ratios) ranged from 0.01 to 2.16 for the mean common CIMT protocols and from 0.02 to 1.46 for the mean maximum CIMT protocols. The highest TP-ratios were found mainly in the mean common CIMT protocols that used measurements of both walls. Mean maximum protocols that included

Table 7: Reproducibility of CIMT measurements at baseline in the **RADIANCE 2** study (n= 521), estimated as Intraclass correlation coefficients (ICC) and the mean difference in CIMT (mm) between duplicate scans and ranking for all protocols

				ICC	Ranking of ICC			Mean difference	SD	Ranking of SD		
Segment	Wall	Angles			Mean common CIMT	Mean maximum CIMT	Overall			Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,771	6		20	-0,0051	0,1114	13		53
			2	0,805	10		33	0,0000	0,1032	15		55
			3	0,743	4		11	-0,0009	0,1243	7		47
			4	0,705	2		4	-0,0020	0,1295	5		45
		2	5	0,836	16		50	0,0007	0,0974	20		60
			6	0,834	15		49	-0,0019	0,0987	19		59
			7	0,797	9		30	0,0010	0,1107	14		54
		3	8	0,843	19		56	-0,0009	0,0988	18		58
			9	0,830	14		47	0,0003	0,1029	16		56
		4	10	0,847	20		58	0,0004	0,1000	17		57
	FW	1	11	0,720	3		7	-0,0100	0,1504	2		42
			12	0,757	5		16	-0,0056	0,1365	3		43
			13	0,782	8		27	-0,0049	0,1269	6		46
			14	0,678	1		1	-0,0074	0,1572	1		41
		2	15	0,821	11		43	-0,0012	0,1209	9		49
			16	0,827	12		44	-0,0066	0,1163	11		51
			17	0,780	7		26	-0,0041	0,1330	4		44
		3	18	0,841	18		52	-0,0042	0,1159	12		52
			19	0,828	13		46	-0,0061	0,1209	8		48
		4	20	0,841	17		51	-0,0040	0,1197	10		50
CCA & BIF	FW & NW	1	21	0,773		17	23	-0,0050	0,1909		21	21
			22	0,775		19	25	0,0152	0,1803		25	25
			23	0,749		10	14	0,0013	0,1880		22	22
			24	0,732		7	10	0,0030	0,1854		23	23
		2	25	0,811		26	36	0,0098	0,1809		24	24
			26	0,821		32	42	0,0115	0,1728		30	30
			27	0,809		25	35	0,0041	0,1706		34	34
		3	28	0,842		36	54	0,0067	0,1738		29	29
			29	0,842		35	53	0,0095	0,1706		33	33
		4	30	0,861		40	60	0,0057	0,1696		35	35
	FW	1	31	0,684		1	2	-0,0074	0,2556		2	2
			32	0,717		4	6	0,0200	0,2374		5	5
			33	0,695		2	3	0,0047	0,2561		1	1
			34	0,721		5	8	0,0073	0,2420		4	4
		2	35	0,743		8	12	0,0150	0,2451		3	3
			36	0,769		14	19	0,0150	0,2315		9	9
			37	0,773		18	24	0,0154	0,2319		8	8
		3	38	0,785		21	29	0,0134	0,2346		6	6
			39	0,814		28	38	0,0181	0,2211		12	12
		4	40	0,814		29	39	0,0162	0,2280		10	10
CCA & BIF & ICA	FW & NW	1	41	0,798		22	31	-0,0053	0,1743		27	27
			42	0,773		16	22	0,0116	0,1738		28	28
			43	0,772		15	21	0,0024	0,1694		36	36
			44	0,760		12	17	-0,0013	0,1709		31	31
		2	45	0,812		27	37	0,0100	0,1769		26	26
			46	0,816		30	40	0,0098	0,1686		38	38
			47	0,821		31	41	0,0061	0,1584		40	40
		3	48	0,845		38	57	0,0093	0,1692		37	37
			49	0,843		37	55	0,0106	0,1643		39	39
		4	50	0,855		39	59	0,0116	0,1708		32	32
	FW	1	51	0,712		3	5	-0,0121	0,2328		7	7
			52	0,732		6	9	0,0113	0,2196		14	14
			53	0,753		11	15	-0,0006	0,2175		15	15
			54	0,745		9	13	0,0073	0,2202		13	13
		2	55	0,768		13	18	0,0114	0,2227		11	11
			56	0,783		20	28	0,0102	0,2163		16	16
			57	0,803		23	32	0,0128	0,2076		19	19
		3	58	0,808		24	34	0,0096	0,2149		17	17
			59	0,827		33	45	0,0183	0,2049		20	20
		4	60	0,833		34	48	0,0174	0,2112		18	18

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment; NW: near wall, FW: far wall. ICC : Intra class correlation; SD : standard deviation; Protocols were ranked from 1 to 66 in an order of low to high reproducibility (increasing ICC and decreasing standard deviation of mean difference)

Table 8: Annual rate of progression of CIMT (mm), its precision (standard errors), absolute ratio of progression-precision and ranking of the ratio for all 60 ultrasound protocols in the **RADIANCE 2** study for the atorvastatin only group (n=268)

							Ranking Ratio		
Segment	Wall	Angle		Progression (mm./year)	SE	Ratio	Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,00289	0,00099	2,91	**5**		**15**
			2	0,00225	0,00136	4,75	14		**48**
			3	0,00405	0,00085	2,43	**4**		**7**
			4	0,00521	0,00116	0,84	**1**		**1**
		2	5	0,00247	0,00102	5,44	**19**		**58**
			6	0,00440	0,00100	5,17	**17**		**55**
			7	0,00084	0,00101	3,47	6		22
		3	8	0,00158	0,00119	5,61	**20**		**60**
			9	0,00474	0,00087	4,72	13		**46**
		4	10	0,00472	0,00115	4,44	11		**40**
	FW	1	11	0,00429	0,00083	1,65	**3**		**3**
			12	0,00539	0,00101	4,51	12		**41**
			13	0,00326	0,00094	4,39	10		38
			14	0,00455	0,00108	1,33	**2**		**2**
		2	15	0,00481	0,00086	4,09	7		31
			16	0,00529	0,00107	5,35	**18**		**57**
			17	0,00414	0,00088	4,21	8		35
		3	18	0,00480	0,00111	4,93	15		**51**
			19	0,00471	0,00092	4,34	9		37
		4	20	0,00505	0,00114	5,13	**16**		**54**
CCA & BIF	FW & NW	1	21	0,00456	0,00180	2,54		**6**	**10**
			22	0,00551	0,00226	3,73		21	27
			23	0,00560	0,00150	2,43		**4**	**8**
			24	0,00713	0,00206	2,26		**2**	**5**
		2	25	0,00411	0,00169	4,27		28	36
			26	0,00515	0,00223	4,18		27	34
			27	0,00398	0,00176	3,74		22	28
		3	28	0,00424	0,00229	4,51		30	**42**
			29	0,00675	0,00158	4,40		29	39
		4	30	0,00745	0,00218	4,62		**31**	**43**
	FW	1	31	0,00627	0,00150	2,44		**5**	**9**
			32	0,00769	0,00203	3,47		17	23
			33	0,00620	0,00166	2,30		**3**	**6**
			34	0,00650	0,00221	1,85		**1**	**4**
		2	35	0,00697	0,00155	3,42		15	**20**
			36	0,00780	0,00216	3,78		23	29
			37	0,00703	0,00160	2,93		11	**16**
		3	38	0,00763	0,00214	3,62		20	26
			39	0,00766	0,00166	3,57		18	24
		4	40	0,00796	0,00223	3,57		19	25
CCA & BIF & ICA	FW & NW	1	41	0,00462	0,00171	2,70		**7**	**11**
			42	0,00625	0,00218	3,32		13	**18**
			43	0,00463	0,00139	3,29		12	**17**
			44	0,00631	0,00188	2,81		**9**	**13**
		2	45	0,00509	0,00155	4,14		25	32
			46	0,00707	0,00205	4,80		**35**	**49**
			47	0,00470	0,00167	4,69		**33**	**45**
		3	48	0,00594	0,00213	4,88		**36**	**50**
			49	0,00645	0,00156	5,44		**40**	**59**
		4	50	0,00838	0,00212	5,29		**39**	**56**
	FW	1	51	0,00667	0,00139	2,87		**10**	**14**
			52	0,00874	0,00185	3,36		14	**19**
			53	0,00727	0,00155	3,44		16	21
			54	0,00870	0,00210	2,79		**8**	**12**
		2	55	0,00737	0,00151	3,95		24	30
			56	0,00949	0,00205	4,73		**34**	**47**
			57	0,00829	0,00152	4,14		26	33
		3	58	0,00978	0,00197	4,63		**32**	**44**
			59	0,00858	0,00162	4,95		**38**	**53**
		4	60	0,01057	0,00214	4,94		**37**	**52**

CCA : common carotid artery segment; BIF : bifurcation segment; ICA : internal carotid artery segment; SE: standard error; ratio = calculated as progression / SE, protocols were ranked in an order of lowest to highest ratio

measurements of only the common carotid and the bifurcation segment showed higher TP-ratios than 3 segment protocols. Protocols with measurements of the near and far wall had higher ratios in both the mean common and the mean maximum protocols.

Overall Ranking based on Reproducibility, Rate of CIMT Progression and Effect of Treatment on CIMT Change over Time

Mean common CIMT protocols provided the best balance between high reproducibility, large and precise rates of CIMT progression and effect of treatment (table 10). Mean common CIMT protocols that measured both walls had higher total rank scores than single wall protocols. The highest total rank scores were found in mean maximum CIMT protocols that used measurements of the near and far wall. Mean maximum CIMT protocols that included measurements of all 3 segments at multiple angles had the highest total rank scores.

Discussion

Mean common CIMT that was assessed through measurements of both the near and far wall of the common carotid artery segment at multiple (≥2 angles) provided the best balance between high reproducibility, large estimates of CIMT progression over time, large change in progression over time induced by the intervention and their associated precision. With regards to mean maximum CIMT, protocols that included measurements of both near and far wall of all three carotid segments performed best.

Some aspects of this study need to be addressed. Firstly, all CIMT measurements in our study were fixed with regard to the angle of insonation through use of the Meijer's Carotid Arc. This guaranteed that consecutive scans could be performed at approximately the same angle. Our results, however, might differ from CIMT ultrasound protocols that are dedicated to finding the previous image (insight / memory) or those that interrogate the maximum measurements (independent of angles), or interrogate from a single angle that visually measures the best image from which CIMT should be measured. However, there are no data available to indicate how protocols with absence of angle approaches would rank in our tables.

