Ankle brachial index, C-reactive protein, and central augmentation index to identify individuals with severe atherosclerosis

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Objectives We examined the ability of ankle brachial index, C-reactive protein and central augmentation index to identify individuals in the general population with severe atherosclerosis, diagnosed as those with ischaemic cardiovascular disease.

Methods and results We examined 4159 randomly sampled individuals from the Danish general population, of which 250 had severe atherosclerosis. After adjustment for gender and age, individuals with ankle brachial index of 0.71–0.90 and 0.70 vs. 0.91–1.10 had odds ratios for severe atherosclerosis of 1.6 (95%CI:1.1–2.3) and 2.9 (1.9–4.6), respectively. C-reactive protein of 3.0 or 1.0–3.0 mg/L vs. 1.0 mg/L as well as central augmentation index in quintiles did not identify individuals with severe atherosclerosis, and did not improve further the ability of ankle brachial index to identify such individuals. On a continuous scale using receiver operating characteristics curves, presence of severe atherosclerosis was predicted by ankle brachial index (P = 0.00000003), C-reactive protein (P = 0.000003), as well as central augmentation index (P = 0.001); these three curves did not differ.

Conclusion Ankle brachial index < 0.9 identify individuals with severe atherosclerosis in the general population, while C-reactive protein in three groups and central augmentation index in quintiles did not. On a continuous scale, all three variables predicted severe atherosclerosis.

KEYWORDS
Atherosclerosis; Ankle brachial index; C-reactive protein; Central augmentation index

Introduction

Risk models for ischaemic cardiovascular disease, like the Framingham coronary risk model1 and the SCORE system,2 have been developed in an attempt to identify those who will have the highest benefit of preventive treatment. Such risk models are mainly based on age, gender, smoking status, systolic blood pressure, cholesterol, and whether the patient has symptomatic ischaemic cardiovascular disease or diabetes. However, even using these risk models, a large proportion of individuals are not identified before they have developed ischaemic cardiovascular disease.4 Therefore, non-invasive tests to detect individuals with atherosclerosis, preferably before they develop ischaemic cardiovascular disease, may improve selection of individuals for preventive treatments.

At least three inexpensive tests that potentially can detect atherosclerosis non-invasively already exist. First, reduction of systolic blood pressure at ankle level, caused by peripheral atherosclerosis and identified as reduced ankle brachial index (ABI), is associated with an increased risk of ischaemic cardiovascular disease.3–5 ABI may be reduced in individuals without symptoms, and among individuals older than 65 years, 17% had an ABI < 0.9 but only 9% of those had symptoms of peripheral atherosclerosis.6 Secondly, in 2003, the Centres for Disease Control and Prevention and the American Heart Association7 recommended that C-reactive protein was to be used in risk assessment for primary prevention of ischaemic cardiovascular disease. This is because inflammation is an important part of atherosclerosis8,9 and because increased levels of the acute phase reactant C-reactive protein is associated with increased risk of ischaemic cardiovascular disease.10 Thirdly, pulse wave analysis, expressed as central augmentation index, suggests that atherosclerosis in aorta and peripheral arteries may be detected as increased arterial stiffness, even before patients develop ischaemic cardiovascular disease.11,12

We examined the ability of ABI, C-reactive protein, and central augmentation index to identify individuals in the general population with severe atherosclerosis, diagnosed as those with ischaemic cardiovascular disease (ischaemic heart disease and ischaemic stroke). For this purpose, we studied 4159 randomly selected individuals from the Danish general population.

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Methods

The Copenhagen City Heart Study is an ongoing cardiovascular epidemiological survey started in 1976. From the Danish general population of Copenhagen, by use of the Danish Central Person Registry Number, 12,600 randomly chosen citizens were invited to participate in the fourth examination of the Copenhagen City Heart Study from September 2001 to July 2003. Of these, 6,038 were examined, 3,462 women, and 2,576 men (response rate 50%). Invited participants were stratified on age and gender such that 20 to 80+ years old women and men were represented, with the main emphasis on those above 50 years, the age range with most cardiovascular disease. Of the 6,038 participants examined, 1,879 individuals were excluded from the present study due to missing data on either ABI (n = 375) or C-reactive protein (n = 59) or central augmentation index (n = 1,477). Of the 375 participants with missing data on ABI, 314 was due to problems with the Doppler, while on 61 individuals the examiners could not measure the systolic blood pressure of the posterior tibial arteries; of these 61 individuals, non-compressible arteries could have been the problem, particularly in the eight participants with diabetes mellitus within this group. The 1,477 participants with missing data on central augmentation index were all due to problems with the SphygmoCor apparatus; of these, 198 had a measurement performed but the quality index was so low that central augmentation index could not be derived. The 59 individuals with missing data on C-reactive protein were because blood samples were not draw or because of technical problems with the assay.

