Dysfunction of stress responsive systems in somatization
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Is high-sensitive C-reactive protein a biomarker for functional somatic symptoms?
A population-based study

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ABSTRACT

Functional somatic symptoms (FSS) are symptoms unexplained in terms of underlying organic pathology. Alterations in the immune system function may be associated with FSS via induction of sickness behavior. We aimed to investigate whether low-grade immune system activation is positively associated with FSS in a population-based cohort of 881 adults (46% male, mean age 53.0, SD 11.4). Participants completed the somatization section of the Composite International Diagnostic Interview surveying the presence of 43 FSS. Innate immune function was assessed by measuring high-sensitive C-reactive protein (hs-CRP). Follow-up measurements of hs-CRP and FSS were performed approximately 2 years later. Regression analyses, with adjustments for gender, age, body mass index, anxiety, depression, smoking, alcohol use, and frequency of exercise, did not reveal a cross-sectional association (β = 0.01, t = 0.40, p = 0.693) or longitudinal association (β = -0.03, t = -0.93, p = 0.352) between hs-CRP and the total number of FSS. When examining different bodily clusters of FSS, hs-CRP was not associated with the gastrointestinal FSS cluster, but the association approached statistical significance for the general FSS cluster (OR 1.08, 95% CI 0.98 to 1.18) and musculoskeletal FSS cluster (OR 1.08, 95% CI 0.99 to 1.17). For the latter association, exploratory analyses revealed that mainly the pure musculoskeletal complaints were responsible (OR 1.12, 95% CI 1.03 to 1.21). We conclude that the level of hs-CRP is not a biomarker for the total number of FSS in the general population. The association between hs-CRP and musculoskeletal and general FSS needs further study.
INTRODUCTION

Functional somatic symptoms (FSS), symptoms unexplained by underlying organic pathology, are multifactorially caused (Mayou & Farmer 2002). Functional somatic disorders (FSD), such as chronic fatigue syndrome, fibromyalgia, and irritable bowel syndrome, can be regarded as clusters of several FSS and are likely to share a common etiology (Wessely et al. 1999). Virtually all explanatory models on the etiology of FSS and FSD assign a role for psychosocial stress (Deary et al. 2007). One of the psychobiological mechanisms that may link psychosocial stress to FSS is the immune system (Segerstrom & Miller 2004).

Different pathways may be etiologically involved in this link. Firstly, the immune system may be related to FSS via sickness behavior, a constellation of non-specific symptoms induced by pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α (Dantzer 2001, Wieseler-Frank et al. 2005). These non-specific sickness behavior symptoms, including fatigue, weakness, malaise, hyperalgesia, and increased focus on own body, are core characteristics of persons having FSS and FSD (Dantzer 2005). Secondly, immune activation may be a marker for activation of other stress responsive systems that are possibly involved in FSS and FSD, namely, the autonomic nervous system and the hypothalamic-pituitary-adrenal axis (Heim et al. 2000a, Tak & Rosmalen 2007), as both systems closely interact with the immune system (McEwen et al. 1997, Araujo et al. 2006).

Indeed, numerous abnormalities in immune cell quantity and function have been identified in several FSD (Klimas & Koneru 2007, Liebregts et al. 2007). However, those abnormalities are rarely replicated, which is illustrated by a critical review on immunological abnormalities in chronic fatigue syndrome (Lyall et al. 2003). Furthermore, a study combining patients with different FSD did not find differences in pro- and anti-inflammatory cytokines between patients and healthy controls (Houtveen et al. 2007a). These inconsistent findings may be due to the fact that relevant alterations in central cytokine levels, if present, are not adequately reflected in peripheral blood measurements (Dantzer 2001).

IL-6 and TNF-α have a central role in stimulating the liver to produce C-reactive protein (CRP), which might be a more integrated and accurate peripheral marker for innate immune system activation. CRP is a particularly suitable measure for large epidemiological studies because assessment is relatively easy and reference values are known. Although historically long been considered as clinically irrelevant, minor elevations of CRP (3 - 10 mg/L) have been reported to be associated with psychosocial stress (McCabe et al. 2006) and a variety of somatic as well as psychiatric conditions (Kushner et al. 2006). Ultrasensitive assays can detect CRP in this subclinical range as high-sensitive CRP (hs-CRP). Of associations of circulating concentrations of CRP and pro-inflammatory cytokines
in subjects of the general population without active infection, those between IL-6 and CRP have been most frequently investigated and are generally strongest, with reported significant correlation coefficients ranging from 0.24 to 0.50 (Ridker et al. 2000, Cesari et al. 2003, Piche et al. 2005, Berrahmoune et al. 2007, Stewart et al. 2008, Milaneschi et al. 2009). In the study with the strongest correlation between IL-6 and CRP, which included 991 subjects aged 65 years and older, correlations of CRP with other investigated cytokines were lower: significant correlations of 0.09 and 0.19 were found for IL-1β and IL-18 respectively, and an non-significant correlation of 0.06 was found for TNF-α (Milaneschi et al. 2009).