Secondly, reproducibility was assessed by the ICC and the standard deviation of the mean difference. The ICC is a commonly used and well known measure to evaluate reproducibility. It quantifies measurement reliability by comparing the variability of different measurements of the same subject to the total variation across all measurements and all subjects. One characteristic of the ICC is that it depends on the magnitude of the variation between individuals. If the variation between individuals is large, relative to the measurement error, the ICC will become high. In contrast, if the variation between individuals is small and CIMT is measured with the same measurement error, the ICC will become small. Multiple segment protocols could therefore produce higher ICC's than single segment protocols , based only on this difference in between individual variation. Therefore, protocols were also ranked on the standard deviation of the mean difference of the duplicate scan. This measure is a suitable test to assess reproducibility and is less affected by between individual variations. In the assessment of

Table 9. Effect of treatment on CIMT rate of progression over time in **RADIANCE 2** (n=521)

Segment	Wall	Angle		treatment effect (mm. /year)	SE	Absolute ratio	Ranking of ratio		
							Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	0,00199	0,00151	1,32	**16**		**1**
			2	0,00019	0,00132	0,14	**3**		**55**
			3	0,00156	0,00137	1,14	15		**10**
			4	0,00327	0,00152	2,14	**19**		**54**
		2	5	0,00014	0,00131	0,11	**2**		**59**
			6	0,00098	0,00122	0,8	13		**7**
			7	0,00190	0,00134	1,41	**18**		**46**
		3	8	0,00111	0,00126	0,88	14		**57**
			9	0,00098	0,00129	0,76	12		**51**
		4	10	0,00071	0,00133	0,53	9		**42**
	FW	1	11	0,00274	0,00197	1,39	**17**		32
			12	-0,00088	0,00169	0,52	8		**56**
			13	-0,00101	0,00149	0,68	11		31
			14	0,00400	0,00185	2,16	**20**		38
		2	15	0,00037	0,00164	0,22	**4**		**60**
			16	-0,00065	0,00144	0,45	7		**14**
			17	0,00089	0,00157	0,56	10		26
		3	18	0,00002	0,00154	0,01	**1**		34
			19	0,00040	0,00152	0,26	**5**		**16**
		4	20	0,00046	0,00160	0,29	6		**19**
CCA & BIF	FW & NW	1	21	0,00283	0,00279	1,02		**39**	**53**
			22	0,00023	0,00229	0,10		**5**	**6**
			23	0,00154	0,00227	0,68		28	39
			24	0,00232	0,00248	0,94		**38**	**52**
		2	25	0,00108	0,00241	0,45		20	27
			26	0,00025	0,00222	0,11		**6**	**8**
			27	0,00185	0,00240	0,77		**32**	**44**
		3	28	0,00063	0,00234	0,27		**13**	**18**
			29	0,00033	0,00241	0,14		**8**	**11**
		4	30	0,00027	0,00252	0,11		**7**	**9**
	FW	1	31	0,00303	0,00362	0,84		**36**	**49**
			32	-0,00027	0,00313	0,09		**3**	**4**
			33	0,00048	0,00306	0,16		**10**	**13**
			34	0,00486	0,00333	1,46		**40**	**58**
		2	35	0,00083	0,00318	0,26		**12**	**17**
			36	-0,00178	0,00297	0,60		26	36
			37	0,00265	0,00323	0,82		**35**	**48**
		3	38	-0,00092	0,00303	0,30		**14**	**20**
			39	-0,00014	0,00316	0,05		**2**	**3**
		4	40	0,00006	0,00318	0,02		**1**	**2**
CCA & BIF & ICA	FW & NW	1	41	0,00143	0,00253	0,57		25	35
			42	0,00172	0,00217	0,80		**34**	**47**
			43	-0,00071	0,00211	0,34		**15**	21
			44	0,00031	0,00227	0,14		**9**	**12**
		2	45	0,00101	0,00236	0,43		18	24
			46	-0,00100	0,00210	0,48		22	29
			47	-0,00052	0,00218	0,24		**11**	**15**
		3	48	-0,00098	0,00228	0,43		19	25
			49	-0,00144	0,00226	0,63		27	37
		4	50	-0,00132	0,00242	0,54		24	33
	FW	1	51	0,00144	0,00315	0,46		21	28
			52	0,00218	0,00289	0,75		30	**41**
			53	-0,00217	0,00281	0,77		**33**	**45**
			54	0,00218	0,00300	0,73		29	40
		2	55	0,00103	0,00301	0,34		**16**	22
			56	-0,00231	0,00275	0,84		**37**	**50**
			57	-0,00025	0,00300	0,09		**4**	**5**
		3	58	-0,00222	0,00291	0,76		**31**	**43**
			59	-0,00113	0,00298	0,38		17	23
		4	60	-0,00154	0,00311	0,50		23	30

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; SE: standard error; treatment effect: regression coefficient of CIMT: represent the amount of increase in CIMT per year due to treatment with torcetrapib; ratio: calculated as absolute value of beta divided by the SE; the regression model used: 'Progression Rate = a + beta (treatment)'

Table 10: Ranking of the ultrasound protocols based on summation of the transformed rank scores on reproducibility, progression-precision ratio and treatment-precision ratio for the RADIANCE 2 study

Segments	Walls	Angle		RADIANCE 2		
				Mean common CIMT	Mean maximum CIMT	Overall
CCA	FW & NW	1	1	12		11
			2	12		**21**
			3	**8**		**9**
			4	**9**		13
		2	5	**15**		**23**
			6	**18**		**18**
			7	**14**		**17**
		3	8	**19**		**24**
			9	**15**		**22**
		4	10	**16**		**21**
	FW	1	11	**8**		11
			12	**8**		**17**
			13	10		**16**
			14	**8**		11
		2	15	**9**		**20**
			16	13		**19**
			17	**8**		15
		3	18	13		**22**
			19	11		**16**
		4	20	**14**		**19**
CCA & BIF	FW & NW	1	21		12	13
			22		11	**10**
			23		**10**	**10**
			24		**10**	11
		2	25		14	14
			26		13	13
			27		**16**	**16**
		3	28		**15**	**16**
			29		**15**	**16**
		4	30		**15**	**16**
	FW	1	31		**8**	**8**
			32		**6**	**6**
			33		**5**	**5**
			34		**8**	**9**
		2	35		**6**	**7**
			36		11	**10**
			37		11	11
		3	38		**9**	**9**
			39		**10**	**10**
		4	40		**10**	**9**
CCA & BIF & ICA	FW & NW	1	41		12	13
			42		13	13
			43		11	12
			44		**10**	**10**
		2	45		**15**	14
			46		**17**	**16**
			47		**16**	**16**
		3	48		**18**	**18**
			49		**19**	**20**
		4	50		**17**	**20**
	FW	1	51		**7**	**7**
			52		**9**	**9**
			53		11	12
			54		**9**	**10**
		2	55		**9**	**10**
			56		**15**	15
			57		11	11
		3	58		14	**16**
			59		**16**	**16**
		4	60		**16**	**16**

CCA: common carotid artery segment, BIF: bifurcation segment, ICA: internal carotid artery segment

reproducibility the mean absolute difference (MAD) of the duplicate baseline scan was also evaluated (results not shown). Ranking of the protocols on the absolute mean difference provided similar results as the ranking based on SD of the mean difference. The correlation between these two parameters was close to 1, indicating that these two parameters assess the same outcome. Thirdly, protocols were ranked based on the absolute progression–precision ratio in the atorvastatin monotherapy group. Most ideally, one would like to rank protocols on progression-precision in a placebo-group as it is then clear which direction the CIMT progression rate is assumed to go: progression of CIMT rates over time. However in the group that received atorvastatin, the direction was not known. As previous studies showed that statin treatment produces at most a slowing down of the CIMT progression rate that is close to zero [14,16], the absolute PP-ratio was used ranking on magnitude of progression rate (either in a negative direction or in a positive direction) and precision. Yet, in our study negative progression rates in both the mean common and the mean maximum CIMT protocols were observed. We also ranked the protocols based on direction of CIMT progression, i.e. ranking protocols with negative progression rates and high precision higher, (results not shown) however this only had a minor effect on the ranking. Finally, we observed the same difficulty of direction of progression in the ranking of treatment effect on progression of CIMT. Although the event trial with atorvastatin and torcetrapib showed increased risk in the torcertrapib arm as compared to atorvastatin monotherapy [22], we decided to rank the ultrasound protocols as if treatment with torcetrapib was to slow down CIMT progression. This was done for two reasons. One, CIMT trials are designed to show benefit of treatment and if treatment is expected to cause harm a CIMT trial would not be conducted beforehand. Two, the event trial showed that torcetrapib therapy resulted in an increased risk of mortality and morbidity of an unknown mechanism, with evidence of an off-target effect of torcetrapib[22]. Currently trials with other CETP inhibitors are being conducted, and these will provide information whether this class of drugs yields effect on CIMT progression. To overcome this difficult problem, we ranked based on the absolute TP-ratio that corresponds to the magnitude of effect with its precision, whilst discarding the direction of effect of treatment (positive or negative direction).

The generalizability of the results of this study may depend on the study populations since reproducibility, progression and its precision and effect of treatment and its precision may be affected by the level of atherosclerosis. However, the implications on choice of ultrasound protocol seem to be equal for both study populations. For the FH populations the best balance between the three parameters were found in mean common CIMT protocols, with increasing ratios if both walls were measured at more angles. This also applies to the MD populations, yet in the MD population, some mean maximum CIMT protocols also ranked favorably.

For decades a lively discussion is ongoing regarding the desirability of performing near wall CIMT measurements [12]. The reluctance is based on the physics of ultrasound indicating that the near wall CIMT is at best an approximation of the true value, whereas the advocates indicate that the addition of the near wall measurements increase precision and hence increase precision in risk prediction and evaluation of drug effects. Our study indicated that the best protocols with regard to reproducibility, PP-ratio and TP ratio were all predominantly based on data collected from both the far and the near wall.

The results of our study support the view that ultrasound protocols that measure both walls should be preferred above protocols that measure only the far wall.

In every study using CIMT measurements, whether a cohort study or a randomized controlled trial, a choice needs to be made based on the costs and the benefits. Benefits relate to high reproducibility, precise measurements, and when appropriate a high progression-precision balance, and thus a high likelihood of success. Costs relate to the time needed to acquire the CIMT information (ultrasound scanning and reading) and the financial expenses related to that process. The balance between quality and costs should be evaluated and considered in the decision making process. We feel that the present analyses provide sufficient information to estimate that balance in an evidence based way.

To the best of our knowledge this type of data has never been published before, most likely since no other study had such a wealth of detailed information on CIMT measurements as was available in the RADIANCE 1 and RADIANCE 2 study. Data from other studies with similar extensive ultrasound protocols were analyzed and provided information on the best ultrasound protocol for trials in other population groups. In healthy middle-aged subjects, the OPAL study [23], mean common CIMT ultrasound protocols that included measurements of both the near and far wall at multiple angles performed best on reproducibility and providing a large rate of CIMT progression with a high precision [24]. In low risk middle-aged subjects with asymptomatic atherosclerosis, the METEOR study [25], mean maximum CIMT protocols with measurements of both the near and far wall performed best in providing the best balance between reproducibility, large rate of CIMT progression with a high precision and a precise and large estimate of effect of treatment on change of CIMT over time [26].

In conclusion, our findings support the opinion that the number and specific combination of segments, angles and walls interrogated are associated with differences in reproducibility; magnitude and accuracy of progression of CIMT over time. Given these findings, the choice for an ultrasound protocol should depend on a well-considered balance of these parameters before the study is carried out.

Common CIMT assessed at both walls at multiple angles resulted in best balance between high reproducibility, large rate of CIMT progression and large effect of treatment, all measured with high precision, in particular in FH patients.