A non-fasting venous blood sample was analysed by nephelometric and colorimetric assays for plasma levels of high sensitive C-reactive protein (Dade Behring, Rødovre, Denmark), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and glucose (All Konelab, ILS Laboratories Scandinavia, Allerød, Denmark). Plasma triglycerides levels did not differ as a function of time of blood samples after the last meal (0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, 7–8, or ≥ 8 h) (analysis of variance: P = 0.85; n = 4,065). A physical examination was performed including recording of standard brachial systolic and diastolic blood pressure on both arms, and systolic ankle blood pressure of the posterior tibial artery on both legs obtained by Doppler (Huntleigh Mini Duplex D900, Huntleigh, UK); ABI was the lowest ankle systolic blood pressure divided by the highest brachial systolic blood pressure. Pulse wave analysis was measured using a SphygmoCor model BPAS-1/MM2 (SphygmoCor, West Ryde, Australia); the principle of this non-invasive method consists in the registration of a pulse waveform at the radial artery and its derivation at the ascending aorta by means of a mathematical transformation, expressed as the central augmentation index. The central augmentation index is the proportion of central pulse pressure that results from arterial wave reflection and is a measure of arterial stiffness.

Table 1 Characteristics of participants from the general population at 2001–2003 examination

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Severe atherosclerosis*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>3,909</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>2,291 (59)</td>
<td>103 (41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>58 ± 16</td>
<td>71 ± 9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>2,544 (66)</td>
<td>200 (81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>2,180 (56)</td>
<td>207 (84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>191 (5)</td>
<td>32 (13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26 ± 4</td>
<td>27 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.5 ± 1.2</td>
<td>3.3 ± 1.1</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.5 ± 1.0</td>
<td>1.3 ± 0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.5 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.5 ± 1.3</td>
<td>1.8 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>156 (4)</td>
<td>70 (29)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P-values were calculated using Student’s t-test, χ² test or Mann-Whitney U test. Variables are shown as mean ± SD or frequencies.

*Defined as ischaemic heart disease or ischaemic stroke.

Figure 1 Ankle brachial index, C-reactive protein, and central augmentation index as a function of age in 4,159 individuals from the general population. Values are shown as medians with 2.5 and 97.5%. For ankle brachial index and C-reactive protein, levels of 0.9 and 3.0 mg/L are shown as horizontal lines. *P < 0.05, **P < 0.01 on Mann-Whitney U test vs. 20–29 years old after Bonferroni correction for multiple comparison.
Severe atherosclerosis was defined as ischaemic heart disease (myocardial infarction, previous coronary artery bypass, percutaneous transluminal coronary angioplasty, and angina pectoris) and ischaemic stroke. Diagnoses were gathered from the Danish National Hospital Discharge Registry identifying all hospital admissions in Denmark from 1976 to 2000 and from records from hospitals and general practitioners. Myocardial infarction was classified according to the World Health Organization International Classification of Diseases eight edition (ICD-8) code 410 until December 1993 or 10th edition (ICD-10) codes I21–I22 from January 1994 onwards. Angina pectoris was ICD-8 codes 411–413 or ICD-10 codes I20 and I25. Ischaemic stroke was ICD-8 codes 432–434 or ICD-10 code I63, excluding transient ischaemic attack and computed tomography proved cerebral and subarachnoidal haemorrhage.

Diabetes mellitus was self reported disease or a non-fasting plasma glucose $>11.0$ mmol/L. Smokers were current or former smokers. Hypertension was treatment with antihypertensive medication or a systolic or diastolic blood pressure above 140/90 mmHg. Body mass index was weight (kg) divided by height squared (m$^2$).

