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Original Article

Association between C-reactive protein and chronic fatigue syndrome: a meta-analysis

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Abstract: Chronic fatigue syndrome (CFS) is an agnogenic disease, which has recently been linked to inflammation. Several studies have found an association between inflammatory factors such as C-reactive protein (CRP) and CFS. However, these studies have shown inconsistent results. PubMed, Embase, and CBM (Chinese Biomedical Literature Database) were searched for relevant studies published as of August 2016. A total of 8 studies were included in the meta-analysis and trial sequential analyses (TSA). Meta-analysis revealed a mean difference (MD) of 0.39 μg/mL (95% CI: 0.15-0.64) in CRP levels between the CFS patients and healthy controls. Subgroup analysis revealed that CRP levels were not elevated in teenagers [MD 0.10 μg/mL (95% CI: -0.04-0.24)]. There was a statistically significant between-group difference with respect to CRP levels between adult European population [MD 1.58 μg/mL (95% CI: 0.88-2.27)] and adult American population [MD 0.34 μg/mL (95% CI: 0.16-0.51)]. TSA results showed that the trial sequential monitoring boundary (TSBM) was crossed only in the group of European adults, while the group of European teenagers did not cross TSBM and the traditional futility boundary. The group of American adults crossed the traditional boundary, but not TSBM. These findings suggest that baseline CRP levels are greater in CFS patients with the exception of European teenage patients, which could provide insights into the causality of CFS. However, considering the sample size, further studies with larger sample size and more robust design are needed to validate the association between CRP and CFS.

Keywords: Chronic fatigue syndrome, C-reactive protein, TSA, inflammation, meta-analysis

Introduction

Chronic fatigue syndrome (CFS) is a clinical syndrome characterized by persistent and unexplained fatigue that is worsened by physical and mental exertion and typically lasts for ≥ 6 months [1, 2]. The fatigue is typically not alleviated by rest, and is accompanied by at least four of the following eight symptoms: sore throat, tender lymphadenopathy, impaired memory or concentration, myalgia, arthralgia, unrefreshing sleep, post-exertional malaise, and headache [3].

CFS imposes a considerable burden on the affected families and the society at large. Approximately 836,000 to 2.5 million Americans suffer from CFS [4]. The consequent economic burden is estimated to be substantial ($17-24 billion annually) (https://prevention.nih.gov/programs-events/pathways-to-prevention/workshops/mecfs/workshopresources). The condition severely affects the health related quality of life (particularly of the affected adolescents) [5] and cognitive function [6]. CFS commonly involves adults, but may also affect children and adolescents [7]. Athough several theories have been formulated to explain the pathogenesis of CFS, such as viral infection [8], endocrinal dysfunction [9, 10], immune dysfunction [11] and genetic factors [12], CFS is still an agnogenic disease.

The diagnostic criteria for CFS point towards the inflammatory nature of the disease [3]. Moreover, several studies have documented altered expression of inflammatory factors in these patients. A systematic review of 38 studies [13] conducted on patients with CFS could not reach definitive conclusion owing to inconsistent results from the included studies. For example, increased expression of interleukin-2 (IL-2) in patients with CFS was found in 3 studies, decreased expression of IL-2 was found in 3 studies, while no significant association was found in 9 studies. Moreover, C-reactive protein

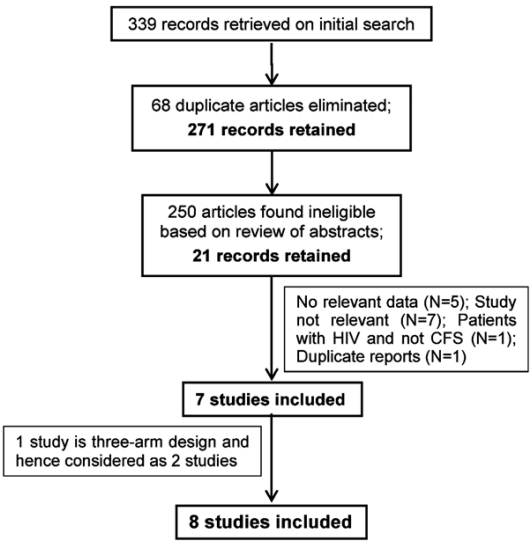

Figure 1. Schematic illustration of the study selection criteria for the meta-analysis.