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Chapter 6

Effect of batch reading on carotid intima-media thickness progression rates in randomized controlled trials

Abstract

Background

Theoretically, batch reading reduces measurement variability of carotid intima-media thickness (CIMT) estimates of change over time by preventing drift (change in reading habits of readers) as compared to consecutive reading approaches. Yet, published data on this issue are lacking. We assessed the effect of two different reading approaches of CIMT images on progression rates and treatment effect.

Methods

After completion of the Folic Acid and Carotid Intima-media Thickness (FACIT) study, a single-center, randomized, double blind, placebo-controlled trial in 819 older adults with initially elevated concentrations of total homocysteine (Clinical trial registry: NCT00110604), all carotid B-mode ultrasound examinations were re-read in batch fashion; i.e. all study scans of a participant were read in a short time window by one person. Progression rates and treatment effects were estimated using linear regression analyses for each reading approach.

Results

Three-year CIMT progression in the non-batch approach was -0.012 mm (95% confidence interval (95%CI) -0.018;-0.007) in the Folic Acid group and -0.009 mm (95%CI -0.014; -0.004) in the placebo group. In the batch reading approach these were 0.005 mm (95%CI -0.001; 0.010) and 0.004 mm (95%CI -0.001; 0.010), respectively. Treatment effects were similar: mean difference in CIMT progression across groups was -0.001 mm./year (95%CI -0.003; 0.003) in the non batch approach and 0.001 mm/year (95%CI -0.002; 0.003) for the batch approach.

Conclusion

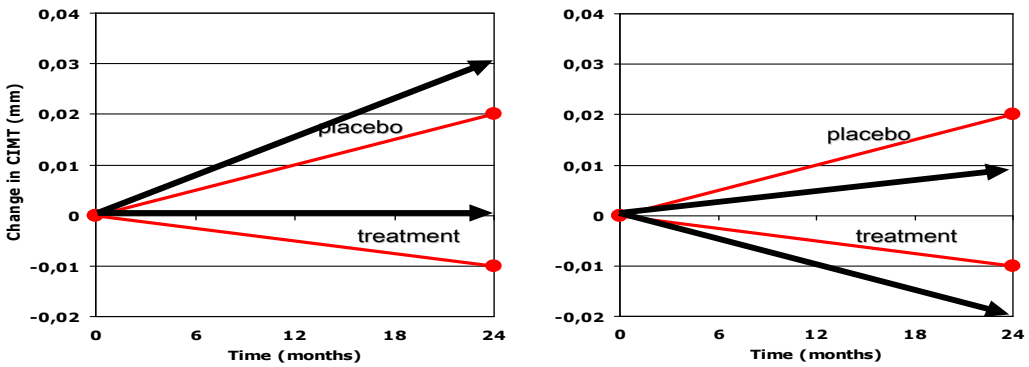
The quantification of the effect of an intervention appears not to be affected by the type of reading approach (batch or non-batch). In contrast, the absolute observed CIMT progression rates might be biased using a non-batch approach.

Introduction

Change in carotid intima-media thickness (CIMT) over time is used in trials as an alternative, surrogate end point for cardiovascular events to evaluate the efficacy of interventions[1-13]. Typically, after obtaining carotid ultrasound scans, CIMT images are collected in central core laboratories (specialized vascular imaging centers) where CIMT is measured. There are two approaches to read CIMT from images: random continuous readings (non-batch) and batch readings. In the non-batch approach CIMT measurements are performed continuously over the course of the study, by randomly allocating a reader to a scan that is received at the core lab. In batch reading one reader reads all the scans of a certain participant in a short time-period after collection of the last scan. A logistic advantage of non-batch reading is efficiency and short time-lag between completion of the trial and data availability. A disadvantage of non-batch reading, however, may be the temporal component. In studies that last several years between the first CIMT measurements and the last CIMT measurements, theoretically a bias may occur in the estimates of the progression, due to change over time in measuring habits of reading personnel of the core laboratory. This is illustrated in figure 1, where the light-coloured lines represent unbiased progression rates of CIMT over time in the placebo and the treatment group. If there is a positive drift in reading habits (thick black arrows in the left figure) progression rates of both treatment group and placebo group are measured thicker, which leads to a shift towards more pronounced progression in both groups. In case of negative drift (thick black arrows in the right figure) progression rates are measured thinner, which lead to a shift towards negative progression (regression) in both groups. Drift therefore may affect progression rates in theory. Drift should not affect treatment effects, as readers are blinded for assignment of the intervention, and thus potential drift is likely to affect both treatment arms and thereby the estimated difference between the treatment arms should remain equal. Empirical data on this issue is however scarce.

Our objective was to study whether batch reading affects CIMT progression rates and whether the estimated treatment effect is different in batch reading compared to non-batch reading.

Figure 1, theoretical representation of the effect of change in measureing habits on CIMT progression estimates with readers that are blinded for the treatment allocation.



Light coloured lines = correct (unbiased) CIMT progression in treatment and placebo group; black arrows = left figure, CIMT affected by positive drift (reading thicker over time); right figure, CIMT affected by negative drift (reading thinner over time). Treatment effect in both situations is equal to the unbiased effect, which is 0.03 mm

Methods

General

We used data of 'Folic Acid and Carotid Intima-media Thickness (FACIT) trial (unpublished), a double-blind, placebo-controlled study that took place between November, 1999, and December, 2004, in the Netherlands that investigated the effect of folic acid supplementation on atherosclerotic progression. 819 participants, men and post-menopausal women aged 50–70 years from the Gelderland region in the Netherlands, were randomly assigned to 800 μg daily oral folic acid (FA) or placebo for 3 years [14,15]. The Medical Ethics Committee of Wageningen University approved the study and subjects gave written informed consent. This trial is registered with clinical trials.gov; trial number NCT00110604 |(http://clinicaltrials.gov).

Carotid Ultrasound examinations

High-resolution B-mode carotid ultrasonography was performed with a 7.5 MHz linear-array transducer (ATL Ultramark IX, Bothell, Washington) at two separate visits at baseline and at two subsequent visits at the end of study. Longitudinal images of the distal common carotid arteries were obtained at four predefined angles of 30º steps (90º to 180º on the right side and 270º to 180º on the left side). Images were frozen on the top of the R-wave of the electrocardiogram and recorded on VHS tape. The videotapes were collected in a central core laboratory, the Vascular Imaging Center, in Utrecht, the Netherlands.

CIMT measurements were performed in the distal 10 mm of the right and left common carotid arteries with a semi-automated edge detection program, the Artery Measurement System [16] according to a uniform reading protocol. The automatically derived interfaces could be modified manually by the reader if necessary. For CIMT measurements, trailing edges were traced on the near wall boundaries and leading edges on the far wall boundaries. The values of the mean intima-media thickness of all the angles were averaged for each individual, similar to earlier trials[17,18]. The primary outcome was change in mean common CIMT over time (mm/yr).

Reading procedure: batch & non-batch approach

CIMT measurements at the core laboratory were performed initially in a non-batch random continuous fashion. When videotapes arrived at the core laboratory one of 2 readers was randomly assigned to read the particular ultrasound examination. The readers were blinded to the treatment allocation. After completion of the study all CIMT scans were read in batch fashion by one single reader in a short time period (June 2005 – December 2005). Each batch constituted of 100 subjects (duplicate baseline and duplicate follow-up scans). The order of the four visits was randomized and the reader was blinded to the treatment allocation.

Statistical analyses

A mean baseline CIMT value was calculated as the mean of the duplicate scans at baseline. The same was done for the duplicate scans at the end of the study. Total CIMT progression over 3 years

was estimated by subtracting the mean baseline CIMT value from the mean end of study CIMT value. The difference in CIMT progression rate between the two treatment arms was assessed using linear regression models without any adjustments based on the intention to treat principle. Differences of rate of progression between the treatment groups were presented with 95% confidence intervals.

Results

General characteristics of the study population are presented in table 1. The risk factors were well balanced across the two groups. Table 2 shows progression rates per group of 100 participants for both reading approaches. Three year CIMT change in the non-batch reading approach went into a negative direction (regression) for both the folic acid group with -0.012 mm (95% confidence interval (95%CI) -0.018;-0.007) and the placebo group with -0.009 mm (95%CI -0.014; -0.004). In the batch reading approach CIMT change was in a positive direction (progression) with 0.005 mm (95%CI -0.001; 0.010) in the folic acid group and 0.004 mm (95%CI -0.001; 0.010) in the placebo group. The mean difference in CIMT progression rates between the treatment groups did not differ between the batch and non-batch reading approaches and was consistent over the batches (figure 2). The pooled variance estimate and width of the 95% confidence intervals for treatment effect were slightly smaller for the batch readings.

Table 1: Mean (standard deviation) or number (percentage) of the characteristics of the folic acid and placebo group at the start of the study

	Folic acid (*n*=406)	**Placebo (*n*=413)**
Age, year	60 (6)	60 (6)
Male, *n*	294 (72)	292 (70)
Creatinine, µmol/L	93 (13)	92 (12)
Total cholesterol, mmol/L	5.81 (1.12)	5.84 (1.11)
LDL cholesterol, mmol/L (1)	4.02 (1.00)	4.02 (0.96)
HDL cholesterol, mmol/L	1.22 (0.34)	1.25 (0.38)
Body mass index, kg/m^2 (2)	26.6 (3.6)	26.5 (3.6)
Current smokers, n (%)	84 (21%)	83 (20%)
Diabetes mellitus, n (%)	12 (3%)	14 (3%)
Mean common CIMT, mm (Standard Error)	0.79 (0.006)	0.77(0.005)

The body mass index is the weight in kilograms divided by the square of the height in meters

Table 2: Three year progression of mean common CIMT (in mm) with corresponding standard errors, by treatment group and by reading mode (ongoing or batch) in increasing numbers of batches

