Micro-inflammatory changes in asymptomatic healthy adults during bouts of respiratory tract infections in the community: Potential triggers for atherothrombotic events

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\textbf{A R T I C L E  I N F O}

\textbf{Article history:}
Received 12 July 2008
Received in revised form 28 January 2009
Accepted 29 January 2009
Available online xxxx

\textbf{Keywords:}
Inflammation
Acute respiratory tract infection
Atherothrombotic events
C-reactive protein
Fibrinogen

\textbf{A B S T R A C T}

\textbf{Objective:} To explore the possibility that mild inflammatory changes exist in asymptomatic adults during bouts of acute respiratory tract infections in the general population, and may mediate atherothrombotic events.

\textbf{Methods:} An epidemiological study that enrolled 5315 males and 2795 females attending a routine screening health program between the years 2003 and 2007. We correlated the concentrations of high sensitivity C-reactive protein (hs-CRP) and quantitative fibrinogen in completely asymptomatic and non-inflamed adults to the weekly epidemiological data for the incidence of acute respiratory tract infections in the community.

\textbf{Results:} Significant seasonal variations in the inflammatory variables were found for both genders. The population’s weekly rates of acute respiratory tract infection had a significant epidemiological influence on the inflammatory biomarkers in the asymptomatic cohort. The magnitude of this influence could reach as much as 12% (3–22%) in hs-CRP concentrations in women and 0.30\,\mu\text{mol/L} (0.20–0.41) in fibrinogen concentrations in men, for the change between the mean August and the mean January population’s respiratory illness burden.

\textbf{Conclusion:} Increase in the concentrations of two inflammation-sensitive biomarkers can occur in completely asymptomatic adults at times of increased burden of acute respiratory tract infection in the general population. The possibility exists that these inflammatory changes represent occult and asymptomatic infections that could by themselves trigger acute atherothrombotic events.

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1. Introduction

The debate whether acute phase reactants, such as C-reactive protein (CRP) and quantitative fibrinogen, are causal promoters of atherosclerosis or innocent bystanders in its pathogenesis, still remains [1–3]. Support for the role of CRP and quantitative fibrinogen in the pathogenesis of atherosclerosis comes largely from epidemiologic studies that have consistently observed an association between elevated inflammatory biomarker levels and cardiovascular events [4,5].

Cardiovascular events are known to have a seasonal pattern, generally assumed to be related to influenza epidemics and increased rates of acute infectious respiratory diseases [6,7]. In fact, influenza vaccination has been shown to be associated with a decrease in recurrent coronary events [8,9]. Destabilization and rupture of the atherosclerotic plaque in proximity to an acute respiratory infection has been proposed as one of the possible mechanisms to explain this seasonality [10–14].

We have presently explored the question of whether inflammatory changes can occur in a cohort of completely asymptomatic and non-inflamed individuals during bouts of respiratory tract infections in the general population. The concentrations of high sensitivity CRP (hs-CRP) and quantitative fibrinogen in a large sample of asymptomatic individuals were analyzed during a four-year period, and correlated to epidemiological data of the acute respiratory disease burden in the general population, using data obtained from the Israel center for disease control (ICDC) influenza surveillance system.
2. Methods

2.1. Study population

In the present study we have analyzed the data collected as part of the Tel Aviv Medical Center Inflammation Survey (TAMCIS) [15–17]. Between November 2002 and May 2007 a total of 11,274 subjects, on their first ever visit to the TAMCIS, gave their informed consent (7095 males and 4179 females) to participate in the survey.

We initially excluded 2627 subjects due to known inflammatory diseases, pregnancy, steroidal or non-steroidal treatment, any current infection or recent invasive procedures during the preceding six months. Two hundred and twenty-five subjects were later excluded from the analysis due to missing data for one of the inflammatory biomarkers. Finally, in order to exclude acute occult inflammation and/or infections, we excluded 312 subjects with hs-CRP concentrations above 10 mg/L. Following these exclusions the study group comprised 8110 individuals (5315 males and 2795 females). All of the remaining individuals reported no signs or symptoms of an intermittent infection and this lack of symptomatology was confirmed by a detailed questionnaire with specific questions regarding the possibility of a recent episode of infection/inflammation.

