Challenging queries of Q fever

emphasizing Q fever fatigue syndrome

Stephan P. Keijmel

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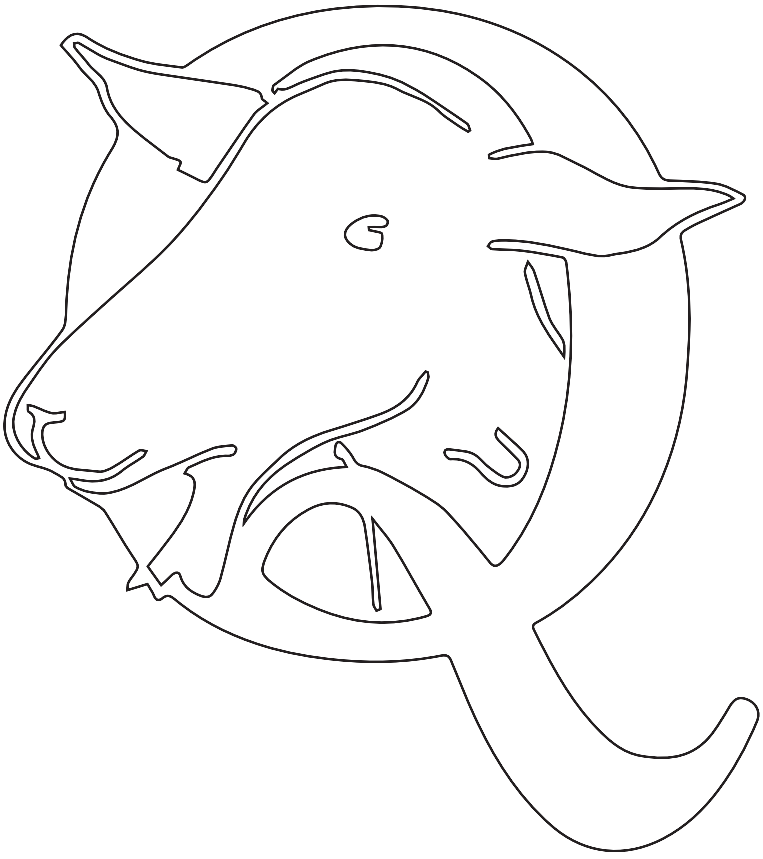
“On the difficult days, when the world’s on your shoulders,
remember that diamonds are made under the weight of mountains”
- Beau Taplin



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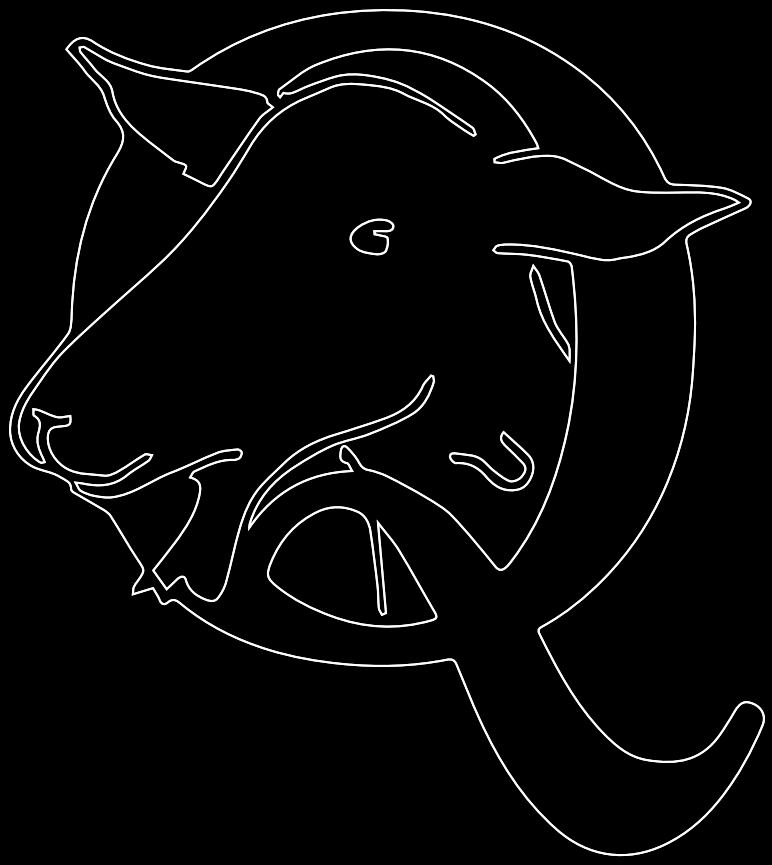
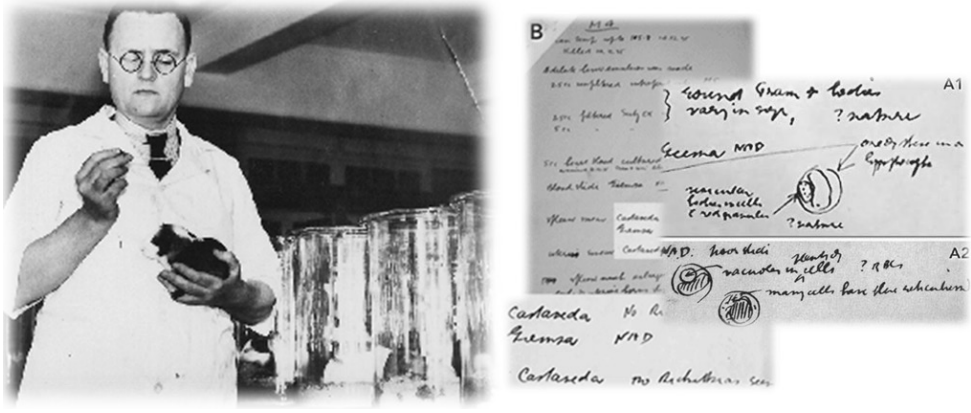


CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

GENERAL INTRODUCTION

After an outbreak of a flu-like illness among Australian abattoir workers in 1935, Edward Holbrook Derrick, who investigated the outbreak, was the first who described Q fever in 1937 [1]. As the causative pathogen of this flu-like illness was unknown at that time, the disease was called "Query (Q) fever". In subsequent years, the causative agent of Q fever was identified, and was named *Coxiella burnetii*, derived from a combination of Frank Macfarlane Burnet (Australia) and Herald Rea Cox (USA), because of their effort in the discovery of the bacterium [2, 3]. This discovery did not result in adaptation of the name of the disease, which is understandable as still several queries around this disease exist.



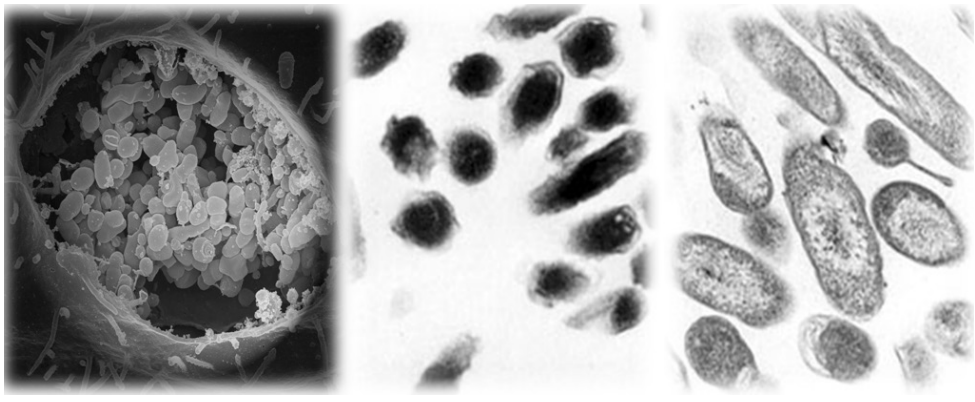
Edward Holbrook Derrick [Queensland, Australia] (left), and his first notes on *C. burnetii* (right) [4].

The causative agent of Q fever

C. burnetii, the aetiological pathogen, is a small Gram-negative intracellular coccobacillus. Even though historically classified in the *Rickettsiaceae* family, phylogenetic investigations, mainly based on 16s rRNA sequence analysis [5] and genome sequencing [6], led to the reclassification into the *Coxiellaceae* family in the order *Legionellales* of the gamma subdivision of *Proteobacteria*. *C. burnetii* exhibits a developmental cycle that contains two morphologically distinct forms, a small cell variant (SCV) and a large cell variant (LCV) [7, 8]. The SCV, a metabolically inactive, small, dense, highly resistant spore-like form, is resistant to adverse conditions that may be encountered by the pathogen while in the extracellular environment [8, 9]. Following passive entry into the host-cell, the SCV becomes located in an acidic cytoplasmic vacuole, and eventually prevents fusion with lysosomes enhancing the pathogen's survival [9, 10]. This process triggers the transformation to the LCV, which is in contrast to SCV a metabolically active, relatively fragile cell type [11]. To complete the life cycle, the bacterial population eventually transforms into SCVs, which are released upon lysis of the host cell [9]. Another essential characteristic is that *C. burnetii* displays two antigenic forms, namely phase I and phase II. Phase variation is related mainly to variation in the lipopolysaccharide (LPS) on the outer side of the membrane of the bacterium [12]. The

highly infectious phase I refers to *C. burnetii* with full-length LPS molecules with O-chains, as found in naturally infected animals, arthropods, and humans. In contrast, phase II is considered avirulent and possesses LPS with truncated O-side chains and is only obtained in the laboratory following serial passage in cell cultures or embryonated egg cultures [9, 13]. In daily clinical practice, this phase variation and the subsequent antibody response is used to differentiate between a past infection, acute Q fever, and chronic Q fever.

Q fever is a zoonosis, i.e. transmission to humans occurs through an animal reservoir, and a wide variety of animal species are reservoirs of *C. burnetii* in nature. Even though domestic ruminants (cattle, sheep, and goats) are considered as the main reservoir for the pathogen [5, 14], the bacterium has been found in a variety of other animal species [15-17]. Animals shed *C. burnetii* in milk, faeces, urine, and in birth by-products [18-21]. Especially during parturition, high amounts of bacteria enter the environment, resulting in a wind-borne spread of *C. burnetii* over a large area [22-24]. The bacterium is highly infective, as low doses already induce asymptomatic seroconversion [25]. Inhalation of contaminated aerosols are the main route of human infection [5]; however, the ingestion of contaminated dairy products has also been associated with seroconversion in humans [26]. Even though human-to-human transmission has been described [27-31], this is rarely seen and not considered to be an important route of transmission.



C. burnetii (left), the causative agent of Q fever, and the morphologically distinct forms: SCV (middle) and LCV (right). Courtesy: National Institute of Allergy and Infectious Diseases

The occurrence of Q fever in the Netherlands

After its first documentation in 1937, Q fever appeared to be common all over the world [32], except for New-Zealand [33]. For a long time, Q fever was considered an occupational disease, mainly among farmers, veterinarians, and laboratory workers. However, numerous human Q fever outbreaks have been reported in many countries [34-47], of which many were associated with livestock farming. In the Netherlands, the first three human cases of Q fever were identified in 1956 [48, 49], and it became a notifiable disease for humans in 1975

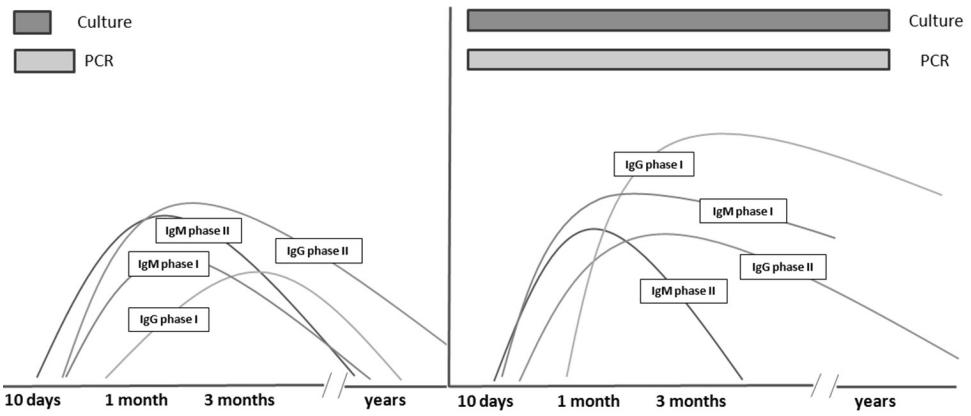
[50]. Until 2007, an annual number of human cases up to 32 per year were notified nationally [39], with an estimated seroprevalence, i.e. an indication of past infections, of 2.4% [51, 52]. From 2007 until 2010, the Netherlands experienced an exceptionally large Q fever outbreak among humans. During this period, over 4000 patients with symptomatic acute Q fever were notified, and it was estimated that at least 32,200 individuals experienced a latent infection [53, 54]. Subsequently, the seroprevalence of Q fever increased to approximately 12.2%-20.4% in 2009 [55, 56]. Preceding this major outbreak, an increased abortion rate among goats was observed in several provinces, particularly in the southern part of the Netherlands. As most patients lived in densely-populated urban areas with intensive goat farming, and abortions are accompanied by the spreading of high loads of *C. burnetii*-contaminated aerosols in the air [5, 16, 57], dairy goats were identified to be the source of the epidemic [45, 58]. Compared to 2007, outbreaks of similar size had been reported in other countries; however, the number of notifications kept increasing to 1000 and 2354 cases in 2008 and 2009, respectively [59]. To prevent further spread, drastic measures were taken, including the culling of pregnant goats and sheep at infected farms and a vaccination programme [39]. Eventually, this resulted in a massive reduction in the number of new patients from 2010 onwards. At present, a similar number of notified cases is seen as before 2007 [59].