Non-batch reading

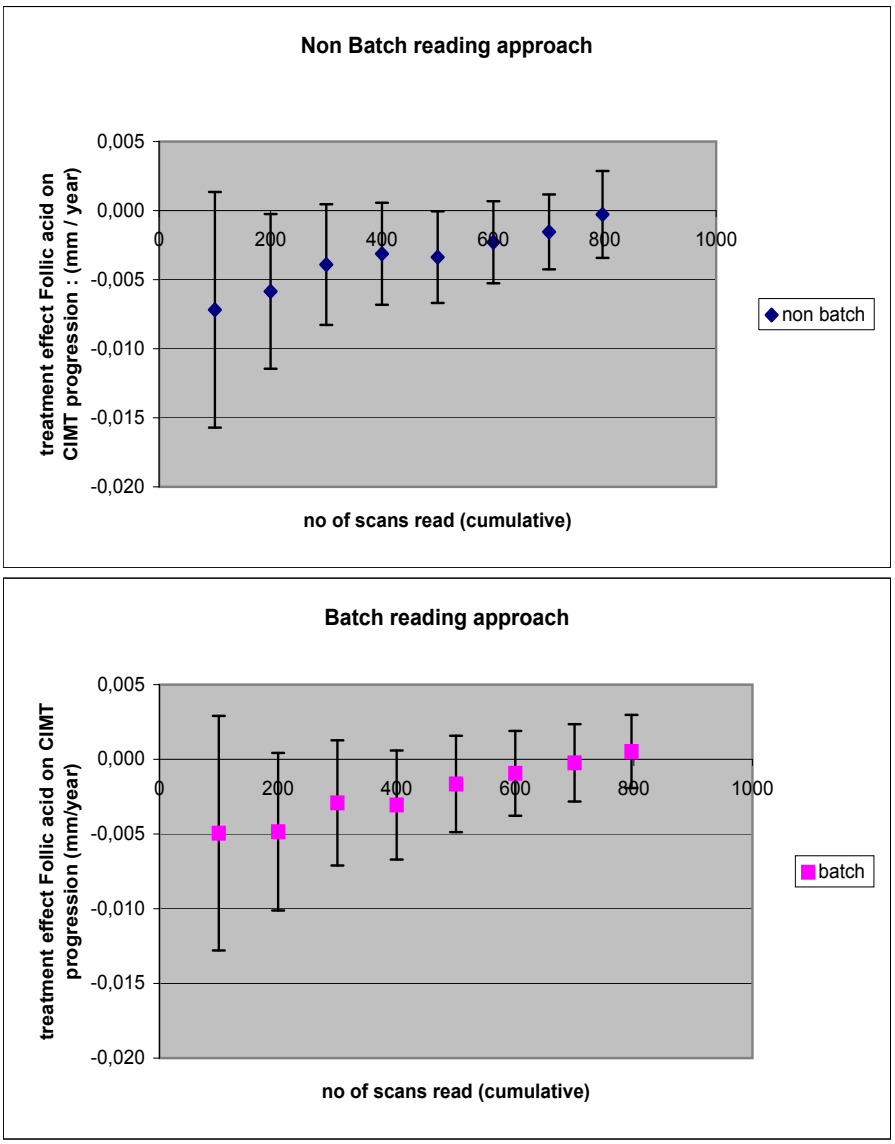
	Folic Acid		**Placebo**			
No of consecutive participants	mean progression (mm)	SE	mean progression (mm)	SE	Difference intervention- placebo	SE
100	-0.0166	0.0098	0.0049	0.0087	0.0215	0.0092
200	-0.0180	0.0062	-0.0005	0.0059	0.0175	0.0061
300	-0.0182	0.0049	-0.0065	0.0046	0.0117	0.0047
400	-0.0175	0.0040	-0.0082	0.0040	0.0094	0.0040
500	-0.0167	0.0036	-0.0066	0.0035	0.0101	0.0036
600	-0.0151	0.0033	-0.0082	0.0031	0.0069	0.0032
700	-0.0136	0.0030	-0.0089	0.0029	0.0046	0.0029
796	-0.0122	0.0028	-0.0090	0.0027	0.0032	0.0028

Batch reading

	Folic Acid		**Placebo**			
No of consecutive participants	mean progression (mm)	SE	mean progression (mm)	SE	Difference intervention- placebo	SE
100	0.0005	0.0098	0.0154	0.0072	0.0148	0.0085
200	-0.0013	0.0063	0.0133	0.0051	0.0145	0.0057
300	-0.0016	0.0050	0.0072	0.0041	0.0087	0.0046
400	-0.0012	0.0042	0.0080	0.0037	0.0092	0.0040
500	0.0022	0.0037	0.0071	0.0033	0.0049	0.0035
600	0.0026	0.0033	0.0054	0.0029	0.0028	0.0031
700	0.0040	0.0030	0.0047	0.0026	0.0007	0.0028
796	0.0047	0.0029	0.0044	0.0025	-0.0003	0.0027

SE : standard error

Figure 2. Treatment effect on CIMT progression (mm/year) with corresponding 95% confidence intervals for continuous reading approach (non-batch) and batch reading approach.



Discussion

We have presented empirical data that supports the theoretical concept of the effect of readers drift on the rate of CIMT progression and on its effect on the assessment of treatment effects. In our study a negative drift was present (towards thinner readings), that affected progression rates in both treatment arms without altering the effect of treatment on CIMT progression over time in any appreciable manner.

In a randomized trial the main interest lies in the assessment of a true treatment effect on the primary outcome. For a CIMT trial the primary outcome would be the rate of change over time in CIMT. The measurement of change over time in CIMT is susceptible to drift, i.e., the CIMT measurements are read in different way at the start of the trial compared to the beginning of the trial. In general, this drift has been looked upon as undesirable, and the recommended solution is performing a batch reading. In batch reading all scans of one individual from which CIMT should be measured, are collected at the end of the trial and are read in a short time window (days-weeks) by one 'reader'. This approach minimizes drift, and also removes differences between readers from the estimation of change within an individual. However, from a theoretical point of view, when drift is a random phenomenon and happens across both treatment arms (figure 1), the net effect of drift on the estimated difference in rate of change between treatment arms is not affected. Indeed, with empirical data we have confirmed this concept. So in principle, unbiased treatment effects can be obtained with a non-batch reading fashion only when readers are blinded for exposure information (e.g. treatment allocation).

In our study the batch reading was performed by a single reader. By removing between reader variation from the estimation of CIMT change within subjects, batch reading should also reduce the variance and increase the power of tests of treatment effects. We did observe a reduction in the standard errors of treatment effects for batch readings in this study, but that reduction was small and not practically important. In studies using a larger number of readers, however, the magnitude of this effect might be increased.

In the design phase of a trial one should balance the benefit of batch reading (finding a true valid estimate of the difference between treatment groups) and the down side of batch reading. The latter involves delays in reading activity as one has to wait for readings of the main study until a participant has completed the study. The batch approach therefore extends study timelines and has impact on staffing (depending on the amount of scans to be read at the end of the study) Furthermore it requires excellent data flow processes and administration skills to organize batches correctly. All processes should be processed by a clear standard operating procedure, which leaves no place for administrative errors. Batch reading also poses a challenge for ongoing quality assurance / quality control activities while scans are being performed. Often, the core laboratory needs to read some portion of scans in a non-batch fashion to allow for quantitative assessment of sonographer performance and potential intervention when quality of CIMT scanning is too low.

The main interest of a randomized trial is often obtaining an effect on rates of CIMT progression over time in the intervention group as compared to the placebo group. However when the main interest is in obtaining 'correct' rates of change of CIMT, our data suggest that a batch approach may help in obtaining

unbiased rates of change. Indeed, previous studies have detected temporal bias in progression rates of CIMT. In the MIDAS trial [19], a large randomized double-blind clinical trial that compared effects of isradipine and hydrochlorothiazide on CIMT progression, a significant upward drift occurred at the 36-month visit in both treatment groups. In this study the investigators calculated corrected CIMT estimates for the 36-month visit and used these instead of the biased ones. Overall the drift did not lead to difference in treatment effect and only influenced the magnitude of rate of progression.

A solution that has been proposed to prevent drift is the use of an automated edge detection program to measure CIMT. Automated measurement systems in theory should prevent reader drift as no change of reading habits over time occurs. Automated edge detection programs work very well in excellent imaged carotid segments, predominantly in the common carotid artery. In addition, considerable progress has been made is the automated detection of intima-media thickness from carotid bifurcation images or internal carotid imaging. Yet, in a considerable proportion, a manual override can be used by the reader to adjust the traced boundaries. Such an approach introduces a reader to the automated process, and thus drift may occur. Moreover, even in fully automated readings, readers may still influence the measurements obtained through frame selection or identification of the region within the image in which measurements will be collected. In our study, despite the use of a semi-automated measuring system, drift occurred. In a recent comparative study [20], between an updated and a older version of DICOM-software program, observer biases caused differences in mean CIMT of a magnitude commonly reported as significant in intervention trials. This shows the importance of concurrent controls to detect observer drift between baseline and follow-up measurements.

Apart from the implications for the design of future CIMT trials, our findings point towards the arbitrary definition of progression and regression, i.e., a rate of change in CIMT above or below zero, preferably with 95% confidence interval not including zero. Had we used the non batch approach then we might have concluded that CIMT had regressed in the intervention arm. But, in this case, the placebo group would have 'regressed' in atherosclerosis as well. The results of our batch reading point towards the potential of drift in these estimates. Given that apart from the METEOR study[18], lipid lowering CIMT trials performed so far have not used a batch reading approach, the absolute CIMT estimate of rate of change from these trials may have been subject to drift. Pooling of these absolute estimates of rate of change is therefore potentially incorrect. Apart from differences between studies in ultrasound protocol, reading equipment used, patient populations, differences in drift may enhance incomparability in progression rates. Therefore pooling of CIMT trial findings is best served with pooling the observed differences in CIMT progression between treatment arms.

In conclusion, both batch and non-batch reading provide correct estimates of a treatment effect. Batch reading minimizes bias in the estimation of (correct direction of) CIMT rate of progression over time, but this approach is expensive and operationally challenging. A thorough consideration of the need of obtaining unbiased CIMT rate of progression and the time and financial burden associated with the batch reading approach should be made before choosing a reading approach.

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Chapter 7

Intensive lipid lowering reduces progression of carotid atherosclerosis within 12 months of treatment: the METEOR study

Abstract

Background

In several statin trials vascular event rates for treatment groups begin to separate 1 year after commencement of treatment. For atherosclerosis progression, the temporal sequence of the effect has not been defined. We used data from the METEOR trial to determine the earliest time point at which significant differences in atherosclerosis progression rates could be detected after initiation of statin therapy.

Methods

METEOR was a double-blind, randomized placebo-controlled trial that studied the effect of low density lipoprotein cholesterol (LDL-C) lowering with 40 mg Rosuvastatin on the rate of change of carotid intima media thickness (CIMT) measured by B-mode ultrasound among 984 low risk subjects. Ultrasound assessments were made at baseline and every 6 months up to two years.

Results

The difference in rate of maximum CIMT progression between the Rosuvastatin and placebo groups was not statistically significant after 6 months (0.0023 mm/yr and 0.0106 mm/yr, respectively p=0.34). After 12 months CIMT progression rates were significantly different between the groups: 0.0032 mm/yr and 0.0133 mm/yr in the Rosuvastatin treated and placebo treated groups, respectively (p=0.049). This divergence grew with further follow-up: -0.0009 mm/yr and 0.0131 mm/yr after 18 months ($p < 0.001$) and -0.0014 mm/yr and 0.0131 mm/yr after 24 months of treatment ($p < 0.001$). Results were more pronounced for mean common CIMT progression.

Conclusion

Aggressive LDL-C lowering exerts its beneficial effect on atherosclerosis progression during the first 12 months of treatment. This parallels the timing of event reduction seen in clinical trials and suggests that the efficacy of lipid lowering treatment on CIMT progression can be evaluated in trials conducted over 1 year, given sufficient sample size, high precision of measurements and a treatment effect comparable to that seen in METEOR.

Introduction

Results from observational studies across a variety of populations indicate a continuous positive relationship between low density lipoprotein cholesterol (LDL-C) and risk of coronary heart disease[1]. The use of 3-hydroxy-3-methylglutaryl-lowering co-enzyme A (HMG-CoA) inhibitors (statins) results in considerable reduction of LDL-C[2]. Furthermore, randomized controlled trials have consistently shown that statins reduce the risk of coronary heart disease[3]. This risk reduction has been demonstrated in a wide range of populations and patient groups and its magnitude appears to be proportional to the absolute reduction in LDL-C[4]. However, it is not clear how rapidly benefits emerge after initiation of statin therapy. Some trials report no benefit within the first year, and others report more rapid benefit. A recent meta analysis, based on over 90.000 individual patients participating in randomized controlled trials of statins, indicated a significant 14% reduction in major vascular events within the first year of treatment[4].

