Arterial stiffness is one of the characteristics of vascular aging. Increases in pulse pressure, which reflect an increase in the stiffness of the large arteries, are associated with elevated C-reactive protein (CRP) levels. This may suggest a role of inflammation in the development of arterial stiffness. We investigated the relation between measures of arterial stiffness and CRP within the framework of the Rotterdam Study, a population-based cohort study including subjects aged 55 years and older. The carotid-femoral pulse wave velocity and the distensibility coefficient of the carotid artery were used as measures of arterial stiffness. Data on both arterial stiffness and CRP were available for 866 participants. In adjusted models, levels of CRP were linearly associated with pulse wave velocity (regression coefficient 0.088, 95% CI 0.006–0.170). Adjusted mean values of pulse wave velocity were significantly different across tertiles of CRP, being higher in the highest tertile of CRP. However, no significant association between CRP and carotid distensibility was observed.

1. Introduction

Arterial stiffness increases with age [1], but also hypertension [2], atherosclerosis [3] and diabetes mellitus [4] are conditions associated with increased vessel wall stiffness. Several studies have suggested that subjects with cardiovascular disease have increased arterial stiffness compared to subjects without cardiovascular disease [5,6]. Moreover, high arterial stiffness has been shown to be an independent predictor of cardiovascular morbidity and mortality in patients with essential hypertension [7] and end-stage renal disease [8].

Increased levels of inflammatory markers, particularly C-reactive protein (CRP) are associated with atherosclerosis [9,10] and higher risk of cardiovascular events [11–13]. A recent study found that increases in pulse pressure, which reflect a gradual increase in the stiffness of the large arteries, are associated with elevated CRP levels [14]. This association might also suggest a role of inflammation in the pathogenesis of arterial stiffness.

To study the hypothesis that high CRP levels are associated with arterial stiffness, we conducted a cross-sectional study examining the association between serum CRP levels and measures of arterial stiffness.
2. Subjects and methods

2.1. Study subjects

This study was conducted within the framework of the Rotterdam Study, an ongoing prospective population-based cohort study composed of 7983 men and women aged 55 years and over, living in Ommoord, a suburb of Rotterdam, The Netherlands. Its overall aim is to investigate the incidence and determinants of chronic disabling diseases. The rationale and design of the Rotterdam Study have been described elsewhere [15]. Baseline data were collected from 1990 to 1993. The third examination phase took place from 1997, until 1999. During this phase, information on cardiovascular risk factors was collected, measurements of arterial stiffness and atherosclerosis were obtained and blood samples were taken for the measurement of CRP. The Medical Ethics Committee of the Erasmus Medical Center approved the study and written consent was obtained from all participants.

2.1.1. Examples of chemical reactions

The base Na2CO3 provided excellent results and, unlike much of our earlier annulation chemistry and Catellani’s procedure, we only needed to use catalytic amounts of n-Bu4NCl to get high yields.

\[
\text{C}_{10}H_{11}O_2 + 2 \text{C}_{10}H_{12}O_2 \rightarrow \text{C}_{10}H_{12}O_2 + \text{C}_{10}H_{12}O_2
\]

(1)

The reaction conditions used on norbornene produced only marginal success when applied to the reaction of 2-iodophenol and the bicyclic alkene indene product \( \text{C}_{10}H_{11}O_2 \) remained the favored isomer and a decrease in the yield of heteroannulation product \( \text{C}_{10}H_{12}O_2 \) was observed.

2.2. Arterial stiffness

Arterial stiffness was measured by two different methods, i. e. the distensibility coefficient (DC) of the common carotid artery as a measure of common carotid arterial stiffness and the carotid-femoral pulse wave velocity (PWV) as a measure of aortic stiffness. Both measures were obtained on the same day, in the same room. Subjects were instructed to refrain from smoking and from taking coffee, tea or pain medications on the day of measurement, and from taking alcohol on the day of measurements and the day before. In a reproducibility study in 47 subjects the intra-class correlation coefficient was 0.80 both for the DC and the carotid-femoral PWV.