Clinical manifestations

Acute Q fever

The incubation period ranges from four days up to six weeks [60, 61], with most cases occurring 2-3 weeks after exposure [16, 62, 63]. Infection with *C. burnetii* causes symptomatic disease in approximately 40% of all patients [5]. The presentation varies from a mild self-limiting flu-like illness to pneumonia or a hepatitis-like syndrome [57, 64, 65]. Signs and symptoms are usually non-specific and compatible with many infectious diseases, and can differ per region [5, 16, 17, 57, 61, 62]. Therefore, the diagnosis is often missed and the incidence of Q fever among humans is probably underestimated [66]. Rarely, more severe manifestations are described [62], and the case fatality rate of acute Q fever is approximately 1%-2% [16, 57, 67]. A hospitalisation rate of 2%-5% has been reported throughout literature [16, 34, 57]. In the Netherlands, however, a hospitalisation rate of 50% was registered in 2007, which stabilized around 20% in the years after [68]. Diagnosis is mainly based on (recognizing) the clinical presentation in combination with laboratory test results. In case of suspicion of acute Q fever, it is recommended to perform polymerase chain reaction (PCR) and serological evaluation [69]. *C. burnetii* DNA can be detected in serum in the early acute phase of disease using PCR [70]. However, the sensitivity of PCR in detecting DNA decreases when antibodies against *C. burnetii* develop, if no development to chronic Q fever occurs. Antibodies appear in the first two weeks after the initial symptoms [61, 62]. Several serological techniques exist to diagnose acute Q fever of which immunofluorescence assay (IFA) is the reference method, but other suitable techniques are available, such as complement fixation test (CFT) and enzyme-linked immunosorbent assay (ELISA) [62, 71]. In the classical serological response of acute Q fever, antibodies directed against phase II antigens are first detectable, shortly thereafter followed by antibodies against phase I. In general, antibodies to phase

II predominate in the acute stage of disease and after convalescence from acute Q fever without signs of chronic infection, whereas high levels of IgG antibodies to phase I more than 3 months after the primary infection are found in chronic Q fever [62, 72]. Besides a positive PCR in the early stage of disease, the diagnosis of acute Q fever can made using two serum samples with an interval of at least two weeks, showing seroconversion or a fourfold rise in antibodies. Although IgG antibodies tend to be more persistent than IgM antibodies, all frequently persist for months to even years after the initial infection [73, 74].



Reproduction of the antibody responses (IFA), culture, and PCR in acute Q fever (left) and chronic Q fever (right)

Treatment should be started as soon as Q fever is suspected. Although *C. burnetii* is known for its self-limiting character, treatment of the acute infection decreases the duration of fever [75], reduces the risk of hospitalisation [76], and shortens the recovery period from pneumonia [77]. The treatment of choice in the acute setting is doxycycline 200 mg/day for 2-3 weeks [76], whereas moxifloxacin 400mg/day for 2-3 weeks is advised in case of doxycycline intolerance [78]. Most patients with symptomatic acute Q fever recover completely with only a serological scar left, but infection with *C. burnetii* is notorious for causing long-term sequelae, i.e. chronic Q fever and Q fever fatigue syndrome (QFS). In case of clear risk factors for development of chronic Q fever, prophylactic treatment might prevent persistent infection [79-81].

Chronic Q fever

Following initial infection, chronic Q fever develops in 1%–5% of *C. burnetii*-infected patients [5, 82], and is characterised by the persistence of viable *C. burnetii*. Most patients do not recall an acute Q fever episode, indicating that asymptomatic primary infections can also result in development of chronic Q fever [83]. It mostly manifests within the first year following infection, but the disease can also present itself several years later [79, 82, 84]. Chronic Q fever usually develops insidiously and most patients are asymptomatic or report only non-specific symptoms such as low-grade fever, night sweats, and weight loss [5, 84,

85]. Frequently, this causes a delay in diagnosis with subsequently a more severe clinical presentation at diagnosis [65, 86]. Chronic Q fever presents mainly as vascular infection [83], including mycotic aneurysms and infections of vascular prosthesis, and endocarditis [17], followed by less frequently reported manifestations such as osteomyelitis, pericarditis, and hepatitis [85]. Clear risk factors for the development of chronic Q fever are heart valve pathology, including valve prostheses and pre-existent valvulopathy, vascular prostheses, and aneurysms [65, 79, 87, 88]. Other factors that might be associated with an increased risk are immunosuppresion, older age, pregnancy, and (mild) renal insufficiency [46, 57, 87]. Diagnosing chronic Q fever has proven to be challenging. Routine blood cultures remain negative. In addition, culturing *C. burnetii* is difficult and time-consuming, requires a level 3 biosafety laboratory, and lacks sensitivity [89]. Both serology and DNA detection in blood or tissue using PCR aid the laboratory diagnosis of chronic Q fever [82]. A positive PCR or culture of *C. burnetii* in blood or tissue, in the absence of a serologic profile for acute Q fever, is considered diagnostic for chronic Q fever, although sensitivity of these techniques is low [83, 90]. Serological analysis is therefore essential. Because chronic Q fever is characterized by persistent high titres of IgG antibodies against *C. burnetii* phase I antigens [57, 91], the IgG phase I titre is used as standard for the serological diagnosis of chronic Q fever. The cut-off titre depends on the used method, and varies between in-house-developed IFA and commercially available IFA [72, 82, 92]. However, in case of absence of PCR positivity, serology alone is insufficient for diagnosing chronic Q fever, and clinical data should be included [93]. Furthermore, localisation of infectious foci is important, because, in addition to prolonged antimicrobial therapy, adjuvant therapeutic measures such as surgical drainage or graft replacement are often necessary [85, 94, 95]. In conclusion, the diagnosis currently relies on a combination of symptoms, risk factors, microbiological findings, and imaging techniques. Long-term antibiotic treatment, preferably doxycycline combined with hydroxychloroquine, for at least 18-24 months, sometimes in combination with surgery, is necessary to reduce morbidity and mortality, which is up to 60% of patients if left untreated [88, 96]. However, even in case of adequate antibiotic treatment, chronic Q fever still has a high case fatality rate [83]. In addition, the antibiotic treatment itself sometimes causes mortality [83], and at least frequently causes important side effects, including gastrointestinal complaints and severe photosensitivity.

QFS

This thesis especially focuses on QFS, occurring in approximately 20% of cases following a symptomatic acute Q fever infection. In contrast to chronic Q fever, which also occurs after asymptomatic *C. burnetii* infection, no viable *C. burnetii* is present. Already in 1960, fatigue was notified as complaint following acute Q fever [97]. However, it was until 1992 before the first reference to QFS, referred to as “post Q fever fatigue syndrome”, appeared in the scientific literature [98]. Ever since, QFS has been recognised and described all over the world [99-103]. Following the major Q fever outbreak in the Netherlands, several reports were published showing a high rate of severe fatigue and decreased health status in the years after infection [104-107]. Although the existence of QFS is debated by some [108], and fatigue following infection with *C. burnetii* might not be specific compared to fatigue

following other infectious diseases, it occurs frequently and has important clinical and economical consequences. Therefore, this sequel should be taken seriously, as it has major implications for both patients and treating physicians [109, 110], especially in the case of an outbreak. Subsequently, QFS appeared to be the major cause of the Q fever-related economical burden of the Dutch outbreak [111]. At present, Q fever is endemic almost all over the world, and it can be anticipated that new outbreaks will occur in the future, leading to a growing number of patients with long-term sequelae. Like in chronic fatigue syndrome (CFS) and patients with fatigue following Lyme disease, a vast medical consumption can be anticipated in the absence of an accessible and effective intervention and clear guidelines. With an increasing number of QFS patients in the aftermath of the outbreak, and the societal need for uniform criteria for the syndrome, a national guideline on QFS was formulated and published in 2012 [112]. However, several knowledge gaps existed and still exist with regard to QFS. Despite the lack of a formal comparison, this consensus guideline was therefore partly based on the diagnosis and treatment of CFS, as QFS and CFS at least partly overlap in symptoms. Furthermore, as for other forms of chronic fatigue [113], patients frequently report accompanying symptoms [98, 114, 115]. According to the Dutch guideline, the diagnosis of QFS can be made after a uniform diagnostic work-up, and the definition comprises a severe fatigue related to an acute Q fever infection, which lasts for at least six months and causes significant disabilities in daily functioning. The fatigue should be of new onset or should increase significantly due to the acute Q fever infection. Finally, chronic Q fever and other causes of fatigue, somatic or psychiatric, need to be excluded [112]. However, international consensus has not been reached yet. Although QFS increasingly received attention in previous years, the underlying pathophysiological mechanism remains to be elucidated, hampering treatment based on aetiological insight. Several hypotheses regarding the aetiology of QFS exist [74, 103, 116-118], all requiring further confirmation as contradictory results have been published. Also evidence-based information concerning the treatment of QFS patients is lacking, as no randomised controlled trials have been done. The published reports concerning treatment of QFS included mostly patients without clear QFS definition, and are mostly case-reports or suffer from other major limitations [102, 103, 119-121], limiting the extrapolation of findings. Finally, information on prevention and prognosis is underrepresented in the international literature. The Q fever outbreak in the Netherlands provided a unique opportunity to investigate QFS more thoroughly. The studies described in this thesis contribute to the knowledge on QFS and challenges in both acute and chronic Q fever, and will hopefully lead to improvement of clinical care for Q fever patients, especially for those with QFS.

OUTLINE OF THE THESIS

The primary aim of this thesis was to increase the recognition of QFS, to reveal new insights in the pathophysiology of QFS, and to evaluate the efficacy of treatment with cognitive behavioural therapy and long-term doxycycline (*part I*). A secondary aim of this thesis was to investigate diagnostic and treatment challenges in both acute and chronic Q fever (*part II*).

In order to perform research into aetiology and treatment, awareness and recognition of QFS is mandatory. *Part I* of this thesis starts with **chapter 2**, which contains a systematic review of the available literature regarding fatigue following acute Q fever. In **chapter 3**, a comparison is made between QFS patients and CFS patients, with a focus on inflammatory markers and possible fatigue perpetuating cognitions and behaviour. In **chapter 4** the question was addressed whether there is an aberrant antigen-specific IFNy-production and IFNy/IL-2 ratio in QFS patients. This might provide insight in the potential pathophysiological mechanisms underlying this debilitating long-term complication, which remain unclear at present. Furthermore, it is still unclear whether effective treatment for QFS is possible. **Chapter 5** contains the study protocol to assess the efficacy of both cognitive behavioural therapy and long-term doxycycline in QFS patients. The results of this randomised placebo-controlled trial (the Qure study) are presented in **chapter 6**.

Part II of this thesis starts with the challenge of differentiating acute Q fever from other pathogens in patients presenting to hospitals, described in **chapter 7**. Furthermore, outcome of patients hospitalised with acute Q fever was evaluated, and the effect of prophylactic treatment for those patients with an indication to prevent development of chronic Q fever was analysed. Another challenging query is to localise the infection in case of chronic Q fever. In **chapter 8**, the value of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET/CT) and echocardiography in detecting the localization of infection in chronic Q fever patients was evaluated. Once chronic Q fever has been diagnosed, it often requires intensive and prolonged antibiotic treatment, which frequently causes serious side effects. In **chapter 9**, a series of patients is described with treatment-induced cutaneous hyperpigmentation, a relatively rare phenomenon. But even in case of adequate treatment, chronic Q fever remains an unpredictable disease with a high mortality rate. In **chapter 10**, the severity of this disease and the diversity of signs and symptoms that may occur is underlined, in which a fatal case of an immunocompromised patient with an unusual disseminated chronic Q fever infection is described.

In **chapter 11**, a general discussion and future perspectives are provided, followed by the summary and conclusions in **chapter 12** (English) and **chapter 13** (Dutch).

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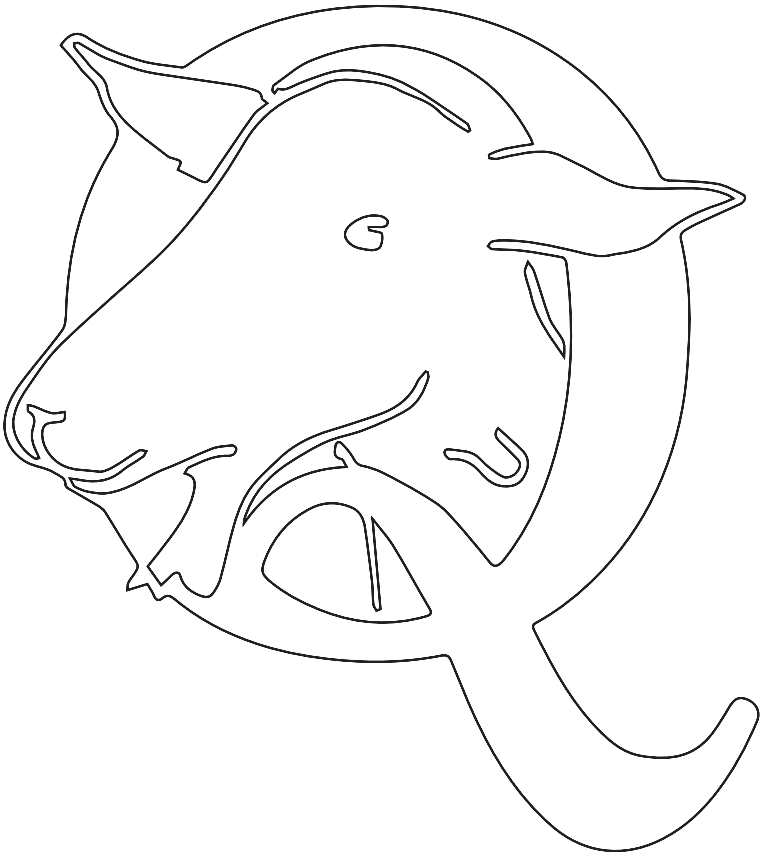
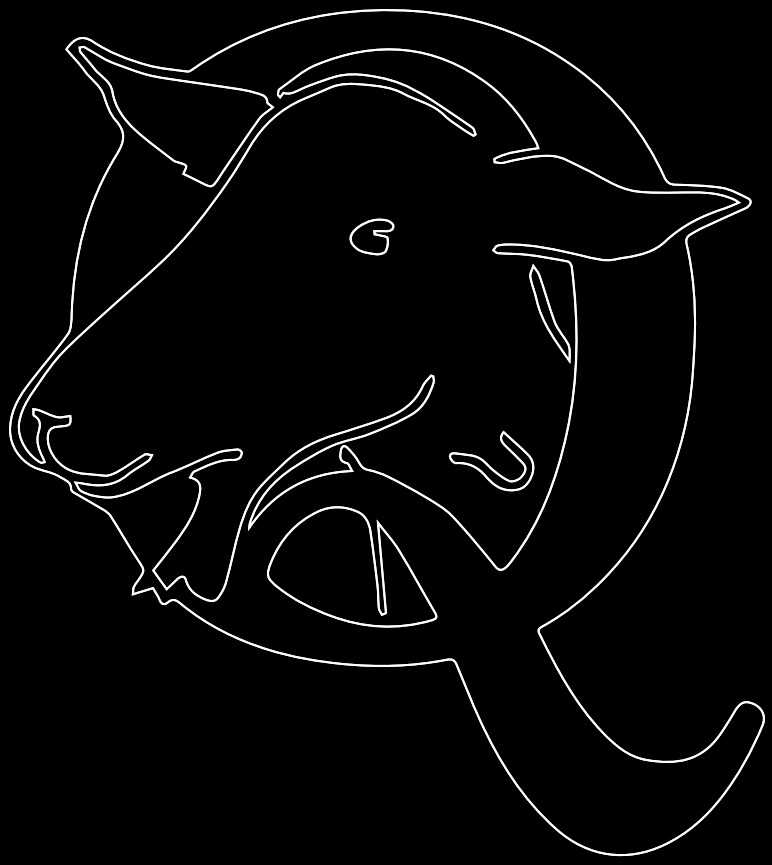


PART I

RECOGNITION AND TREATMENT OF Q FEVER FATIGUE SYNDROME (QFS)



CHAPTER 2

FATIGUE FOLLOWING ACUTE Q-FEVER: A SYSTEMATIC LITERATURE REVIEW

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ABSTRACT

Background: Long-term fatigue with detrimental effects on daily functioning often occurs following acute Q-fever. Following the 2007-2010 Q-fever outbreak in the Netherlands with over 4000 notified cases, the emphasis on long-term consequences of Q-fever increased. The aim of this study was to provide an overview of all relevant available literature, and to identify knowledge gaps regarding the definition, diagnosis, background, description, aetiology, prevention, therapy, and prognosis, of fatigue following acute Q-fever.