2.2. Laboratory methods

Fibrinogen was quantified by the method of Clauss [18] by a Sysmex 6000 (Sysmex Corporation, Hyaga, Japan) autoanalyser and Dade thrombin reagent (Dade Behring, Marburg, GMBH). Using normal plasma pool (mean concentration of 8.23 μmol/L) the intra-assay coefficient of variation (CV) was 3.5% and the inter-assay CV was 2.3%. The hs-CRP was measured by a Behring BN II nephelometer [19] and CardioPhase reagent (Dade Behring, Marburg, Germany). For hs-CRP levels below 15 mg/L, the intra-assay CV was in the range of 3.1–4.0% and the inter-assay CV was in the range of 2.1–3.8%.

2.3. Definitions of atherothrombotic risk factors

Diabetes mellitus was defined as a fasting blood glucose of ≥7.0 mmol/L, the use of insulin or oral hypoglycaemic medications, or self-reporting of diabetes. Hypertension was defined as a resting blood pressure of ≥140/90 mmHg on two separate measurements or the use of anti-hypertensive medications. Dyslipidaemia was defined by a low-density lipoprotein cholesterol (LDL-C) as being above the recommended levels according to the risk profile defined by the updated ATP III recommendations [20] or the use of lipid altering medications. According to the same recommendations, we also defined individuals as dyslipidemic if they displayed triglyceride concentrations of ≥2.26 mmol/L and non-high-density lipoprotein cholesterol (non-HDL-C) concentrations above the recommended levels [20].

2.4. Burden of acute respiratory infections data

The Israel center for disease control has an ongoing surveillance system attaining daily data regarding nationwide outpatient clinic visits, including three groups of respiratory diagnoses: pneumonia, upper respiratory tract infections (URTIs) and influenza-like illness (ILI). The pneumonia group includes such diagnoses as viral pneumonia, bacterial pneumonia and complications influencing influenza. The URTI group includes diagnoses such as acute pharyngitis, acute tonsillitis, common cold and influenza. The ILI group only consists of the diagnosis influenza. The method used to obtain the data has been previously described [21].

In our study, the burden of acute respiratory infections in the general population was assessed using the component of the IDC's surveillance system which is based on data gathered from Maccabi healthcare services, Israel's second largest HMO and the largest HMO in the Tel Aviv metropolitan area where our survey took place. We included only visits of individuals aged 19–64 in order to match the study population. The data was analyzed according to weekly rates of the above three groups of diagnoses per 100,000 individuals.

2.5. Statistical analysis

All data were summarised and displayed as mean (standard deviation [S.D.]) for the continuous variables and as number and percentage of patients in each group for the categorical variables. Since hs-CRP concentrations displayed an irregular distribution, we used a logarithmic transformation for all statistical procedures in order to obtain a normal distribution. Due to the high Pearson's partial correlations found between all three rates used to measure the acute respiratory infection burden, and in order to avoid colinearity in our models, we used factor analysis to produce one common variable that represents the total illness burden for the purpose of further analyses.

Firstly, in order to evaluate whether there were seasonal differences in the inflammatory variables and to compare between the years, we divided our data into four full years starting at the beginning of Summer (late June) and ending at the end of the following Spring (mid June), and calculated for each gender and for each year separately the age-adjusted sinusoidal pattern of the inflammatory variables using multiple linear regression models.

The sinusoidal model used:

\[ Y(t) = a + b \times \sin \left( \frac{2\pi t}{n} \right) + c \times \cos \left( \frac{2\pi t}{n} \right) + d \times \text{AGE}_t + \epsilon \]

where \(Y(t)\) is the level of the inflammatory variable at week \(t\), \(a\) is the overall mean (also termed rhythm-adjusted mean, or MESOR) and \(n\) is the number of weeks in a specific year. The seasonal amplitude is \(\sqrt{b^2 + c^2}\) and it was tested for being different than zero using the \(F\)-test. The seasonal variation is two times that amplitude. \(\epsilon\) is the residual error from the regression model assumed to be normally, independently and identically distributed with zero mean and variance, \(\sigma^2\). Normal plots of the residuals showed acceptable normal distribution for all models.

