

## CONCISE REPORT

## Serum cytokine levels related to multiple dimensions of fatigue in patients with primary Sjögren's syndrome

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**Objective:** To test whether serum levels of selected cytokines relate to different dimensions of fatigue in patients with primary Sjögren's syndrome (pSS).

**Methods:** Sixty female patients with pSS filled out a questionnaire to assess multiple dimensions of fatigue. Scores were compared with values in a population based control group (n=139). Levels of interleukin (IL)1 $\beta$ , IL2, IL6, IL10, and tumour necrosis factor  $\alpha$  were measured in serum with commercial sandwich ELISAs. The relationship between self reported dimensions of fatigue and these serum cytokine levels was determined.

**Results:** Patients with pSS had high scores at all dimensions of fatigue (p<0.001): general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. Fatigue levels were not related to serum cytokine levels. The incidental finding that reduced motivation was higher in patients with detectable serum levels of IL10 (p=0.04) disappeared after correction for multiple testing.

**Conclusion:** Fatigue is prominent in patients with pSS and involves all dimensions of fatigue. The findings do not suggest a widespread effect of circulating cytokines on multiple aspects of fatigue.

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease that primarily affects the exocrine glands. The disease has a female preponderance and manifests itself most commonly in the fourth and fifth decade of life. The clinical picture varies from mild complaints of ocular and oral dryness to severe keratoconjunctivitis sicca and a variety of extraglandular features.<sup>1</sup> For many patients with pSS fatigue is a prominent and disabling feature.<sup>2</sup>

In pSS, both cells constituting the characteristic glandular periductal infiltrates as well as the ductal epithelial cells actively produce a variety of (proinflammatory) cytokines. Compared with healthy controls, patients with pSS have increased serum levels of interleukin (IL)2,<sup>3</sup> IL6,<sup>3,4</sup> and IL10.<sup>3,5</sup> Several observations link cytokines to fatigue. In animal studies administration of IL1 $\beta$ , tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), or lipopolysaccharide leads to decreased activity and increased somnolence.<sup>6</sup> In man, cytokine treatment with TNF $\alpha$ ,<sup>7</sup> IL2,<sup>8</sup> or IL6,<sup>9</sup> is associated with flu-like symptoms, including fatigue, depressed mood, and cognitive disturbances. In patients with rheumatoid arthritis fatigue is reduced with TNF $\alpha$  blocking therapy.<sup>10</sup> These data strongly suggest a role for cytokines as triggers for a complex set of events leading to physiological, behavioural, affective, motivational, and cognitive changes known as sickness behaviour.<sup>6</sup> The finding of a significant association between serum cytokine levels and fatigue provides a rationale to direct future treatments at proinflammatory cytokines. In this study

we test whether serum levels of selected cytokines relate to multiple dimensions of fatigue in patients with pSS.

## METHODS

Participants were 60 patients from the departments of rheumatology and clinical immunology of the University Medical Centre, Utrecht and the University Hospital, Groningen, The Netherlands, who consecutively gave informed consent to participate in a placebo controlled study on the effects of administration of dehydroepiandrosterone (DHEA) on fatigue and general wellbeing. Inclusion criteria were female sex, a focus score  $\geq 1$  on minor salivary gland biopsy, meeting at least four of the European criteria for the classification of primary Sjögren's syndrome,<sup>11</sup> normal serum creatinine and thyroid stimulating hormone levels, and no use of corticosteroids in the preceding year. The mean (SD) age of patients was 53.3 (13.1) years. The current study took place before the start of treatment with DHEA or placebo.

All patients completed the Multidimensional Fatigue Inventory (MFI),<sup>12</sup> a 20 item, self report questionnaire covering five dimensions of fatigue: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. This scale has been used and validated in a variety of conditions, including pSS.<sup>2</sup>

As surrogate parameters of disease activity erythrocyte sedimentation rate (ESR), haemoglobin concentration, and total serum IgG were used.

Levels of IL1 $\beta$ , IL2, IL6, IL10, and TNF $\alpha$  were measured in undiluted serum samples by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BioSource International, Inc, Camarillo, Ca, USA). The range of detection for the assay used is for IL1 $\beta$ : 0.19–20 pg/ml, for IL2: 0.1–30 U/ml, for IL6: 0.104–10 pg/ml, for IL10: 0.2–50 pg/ml, and for TNF $\alpha$ : 0.09–32 pg/ml.