Design: A systematic review was conducted through searching Pubmed, Embase, and PsycInfo for relevant literature up to 26th May 2015. References of included articles were hand searched for additional documents, and included articles were quality assessed.

Results: Fifty-seven articles were included and four documents classified as grey literature. The quality of most studies was low. The studies suggest that although most patients recover from fatigue within 6-12 months after acute Q-fever, approximately 20% remain chronically fatigued. Several names are used indicating fatigue following acute Q-fever, of which Q-fever fatigue syndrome (QFS) is most customary. Although QFS is described to occur frequently in many countries, a uniform definition is lacking. The studies report major health and work-related consequences, and is frequently accompanied by nonspecific complaints. There is no consensus with regard to aetiology, prevention, treatment, and prognosis.

Conclusions: Long-term fatigue following acute Q-fever, generally referred to as QFS, has major health-related consequences. However, information on aetiology, prevention, treatment, and prognosis of QFS is underrepresented in the international literature. In order to facilitate comparison of findings, and as platform for future studies, a uniform definition and diagnostic work-up and uniform measurement tools for QFS are proposed.

INTRODUCTION

Q-fever, caused by the Gram-negative intracellular coccobacillus *Coxiella burnetii,* is a zoonosis that occurs worldwide [1]. Between 2007 and 2010 the largest Q-fever outbreak ever described in the literature occurred in the Netherlands, resulting in 4107 notifications [2].

Fatigue following acute Q-fever, also referred to as Q-fever fatigue syndrome (QFS), has been described worldwide in up to 20%-30% of patients [3-8] and may last up to ten years or longer [7, 9]. Although some debated the term QFS [10], it has been frequently used throughout literature. QFS patients experience an impaired health status, pulmonary disorders, and impairment of general and social functioning [3, 7-9, 11, 12], and QFS accounted for major Q-fever-related economic cost during the Dutch outbreak [13]. Therefore, although not always recognised as a (diagnostic) problem, this sequel has major implications. The word "syndrome" refers to other frequently accompanying nonspecific symptoms [3, 8, 9, 14] resembling chronic fatigue syndrome (CFS) [15, 16]. However, in CFS the cause is usually unknown, while in QFS a *C. burnetii* infection can be identified as the trigger. Furthermore, QFS has a sudden onset of fatigue, while in CFS this is often not the case. Several queries regarding QFS without clear answers exist. A uniform international definition is not available, and tools to assess this syndrome and its consequences vary [5, 6, 17]. Hypotheses on aetiology appear contradictory [18], and vary from altered cytokine production [6, 19], development of symptoms determined by host and genetic factors [19-21], to the perpetuation of symptoms due to psychogenic factors and behaviour [8]. Furthermore, opinions on possible treatment of QFS differ [5, 6, 17], and questions exist regarding prevention and prognosis.

The aim of this first systematic review regarding fatigue after acute Q-fever in humans is to provide an overview of all relevant available literature, and to identify knowledge gaps regarding the definition, diagnosis, background, description, aetiology, prevention, therapy, and prognosis. This provides an evidence map both for physicians and patients.

METHOD

Search strategy and selection criteria

Relevant articles were identified through a systematic literature search in the scientific databases Medline, Embase and PsycInfo up to the 26th of May 2015 (*Table 1*). As Pubmed was used to search in Medline, only Pubmed is mentioned in this article. There were no restrictions on year of publication, language, and article or study type. Abstracts without full-text were excluded, as well as non-human studies. During the first selection step, potentially relevant references were selected based on screening of titles and or abstracts by two investigators independently (GM and SPK, both content area experts). Potentially relevant articles were included for full-text assessment. Articles on fatigue following acute Q-fever that could provide information on the following domains: diagnosis (i.e. definition and/or diagnosis), background/descriptive (i.e. incidence, prevalence, the course of fatigue and the role of co-morbidity, and other complaints besides fatigue), aetiology (i.e. pathophysiology, predictors), prevention/therapy, and prognosis, were selected.

During the full-text assessment, articles without original or relevant data were excluded, upon an independent decision of each investigator, followed by consensus if needed. In case of any disagreement, the verdict of a third independent investigator was conclusive. If GM or SPK was a (co-)author of a potentially relevant article, a third independent investigator assessed and decided (both selection steps) on inclusion. GM and SPK translated non-English articles, if needed, native speakers where sought. If native speakers were unavailable, the corresponding author was contacted. If this yielded no response, the article was excluded. Reference lists of included full-text articles were hand searched for additional relevant publications. If the title (or keyword in the title) suggested potential information on the topic, retrieval and full-text assessment followed. Finally, the World Health Organization, Centres for Disease Control and Prevention (CDC), Queensland Health, and gov.uk websites were searched for guidelines. Documents with relevant information that were identified during the search, but not classified as peer-reviewed articles, were included as grey literature.

Table 1. Search strategy used in Pubmed, Embase, and PsycInfo.

Pubmed	***Search terms*** †	***Hits***
6-5-2014	("coxiella burnetii" OR "Q fever" OR "coxiella" OR "Q-fever" OR "rickettsia burnetii" OR "rickettsia burnetti" OR "rickettsiosis infection" OR "rickettsiosis rickettsia" OR "australian Q fever")	
	AND	
	("fatigue" OR "syndrome" OR "Q fever Fatigue Syndrome" OR "Q-fever Fatigue Syndrome" OR QFFS OR QFS OR persisten* OR progress* OR "long term" OR "long-term" OR consequence* OR "chronic fatigue" OR tired*)	**494**
26-5-2015		**537**
Embase	***Search terms*** †	***Hits***
6-5-2014	(exp Q fever/ OR Q fever.tw. OR exp Coxiella/ OR coxiella.tw. OR rickettsia burnetii.tw. OR rickettsiosis.tw.)	
	AND	
	(exp fatigue/ OR exp Fatigue Impact Scale/ OR exp chronic fatigue syndrome/ OR exp Fatigue Severity Scale/) OR fatigue.tw. OR QFFS.tw. OR QFS.tw. OR exp persistent infection/ OR (persistence or persistent).tw. OR (progression or progressive or consequence or consequential).tw. OR exp chronic fatigue syndrome/ OR (tired or tired' or tiredeness or tireding or tiredness or tiredness).tw.	**440**
26-5-2015		**489**
PsycInfo	***Search terms***	***Hits***
6-5-2014	(Q fever OR coxiella OR rickettsia burnetii OR rickettsia burnetti OR rickettsiosis OR rickettsiosis rickettsia)	**15**
26-5-2015		**18**

Literature search performed on 6th May 2014, updated on 26th May 2015, using the same search terms as in the first search.

† Excluded from the search: Mesh term for rickettsiosis, as this labels for several typhus infections with a total hits of 15600 records; and the word 'chronic', to avoid inclusion of chronic Q-fever articles.

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Quality assessment

The methodological quality of case-control and cohort studies was assessed with the Newcastle-Ottawa Scale (NOS) [22], that evaluates selection (maximum of 4 stars), comparability (maximum of 2 stars), and outcome (maximum of 3 stars). For economic evaluations, the 'Evers checklist' was used [23]. Case-series were assessed with a quality appraisal tool with 18 criteria. A score of ≥14 criteria (≥70%) was considered acceptable [24]. No specific instruments exist to assess the quality of case-reports, which in general is considered to have a low level of evidence. Therefore, the quality was assessed with a method based on the Coordination of Cancer Clinical Practice Guidelines in Europe (CoCanCPG), addressing eight criteria: an appropriate and clearly focused question, representative population, description of the survey method or data collection, outcome measures defined and described, response rate reported, and results valid and applicable to the targeted patient group. Articles could score: -/-, -, +/-, +, or ++ on these items. Although personal opinions were included to obtain a complete overview of all literature, these were not quality assessed as in general the quality is considered low.

Data extraction and presentation

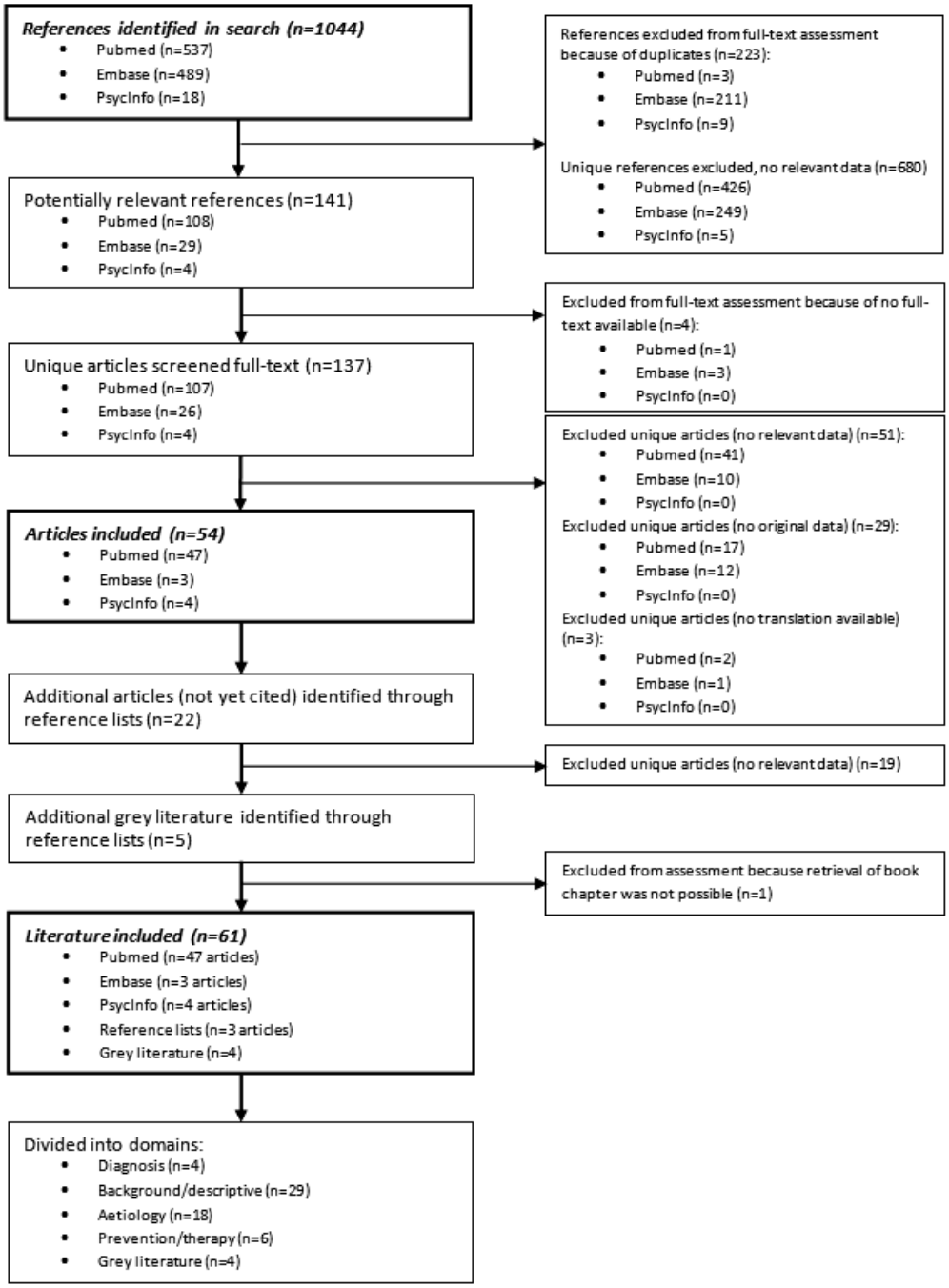
Study populations and definitions per included article were summarised in a separate table (*S1 Table*). Included articles were summarised in main domain tables: diagnosis, background/descriptive, aetiology, prevention/therapy, and prognosis (*S2-S5 Tables*). If articles contained additional information on other domains, this was noted in the main table. The following information was provided per article, if applicable: year of publication in chronological order starting with the oldest articles; first author; country; year of the study; study period and duration; study type; number of patients and controls; patient characteristics; co-morbidity; outcome measurement tools; intervention(s); outcome; conclusion(s)/recommendation(s); and the quality of the article. In case an article could not be assessed with any of the mentioned tools, this was stated in the table in column quality assessment (QA) as not applicable (NA). Grey literature was similarly ordered in a separate table (*S6 Table*).

RESULTS

Inclusion of articles

The search yielded 1044 references (*Fig 1*); Pubmed n=537, Embase n=489, PsycInfo n=18, of which 223 were duplicates. During the first selection phase, 680 references were excluded as not relevant, 141 identified as potentially relevant, and the full-text articles were searched. One full-text article (Spanish) could not be obtained from three different libraries and as the author could not be reached, the article was excluded. Three conference abstracts without full-text article were excluded. Of the remaining 137 full-text articles, 51 articles were deemed not relevant, 29 had no original data, and for three no translation was available (two Russian, one Japanese). The remaining 54 articles were included and hand searching their reference lists yielded 22 potentially relevant articles, of which three were included after full-text assessment. From the reference lists of included articles, we identified one guideline, one dissertation, two book chapters, and one economic report. After confirmation of relevance, these were included as grey literature except for one book

Figure 1. Flow diagram of identified literature.



chapter as retrieval was not possible. In total, we included 57 articles and four grey literature documents.