2.2.1. Carotid distensibility

Common carotid distensibility was assessed with the subjects in supine position, the head tilted slightly to the contralateral side for the measurement in the common carotid artery. The vessel wall motion of the right common carotid artery...
was calculated according to the following equation: distensibility coefficient = 2(D/D_{1}) / \Delta P + 1/3 (systolic blood pressure - diastolic blood pressure). The cross-sectional arterial wall distensibility coefficient was calculated according to the following equation: distensibility coefficient = 2(D/D_{1}) / \Delta P (10^{-3} kPa) [18]. In the present study, measurements were restricted to the right side to save time. In previous studies no differences were detected between arterial wall properties of the right and left common carotid artery (SK Samijo, unpublished results, 1997).

2.2.2. Pulse wave velocity
Carotid-femoral pulse wave velocity (PWV) was measured with the subjects in supine position. Blood pressure was measured twice at the upper arm with a Dinamap automatic blood pressure recorder during the measurement session. The mean arteriogram was measured twice with a sphygmomanometer after five minutes of rest, and the mean was taken as the subject's reading.

2.2.3. Examples of equations
The kinematic and dynamical parameters of the space manipulator can be mapped to those of the DEM as follows [5]:

\[
\begin{align*}
\mathbf{m}_i &= \mathbf{m}_0 \left( \sum_{k=0}^{i-1} \kappa_k \right)^2, \\
\mathbf{i}_k &= \mathbf{i}_0 \left( \sum_{k=0}^{i-1} \kappa_k \right), \\
\mathbf{w}_i &= \mathbf{w}_0 \left( \sum_{k=0}^{i-1} \kappa_k \right), \\
\mathbf{w}_i &= \mathbf{w}_0 \left( \sum_{k=0}^{i-1} \kappa_k \right).
\end{align*}
\]

Proof. From the definition of the tracking error this can be written as

\[
\dot{\mathbf{e}} = \dot{\mathbf{M}}(\mathbf{q}) \dot{\mathbf{q}} + \dot{\mathbf{K}} \mathbf{e} + \dot{\mathbf{P}} \mathbf{e} + \ddot{\mathbf{M}}(\mathbf{q}) \ddot{\mathbf{q}} + \dddot{\mathbf{C}} \mathbf{q}.
\]

Substitution yields

\[
\dot{\mathbf{e}} = \dot{\mathbf{M}}(\mathbf{q}) \dot{\mathbf{q}} + \dot{\mathbf{K}} \mathbf{e} + \dot{\mathbf{P}} \mathbf{e} + \ddot{\mathbf{C}} \mathbf{q} + \dddot{\mathbf{C}} \mathbf{q}.
\]

Thus, the tracking error system can be written in the form

\[
\dot{\mathbf{e}} + \dot{\mathbf{K}} \mathbf{e} + \dot{\mathbf{P}} \mathbf{e} + \ddot{\mathbf{C}} \mathbf{q} + \dddot{\mathbf{C}} \mathbf{q} = 0,
\]

where \( \mathbf{e} \) is the parameter error vector and \( \dddot{\mathbf{C}} \) a vector of the original values of the parameters.

2.3. Cardiovascular risk factors
Data on drug use and smoking habits were obtained during the home interview. Smoking status was classified as current, past or never smoker. At the research center, blood pressure was measured twice on the right arm using a random-zero
sphygmomanometer. Body mass index [(weight (kg)/height\(^2\) (m)) was calculated. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol values were determined by an automated enzymatic procedure (Boehringer Mannheim System). Diabetes mellitus was defined as the use of anti-diabetic medication and/or a fasting serum glucose level ≥11 mmol/L. Prevalent cardiovascular disease was defined as a history of myocardial infarction or stroke. Information on cardiovascular disease was assessed during a home interview. A history of myocardial infarction and stroke was confirmed by reviewing the medical records from the general practitioner and/or medical specialist or by ECG. Occurrence of myocardial infarction or stroke was reported by general practitioners in the research area. Research physicians verified all information by checking patient records of the general practitioner.

In addition, discharge reports and letters of medical specialists were obtained for hospitalized patients.

2.4. C-reactive protein

Non-fasting blood was collected in tubes containing 0.129 mol/L of sodium citrate. The ratio of blood to sodium citrate was 9:1. Plasma was collected after centrifugation for 10 min at 3000 rotations per minute (rpm) at 4 °C. Subsequently, platelet-free plasma was obtained by centrifugation for 10 000 rotations per minute and was immediately frozen and stored at −80 °C. All tubes were stored on ice before and after blood sampling. C-reactive protein was measured by a nephelometric method (Dade-Behring). The detection limit of the assay was 0.2 mg/L; the inter- and intra-assay coefficient of variation for the method used were both 3.24%.

