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Chronic fatigue syndrome and circulating cytokines: A systematic review

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ABSTRACT

There has been much interest in the role of the immune system in the pathophysiology of chronic fatigue syndrome (CFS), as CFS may develop following an infection and cytokines are known to induce acute sickness behaviour, with similar symptoms to CFS. Using the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) guidelines, a search was conducted on PubMed, Web of Science, Embase and PsycINFO, for CFS related-terms in combination with cytokine-related terms. Cases had to meet established criteria for CFS and be compared with healthy controls. Papers retrieved were assessed for both inclusionary criteria and quality. 38 papers met the inclusionary criteria. The quality of the studies varied. 77 serum or plasma cytokines were measured without immune stimulation. Cases of CFS had significantly elevated concentrations of transforming growth factor-beta (TGF-β) in five out of eight (63%) studies. No other cytokines were present in abnormal concentrations in the majority of studies, although insufficient data were available for some cytokines. Following physical exercise there were no differences in circulating cytokine levels between cases and controls and exercise made no difference to already elevated TGF-β concentrations. The finding of elevated TGF-β concentration, at biologically relevant levels, needs further exploration, but circulating cytokines do not seem to explain the core characteristic of post-exertional fatigue.

1. Introduction

Chronic fatigue syndrome (CFS) is a chronic, disabling disorder that affects 0.3–2.5% of the population, depending on the definition used (Prins et al., 2006). The term CFS is usually thought to encompass what is known as myalgic encephalomyelitis, although some regard these as separate conditions. The diagnosis is made on clinical grounds with alternative diagnoses excluded. Although CFS was only first given a case definition in 1988 (Holmes et al., 1988), there have been several similar syndromes described with different names in the past (Acheson, 1959). Since 1988, there have been a number of operationalised criteria (Brurberg et al., 2014). One of the most commonly used definition is the 1994 Centers for Disease Control (CDC) led definition (Fukuda et al., 1994) revised in 2003 (Reeves et al., 2003), which requires six months of disabling fatigue with four of eight possible accompanying symptoms including: headaches, sleep disturbance, post-exertional malaise, poor concentration, myalgia, joint pain, sore throat and tender lymph nodes.

There are no established biomarkers in CFS, but there has been a long-standing interest in the role of the immune system in CFS pathogenesis and pathophysiology, and the role that cytokines might play (Ur et al., 1992). This focus on the immune system was borne out of several findings. Firstly, CFS may develop following an infection (Hickie et al., 2006). Secondly, the role that cytokines play in acute sickness behaviour is well-described and some symptoms are similar to those of CFS (Dantzer et al., 2008). Finally, the interaction between neuroendocrine and immune systems is well documented (Glaser and Kiecolt-Glaser, 2005) and there is evidence of hypothalamic-pituitary axis down-regulation in CFS (Papadopoulos and Cleare, 2012).

Cytokines are protein mediators of the immune system produced by both leukocytes and non-immune cells. They coordinate appropriate immune responses in infection and inflammation. Abnormal and inappropriate production are seen in many diseases, for example tumour necrosis factor-alpha (now known as TNF) in rheumatoid arthritis (Chauffier et al., 2012) and transforming growth factor (TGF)-β in systemic sclerosis (Lafyatis, 2014). In addition, fatigue is a common disabling symptom in RA (Nikolaus et al., 2013) and is the most common adverse effect of interferon (IFN)-α treatment in hepatitis C (Hauser et al., 2014). Cytokines can be classified dependent upon their role in the inflammatory

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response, pro-inflammatory or anti-inflammatory, or the subset of cells that primarily produce the cytokine, for example Th1 or Th2 subset of T lymphocytes. Although, this classification may appear to be overly simplistic, there are transcriptional regulators directing cells along these largely cytokine determined lineages (Kanhere et al., 2012). The role of cytokines may therefore be in the induction of the pathophysiology of chronic fatigue, via expansion of specific cell subsets or its maintenance following immune activation by an innocuous stimulus.

In addition, cytokines may serve as potential biomarkers that may aid diagnosis, subtyping, monitoring and determining the prognosis of CFS. In rheumatoid arthritis, several biomarkers, including cytokines have been used to create a multi-biomarker disease activity score, which has been shown to significantly correlate with disease activity (Centola et al., 2014). As CFS is a clinical diagnosis, biomarkers could be used as objective markers of disease status.

There have been many studies that focus on the role cytokines may play in CFS and a narrative review has been published (Lorusso et al., 2009). This included comparisons of circulating blood cytokines, assessing the ability of circulating lymphocytes to produce cytokines and gene expression of cytokines. As many of these papers have conflicting results, it is important to carry out a systematic review to assess the available literature. A previous systematic review examined all immune aspects of CFS making it difficult to answer specific questions (Lyall et al., 2003). Nijs et al. (2014) systematically reviewed immune responses to exercise in CFS, finding some evidence of an abnormal immune response, but normal concentrations of cytokines. Here we focus on circulating cytokines and we seek to determine whether a pro-inflammatory circulating cytokine profile exists in patients with CFS in comparison to controls and how this cytokine profile differs from controls following stimulation such as exercise.

2. Method

The PRISMA (the Preferred Reporting Items for Systematic reviews and Meta-analyses) guidelines were used to carry out this systematic review (Moher et al., 2009).

2.1. Search strategy

A search was conducted on three general databases (PubMed, Web of Science and Embase) and one psychiatry specific database (PsycINFO). CFS-related terms used were myalgic encephalomyelitis, post-viral fatigue syndrome, chronic fatigue immune dysfunction syndrome, post-infectious fatigue syndrome, neurasthenia and chronic mononucleosis. Cytokine-related terms included chemokines, interleukin, interferon, tumour necrosis factor, transforming growth factor. CFS-related terms were combined with cytokine related terms by use of the Boolean 'AND'. These terms were chosen following discussion with a bibliometrist, a CFS specialist (PW) and immunologist (MB). A restriction was placed on the date of publication from 01/01/1988, the year that the first diagnostic criteria for CFS were published (Holmes et al., 1988), until 31/3/2015. Only articles published in the English language were retrieved.

2.2. Study selection and criteria for inclusion

The papers retrieved from the initial search of the four databases were then checked for duplicates. The remaining papers were assessed in three rounds: titles, abstracts and then full texts. Titles were screened to include papers that firstly had any term relating to CFS (as given in the search terms in initial search), secondly any term relating to the immune system, thirdly any animal studies were excluded, and finally, any papers that were designated as a review or meeting abstract were also excluded. Abstracts were then screened to determine whether patients with CFS were included in the study, whether controls could be identified and finally where cytokines may have been measured. Finally, full texts were screened to ensure they met the inclusionary criteria and no exclusions. The inclusionary criteria were CFS defined by established operational criteria (as a measure of the quality of clinical assessments), case-control comparisons, healthy controls, human studies, serum/plasma cytokines and published in the English language. Because we were interested in whether cytokines were involved in the pathophysiological explanations of symptoms, we only included studies of cytokines measured in vivo, and excluded those that only measured cytokines following *in vitro* immune stimulation. Any studies that duplicated previously published data were excluded. The references of the included papers and reviews were hand searched to identify any other papers that might have been relevant to the systematic review.

2.3. Data extraction

Data extracted from each paper that met the inclusion criteria included: the year study was published, the number of patients and controls, proportions of males and females, the CFS definition used, matching of controls, comorbidities excluded, drugs excluded/included, smoking status, number and nature of the cytokines measured, assays used, sample handling, and differences between case and controls for each cytokine. The data were collated into an Excel spreadsheet. Data extraction was done independently by two authors, each masked to the other's work. The results were compared and any discrepancies were discussed and consensually resolved. In the circumstance where differences could not be resolved a third opinion would have been sought, but proved unnecessary.

2.4. Assessment of quality

The Newcastle-Ottawa scale (NOS) (Wells et al.) of quality was used to assess the quality of the studies included and to identify biases within studies. The NOS uses a star system to assess case-control (or cohort) studies on three broad perspectives: the selection of the study groups, the comparability of the groups and the ascertainment of outcome of interest (see Supplementary item 1). The NOS manual was used to guide this process and a full explanation is given in Appendix 1. Two authors assessed quality independently, masked to each other's work. A third author determined final scores when discrepancies were not resolved following re-review of initial scores.

In order to interpret differences and draw conclusions we decided a priori that a minimum of five studies was required to draw a reasonable conclusion from the results. Cytokines were classified by their main role in the inflammatory process: pro- versus anti-inflammatory.