## Statistical analyses

The fatigue scores and disease activity parameters were normally or nearly normally distributed; the skewness of the distribution of the scores was between 0.16 for "reduced motivation" and -1.57 for "general fatigue". It was decided not to resort to non-parametric statistics or improve normality by transforming variables, because it was considered invalid to change these scores by transformation and because of the impossibility of adjusting non-parametric scores for the effect of age. Univariate analysis of variance was used to compare the disease activity and fatigue scores for patients with cytokine levels below and above the detection limit of the assay used. As cytokine levels, surrogate measures of disease activity, and fatigue levels may all depend on age, analyses were adjusted for age.

**Abbreviations:** DHEA, dehydroepiandrosterone; ELISA, enzyme linked immunosorbent assay; ESR, erythrocyte sedimentation rate; IL, interleukin; MFI, Multidimensional Fatigue Inventory; pSS, primary Sjögren's syndrome; TNF $\alpha$ , tumour necrosis factor  $\alpha$

**Table 1** Mean (SD) fatigue levels of patients with serum cytokine levels below and above the detection limit

	IL2 (U/ml)			IL6 (pg/ml)			IL10 (pg/ml)			TNF $\alpha$ (pg/ml)		
	Below	Above	p	Below	Above	p	Below	Above	p	Below	Above	p
General fatigue	16.7 (4.4)	18.0 (3.0)	0.38	16.7 (4.3)	18.0 (3.5)	0.55	16.5 (4.7)	17.5 (2.8)	0.56	17.0 (3.6)	16.8 (4.4)	0.73
Physical fatigue	14.9 (3.9)	15.5 (4.1)	0.67	14.9 (4.0)	15.6 (2.7)	0.72	14.6 (4.4)	15.7 (2.5)	0.39	15.6 (4.4)	14.8 (3.8)	0.52
Reduced activity	13.6 (4.3)	13.8 (4.9)	0.90	12.5 (4.3)	14.4 (5.3)	0.69	13.3 (4.7)	14.3 (3.6)	0.44	14.3 (5.3)	13.5 (4.2)	0.54
Reduced motivation	11.5 (3.8)	12.4 (4.7)	0.58	11.4 (3.9)	14.2 (2.9)	0.11	11.0 (3.8)	12.9 (3.8)	0.04	12.3 (3.6)	11.5 (4.0)	0.64
Mental fatigue	12.6 (5.0)	12.9 (5.9)	0.88	12.9 (5.0)	8.8 (4.4)	0.08	12.5 (5.5)	12.9 (4.2)	0.74	13.0 (5.2)	12.5 (5.1)	0.80

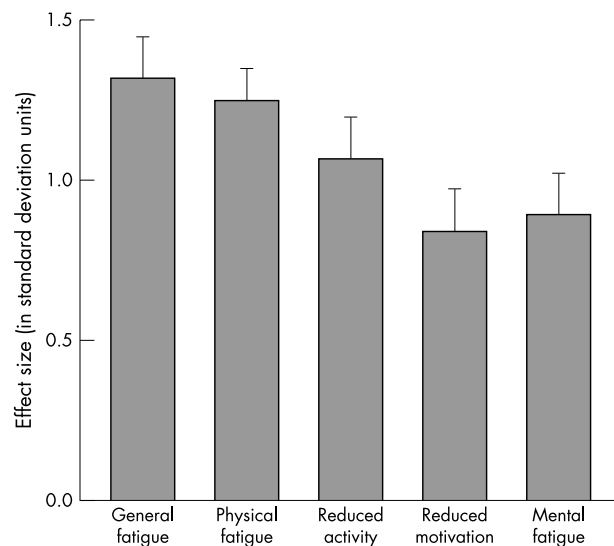
The numbers of patients with serum cytokine levels below and above the detection limits of the assays were 52 and 8 for IL2, 55 and 5 for IL6, 41 and 19 for IL10, and 10 and 50 for TNF $\alpha$ , respectively; the range of the fatigue subscales is 4–20.

The fatigue scores of our patients were compared with the scores of a normal healthy control population as described by the investigators who developed the MFI questionnaire.<sup>13</sup> This group comprised 78 women and 61 men with a mean (SD) age of 46 (16) years. Effect sizes were computed to quantify the extent to which the scores of our group deviated from scores of the control group.<sup>14</sup> An individual score of a patient (X) minus the control group average (M) divided by the standard deviation of a control group results in the effect size: (X–M)/SD. Effect sizes of 0.2, 0.5, and 0.8 indicate small, moderate, and large deviations from the norm, respectively. Univariate analyses of variance with age as covariate were applied to compare patients with cytokine levels below and above the lower detection limit for disease activity measures and fatigue scores.

Analyses were done with SPSS. All tests were two sided, and p values <0.05 were considered significant. To take account of multiple testing, the Bonferroni criterion (the normal  $\alpha$  divided by the number of tests) was used to interpret findings and determine significance.