(CRP) level was not included in the systematic review. CRP is an acute phase protein and a non-specific biochemical marker of chronic inflammation [14]. It is synthesized by hepatocytes and adipocytes in response to increased levels of pro-inflammatory cytokines (such as TNF-α and IL-6) in the peripheral circulation [15, 16]. Serum or plasma level of CRP is a clinically relevant indicator of systemic pro-inflammatory activity [17].

To date, several studies have shown an association between high baseline levels of CRP, a pro-inflammatory biomarker, and CFS [14, 18-23]. However, the results of different studies have largely been inconsistent. Therefore, to further clarify the association between CRP and CFS, we conducted this meta-analysis of relevant studies.

Materials and methods

Search methods

Original articles published before August 2016 that evaluated the association between CRP and CFS were searched on PubMed, Embase, and CBM (Chinese Biomedical Literature Database) databases. The medical subject headings and keywords used for the search were "C-reactive protein", "CRP", "chronic fatigue syndrome", "Myalgic Encephalomyelitis", "chronic mononucleosis", "post-infectious fatigue syndrome", "chronic fatigue immune dysfunction syndrome", "post-viral fatigue syndrome", and "neurasthenia". The reference lists of all retrieved publications were manually searched for additional studies.

Inclusion and exclusion criteria

Studies that qualified all the following criteria were eligible for inclusion: (1) original papers; (2) case-control studies; (3) studies that evaluated the association between CRP levels and CFS; (4) controls were healthy subjects; (5) clear diagnostic criteria employed for CFS.

The exclusion criteria were: (1) overlapping data; (2) case-studies; (3) literature reviews; (4) CRP levels not reported. Two researchers extracted data independently; any difference of opinion was resolved by consensus.

Quality assessment and data extraction

Based on the Newcastle-Ottawa scale, two reviewers independently assessed the studies included in the meta-analysis. Any disagreement was resolved by discussion with the third author.

Data on the following variables were extracted: (1) First author's last name, publication year, origin of the study population; (2) characteristics of study population: sample size, age, gender, diagnoses, and methods of CRP measurement; (3) mean (SD) CRP levels in each group. Studies with a three-arm design were considered as two studies based on the design. For studies that reported data as median and quartiles, the median was treated as the mean. The distribution was assumed to be normal, with a *z*-value of ± 0.68 that corresponded to the reported 25^{th} and 75^{th} percentiles [24]. In this manner, the mean and standard deviation values were obtained.

Statistical analyses

For better characterization of the difference in CRP levels between CFS patients and healthy controls, the strength of association in the pooled data was measured by mean difference [MD] at 95% CI [25]. The significance of pooled MD was tested by *z*-test ($P < 0.05$ was considered statistically significant). The I^2 value was calculated as a measure of heterogeneity for each outcome analysis, where 0% to 25% indicated no observed heterogeneity, while larger values indicated increasing heterogeneity; 25% to 50% was regarded as low, 50% to 75% as

Table 1. Characteristics of studies included in the meta-analysis

First author	Year	Diagnostic criteria	Country	Case/Control (N)	Detection methods	Is the method high-sensitive?	Specimen type
Sulheim, Dag	2014	Clinical guidelines	Norway	60/60/68[a]	Not mentioned	No	Serum
Groeger, David	2013	CDC1994 [3]	Ireland	48/35	Electrochemiluminescence	Yes	Plasma
Kennedy, Gwen	2010	CDC1994	United Kingdom	25/23	ELISA	Yes	Plasma
Raison, Charles L.	2009	CDC1994	United States	96/111	Turbidimetric assay	Yes	Plasma
Spence, Vance A.	2008	CDC1994	United Kingdom	41/30	ELISA	Yes	Serum
Richards, R. S	2000	CDC1988 [4]	United Kingdom	24/20	Not mentioned	No	Plasma
Buchwald, D	1997	CDC1988	United States	98/51	Enhanced immuNonephelometry	No	Serum

[a]: stands for case group 1, case group 2 and control group.

moderate, and 75% to 100% as high heterogeneity [26]. Fixed effects model was used in case of low heterogeneity ($P < 0.05$); otherwise random effects model was used.

Publication bias was assessed by funnel plot [27], Egger's linear regression test [28] and Begger's rank correlation test [29], if appropriate. Cumulative analysis of the extracted data was performed using a pooled random effects model with the sample sizes arrayed in ascending order so as to ascertain the tendency of pooled results. In the event of obvious heterogeneity, subgroup analysis was performed. Meta-analysis was performed using the "meta" package [30] of the R software [31].

