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Influenza and pneumococcal vaccination as a model to assess C-reactive protein response to mild inflammation

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Abstract

This study was set up to examine whether an influenza vaccine or an influenza vaccine in combination with pneumococcal vaccine can be used as a model to study responses to mild stimulation of the inflammatory system.

In this study, 19 subjects received the influenza vaccine, 20 subjects the combination of influenza and pneumococcal vaccine. CRP and prothrombin fragment 1 and 2 (F1 + 2) were measured at baseline, and two times after vaccination. Influenza vaccination increased CRP by 0.20 mg/L, and influenza in combination with pneumococcal vaccine increased CRP by 0.60 mg/L. F1 + 2 increased 0.15 nmol/L after the combined vaccination; an increase in response to the influenza vaccination was not statistically significant.

Our findings show that the influenza vaccine alone as well as the combination of the influenza and pneumococcal vaccine increases CRP-levels with a peak 2 days after vaccination.

Keywords: C-reactive protein; Influenza vaccine; Pneumococcal vaccine

1. Introduction

Inflammation plays an important role in cardiovascular disease [1]. Both in the acute and the chronic phase of cardiovascular diseases, inflammatory processes are involved [2]. Firstly, macrophages and T-lymphocytes play a part in atherosclerosis [1,3,4]. But there is also evidence that inflammation promotes endothelial dysfunction and vasoreactivity of unstable atherosclerotic plaques [5–7].

Repeatedly it has been shown that C-reactive protein (CRP), a reliable marker of inflammation, is an important predictor of cardiovascular events [3,8–10]. There is also evidence that C-reactive protein (CRP) may play a direct role in atherogenesis and may be produced by atherosclerotic plaques [8,11].

It has been suggested that subjects with a more pronounced acute-phase response (high-responders) are at increased risk for acute cardiovascular events [12,13]. This may be mediated by activation of the coagulation system. To further explore this possibility, we need a method to assess small differences in inflammatory responses. Several vaccines have been used in in vivo models to induce a mild inflammatory response [5,12,14,15]. It is not clear whether influenza and pneumococcal vaccines increase serum CRP concentrations [16–19].

This study was set up to examine the magnitude and time patterns of CRP response and thrombin activation after administration of an influenza vaccine with or without a pneumococcal vaccine in older subjects.

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2. Materials and methods

This study was performed in the surgery of four general practitioners in Utrecht, the Netherlands. Subjects aged 65 years and older visiting their general practitioner for their yearly influenza vaccination in November 2002 were invited to participate in this study. Patients with symptoms of chronic infectious or inflammatory diseases, acute infections, or symptomatic cardiovascular diseases in the last three months were excluded. Subjects that had received a pneumococcal vaccine within 5 years before this study were also excluded. Thirty-nine subjects were included and gave written informed consent. Questionnaires were given to the participants to elicit information on risk factors, medical history, and medication. The study was approved by the Medical Ethical Committee of the University Medical Center Utrecht. Baseline blood samples were collected from all participants. Immediately thereafter participants were randomly assigned to receive either an influenza vaccination (Influvac, 0.5 ml, Solvay Pharma) alone or to an influenza vaccination with an additional pneumococcal vaccination (Pneumovax, 50 mg/ml, 0.5 ml, PMMSD, polyvalent from 23 types). The vaccines were injected in the deltoid muscle of the arm. Further blood samples were collected 2 or 3 days after vaccination and 4 or 5 days after vaccination; in total three samples of each participant, depending on availability of subjects on sampling days. A third blood sample was missing for one subject. Blood samples were centrifuged at 3000 rpm and at room temperature for 10 min. Samples were stored at −70 °C and high sensitivity CRP was assessed (BN II N High Sensitivity CRP Assay, by Dade Behring, Marburg, Germany) a few days after the last blood sample was collected. Prothrombin fragments (F1 + 2) were measured by ELISA (Enzygnost F1 + 2; Dade Behring, Marburg, Germany). The intra-assay coefficient of variation was 5–7% and the inter-assay coefficient of variation was 6–13%. The detection limit was 0.04 nmol/L.