At the next stage, linear regression models were performed in order to quantify the epidemiological contribution of the population's illness burden to the variability of the different inflammation-sensitive biomarkers. All models were adjusted for various confounders including age, waist circumference, body mass index (BMI), complete lipid profile (LDL cholesterol, HDL cholesterol and triglycerides), diastolic and systolic blood pressure measurements, blood glucose concentration, alcohol consumption, physical activity, medications (aspirin, alpha blockers, beta blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, statins, fibrates and oral contraceptives or hormonal replacement therapy) and cardiovascular risk factors including smoking status, family history of coronary heart disease and a history of a proven atherothrombotic event (including myocardial infarction, history of coronary intervention, cerebrovascular event or peripheral arterial disease). In all linear regression models we tested the distribution of the residuals for normality. The results are presented as the mean and the 95% confidence interval (CI) of the change in the inflammatory variable for the change between the mean January and mean August levels of the values of the illness burden variable. According to the logarithmic transformation of hs-CRP, the expected change in the linear model with hs-CRP reflects relative change and is presented as.
percent of change rather than absolute change, as is presented for the concentration of fibrinogen. The results are presented as crude, age-adjusted and multi-variable adjusted estimate change.

All above analyses were considered significant at $P<0.05$ (two-tailed). The SPSS statistical package was used to perform all statistical evaluations (SPSS Inc., Chicago, IL, USA).

### 3. Results

We have presently analyzed the data of 8110 apparently healthy individuals (5315 males and 2795 females) with a mean (S.D.) age of 45 (11). The study population characteristics are described in Table 1, while the respective cardiovascular risk factors as well as the frequency of medications used by the two genders, are described in Table 2. Fig. 1 demonstrates the distribution of the outpatient visits rates due to respiratory infection illnesses during the time period of the study.

Using the age-adjusted sinusoidal pattern regression, we found significant seasonal variations in fibrinogen and hs-CRP in both genders and during most of the years tested, with greater differences in fibrinogen concentrations (Table 3). For hs-CRP, the seasonal variation in the concentrations reached a maximum of 0.64 mg/L and for fibrinogen, a maximum of 1.72 μmol/L. In addition, whenever a significant seasonal variation existed, there was a strong correlation between the time of maximal point of the fitted sinusoidal wave and the time of maximal respiratory disease activity (Fig. 1).

Following these results and in order to assess whether the inflammatory status of the apparently healthy individuals is influenced by the weekly burden of respiratory diseases, we calculated the multiple adjusted linear regressions of the inflammatory parameters. The results are displayed in Table 4 and demonstrate that even after adjustment for many known and possible confounders, the burden of the acute respiratory diseases had a significant influence on the variability of the different inflammatory biomarkers which reached as much as an 12% (3–22%) change in hs-CRP in women and as much as 0.30 μmol/L (0.20–0.41) change in fibrinogen concentration in men ($P<0.01$) for the change between the mean January and the mean August population's illness burden.

In order to investigate whether subjects who had more atherogenic risk factors had greater changes in the inflammatory markers, we divided our sample into low- and high-risk patients based on history of atherothrombotic event, diabetes mellitus, metabolic syndrome, >2 risk factors or Framingham coronary risk score >10%. The results (not shown) demonstrated greater changes in some of the high-risk group (19% vs. 10% for hs-CRP in women, and 0.35 vs. 0.27 μmol/L for fibrinogen in men), although the differences between the groups were not statistically significant.
Table 3