## RESULTS

The mean scores of the patients showed for all dimensions of fatigue an effect size deviation of the control group that was large and highly significant ( $p < 0.001$ ; fig 1). Taking a large effect size deviation of 0.8 standard deviation from the norm or more as classification criterion for fatigue for each MFI subscale, general fatigue and physical fatigue were present in 45 (75%), reduced activity in 38 (63%), and reduced motivation and mental fatigue in 31 (52%) patients.



**Figure 1** Effect sizes (mean (SEM)) at five dimensions of fatigue of the MFI. Effect sizes compare scores assessed in 60 patients with primary Sjögren's syndrome with a normal control population.

Within the group of 60 patients a cytokine level above the lower range of detection of the assay used was present in 8 (13%) patients for IL2, 5 (8%) for IL6, 19 (32%) for IL10, and 50 (83%) for TNF $\alpha$ . No sample had detectable levels of IL1 $\beta$ . The mean (SD) ESR, haemoglobin, and IgG levels were 32.4 (26.1) mm/1st h, 8.1 (0.7) mmol/l, and 18.3 (8.3) g/l, respectively. Mean levels of ESR, haemoglobin, and IgG were significantly higher in patients with detectable IL2, IL6, IL10, and TNF $\alpha$  levels than in patients with levels below the lower detection limit ( $p \leq 0.01$ ), except for mean IgG levels in patients below and above the detection limit for TNF $\alpha$  ( $p = 0.06$ ). Fatigue scores did not correlate with ESR, IgG, or haemoglobin, except for a correlation between the dimension reduced motivation and IgG ( $r = 0.27$ ,  $p = 0.04$ ).

Table 1 shows the mean fatigue scores of patients with or without detectable levels of IL2, IL6, IL10, and TNF $\alpha$ . The only significant association between reduced motivation and IL10 disappeared when the level of significance was set to the Bonferroni criterion.

## DISCUSSION

Our study confirms that many patients with pSS have abnormally high fatigue levels at all dimensions of fatigue. We suggested that in the chronic inflammatory disorder pSS, persistent fatigue is mediated by overproduction of cytokines and that serum levels of cytokines might relate to fatigue. As expected, we found an association between serum levels of cytokines and markers of disease activity in pSS—namely, ESR, haemoglobin, and IgG. We consider this finding as a sign of the validity of our data on serum cytokine levels.

There are only a limited number of studies on serum cytokine levels with which our data can be compared. With the assays used we found levels above the lower detection limit in none of the patients for IL1 $\beta$ , in 8–32% for IL2, IL6, and IL10, and in 83% for TNF $\alpha$ . Levels above the detection limit of the assay used were reported in 29/31 (94%) patients with pSS for IL6<sup>4</sup> and 31/53 (58%) patients with pSS for IL10.<sup>5</sup> The frequency for detectable levels of IL6 (8%) in our study was relatively low. Possible explanations are the different sensitivities of sandwich ELISAs used and differences in pSS populations studied. It has been reported that patients with pSS have higher mean serum levels of IL2,<sup>3</sup> IL6,<sup>4</sup> and IL10<sup>5</sup> than healthy controls, and that mean TNF $\alpha$ <sup>3</sup> levels are similar. However, these studies did not indicate how many patients had undetectable levels.<sup>3–4</sup>

Few studies have investigated the possible association between fatigue and serum cytokine levels. In patients with systemic lupus erythematosus, no association was found between fatigue and circulating levels of cytokines,<sup>15</sup> but in fatigued breast cancer survivors higher levels of serum markers of proinflammatory cytokine activity were found than in non-fatigued survivors.<sup>16</sup> In contrast with our study, these studies did not use multidimensional fatigue assessments, which have been shown to be useful in patients with breast cancer during radiotherapy, where physical and cognitive fatigue were increased, while affective fatigue was

not changed.<sup>17</sup> We found that although patients with pSS are fatigued at all dimensions, levels of serum cytokines were definitely not related to physical dimensions of fatigue. The small number of patients with detectable IL6 and IL10 levels might have reduced the possibility of finding a significant association between levels of these cytokines and reduced motivation.

The absence of an association between serum levels of cytokines and dimensions of fatigue does not necessarily mean that the theory that cytokines are related to fatigue is wrong. The pronounced physiological and behavioural changes noted when cytokines are given to patients<sup>7-9</sup> might be due to the much higher levels of circulating cytokines that are thus induced. Furthermore, levels of circulating, peripherally produced cytokines may not reflect the local situation in the central nervous system as proinflammatory cytokines can induce synthesis and release of cytokines by glial, vascular, and immune cells in the brain itself.<sup>6</sup>

In conclusion, with the exception perhaps of the motivational aspect of fatigue, our findings do not reflect a widespread effect of serum cytokines on multiple aspects of fatigue.

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