Trial sequential analyses (TSA)

Trial sequential analyses (TSA) was performed to better understand the power of the meta-analysis, to gauge the reliability of evidence, and to avoid potential false positive results owing to insufficient sample size [32, 33]. The sample size needed for a reliable meta-analysis is at least as large as that required for a single optimally powered randomized controlled trial. Briefly, TSA is similar to interim analyses and is used to decide whether a particular randomized trial could be terminated early because of the *P* value being sufficiently small to show an effect or sufficiently large to show potential futility by monitoring boundaries. Unlike cumulative meta-analysis, it is at risk of producing random errors because of limited data and repetitive testing of accumulating data, and because the information size requirement analogous to the sample size of one optimally powered clinical trial may not be met [32, 33]. TSA was performed using TSA software version 0.9 Beta [34] (http://www.ctu.dk/tsa/).

Results

Study selection

A schematic illustration of the study selection criteria is presented in **Figure 1**. A total of 7 articles [14, 18-23] (8 studies) were eligible for inclusion in the meta-analysis. Combined study population consisted of 452 cases and 406 controls. Six studies were conducted in Europe (Norway, Ireland and United Kingdom) and two in North America (United States of America).

Description of clinical studies

Characteristics of the included studies are presented in **Table 1**. Quality assessment of the seven included studies (**Table 2**) indicated medium quality of the included studies. Four studies showed greater baseline CRP levels in CFS patients as compared to that in controls, while no significant between-group difference was observed in the other four studies.

Quantitative data synthesis

Overall, a significant positive association was found between CRP concentration and CFS. The MD in the CRP levels between the CFS patients and controls was 0.39 μg/mL (95% confidence interval [CI]: 0.15-0.64) using a random effects model (z-score: 3.12; $P = 0.002$) for the overall effect and 69.8% heterogeneity I^2 ($P = 0.002$).

A forest plot based on the meta-analysis is shown in **Figure 2A**. A cumulative meta-analysis was also conducted after listing the studies in the ascending order of the sample sizes; the pooled MD at 95% CI started to show statistical significance at 1.24 (95% CI: 0.41-2.07) from the third study, and a gradual stabilization was

Table 2. Quality assessment of involved studies using Newcastle-Ottawa scale

Author	Year	Selection				Comparability		Exposure			score
		1	2	3	4	1	2	1	2	3	
Sulheim, D.	2014	1	1	0	1	0	1	1	1	0	6
Groeger, David	2013	1	0	0	1	0	1	0	1	1	5
Kennedy, Gwen	2010	1	0	1	0	1	0	1	1	1	6
Raison, Charles L.	2009	1	1	1	1	0	1	1	1	1	8
Spence, Vance A.	2008	1	0	1	0	0	0	1	1	0	4
Richards, R. S.	2000	1	0	0	1	1	0	1	1	0	5
Buchwald, D.	1997	1	1	0	1	1	0	1	1	0	6

observed thereafter. Therefore, the possibility that the results may have been influenced by sample size cannot be ruled out (**Figure 2B**).

Heterogeneity analysis

Considering the moderate heterogeneity revealed by the analysis (I^2 = 69.8%, P = 0.002), sensitivity analysis and subgroup analysis were performed to identify the source of heterogeneity. Sensitivity analysis showed no change in heterogeneity after sequential exclusion of one article at a time. However, on subgroup analysis based on the type of specimen used for CRP test (plasma or serum), the method used for measurement of CRP (regular or high-sensitivity CRP assay) and the age-group of the population (adults or teenagers), the heterogeneity was found to have reduced to some extent. The MD between the plasma sample and serum sample subgroups were 0.97 μg/mL (95% CI: 0.25-1.69) and 0.20 μg/mL (95% CI: 0.00-0.41), respectively (**Figure 3**). The MD in CRP levels between the regular CRP assay and high-sensitivity CRP assay groups were 0.18 μg/mL (95% CI: 0.07-0.28) and 1.09 μg/mL (95% CI: 0.36-1.83), respectively (**Figure 4**). The MD in the adult and teenaged groups were 0.75 μg/mL (95% CI: 0.28-1.21) and 0.10 μg/mL (95% CI: -0.04-0.24), respectively (**Figure 5**). The between-group differences in all subgroup analyses were statistically significant. Furthermore, we also performed subgroup analyses based on gender (M/F > 1 or < 1) and year of publication (before 2010 vs. after 2010), and found that these factors were not statistically associated with heterogeneity and had almost no effect on our results. An important finding was that the change in CRP level was not significant in the teenaged group. We further analyzed the ethnicity of population (European or American) and found that there was no heterogeneity in the American group. However, high heterogeneity was observed in the European group. Therefore, we performed subgroup analysis based on the ethnicity of population (European or American) and age of population (adults or teenagers), the results of which indicated that no heterogeneity existed in the three groups (there was no relevant studies with teenage American population) (**Figure 6**).