Because CRP and F1 + 2 values were skewed toward higher levels, median concentrations and ranges are presented. The significance of difference in CRP and F1 + 2 was assessed using Wilcoxon Signed Ranks Test.

3. Results

The average age of the 39 subjects was 72 years. Median baseline CRP was 1.90 (range 0.30–8.90) mg/L, median baseline of F1 + 2 was 1.15 (range 0.27–5.47) nmol/L. Other baseline characteristics of the participants are displayed in Table 1.

The highest CRP levels were observed 2 days after the vaccinations. The changes in CRP from days 0 to 2, was 0.20 mg/L (−0.60 to 2.50, $P = 0.091$) in those who received influenza, and 0.75 mg/L (0.00–3.90, $P = 0.018$) in subjects who received the influenza with the additional pneumococcal vaccine (Table 2).

The change in CRP between baseline and second blood sample (i.e. days 2 and 3 together) was 0.20 mg/L (−0.60 to 2.50, inter-quartile range (IQR) 0.00–0.90, $P = 0.029$) for influenza alone, and 0.60 mg/L (−1.00 to 29.70, IQR 0.40–2.15, $P = 0.001$) for combined influenza and

Table 1
Baseline characteristics of study participants according to type of vaccination

	Influenza vaccine ($n = 19$)	Influenza + pneumococcal vaccine ($n = 20$)	Total ($n = 39$)
Sex (male, %)	11 (57.9)	13 (65.0)	24 (61.5)
Age (years)	72.9 ± 5.96	72.0 ± 4.38	72.4 ± 5.16
Hypertension (%)	10 (52.6)	6 (30.0)	16 (41.0)
Diabetes (%)	1 (5.3)	4 (20.0)	5 (12.8)
Hypercholesterolaemia (%)	2 (10.5)	6 (30.0)	8 (20.5)
Smoking status			
Current (%)	3 (15.8)	4 (20.0)	7 (17.9)
Past (%)	10 (52.6)	11 (55.0)	21 (53.8)
Never (%)	6 (31.6)	5 (25.0)	11 (28.2)
Body mass index (kg/m^2)	25.45 ± 3.28	25.37 ± 4.56	25.41 ± 3.93
History of cardiovascular diseases (%)	3 (15.8)	7 (35.0)	10 (25.6)
CRP (mg/L)[a]	1.70 (0.30–8.90)	2.65 (0.30–5.00)	1.90 (0.30–8.90)
Prothrombin fragments 1 + 2[a]	1.12 (0.27–5.47)	1.15 (0.53–3.11)	1.15 (0.27–5.47)

Values are numbers (%) or mean ± standard deviation.
[a] Values of CRP and F1 + 2 are medians (range).

Table 2
The medians of the concentrations of C-reactive protein (mg/L) according to the vaccination with influenza vaccine alone or with an additional pneumococcal vaccine

	Day 0[a]	Day 2	Day 3	Day 4	Day 5
Influenza vaccine (range)	1.70 (0.30–8.90)	2.70 (0.70–8.30)	2.10 (0.50–8.00)	1.10 (0.40–7.90)	1.35 (0.60–6.80)
Influenza and pneumococcal vaccine (range)	2.65 (0.30–5.00)	3.70 (0.50–8.90)	3.30 (0.70–33.80)	3.20 (0.30–19.40)	2.70 (0.40–4.30)

[a] Samples were taken at three times: immediately before vaccination (sample 1 = day 0), 2 or 3 days later (sample 2) and at days 4 or 5 (sample 3).