Classification in domains

The 57 included articles were classified into one of the main domains: diagnosis (n=4, *S2 Table*), background/descriptive (n=29, *S3 Table*), aetiology (n=18, *S4 Table*), and prevention/therapy (n=6, *S5 Table*). As none of the included articles described the course of fatigue in QFS, no articles were classified into the domain prognosis. Grey literature (n=4) is presented in *S6 Table*.

Quality of included literature

From the four articles in the table diagnosis, one article was assessed with the NOS and scored 4/9 possible stars [25]. The remaining items (five stars) could not be assessed, as these items were not applicable for this study. The other three articles were personal opinions [10, 26, 27].

The quality of 21/29 articles in the domain background/descriptive was assessed with the NOS. Most articles had a moderate quality; however, none scored on all specific applicable criteria, mostly because of inadequate controls in the design or analysis. For four articles, not all items could be assessed, as these were not applicable for these studies. The quality of three case-reports (n=1) was low [28-30]. The quality of one study regarding burden of disease was not assessed [31], as no standard quality assessment checklist was available for this study category. One economic evaluation scored well (16/19) [32]. Two articles were personal opinions [33, 34], and one was a personal observation [35].

The quality of 15/18 articles on aetiology was assessed with the NOS. Although none scored on all specific applicable criteria, the quality of the articles was considered moderate. Seven articles did not score on comparability although applicable, as they lacked a correction for other factors that might explain the outcome. For four articles, not all possible stars could be retrieved, as these items were not applicable for these studies. Two laboratory case studies were not quality assessed [36, 37], and one article was a personal opinion [38].

The quality of 2/6 prevention/therapy articles was assessed with the NOS. One study scored 4/9 stars, but none on comparability [39], while the other scored on 4/5 applicable items [6]. The quality of two case-reports (n=1) [40, 41] was below average, as was that of the case-series (n=3) [5], that scored on only 9/18 criteria. One article, a study protocol, was not quality assessed [42]. The Dutch QFS guideline was developed based on the AGREE criteria [43], and therefore considered to be of good quality [17]. The quality of the other grey literature was not assessed.

Definition and diagnosis

Nineteen articles contained information on diagnosis of which four were classified in the main table diagnosis (*S2 Table*) [10, 25-27].

Terminology

The name QFS was introduced in 1992 [44]. Ever since, it has been debated whether fatigue

following acute Q-fever is a separate entity compared to other forms of post-infective fatigue or CFS [27]. Some argue that chronic fatigue is a non-specific subjective state or symptom after Q-fever rather than a diagnosis [27]. Other consider QFS as a description of CFS implicating a specific micro-organism, and that this terminology might result in increased health-care costs [10]. Others stated that due to convincing evidence of a causal factor, QFS is a causally-defined subset of CFS, and that this factor should take precedence in the diagnostic statement [26]. Names used to indicate fatigue following acute Q-fever, include: residual asthenia following Q fever [38], postinfective fatigue or postinfective fatigue syndrome [10, 12, 18, 31, 45-47], postinfectious chronic fatigue [11], post-Q-fever debility syndrome [35], post-Q-fever chronic fatigue syndrome [35], qCFS [36], Q fever induced chronic fatigue syndrome [48], post-Q-fever fatigue or post-Q-fever fatigue syndrome [36, 49], post-(acute) Q-fever (fatigue) syndrome [5, 14, 26, 28, 33, 50], and most frequently Q-fever fatigue syndrome (QFS or QFFS) [6, 8, 10, 19-21, 26, 30, 33, 36, 39, 42, 50-52].
In conclusion, the term QFS has been used for years and seems generally accepted.

Definition of QFS
An overview of the study populations and definitions used is provided for articles (*S1 Table*) and grey literature (*S6 Table*). Seven articles lacked a definition of the study population or of QFS [10, 26, 27, 33-35, 38]. In 32 articles the study population was defined but QFS was not [3, 7, 9, 11, 12, 14, 18, 25, 31, 32, 36, 37, 45-47, 49, 52-67]. In five articles individual patients were considered to have QFS, without providing a definition [5, 28-30, 40]. Six articles provided a definition of QFS [6, 8, 19, 39, 42, 48], which has been used in articles in subsequent years [20, 21, 50, 51]. A detailed description of QFS is published in a thesis [44], but is based on a retrospective comparative-cohort study and is not available online. In the Dutch QFS guideline [17], QFS is defined as: a severe fatigue causing significant disabilities in daily life present for at least 6 months, with a temporal relationship with acute Q-fever, and not caused by co-morbidity. Fatigue should be absent before acute Q-fever or should have significantly increased since the infection.
In conclusion, there is no international uniform definition for QFS.

Diagnosis
No articles provided complete information on the diagnostic work-up. The Dutch guideline on QFS bases diagnosis on a combination of history, physical examination and laboratory examination excluding other causes of fatigue, and should at least include erythrocyte sedimentation rate, C-reactive protein (CRP), creatine kinase, thyroid stimulating hormone, leukocytes with differentiation, creatinine, alkaline phosphatase, alanin aminotransferase, calcium, glucose, ferritin, and a urinary sediment. Through the use of validated questionnaires fatigue severity should be objectified. Morbid obesity (BMI>40) and substance abuse should lead to refraining from diagnosing QFS. It is not possible to diagnose QFS in case of: depression (if this preceded current symptoms), schizophrenia, psychosis, dementia or eating disorders (unless already resolved for a minimum of 5 years) [17].
In conclusion, the Dutch guideline on QFS provides a clear diagnostic work-up.

Background/descriptive

Of the 40 articles containing background/descriptive information, 29 were classified in the main table background/descriptive (*S3 Table*) [3, 7-9, 11, 12, 14, 28-35, 52, 53, 56-59, 61, 62, 64-69].

Incidence and prevalence of fatigue following C. burnetii infection

Fatigue following acute Q-fever was first described in 1960 [68]. Without indicating a time-relation with acute Q-fever, it was noted in 1990 that 4% of acute Q-fever cases had prolonged fatigue [53]. In 1992, it was stated that approximately 23% of study subjects developed QFS within 12 months following acute Q-fever [44]. Ever since, several studies on fatigue following acute Q-fever reported different prevalences. It was stated that 5-10% of patients experience residual asthenia six months after acute Q-fever and only few after one year [38]. In a reaction, it was underlined that a substantial proportion of acute Q-fever patients have symptoms similar to QFS for 6-9 months after the acute infection and then recover, but 8-10% of patients exhibit symptoms for at least a year [33]. This is similar to other reports, showing persistent symptoms for longer than two years [3], up to six years after the infection with 66% of patients reporting fatigue [14]. In Australia, QFS is the most common sequel of acute Q-fever reported to affect 10-15% of patients [70]. Higher percentages were described, with up to 28% of patients meeting the Centres for Disease Control and Prevention criteria for CFS 5 to 14 years after acute Q-fever, compared to none in the control group [8, 15]. The highest percentage of reported fatigue was 69% five years after acute Q-fever [9]. CFS criteria were met by 42% of *C. burnetii*-infected patients and 26% of controls [9, 15]. Ten years after acute Q-fever, 68% of patients reported fatigue of any duration [54], of whom 20% met the CFS criteria [15]. Excluding co-morbidity, 8% of patients met the CFS criteria compared to none of the controls [54]. *C. burnetii*-exposed compared to non-exposed subjects reported ten years later a fatigue prevalence of 65% vs. 35%, respectively, and 19% vs. 4% met the CFS criteria [7, 15]. In accordance, later results demonstrated fatigue to be more common after Q-fever compared to controls [58], up to two [61] and six years later [49, 69].

Post-infective fatigue following *Epstein-Barr virus*, *Ross River virus* or *C. burnetii* infection, was reported in 35% of cases after six weeks, 27% after three months, 12% after six months, and 9% after 12 months, regardless of the infective agent [12]. And, although not significantly different, 12 months after acute Q-fever, patients were more fatigued than after Legionnaires' disease, while being younger and having less pre-existing health problems [11]. In patients with a lower respiratory tract infection who were *C. burnetii* seropositive 10-19 months after the acute illness, 40% reported clinically relevant fatigue, compared to 64% of seronegatives, concluding that patients have long-term health problems after a lower respiratory tract infection in general [64].

In conclusion, fatigue following acute Q-fever might not be specific but occurs frequently and may persist for years. A large variance in prevalence of fatigue after Q-fever is reported between countries, due to differences in definitions, study designs and populations, and measurement tools, which impairs direct comparisons.

Health status, burden of disease and economic impact

A sustained decrease in health status or health-related quality of life was reported [3, 58, 61]. Twelve months after acute Q-fever, 50% of patients had a reduced general quality of life [11]. Other studies show a significant linear improvement in health status after acute Q-fever, but it was still reduced after 24 months in more than one third of all patients [67]. Twenty-seven months after acute Q-fever, 52% of patients reported persistent symptoms and lower scores on 5/8 Short Form 36 (SF-36) scales [71] compared to uninfected controls [3]. Four years after acute Q-fever, patients also had a significantly reduced health status compared to healthy controls [65]. To obtain a detailed overview of the patients' health, a combination of the complete Nijmegen Clinical Screening Instrument (NCSI) [72] with subdomains (Role Physical, Bodily Pain, Social Functioning, and Role Emotional) of the SF-36 was advised [25]. Two studies focus on the burden of disease of fatigue following acute Q-fever [31, 32], one also assessed the economic impact of the outbreak in the Netherlands [13]. In 1992, for Australian *C. burnetii*-infected abattoir workers the costs per year for medical care and loss of wages for endocarditis and for QFS were calculated [44]. QFS represented the largest burden of disease [32, 44]. Furthermore, others found that, although the number of disability adjusted life years was higher for influenza, on a per case basis, Q-fever was more severe, and overall the burden of disease was more than eight times higher than for influenza, due to long-term sequelae [31]. The estimated income loss was largest due to the accumulation over time as a consequence of the projected duration of sick leave, and QFS was estimated to be one of the major Q-fever-related economic cost during the Dutch outbreak [13].

In conclusion, there are clear indications that fatigue following acute Q-fever results in a high burden of disease, a major negative impact on the health status of patients, and has significant economic implications.

Work-related consequences

In 1960, it was noticed that the majority of acute Q-fever patients recovered within weeks and returned to work [68]. However, this convalescence period was prolonged in 25% of cases who were absent from work for more than 6 weeks, 20% longer than 8 weeks, up to 23 weeks [68]. The mean period of sick-leave increased with age [68]. Later studies revealed that following acute Q-fever, 40% of patients were absent from work for more than one month [62]. After 12-26 months 9% was unable to function at premorbid levels due to fatigue and diminished concentration while more than 30% had not fully resumed daily activities, in 81% due to fatigue [62]. Besides work-related consequences, patients were more likely to report functional impairment in performing daily activities than healthy controls [46]. Q-fever patients showed a reduced work participation, from 45% after three months to 19% after 12 months, versus 15% of patients with Legionnaires' disease after 12 months [66]. Factors associated with reduced work participation were: having symptoms; a higher level of sorrow; being a former smoker (compared to never smoking); not consuming alcohol; and receiving treatment for health-related effects of Q-fever [66].

In conclusion, the majority of patients return to work within the first 12 months after acute Q-fever, although up to 20% reported reduced work participation.

Course of fatigue following acute Q-fever and the role of co-morbidity
Following acute Q-fever, 69% of patients self-reported fatigue, which dropped to 52% at six months to 26% at 12 months [57]. Studies using the NCSI found that severe fatigue following acute Q-fever improved from 73% at three months, to 60% at 12 months [11, 67]. Twelve to 26 months after acute Q-fever up to 59% of patients reported fatigue of which 44% had severe fatigue [59], whilst after 24 months 37% of patients compared to 3% of healthy controls, reported severe fatigue [67]. Higher rates of 51% were described four years after infection [65]. Most articles describe a continuous fatigue syndrome, up to 74 months after the initial infection [19], while relapsing or remittent fatigue patterns also seemed to occur [3], up to 57 months [19] after acute Q-fever. One article reported a fatigue free period of 2-4 months after acute Q-fever, eventually followed by QFS [5]. A disease period up to 20 years has also been reported [44]. Pre-existing health problems were associated with a long-term reduced health status including fatigue [59, 62, 67].
In conclusion, the percentage of patients who experience severe fatigue following acute Q-fever slowly decreases over time, mainly in the first 6-12 months. Fatigue remains a persistent complaint in approximately 20% of patients, with varying percentages and variability in the reported course of fatigue following acute Q-fever, and may persist for up to 20 years.

Complaints besides fatigue
QFS is frequently compared to CFS, and patients who fulfil the international CFS criteria by definition have multiple symptoms [15, 16]. The mean number of symptoms was higher in Q-fever exposed subjects 10 years after exposure compared to controls [7]. Patients with post-infective fatigue, including Q-fever-related post-infective fatigue, reported more symptoms in general and fatigue-related symptoms in particular [46]. Twelve to 26 months after acute Q-fever 40% of patients reported additional complaints [62]. An overview of frequently reported complaints besides fatigue after acute Q-fever is given below.
Musculoskeletal complaints. Myalgia and arthralgia were frequent complaints of patients considered to have QFS [5, 6, 17, 28, 39, 40, 44, 70]. Musculoskeletal pain accompanied fatigue 12 months after several infections [12], and was associated with a higher age [18]. Myalgia was significantly more often present 5-14 years after acute Q-fever compared to controls [8]. Twelve to 26 months after acute Q-fever, 4% of patients reported myalgia [62]. Myalgia was a major complaint in 23% of working patients 12 months after acute Q-fever [66]. Arthralgia was reported by 69% of patients up to six years after acute Q-fever [14], and was more severe compared to controls [9]. Both myalgia and arthralgia were also described in up to 70% of patients after a laboratory documented *C. burnetii* infection [52]. Compared to controls, presumed QFS patients had a higher pain score [48].

Neurocognitive problems. Although some authors found no association between *C. burnetii* seropositivity and concentration difficulties [56], neurocognitive difficulties were described in patients with post-infective fatigue, including QFS patients, 12 months after primary infection [12]. In addition, older subjects reported more neurocognitive symptoms [18]. Twelve to 26 months after acute Q-fever, 4% of patients had difficulties concentrating [62].

Concentration and memory problems were also shown to be a major complaint in 24% of working Q-fever patients 12 months after the infection [66]. Although no difference was found in the frequency of memory problems between cases and controls, the severity was significantly higher after Q-fever [9]. A lack of concentration and short memory impairment within a year following acute Q-fever was also reported [17, 44], while another study found decreased concentration and mental acuity that could last up to 5-10 years [70].

Sleeping problems. Six years after acute Q-fever, 65% of patients reported a disturbed sleep pattern, which was significantly more frequent than in controls [14]. This was also reported by others [17, 29, 44, 70], including unrefreshing sleep [5].