Publication bias and sensitivity analysis

The potential effect of publication bias on the observed association between CRP and CFS was assessed. An asymmetrical distribution was found in the funnel plot. In addition, Egger's test revealed a significant publication bias (t = 2.83, *P* = 0.03), while Begger's test did not reveal any significant publication bias (*z* = 0.62, *P* = 0.53). These findings indicate that our results may have been influenced by publication bias. Further, on sensitivity analysis, no significant effect of any one study on the pooled MD was observed based on *p*-values after sequential exclusion of individual studies.

Trial sequential analyses

To determine the optimal sample size for each subgroup, we assumed 0.1, 1.57, 0.22 as the mean difference for European teenagers, European adults and American adults group, with a variance of 0.77, 12.42 and 1.96, (the MD and variance were calculated by TSA software based on the trials included), statistical power of 80% and a two sided *P* value < 0.05 for type 1 error. Based on these assumptions, sample sizes of 2625, 159, and 5567 were needed to reliably detect a plausible effect for each group. However, in the present meta-analysis, only the group of European adults reached the optimal sample size calculated by TSA. We used the optimal sample size to help construct the trial sequential monitoring boundary. The cumulative *z* curve for American adults did not cross the trial sequential monitoring boundary (TSBM), but did cross the traditional futility

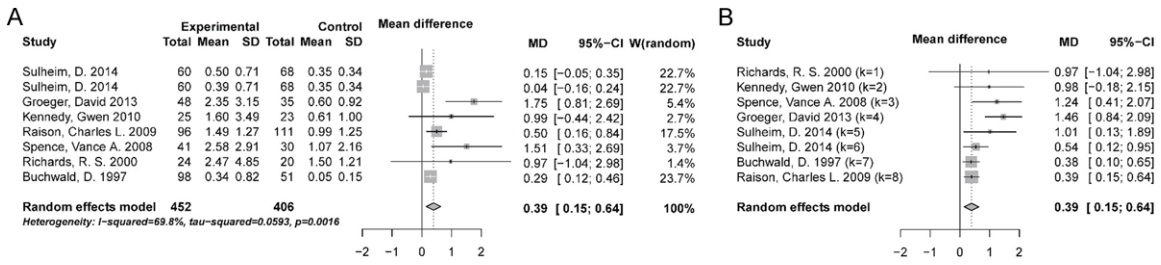

Figure 2. Forest plot of increased baseline C-reactive protein levels in CFS patients compared with healthy controls (random-effects model). A. Standard technique; B. Cumulative technique.

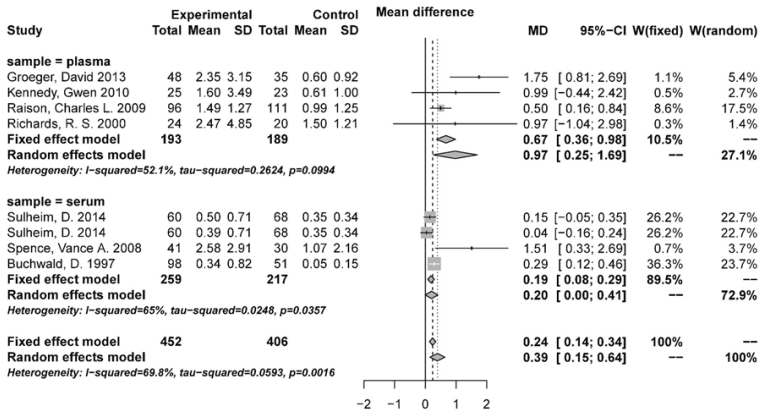

Figure 3. Forest plot of increased baseline C-reactive protein levels in patients with CFS, compared with healthy controls: subgroup analyses by sample type (plasma *vs.* serum).