3. Results

3.1. Search and study selection

The initial search retrieved 412 papers from PubMed, 607 papers from Embase, 666 papers from Web of Science and 63 papers from PsycINFO. Once duplicates were removed, papers were subjected to the screening process to identify papers suitable for the systematic review. Fig. 1 shows the number of papers excluded at each round of screening and the reasons for their

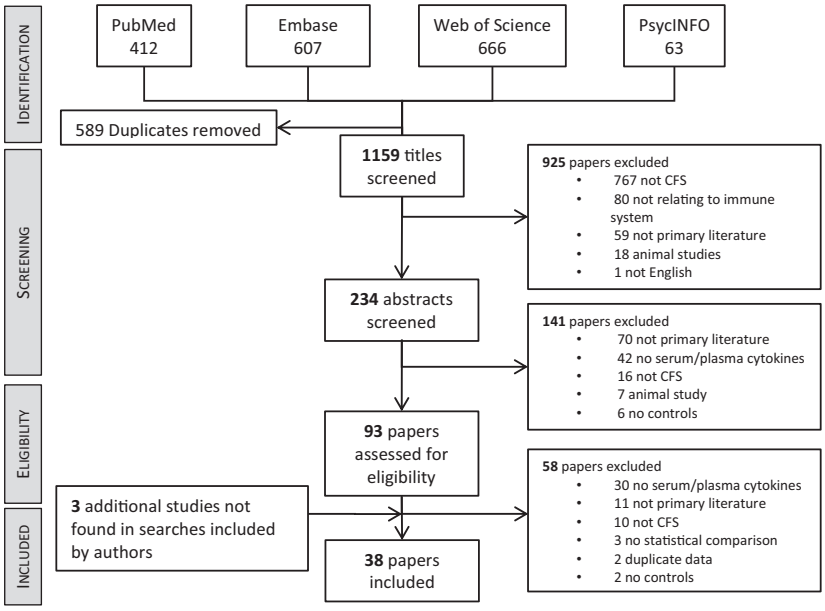

Fig. 1. Flow diagram of the flow of information through the stages of the systematic review.

exclusion. 93 full texts were assessed for eligibility for inclusion. 58 papers were excluded. The reasons that the 58 papers failed to be eligible are shown in Fig. 1. The most common reason for non-inclusion was the lack of patients with CFS in the paper, which included studies that failed to define their patients with CFS by established diagnostic criteria. Hand searching through both the papers included and reviews did not reveal any additional studies. However, authors' knowledge of other studies involving cytokine measurement in CFS provided an additional three studies (Garcia et al., 2014; Hornig et al., 2015; Nakatomi et al., 2014).

3.2. Study characteristics

There were a total of 38 papers that fulfilled our inclusionary criteria and did not meet the exclusionary criteria (Table 1 and Supplementary item 2). These were published between 1989 and 2015. The number of cases ranged from 9 to 298 cases, using a range of case definitions. Fifteen studies explicitly stated that psychiatric disorders were excluded (for full details, Supplementary Fig. 5). In spite of using the CDC 1994 definition (Fukuda et al., 1994), 10 studies did not explicitly state that they excluded a comorbid psychiatric disorder. In 12 studies there was no matching of controls to cases (Buchwald et al., 1997; Garcia et al., 2014; Khaiboullina et al., 2014; Linde et al., 1992; Maes et al., 2013, 2012; Neu et al., 2014; Patarca et al., 1994; Smylie et al., 2013; Spence et al., 2008; White et al., 2010; Wyller et al., 2015). The factors that were matched including age, activity level, gender, body mass index, ethnicity, neighbourhood and menstrual cycle stage. Table 1 gives a summary of the results of the studies included.

3.3. Quality assessment

There was a range of quality scores ranging from two to nine out of a maximum score of ten. In terms of selection, all 38 studies used a case definition of CFS, as this was an inclusionary criterion. However, only 17 studies explained how the diagnosis was confirmed (Table 2). Only 11 studies were considered to adequately represent the CFS population and be free from selection bias. Reasons for failure to represent the CFS population included all female cohorts (Broderick et al., 2012; Fletcher et al., 2009; Nakamura et al., 2013; Scully et al., 2010) all male cohorts (Lloyd et al., 1994), adolescent/children cohorts (Wyller et al., 2015) or cohorts where there were more males than females (Jammes et al., 2009; Smylie et al., 2013). There was a selection bias when patients were only recruited from one source (Bennett et al., 1997; Buchwald et al., 1997; Cannon et al., 1999; Maes et al., 2013, 2012; Nakatomi et al., 2014; Nas et al., 2011; Peterson et al., 1994). One of these studies required patients to fund their own tests (Maes et al., 2012). Seven papers did not state how patients were selected and therefore were unable to score positively for representation (Chao et al., 1991; Kennedy et al., 2004; Neu et al., 2014; Nijs et al., 2009; Patarca et al., 1994; Straus et al., 1989; White et al., 2010, 2004).

In terms of control selection, 15 studies recruited from the same community as cases (Table 2). Three studies recruited controls from the hospital setting, including outpatients (Jammes et al., 2009; Nas et al., 2011; Smylie et al., 2013). It was unclear whether control communities were the same as case communities in five studies (Bennett et al., 1997; Fletcher et al., 2009; Lloyd et al., 1994; Maes et al., 2012; Patarca et al., 1994) and there was no description of where controls were recruited from in 13 studies (Cannon et al., 1999; Cheney et al., 1989; Kennedy et al., 2004; Khaiboullina et al., 2014; Lattie et al., 2012; Linde et al., 1992; Nakamura et al., 2010; Nakatomi et al., 2014; Neu et al., 2014; Robinson et al., 2010; Scully et al., 2010; Straus et al., 1989; White et al., 2010). The definition of controls was the most poorly reported with only five studies explicitly stating that the controls had never had a history of CFS. Few studies ensured that their case and control populations were comparable. Only six studies controlled for activity level (Table 2). 13 studies excluded psychiatric illness that could affect cytokine levels, such as depression (Supplementary item 2).

Studies scored well with regards to outcome measures of cytokine concentrations. Only one study did not publish the exact concentrations of cytokines (Visser et al., 2001). Of the 38 studies only two stated that they blinded their laboratory staff to the samples when carrying out the assays (Hornig et al., 2015; MacDonald et al., 1996). All of the studies used the same methods to measure cytokines in both cases and controls. 22 papers scored for adequate

Table 1
Study characteristics of the studies included.