Part of the beneficial effect of LDL-C lowering on CHD risk has been attributed to its influence on atherosclerosis progression. Indeed, several randomized controlled trials have shown that statins lead to reduced progression, or even regression, of atherosclerosis[5-10]. For atherosclerosis progression, the temporal sequence of the beneficial effect has not yet been defined. This is partly because, in trials using quantitative coronary angiograms or coronary intravascular ultrasound as tools to assess atherosclerosis progression, follow-up measurements were generally obtained 18 or 24 months after the start of the study[6-8]. Also, trials using magnetic resonance imaging to assess atherosclerosis progression over time used repeated measurement after 18 months[9]. In contrast, in several lipid lowering trials where ultrasound-assessed carotid intima-media thickness (CIMT) was used as indicator of atherosclerosis progression, atherosclerosis was evaluated at baseline and every 6 months over a period of two or three years[10-14]. These CIMT trials allow assessment of the temporal sequence of statin effects on atherosclerosis progression more precisely. Using data from one of these trials, the Measuring Effects on intima media Thickness: an Evaluation Of Rosuvastatin (METEOR) study [14] we sought to determine the earliest time point after initiation of statin therapy at which significant differences in atherosclerosis progression rates could be detected.

Methods

The rationale, design and main findings of the METEOR study have been detailed elsewhere[14,15]. In short METEOR was a 2-year, double-blind, placebo-controlled trial that compared Rosuvastatin 40 mg with placebo treatment in middle-aged asymptomatic subjects with moderately elevated cholesterol and low risk of cardiovascular disease according to the National Cholesterol Educational Program (NCEP) Adult Treatment Panel (ATP) III criteria (0-1 risk factor or ≥2 risk factors with a 10-year coronary heart disease (CHD) risk <10%). The study was conducted in accordance with the ethical principles in the Declaration of Helsinki, the International Conference on Harmonization of Good Clinical Practice guidelines, and appropriate regulatory requirements. The study protocol was approved by the appropriate Institutional Review Board and/or Independent Ethics Committee at each site. All participants provided written informed consent. Main inclusion criteria were: age 45-70 years (male) or 55-70 years (female); screening

LDL-CC 120-190 mg/dL (3.1-4.9 mmol/L) for those with only age as a CHD risk factor, or 120-160 mg/dL (3.1-4.1 mmol/L) for individuals with 2 or more CHD risk factors and a 10-year risk of CHD events <10%; HDL-C ≤60 mg/dL (1.6 mmol/L); TG <500 mg/dL (5.7 mmol/L); and at least one maximum CIMT measurement >1.2 mm and no measurement >=3.5 mm from 2 separate ultrasound examinations. This lower boundary for CIMT measurement actually identifies subjects with relatively thick walls compared to the general population. Thus, clinically these participants were at "low risk", although their IMT indicated the presence of subclinical atherosclerosis. Eligible participants were randomized to either the placebo or Rosuvastatin groups in blocks of seven (5 Rosuvastatin, 2 placebo) at each clinical site. Carotid ultrasound examinations were performed twice before randomization, once each at 6, 12 and 18 months after randomization, and then twice at the end of 24 months of study treatment. At each visit sonographers obtained standardized longitudinal B-mode images of the left and right and near and far walls of the 3 segments of the carotid artery, as detailed elsewhere[15]. The common carotid artery (CCA) was defined as the segment extending from 10 to 20 mm proximal to the tip of the flow divider. The carotid bifurcation was defined as the segment beginning at the tip of the flow divider and extending 10 mm proximal. The internal carotid artery (ICA) was defined as the segment beginning at the tip of the flow divider and extending 10 mm distally. Meijer's Carotid Arc® was used to image the artery at pre-specified angles[16]. All ultrasound scans were read with Image Pro® software using a uniform reading protocol that ensured standardized settings across reading stations and core laboratories. The image boundaries were marked manually. For CIMT measurements, trailing edges were traced on the near wall boundaries and leading edges on the far wall boundaries Measurements were performed on images from selected predefined angles: for the right carotid artery – 60, 90, 120, 150, and 180 degrees on the Meijer's Carotid Arc; for the left carotid artery – 300, 270, 240, 210, and 180 degrees. For the near and far walls of the right and left carotid bifurcation and ICA, measurements were made only of the maximum CIMT at all selected angles. For the CCA, measurements were made of both the mean and maximum CIMT of each wall at all selected angles. All readers completed a uniform training program. A single reader read all 7 scans in random order and in a batch fashion after each individual had finished the study. Reproducibility of the measurement was excellent[14,15].

Data analysis

CIMT data were analyzed according to the intention-to-treat (ITT) principle in all individuals with a baseline reading and at least 1 post-baseline CIMT reading. For the present analysis the endpoints were 1) rate of change in maximum CIMT based on all scans performed over the study period from each of the 12 carotid artery sites (near and far walls of the right and left common carotid artery (CCA), carotid bulb, and internal carotid artery (ICA)) and 2) rate of change in mean common CIMT for the near and far walls of the right and left CCA and 3) rate of change in mean common CIMT based on only the far walls of the right and left CCA.

To study treatment effects on CIMT progression a multi-level, repeated measures, linear mixed-effects model was used as described earlier[14]. Levels used for the data were subject, and carotid artery site within subject; the repeated measure was time. The model was specified in terms of fixed effects for

carotid artery site, age, sex, reader, ultrasound machine, randomized-treatment group, time, and the interaction of randomized-treatment group and time. Time was defined as the interval from date of randomization to date of CIMT measurement. To assess linearity of changes in CIMT values across the study measurements, time-squared terms were included in the model. Random effects within the model were intercepts and slopes for both subjects and sites-within-subjects.

To study the time sequence of the difference in CIMT progression rates between treatments, CIMT progression rates were estimated based on baseline and 6 month measurements only, on baseline, 6 month and 12 month assessments only, on baseline, 6 month, 12 month and 18 month assessments only, and on the full dataset up to assessments at 24 months. Furthermore, based on the findings in this study, sample size estimations were performed to define the size needed for a trial when CIMT measurements were done up to 6 months, up to 12 months, up to 18 months, or up to 24 months after baseline.

Results

The general characteristics of the study population are given in table 1, by treatment assignment. Characteristics were similar between the two treatment arms. Rosuvastatin treatment was associated with a 49% reduction in LDL-C-C, a 34% reduction in TC, an 8.0% increase in HDL-C, and a 16% reduction in TG (all p <.0001 compared with placebo)[14].

Table 1. General characteristics of the METEOR study populations by treatment group

	Rosuvastatin (n = 702)	Placebo (n = 282)
Age (SD), years	57 (6.2)	57 (6.0)
Men, n (%)	421 (60)	167 (59)
Race (n, % Caucasian)	659 (94)	268 (95)
Body mass index (SD), kg/m2	27.1 (4.0)	27.5 (4.0)
Systolic blood pressure, mmHg (SD)§	124 (13.4)	125 (13.6)
Diastolic blood pressure, mmHg (SD)§	77 (8.2)	78 (8.5)
Hypertension (≥140/90 mmHg or antihypertensive medication)	138 (20)	58 (21)
Fasting blood glucose, mg/dL (SD) §	95 (0.68)	97 (0.80)
Total cholesterol, mg/DL (mean [SD])¶	229 (29)	230 (28)
LDL-cholesterol, mg/DL (mean [SD])¶	154 (24)	154 (24)
HDL-cholesterol, mg/DL (mean [SD])¶	49.7 (9.0)	49.0 (9.2)
Triglycerides, mg/dL	126 (64.3)	134 (67.8)
Family history of premature CHD†	65 (9)	31 (11)
Smoking (during the previous month)	22 (3)	16 (6)
MeanMax‡‡ of all 12 CIMT sites, mm (mean [SD])¶	1.15 (0.19)	1.17 (0.20)
MeanMean of the CCA, mm (mean [SD])¶	0.76 (0.12)	0.76 (0.12)

CCA, common carotid artery; CIMT, carotid intima media thickness; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation. †Defined as CHD in a first-degree male relative <55 years old or in a first-degree female relative <65 years old. §Values based on randomized safety population (n=700 Rosuvastatin, n=281 placebo). ¶Values based on ITT population (n=624 Rosuvastatin, n=252 placebo). ‡‡MeanMax=mean of maximum CIMT measurements at different carotid sites

In table 2 the CIMT progression rates based on different CIMT assessments are given by treatment group. The difference in rate of maximum CIMT progression between the Rosuvastatin and placebo groups was not statistically significant 6 months after baseline (0.0023 mm/yr and 0.0106 mm/yr, respectively p=0.34). After 12 months CIMT progression rates were significantly different between groups: 0.0032 mm/yr and 0.0133 mm/yr (p=0.049). This divergence grew and statistical significance increased with further follow-up: -0.0009 mm/yr and 0.0131 mm/yr after 18 months (p < 0.001) and -0.0014 mm/yr and 0.0131 mm/yr after 24 months of treatment (p < 0.001). Results were similar for common CIMT progression when based on the combined near and far wall measurements or when based on the far wall measurements only (table 2). We found a strong and highly statistically significant difference in mean common CIMT progression between the two treatments 12 months after initiation of statin therapy.

Table 2. CIMT progression by time after baseline, for the Rosuvastatin groups and the placebo group and differences in CIMT progression between treatments.*

	CIMT progression (mm/yr)			
	Rosuvastatin	Placebo	Difference in progression between treatment groups (mm/yr)	P- value for the difference
		Mean maximum CIMT (near and far wall combined))		
6 months	0.0023	0.0106	-0.0083	0.34
12 months	0.0032	0.0133	-0.0101	0.049
18 months	-0.0009	0.0131	-0.0140	< 0.001
All time points	-0.0014	0.0131	-0.0145	< 0.001
		Mean common CIMT (near and far wall combined)		
6 months	-0.0005	0.005	-0.0056	0.29
12 months	-0.0011	0.0062	-0.0073	0.013
18 months	-0.0012	0.0084	-0.0097	<0.001
All time points	0.0004	0.0089	-0.0085	< 0.001
		Mean common CIMT (far wall only)		
6 months	-0.0014	0.0025	-0.0039	0.51
12 months	-0.0040	0.0056	-0.0096	0.004
18 months	-0.0037	0.0065	-0.0102	<0.001

* progression estimates are based on data from duplicate baseline scans, intermediate scans every six months, and duplicate end of study scans, as appropriate. A later time point also includes the earlier time points for calculation the rate of change

Based on the METEOR design (i.e., duplicate baseline and end of study scans with intermediate scans every six months as appropriate and reading of the scans in a batch fashion), and on the METEOR main findings (i.e., an expected difference in mean maximum CIMT progression rates of 0.0145 mm/yr), a similar study with 1:1 randomization would have 90% power to show a difference between treatments arms at the p<0.05 level with a total number of subjects of 115 subjects per arm in a 2 year study, 175/ arm in a 1.5 year study, 355/arm in a 1 year study, or 1285/arm in a 6 month study. These estimates

do not include drop-outs. Total number of subjects in treatment arms based on mean common CIMT measurements only, with an expected difference in mean common CIMT of -0.0085 mm/yr would be 100 subjects/arm in a 2 year study, 155/arm in a 1.5 year study, 325/arm in a 1 year study, or 1215/arm in a 6 month study.