Headache. Headache was frequently reported [5, 6, 17, 28, 30, 39, 52, 68, 70]. Twelve months after acute Q-fever, 24% of working patients reported frequent headaches [66]. Another study reported headache in 47% of patients six years after acute Q-fever [14]. Although the frequency of headache was similar to controls, the same authors found that the severity of headache was more profound in those after Q-fever [9].

Blurred vision. Blurred vision six years after acute Q-fever was similar to controls [14], but was more prevalent and more severe five years after acute Q-fever compared to controls in another study (34% vs. 18%) [9]. Blurred vision was also reported by others [17, 44]. Visual complaints were noted by 2% of patients 12 to 26 months after acute Q-fever [62].

Increased (night) sweating. Night sweats starting 6-12 months after acute Q-fever were described [70]. Twelve to 26 months after acute Q-fever, 3% of patients reported night sweats [62]. In comparison to controls, night sweats were more common after acute Q-fever [17, 44, 70]. Most QFS patients had this symptom for 5-10 years [70], up to 14 years [8, 28]. A combination of night sweating and increased sweating was also reported [30]. Increased sweating occurred with 53% more frequent after acute Q-fever compared to controls [14]. Others reported 53% of cases with increased sweating [5, 9]. Some authors considered abnormal sweating at least ten times a year as major QFS symptom [44].

Respiratory tract problems. Following acute Q-fever, 9% of patients complained of persistent chest symptoms [53]. Others reported that 47% of presumed QFS patients complained of cough and a sore throat with a mean symptom duration of four years [52]. Others reported these complaints also [17, 28-30, 39]. Five years after acute Q-fever, 51% of cases complained of breathlessness on exertion [9], compared to 32% of controls. Six years after acute Q-fever, 59% of patients complained of cough, 49% of breathlessness, and 51% of chest pain, all significantly more frequently than controls [14]. Furthermore, an association between QFS and bronchial asthma has been suggested [30].

Mood disorders. Patients with fatigue after acute Q-fever have been reported to experience increased irritability [14], mood disturbances [12, 17], and anger [70]. Mental problems, e.g. depression and unstable moods, can occur within a year following acute Q-fever [44],

whereas, with regard to depression, most subjects were healthy before the infection [44]. Two years after acute Q-fever more psychosocial complaints were observed compared to controls [61]. Common symptoms of psychological distress were reported significantly more in patients with post-infective fatigue, including QFS patients, compared to healthy controls [46]. Others hypothesise that Q-fever-related fatigue might be explained by psychological distress, caused by uncertainty about their illness and repeated medical contacts that reinforce perceptions of ill health [7]. Some contradict this hypothesis [67]. Infection with *C. burnetii* was followed by depression in 10% of cases [53]. Three case-reports (all n=1) [28-30] reported a *C. burnetii*-triggered depression, leading to thoughts of death [28], a near suicide attempt [30], and suicide [29]. The suggestion was that cytokine network abnormalities after a *C. burnetii* infection might underlie the onset of depression [28, 29, 73]. Although a possible relationship between high IgG phase II *C. burnetii*-antibodies and depression was suggested [69], others found no association between seropositivity, and depression, depressive ideas or overall psychiatric morbidity [56].

Other complaints. Other reported symptoms accompanying prolonged fatigue after Q-fever are severe malaise [40, 41], setback upon exertion and the need for prolonged rest after simple tasks [5, 8, 68], poor appetite [30, 68], gastrointestinal symptoms [6, 17, 29, 30, 44, 70], muscle fasciculation or spasms [8, 17, 41, 44, 70], dizziness [14, 17, 30], light intolerance [8, 19], tinnitus [28], taste disturbance [28, 29], loss of libido [17, 19], nasal and bronchial congestion [8, 17], and enlarged or painful lymph nodes [17, 70]. Bradycardia was postulated as a sign of QFS [35], and palpitations were described [30]. Even though reported in several studies [8, 17, 19, 44], alcohol intolerance was not statistically more frequent in the Q-fever group six years after acute Q-fever when compared to controls [14]. A slightly elevated body temperature (below 38 degrees Celsius) was described in QFS patients [5, 6, 28, 30, 39-41, 44, 70]. Up to 53% of assumed QFS patients felt feverish for four years [52].

In conclusion, besides fatigue as the main complaint, several nonspecific symptoms accompanying fatigue following *C. burnetii* infection were described. Commonly reported symptoms include musculoskeletal complaints, neurocognitive symptoms, sleeping problems, headaches, blurred vision, increased (night) sweating, respiratory complaints, and mood disorders.

Aetiology

Of the 28 articles that contained information on aetiology, 18 were classified in the main table aetiology (*S4 Table*) [18-21, 36-38, 45-51, 54, 55, 60, 63].

Pathophysiology

Genetic variance and relationship with fatigue. No relation [3] or correlation [47] between genetic factors and QFS was found. A lack of a coherent set of gene expression correlating across cohorts argued against the genetic signature for post-infective fatigue or CFS [47]. In contrast, another study found similar gene expression patterns for QFS and CFS patients [48]. The frequency of human leukocyte antigen – group DR (HLA-DR)-11 was significantly

increased in QFS patients compared to controls. Also, more polymorphic variants within the NRAMP1 gene differing from the wild type were found, as well as significant differences in allelic variant frequencies within interferon-γ (IFNγ) genes, but effects were thought to be multigenic and cumulative. It was hypothesised that QFS might result from individual variations in immune response to *C. burnetii* [50]. QFS patients differed in the frequency of HLA-DRB1*11 carriage and the 2/2 genotype of the IFNγ intron 1 microsatellite compared to control groups [51]. Carriage was associated with reduced IFNγ and interleukin(IL)-2 responses from stimulated peripheral blood mononuclear cells (PBMC) [51].
In conclusion, results regarding genetic variations in host immune responses in QFS were contradictory.

Immunological aspects. An immunological basis for QFS or other post-infective fatigue syndromes was debated in several articles. A reduction in reported fatigue correlated with improvement in the delayed-type hypersensitivity skin response and general health scores [45]. Resolving fatigue after acute infection seemed associated with improved cell-mediated immunity, supporting an immunological basis for post-infective fatigue [45]. Upregulation of 2',5'-oligoadenylate synthetase (2-5AS) activity in PBMC of CFS patients was present, but a relation between *C. burnetii* antibody titres and 2-5AS activities lacked [55]. It was however suggested that *C. burnetii* infection is associated with 2-5AS activities in some CFS patients, as 2-5AS activities changed from positive to negative in one CFS patient when *C. burnetii* antibodies disappeared [55]. In acute Q-fever IL-6 and CRP seemed predictive of more severe disease, but no support was found that these were associated with prolonged fatigue [63]. Markers of inflammation and pro-inflammatory cytokine concentrations did not remain altered in patients with post-infective fatigue [12, 18].
In conclusion, no clear evidence exists with regard to an immunological basis involving 2-5AS, IL-6, and CRP for the development of QFS.

Immunomodulatory complex and cell-mediated immunity. Persistence of *C. burnetii* or its antigens resulting in chronic immune stimulation with subsequent fatigue [8, 19-21, 36, 37, 49], or causing dysregulation of the macrophage/T-lymphocyte axis with subsequently aberrant monokine and lymphokine production mediating symptoms [8], was hypothesised. Cytokine release patterns of PBMC of QFS patients were aberrant with an accentuated IL-6 release, a decreased number of IL-2 responders, and an increased number of IFNγ responders [19]. *In vitro*, using human samples, an increased cellular immune response and cytokine dysregulation was found with increased levels of IL-6 and IL-10, and decreased level of IL-2 [70]. A significant correlation between IL-6 and scores for key and total symptoms was found [19]. The detection of low levels of *C. burnetii* DNA in bone marrow aspirates, thin needle liver biopsies, and blood mononuclear cells, supports cytokine dysregulation and immunomodulation caused by *C. burnetii* persistence [20]. Others showed a more complex interaction between host-regulated disease and persistent *C. burnetii* DNA carriage - either live, dormant, or dead but with undegraded DNA - in bone marrow, irrespective of clinical state [21]. An additional but variable factor of host regulation of cell-mediated immunity was postulated, determining the level of persistence and symptomatic outcomes.

It was hypothesised that in Q-fever without sequelae, the process of multiplication of live *Coxiella* was largely confined to bone marrow, in contrast to QFS, in which a modulated immune response caused increased levels of *C. burnetii* genome in bone marrow with increased shedding into peripheral blood [21]. Subsequently, one of the core hypotheses postulated included the presence of an immunomodulatory complex, consisting of non-viable undegraded *C. burnetii* DNA or its antigens, causing an abnormal cell-mediated immune response via damaged macrophages [37]. This stops the patient from clearing the microbe completely, leading to ongoing production of pro-inflammatory cytokines and subsequently fatigue. In contrast to QFS patients, those who fully recovered from acute Q-fever had no immunomodulatory complex [37]. The bacteraemia is restricted by humoral and cell-mediated immunity, by clearing of *C. burnetii* DNA containing components with an immunomodulatory effect of cell-mediated immunity and dendritic cells causing dysregulation, cytokines and other immune mediators, giving rise to symptoms [70]. The complexes appeared more likely to be a residue of the original heavy seeding during the bacteraemia of the acute infection, rather than the product of an ongoing multiplication, destruction and renewal of infection [21]. QFS follows clinical overt infection, rarely subclinical infection, and the systemic symptoms of QFS may reflect a wide distribution of parasitized mononuclear phagocytes [36, 37]. In other patient cohorts, neither viable *C. burnetii* nor DNA in PBMC was detected [49].
In conclusion, several studies point towards cytokine dysregulation mediating symptoms in QFS. This may originate from an immunomodulatory complex consisting of non-viable undegraded *C. burnetii* DNA or its antigens. However, results regarding remnant *C. burnetii* DNA were contradictory.

Cardiac involvement in QFS. No ECG abnormalities excess in the *Coxiella*-exposed cohort with fatigue was found in comparison to controls [54]. Post-infective fatigue was associated with higher heartbeat discrimination accuracy, increased resting heart rate with decreased heart rate variability, and a lower pressure pain threshold [46]. The altered cardiac response was believed to be a stress response portraying an over-responsive system lacking dynamic flexibility [46]. Heightened interoceptive sensitivity with strong symptom correlation was also found. This suggests physiological hyper-vigilance and response inflexibility in post-infective fatigue [46].
In conclusion, there is no evidence for direct cardiac involvements in QFS, but there is some evidence for physiological hyper-vigilance and response inflexibility in patients with post-infective fatigue.

(Bio)psychological origin of QFS. It is unknown whether chronic fatigue following Q-fever is directly caused by the bacterium or if it is (bio)psychological in origin [38]. As subjective symptoms are difficult to quantify, it was stated that they might reflect an observational bias, *C. burnetii* strain or cultural differences, or genetic susceptibility [38]. In addition to the immune stimulation hypothesis, interpretations range from compensation-driven through psychogenic perpetuation of original symptoms or depression [8]. Q-fever patients with fatigue symptoms had higher somatisation scores, a higher tendency for hypochondriac

worries and beliefs, a higher level of psychosocial complaints, and reduced quality of life [61]. The non-proven presumption was that Q-fever triggered fatigue development and that the risk of developing symptoms might be increased by hypochondriac features and a tendency to somatisation, supporting a biopsychological aetiology [61].
In conclusion, some studies supported the view of a biopsychological aetiology of QFS.

Predictors of post-infective fatigue syndrome, including QFS
Psychological factors and demographics. Post-infective fatigue appeared to be stereotyped across different infective triggers, and it was suggested that the host response rather than psychological or microbial factors determined ongoing symptoms [18]. No source of exposure was associated with developing persistent symptoms [3]. Premorbid and intercurrent psychiatric disorders were not predictive for post-infective fatigue [12]. In contrast to the biopsychological aetiology [61], it was recently suggested that psychological distress was not an important factor in explaining increased fatigue levels after acute Q-fever [67]. Although some found that gender was not a predictor [12], others found an overrepresentation of women in high severity groups for fatigue, mood disturbance and neurocognitive difficulties [60]. Being female or a young adult, and smoking were characteristics significantly associated with long-term reduced health status including fatigue [62, 67]. In contrast, another study found no association between fatigue and age [59].
In conclusion, neither psychological nor microbial factors seem to predict post-infective fatigue, including QFS.

Severity of the acute illness. It was stated that one of the key risk factors for the development of post-infective fatigue, including QFS patients, is the severity of the acute illness [12]. Patients with post-infective fatigue had a longer mean duration of the acute illness, and more days in bed and days out of role during the acute phase compared to controls [18].The clinical expression of acute Q-fever seemed an essential factor in the subsequent sustained decrease in health status [58], which is supported by the finding that QFS usually follows acute Q-fever and rarely if ever asymptomatic infection [70]. Pre-existing health problems [62, 67], and hospitalisation, as an indicator of the severity of the initial infection, were also fatigue predictors [59, 62]. No symptoms during the acute Q-fever infection were predictors for persisting symptoms [3], nor did these determine the long-term health status [65]. Neither IL-6 and CRP levels nor antibiotic treatment during the acute infection were predictors for the development of prolonged fatigue [3, 63]. No relationship was found between fatigue and antibody titres six years after the Q-fever infection [49].
In conclusion, the severity of the acute Q-fever infection seems a key factor for worse long-term health status, including fatigue and QFS.

Genetic factors in predicting fatigue. A single nucleotide polymorphism (SNP) of the T allele IFNy+874T/A appeared to be the best predictor of increased fatigue after the acute phase of several infections, including *C. burnetii* [60]. While the C allele of IL-10-592C/A SNP exerted a protective effect on neurocognitive difficulties, the A allele IL-10-592 SNP and G allele IL-6-174G/C SNP were associated with increased mood disturbance [60].

In conclusion, as evidence is scarce, more research is needed regarding genetic factors predicting fatigue in QFS.

Prevention/therapy

Eleven articles contained information on prevention/therapy of which six are classified in the main table prevention/therapy (*S5 Table*) [5, 6, 39-42].

Prevention

No articles on the prevention of QFS were found. The Dutch guideline on QFS proposes to advice patients within the first six months after acute Q-fever or after established QFS to: i) stay mentally and physically as active as possible, adjust pace if necessary; ii) alternate activities, also within activities; iii) keep fulfilling the role in daily life; iv) maintain a regular sleep-wake pattern; v) avoid focusing on fatigue; and vi) focus on feasible activities and appreciate accomplishments [17]. It is also proposed to explain that most patients recover within the first 6-12 months following acute Q-fever.