In an elderly population of 2225 subjects, TNF-α and CRP were found to have a weak but significant correlation (correlation coefficient 0.13) (Cesari et al. 2003). In smaller studies of 112 postmenopausal women and of 315 adults, correlations of TNF-α with CRP were non-significant (Piche et al. 2005, Berrahmoune et al. 2007).

In this study, our hypotheses are that hs-CRP is positively associated with the number of FSS in a large population cohort, and that hs-CRP elevations predict development of FSS in a two-year follow-up period. Additionally, we explore whether hs-CRP levels are differentially related to different bodily clusters of FSS.

METHODS

Study population
Our study has been performed in a population cohort in Groningen, The Netherlands. The selection procedure and sample characteristics have been extensively outlined elsewhere (Tak et al. 2009b). Baseline measurements in the 2001 - 2002 wave were completed by 1094 participants (aged 33 - 79 years). Follow-up measurements in the 2003 - 2004 wave were completed by 976 participants. The study was approved by the local medical ethics committee and all subjects gave written consent to participate.

C-reactive protein
Fasting blood samples were collected in all participants during a visit to the research facilities. In case of flu or a febrile temperature, blood collection was postponed to a later time (available blood samples at baseline N = 1024, at follow-up N = 983). High-sensitive CRP (hs-CRP) was determined by nephelometry with a threshold of 0.18 mg/L and intra- and inter-assay coefficients of less than 4.4 and 5.7%, respectively (BNIIN, Dade Behring, Marburg, Germany). CRP levels below the detection level were scored as 0.18 mg/L. One-time measurement is a reliable measure of CRP over an extended period of time in healthy individuals (Macy et al. 1997). Since concentrations of CRP increase dramatically as part of acute inflammatory response, it has been recommended to
discard plasma CRP concentrations above 10 mg/L in studies on CRP as a risk factor for cardiovascular disease (Pearson et al. 2003). Likewise, CRP levels above 10 mg/L may obscure the association between CRP elevations and FSS. Therefore, we eliminated subjects with CRP levels above 10 mg/L from our analyses (N = 44 at baseline, N = 25 at follow-up).

**Functional somatic symptoms**

FSS were measured by the somatization section of the Composite International Diagnostic Interview (CIDI), for which the procedure has been outlined elsewhere (Tak et al. 2009b). In brief, 43 symptoms are assessed through asking “have you had” this symptom. In the CIDI, symptoms are considered present when they meet severity criteria, i.e., provoked a health care visit. If these criteria are met, the interview assesses in a hierarchical fashion whether a physician diagnosed a symptom as due to physical illness or injury, or whether a symptom was caused by the use of medication, drugs, or alcohol. If these inquiries are negative for medical explanations, the symptom is scored as a FSS. The CIDI has adequate test-retest reliability and validity (Andrews & Peters 1998). Participants first completed the CIDI lifetime version, measuring lifetime FSS (N = 1088 completed CIDIs at baseline). Two years later, participants completed the CIDI 12-months version (N = 964 completed CIDIs at follow-up), surveying the same 43 symptoms in the previous year. As a measure of FSS, we summed all FSS reported in the 12-months interview. Furthermore, we created a measure of new-onset FSS by summing the FSS that were only reported in the 12-months interview but not in the lifetime interview.

Additionally, we constructed bodily clusters of FSS, based on symptom clusters previously identified in a large study on the classification of FSS (Fink et al. 2007). We generated a cardiopulmonary cluster (including questions about chest pain and shortness of breath), a musculoskeletal cluster (including questions about back pain, joint pain, pain in extremities, loss of touch or pain sensation, muscle weakness, and numbness or tingling sensations), a gastrointestinal cluster (including questions about abdominal pain, nausea, diarrhea, feeling bloated or flatulence, and food intolerance), and a general symptom cluster (including questions about headache, trouble with balance and walking, and dizziness). For all clusters, a dichotomous score was generated, with a score of 0 if no FSS in the symptom cluster was scored present and a score of 1 if one or more FSS in the symptom cluster was scored present.