	Cases		Controls		Study serum/plasma cytokine results		
	Number	CFS definition	Number	Matching	Higher in CFS subjects	Lower in CFS subjects	No significant difference
Khaiboullina et al. (2014)	67	CDC 1994 or Carruthers	42	Not mentioned	CCL1, CCL2, CCL20, CCL3, CXCL10, IFNγ, IL-1, IL-10, IL-13, IL-1β, IL-25, IL-31, IL-4, IL-6, IL-7, IL12 (p75), TNF	CCL11, CCL17, CCL19, CCL21, CCL25, CCL26, CCL3, CCL4, CCL5, CCL8, CSF1, CSF3, CX3CL1, CXCL1, CXCL13, CXCL6, CXCL8, HGF, IL-17F, IL-5, IL-9, LIF, MIF, PDGF, TRAIL, VEGF	CCL13, CCL22, CCL23, CCL24, CCL27, CCL7, CXCL11, CXCL12a, CXCL12ab, CXCL13, CXCL16, CXCL2, CXCL5, CXCL9, FGF, GM-CSF, IFN-α, IL-12 (p40), IL-15, IL-16, IL-17A, IL-18, IL-1RA, IL-1α, IL-1β, IL-2, IL-21, IL-22, IL-23, IL-3, IL-33, IL-6, IL-2RA, LIF, sCD40L, SCF, SCGF-β, TNF-β*, β-NGF
Wyller et al. (2015)	87	CDC 1994	68	No			IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7. IL-8, IL-9, IL-10, IL-12, IL-13, IL-17, IFN-γ, CCL2, CCL3, CCL4, CCL5, CXCL10, PDGF-BB, VEGF, FGF, TNF
Hornig et al. (2015)	298	CDC 1994 or Carruthers	348	Age, gender, geography, ethnicity, season of sampling,	Leptin	Il-6, IL-8, IL-10, LT-α, IL-17A, sFasL, CXCL10, MCSF,	TGF-β, IL-1β, IL-1α, TNF, IFN-α, IL-2, IL-12, IFN-γ, IL-4, IL-13, IL-5, IL-15, IL-7, IL-13, IL-9, GMCSF, LIF, CD40L, TRAIL, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CXCL1, CXCL5, CXCL9, PDGF-BB, VEGFA, sICAM-1, VCAM-1, TGF-α, FGFβ, βNGF, HGF, SCF, GCSF,
Neu et al. (2014)	16	CDC 1994	14	Not mentioned	IL-1β, TNF, IL-8, IL-10	IL-6, IFNγ	
Nakatomi et al. (2014)	9	CDC 1994 and Carruthers	10	Age, gender			IL-1β, IL-6, TNF, IFN-γ
Garcia et al. (2014)	28	CDC 1994	96	No	IL-6, IL-2, IL-12, IFN-γ, GMCSF, CXCL10		IL-1β, IL-1α, IL-6, TNF, IL-8, IL-10, IFN-α, IL-4, LT-α, IL-5, IL-7, CCL2, CCL3, CCL4,
Nakamura et al. (2013)	26 F	CDC 1994	24 F	Sex			IL-1β, IL-6, TNF, IL-8, IL-10,IL-4
Maes et al. (2013)	115	CDC 1994	35	No	IL-1β, IL-1α, IFN-α		
Lattie et al. (2012)	117	CDC 1994	95	Age, gender	IL-1β, IL-6		TNF, IL-10, IL-2
Smylie et al. (2013)	22	CDC 1994	30	Sedentary controls	*Males*: IL-2, IL-23		*Females*: IL-1β, IL-1α, IL-6, TNF, IL-8, IL-10, IL-2, IL-12, IFN-γ, IL-4, IL-13, TNF-β, IL-5, IL-23, IL-17, IL-15 *Males*: IL-1β, IL-1α, IL-6, TNF, IL-8, IL-10, IL-12, IFN-γ, IL-4, IL-13, TNF-β*, IL-5, IL-17, IL-15
Broderick et al. (2012)	9 F	Reeves et al. and Jason et al.	12F	Age tanner stage	IL-8	IL-23	IL-1β, IL-1α, IL-6, TNF, IL-10, IFN-α, IL-2, IL-12, IFN-γ, IL-4, IL-13, TNF-β*, IL-5, IL-17, IL-15
Maes et al. (2012)	107	CDC 1994	20	No	IL-1β, TNF		
Nas et al. (2011)	25	CDC 1994	20	Age, gender	IL-6		IL-8
White et al. (2010)	19	CDC 1994	17	No			IL-1β, IL-6, TNF, IL-8, IL-10, IL-2, IL-12, IFN-γ, IL-4, IL-13
Nakamura et al. (2010)	20 F	CDC 1994	19 F	Age, BMI, gender			IL-1β, IL-6, TNF, IL-8, IL-10, IL-4
Nijs et al. (2009)	22 F	CDC 1994	22F	Gender, sedentary controls			IL-1β (not detected)
Robinson et al. (2010)	33	CDC 1994	33	Age, BMI, gender, sedentary controls			IL-6
Scully et al. (2010)	35 F	CDC 1994	54F	Age, gender	IL-1β, IL-6, IL-8		TNF, IL-10, IFN-γ, IL-12p70, IL-13
Fletcher et al. (2009)	40 F		59F	gender	IL-1β, IL-1α, IL-6, IL-12, IL-4, IL-5,	IL-8, TNF-β*, IL-15	TNF, IL-10, IL-2, IFN-γ, IL-13, IL-23, IL-17
Jammes et al. (2009)	18	CDC 1994	9	Age, gender, weight, ethnicity, sedentary controls			IL-6, TNF
Nater et al. (2008)	28	CDC 1994	39	Age, BMI, gender, ethnicity			Il-6
Spence et al. (2008)	41	CDC 1994	30	No			IL-1β, TNF
Vollmer-Conna et al. (2007)	22	CDC 1994	42	Age			IL-1β, IL-2, IL-6, IL-10, IL-12, TNF, IFN-γ (below detection limit)
Kennedy et al. (2004)	47	CDC 1994	34	Age, gender	TGF-β		
White et al. (2004)	9	CDC 1994	9	Age, gender, exercise	TGF-β		IL-α, TNF,

(*continued on next page*)

Table 1 (*continued*)

	Cases		Controls		Study serum/plasma cytokine results		
	Number	CFS definition	Number	Matching	Higher in CFS subjects	Lower in CFS subjects	No significant difference
Visser et al. (2001)	59	CDC 1994	56	Age, gender, ethnicity			TNF, IL-10, IL-12 (not detectable), IFN-γ
Cannon et al. (1999)	10 F	CDC 1988	11F	Age, gender, activity			IL-6
Buchwald et al. (1997)	98	CDC and CDC	19	No			IL-6
Bennett et al. (1997)	93	CDC 1988	80	Age, gender	TGF-β		
MacDonald et al. (1996)	47	CDC 1988	47	Age, gender, neighbourhood			TGF-β, IL-1β, IL-6, TNF
Swanink et al. (1996)	76	Sharpe 1991	69	Age, gender, neighbourhood			TGF-β, IL-1β, IL-1α, TNF
Peterson et al. (1994)	10	CDC 1988	10	Age	TGF-β		IL-1β (not detectable), IL-6 (not detectable), TNF (not detectable)
Patarca et al. (1994)	70	CDC 1988	53	No	TNF		IL-1β, IL-1α, IL-6, IL-2, IL-4
Lloyd et al. (1994)	12 M	Lloyd	13 M	Age, weight, height, training status			IL-1β, TNF, IFN-α, IFN-γ
Linde et al. (1992)	35	CDC 1988	20	No	IL-1α,		IL-1β, IL-6, IFN-γ
Chao et al. (1991)	10	CDC 1988	10	Age, gender	TGF-β		IL-1β, IL-6, TNF, IL-2 (not detectable), IL-4 (not detectable)
Straus et al. (1989)	25	CDC 1988	25	Age, gender			IL-1β, TNF, IFN-α, IL-2, IFN-γ
Cheney et al. (1989)	104	CDC 1988	22	Age, gender	IL-2		

TNF-β is now known as lymphotoxin-α. βNGF (β-nerve growth factor), CC chemokines (CCL2, CCL3, CCL4, CCL5, CCL7, CC11), CXC chemokines (CXCL1, CXCL5, CXCL8, CXCL9, CXCL10), G-CSF (granulocyte colony-stimulating factor), HGF (hepatocyte growth factor), M-CSF (macrophage colony-stimulating factor), PDGF-BB (platelet-derived growth factor-BB), SCF (stem cell factor), sICAM-1 (soluble intercellular adhesion molecule–1), TRAIL (Tumour necrosis factor-related apoptosis-inducing ligand), VCAM-1 (vascular cell adhesion molecule–1), VEGF (vascular endothelial growth factor), FGFb (fibroblast growth factor, basic).

non-response rates. Failure to score this point was most commonly due to not stating how many of the cases and controls that were included in the study also had cytokine concentrations measured (Bennett et al., 1997; Cannon et al., 1999; Jammes et al., 2009; Kennedy et al., 2004; Lloyd et al., 1994; Nater et al., 2008; Spence et al., 2008; Swanink et al., 1996; Visser et al., 2001) or differences between the proportions of cases and controls who had their cytokine levels measured (compared to the total number included in the study) (Buchwald et al., 1997; Linde et al., 1992; Patarca et al., 1994).

3.4. Cytokines assayed

The 38 studies measured between one and 77 serum or plasma cytokines. With the exception of transforming growth factor-beta (TGF-β), the large majority of the studies showed no statistically significant differences between case and controls. At baseline, i.e. at rest, 17 cytokines were measured in five or more studies (Table 3). Of these cytokines, the pro-inflammatory cytokines interleukin-1 beta (IL-1β), TNF and interleukin-6 (IL-6) were most frequently measured, which is likely to reflect the hypothesis that pro-inflammatory cytokines contribute to the symptoms of CFS. There were no significant differences between cases and controls in 75%, 71% and 80% of the studies measuring IL-1β, IL-6 and TNF, respectively (Table 3).

The three pro-inflammatory cytokines IL-1β, TNF and IL-6 were the only cytokines that were measured in more than five studies after exercise (Table 4). For these three cytokines the majority of the studies showed no significant difference between cases and controls. Five out of eight (63%) studies measuring TGF-beta showed significantly increased concentrations of TGF-β in cases compared to controls (Table 5). These studies were published between 1991 and 2015. Four used immuno-assays and four used the HT-2 murine cell proliferation bio-assay. Of the three that did not show any difference in TGF-β levels, two used an immune-assay and one used a bio-assay.