Discussion

The Lipid Research Clinics (LRC) trial, one of the first to test the cholesterol hypothesis, suggested that cholesterol lowering therapy took at least two years to exert its effect on event reduction[17,18]. Subsequently, data from the Scandinavian Simvastatin Survival Study suggested that a statin that lowered LDL-C by 35% began to exert its effect on event reduction between one and two years after treatment initiation[19]. As the occurrence of an event is an interplay between atherosclerotic abnormalities and a variety of factors that trigger the event to occur, statin use may affect both processes in a different manner and in a different time window. We focused on the time course of initiation of effects of aggressive lipid lowering therapy on atherosclerosis progression in humans. This may come from studies where repeated assessments of atherosclerosis progression have been performed. Trials using imaging modalities for atherosclerosis progression, such as IVUS or MRI, mostly have only two measurements with at least an 18 month period between the assessments[5-9]. At present, availability of longitudinal repeated data seem to be restricted to randomized controlled trials of statins using change in CIMT over time as indicator of atherosclerosis progression, since in several of these trials CIMT assessments were done every 6 months after baseline[10-14]. A basic assumption in these analyses is that change in CIMT over time is a linear phenomenon in statin and no-statin users. Based on existing data and exploratory analyses using the METEOR data[14], this assumption seems to be holding [11-13]. Of all the lipid lowering trials using CIMT progression, none have specifically addressed the subject of this communication: i.e., the earliest time to benefit of lipid lowering therapy on CIMT progression. Although in several CIMT trials information on timing of treatment effect can be retrieved from the published reports, it should be noted that some CIMT trials had a duration of 12 months only[20-22]. Others, although designed as 2 to 4 year intervention studies, had only a first re-measurement at 18-24 months [23-25]. Other reports are too restricted to extract information on potential early treatment effects[26-31].

In the ARBITER trial, where 161 high risk patients were randomized to pravastatin (40 mg) or atorvastatin (80 mg), no difference in common CIMT progression was found at 6 months, but a significant difference was reported at 12 months[20]. Unfortunately, no information on 18 months or 24 months CIMT progression was collected to substantiate the 12 month finding. Furthermore, the observed difference in CIMT progression constituted one of the largest found in the trials performed so far. In ASAP, 325 mainly untreated patients with familial hypercholesterolemia were randomized to simvastatin (40 mg) or atorvastatin (80 mg)[32]. From that report the mean maximum and mean common CIMT progression rates appear to be significantly different after one year of treatment, and this was more pronounced after 2 years. However the precise estimates and statistical evaluation were not presented. In the MARS study, where 188 patients with angiographically documented coronary heart

disease were randomized to lovastatin or placebo on top of a diet, there was a clear divergence of the common CIMT after 6 months and 12 months, which further extended during the next 3 years in a linear fashion[33]. Unfortunately the publication does not provide information on CIMT progression rates and their precision. The examination of high risk patients in the above mentioned studies provides a population in whom the extent of disease and the potential to demonstrate changes due to different therapeutic interventions may be greater than that anticipated for lower risk patients, as studied in METEOR. Yet, the results from these high risk population studies are in line with our findings, i.e., a benefit of aggressive lipid lowering on atherosclerosis progression within 12 months. Importantly, we expand the evidence into an asymptomatic population at reasonably low CHD risk.

The data presented in this report may have important implications for the design of new lipid modifying treatments, in particular when investigators want to have an early indication of benefit of their treatment before embarking on a larger more costly morbidity and mortality trial. Trials using imaging to assess progression of atherosclerosis as the primary endpoint have been proposed for this purpose since such trials can be performed in a smaller number of subjects and generally are of shorter duration. The present analysis shows that for trials that assess the efficacy of lipid modifying treatment on CIMT progression, a duration of 12 months may be sufficient, given appropriate sample size, high precision of measurements and a treatment effect comparable to that seen in the METEOR trial.
In conclusion, aggressive LDL-C lowering with Rosuvastatin exerts its beneficial effect on atherosclerosis during the first 12 months of treatment. This parallels the timing of event rate reduction seen in some clinical trials. The finding suggests that in trials on the efficacy of lipid lowering treatment on CIMT progression, a duration of 12 months may be adequate, given sufficient sample size, high precision of measurements and treatment effect.

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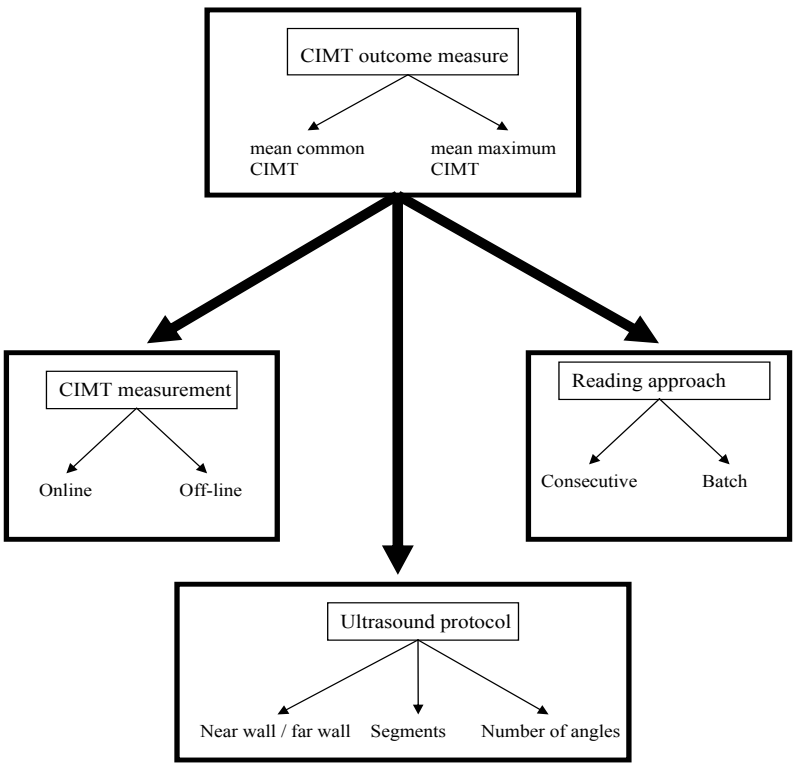
Chapter 8
General discussion

Carotid artery intima-media thickness (CIMT) is widely used in observational and intervention studies to study determinants of atherosclerosis and its consequences for symptomatic cardiovascular events [1-4]. Change in CIMT over time is increasingly used as an alternative outcome for cardiovascular events (morbidity and mortality) and a primary outcome in intervention studies to study efficacy of interventions. Despite the long history and its frequent use there is little uniformity in the measurement of CIMT. The choice of an ultrasound protocol and the measurement approach is generally based on expert opinion and personal preference rather than on published evidence on the optimal strategy. In designing a study, either an observational study or an intervention study, there are several aspects to consider, illustrated in figure 1.

Figure 1: Aspects that need to be considered in designing a study using CIMT measurements as primary outcome parameter.



First a primary outcome measure has to be chosen. One may choose the mean common CIMT, based on only the common carotid artery segment or a mean maximum CIMT based on values obtained from all the three carotid segments: the common carotid segment, the bifurcation segment and the internal carotid artery segment. The acquisition of the ultrasound images, and thus the ultrasound protocol to measure CIMT, follows the choice of the primary outcome measurement.

Measurements can be performed on-line (during acquisition, real-time) or off-line. Online, CIMT can be measured manually by the sonographer while performing the ultrasound scan or it can be measured using an on-line automated edge detection software program that is incorporated in the ultrasound equipment. The alternative for online measurements is off-line measurement, where images are collected during the ultrasound scan and stored on a medium (digital storage or analog on videotape) and sent to a core-laboratory, where the actual CIMT measurements are performed. Off-line measurements can be performed manually by the reader who places calipers on the boundaries of the arterial walls or automated by use of an edge detection software program. In both the online and off-line approach one has to decide which walls, segments and angles will be used to measure CIMT by determining the ultrasound protocol.

In the off-line approach an additional choice has to be made regarding reading approaches. After collection of the ultrasound scans in the core-laboratory CIMT can be measured in batch or in continuous and consecutive order. In batch-reading, one reader measures all the scans of a certain participant in a short time-period after collection of these scans, whereas in the consecutive reading approach ultrasound scans are measured continuously over time by randomly allocating a reader to a scan as soon as it is received at the core laboratory.

All these aspects are important to consider in the designing phase of a study. Choices will affect conduct, size, costs and outcome of the study. In this chapter we will summarize the pros and cons of these choices and indicate where the results from studies in this thesis may assist in making a decision.

Mean common CIMT versus mean maximum CIMT

The arguments that have generally been used in favor of the mean common CIMT as the outcome measure generally include higher reproducibility, more complete measurement, an equally strong relation with future events, a stronger relation between progression rates and lipid levels, a higher response to lipid lowering treatment and most importantly a more rapid ultrasound protocol to acquire CIMT images as compared to measurement of the mean maximum CIMT (table 1).

Arguments to favor the mean maximum CIMT include the view that reproducibility, completeness of measurements, risk prediction, and relations with lipid levels are similar to that of the common CIMT. But in addition, mean maximum CIMT provides a more complete coverage of the extent of local carotid atherosclerosis as compared to the mean common CIMT in which usually local atherosclerosis is far less prevalent. Furthermore it is often unclear on which segments of the carotid artery an intervention will exert its effect on atherosclerosis progression [5-8]. The mean maximum CIMT thus, from a conceptual point of view, provides a higher probability of detecting a treatment effect. A disadvantage of the mean maximum CIMT is the extensiveness of the protocol. Data

acquisition and the actual measurement of the CIMT take up considerably more time than in mean common CIMT (table 1).

The studies presented in this thesis provided evidence to support some and discard other previously used arguments. If most things (apart from logistics) between mean common and mean maximum are equal, the decisive aspect that remains is the congruency of the result of the CIMT measurement with event data. In our analyses, the congruency with event data showed a complementary value of both outcome measures which thus favors both outcome measures to be measured. Since the measurement of the mean maximum CIMT also includes measurement of the mean common, the balance tips towards the mean maximum CIMT measurement as the choice of outcome (chapter 3).

It is important to note that our findings are based on studies on the effect of lipid lowering drugs. To what extent our results apply to CIMT imaging trials that study the effects of other classes of drugs that are used in the treatment of atherosclerosis (e.g. blood pressure lowering medication or glucose lowering medication) remains to be established in future studies although one would assume that findings are likely to be largely similar for interventions such as blood pressure reduction or smoking cessation.