<table>
<thead>
<tr>
<th>hs-CRP</th>
<th>Year</th>
<th>MESOR (mg/L)</th>
<th>Amplitude (mg/L)</th>
<th>Significance</th>
<th>Max week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Summer 2003–Spring 2004</td>
<td>1.62</td>
<td>0.25</td>
<td>0.076</td>
<td>Late October 2003</td>
</tr>
<tr>
<td></td>
<td>Summer 2004–Spring 2005</td>
<td>1.44</td>
<td>0.32</td>
<td>0.002</td>
<td>Late December 2004</td>
</tr>
<tr>
<td></td>
<td>Summer 2005–Spring 2006</td>
<td>1.41</td>
<td>0.17</td>
<td>0.091</td>
<td>Early December 2005</td>
</tr>
<tr>
<td></td>
<td>Summer 2006–Spring 2007</td>
<td>1.46</td>
<td>0.21</td>
<td>0.035</td>
<td>Mid March 2007</td>
</tr>
<tr>
<td>Men</td>
<td>Summer 2003–Spring 2004</td>
<td>1.39</td>
<td>0.06</td>
<td>0.208</td>
<td>Mid November 2003</td>
</tr>
<tr>
<td></td>
<td>Summer 2004–Spring 2005</td>
<td>1.36</td>
<td>0.20</td>
<td>0.007</td>
<td>Early December 2004</td>
</tr>
<tr>
<td></td>
<td>Summer 2005–Spring 2006</td>
<td>1.31</td>
<td>0.18</td>
<td>0.006</td>
<td>Late February 2006</td>
</tr>
<tr>
<td></td>
<td>Summer 2006–Spring 2007</td>
<td>1.38</td>
<td>0.18</td>
<td>0.087</td>
<td>Late January 2007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibrinogen</th>
<th>Year</th>
<th>MESOR (μmol/L)</th>
<th>Amplitude (μmol/L)</th>
<th>Significance</th>
<th>Max week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Summer 2003–Spring 2004</td>
<td>8.44</td>
<td>0.07</td>
<td>0.324</td>
<td>Late July 2003</td>
</tr>
<tr>
<td></td>
<td>Summer 2004–Spring 2005</td>
<td>8.85</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td>Late December 2004</td>
</tr>
<tr>
<td></td>
<td>Summer 2005–Spring 2006</td>
<td>9.11</td>
<td>0.61</td>
<td>&lt;0.001</td>
<td>Early March 2006</td>
</tr>
<tr>
<td></td>
<td>Summer 2006–Spring 2007</td>
<td>9.47</td>
<td>0.78</td>
<td>&lt;0.001</td>
<td>Late September 2006</td>
</tr>
<tr>
<td>Men</td>
<td>Summer 2003–Spring 2004</td>
<td>7.4</td>
<td>0.13</td>
<td>0.101</td>
<td>Late March 2004</td>
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<td></td>
<td>Summer 2004–Spring 2005</td>
<td>8.11</td>
<td>0.47</td>
<td>&lt;0.001</td>
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<td></td>
<td>Summer 2005–Spring 2006</td>
<td>8.14</td>
<td>0.86</td>
<td>&lt;0.001</td>
<td>Late March 2006</td>
</tr>
<tr>
<td></td>
<td>Summer 2006–Spring 2007</td>
<td>8.61</td>
<td>0.61</td>
<td>&lt;0.001</td>
<td>Early October 2006</td>
</tr>
</tbody>
</table>

a MESOR—the rhythm-adjusted mean.
b Amplitude of the fitted sinusoidal curve (difference between the MESOR and the maximum or minimum point).
c The significance of the amplitude being equal to zero.
d Max week is the time period with maximal inflammatory concentrations in the fitted sinusoidal curve.

Fig. 1. Smoothed curves of the weekly rates of outpatient visits due to respiratory illness during November 2002–May 2007. URTI: upper respiratory tract infections; ILI: influenza-like illness.

Table 4
Expected mean change (95% CI) in the inflammatory variables for the change between the mean August and the mean January population’s illness burden compound variable, according to the linear regression models.

<table>
<thead>
<tr>
<th>Units</th>
<th>Adjustments</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP %</td>
<td>Crude</td>
<td>14 (7–22)</td>
<td>19 (7–32)</td>
</tr>
<tr>
<td></td>
<td>Age-adjusted</td>
<td>13 (6–20)</td>
<td>19 (7–32)</td>
</tr>
<tr>
<td></td>
<td>Multi-adjusted</td>
<td>7 (1–14)</td>
<td>12 (3–22)</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Crude</td>
<td>0.38 (0.27–0.49)</td>
<td>0.27 (0.11–0.43)</td>
</tr>
<tr>
<td></td>
<td>Age-adjusted</td>
<td>0.34 (0.24–0.45)</td>
<td>0.27 (0.12–0.43)</td>
</tr>
<tr>
<td></td>
<td>Multi-adjusted</td>
<td>0.30 (0.20–0.41)</td>
<td>0.19 (0.04–0.34)</td>
</tr>
</tbody>
</table>

a Adjusted to the complete list of potential confounders evaluated.
b P < 0.01.
c 0.01 ≤ P < 0.05.
4. Discussion

There are multiple lines of evidence to support the notion that atherosclerosis is a chronic inflammatory disease [22,23]. As such, it is often accompanied by increases in the concentration of inflammatory biomarkers. The concentration of these biomarkers can further increase during bouts of acute infection/inflammation as well as clinically relevant atherothrombotic events including acute coronary syndromes and stroke [1,5,24]. A well-known observation in atherosclerotic individuals is the seasonality of their clinical events which is associated with bouts of respiratory tract infections [12,14]. However, it is not entirely clear whether these associations are also relevant for individuals who are entirely asymptomatic.