The statistical software package SPSS (SPSS for windows release 12.0.2, SPSS Inc., Chicago, IL, USA) and STATA (STATA SE 8.0 for windows, College Station, TX, USA) were used. We used Mann–Whitney U test, Student’s t-test, analysis of covariance (for the association between ABI, C-reactive protein, and central augmentation index adjusted for age), and $\chi^2$ test. Logistic regression models were adjusted for gender and age, or multivariable for gender, age, use of statin, smoking, hypertension, diabetes mellitus, body mass index, LDL cholesterol, HDL cholesterol, and triglycerides; these covariates were chosen because they are well-known risk factors for atherosclerosis. In the multivariable logistic regression models, data were missing on smoking in 50 participants, hypertension in 28, diabetes mellitus in 51, body mass index in 2, LDL cholesterol in 47, HDL cholesterol in 51, and triglycerides in 63, excluding transient ischaemic attack and computed tomography proved cerebral and subarachnoidal haemorrhage.

Table 2 shows the characteristics of the 4159 participants from the general population of Copenhagen, Denmark. Of these, 250 had severe atherosclerosis, defined as ischaemic cardiovascular disease, whereas 3909 were controls free of this disease.

### Results

Table 1 displays the characteristics of the 4159 participants from the general population of Copenhagen, Denmark. Of these, 250 had severe atherosclerosis, defined as ischaemic cardiovascular disease, whereas 3909 were controls free of this disease.

#### ABI, C-reactive protein, and central augmentation index as a function of age

ABI was similar between 20 and 59 years, but decreased from 50 to 59 years in the following three older 10-year age groups (Figure 1, upper panel). The fraction of participants with ABI below 0.9 increased with age from 7–10\% in 20–29 years old to 34\% in 80+ years old (Table 2).

C-reactive protein increased from age 40–49 years to 80+ years (Figure 1, middle panel). At the age of 20–29 years, 21\% of the population had C-reactive protein $>3$ mg/L, which increased to 39\% at the age above 80 years (Table 2). Central augmentation index increased with age from 20–29 years to 80+ years (Figure 1, lower panel).

#### Association between ABI, C-reactive protein, and central augmentation index

When ABI dropped below 0.9, C-reactive protein increased (Figure 2, upper panel). C-reactive protein also changed as a function of central augmentation index (Figure 2, middle panel). Central augmentation index increased slightly as a function of decreased ABI (Figure 2, lower panel).

#### ABI, C-reactive protein, and central augmentation index to identify individuals with severe atherosclerosis

After adjustment for gender and age, individuals with ABI of 0.71–0.9 and $<0.7$ vs. 0.91–1.1 had odds ratios for severe atherosclerosis of 1.6 (95\% CI: 1.1–2.3) and 2.9 (1.9–4.6) (Figure 3, upper part). After multivariable adjustment, ABI of 0.71–0.9 and $<0.7$ vs. 0.91–1.1 had odds ratios for severe atherosclerosis of 1.4 (1.0–2.1) and 2.2 (1.4–3.6).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Percent of individuals in the general population with low ABI or high levels of C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20–29 (n = 304)</td>
</tr>
<tr>
<td>ABI &lt; 0.9 %</td>
<td>10 (30)</td>
</tr>
<tr>
<td>C-reactive protein $&gt;3.0$ mmol/L %</td>
<td>21 (63)</td>
</tr>
</tbody>
</table>
C-reactive protein divided into three groups was not associated with severe atherosclerosis after adjustment for gender and age or after multivariable adjustment (Figure 3, middle part). Central augmentation index divided into quintiles was not associated with severe atherosclerosis after adjustment for gender and age or after multivariable adjustment (Figure 3, lower part). When statin use was excluded for the multivariable adjusted models, the odds ratios for severe atherosclerosis for ABI, C-reactive protein, and central augmentation index were similar to those shown in Figure 3 (data not shown).

On a continuous scale, an increment of one standard deviation in ABI was associated with an odds ratio for severe atherosclerosis of 0.80 (95% CI: 0.71–0.89) after adjustment for gender and age and 0.89 (0.78–1.00) after multivariable adjustment like in Figure 3. Neither C-reactive protein nor central augmentation index on a continuous scale was associated with severe atherosclerosis (data not shown).