2.5. Measures of atherosclerosis

Ultrasonography of both carotid arteries was performed with a 7.5 MHz linear-array transducer and a duplex scanner (ATL UltraMark IV). Common carotid intima-media thickness (IMT) was determined, as previously described [20].

Aortic atherosclerosis was diagnosed by radiographic detection of calcified deposits in the abdominal aorta on a lateral abdominal film. The extent of abdominal aortic atherosclerosis was scored according to the length of the involved area (with scores 0–5 corresponding to 0, ≤1, 1–2.5, 2.5–4.9, 5.0–9.9 and ≥10.0 cm) [21].

2.5.1. Example of a displayed quote

The text below is an example of a displayed quote [taken from The Cloister and the Hearth, Charles Reade, 1814–1884].

Not a day passes over the earth, but men and women of no note do great deeds, speak great words, and suffer noble sorrows. Of these obscure heroes, philosophers, and martyrs, the greater part will never be known till that hour, when many that are great shall be small, and the small great; but of others the world’s knowledge may be said to sleep: their lives and characters lie hidden from nations in the annals that record them.

2.6. Population for analysis

Of the 4024 subjects, who underwent the physical examination of the third phase of the Rotterdam Study, aortic stiffness measured as PWV was measured in 3550 subjects, whereas common carotid distensibility was measured in 3098 subjects. Missing information on both measures was almost entirely due to logistic reasons. For the present study, levels of CRP were assessed in a randomly selected age- and sex-stratified sample of 970 participants. Outliers’ values (>3 S.D. of the population distribution) of logarithmically transformed CRP (n = 21) were excluded, since they might indicate the presence of an active inflammatory disease leaving 949 subjects for analysis. Finally, data on C-reactive protein and PWV were available for 866 subjects; data on C-reactive protein and the carotid distensibility were available for 677 subjects.

2.6.1. Some more examples of chemical reactions

Some more examples of chemical reactions (see Scheme 1).

2.7. Data analysis

The association between C-reactive protein and arterial stiffness was investigated by linear regression analysis (with PWV and DC as the dependent variable) and next investigated by analysis of covariance, where mean values of PWV were compared across tertiles of CRP levels. Cut-off points for tertiles of CRP were 1.67 and 3.40 mg/L. Analyses were adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, smoking status (current, past or never smoker) and previous cardiovascular disease. Additional adjustments were made for intima media thickness and aortic calcification in the analyses of PWV, only for intima media thickness in the analyses of DC.
3. Results

Baseline characteristics of the population are shown in Table 1. After adjustment for age and gender, levels of CRP were linearly associated with PWV (regression coefficient 0.138, 95% confidence interval, 0.063–0.213) and remained significant after adjustment for confounders and after additional adjustment for measures of atherosclerosis (Table 2). C-reactive protein was not associated with carotid distensibility (Table 2). Increasing tertiles of CRP were significantly associated with increasing levels of PWV (Fig. 1). Mean values of PWV adjusted for age and gender were 12.98 m/s (95% confidence interval, 12.66–13.30) in the first tertile of CRP, 13.49 m/s (95% confidence interval, 13.17–13.81) in the second tertile and 13.82 m/s (95% confidence interval, 13.50–14.15) in the last tertile. Corresponding mean values of PWV in fully adjusted models were 13.08 m/s (95% confidence interval, 12.77–13.45) in the first tertile of CRP, 13.56 m/s (95% confidence interval, 13.24–13.89) in the second tertile and 13.59 m/s (95% confidence interval, 13.24–13.94) in the last tertile of CRP. Associations of cardiovascular risk factors and measures of atherosclerosis with pulse wave velocity are given in Table 3. Also of interest is Fig. 2.