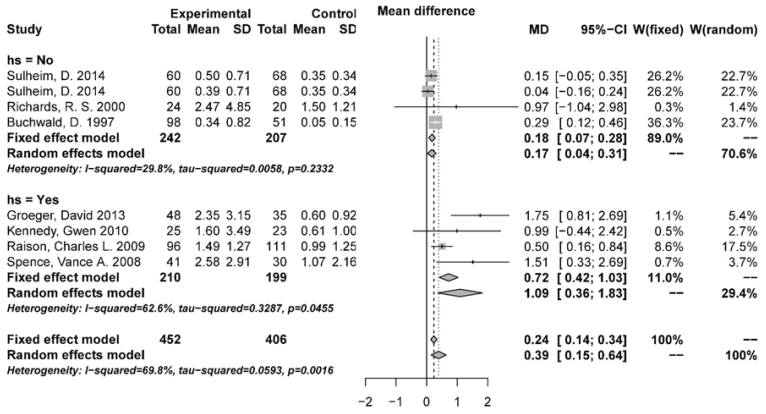

Figure 4. Forest plot of increased baseline C-reactive protein levels in patients with CFS, compared with healthy controls: subgroup analysis by method used for measurement (whether or not high-sensitive methods).

boundary (FB), which indicated that the cumulative evidence may be unreliable and inconclusive. The cumulative *z* curve for European teenagers did not cross the trial sequential monitoring boundary (TSBM), and also did not cross the traditional futility boundary (FB), which indicated that the cumulative evidence may not be true negative. The cumulative *z* curve for European adults did cross the trial sequential monitoring boundary (TSBM), and did cross the traditional futility boundary (FB), which indicated that the cumulative evidence may be really positive (Supplementary File).

Discussion

In our meta-analysis, we included 7 trials which revealed higher CRP levels in patients with CFS as compared to that in healthy controls; the difference in adults (but not in teenagers) were significant, and the differences between European and American adults were statistically significant. However, considering the sample size of meta-analysis obtained from TSA, the results need further confirmation.

As the pooled result indicated obvious heterogeneity, many factors were taken into consideration for subgroup analysis. We finally reached the conclusion that the age of study population (adults vs. teenagers) and the ethnicity of population (European or American) were likely to be main sources of heterogeneity; while the sample type (plasma *vs.* serum) and measure type (whether or not high-sensitive methods) probably also contributed to the heterogeneity to some extent. Some other possible reasons may contribute to the heterogeneity. For example,

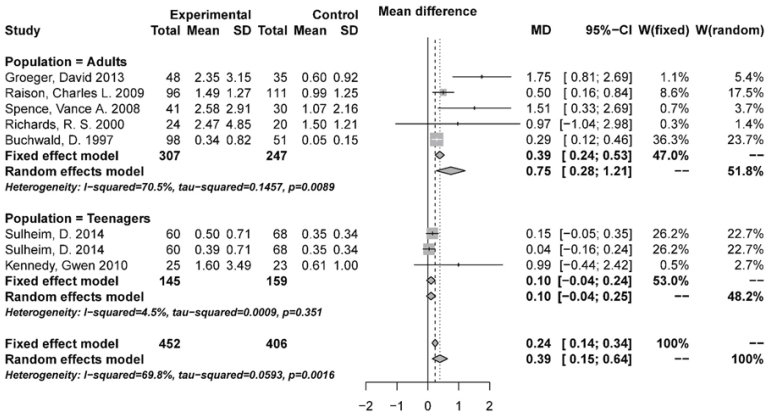

Figure 5. Forest plot of increased baseline C-reactive protein levels in patients with CFS, compared with healthy controls: subgroup analysis by study population (adults *vs.* teenagers).