Antibiotic treatment

Four articles reported on the effect of long-term antibiotic treatment in assumed QFS patients [5, 6, 39, 40]. No randomised controlled trial (RCT) was found. Treatment with either 3 months of minocycline 200mg/day (n=18), levofloxacine 200mg/day (n=1), or erythromycin 400mg/day (n=1), improved performance status and reduced fatigue [6], concluding that minocycline was useful in treating QFS [6]. In a pilot-study, treatment with three months of minocycline 100mg/day (n=29), doxycycline 100mg/day (n=26), or levofloxacin 200mg/day (n=3), showed improvement in performance status, headache, and mean weekly temperature [39]. A case-series (n=3) [5] and case-report (n=1) [40] showed inconsistent results of treatment with long-term antibiotics. According to others, the positive effect of antibiotic treatment for QFS is not confirmed nor advised [17]. The efficacy of long-term antibiotic treatment is now tested in a RCT but results are not yet available [42].

In conclusion, available data on long-term antibiotic treatment for QFS are scarce and inconsistent.

Cognitive behavioural therapy (CBT) and graded exercise therapy (GET)

CBT proved effective in reducing symptoms and improving functioning in CFS patients [74, 75], and in chronic fatigue in chronic illnesses [76-78]. It was suggested as treatment option for QFS patients who experience psychological distress [61]. Based on CFS literature and similarities between CFS and QFS, CBT is advised in the Dutch QFS guideline, although suspected not to be beneficial for all patients [17]. The effectiveness of CBT treatment for QFS is currently under investigation [42]. Also GET is recommended for QFS patients, as proven effective in reducing fatigue in CFS [17].

In conclusion, although evidence is lacking, CBT and GET might be effective in reducing fatigue in QFS patients.

Treatment of QFS-related symptoms

Three articles (all n=1) reported treatment of QFS-related symptoms [28-30]. The authors concluded that education and counselling about QFS and QFS-related symptoms should be provided to QFS patients [28]. Attention to the patient's mental state is necessary in order to recognise accompanying symptoms, e.g. depressive thoughts, that should be treated [30], and involving a psychiatrist early ought to be considered [29]. This has been recognised before, where tricyclic antidepressants were beneficial treatment of mental problems after acute Q-fever [44].

In conclusion, education and counselling of patients about QFS and QFS-related symptoms seems important, as well as considering a patient's mental state.

Alternative treatment

Alternative therapies for QFS patients were described (both n=1), including Kampo formula Tsumura Hochu-ekki-To granules, which appeared not to be effective [40], and Kampo formula Shakuyaku-Kanzo-To granules, which resulted in alleviation of stiffness in hand and arm [41].

At present, evidence for the use of alternative treatment lacks.

DISCUSSION

This first systematic review on fatigue following acute Q-fever, includes 57 articles and four grey documents up to the 26th of May 2015. The main limitation is the lack of a uniform definition of fatigue after Q-fever and the absence of a standardized diagnostic tool. In addition, the terminology both for fatigue and *C. burnetii*-related fatigue differed between publications and in time. Consequently, comparison of outcomes is difficult or impossible. Although not all articles could be quality assessed, these were nevertheless included as their information was considered valuable.

An international uniform definition of QFS, discriminating fatigue caused by *C. burnetii* from other post-infective fatigue syndromes and CFS is unavailable [19, 26, 36]. As the Dutch QFS guideline provides the most detailed description of QFS [17], we propose to use its definition and diagnostic work-up internationally. An international uniform definition provides the opportunity to achieve uniformity in diagnosis, treatment, and comparison of research results. It also provides recognition for physicians and acknowledgement for patients, reducing fear concerning uncertainty about their disease, providing an opportunity to continue their path to recovery [79, 80].

Whether fatigue following acute Q-fever is a separate entity compared to other forms of post-infective fatigue is debatable [10, 12, 18, 27, 44, 47, 81], but should not hamper the use of the term QFS.

Although differences in incidence and prevalence were reported, approximately 20% of patients remain chronically fatigued following an acute Q-fever infection. These differences can be explained by lack of recognition, uniform definition and diagnostic work-up, follow-up, and assessment tools. Using similar validated screening instruments is essential to compare studies [34]. Therefore, we advocate using validated screening instruments for measuring fatigue severity and disabilities, preferably with international available instruments [82],

such as the Checklist Individual Strength or Chalder Fatigue Scale for fatigue [83, 84], and the NCSI, SF-36, or Sickness Impact Profile for disabilities [71, 72, 85]. This also helps to map the impact of QFS. The cut-off period of 6 months to diagnose QFS has been proposed as most patients recover spontaneously within this period, which corresponds with the internationally accepted definition for CFS [15, 16]. In QFS, fatigue frequently lasts beyond a year and mostly more than 5 to 10 years [8, 14]. Many nonspecific symptoms described accompanying fatigue in QFS were not systematically monitored as prospective data were unavailable. Most studies did not report the time-relation between these symptoms, fatigue, and the Q-fever infection, nor the frequency of occurrence. Therefore, it was not possible to list all symptoms possibly related to fatigue following *C. burnetii* infection nor provide a temporal or causal relationship. However, guidelines with regard to the examination of chronic fatigue should be followed to rule out other diseases which can cause chronic fatigue.

Several hypotheses regarding the underlying pathophysiological mechanism of QFS were proposed, but no conclusive answers have been identified yet. Research on the relationship between genetic factors and QFS is contradictory and scarce. Several studies point towards cytokine dysregulation mediating symptoms in QFS, including an immunomodulatory complex consisting of non-viable undegraded *C. burnetii* DNA and or its antigens. However, these results need further confirmation, as most studies regarding this topic have been done by the same study group and contradictory results exist with regard to the presence of *C. burnetii* DNA in QFS. Several queries exist regarding predictors of QFS. Neither psychological nor microbiological factors seemed to predict post-infective fatigue. Only the severity of the acute Q-fever infection appears a predictor of long-term reduced health status.

No uniformity exists regarding optimal treatment for QFS. Results from RCTs using long-term antibiotics are not available, and the available studies all suffer from several important limitations, such as the lack of a clear QFS description, the inclusion of patients with a symptom duration of 1-4 months, and the inclusion of patients with positive *C. burnetii* PCR at baseline, possibly indicating chronic Q-fever, and can therefore not be generalized. As the evidence of beneficial antibiotic treatment in QFS patients lacks, it should not be prescribed for QFS patients. The recommended treatment after diagnosis of QFS in the Dutch QFS guideline is based on CFS literature, and consists of CBT and, if available GET. The effectiveness of these treatments in QFS has not been proven yet. A randomised placebo-controlled trial in order to evaluate the efficacy of both long-term doxycycline and CBT in QFS patients is currently performed [42]. Treatment should at least focus on the provision of medical care, physical rehabilitation and additional psychological support [81]. Furthermore, physicians should be aware of accompanying complaints, especially depressive thoughts, which require treatment at an early stage [29]. Alternative treatments were only effective in one case-report and are therefore not recommended. Finally, the prognosis of QFS patients is unclear regardless if treated or not.

In conclusion, the occurrence and long-term persistence of fatigue following acute Q-fever, generally referred to as QFS, has major health-related consequences. Information on aetiology, prevention, treatment, and prognosis of QFS is underrepresented in the international literature. In order to facilitate comparison of findings, and as a platform

for future preferably prospective studies, we propose a uniform definition of QFS and the use of uniform measurement tools. In addition, in order to facilitate comparison of long-term sequelae following several infectious agents, and as a platform for further preferably prospective studies, an international collaboration and a research agenda are desirable with regard to micro-organisms known for causing post-infective fatigue, in which *C. burnetii* should undoubtedly be included.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: GM SPK AT CPBR ML. Performed the experiments: GM SPK. Analyzed the data: GM SPK AT CPBR. Contributed reagents/materials/analysis tools: Not applicable. Wrote the paper: GM SPK. Critical revisions during the drafting of the manuscript: CED GB ML AT CPBR. Assisted in the search strategy: ML.

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SUPPORTING INFORMATION

Table of Contents

S1 Table. Overview of study populations and used definitions

Included articles	Study populations and used definitions
1960, O. Powell [1]	1-2 yrs post AQF (AQF confirmed by demonstration CFT to *C.b.* with either a rise from zero to ≥1:32, or a titre in a single specimen of ≥1:256 in patients admitted to hospital or suspected of infection late in the illness) from Princess Alexandra Hospital, Brisbane. No definition for QFS or fatigue
1990, S. Reilly [2]	All AQF cases diagnosed and monitored by the Public Health Laboratory in Plymouth between 1972 and 1988 out of FUO, respiratory infections, CNE, and hepatitis cases. Clinical and serological status assessed in 1989. AQF: ≥fourfold rise in phase II titre, or by a stable phase II titre ≥80 if there was strong clinical evidence of AQF. Past infection: evidence of past exposure to *C.b.* by single or sustained phase II titres ≥10 to ≤40, with QF not being considered to be causally related to the presenting complaint. No definition for fatigue
1995, P. Harvey-Sutton [3]	No study population or QFS definition
1996, B. Marmion [4]	5-14 yrs post laboratory-proven AQF (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies), QFS defined as: 1) incapacitating fatigue requiring prolonged rest after simple tasks; 2) nausea, persistent headache; 3) feeling feverish with profuse, odoriferous sweats at night, usually afebrile; 4) myalgia in any muscle group; 5) intermittent fasciculation of muscle fibres and muscle tenderness on palpation; 6) arthralgia without swelling, in any joint including costochondrals; 7) ethanol intolerance compared with capacity before AQF; and 8) interrupted sleep patterns, excessive and unreasonable irritability, unreliable short-term memory, and poor concentration. Less frequent complaints: bloating, irritable bowel syndrome, nasal and bronchial congestion, blurred vision, bright light intolerance, and enlargement and pain in lymph nodes. Definition CFS: according to the 1994 international CFS criteria [5]
1996, J. Ayres [6]	6 yrs post AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies), no QFS definition, but rather description of complaints being significantly more prevalent in past QF cases i.c.w. controls: joint pains, sleep disturbance, cough, sweating, irritability, chest pain, breathlessness, and dizziness
1998, J. Ayres [9]	5 yrs post AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies), no QFS definition, but rather description of complaints being significantly more prevalent in past QF cases i.c.w. controls: fatigue, sweating, breathlessness on exertion, blurred vision, with symptom severity in QF cases being higher for fatigue, blurred vision, sweating, memory deterioration, joint pains and headaches
1998, B. Bennet [10]	PIFS patients from DIOS or from the University Health Service at the University of New South Wales whose symptoms have been present ≤4 wks
1998, K. Kato [11]	Patients with chronic nonspecific symptoms, such as fatigue, joint aches, sleep disturbance, night sweats, myalgia affecting various muscle groups, nausea, persistent headache, and so on, without diagnosis or treatment history of QF and living in close contact with animals, presented between March 1996 and April 1997 to the Department of Internal Medicine and Psychosomatic Medicine, Nihon University Health Science Centre. Healthy controls: without/few complaints, who received annual examinations at the same hospital

S1 Table continued. Overview of study populations and used definitions

Included articles	Study populations and used definitions
1998, I. Penttila [12]	Definition QFS patients: 1) severe incapacitating fatigue ≥6 mo post AQF, with symptom score >100; 2) presence of myalgia and arthralgia; and 3) abnormal sweats, particularly at night. In addition, most patients had other symptoms such as inappropriate exhaustion on minor exertion, muscle fasciculation, headaches, bright light intolerance, ethanol intolerance, interrupted and unrefreshing sleep patterns, irrational irritability, loss of libido, depression, impairment mental concentration and short-term memory. Resolving QFS: recruited in similar way after several yrs observation, but symptom score dropped from values >100 to ≤95. QF without QFS: 6 mo post AQF without complex of symptoms and low symptom score (1-35)
1999, J. Scadding [13]	No study population or QFS definition
2000, R. Harris [14]	Definition of QFS patients: conform [12]. Controls: conform [12]
2002, J. Ayres [15]	10 yrs post laboratory-proven AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies). Controls: no serological evidence of past exposure to *C.b*. Definition fatigue: according to the 1994 international CFS criteria [5, 16]
2002, M. Wildman [17]	10 yrs post laboratory-proven AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies). Definition fatigue: score ≥4 using the traditional scoring system for the fatigue questionnaire [18]. Definition ICF: fatigued and describing fatigue >50% of the time for 6 mo. Definition CFS: ICF and functional impairment and ≥4 additional symptoms according to the 1994 international diagnostic criteria [5]. Controls: no serological evidence of past exposure to *C.b.*
2002, D. Raoult [19]	Definition QFS patient: residual asthenia following QF at 6 mo post AQF
2002, B. Marmion [20]	No study population or QFS definition
2002, M. Wildman [21]	No study population or QFS definition. Definition fatigue: according to the 1994 international CFS criteria [5, 16]
2003, T. Hatchette [22]	3 and 27 mo post AQF [23], no QFS definition. Controls: without AQF during same outbreak cohort
2003, K. Helbig [24]	Definition QFS patients: conform [12]. Recovered QFS: conform [12]
2003 K. Ikuta [25]	CFS based on the 1988 CDC working case definition [26] and the Ministry of Health and Welfare of Japan, from Tottori University Hospital, Yonago, and from Osaka University Hospital, Osaka, Japan. Healthy controls: from Tottori University Hospital Yonago
2004, Y. Arashima [27]	Definition QFS patients: prolonged nonspecific complaints, with general fatigue of unknown origin, or headache, slightly elevated body temperature (37-37,5˚C), arthralgia, or myalgia, with *C.b.* seropositive defined by IgMII ≥1:32 or IgGII ≥1:128 (or ≥1:64 if antibody for *B. henselae* was negative) and/or detectable *C.b.* DNA, for 3 mo till 4 yrs, between July and November 2001 from the Department of Internal Medicine of the Nihon University School of Medicine, Tokyo
2004, H. Thomas [28]	8 yrs post recruitment in 1991 from a random sample of farmers drawn from the Ministry of Agriculture, Fisheries and Food June Agricultural Census lists of agricultural holdings, with *C.b.* seropositivity defined by IgGII ≥1:32. No QFS definition
2005, B. Marmion [29]	Definition UK cases: 12 yrs post laboratory-proven AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies). Definition fatigue: conform [17]. Definition Australian QFS cases: conform [12, 14], 9 mo-5 yrs post AQF. Definition fatigue: according to the 1994 international CFS criteria [5]