Table 1: Arguments for and against the mean common or the mean maximum CIMT as a primary outcome parameter in intervention studies, and findings reported in this thesis that may help decision making

	Common CIMT	Mean Max CIMT	Findings reported in this thesis
Reproducibility	High	Lower	Nearly identical (chapter 5.1; 5.2; 5.3)
Relations with lipid levels	Strong	Less strong	Equally strong (chapter 3)
Obtaining complete image data	Nearly complete	Many missing data	Common less missing values , but differences were small (chapter 4)
Near and far wall measurements	Only far wall (validity [10,11]	Both near wall and far wall	Protocol with both walls perform better (chapter 5.1; 5.2; 5.3)
Measurement of local atherosclerosis	No local atherosclerosis	Local atherosclerosis	Not addressed here
Prediction of future events	Strong	Stronger	Not addressed here
Response to lipid treatment	High	Lower	Depends on the study population: - Healthy individuals and FH population favors common (chapter 5.1 and 5.3) - Asymptomatic population with subclinical atherosclerosis and MD population equally high (chapter 5.2 and 5.3)
Ultrasound protocol	Short, quick, simple	Laborious, more costly	Not addressed here
Congruency with event trials	Argument never used	Argument never used	Complementary value of both outcome measures (chapter 3)

FH: familial hypercholesterolemia, MD: mixed dyslipidemia

Online measurements versus off line measurements

Online measurement of CIMT is appealing as the technique is widely available, cheap, easy to operate and it provides immediate information (table 2). Furthermore with online automated radio frequency (RF) equipment it is possible to obtain additional arterial wall properties such as distensibility and stiffness that facilitate a detailed study of the intrinsic wall characteristics [9]. Disadvantages of online approaches are that at present there is little published data on the predictive value of future events of the CIMT measured with RF equipment, quality assessment and quality control are more difficult to assure, at present only measurements of the far wall are possible and until recently measurements of only the common carotid artery segment were possible.

In the observational study described in chapter 2 we showed that in patients with manifest arterial disease, the CIMT measured manually with B-mode shows stronger relations with established risk

Table 2: Arguments for and against online or off line reading, and findings reported in this thesis that may help decision making

	On-line	Off-line	Thesis results
Results directly available	Yes	Delayed availability	Not addressed here
No reader difference	Yes (automated)	Yes (automated)	Not addressed here
	No (manual)	No (manual)	
Walls	Only measurements of the far wall CCA	Measurements of both near and far wall possible	Protocols with measurements of near and far wall perform better (chapter 5.1; 5.2; 5.3)
Segments	Only CCA (currently with newer equipment also BIF and ICA)	All three segments (CCA, BIF, ICA)	CCA protocols perform equally as three segment protocols (chapter 5.1; 5.2; 5.3)
QA / QC	Troublesome	Well established	Not addressed here
Prediction of future events	Data available for manual B-mode, none for RF-signal	Consistent supportive data	Not addressed here
RF signal	Available	Not available	Prediction events modest (chapter 2)
Manual B-mode	Possible	Possible	Consistent results on prediction of events (chapter 2)
Automated edge detection	Possible	Possible	Not addressed here
Reproducibility	High	High	Not addressed here

QA: quality assessment; QC: quality control; CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment

factors and is more strongly associated with risk of cardiovascular events in the future (vascular death and ischemic stroke) than CIMT measured with automated RF. Yet, in individuals with a thin CIMT (< 0.9 mm) automated RF provides stronger relations with risk factors and stronger associations with future events.

If CIMT is measured online the study population of interest should be considered in the approach. In a population at high risk, a manual B-mode approach is preferred, whereas in a population likely to have a less progressed stage of atherosclerosis and thus with a relatively thin CIMT (< 0.9 mm) an automated RF approach is appropriate.

Design options for the ultrasound protocol

There exist many different protocols to measure CIMT without, however, much published evidence to help make decisions on which carotid segments, walls and angles to be included for the most valid and meaningful results. In chapter 5 we evaluated four different study populations for their effects of the in- or exclusion of combinations of walls, segments and angles of CIMT. In addition the reproducibility, magnitude and precision of the estimate of CIMT progression rate and magnitude and precision of the effect of intervention on CIMT progression were compared (table 3).

When the mean common CIMT is chosen as the principal outcome, the ultrasound protocol should include measurements of both walls at multiple (≥ 2) angles. If the mean maximum CIMT is chosen, the ultrasound protocol should include measurements of both walls at multiple (≥ 2) angles of all

Table 3: Summary of the results of the three studies that compared different ultrasound protocols

Study population	Segments	Wall	Number of angles
Mean common CIMT protocols			
Healthy individuals	CCA	NW & FW	≥ 2
Subclinical atherosclerosis	CCA	NW & FW	≥ 2
Familial Hypercholesterolemia	CCA	NW & FW	≥ 2
Mixed dyslipidemia	CCA	NW & FW	≥ 2
Mean maximum CIMT protocols			
Healthy individuals	CCA, BIF, ICA	NW & FW	≥ 2
Subclinical atherosclerosis	CCA, BIF, ICA	NW & FW	≥ 2
Familial hypercholesterolemia	CCA, BIF, ICA	NW & FW	≥ 2
Mixed dyslipidemia	CCA, BIF, ICA	NW & FW	≥ 2
Protocol that performed best overall			
Healthy individuals	CCA	NW & FW	≥ 2
Subclinical atherosclerosis	all three segments (CCA,BIF,ICA) or CCA	NW & FW	≥ 2
Familial hypercholesterolemia	CCA	NW & FW	≥ 2
Mixed dyslipidemia	all three segments (CCA,BIF,ICA) or CCA	NW & FW	≥ 2

CCA: common carotid artery segment; BIF: bifurcation segment; ICA: internal carotid artery segment; NW: near wall; FW: far wall; healthy individuals (chapter 5.1); subclinical atherosclerosis (chapter 5.2); familial hypercholesterolemia (chapter 5.3); mixed dyslipidemia: (chapter 5.3)

three carotid segments. If there is no preference beforehand for one or the other outcome measure, one can use the protocol that performed best in the specific study population. In healthy individuals and subjects with familial hypercholesterolemia this was a mean common CIMT protocol with measurements of both walls at multiple (≥ 2) angles. In subjects with subclinical atherosclerosis and subjects with mixed dyslipidemia this was a mean maximum CIMT protocol with measurements of both walls at multiple (≥ 2) angles of all three carotid segments. Based on the studies presented in this thesis it is not possible to make an explicit statement on which specific angles should be included or excluded as we did not quantify the effect of the in/exclusion of specific angles on the parameters described above.

Reading approach: reading in batch or consecutive reading

Reading all ultrasound scans of one participant in batch by one reader has several advantages. It minimizes drift (change of reading habits over time) and provides unbiased estimates of progression rates and unbiased estimates of treatment effect. Furthermore it removes differences between readers from the estimation of change within an individual which leads to higher precision of measurements. A disadvantage is that it may delay reading activity as the measurements can not start until all scans are collected and thus it extends study timelines.
In the alternative approach, reading scans in consecutive order, between reader differences and drift may influence estimates of treatment effect and estimates of CIMT progression rates, hence leading to biased measurements. Yet, in consecutive reading there is no delay and data of the study becomes more quickly available.
The results shown in chapter 6 suggest that absolute estimates of treatment effect are not affected by the choice of reading approach. However, reading in batch provided estimates of CIMT progression rates at higher precision. Therefore reading scans in batch should be the preferred choice in off-line CIMT measurement approaches however one needs to weight the associated increase in expenses (time and costs) versus the beneficial effect on precision and thus on the statistical power of the study, e.g. the sample size.

Practical examples of the effects of choice of the outcome measure on sample size

In this section the effect of the choice of outcome measure on sample size will be shown by estimating sample sizes based on the ‘best’ mean common CIMT protocol and the ‘best’ mean maximum CIMT protocol found for the different study populations (chapter 5.1; 5.2; 5.3). Next, based on these sample sizes the power of the opposite outcome measure will be estimated to test if a study based on one outcome measure provides enough power for the other outcome measure to give results with sufficient precision. First this is done for two studies in which subjects were randomly assigned to intervention or to placebo (chapter 5.1; 5.2), next this is done for the two studies in which

subjects were randomly assigned to intervention + “standard treatment” or to “standard” treatment only (chapter 5.3). The protocol numbers refer to the ultrasound protocols that reached the highest summation scores per study (chapter 5.1 - table 4; chapter 5.2 - table 5; chapter 5.3 - table 6 and table 9).

Intervention versus placebo

In brief the METEOR trial, described in chapter 5.2, examined the effect of rosuvastatin compared to placebo on CIMT progression in low risk subjects with signs of subclinical atherosclerosis. The overall ‘best’ ultrasound protocols in subjects with subclinical atherosclerosis were protocol 8 (mean common CIMT: total score 21) and protocol 52 and 54 (mean maximum CIMT: score 23). As protocol 52 was based on fewer measurements than protocol 54, sample sizes will be estimated with protocol 52. The sample sizes needed to demonstrate a *statistically significant* change in progression rate regression assuming a 2-sided level of significance of .05, 90% power, and a decrease in CIMT of 50%, 75% and 100% would be 916, 406 and 228 subjects respectively for the mean common CIMT protocol and 238,106 and 60 subjects respectively for the mean maximum CIMT protocol (table 4).

If the mean common CIMT is chosen as primary outcome measure, the sample size suffices to demonstrate also a significant effect on progression of the mean maximum CIMT (power of 100% for all expected percentages of treatment effects) with the effects of intervention obtained in the trial. However, if the calculation is based on the progression rate of the mean maximum CIMT, the power becomes too low to demonstrate statistically significant results for the mean common CIMT (31%, 40% and 38% respectively). Similar estimations of the sample size for healthy individuals (chapter 5.1) are presented in table 5.

Intervention + standard treatment versus standard treatment

In brief the RADIANCE studies, described in chapter 5.3, examined the effects of torcetrapib/ atorvastatin compared with atorvastatin alone on CIMT progression in patients with familial hypercholesterolemia (RADIANCE 1) or mixed dyslipidemia (RADIANCE 2). The overall ‘best’ ultrasound protocols in subjects with familial hypercholesterolemia were protocol 9 (mean common CIMT) and protocol 46 (mean maximum CIMT). The sample sizes needed to demonstrate a statistically significant change in progression assuming a 2-sided level of significance of .05, 90% power, and a decrease in CIMT progression of 0.01, 0.02 and 0.03 mm/year, given the intervention effect obtained in the trial, are given in table 6. Similar estimations of the sample size for subjects with mixed dyslipidemia are presented in table 7.

These power calculations show that if one aims to design a study with a sample size that is large enough to demonstrate a statistically significant effect of the intervention on both outcome measures, one needs to consider the differences in magnitude of progression rate and precision of the estimates of both outcome measures.