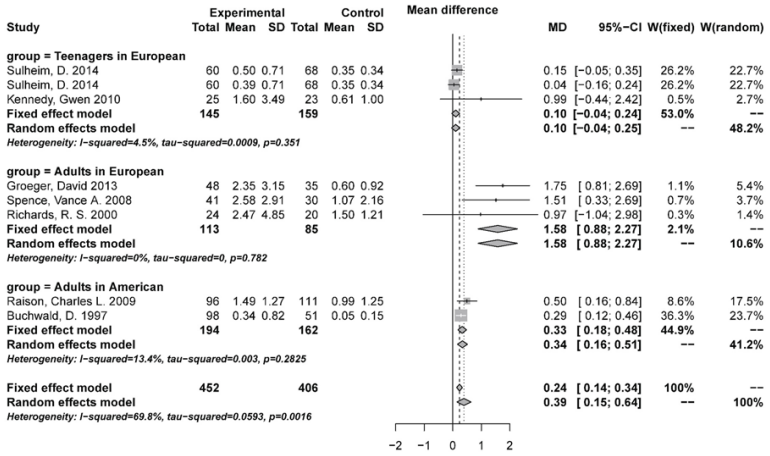

Figure 6. Forest plot of increased baseline C-reactive protein levels in CFS patients compared with healthy controls: subgroup analysis by study population (adults *vs.* teenagers) and ethnicity of population (European *vs.* American).

the different time intervals between infections and test may have been a source of heterogeneity, which was not detected on subgroup analysis. In addition, many other factors, such as the effect of body mass index, depressive status and immune-modulating medications [18], may also affect the CRP levels. Although the diagnostic criteria of CFS are clear, heterogeneity in patients may still exist as the diagnosis is based on symptoms, and not on clinical examination.

In our study, the CRP level was not found to be significantly elevated in teenaged CFS patients. However, considering that the CRP level was slightly higher in patients as compared to that in controls, and that the CRP level could be reduced following treatment with clonidine [22], the pooled results for teenaged patients may be false-negative due to insufficient sample size or due to some other reasons. In addition, that the strength of inflammatory reaction varied with age might be the main reason for this phenomenon [35].

Depression has been shown to significantly correlate with the severity of CFS [36]. CFS patients appeared to be at a heightened risk for development of major depressive disorder (MDD), as indicated by a study that compared the levels in CSF patients with those in non-CFS community samples [37, 38]. In a meta-analysis of 94 trials, tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) and other antidepressants appeared to be effective in treating unexplained somatic symptoms including those of CFS [39]. At the same time, as depression is accompanied by various direct and indirect indicators of a moderate activation of the inflammatory response system (IRS) [40, 41], the pathogenesis of CFS may involve inflammation caused by infections. However, this hypothesis needs to be confirmed. Moreover, clonidine has been reported to lower both plasma norepinephrine and serum CRP levels in patients with CFS [22], which implicates enhanced sympathetic nervous activity in the causation of low-grade systemic inflammation. Therefore, the treatment effect of clonidine may also benefit from enhanced parasympathetic activity [42]. On the other hand, the inflammation in CFS may be due to infections, as many studies have documented altered intestinal microbiota in these patients [43, 44]. In addition, as the reduction of CRP levels has been reported after treatment with *Bifidobacterium infantis* 35624 [19], the inflammatory milieu in CFS patients is

thought to be modulated by intestinal probiotics.

In our meta-analysis, trial sequential analyses (TSA) was used to calculate the optimal sample size. The sample size of our study was lower than the optimal requirement suggested by the TSA, and the results of the meta-analysis may be false-positive. However, our results are the most comprehensive so far.

Although a significant positive association between CRP levels and CFS was observed, certain limitations in the study need to be taken into account while interpreting the results. The small sample size was the foremost limitation of our meta-analysis. Secondly, considering the CRP level can be influenced by many factors, heterogeneity might still exist, even though heterogeneity decreased dramatically after subgroup analyses by ethnicity of population and age of patients. Thirdly, as the meta-analysis was based on observational studies, publication bias may have affected our results, given that studies with positive results are more likely to be published. Fourthly, conversion of data pertaining to the non-normally distributed variables to normally distributed statistics may have introduced bias. Fifthly, considering the big difference observed between adults and teenagers, the CRP level of teenagers in American CFS patients needs studying for the reason that no relevant studies were published. However, this still is the most comprehensive result to evaluate the relationship between CRP and CFS so far.

Conclusion

The present meta-analysis provides the best evidence till date on the association between increased CRP levels and CFS with the exception of European teenage patients with CFS. However, considering the sample size, further well-designed studies with larger sample sizes of European adults and American adults group are required to confirm our findings. In addition, other inflammatory factors, such as IL-6, IL-8 also need to be studied to understand the link between CFS and inflammation.

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Disclosure of conflict of interest

None.