**Statistical analysis**

Given the skewed distribution, the number of FSS and new-onset FSS were log-transformed. We used forced entry linear regression analyses to test whether hs-CRP was cross-sectionally (using hs-CRP and FSS measured at follow-up) or longitudinally (using hs-CRP measured at baseline and FSS measured at follow-up, adjusted for the number of lifetime FSS) associated with the total number of
FSS. Additionally, we tested whether hs-CRP predicted new-onset FSS. Standardized $\beta$'s are given. Logistic regression analyses were performed to test whether hs-CRP is differentially associated with FSS in bodily clusters. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented. Gender, age, body mass index (BMI), depression, anxiety, smoking, alcohol use, and frequency of exercise explain variance in hs-CRP (Visser et al. 1999, Hutchinson et al. 2000, Albert et al. 2003, Bazzano et al. 2003, Liukkonen et al. 2006, Lakoski et al. 2006, Pitsavos et al. 2006, Kuo et al. 2007) and FSS (Kroenke & Spitzer 1998a, Henningsen et al. 2003, Glass et al. 2004, Hasin & Katz 2007, Petry et al. 2008, Neumann et al. 2008). Therefore, all regression analyses were adjusted for gender, age, BMI, presence of depressive disorder (previous-year diagnosis of DSM-IV depressive disorder as assessed by the CIDI), presence of anxiety disorder (previous-year diagnosis of DSM-IV panic disorder with or without agoraphobia, agoraphobia, general anxiety disorder, and social phobia as assessed by the CIDI), smoking (none, 1-5 cigarette(s)/day, 6-10 cigarette(s)/day, 11-15 cigarette(s)/day, 16-20 cigarette(s)/day, >20 cigarette(s)/day), alcohol use (none, 1-4 unit(s)/month, 2-7 unit(s)/week, 1-3 unit(s)/day, >3 units/day), and frequency of exercise (not/hardly, once per week, twice or more per week). We also present results of regression models that are only adjusted for gender and age to illustrate the influence of the covariates. Interaction terms of gender with hs-CRP (hs-CRP $\times$ gender) and age with hs-CRP (hs-CRP $\times$ age) were created. All models were evaluated for absence of multicollinearity. All analyses were repeated after exclusion of participants with medication use (corticosteroids, antihypertensives, analgesics, oral contraceptives) or somatic disease (cardiovascular disease, rheumatoid arthritis, chronic obstructive pulmonary disease, inflammatory bowel disorders, malignancy) that may influence hs-CRP levels. All $p$-values less than 0.05 were considered statistically significant.

RESULTS

At baseline, the mean age of the study population was 53.0 years (SD 11.4), with 46% males. The median hs-CRP concentration was 1.18 mg/L (interquartile range 0.55 - 2.58 mg/L). The median number of FSS in the previous year was 1 (interquartile range 0 - 2 FSS, range in population 0 – 19 FSS). Prevalence of having FSS was 6.7% (N = 60) in the cardiorespiratory cluster, 23.7% (N = 227) in the musculoskeletal cluster, 12.7% (N = 114) in the gastrointestinal cluster, and 17.4% (N = 156) in the general cluster. Of the total population, 38.3% developed at least one new-onset FSS.

In the regression models only adjusted for age and gender, no cross-sectional association between hs-CRP and the total number of FSS was found ($\beta = 0.05$, $t = 1.425$, $p = 0.155$), nor did hs-CRP predict the total number of FSS ($\beta = -0.01$, $t = -
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0.38, \( p = 0.703 \)). After adjustment for several confounders, as shown in Table 1, hs-CRP was not associated with the total number of FSS in the cross-sectional analysis or in the longitudinal analysis. There were no statistical significant interactions of hs-CRP with gender or age (data not shown). Furthermore, hs-CRP did not predict the number of new-onset FSS (\( \beta = -0.03, t = -0.76, p = 0.449 \)).

Table 1. Multivariable linear regression explaining the number of FSS out of hs-CRP and possible confounders.

<table>
<thead>
<tr>
<th>Outcome number of FSS</th>
<th>Cross-sectional analysis</th>
<th>Outcome number of FSS</th>
<th>Longitudinal analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 881</td>
<td>N = 865</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \beta )</td>
<td>( t )</td>
<td>( p \text{ value} )</td>
<td>( \beta )</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>0.01</td>
<td>0.40</td>
<td>0.693</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.16</td>
<td>4.77</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Age</td>
<td>0.05</td>
<td>1.44</td>
<td>0.150</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.03</td>
<td>5.74</td>
<td>0.431</td>
</tr>
<tr>
<td>Anxiety</td>
<td>0.19</td>
<td>5.74</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Depression</td>
<td>0.13</td>
<td>3.74</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.02</td>
<td>0.49</td>
<td>0.628</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>-0.10</td>
<td>-2.95</td>
<td>0.003*</td>
</tr>
<tr>
<td>Frequency of exercise</td>
<td>-0.06</td>
<td>-1.84</td>
<td>0.066</td>
</tr>
<tr>
<td>Lifetime number of FSS</td>
<td>0.44</td>
<td>14.48</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

| Adjusted \( r^2 \) | 0.11 | 0.18 |

Abbreviations: FSS, functional somatic symptoms; hs-CRP, high-sensitive C-reactive protein. \( *p<0.050 \).