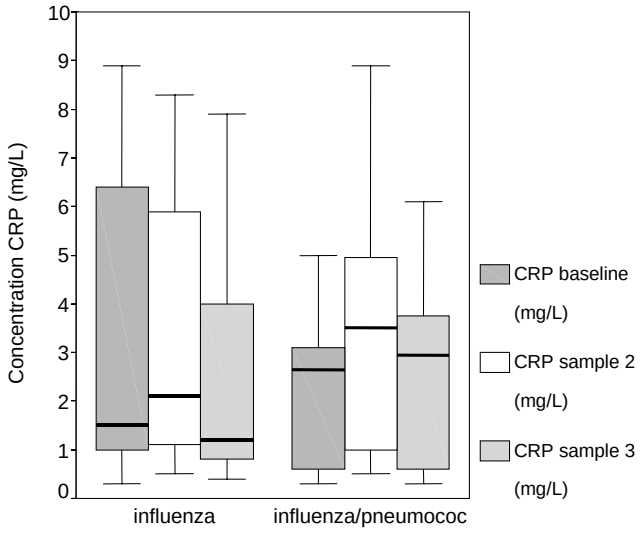

Fig. 1. Medians interquartile ranges and extreme values of CRP concentrations in subjects receiving influenza with or without pneumococcal vaccine. CRP levels were obtained at baseline (just before vaccination, at day 2 or 3 (sample 2), and day 4 or 5 (sample 3).

pneumococcal vaccine. On average we observed higher levels in patients receiving the combined vaccination. The highest medians were seen in sample 2 for both vaccination schemes (Fig. 1).

The change in F1 + 2 between baseline and second blood sample (i.e. day two and day three together) was 0.09 nmol/L (−4.28 to 4.06, IQR 0.05–0.21, $P = 0.091$) for influenza alone, and 0.15 nmol/L (−1.21 to 1.08, IQR 0.01–0.33, $P = 0.023$) for the combination of influenza and pneumococcal vaccine.

4. Discussion

Both influenza vaccine and influenza with the additional pneumococcal vaccine induce an increase in plasma levels of CRP among subjects aged 65 years and older. Peak levels of CRP were observed 2 days after vaccination in both vaccination groups. F1 + 2 increased 0.15 nmol/L after the combined vaccination; an increase in response to the influenza vaccination was not statistically significant.

In this before-after study we included subjects aged 65 years and older. They were our target population because older subjects are probably at highest risk for complications due to a more pronounced inflammatory response [20]. In order to study whether the vaccinations influenced the activation state of the coagulation system we also measured F1 + 2. We found a slight increase in F1 + 2 after the combined vaccination. More research is needed to assess whether and how the coagulation system is involved in the response to mild inflammation.

There were differences in baseline characteristics between the two groups. However, the objective of this study was to describe the response of CRP and F1 + 2 to a mild stimulus (i.e. vaccination). The aim was not to compare the responses between the two groups. Therefore, we did not adjust for unequally distributed baseline characteristics.

We measured CRP with a high sensitivity method. Standard clinical assays for CRP typically have a lower detection limit of 3–8 mg/L. Thus, these assays lack sensitivity within the low-normal range and cannot be used effectively for vascular risk prediction [21]. The Dade Behring BN II N High Sensitivity CRP assay has a lower detection limit of 0.15 mg/L and is therefore appropriate for assessing inflammatory response, within the low-normal range [22].

Other studies suggested highest increases of CRP in the first week after vaccinations [12,18]. We therefore measured CRP at baseline and 2, 3, 4 and 5 days thereafter.

Previous studies using influenza or pneumococcal vaccine have measured CRP longer after vaccination. Pozzetto et al. showed a non-significant increase in CRP 28 days after influenza vaccination while Elkayam et al. found no change at all when measuring CRP one month after pneumococcal vaccination [16,17]. According to our observations, increase in CRP is highest 2 days after vaccination and it decreases in the following days.

Raaska et al. found a non-significant increase of CRP in a group of 12 schizophrenic patients after influenza vaccination and highest levels of CRP at day 2 [18]. CRP was measured before and 2, 4, 7 and 14 days after vaccination. In this small group, however, two dropouts showed a marked increase of CRP.

Dynamic variation in plasma levels of inflammatory molecules may be important. Individuals who respond strongly to inflammatory triggers (hyperresponders) may have regularly slightly elevated plasma levels of inflammatory molecules and may have increased risk of cardiovascular events. Recognition of hyper-responsive individuals may lead to specific prevention and treatment measures [12].

Taken together our findings show that the influenza vaccine is a useful way to induce the production of the acute phase protein CRP. The effect is more marked for the combination of influenza and pneumococcal vaccination. Peak CRP levels are observed 2 days after vaccination. The influenza vaccine may be used to assess inter-individual differences in inflammatory responses.

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