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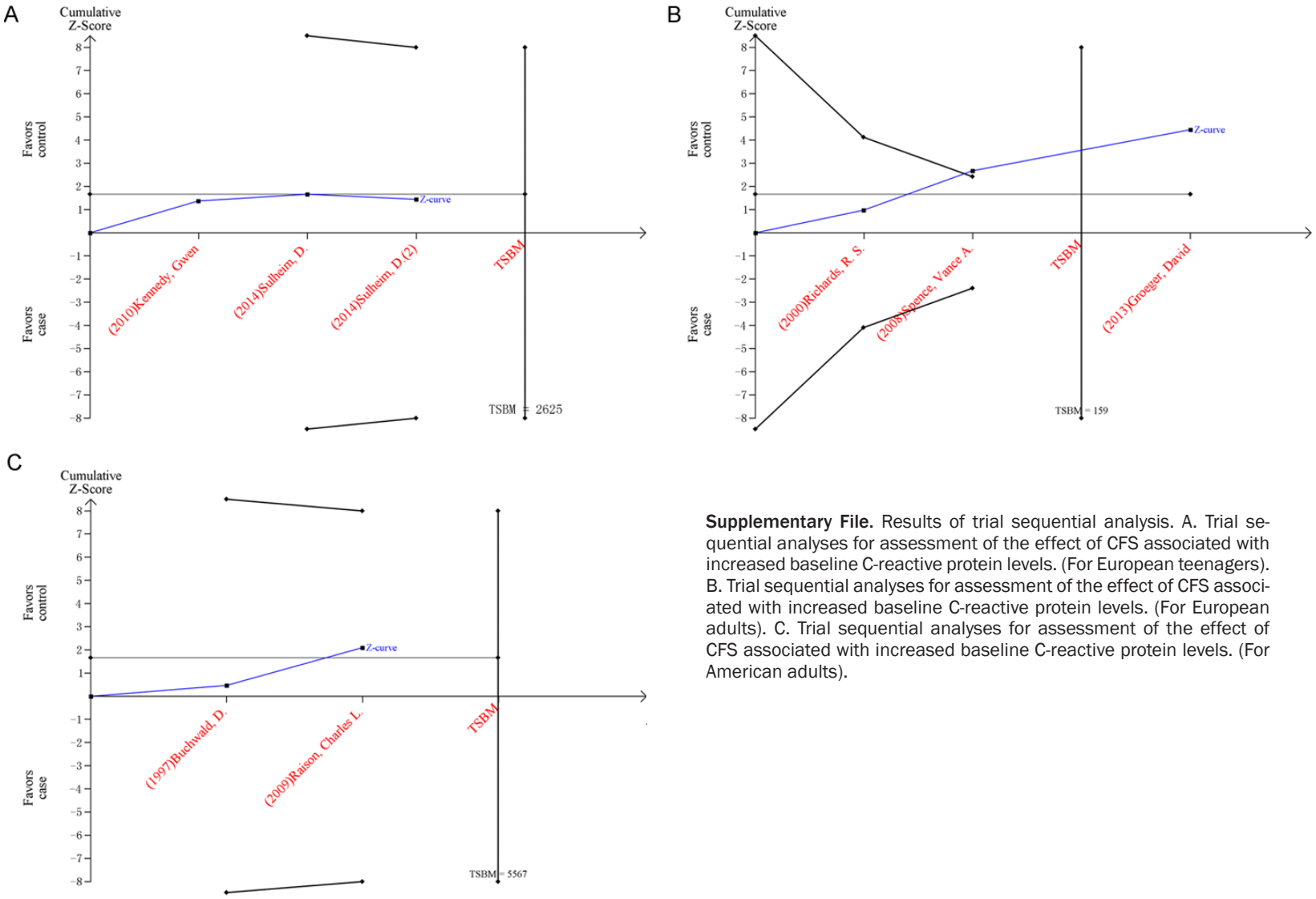

Supplementary File. Results of trial sequential analysis. A. Trial sequential analyses for assessment of the effect of CFS associated with increased baseline C-reactive protein levels. (For European teenagers). B. Trial sequential analyses for assessment of the effect of CFS associated with increased baseline C-reactive protein levels. (For European adults). C. Trial sequential analyses for assessment of the effect of CFS associated with increased baseline C-reactive protein levels. (For American adults).