3.5. Sample handling

Timing of sampling and sample handling is important when measuring plasma or serum cytokines, ensuring that samples are taken at the same time of the day due to the diurnal variation of cytokine levels. Once samples are taken, it is important that the plasma or serum is separated as soon as possible, as *in vitro* cytokine production may occur affecting results. Thirteen studies stated the time that samples were taken at the same time of day with 10 ensuring samples were taken in the morning, one in the evening and two studies stating only that the samples were taken at the same time of the day (Table 6). Fourteen studies stated the time from sample collection to the point of separation of serum or plasma. Six studies separated serum/plasma immediately, two studies within 30 min, five studies within 2 h and one study within four hours (full details in Table 6). Two studies flash/snap froze samples, which can considered the ideal method of sample handling (Nakamura et al., 2010; White et al., 2010).

4. Discussion

In this article we have systematically summarised the available data for circulating cytokine concentrations in CFS subjects in comparison to healthy controls. We found little or no evidence to support the hypothesis that pro-inflammatory circulating cytokines are raised in CFS. The pro-inflammatory cytokines IL-1β, TNF and IL-6 were most frequently studied and the results demonstrate that they were not raised in subjects with CFS in comparison to controls (range of proportions of negative studies between 71% and 100%), either at rest or after exercise. In contrast, five out of eight studies showed a significant elevation in circulating TGF-β at rest.

Table 2
Quality assessment of included studies based upon NOS in Supplementary item 1.

Author	Selection				Comparability		Exposure			Total
	1	2	3	4	1	2	1	2	3	
MacDonald et al. (1996)	*	*	*	*	–	*	**	*	*	9
Nater et al. (2008)	*	*	*	*	–	*	*	*	–	7
White et al. (2004)	–	*	*	–	*	*	*	*	*	7
Hornig et al. (2015)	*	*	*	*	–	–	*	*	*	7
Peterson et al. (1994)	*	–	*	–	–	*	*	*	*	6
Maes et al. (2013)	–	–	*	*	–	*	*	*	*	6
Broderick et al. (2012)	*	–	*	*	–	–	*	*	*	6
Lattie et al. (2012)	*	*	–	–	–	–	*	*	*	5
Nakamura et al. (2013)	*	–	*	–	–	–	*	*	*	5
Maes et al. (2012)	–	–	–	*	–	*	*	*	*	5
Nas et al. (2011)	*	–	–	–	–	*	*	*	*	5
Nakamura et al. (2010)	–	*	–	–	–	*	*	*	*	5
Vollmer-Conna et al. (2007)	–	*	*	–	–	–	*	*	*	5
Chao et al. (1991)	*	–	*	–	–	–	*	*	*	5
Khaiboullina et al. (2014)	*	*	–	–	–	–	*	–	*	4
Wyller et al. (2015)	*	–	*	–	–	–	*	–	*	4
Neu et al. (2014)	*	–	–	–	–	*	*	–	*	4
Nakatomi et al. (2014)	*	–	–	–	–	*	*	–	*	4
Spence et al. (2008)	*	–	*	–	–	–	*	*	–	4
Smylie et al. (2013)	–	–	–	–	*	–	*	*	*	4
Swanink et al. (1996)	–	*	*	–	–	–	*	*	–	4
Nijs et al. (2009)	–	–	*	–	*	–	*	*	–	4
Robinson et al. (2010)	–	*	–	–	*	–	*	*	–	4
Fletcher et al. (2009)	–	–	–	–	–	*	*	*	*	4
Jammes et al. (2009)	*	–	–	–	*	–	*	*	–	4
Visser et al. (2001)	–	*	*	–	–	*	–	*	–	4
Garcia et al. (2014)	*	–	–	–	–	–	*	–	*	3
Cheney et al. (1989)	–	–	–	–	–	–	*	*	*	3
Buchwald et al. (1997)	*	–	–	–	–	–	*	*	–	3
White et al. (2010)	–	–	–	–	–	–	*	*	*	3
Scully et al. (2010)	–	–	–	–	–	–	*	*	*	3
Cannon et al. (1999)	–	–	–	–	*	–	*	*	–	3
Straus et al. (1989)	–	–	–	–	–	–	*	*	*	3
Lloyd et al. (1994)	–	–	–	–	*	–	*	*	–	3
Kennedy et al. (2004)	–	–	–	–	–	–	*	*	–	2
Bennett et al. (1997)	–	–	–	–	–	–	*	*	–	2
Patarca et al. (1994)	–	–	–	–	–	–	*	*	–	2
Linde et al. (1992)	–	–	–	–	–	–	*	*	–	2

The quality assessment showed that half of the studies would be considered poor quality (score 4 or less). Many of the studies failed to account for confounding factors that can affect cytokine concentrations, such as age (Alvarez-Rodriguez et al., 2012), activity level (Shephard, 2002), gender, BMI (Ouchi et al., 2011), stage in the menstrual cycle (Al-Harthi et al., 2000) and co-morbid diseases including psychiatric disorders (Felger and Lotrich, 2013). Anti-depressants have also been shown to affect cytokine levels; serotonin reuptake inhibitors (SSRIs and SNRIs) can increase IL-6, and serotonin agonists can increase the production of IL-6 and TNF (Kubera et al., 2004, 2005). There is a diurnal variation in cytokine levels (Al-Harthi et al., 2000), and therefore it is important to sample cytokines at the same time in the day for both cases and controls. Twenty-five of the studies' patient groups were not representative of CFS patient groups, due to being all of one gender or another selection bias. Sample sizes were often small with five studies containing 10 or fewer patients. Given that the CFS patient population is heterogeneous, large sample groups are important in order to demonstrate an association. Different assays were used across studies. This reflects changed assays used over time and the use of different suppliers across continents. As a result, we were unable to do a meta-analysis of the results.

We reduced source selection bias through the use of four databases, with the addition of hand-searching through gathered references and recent reviews. There was a risk of recruitment bias as a result of recruitment from tertiary care, particularly when the

Table 3
Summary of study results for cytokines at baseline/resting.

Cytokine		Higher in CFS subjects N studies (%)	Lower in CFS subjects N studies (%)	No difference N studies (%)
Mixed pro/anti	TGF-β	5 (63%)	0 (0%)	3 (38%)
Pro-inflammatory	IL-1β	7 (25%)	0 (0%)	21 (75%)
	TNF	5 (20%)	0 (0%)	23 (80%)
	IL-6	6 (21%)	2 (7%)	20 (71%)
	IL-1α	3 (27%)	0 (0%)	8 (73%)
	LT-α	2 (33%)	1 (17%)	3 (50%)
	INF-α	1 (20%)	0 (0%)	4 (80%)
Anti-inflammatory	IL-10	2 (13%)	1 (6%)	13 (81%)
	IL-13	1 (9%)	2 (18%)	8 (73%)
Th1	IL-2	3 (20%)	3 (20%)	9 (60%)
	IL-12	2 (18%)	0 (0%)	9 (82%)
	IFN-γ	2 (12%)	1 (6%)	14 (82%)
	IL-15	0 (0%)	1 (20%)	4 (80%)
Th2	IL-5	1 (13%)	1 (13%)	6 (75%)
	IL-4	2 (15%)	0 (0%)	11 (85%)
Th17	IL-23	1 (25%)	1 (25%)	2 (50%)
	IL17	0 (0%)	2 (25%)	6 (75%)
NK-cell attracting	IL-8	4 (29%)	2 (14%)	8 (57%)

Cytokines shown when four or more studies measured them; less frequently studied cytokines shown in Table 1.

Table 4
Summary of study results for serum/plasma cytokines following exercise.

Cytokine		Higher in CFS subjects N studies (%)		Lower in CFS subjects N studies (%)		No difference N studies (%)		Total
Mixed pro/anti	TGF-β	2	100%	0	0%	0	0%	2
Pro-inflammatory	TNF	1	14%	1	14%	5	71%	7
	IL-6	0	0%	0	0%	7	100%	7
	IL-1β	0	0%	0	0%	6	100%	6
	IL-1α	0	0%	0	0%	3	100%	3
	LT-α	0	0%	0	0%	2	100%	2
	IL-13	0	0%	0	0%	2	100%	2
Anti-inflammatory	INF-α	0	0%	0	0%	1	100%	1
	IL-10	0	0%	0	0%	3	100%	3
Th1	IL-2	2	100%	0	0%	0	0%	2
	IFN-γ	0	0%	0	0%	3	100%	3
	IL-12	0	0%	0	0%	2	100%	2
	IL-15	0	0%	0	0%	2	100%	2
Th2	IL-5	0	0%	0	0%	2	100%	2
	IL-4	0	0%	1	100%	0	0%	1
Th17	IL-23	1	50%	0	0%	1	50%	2
	IL17	0	0%	0	0%	2	100%	2
NK-cell attracting	IL-8	0	0%	0	0%	3	100%	3

patients were recruited from a single speciality clinic, although there are some studies suggesting the risk is low (White et al., 2002). Population-based recruitment are the gold standard method to avoid such biases (Raison et al., 2009). In addition, patients too unwell to attend the clinics would not be represented in such studies. As with all scientific literature, there is likely to be an associated reporting bias, a tendency to report the positive finding, and a failure to focus on the negative findings.