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>n = 866</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70 ± 5.4</td>
</tr>
<tr>
<td>Pulse wave velocity (m/s)</td>
<td>13.4 ± 3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80 ± 12.6</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>75 ± 15.1</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.8 ± 3.7</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>25.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.8 ± 0.95</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.39 ± 0.39</td>
</tr>
<tr>
<td>Previous cardiovascular disease (%)</td>
<td>13.9</td>
</tr>
<tr>
<td>C-reactive protein (ng/mL)</td>
<td>2.74 (1.54–3.52)</td>
</tr>
<tr>
<td>Carotid intima-media thickness (mm)</td>
<td>0.87 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± S.D. for continuous variables and percentages for dichotomous variables.

(loss) Per data with a skewed distribution, the median and interquartile range are shown.

(loss) Defined as a calcification score (range 0–5) larger than 3.
Table 2

Multiple linear regression (β) coefficients and 95% confidence intervals describing the association between C-reactive protein (independent variable) and measures of arterial stiffness (dependent variable).

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulse wave velocity (m/s)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.136</td>
<td>(0.063, 0.213)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.099</td>
<td>(0.024, 0.172)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.115</td>
<td>(0.039, 0.190)</td>
</tr>
<tr>
<td>Model 4</td>
<td>0.088</td>
<td>(0.000, 0.170)</td>
</tr>
<tr>
<td><strong>Distensibility coefficient (10^{-3} kPa)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.005</td>
<td>(-0.175, 0.064)</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.003</td>
<td>(-0.104, 0.097)</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.003</td>
<td>(-0.110, 0.094)</td>
</tr>
<tr>
<td>Model 4*</td>
<td>0.001</td>
<td>(-0.052, 0.122)</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age and gender. Model 2: adjusted for age, gender, mean arterial pressure and heart rate. Model 3: adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, smoking and previous cardiovascular disease. Model 4: adjusted for age, gender, mean arterial pressure, heart rate, body mass index, total cholesterol, HDL cholesterol, diabetes mellitus, smoking, previous cardiovascular disease, intima media thickness and aortic calcification score.

4. Discussion

In this population-based study we found that in older adults CRP levels are associated with PWV, a measure of arterial stiffness. No association was found between CRP levels and DC.

Some aspects of this study need to be discussed before interpreting these data. Firstly, the cross-sectional design may limit our ability to infer a causal relationship between measures of aortic stiffness and inflammatory mediators. Secondly, serum levels of CRP were measured only once, therefore intra-individual variation, as has been reported [22], cannot be taken into account. However, such variation will likely result in an underestimation of the true relationship. Thirdly, measures on arterial stiffness were not available for all participants. Because this was primarily due to logistic reasons and therefore random, we believe that this will not have biased the results.

In the present study, we found that high CRP levels were independently associated with increased arterial stiffness. These results are in agreement with the ones of a recent study [14], which showed that wide pulse pressure, a consequence of increased stiffness of the large arteries, is associated with elevated CRP levels. However, pulse pressure is considered to be a surrogate measure of arterial stiffness; in our study we used the PWV and the DC of the common carotid artery which are direct measures of arterial stiffness.

Several mechanisms may explain the role of CRP in the pathophysiology of arterial stiffness. Increased levels of CRP have been found to be associated with insulin resistance variables [23], diabetes mellitus [24,25], and high blood pressure levels [26], which are major determinants of arterial stiffness. High CRP levels, therefore, could contribute to increased PWV,
by being responsible for metabolic and hemodynamic changes that lead to arterial stiffness. However, after inclusion of cardiovascular risk factors in the analyses, the estimates remained statistically significant. This indicates an independent association between CRP levels and PWV.

Another possible mechanism may be the effect of CRP on endothelial dysfunction. Several studies have shown that increased levels of inflammation markers, particularly CRP, are associated with endothelial dysfunction [23,27], and the inflammation reaction is known to inhibit the endothelium-dependent vasodilatation [28]. The vascular endothelium releases vasactive substances; one of these, nitric oxide, has a major influence on basal arteriolar tone and blood pressure [29,30].