The relevant finding of the present study for individuals with an eventual underling atherosclerotic milieu is that despite being completely asymptomatic, they harbored a heightened inflammatory response that correlated significantly with documented bouts of respiratory infections in the general population. The link to potential atherothrombotic events has been shown in the past and is mainly related to the erosion of the atheroma shoulder and the ensuing plaque rupture. The stability or instability of these atherothrombotic processes have been discussed [25]. Nevertheless, elevated concentration of either fibrinogen or CRP might not be necessarily harmless regarding the evolving atherothrombotic process per se. In fact, the possible fibrinogen–atheroma–fibrinogen sequence has been recently discussed by Meade [26]. Although still debatable [27] one cannot exclude the possibility that increased concentrations of fibrinogen do promote the progression of the atherosclerotic disease. The same stands for CRP [2].

Our current results confirm those of previous publications regarding the seasonality of fibrinogen concentrations [28–30] observing higher concentrations during Winter months. Regarding CRP, there were conflicting results in the literature, with most results being borderline [30] or negative including those of a previous publication by our group [15]. In the present analysis, beside the first year tested which was not significant also for fibrinogen, the other three years demonstrated seasonality which was statistically significant, or if not had a maximal P value of 0.091. Due to the consistency of the results between the years tested and between fibrinogen and hs-CRP, we believe the seasonality observed is real and the overall chances of causality are low, although not negligible. It is reasonable to assume that the borderline seasonality previously reported in the literature, could have arisen from the merging of hs-CRP values over several years with different time courses of respiratory infections. The present study included data collected over a four-year period and has shown an agreement between the timing of the population’s maximal illness burden and the timing of the maximal point in the sinusoidal pattern. This urged us to look for the short time linear associations between the respiratory illness burden and the inflammatory variables. The present study thus has the advantage of looking not only at the overall seasonal variation, but in addition, at the year-to-year variation and in accordance with the weekly burden of respiratory tract infections in the community. Our results emphasize therefore, the importance of analyzing each year separately as well as the importance of the interpretation of inflammatory biomarker seasonality in relation to the background respiratory disease in each year.

We note two main strengths to the present study, the first being the multiple adjustments for the relatively large number of possible confounders that can affect the concentrations of the inflammation-sensitive biomarkers hereewith reported. An additional strength is the fact that we excluded individuals who presented with concentrations of hs-CRP above 10 mg/L. Although it is possible that by this act we actually attenuated the observations of the present study, we do feel that this criterion was essential for the exclusion of individuals who have an unnoticed inflammatory, infectious or malignant disease.

The main limitation of the study is that we were not able to report clinical outcomes. Despite the fact that we did not follow our cohort for the eventual appearance of clinical events, our findings do support the possibility that clinically occult infection might exist in asymptomatic individuals. Thus, preventive measures such as influenza vaccinations, which in some epidemiological studies have been shown to be associated with reduced cardiovascular morbidity, [8,9] might be applied to the entire population in order to prevent those potential harmful inflammatory elevations and possible subsequent atherothrombotic events.

We conclude that bouts of respiratory tract infections in the general population are associated with a silent acute phase inflammatory response in completely asymptomatic individuals. This epidemiological information supports the notion that events of low grade inflammation do exist in asymptomatic individuals, and may contribute to the atherosclerotic process. It remains to be seen if the degree of inflammation in atherosclerotic lesions does show a similar seasonality to the one that was presently shown in the peripheral venous blood.

Conflict of interest

All authors declare having no known conflict of interests.

Acknowledgements

The authors wish to thank Zeev Rogowski, Ph.D. for his contribution to the analyses in this paper.

Source of funding: None.

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