On a continuous scale using receiver operating characteristic curves, presence of severe atherosclerosis was predicted by ABI ($P = 0.00000003$), C-reactive protein ($P = 0.0000003$), and central augmentation index ($P = 0.001$) (Figure 4). Although these $P$-values differ, the diagnostic accuracy to identify people with severe atherosclerosis for ABI, C-reactive protein, and central augmentation index over the entire continuous scale did not differ ($P$-values AUC$_1$ vs. AUC$_2$).

### ABI, C-reactive protein, and central augmentation index combined to identify individuals with severe atherosclerosis

The combination of increasing C-reactive protein with decreasing ABI did not appear to potentiate the ability to identify individuals with severe atherosclerosis (data not shown). This was true after adjustment for gender and age as well as after multivariable adjustment. Likewise, central augmentation index together with ABI and/or C-reactive protein did not improve the ability to identify individuals with severe atherosclerosis (data not shown). Multicollinearity was not found likely in any of the models, because the variance inflation factor was $< 2.5$ (ABI 1.035, C-reactive protein 1.024, and central augmentation index 1.030).

### Discussion

In this study, we demonstrate that ABI $< 0.9$ is superior to C-reactive protein in three groups and central augmentation index in quintiles in identifying individuals in the general population with severe atherosclerosis, defined as ischaemic cardiovascular disease. However, on a continuous scale using receiver operating characteristic curves, all three variables predicted severe atherosclerosis and did not differ in diagnostic accuracy.

### ABI, C-reactive protein, and central augmentation index as a function of age

When compared with the latest survey from the United States, our prevalence of ABI $< 0.9$ is higher for all age groups, but similar to those found in the German GetABI study from Germany and in the Rotterdam Study. The difference between these prevalences in the United States and ours in Copenhagen, is most likely due to differences in the methods used: Selvin and Erlinger determined ABI by dividing mean systolic blood pressure of the right and left ankle with the brachial systolic pressure of the right arm, while we divided the lowest of left and right ankle systolic pressure with the highest of left and right brachial systolic pressure. Reduced ankle pressure is known to be a segmental disease often only affecting a single leg. If so,
a normal pressure of one leg will raise the mean pressure even if one leg is severely affected, thereby misclassifying the patients as having normal ABI.

C-reactive protein is known to increase with age,\textsuperscript{19–21} and our results are thus in agreement with previous studies. Studies of a cohort from the general population with pulse wave analysis, expressed as central augmentation index, have to our knowledge not been published previously. We showed a clear association between advancing age and increasing central augmentation index, which is in agreement with previous findings from small studies.\textsuperscript{22,23}

**Association between ABI, C-reactive protein, and central augmentation index**

As inflammation is involved in atherosclerosis,\textsuperscript{8} it was expected that individuals with reduced ABI, as a sign of severe atherosclerosis in the legs, should have increased C-reactive protein, exactly as we observed. The trend toward increase in C-reactive protein in individuals with ABI above 1.3 can be explained by that many individuals have diabetes and/or renal disease with concomitant media-sclerosis of the ankle arteries.\textsuperscript{24}

If central augmentation index is an expression for arterial stiffness\textsuperscript{11} as a consequence of atherosclerosis, increasing C-reactive protein as a function of increasing central augmentation index should be expected, as we observed. This is also in accordance with the study of Kampus et al.,\textsuperscript{25} who showed in a group of healthy people that C-reactive protein $>1.0$ mg/L was associated with increased central augmentation index.

Reduced ABI is associated with larger plaques of the aortic arch\textsuperscript{26} and increased arterial stiffness is associated with an increased amount of aortic calcification.\textsuperscript{27} Therefore, our results are consistent with previous findings that reduced ABI is associated with increased arterial stiffness due to calcified arteries.\textsuperscript{27}

**ABI, C-reactive protein, and central augmentation index to identify individuals with severe atherosclerosis**

This is not a conventional epidemiological study searching for causal factors for severe atherosclerosis, defined as ischaemic cardiovascular disease. The focus of this article was instead to examine the ability of three non-invasive tests to identify people in the general population with severe atherosclerosis, diagnosed as having already suffered from ischaemic cardiovascular disease. An explanation for why, on categorical scales, only ABI below 0.9 was significantly associated with ischaemic cardiovascular disease, could therefore be that reduced ABI is not a risk factor for, but rather a sign of having severe atherosclerosis. This would be consistent with the study by Hertzer et al.,\textsuperscript{28} showing that among patients referred for peripheral revascularisation, 90% had significant atherosclerosis of the coronary arteries as assessed by coronary angiography.