Table 4. Calculation of sample size based on progression rates as observed with the best performing mean common CIMT and mean maximum CIMT protocol in subjects with sub clinical atherosclerosis (chapter 5.2)

	Mean common CIMT protocol 8			**Mean maximum CIMT protocol 52**		
CIMT progression (mm/year)	0.009			0.016		
Standard deviation of progression	0.021			0.019		
2-sided alpha	0,05			0,05		
Power	90%			90%		
Expected treatment effect (% reduction)	50%	75%	100%	50%	75%	100%
No. of cases (n=)	458	203	114	119	53	30
No. of controls (n=)	458	203	114	119	53	30
Total sample size (n=)	**916**	**406**	**228**	**238**	**106**	**60**

Power of the alternative protocol, based on a sample size calculated with the other protocol

	Mean maximum CIMT protocol 52			**Mean common CIMT protocol 8**		
Power	100%	100%	100%	38%	38%	38%

Table 5. Calculation of sample size based on progression rates as observed with the best performing mean common CIMT and mean maximum CIMT protocol in healthy subjects (chapter 5.1)

	Mean common CIMT protocol 6			**Mean maximum CIMT protocol 49**		
CIMT progression (mm/year)	0,006			0,008		
Standard deviation of progression	0,021			0,037		
2-sided alpha	0,05			0,05		
Power	90%			90%		
Expected treatment effect (% reduction)	50%	75%	100%	50%	75%	100%
No. of cases (n=)	1030	458	257	1798	799	450
No. of controls (n=)	1030	458	257	1798	799	450
Total sample size (n=)	**2060**	**916**	**514**	**3596**	**1598**	**900**

Power of the alternative protocol, based on a sample size calculated with the other protocol

	Mean maximum CIMT protocol 49			**Mean common CIMT protocol 6**		
Power	70%	70%	70%	99%	99%	99%

Table 6. Calculation of sample size based on progression rates as observed with the best performing mean common CIMT and mean maximum CIMT protocol in subjects with familial hypercholesterolemia (chapter 5.3)

	Mean common CIMT protocol 9			**Mean maximum CIMT protocol 46**		
CIMT progression (mm/year)	-0,002			0,001		
Standard deviation of progression	0,012			0,017		
2-sided alpha	0,05			0,05		
Power	90%			90%		
Expected treatment effect (absolute reduction of CIMT progression mm/year)	0,01	0,02	0,03	0,01	0,,02	0,03
No. of cases (n=)	30	8	3	61	15	7
No. of controls (n=)	30	8	3	61	15	7
Total sample size (n=)	**60**	**16**	**6**	**122**	**30**	**14**

Power of the alternative protocol, based on a sample size calculated with the other protocol

	Mean maximum CIMT protocol 46			**Mean common CIMT protocol 9**		
Power	62%	65%	58%	100%	100%	100%

Table 7. Calculation of sample size based on progression rates as observed with the best performing mean common CIMT and mean maximum CIMT protocol in subjects with mixed dyslipidemia (chapter 5.3)

	Mean common CIMT protocol 8			**Mean maximum CIMT protocol 49**		
CIMT progression (mm/year)	0,002			0,006		
Standard deviation of progression	0,019			0,026		
2-sided alpha	0,05			0,05		
Power	90%			90%		
Expected treatment effect (absolute reduction of CIMT progression mm/year)	0,01	0,02	0,03	0,01	0,,02	0,03
No. of cases (n=)	76	19	8	142	36	16
No. of controls (n=)	76	19	8	142	36	16
Total sample size (n=)	**152**	**38**	**16**	**284**	**72**	**32**

Power of the alternative protocol, based on a sample size calculated with the other protocol

	Mean maximum CIMT protocol 49			**Mean common CIMT protocol 8**		
Power	33%	66%	64%	99%	99%	99%

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Chapter 9.1

Summary

Carotid artery intima-media thickness (CIMT) is widely used in observational and intervention studies. Despite the long history and its frequent use there is little uniformity in the measurement of CIMT. This diversity may have substantial effects on published results of studies and on the interpretation thereof. Therefore we studied several methodological aspects of CIMT measurements to facilitate an evidence-based decision making with respect to CIMT measurements.

In **chapter 2**, two online techniques to measure CIMT, an automated Radio-Frequency (RF) approach and a manual B-mode approach, were compared. CIMT measured with B-mode showed stronger relations with established risk factors and was more strongly associated with risk of future cardiovascular events than CIMT measured with automated RF. In individuals with a thin CIMT (< 0.9 mm) automated RF provided stronger relations with risk factors and stronger associations with future events, indicating that the type of study population and the expected presence of local atherosclerotic abnormalities should be considered in the choice of technique.

The pros and cons of two different CIMT outcome measures (the mean common CIMT and mean maximum CIMT) were considered in **chapter 3**. As most parameters (apart from logistics) between both measures were equal, the decisive aspect that remained was the congruency of the result of the outcome measure with event data. The congruency showed a complementary value of both measures, favoring the use of both measures. Since the measurement of the mean maximum CIMT also includes measurement of the mean common CIMT, the balance tips towards the mean maximum CIMT measurement as the choice of outcome.

Completeness of CIMT data at different walls, segments and angles of the carotid artery was studied in a population with familial hypercholesterolemia (FH) and in a population with mixed dyslipidemia (MD) in **chapter 4**. With the current ultrasound protocols and equipment it is possible to obtain high levels of complete CIMT information from nearly all walls, segments and angles. Apart from the study population, the completeness depended on the body mass index and waist circumference.

In **chapter 5** ultrasound protocols were compared on reproducibility, progression rates of CIMT and treatment effects in four different study populations. Ultrasound protocols that included measurements of both walls at multiple (≥ 2) angles for both the mean common CIMT and the mean maximum CIMT overall provided the best balance of the parameters mentioned above.

In **chapter 6**, we showed that the quantification of the effect of an intervention did not appear to be affected by the type of reading approach (in batch versus in consecutive order). However, the absolute observed CIMT progression rates might be biased in a non-batch approach.

The earliest time point at which significant differences in atherosclerosis progression rates could be detected after initiation of statin therapy was studied in the METEOR trial, **chapter 7**. Given

sufficient sample size, high precision of measurements and a treatment effect comparable to that seen in METEOR, an effect of lipid lowering treatment on CIMT progression can be observed during the first 12 months of treatment.

Finally in **chapter 8**, all the findings were put in perspective and the implications of choices on sample size were discussed. We concluded that if one aims to design a study with a sample size that is large enough to demonstrate a statistically significant effect of the intervention on both the mean maximum CIMT as the mean common CIMT, one needs to consider the differences in magnitude of progression rate and precision of the estimates of both outcome measures.

Chapter 9.2

Samenvatting

De vaatwanddikte van de halsslagaders gemeten middels echografie, de intima-media dikte van de arteria carotis (CIMT), wordt gebruikt in observationele studies en interventie studies als een indicator van atherosclerose. Ondanks de lange voorgeschiedenis en het veelvuldige gebruik is er weinig overeenstemming over deze meting. De verscheidenheid aan verschillende meetmethoden kan van invloed zijn op de resultaten van studies en hun interpretatie. Om deze redenen hebben we diverse methodologische aspecten van de CIMT meting bestudeerd om bewijzen aan te leveren die gebruikt kunnen worden tijdens het besluitvormingsproces over de keuze van meetmethoden en uitkomstmaten.

In **hoofdstuk 2** werden twee online (real-time) technieken om CIMT te meten vergeleken; een automated RF en een manual B-mode benadering. CIMT welke gemeten was met B-mode toonde sterkere relaties met traditionele risico factoren en was sterker geassocieerd met het optreden van een nieuwe uiting van vaatziekte (cardiovasculaire aandoeningen) dan CIMT welke gemeten was met automated RF. In individuen met een dunnere vaatwanddikte (CIMT < 0.9 mm) bood de automated RF benadering sterkere relaties met traditionele risicofactoren en een sterkere associatie met het optreden van een nieuwe uiting van vaatziekte. Dit duidt aan dat de studie populatie en de verwachtte mate van lokale atherosclerotische afwijkingen dienen te worden overwogen in de keuze van de meetmethode.

De voor- en nadelen van twee verschillende CIMT uitkomst maten; de mean common CIMT en de mean maximum CIMT, werden kritisch beoordeeld in **hoofdstuk 3**. Omdat de meeste parameters waarop de twee uitkomstmaten werden vergeleken gelijk waren (behalve de logistieke factoren) was de doorslaggevende factor in deze vergelijking de overeenstemming (congruentie) van beide uitkomstmaten met de resultaten van clinical event studies. De congruentie toonde een aanvullende waarde van beide uitkomstmaten. Echter aangezien met de mean maximum CIMT metingen tevens gegevens worden verzameld waarmee eveneens de mean common CIMT kan worden bepaald, dient de voorkeur gegeven te worden aan de mean maximum CIMT als uitkomstmaat van keuze.

In **hoofdstuk 4** bestudeerden we de compleetheid van CIMT metingen van de wanden, segmenten en hoeken van de arteria carotis in een populatie met familiaire hypercholesterolemie (FH) en in een populatie met mixed dyslipidaemie (MD). Met de huidige ultrasound protocollen en apparatuur is het mogelijk om hoge percentages van complete data te bemachtigen van bijna alle wanden, segmenten en hoeken. De compleetheid van CIMT metingen hing behalve van de studie populatie tevens af van de maten voor overgewicht: Body Mass Index en de taille-heup-verhouding.

In **hoofdstuk 5** vergeleken we echografie protocollen die gebruikt worden om CIMT metingen te verrichten, op reproduceerbaarheid, grootte en precisie van CIMT progression rates en effect van de interventie op CIMT progressie in vier verschillende studiepopulaties. Echo protocollen die metingen van beide wanden (de near en de far wall) op meerdere (≥ 2) hoeken includeerden boden, voor

zowel de mean common CIMT als de mean maximum CIMT, in het algemeen de beste balans tussen de eerder genoemde parameters.

In **hoofdstuk 6** hebben we aangetoond dat de leesbenadering (de scans in batch lezen of op willekeurige wijze, niet in batch, meteen na ontvangst van de scans) de quantificatie van het effect van de interventie op de CIMT progressie niet leek te beinvloeden. Echter, de absolute waarden van de geobserveerde CIMT progression rates zouden kunnen worden beinvloed door drift van de lezers (verandering van meetgewoonte over tijd) en leiden tot een temporal bias.

Het meest vroege tijdstip waarop significante verschillen in CIMT progression rates (progressie van atherosclerose) gedetecteerd kan worden na de start van behandeling met statines hebben we onderzocht in de METEOR studie, beschreven in **hoofdstuk 7**. Indien het aantal deelnemers voldoende is, dat de CIMT metingen voldoende nauwkeurig gemeten worden en dat het effect van de interventie op de CIMT progressie vergelijkbaar is aan het effect in de METEOR studie, kan een significant effect van lipide verlagende behandeling op CIMT progressie geobserveerd worden gedurende de eerste 12 maanden van behandeling.

Tenslotte worden in **hoofdstuk 8** alle bevindingen, beschreven in dit proefschrift, in perspectief geplaatst en worden de implicaties van de keuze van uitkomstmaat op de sample size besproken. We conluderen dat indien men tracht een studie te ontwerpen met een sample size dat groot genoeg is om een statistisch significant effect van de interventie aan te tonen op de progressie van én de mean common CIMT én de mean maximum CIMT, men het verschil in grootte van de progressie rates en de nauwkeurigheid van meting van beide uitkomstmaten in ogenschouw dient te nemen.

Chapter 9.3

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Chapter 9.4

Curriculum Vitae

CURRICULUM VITAE

Soner Doğan was born on May 12th, 1978, in Hengelo (Overijssel), the Netherlands. After graduating from secondary school at the Bataafse Kamp in Hengelo (1996 Gymnasium), he started his university training in Technical Business Administration at the Technical University Twente in Enschede. In 1997 he started his medical training at the University of Utrecht.

After receiving his medical degree in august 2004, Soner Doğan started his clinical career as a resident at the psychiatric hospital Willem Arntzs Huis – Altrecht in Utrecht. In November 2005 he started the work described in this thesis at the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, under supervision of prof.dr.D.E.Grobbee and dr.M.L.Bots.

In January 2008, Soner Doğan obtained his Master of Science Degree in Clinical Epidemiology at the University of Utrecht. As of October 2008 he will start his training in Psychiatry at Altrecht in Utrecht (supervised by dr. R.W.Kupka).

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