Our findings are consistent with those of Nijs et al. (2014). The negative findings for pro-inflammatory cytokines may be explained by two possible reasons. Firstly, CFS patients are a heterogeneous group (Vollmer-Conna et al., 2006; Wilson et al., 2001), and at different points in the trajectory of their illness. Our lack of understanding of these groups may limit our ability to identify the group/groups of patients where cytokines and the immune system may play a central role in disease pathogenesis (Hornig et al., 2015). Secondly, cytokines are released by immune cells in small amounts and have a broad range of paracrine and even autocrine effects (Lafyatis, 2014; Wahl, 2007). Therefore, we may not be able to detect clinically important concentrations of circulating cytokines, as the majority of their effects are likely to be local. Chronic pain illustrates how cytokines can act locally to modulate synaptic transmission (Ji et al., 2013). In an adult rat model of post-nerve injury neuropathic pain-like hypersensitivity, expression of IFN-γ in the dorsal horn of the spinal cord was upregulated and prerequisite for the development of hypersensitivity (Costigan et al., 2009). Cytokines levels have rarely been measured in the cerebrospinal fluid of CFS subjects (Lloyd et al., 1991; Natelson et al., 2005). Finally, it is likely that cytokines are one factor in a complex network. For example, TNF blockade in rheumatoid arthritis only has a small effect on fatigue in comparison to placebo (Chauffier et al., 2012), suggesting that other aspects need to be considered. One aspect could be genetic polymorphisms; functional polymorphisms of IFNγ and IL-10 affect not only cytokine production but also symptom severity and illness duration of an acute infection (Vollmer-Conna et al., 2008).

With regards to TGF-β, almost all studies were published before 2004. This may reflect the subsequent increased use of multiplex enzyme-linked immunosorbent assay (ELISA) systems, which usually exclude TGF-β. In the included studies, TGF-β was measured using two different types of assays: an older murine Helper T-cell (HT)-2 cell proliferation bioassay and an ELISA. The studies' methodologies not only differed in the choice of assay but also the blood component in which TGF-β was measured: plasma or serum. TGF-β is released from activated platelets (Lafyatis, 2014; Wahl, 2007) and therefore TGF-β measured in serum will also indirectly assess platelet production, as platelets are activated when the blood coagulates to produce serum. In addition, the

Table 5
Studies measuring TGF-β.

Author	Cases/controls (N)	Difference between cases/controls	Assay	Sample	Mean TGF-β value (unless stated otherwise)	TGF-β subtype
Hornig et al. (2015)	298/348	No difference	Immunoassay	Plasma	Cases 36.3 Controls 38.8	All TGFβ
Kennedy et al. (2004)	47/34	Higher in cases	Immunoassay	Platelet poor plasma	Cases 2.4 pg/ml Controls 1.89	TGF-β1
White et al. (2004)	9/9	Higher in cases	Immunoassay	Plasma	Cases 904 pg/ml (median) Controls 50	TGF-β1
Bennett et al. (1997)	93/80	Higher in cases	Bioassay	Serum	Cases 254 pg/ml Controls 149.6 pg/ml	All TGF-β – measured TGF-β bioactivity
MacDonald et al. (1996)	47/47	No difference	Immunoassay	Unclear methods	Cases 47.7 pg/ml, Controls 31.1 pg/ml	?
Swanink et al. (1996)	76/69	No difference; active TGF-β not detectable	Bioassay	Serum	Cases 1.25 ng/ml Controls – 1.2 ng/ml	Total TGF-β and active TGF-β
Peterson et al. (1994)	10/10	Higher in cases	Bioassay	Serum	Cases 287 pg/ml Controls 115 pg/ml	TGF-β all
Chao et al. (1991)	10/10	Higher in cases	Bioassay	Serum	Cases 290 pg/ml, Controls 104 pg/ml	TGF-β all

Table 6
Blood sample handing in the 38 included studies.

Study	Time samples taken	Time to plasma/serum extraction	Storage
Hornig et al. (2015)	Samples collected 10am – 2 pm	No mention	Stored at −80 °C
Khaiboullina et al. (2014)	No mention	Serum separated immediately	Stored at −80 °C
Wyller et al. (2015)	No mention	Placed on ice and centrifuged within 30 min	Stored at −80 °C
Garcia et al. (2014)	No mention	No mention	No mention
Nakatomi et al. (2014)	No mention	Serum extracted within 30 min	Stored at −20 °C
Neu et al. (2014)	No mention	No mention	Stored at −20 °C
Lattie et al. (2012)	No mention	Centrifuged within 4 h	
Maes et al. (2013)	Samples collected 8.30am –11.30am	No mention	No mention
Nakamura et al. (2013)	No mention	No mention	No mention
Smylie et al. (2013)	No mention	Centrifuged within 2 h	
Broderick et al. (2012)	Morning fasting samples	Centrifuged within 2 h	Stored at −80 °C
Maes et al. (2012)	Samples collected 8.30am–11.30am	No mention	No mention
Nas et al. (2011)	No mention	No mention	No mention
Nakamura et al. (2010)	Samples taken at night	Centrifuged immediately	Snap frozen on dry ice and stored at −80 °C
Nijs et al. (2009)	No mention	No mention	No mention
Robinson et al. (2010)	No mention	Centrifuged immediately	Stored at −80 °C
Scully et al. (2010)	No mention	Centrifuged immediately	Stored at −80 °C
White et al. (2010)	No mention	Centrifuged immediately	Flash frozen within 18 min and stored at −80 °C
Fletcher et al. (2009)	Morning sample	Centrifuged within 2 h	Stored at −80 °C
Jammes et al. (2009)	No mention	No mention	No mention
Nater et al. (2008)	Morning sample	No mention	Stored at −80 °C
Spence et al. (2008)	Same time of day	No mention	Stored at −70 °C
Vollmer-Conna et al. (2007)	No mention	No mention	No mention
Kennedy et al. (2004)	Same time of day	No mention	No mention
White et al. (2004)	No mention	No mention	No mention
Visser et al. (2001)	Samples collected 7am–10am	No mention	No mention
Cannon et al. (1999)	No mention	No mention	No mention
Bennett et al. (1997)	No mention	No mention	Stored at −20 °C
Buchwald et al. (1997)	No mention	No mention	No mention
MacDonald et al. (1996)	Samples collected 7am–10am	No mention	No mention
Swanink et al. (1996)	Samples collected 8.30am–11.00am	Centrifuged immediately	No mention
Lloyd et al. (1994)	No mention	Clotted and frozen within 2 h	Stored at −70 °C
Patarca et al. (1994)	Samples collected 7.30–10.30am	No mention	Stored at −20 °C
Peterson et al. (1994)	No mention	Centrifuged within 2 h	Stored at −70 °C
Linde et al. (1992)	No mention	No mention	No mention
Chao et al. (1991)	Samples collected 8am–9am	No mention	Stored at −20 °C
Cheney et al. (1989)	No mention	No mention	No mention
Straus et al. (1989)	No mention	No mention	Stored at −20 °C

heterogeneous nature of CFS was possibly reflected in one study. Peterson et al., 1994, found a significant increase in serum TGF-β in CFS subjects in comparison to controls both before and after exercise. When the individual patient TGF-β levels were examined, it was clear that two outliers accounted for this difference in the pre-exercise group and three outliers in the post-exercise group of the 10 CFS subjects. This suggests that TGF-β may be a potential biomarker for phenotyping CFS subjects into sub-groups.