Next, we tested whether hs-CRP was differentially associated with FSS in any of the bodily clusters (see Table 2). Due to the low number of participants having FSS in the cardiorespiratory cluster, this cluster was not suitable for logistic regression analyses. In the logistic models only adjusted for gender and age, hs-CRP was significantly associated with FSS in the musculoskeletal factor. When adjusting for several confounders, hs-CRP was not associated with FSS in the gastrointestinal cluster, whereas hs-CRP tended to be associated with FSS in the general cluster (\( p = 0.124 \)) and musculoskeletal cluster (\( p = 0.085 \)). In an exploratory analysis, the latter association appeared to be mainly driven by the pure musculoskeletal FSS, namely, back pain, joint pain, pain in extremities, and muscle weakness (model only adjusted for gender and age OR 1.14, 95% CI 1.06 to 1.23, \( z = 11.53, p = 0.001 \), fully adjusted model OR 1.12, 95% CI 1.03 to 1.21, \( z = 6.80, p = 0.009 \)) and not by the more neurological FSS in this factor. Results of the longitudinal analyses showed the same positive, although weaker association for musculoskeletal FSS, whereas the association with general FSS disappeared (Table 2).
Chapter 8

Table 2. Multivariable logistic regression analyses on the association of hs-CRP and bodily clusters of FSS.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Outcome bodily clusters</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Musculoskeletal FSS</td>
<td>Gastrointestinal FSS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.11 (1.03 - 1.20)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.08 (0.99 - 1.17)</td>
</tr>
<tr>
<td>Longitudinal analysis</td>
<td>hs-CRP</td>
<td>1.07 (0.98 - 1.16)</td>
</tr>
<tr>
<td></td>
<td>Adjusted hs-CRP</td>
<td>1.04 (0.95 - 1.13)</td>
</tr>
</tbody>
</table>

Abbreviations: FSS = functional somatic symptoms, hs-CRP = high-sensitive C-reactive protein, OR = odds ratio, 95% CI = 95% confidence interval. ** Adjusted for gender and age. * Adjusted for gender, age, BMI, smoking, alcohol use, depression, anxiety, and exercise frequency. + Adjusted for the lifetime number of FSS at baseline. **p<0.01.

All analyses were repeated after exclusion of participants with medication use (corticosteroids, antihypertensives, analgesics, oral contraceptives) or somatic disease (cardiovascular disease, rheumatoid arthritis, chronic obstructive pulmonary disease, inflammatory bowel disorders, malignancy) that may influence hs-CRP levels. These secondary analyses did not essentially change the results; therefore, only results of the total sample are shown for maximal generalizability.

DISCUSSION

In this population-based study, we did not find evidence for an association between hs-CRP and the total number of FSS. When differentially exploring bodily clusters of FSS, hs-CRP tended to be associated with general and (pure) musculoskeletal FSS.