Increased CRP levels play an role in the development of atherosclerosis [9,10], which has also been related to arterial stiffness [3]. As reported in previous studies, measures of common carotid intima-media thickness and radiographically assessed calcifications of the abdominal aorta reflect atherosclerosis [21,38]. In the present study, measures of intima-media thickness and the aortic calcification score were used as indicators of atherosclerosis in adjusted models. To evaluate whether the association between CRP levels and arterial stiffness was independent of atherosclerosis, we included both variables in models where PWV was tested. Because carotid stiffness is a local measure of arterial stiffness, we included only intima-media thickness in models where carotid stiffness was tested. After adjustment for atherosclerosis, the association between CRP levels and increased PWV remained statistically significant, supporting that the association of CRP with arterial stiffness is independent of atherosclerosis.

![Convergence with supercell size of the guided-mode eigenfrequency for an index-guiding PCF with the air holes arranged in a triangular structure with d = 0.6. The graph shows the normalized frequency as a function of N with \( \beta = 1 \). The calculations for the CR structure [39, 1, 0] (right) all show better convergence compared to \( \beta = 1 \) calculations (left).](image-url)
4.1. Some more examples of equations

If \( \mathbf{R} \) is taken to lie along the \( x \)-axis, it is readily shown (by reflection in the \( x \)-axis) that if \( \mathbf{K} = 0 \). For \( \mathbf{R} \) vectors lying in other directions, the same symmetry argument can be applied by expressing the angular functions in a coordinate system with the \( x \)-axis parallel to \( \mathbf{R} \):

\[
\begin{align*}
    x &= \frac{X}{R} \cos \phi - \frac{Z}{R} \sin \phi \\
    y &= \frac{Y}{R} \cos \phi + \frac{Z}{R} \sin \phi \\
    z &= \frac{Z}{R} \cos \phi + \frac{X}{R} \sin \phi \\
    x' &= \frac{X'}{R'} \cos \phi - \frac{Z'}{R'} \sin \phi \\
    y' &= \frac{Y'}{R'} \cos \phi + \frac{Z'}{R'} \sin \phi
\end{align*}
\]

where \( \phi \) is the angle between \( \mathbf{R} \) and the \( x \)-axis. Finally, a change of basis turns out to be convenient,

\[
\begin{align*}
    e_1 &= \frac{1}{\sqrt{2}}(e_1 + e_2), \quad e_2 &= \frac{1}{\sqrt{2}}(e_1 - e_2).
\end{align*}
\]

and similarly for the \( \mathbf{b} \)-fields. In this representation, one may derive

\[
\begin{align*}
    \mathbf{t}_1(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_1(\mathbf{r}) &= t - t' \sin 2\phi \\
    \mathbf{t}_2(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_2(\mathbf{r}) &= t + t' \sin 2\phi \\
    \mathbf{t}_1(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_2(\mathbf{r}) &= t' \cos 2\phi \\
    \mathbf{b}_1(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_1(\mathbf{r}) &= 0 - O' \sin 2\phi \\
    \mathbf{b}_2(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_2(\mathbf{r}) &= O + O' \sin 2\phi \\
    \mathbf{b}_1(\mathbf{r} - \mathbf{R}) \cdot \mathbf{n}_2(\mathbf{r}) &= O' \cos 2\phi
\end{align*}
\]

Here, the parameters \( t, t', O' \) and \( O, O' \) depend on the radial field distributions of the guided modes, and we cannot make general inferences about their magnitudes. From the theory of step-index fibers, we expect that \( t, O \) (which have contributions from the \( f_1(\cdot) \)-components of the transverse fields) will dominate the other coupling parameters, so these couplings will be the most important to eliminate. Note that the \( I_2 \) and \( O_2 \) matrices have a similar structure with respect to the dependence on \( a \). It is also worth noting that the matrices are not diagonal, meaning that for a general choice of \( k_\perp \)-point, two polarization eigenstates of different frequency will be found, or in other words, a spurious birefringence is introduced by the choice of a symmetry-breaking \( k_\perp \)-point in a supercell calculation. It is an important goal of the present work to determine the magnitude of the birefringence induced by the choice of \( \mathbf{R} \)-\( k \)-points.
In conclusion, in this population-based study, we found that increased levels of CRP were associated with arterial stiffness independently of cardiovascular risk factors and atherosclerosis. Considering the cross-sectional design of the present study, mechanisms remain speculative and required further study. However, the association we found between CRP levels and arterial stiffness may be useful to understand better the biochemical mechanisms responsible for the development of arterial stiffness.

Acknowledgements

Any acknowledgement text would be placed here.

References