TGFβ is part of a superfamily of cytokines. It is secreted as a latent complex, requiring proteolytic cleavage to the active TGF-β (Wahl, 2007). There are 3 isoforms (TGF-β1, TGF-β2 and TGF-β3), all of which interact with the same receptor but have different roles, as demonstrated by knockout-mice and in human pathology. TGF-β has a broad range of activities and therefore is involved in a broad range of diseases. These roles include cellular proliferation, where it is dysregulated in cancer (Flavell et al., 2010), immune response/inflammation, embryogenesis, normal vascular integrity and extracellular matrix deposition which is abnormal in fibrotic disease such as pulmonary fibrosis and systemic sclerosis (Lafyatis, 2014).

The possible biological significance of a raised TGF-β in CFS is unknown, but comparison with concentrations found in other conditions may be instructive. The mean/median elevations of TFG-β observed in all 5 "positive" papers were greater than 30 pg/ml and some were reported as high as 1 ng/ml (Table 5). In cancer studies, serum levels of TGF-β greater than 30 pg/ml are associated with more aggressive disease and at 80 pg/ml has prognostic significance (Papadopoulou et al., 2008). In juvenile diabetes, elevations of TGF-β above 400 pg/ml are associated with increased risk of proliferative retinopathy (Zorena et al., 2013). In inflammatory bowel disease, where over expression of SMAD7 signalling is hypothesised (Feagins, 2010), elevations of TGF-β as high as 2 ng/ml are observed in untreated ulcerative colitis (Sambuelli et al., 2000). In advanced Alzheimer's disease significantly elevated serum and CSF TGF-β were observed in advanced disease (circa 50 pg/ml)(Chao et al., 1994). The observations therefore seem to be consistent with a biologically significant elevation in the cytokine concentration in CFS. The clinical significance of a raised TGF-β is unclear in CFS, but its associations with clinical symptoms, severity and prognosis merits further evaluation.

As an immune mediator, TGF-β has both pro- and anti-inflammatory effects. The intracellular signal transduction, following engagement of the TGF-β receptor, depends upon the cell type and context in which the cell is activated. Naïve or newly recruited leukocytes will be activated, whereas TGF-β will have an inhibitory effect on activated leukocytes. The complex nature of TGF-β makes it difficult to understand how and where it would have its effect in CFS. However, the central nervous system may be a starting point. In murine models exogenous TGF-β is associated with alterations in cerebral blood flow (Gaertner et al., 2005). In a sedentary mouse model Inoue et al. (1999) showed that intracranial injection of TGF-β depressed motor activity in a dose dependent manner and that increased exercise load in rats was associated with increased TGF-β levels in the cerebrospinal fluid (Inoue et al., 1999).

Future research should include studies of cytokines in different body fluids, such as cerebrospinal fluid, as well as sufficiently large studies to enable testing associations between certain cytokines

and sub-groups of CFS. We also need to know the relationship between circulating TGF-β and condition status (before and after remission), severity, and possible confounders, such as medication and comorbid conditions.

5. Conclusion

Our systematic review has highlighted the limited quality of published studies. It has also demonstrated that there is insufficient evidence to support a role for circulating cytokines in CFS, with the exception of TGF-β, which needs further exploration.

Conflict of interest

PDW does consultancy work for the UK government and a re-insurance company. The other authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2015.07.004.

References

Acheson, E.D., 1959. The clinical syndrome variously called benign myalgic encephalomyelitis, Iceland disease and epidemic neuromyasthenia. Am. J. Med. 26, 569–595.
Al-Harthi, L., Wright, D.J., Anderson, D., Cohen, M., Matity Ahu, D., Cohn, J., Cu-Unvin, S., Burns, D., Reichelderfer, P., Lewis, S., Beckner, S., Kovacs, A., Landay, A., 2000. The impact of the ovulatory cycle on cytokine production: evaluation of systemic, cervicovaginal, and salivary compartments. J. Interferon Cytokine Res. 20, 719–724.
Alvarez-Rodriguez, L., Lopez-Hoyos, M., Munoz-Cacho, P., Martinez-Taboada, V.M., 2012. Aging is associated with circulating cytokine dysregulation. Cell. Immunol. 273, 124–132.
Bennett, A.L., Chao, C.C., Hu, S.X., Buchwald, D., Fagioli, L.R., Schur, P.H., Peterson, P.K., Komaroff, A.L., 1997. Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. J. Clin. Immunol. 17, 160–166.
Broderick, G., Katz, B.Z., Fernandes, H., Fletcher, M.A., Klimas, N., Smith, F.A., O'Gorman, M.R.G., Vernon, S.D., Taylor, R., 2012. Cytokine expression profiles of immune imbalance in post-mononucleosis chronic fatigue. J. Transl. Med. 10.
Brurberg, K.G., Fønhus, M.S., Larun, L., Flottorp, S., Malterud, K., 2014. Case definitions for chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME): a systematic review. BMJ Open 4, e003973.
Buchwald, D., Wener, M.H., Pearlman, T., Kith, P., 1997. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. J. Rheumatol. 24, 372–376.
Cannon, J.G., Angel, J.B., Ball, R.W., Abad, L.W., Fagioli, L., Komaroff, A.L., 1999. Acute phase responses and cytokine secretion in chronic fatigue syndrome. J. Clin. Immunol. 19, 414–421.
Centola, M., Cavet, G., Shen, Y., Ramanujan, S., Knowlton, N., Swan, K.A., Turner, M., Sutton, C., Smith, D.R., Haney, D.J., Chernoff, D., Hesterberg, L.K., Carulli, J.P., Taylor, P.C., Shadick, N.A., Weinblatt, M.E., Curtis, J.R., 2014. Development of a multi-biomarker disease activity test for rheumatoid arthritis. PLoS ONE 8, e60635.
Chao, C.C., Ala, T.A., Hu, S., Crossley, K.B., Sherman, R.E., Peterson, P.K., Frey, W.H., 1994. Serum cytokine levels in patients with Alzheimer's disease. Clin. Diagn. Lab. Immunol. 1, 433–436.
Chao, C.C., Janoff, E.N., Hu, S.X., Thomas, K., Gallagher, M., Tsang, M., Peterson, P.K., 1991. Altered cytokine release in peripheral-blood mononuclear cell-cultures from patients with the chronic fatigue syndrome. Cytokine 3, 292–298.
Chauffier, K., Salliot, C., Berenbaum, F., Sellam, J., 2012. Effect of biotherapies on fatigue in rheumatoid arthritis: a systematic review of the literature and meta-analysis. Rheumatology (Oxford) 51, 60–68.
Cheney, P.R., Dorman, S.E., Bell, D.S., 1989. Interleukin-2 and the chronic faitgue syndrome. Ann. Intern. Med. 110, 321.
Costigan, M., Moss, A., Latremoliere, A., Johnston, C., Verma-Gandhu, M., Herbert, T.A., Barrett, L., Brenner, G.J., Vardeh, D., Woolf, C.J., Fitzgerald, M., 2009. T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. J. Neurosci. 29, 14415–14422.
Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.
Feagins, L.A., 2010. Role of transforming growth factor-β in inflammatory bowel disease and colitis-associated colon cancer. Inflamm. Bowel Dis. 16, 1963–1968.
Felger, J.C., Lotrich, F.E., 2013. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience 246, 199–229.
Flavell, R.A., Sanjabi, S., Wrzesinski, S.H., Licona-Limon, P., 2010. The polarization of immune cells in the tumour environment by TGFbeta. Nat. Rev. Immunol. 10, 554–567.
Fletcher, M.A., Zeng, X.R., Barnes, Z., Levis, S., Klimas, N.G., 2009. Plasma cytokines in women with chronic fatigue syndrome. J. Transl. Med. 7, 96.
Fukuda, K., Straus, S.E., Hickie, I., Sharpe, M.C., Dobbins, J.G., Komaroff, A., 1994. The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. Ann. Intern. Med. 121, 953–959.
Gaertner, R.F., Wyss-Coray, T., Von Euw, D., Lesne, S., Vivien, D., Lacombe, P., 2005. Reduced brain tissue perfusion in TGF-beta 1 transgenic mice showing Alzheimer's disease-like cerebrovascular abnormalities. Neurobiol. Dis. 19, 38–46.
Garcia, M.N., Hause, A.M., Walker, C.M., Orange, J.S., Hasbun, R., Murray, K.O., 2014. Evaluation of prolonged fatigue post-West Nile virus infection and association of fatigue with elevated antiviral and proinflammatory cytokines. Viral Immunol. 27, 327–333.
Glaser, R., Kiecolt-Glaser, J.K., 2005. Stress-induced immune dysfunction: implications for health. Nat. Rev. Immunol. 5, 243–251.
Hauser, G., Awad, T., Brok, J., Thorlund, K., Stimac, D., Mabrouk, M., Gluud, C., Gluud, L.L., 2014. Peginterferon plus ribavirin versus interferon plus ribavirin for chronic hepatitis C. Cochrane Database Syst. Rev. 2, CD005441.
Hickie, I., Davenport, T., Wakefield, D., Vollmer-Conna, U., Cameron, B., Vernon, S.D., Reeves, W.C., Lloyd, A.Dubbo Infection Outcomes Study G., 2006. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 333, 575.
Holmes, G.P., Kaplan, J.E., Gantz, N.M., Komaroff, A.L., Schonberger, L.B., Straus, S.E., Jones, J.F., Dubois, R.E., Cunningham-Rundles, C., Pahwa, S., et al., 1988. Chronic fatigue syndrome: a working case definition. Ann. Intern. Med. 108, 387–389.
Hornig, M., Montoya, J.G., Klimas, N.G., Levine, S., Felsenstein, D., Bateman, L., Peterson, D.L., Gottschalk, C.G., Schultz, A.F., Che, X., Eddy, M.L., Komaroff, A.L., Lipkin, W.I., 2015. Distinct plasma immune signatures in ME/CFS are present early in the course of illness. Sci. Adv. 1, e1400121.
Inoue, K., Yamazaki, H., Manabe, Y., Fukuda, C., Hanai, K., Fushiki, T., 1999. Transforming growth factor-beta activated during exercise in brain depresses spontaneous motor activity of animals. Relevance to central fatigue. Brain Res. 846, 145–153.
Jammes, Y., Steinberg, J.G., Delliaux, S., Bregeon, F., 2009. Chronic fatigue syndrome combines increased exercise-induced oxidative stress and reduced cytokine and Hsp responses. J. Intern. Med. 266, 196–206.
Ji, R.R., Berta, T., Nedergaard, M., 2013. Glia and pain: is chronic pain a gliopathy? Pain 154 (Suppl. 1), S10-28.
Kanhere, A., Hertweck, A., Bhatia, U., Gokmen, M.R., Perucha, E., Jackson, I., Lord, G.M., Jenner, R.G., 2012. T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Nat. Commun. 3, 1268.
Kennedy, G., Spence, V., Underwood, C., Belch, J.J., 2004. Increased neutrophil apoptosis in chronic fatigue syndrome. J. Clin. Pathol. 57, 891–893.
Khaiboullina, S.F., DeMeirleir, K.L., Rawat, S., Berk, G.S., Gaynor-Berk, R.S., Mijatovic, T., Blatt, N., Rizvanov, A.A., Young, S.G., Lombardi, V.C., 2014. Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis. Cytokine 72, 1–8.
Kubera, M., Kenis, G., Bosmans, E., Kajta, M., Basta-Kaim, A., Scharpe, S., Budziszewska, B., Maes, M., 2004. Stimulatory effect of antidepressants on the production of IL-6. Int. Immunopharmacol. 4, 185–192.
Kubera, M., Maes, M., Kenis, G., Kim, Y.K., Lason, W., 2005. Effects of serotonin and serotonergic agonists and antagonists on the production of tumor necrosis factor alpha and interleukin-6. Psychiatry Res. 134, 251–258.
Lafyatis, R., 2014. Transforming growth factor beta-at the centre of systemic sclerosis. Nat. Rev. Rheumatol. 10, 706–719.
Lattie, E.G., Antoni, M.H., Fletcher, M.A., Penedo, F., Czaja, S., Lopez, C., Perdomo, D., Sala, A., Nair, S., Fu, S.H., Klimas, N., 2012. Stress management skills, neuroimmune processes and fatigue levels in persons with chronic fatigue syndrome. Brain Behav. Immun. 26, 849–858.
Linde, A., Andersson, B., Svenson, S.B., Ahrne, H., Carlsson, M., Forsberg, P., Hugo, H., Karstorp, A., Lenkei, R., et al., 1992. Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in the patients with chronic fatigue syndrome. J. Infect. Dis. 165, 996–1000.
Lloyd, A., Gandevia, S., Brockman, A., Hales, J., Wakefield, D., 1994. Cytokine production and fatigue in patients with chronic fatigue syndrome and healthy control subjects in reponse to exercise. Clin. Infect. Dis. 18, S142–S146.
Lloyd, A., Hickie, I., Brockman, A., Dwyer, J., Wakefield, D., 1991. Cytokine levels in serum and cerebrospinal fluid in patients with chronic fatigue syndrome and control subjects. J. Infect. Dis. 164, 1023–1024.