S1 Table continued. Overview of study populations and used definitions

Included articles	Study populations and used definitions
2005, K. Helbig [30]	Definition QFS patients: as in [12, 29]. Definition AQF with asymptomatic recovery: 12 yrs post laboratory-proven AQF [7, 8] (AQF defined as a CFT titre of ≥1:256 or a 4-fold rise in phase II antibodies), with complete recovery without QFS or other chronic sequel. Definition QIE: clinical evidence of endocarditis by observation of vegetations on ultrascan or on histopathological examination of the diseased valve, and a compatible serological profile defined by IgGI and II >320, low or no IgM and IgAI ≥160, and PCR positive examination of valve vegetation specimens and in some instances by isolation of *C.b.* in cell culture or laboratory animals, Caucasians mainly from New South Wales and Queensland
2005, E. Iwakami [31]	Definition CFS patients: according to the 1994 international CFS criteria [5, 32], in combination with proven *C.b.* infection defined by IgG ≥1:128 (or ≥1:64 if *B. henselae* was negative), or IgM ≥1:32, and/or detectable *C.b.* DNA, for 8 mo till 11 yrs. Definition QFS patients: nonspecific complaints such as CF, slightly elevated body temperature, headache, arthralgia and myalgia of unknown origin for several mo or longer, but not meeting the 1994 international CFS criteria, in combination with a confirmed *C.b.* infection defined by IgG ≥1:128 (or ≥1:64 if *B. henselae* was negative), or IgM ≥1:32, and/or detectable *C.b.* DNA by n-PCR, regardless of the presence or absence of pre-existing infection, for 1 mo till 10 yrs
2006, I. Hickie [33]	Patients from DIOS with symptoms ≤6 weeks assessed at 3 and 6 wks, and 3 and 12 mo post AI, without pre-existing medical disorders or drug use likely to be associated with prolonged fatigue. Provisional PIFS: if SOMA scores at all time points up to and including 3 mo exceeded the established threshold score [34]. Confirmed PIFS: CFS at 6 mo post AI according to the 1994 international CFS criteria [5]. Controls: recovered promptly from the same infection
2007, D. Ledina [35]	Definition QFS patients: between January 2000 and December 2004 at Split University Hospital, Croatia. 1) 12 mo post AQF complaints of morning fatigue, disrupted sleep, headache, prolonged fatigue >24 hours post exertion, muscle pain, persistent slightly elevated body temperature, without CQF, meeting the 1994 international CFS criteria [5]. 2) 2 mo post AQF no symptoms, than start neck pain with 6 mo post AQF start of fatigue, insomnia, headache, sweating, unrefreshing sleep, for 12 mo, meeting the 1994 international CFS criteria [5] with positive ELISA IgG 1.6 and IgA 1.4. 3) 4 mo post AQF start symptoms of fatigue, disrupted sleep, headaches, muscle and joint pain, for 7 mo, meeting the 1994 international CFS criteria [5], with positive ELISA IgG 2.4 and IgA 1.5
2007, U. Vollmer-Conna [36]	PIFS patients from DIOS assessed at 1, 2, 3, 6, and 12 mo post AI, with confirmed PIFS if symptoms persisted beyond 6 mo with a score of ≥3 at all time points on the empirically derived subscale SOMA, without alternative explanations for ongoing illness and meeting the 1994 international CFS criteria [5]
2009, B. Marmion [37]	Samples from 11 patients ≥12 yrs post laboratory-proven AQF [7, 8], of whom 1 patients had slightly elevated body temperature, late-stage QIE
2009, L. Zhang [38]	Definition CFS/ME: idiopathic CFS/ME according to the 1994 international CFS criteria [5], from Bristol, London, and New York, and CFS/ME from [39, 40]. Definition Q-CFS/ME: CFS/ME according to the 1994 international CFS criteria [5] triggered by laboratory documented QF, from Birmingham. Definition endogenous depression: fulfilled DSM-IV criteria, from Bristol and surrounding area. Definition healthy blood donors: from Dorset National Blood Service [41]. Excluded were psychiatric diseases, smoking previous yr, alcohol or drugs abuse, current use or ≤3 mo of antibiotics, steroids, cytotoxic drugs or antidepressant
2010, Y. Kadota [42]	PIFS patients from DIOS or from a tertiary referral assessment clinic at a public teaching hospital in Sydney, and patients' current symptom profiles had to fulfill the 1994 international CFS criteria [5]

S1 Table continued. Overview of study populations and used definitions

Included articles	Study populations and used definitions
2010, O. Sukocheva [43]	Samples from patients 12 yrs post laboratory-proven AQF [7, 8], classification of patients into clinical groupings according to asymptomatic recovery or presence of QFS with or without other co-morbidity [17, 44], with a chosen subset from 1) recGr3, AQF with asymptomatic recovery; 2) QFSGr5, AQF followed by QFS without co-morbidity; 3) QFSGr6, AQF followed by QFS with fatigue-associated co-morbidity
2010, G. Limonard [45]	12 mo post laboratory-proven AQF (AQF defined as any inhabitant of the outbreak cluster area who presented with compatible clinical symptoms and a positive IFA serology, with an IgMII and IgGII ≥1:64 or seroconversion with 4-fold rise in antibody titre during FU). Controls: from neighbourhood of QF patient without QF history, with negative QF serology
2010, G. Limonard [46]	Post laboratory-proven AQF (AQF defined as any inhabitant of the outbreak cluster area who presented with ≥1 compatible clinical symptoms (fever, fatigue, chills, headache, myalgia, sweats, cough) and the demonstration of *C.b.* infection, as evidence by: 1) seroconversion or 4-fold rise in antibody titre using CFT in samples taken ≥14 days apart; 2) presence of IFA IgMII and IgGII ≥1:64; or 3) a positive serum PCR) assessed at baseline, 3, 6, 12 mo. Definition CQF: any inhabitant of outbreak cluster area with clinical entity compatible with endocarditis, vascular infection, osteoarticular infection, chronic hepatitis, or pregnancy, with an IgGI ≥800, for ≥6 mo post AQF
2011, G. Morroy [47]	12-26 mo post AQF (AQF according to the Dutch notification criteria [48] defined as a laboratory confirmation of QF with a seroconversion or a 4-fold rise in antibody titre between 2 subsequent tests with 2-4 wks time interval using CFT or IFA, and clinical presentation of fever, pneumonia or hepatitis, ≥18 yrs, notified in 2007/2008. Excluded: unknown onset of QF infection, incomplete questionnaires and questionnaires completed by another person
2011, H. van Woerden [49]	6 yrs post AQF (AQF defined as those who had clinical symptoms and serological evidence of AQF as demonstrated by an IgMII ≥80, or a fourfold rise on sequential CFT in 2002). Definition controls: who worked in the same factory but had no symptoms of AQF and no serological evidence of infection with no IgM, no CFT and no IgGI or IgGII at the time of the outbreak
2011, S. Galbraith [50]	Caucasian PIFS patients from DIOS with unexplained illness persisting ≥6 mo with a score of ≥3 at all time points on the empirically derived subscale SOMA, without alternative explanations for ongoing illness and meeting the 1994 international CFS criteria [5]. Controls: recovered promptly from the same infection
2012, B. Piraino [51]	Caucasian adult PIFS patients from DIOS [33] assessed at baseline, 2-3 wks, 4-6 wks, followed by 3-mo interval until 12 mo post AI
2012, B. Strauss [52]	2 yrs post laboratory-proven AQF [53]. Controls: without registered indicator for QF infection, from same general practitioners as study patients
2012, G. Morroy [54]	12-26 mo post AQF (AQF according to the Dutch notification criteria [48] defined as a laboratory confirmation of QF and clinical presentation with fever, pneumonia or hepatitis, notified in 2007/2008)
2012, Y. Arashima [55]	Definition QFS patient: 3 mo post AI with general fatigue, slightly elevated body temperature (37˚C or higher), cough, night sweats, arthralgia, noise in his ears, taste disturbance, and headache, without abnormalities in physical examination, laboratory examination including cultures and additional tests (X-rays, abdominal ultrasound, echocardiography, treadmill exercise test), but with positive n-PCR for *C.b.*, IgGII 1:64
2012, D. Raoult [56]	No study population or QFS definition
2012, H. Hussain-Yusuf [57]	Patients 6 yrs post serological evidence of AQF in 2002 [58]. Controls: worked in the same factory but were serologically negative for QF at the time of the outbreak

S1 Table continued. Overview of study populations and used definitions

Included articles	Study populations and used definitions
2012, J. Oosterheert [59]	No study population or QFS definition
2012, S. Yakubo [60]	Definition QFS patients: general fatigue, nausea, stomach pain, abnormal sensation in the mouth, sore throat, and trouble sleeping, with IgGI 1:256
2013, S. Keijmel [61]	Definition QFS patients: according to the Dutch guideline on QFS [62], referred to Radboud university medical center, Nijmegen, the Netherlands; adults (non-pregnant, non-lactating), ≥18 yrs, with laboratory-proven AQF since 2007 and/or positive serology fitting a past infection with *C.b.*, and being severely fatigued (CIS fatigue ≥35) for ≥6 mo, and being disabled because of fatigue (SIP total score ≥450), with a reference to AQF and absence of fatigue before the episode of AQF or a significant increase ever since. Excluded: CQF [63], AQF in the presence of risk factors for developing CQF necessitating prophylactic use of doxycycline, pregnancy or unwillingness to use effective contraceptives during the study, imminent death, inability to give informed consent, allergy or intolerance to doxycycline, somatic or psychiatric illness explaining chronic fatigue, current enrolment in other investigational drug trials or receiving investigational agents, receiving or having received AB >4 wks potentially active against *C.b.*, use of barbiturates, phenytoin, or carbamazepine, moderate or severe liver disease, current engagement in legal procedure for financial benefits
2013, S. Yakubo [64]	Definition QFS patient: 6 yrs post AI with general malaise, spasm left hand, slightly elevated body temperature, without abnormalities in physical examination, laboratory examination including pharyngeal culture and additional tests (chest X-ray, X-ray of larynx/pharynx/ears and paranasal sinuses, ECG, abdominal ultrasound, brain CT, EEG), with negative n-PCR for *C.b.*, IgMI and IgMII <1:16, IgGI <1:16, IgGII 1:32. Six mo after presentation IgGI 1:128
2013, M. van Asseldonk [65]	All notified, hospitalised, deceased and non-reported cases of QF, determined from [66] and [67]
2013, J. van Loenhout [68]	12 mo post AQF, patients ≥18 yrs diagnosed with QF in 2010 and 2011, who fulfilled the Dutch notification criteria for QF [69] were eligible for participation
2013, S. Yakubo [70]	Definition QFS patient: 2 mo post AI with severe fatigue, general malaise, arthralgia, myalgia, persistent slightly elevated body temperature (around 37˚C), whole-body lassitude, without abnormalities in physical examination, laboratory examination including a pharyngeal culture and additional tests (chest X-ray, ECG), but with positive n-PCR for *C.b.*, without positive antibodies
2013, R. Brooke [71]	QF notified patients with onset symptoms between 1 January 2009 and 31 December 2013. A(H1N1)pdm09 notified patients, reflected by influenza-like-illness registration from the Dutch Sentinel General Practice Network for influenza-like-illness from NIVEL Netherlands Institute for Health Services Research between 27 April 2009 and 26 April 2010
2013, Y. Arashima [72]	Definition QFS patients: 18 mo post AI with general fatigue, cough, dyspnoea, sputum, breathing difficulty, slightly elevated body temperature, headache, poor appetite, copious sweating, night sweating, nausea, vomiting, palpitations, and dizziness, without abnormalities on physical examination, laboratory examination (besides slight liver dysfunction), but with positive n-PCR for *C.b.*, IgMII 1:16, IgGII 1:128
2014, M. Kremers [73]	Post laboratory-proven AQF (AQF according to the Dutch notification criteria [48] defined as symptomatic patients with positive PCR for *C.b.* DNA in serum samples before the development of an IgMII antibody response measured by IFA or ELISA), between April 2009 and August 2009, and assessment 4 yrs post AQF, all who were still alive, ≥18 yrs and of whom a 12 mo FU sample was present

S1 Table continued. Overview of study populations and used definitions

Included articles	Study populations and used definitions
2014, J. van Loenhout [74]	Definition QF study population [75]: notified patients 1 yr post AQF in 2010 and 2011 (AQF according to the Dutch notification criteria defined as a laboratory confirmation of QF with a seroconversion or a 4-fold rise in IgG antibody titre in a paired serum sample with ≥2 wks time interval using CFT or IFA, presence of IgMII antibodies, positive PCR or culture in blood or respiratory material, presence of phase I antibodies, combined with a clinical presentation with fever, pneumonia or hepatitis, an onset of illness within previous 90 days [69], and ≥18 yrs. Definition Legionnaires disease study population: notified patients, 1 yr post Legionnaires' disease in 2010 (Legionnaires' disease according to the Dutch notification criteria defined as matching clinical symptoms, usually pneumonia, confirmed by at least 1 but preferably 2 of the laboratory diagnostic test: isolation of Legionella-species from respiratory secretions or blood; Legionella pneumophila-antigen in urine by radio-immuno-assay, ELISA, or immuno-chromatographic assay; Legionella-species by PCR in clinical material; significant titre of IgM by ELISA; significant titre elevation of antibodies. Healthy controls: via advertisements in local newspapers in the city of Nijmegen area. Excluded controls: underlying respiratory illness
2014, A. van Dam [76]	10-19 mo post LRTI as diagnosed by general practitioner between 1 May 2009 and 30 September 2009 in provinces of Northern Brabant and Gelderland, categorized into following ICPC groups: acute bronchitis, influenza, pneumonia, and other LRTI who were initially tested for QF, ≥18 yrs and ≤75 yrs. Definition QF positive: LRTI patients with positive diagnostic tests by either PCR, IFA, or CFT
2015, J. van Loenhout [77]	Over a period of 24 mo (assessed at 3, 6, 9, 12, 18 and 24 mo) post laboratory-proven AQF in 2010 and 2011 (AQF according to the Dutch notification criteria [69]), ≥18 yrs
2015, J. van Loenhout [78]	Definition notified QF patients: 4 yrs post laboratory-proven AQF in 2007 and 2008 (AQF according to the EU case definition [79] with laboratory criteria (isolation of *C.b.* from clinical specimen; detection of *C.b*. nucleid acid in clinical specimen; *C.b*. specific antibody response (IgGII or IgMII)), epidemiological criteria (exposure to common source; animal to human transmission), and clinical criteria (fever, pneumonia and/or hepatitis), onset of disease <90 days, ≥18 yrs. Definition non-notified QF patients: 4 yrs post laboratory-proven QF in 2008 and 2009 (according to the EU case definition, but only fulfilling the laboratory criteria and not the clinical criteria of fever, pneumonia or hepatitis), onset of disease <90 days, ≥18 yrs
2015, J. van Loenhout [80]	Definition QF study population [75]: notified patients assessed 3, 6, 9 and 12 mo post laboratory-proven AQF in 2010 and 2011 (AQF according to the Dutch notification criteria), ≥18 yrs. Definition Legionnaires disease study population [75]: notified patients 12 mo post Legionnaires' disease in 2010 (Legionnaires' disease according to the Dutch notification criteria)