To our knowledge, no previous studies addressed hs-CRP and a large range of FSS in the general population, however, the few clinical and population-based studies that examined hs-CRP in patients with FSD may serve as a reference. In line with our findings on FSS in the gastrointestinal bodily cluster, hs-CRP levels of patients with irritable bowel syndrome were not different from those of healthy controls (Schoepfer et al. 2008). We are unaware of studies specifically focusing on musculoskeletal FSS, such as in fibromyalgia, however, hs-CRP was related to low back pain in a study in young female adults (Shiri et al. 2008) and self-reported pain not due to chronic disease in a study in older adults (Graham et al. 2006). Also in medically explained chronic pain conditions, sickness behavior is often considered to be an important contributor to symptom experience. The
The association between hs-CRP and pure musculoskeletal FSS, which remained after exclusion of participants with somatic conditions such as rheumatoid arthritis, is in line with previous literature documenting an association between low-grade inflammation and chronic pain. The differential associations may be explained by the fact that those FSS are related to sickness behavior, mainly to the hyperalgesia and weakness components. This idea is supported by studies on chronic fatigue syndrome which consistently show that hs-CRP levels are higher in patients compared to healthy controls (Buchwald et al. 1997, Spence et al. 2008, Raison et al. 2009). Authors of the latter study suggest that this increase in peripheral inflammatory signaling might not be specific to chronic fatigue syndrome, but apply to unwellness in general. Alternatively, as the association of hs-CRP with musculoskeletal FSS became weaker and the association with general FSS disappeared in the longitudinal analysis, the association between hs-CRP and those FSS may be confounded by conditions or behavior that influence CRP levels, such as obesity and depression. Of note, our analyses were adjusted for several of such confounders, and hs-CRP remained independently associated with pure musculoskeletal FSS. If hs-CRP is a biomarker of those intermediate mechanisms, however, this analysis might be over adjusted. We have chosen our confounders a priori based on theory, as this strategy will generally constitute stronger scientific evidence than models that were achieved in other ways (Babyak 2004). The drawback of this strategy is inferring that hs-CRP is not causally important because its association with FSS is eliminated by the inclusion of covariates in the adjustment process, while it may only reflect that the covariates treated as confounders are actually critical to the causal chain (Christenfeld et al. 2004). For example, IL-6 and other pro-inflammatory cytokines are not only released by the liver, but have also been shown to be released by adipose tissue (Mohamed-Ali et al. 1997, Pou et al. 2007). In this perspective, BMI may not be a confounder in the potential relation between hs-CRP and FSS, but rather a causal factor inducing low-grade inflammation and sickness behavior. The same kind of conceptual problems apply to anxiety, depression, and health behaviors, such as regularly being engaged in physical activity. To further disentangle this problem, future studies could employ methods that appropriately account for the time-varying nature of the association by repeatedly assessing hs-CRP, FSS, and covariates like BMI, depression, anxiety, and health behaviors, preferably analyzed using multi-level statistical techniques (Christenfeld et al. 2004). This type of studies has more power to establish whether the association between hs-CRP and musculoskeletal FSS is causal, consequential, or epiphenomenonal.

The findings of this our study should be interpreted in light of some limitations. First, we only measured hs-CRP, which is a general biomarker for immunological activation that may not be sufficient to capture all aspects of immune function. In the introduction, we mentioned sickness behavior and activation of stress responsive systems as potential links between the immune system and FSS.
(McEwen et al. 1997, Heim et al. 2000a, Dantzer 2005, Araujo et al. 2006, Tak & Rosmalen 2007). Regarding the former, it may be questioned whether hs-CRP is an adequate biomarker for sickness behavior in the general population. Current evidence for the existence of sickness behavior mainly originates from experimental research in animals and research in patients with medically explained conditions (Hart 1988, Dantzer et al. 2008a), and has focused on cytokines. Pro-inflammatory cytokines that are considered most important in sickness behavior are IL-1β and TNF-α (Dantzer et al. 2008b), while CRP is more strongly associated with IL-6 than with IL-1β and TNF-α (Ridker et al. 2000, Cesari et al. 2003, Piche et al. 2005, Berrahmoune et al. 2007, Stewart et al. 2008, Milaneschi et al. 2009). Thus, it cannot be inferred from the current results that sickness behavior does not play a role in the experience of FSS in the general population. Instead, our negative results might be explained by the fact that hs-CRP is not a valid indicator for cytokines associated with sickness behavior. Second, although the CIDI is a widely-used, validated instrument to diagnose somatoform disorders (Andrews & Peters 1998), we have no data on whether patients interpreted and recalled the physicians’ opinion correctly. For example, headache diagnosed as tension-type headache or muscle pain diagnosed as fibromyalgia may be considered as physical illness by the participant. In that case, the CIDI will score the symptom as explained by a physical illness and not as FSS. We expect that this limitation will result in an underestimation of the true amount of FSS in our population, since patients presumably tend to interpret FSS as medically explained rather than the opposite (Robins et al. 1982). However, we are confident that we have been able to measure FSS with our approach, as the female preponderance and the about equally strong associations with anxiety and depression are similar to previous studies in the general population (Haug et al. 2004) and to a study in primary care having involved a physician to decide whether symptoms are unexplained (Waal de et al. 2004). Third, we did not take into account the severity of the reported FSS. If hs-CRP elevations are only associated with FSS that have reached a certain level of severity, our measurement of the total number of clinically relevant FSS could have diluted this association. The major strength of this study is its large cohort and extensive data collection, providing the possibility to adjust for several confounders.

In conclusion, we did not find evidence for a relationship between hs-CRP levels and the total number of FSS in the general population. Intriguingly, hs-CRP seems differentially related to pure musculoskeletal FSS. This finding needs further attention in future studies that are designed to gain more insight in which variables should be considered as confounders, which as mediators, and which as moderators.