Lorusso, L., Mikhaylova, S.V., Capelli, E., Ferrari, D., Ngonga, G.K., Ricevuti, G., 2009. Immunological aspects of chronic fatigue syndrome. Autoimmun. Rev. 8, 287–291.

Lyall, M., Peakman, M., Wessely, S., 2003. A systematic review and critical evaluation of the immunology of chronic fatigue syndrome. J. Psychosom. Res. 55, 79–90.

MacDonald, K.L., Osterholm, M.T., LeDell, K.H., White, K.E., Schenck, C.H., Chao, C.C., Persing, D.H., Johnson, R.C., Barker, J.M., Peterson, P.K., 1996. A case-control study to assess possible triggers and cofactors in chronic fatigue syndrome. Am. J. Med. 100, 548–554.

Maes, M., Ringel, K., Kubera, M., Anderson, G., Morris, G., Galecki, P., Geffard, M., 2013. In myalgic encephalomyelitis/chronic fatigue syndrome, increased autoimmune activity against 5-HT is associated with immuno-inflammatory pathways and bacterial translocation. J. Affect. Disord. 150, 223–230.

Maes, M., Twisk, F.N.M., Kubera, M., Ringel, K., 2012. Evidence for inflammation and activation of cell-mediated immunity in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS): Increased interleukin-1, tumor necrosis factor-alpha, PMN-elastase, lysozyme and neopterin. J. Affect. Disord. 136, 933–939.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G.P. Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. Open Med. 3, e123–e130.

Nakamura, T., Schwander, S., Donnelly, R., Cook, D.B., Ortega, F., Togo, F., Yamamoto, Y., Cherniack, N.S., Klapholz, M., Rapoport, D., Natelson, B.H., 2013. Exercise and sleep deprivation do not change cytokine expression levels in patients with chronic fatigue syndrome. Clin. Vaccine Immunol. 20, 1736–1742.

Nakamura, T., Schwander, S.K., Donnelly, R., Ortega, F., Togo, F., Broderick, G., Yamamoto, Y., Cherniack, N.S., Rapoport, D., Natelson, B.H., 2010. Cytokines across the night in chronic fatigue syndrome with and without fibromyalgia. Clin. Vaccine Immunol. 17, 582–587.

Nakatomi, Y., Mizuno, K., Ishii, A., Wada, Y., Tanaka, M., Tazawa, S., Onoe, K., Fukuda, S., Kawabe, J., Takahashi, K., Kataoka, Y., Shiomi, S., Yamaguti, K., Inaba, M., Kuratsune, H., Watanabe, Y., 2014. Neuroinflammation in patients with chronic fatigue syndrome/myalgic encephalomyelitis: an 11C-(R)-PK11195 PET study. J. Nucl. Med. 55, 945–950.

Nas, K., Cevik, R., Batum, S., Sarac, A.J., Acar, S., Kalkanli, S., 2011. Immunologic and psychosocial status in chronic fatigue syndrome. Bratisl. Med. J. 112, 208–212.

Natelson, B.H., Weaver, S.A., Tseng, C.L., Ottenweller, J.E., 2005. Spinal fluid abnormalities in patients with chronic fatigue syndrome. Clin. Diagn. Lab. Immunol. 12, 52–55.

Nater, U.M., Youngblood, L.S., Jones, J.F., Unger, E.R., Miller, A.H., Reeves, W.C., Heim, C., 2008. Alterations in diurnal salivary cortisol rhythm in a population-based sample of cases with chronic fatigue syndrome. Psychosom. Med. 70, 298–305.

Neu, D., Mairesse, O., Montana, X., Gilson, M., Corazza, F., Lefevre, N., Linkowski, P., Le Bon, O., Verbanck, P., 2014. Dimensions of pure chronic fatigue: psychophysical, cognitive and biological correlates in the chronic fatigue syndrome. Eur. J. Appl. Physiol. 114, 1841–1851.

Nijs, J., Nees, A., Paul, L., De Kooning, M., Ickmans, K., Meeus, M., Van Oosterwijck, J., 2014. Altered immune response to exercise in patients with chronic fatigue syndrome/myalgic encephalomyelitis: a systematic literature review. Exerc. Immunol. Rev. 20, 94–116.