Because elevated levels of C-reactive protein is a well-known risk marker for ischaemic cardiovascular disease,\textsuperscript{7} it was somewhat surprising that C-reactive protein, grouped as suggested by the Centers for Disease Control and Prevention and the American Heart Association,\textsuperscript{7} was not able to identify individuals with severe atherosclerosis in the general population; however, limited statistical power might partly explain this lack of association. Alternatively, as statin use lowers C-reactive protein\textsuperscript{29} and 29% of those with severe atherosclerosis used statins vs. 4% of the controls, this may also contribute to the lack of association. Finally, our data suggest that although C-reactive protein has strong prognostic utility,\textsuperscript{10} it may have limited diagnostic utility with respect to severe atherosclerosis, at least when using the groups $<1.0$, $1.0$–$3.0$, and $>3.0$ mg/L.\textsuperscript{7} Therefore, and because C-reactive protein on receiver operating characteristic curves showed diagnostic accuracy similar to ABI, different cut-off values for C-reactive protein possibly should be considered to improve its diagnostic utility.

Even though previous studies have shown that ischaemic cardiovascular disease and atherosclerosis are associated with increased arterial stiffness,\textsuperscript{27,30} we could not demonstrate an increased odds ratio for severe atherosclerosis as a function of central augmentation index in quintiles or on a continuous scale; however, despite this, when evaluated using receiver operating characteristics curves, central augmentation index had some diagnostic ability for severe atherosclerosis. Other studies have also questioned the validity of central augmentation index, because it is highly
dependent on vascular tone and beta-adrenergic stimulation. Kelly et al. further investigated associations between central augmentation index and age, diastolic blood pressure, height, pulse wave velocity, total cholesterol, and systolic blood pressure: in a stepwise multiple logistic regression model, age was the only independent predictor of central augmentation index, consistent with our results. Finally, the only previous studies testing the validity of central augmentation index have all been very small, except one study with 88 healthy controls.

Limitations
The major limitations of the present study is that it is cross-sectional, and thus subjects with severe atherosclerosis, defined as ischaemic cardiovascular disease, are identified retrospectively. Correspondingly, risk factors were not independent of outcome, i.e. statin use, cholesterol level, treatment of hypertension, and smoking habits are likely to have changed as occurrence of the event. This is illustrated by the much higher use of statins in the subjects with severe atherosclerosis. Furthermore, owing to the retrospective identifications of people with ischaemic cardiovascular disease, we cannot be definite that the value of ABI, C-reactive protein, and central augmentation index in identifying individuals with severe atherosclerosis and symptomatic ischaemic cardiovascular disease will be identical to the value of these three non-invasive tests in individuals identified in the general population, with severe atherosclerosis before they develop symptomatic ischaemic cardiovascular disease.

In addition, limiting the study is the fact that we had no knowledge about previous interventions for peripheral arterial or carotid arterial revascularisation. Therefore, misclassification of a few participants suffering from peripheral arterial disease into the control group cannot be excluded. However, such misclassification can only lead to a slight underestimation of the findings in this study.

Systolic blood pressure of the ankle was measured on both legs, but only in the posterior tibial artery. Thus, if this pressure had also been measured in the anterior tibial artery in both legs and if the lowest systolic blood pressure of the posterior or anterior tibial artery in either leg had been chosen to calculate ABI, our ABI might have changed slightly. As this might represent a small misclassification of ABI measurements, the findings for ABI could in fact be more pronounced than that observed in this study.

Finally, the large number of participants excluded due to missing data on either ABI or C-reactive protein or central augmentation index could potentially have caused selection bias.

Perspectives
ABI < 0.9 appears to improve markedly the ability to identify people in the general population with severe atherosclerosis. The procedure is inexpensive and easy to perform: beside standard equipment for measurement of brachial blood pressure, the physician only additional needs to buy a hand-held Doppler apparatus costing roughly €450.

Acknowledgement
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Conflict of interest: none declared.