Nijs, J., Van Oosterwijck, J., Meeus, M., Lambrecht, L., Metzger, K., Fremont, M., Paul, L., 2009. Unravelling the nature of postexertional malaise in myalgic encephalomyelitis/chronic fatigue syndrome: the role of elastase, complement C4a and interleukin-1beta. J. Intern. Med. 267, 418–435.

Nikolaus, S., Bode, C., Taal, E., van de Laar, M.A.F.J., 2013. Fatigue and factors related to fatigue in rheumatoid arthritis: a systematic review. Arthritis Care Res. 65, 1128–1146.

Ouchi, N., Parker, J.L., Lugus, J.J., Walsh, K., 2011. Adipokines in inflammation and metabolic disease. Nat. Rev. Immunol. 11, 85–97.

Papadopoulos, A.S., Cleare, A.J., 2012. Hypothalamic-pituitary-adrenal axis dysfunction in chronic fatigue syndrome. Nat. Rev. Endocrinol. 8, 22–32.

Papadopoulou, E., Anagnostopoulos, K., Tripsianis, G., Tentes, I., Kakolyris, S., Galazios, G., Sivridis, E., Simopoulos, K., Kortsaris, A., 2008. Evaluation of predictive and prognostic significance of serum TGF-beta1 levels in breast cancer according to HER-2 codon 655 polymorphism. Neoplasma 55, 229–238.

Patarca, R., Klimas, N.G., Lugtendorf, S., Antoni, M., Fletcher, M.A., 1994. Dysregulated expression of tumor-necrosis-factor in chronic fatigue syndrome - interelations with cellular sources and patterns of soluble immune mediator expression. Clin. Infect. Dis. 18, S147–S153.

Peterson, P.K., Sirr, S.A., Grammith, F.C., Schenck, C.H., Pheley, A.M., Hu, S., Chao, C.C., 1994. Effects of mild exercise on cytokines and cerebral blood flow in chronic fatigue syndrome patients. Clin. Diagn. Lab. Immunol. 1, 222–226.

Prins, J.B., van der Meer, J.W., Bleijenberg, G., 2006. Chronic fatigue syndrome. Lancet 367, 346–355.

Raison, C.L., Lin, J.M., Reeves, W.C., 2009. Association of peripheral inflammatory markers with chronic fatigue in a population-based sample. Brain Behav. Immun. 23, 327–337.

Reeves, W.C., Lloyd, A., Vernon, S.D., Klimas, N., Jason, L.A., Bleijenberg, G., Evengard, B., White, P.D., Nisenbaum, R., Unger, E.R., 2003. Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution. BMC Health Serv. Res. 3, 25.

Robinson, M., Gray, S.R., Watson, M.S., Kennedy, G., Hill, A., Belch, J.J.F., Nimmo, M.A., 2010. Plasma IL-6, its soluble receptors and F-2-isoprostanes at rest and during exercise in chronic fatigue syndrome. Scand. J. Med. Sci. Sports 20, 282–290.

Sambuelli, A., Diez, R.A., Sugai, E., Boerr, L., Negreira, S., Gil, A., Camartino, G., Huernos, S., Doldan, I., Felstiner, D., Bai, J.C., 2000. Serum transforming growth factor-beta1 levels increase in response to successful anti-inflammatory therapy in ulcerative colitis. Aliment. Pharmacol. Ther. 14, 1443–1449.

Scully, P., McKernan, D.P., Keohane, J., Groeger, D., Shanahan, F., Dinan, T.G., Quigley, E.M., 2010. Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. Am. J. Gastroenterol. 105, 2235–2243.

Shephard, R.J., 2002. Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. Crit. Rev. Immunol. 22, 165–182.

Smylie, A.L., Broderick, G., Fernandes, H., Razdan, S., Barnes, Z., Collado, F., Sol, C., Fletcher, M.A., Klimas, N., 2013. A comparison of sex-specific immune signatures in Gulf War illness and chronic fatigue syndrome. BMC Immunol. 14, 29.

Spence, V.A., Kennedy, G., Belch, J.J.F., Hill, A., Khan, F., 2008. Low-grade inflammation and arterial wave reflection in patients with chronic fatigue syndrome. Clin. Sci. 114, 561–566.

Straus, S.E., Dale, J.K., Peter, J.B., Dinarello, C.A., 1989. Circulating lymphokine levels in the chronic fatigue syndrome. J. Infect. Dis. 160, 1085–1086.

Swanink, C.M.A., Vercoulen, J.H.M.M., Galama, J.M.D., Roos, M.T.L., Meyaard, L., van der Ven-Jongekrijg, J., de Nijs, R., Bleijenberg, G., Fennis, J.F.M., Miedema, F., van der Meer, J.W.M., 1996. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. J. Infect. Dis. 173, 460–463.

Ur, E., White, P.D., Grossman, A., 1992. Hypothesis: cytokines may be activated to cause depressive illness and chronic fatigue syndrome. Eur. Arch. Psychiatry Clin. Neurosci. 241, 317–322.

Visser, J., Graffelman, W., Blauw, B., Haspels, I., Lentjes, E., de Kloet, E.R., Nagelkerken, L., 2001. LPS-induced IL-10 production in whole blood cultures from chronic fatigue syndrome patients is increased but supersensitive to inhibition by dexamethasone. J. Neuroimmunol. 119, 343–349.

Vollmer-Conna, U., Aslakson, E., White, P.D., 2006. An empirical delineation of the heterogeneity of chronic unexplained fatigue in women. Pharmacogenomics 7, 355–364.

Vollmer-Conna, U., Cameron, B., Hadzi-Pavlovic, D., Singletary, K., Davenport, T., Vernon, S., Reeves, W.C., Hickie, I., Wakefield, D., Lloyd, A.R., 2007. Postinfective fatigue syndrome is not associated with altered cytokine production. Clin. Infect. Dis. 45, 732–735.

Vollmer-Conna, U., Piraino, B.F., Cameron, B., Davenport, T., Hickie, I., Wakefield, D., Lloyd, A.R.Dubbo Infection Outcomes Study G., 2008. Cytokine polymorphisms have a synergistic effect on severity of the acute sickness response to infection. Clin. Infect. Dis. 47, 1418–1425.

Wahl, S.M., 2007. Transforming growth factor-beta: innately bipolar. Curr. Opin. Immunol. 19, 55–62.

Wells, G., Shea, B., O'Connell, D., Peterson, J., Welch, V., Losos, M., Tugwell, P., The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. <http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp>.

White, A.T., Light, A.R., Hughen, R.W., Bateman, L., Martins, T.B., Hill, H.R., Light, K.C., 2010. Severity of symptom flare after moderate exercise is linked to cytokine activity in chronic fatigue syndrome. Psychophysiology 47, 615–624.

White, P.D., Nye, K.E., Pinching, A.J., Yap, T.M., Power, N., Vleck, V., Bentley, D.J., Thomas, J.M., Buckland, M., Parkin, J.M., 2004. Immunological Changes After Both Exercise and Activity in Chronic Fatigue Syndrome. J. Chronic Fatig. Syndr. 12, 51–66.

White, P.D., Pinching, A.J., Rakib, A., Castle, M., Hedge, B., Priebe, S., 2002. A comparison of patients with chronic fatigue syndrome attending separate fatigue clinics based in immunology and psychiatry. J. R. Soc. Med. 95, 440–444.

Wilson, A., Hickie, I., Hadzi-Pavlovic, D., Wakefield, D., Parker, G., Straus, S.E., Dale, J., McCluskey, D., Hinds, G., Brickman, A., Goldenberg, D., Demitrack, M., Blakely, T., Wessely, S., Sharpe, M., Lloyd, A., 2001. What is chronic fatigue syndrome? Heterogeneity within an international multicentre study. Aust. N. Z. J. Psychiatry 35, 520–527.

Wyller, V.B., Sorensen, O., Sulheim, D., Fagermoen, E., Ueland, T., Mollnes, T.E., 2015. Plasma cytokine expression in adolescent chronic fatigue syndrome. Brain Behav. Immun. 46, 80–86.

Zorena, K., Malinowska, E., Raczynska, D., Mysliwiec, M., Raczynska, K., 2013. Serum concentrations of transforming growth factor-Beta 1 in predicting the occurrence of diabetic retinopathy in juvenile patients with type 1 diabetes mellitus. J. Diabet. Res. 2013, 614908.