Abbreviations: AI= Acute infection, AQF= Acute Q-fever, B. henselae= Bartonella henselae, C.b.= Coxiella burnetii, CDC= Centres for Disease Control and Prevention, CF= Chronic fatigue, CFS(/ME)= Chronic fatigue syndrome (/myeloencephalitis), CFT= Complement fixation test, CIS= Checklist Individual Strength, CNE= Culture negative endocarditis, CQF= Chronic Q-fever, DIOS= Dubbo Infection Outcomes Study, cohort study of subjects ≥16 yrs followed from the onset of a confirmed and documented AI due to EBV; C.b.; or RRV ≤6 wks post AI until complete recovery, DSM-IV= Diagnostic Statistical Manual of Mental Disorders, EBV= EpsteinBarr virus, ECG= Electrocardiography, ELISA= enzyme-linked immunfluorsorbent assay, EU= European Union, FU= Follow-up, FUO= Fever of unknown origin, I.c.w.= In comparison with, ICF= Idiopathic chronic fatigue, ICPC= International classification of primary care, IFA= Immunofluorescence assay, IgA= Anti-phase IgA, IgG= Anti-phase IgG, IgGI= Anti-phase IgG I titre, IgGII= Anti-phase IgG II titre, IgM= Anti-phase IgM, IgMI= Antiphase IgM I titre, IgMII= Anti-phase IgM II titre, LRTI= Lower respiratory tract infection, Mo= Month(s), (n-)PCR= (nested-) Polymerase chain reaction, PIF(S)= Post-infective fatigue (syndrome), Q-CFS(/ME)= Q-fever induced chronic fatigue syndrome (/myeloencephalitis), QF= Q-fever, QF(F)S= Q-fever fatigue syndrome, or Post-Q-fever chronic fatigue syndrome, or Post-Q-fever debility syndrome, or PQFS= Post-(acute)Q-fever (fatigue) syndrome, (Q)IE= (Q-fever induced) Infective endocarditis, Ref= Reference, RRV= Ross River virus, SIP= Sickness Impact Profile, SOMA= Empirically derived subscale of the SPHERE, used to record PIFS or illness duration. This reliably predicts disability and reflects patients' and doctors' reports of reasons for presentation to primary care. Scores ≥3 represents a clinically-significant fatigue state, UK= United Kingdom,Wks= Weeks, Yr(s)= Year(s).

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S2 Table. Domain diagnosis

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-vention	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
1999, J. Scadding [1]	Country unknown, 1999. Duration study NA	PO, comment on [2]	No patients/ controls. Characteristics and co-morbidity: NR	NA	NA	CFS, defined in clinical-descriptive terms, should convey no causal implication; when there is convincing evidence of a causal factor, the case belongs to a causally-defined subset of this syndrome. PQFS conforms to this desideratum	If mechanisms of complaints and specific therapeutic approaches are unknown, the term PQFS/QFS should be used as this leaves no doubt that findings are relevant to a CFS subset	NA	NA
2012, D. Raoult [3]	France, 2012	PO, comment on [4]	No patients/ controls. Characteristics and co-morbidity: NR. Focus on CQF	NA	NA	NA	CF is a non-specific subjective state, not a specific symptom of QF; no treatment is currently effective, it is not a diagnostic problem. Some patients with fatigue have high antibody titres, others not	NA	NA
2012, J. Ooster-heert [5]	Netherlands, 2012	PO, comment on [6]	No patients/ controls. Characteristics and co-morbidity: NR. Focus on terminology of fatigue following QF	NA	NA	NA	Important to underline and recognise PIFS. New terminology QFS not useful; PIF described for many infectious diseases; not causative micro-organism, but disease severity correlates with symptom duration post AI. Can lead to cultivation, attracting patients with other intentions then getting better, ↑ healthcare costs	NA	NA

S2 Table continued. Domain diagnosis

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-vention	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
2013, J. van Loen-hout [7]	Netherlands, 2011-2012, single mea-surement 12 mo post illness onset	CoS	309 AQF patients, no controls. To assess use of NCSI and SF-36 in providing a detailed assessment of health status of QF patients and to evaluate which subdomains measure unique aspects of health status	NCSI, SF-36	NA	NCSI: ↓ intercorrelations subdomains. 4 subdomains showed conceptual similarity (Subjective Pulmonary Symptoms, Subjective Impairment and Dyspnoea Emotions, and between Fatigue and Health Related Quality of Life) with ≥1 SF-36 subdomain (Vitality and General Health, and between Vitality and Mental Health and Social Functioning) and vice versa	Both NCSI and SF-36 can be used to measure health status in QF patients. Combining NCSI and 4 SF-36 subdomains (Role Physical, Bodily Pain, Social Functioning, Role Emotional), is preferred to obtain a detailed overview	NA	★ ☆ ★ ★ ☆ ☆ ☆ ★ ☆

**** Definition of used study population in articles explained in a different table, including definitions of QFS and/or fatigue is applicable. Main information is on diagnosis. Some articles also contain relevant information on other domains: A= Aetiology, B/D= Background/descriptive, P/T= Prevention/therapy.***

Abbreviations: AI= Acute infection, AQF= Acute Q-fever, CF= Chronic fatigue, CFS= Chronic fatigue syndrome, CoS= Cohort study, CQF= Chronic Q-fever, Mo= Month(s), NA= Not applicable, NCSI= Nijmegen clinical screening instrument, originally developed to provide a detailed assessment of health status of COPD patients. It combines a number of existing health status questionnaires, NOS= Newcastle–Ottawa Scale: S= selection (maximum of 4 stars), C= comparability (maximum of 2 stars), O= outcome (maximum of 3 stars); ★: star earned; ☆: item not applicable, NR= Not reported, PIF(S)= Post-infective fatigue (syndrome), PO= Personal opinion, PQFS= Post-(acute)Q-fever (fatigue) syndrome, QA= Quality assessment, QF= Q-fever, QF(F)S= Q-fever fatigue syndrome, Ref= Reference, SF-36= The Short Form (36) Health Survey, a patient-reported survey of patient health to assess quality of life of patients, functional impairment and reduced health related quality of life, Yr(s)= Year(s).

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S3 Table. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
1960, O. Powell [1]	Australia, 1958 July-1959 June	Observa-tional pros. CoS	AQF patients (n=72, all ♂), describe clinical features and FU	NR	NA	Proportion of cases convalescence prolonged, with undue fatigue, setback up on moderate exertion, poor appetite, and occasional headache. 15/61 returned to work >6 wks post AQF, 12 >8wks. Mean period off work: 0-29 yrs 29 days, 30-49 yrs 45 days, 50-69 yrs 68 days. Total amount of time on workers' compensation payment 2013 days	Confirms previous observations that convalescence is more protracted in elderly	NA	★ ★ ★
1990, S. Reilly [2]	UK, 1972-1988, study period 16 yrs	Observa-tional pros. CoS	Seroprevalence *C.b.* assessed after testing all FUO, respiratory infections, CNE, and hepatitis cases. Co-morbidity: NR. Time baseline (AQF) to measurement complaints NR	CFT, IFA (selected cases)	NA	103 *C.b.* infections: 46 AQF, 5 CQF, 52 past infections. Details 61 cases (46 AQF, 5 CQF, 10 past infections). Outcome AQF: 57% uncomplicated, 4% prolonged fatigue (duration unknown), 11% underlying malignancy, 9% neurological sequelae, 9% persistent chest symptoms, 9% hepatic dysfunction. Outcome previous infection: 10% prolonged fatigue, 10% depression, 10% lymphadenopathy, 20% sarcoidosis, 10% polyarthritis nodosa	QF remains unpredictable, with a propensity to follow a protracted course. Prolonged serological and clinical surveillance of all QF cases is suggested	NA	★ ★ ☆ ☆ ★ ★
1995, P. Harvey-Sutton [3]	Australia, yr NR	POB	N unknown. PQDS or PQCFS. No control group	NA	NA	Observation of bradycardia in PQDS patients	Bradycardia may be a sign of PQDS	NA	NA

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)		
1996, B. Marmion [4]	Australia, 1995. Study period: 5-14 yrs post AQF in 1981-89	CC	Post AQF laboratory proven (n=39) with QFS, skin-test / antibody negative vaccinated (n=39), skin-test or antibody positive without QF history (39), seronegative (n=39). Controls matched (sex, ≤10 yrs age). Co-morbidity: NR	54-item questionnaire based on symptoms	NA	Combinations fatigue, night sweats, myalgia, fasciculation, with various minor symptoms more common in post AQF group, in 18-48% depending on number and mix of symptoms used for QFS definition. Met CFS CDC criteria: 11/39 post AQF, 0/39 vaccinees, 0/39 other controls	Interpretations range from compensation-driven through psychogenic perpetuation of original symptoms/ depression, to chronic immune stimulation. Hypothesis persistence *C.b./* its antigens causes dysregulation macrophage/T-lymphocyte axis with aberrant monokine and lymphokine production mediating symptoms	Diag, A	★ ★ ★	★ ★	★ ★
1996, J. Ayres [5]	UK, 1995. Study period 6 yrs post AQF	CC	QF patients (n=83, 70 ♂) vs. matched (age, sex) controls (n=26). Co-morbidity: NR. Assess prevalence chronic symptoms 6 yrs post AQF	Questionnaire as in [4]	NA	QF group: 66% fatigue, 69% joint aches, 65% sleep disturbance, 59% cough, 53% sweats, irritability 54%, chest pain 51%, breathlessness 49%, headaches 47%, dizziness 39%, blurred vision 34%, alcohol intolerance 33%. ↑ Prevalence cases i.c.w. controls: joint pains, sleep disturbance, cough, sweats, irritability, chest pain, breathlessness, dizziness. No difference prevalence fatigue, blurred vision, headaches, alcohol intolerance	Findings support view that chronic PQFS exists which is in many ways similar to CFS	NA	★ ★ ★	★ ★	★ ☆

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)		
1998, J. Ayres [6]	UK, 1994. Study period: 5 yrs post AQF	CC	71 symptomatic *C.b.* (mean age 55, 81.7% ♂, 32.4% current smokers). Matched (sex, age, ethnicity) controls: 142 (55 yrs, 81.7% ♂, 16.9% no febrile illness needing medical attention April-July 1989). Asses CFS symptoms prevalence post AQF	Modified questionnaire [4], including VAS per symptom	NA	QF symptom prevalence: significant ↑ fatigue, sweating, blurred vision, breathlessness on exertion (especially non-smokers) than controls. QF cases symptom severity: ↑ fatigue, blurred vision, sweating, memory ↓, joint pains and headaches. 42.3% QF cases and 26% controls had CFS according to CDC criteria (p=0.025, post-hoc)	A syndrome characterized by undue fatigue, breathlessness on exertion, excessive sweating and blurred vision post *C.b.* infection, persists yrs. Defining questionnaire based syndrome due to QF is dangerous, objective measures needed. Mechanism elusive, subclinical cardiomyopathy/ autonomic dysfunction suggested	NA	★ ★ ★ ★	★ ★	★
1998, K. Kato [7]	Japan, March 1996-April 1997. Period: NA. Single blood samples	CC	52 patients (13 ♂, mean age 41 SD15, range 9-74): fatigue 77%, feeling feverish 44%, joint aches/myalgia 70%, headache 56%, cough/sore throat 42%; duration 4.9 yrs SD1.0, range 0.5-22. 52 healthy controls (35 ♂, mean age 52, SD10, range 38-82), and 70 cord blood samples	n-PCR	NA	Physical examination. CFS: 17/52 *C.b.* positive, amplification 438-bp fragments n-PCR. 52 controls 5/52 and 2/70 cord blood samples positive n-PCR. Mean age patients positive n-PCR 42, SD14, range 9-67. Estimated duration fatigue 77%, feeling feverish 53%, joint aches/ myalgia 70%, headache 41%, cough/sore throat 47% was 4.0 yrs SD1.2. Positive ratio patients nonspecific complaints ↑ i.c.w. healthy controls (p<0.05) and cord blood (p<0.001)	High prevalence *C.b.* infection adult patients with long term, nonspecific complaints i.c.w. healthy controls, and possible existence chronic post AQF syndrome in Japan. Results appear to support the report of [4] and QFS concept	NA	★ ★		★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2002, M. Wildman [8]	UK, 1999	CC	10 yrs post *C.b.* outbreak. 80 matched controls (sex, age, and smoking) random 2 local general practitioners (mean age 55.4, SD11.7, 68 ♂). 108 Q-exposed cases (mean age 55.6, SD11.8, 68 ♂) last contacted 1989/1994. Exclusion controls serology positive *C.b.* 77 matched pairs analysed. Aim: had subjects involved in West Midlands 1989 outbreak ↑ fatigue i.c.w. non-exposed controls 10 yrs later	11-item fatigue questionnaire, GHQ, CIS-R. MOS, SDQ. Laboratory test *C.b.*, spirome-try, ECG, shuttle walk, incremen-tal exercise test	NA	108 Q-exposed, 64.8% fatigue, 34.3% ICF vs. controls 36.3% and 15.0%. 77 matched pairs: fatigue Q-exposed vs. controls: 64.9% vs. 35.1%, p<0.0001. ICF in 32.5% Q-exposed and 14.3% controls, p=0.01. 46.8% GHQ cases Q-exposed vs. 23.4% controls, p=0.004. Matched analysis: fatigue 66.7% Q-exposed, 34.7% controls, p<0.001, ICF 34.7% Q-exposed vs. 13.9% controls, p=0.004. CFS 19.4% Q-exposed vs. 4.2% controls. p=0.003. 47.2% Q-exposed had GHQ vs. 23.6% controls, p=0.004	*C.b.* cases exposed in 1989 had more fatigue than controls, some fulfilled CFS criteria. Uncertain if this is due to ongoing antigen persistence or to psychological effects of prolonged medical follow-up	A	★ ★ ★ ★ ★ ★
2002, B. Marmion [9]	Australia, 2002. Duration study NA	PO, comment on [10]	No patients/controls. Characteristics and co-morbidity: NR	NA	NA	Previous report [11] did not claim persistent infection to cause PQFS. Substantial proportion AQF patients have QFS-like symptoms to QFS (milder version of acute phase symptoms without fever) for 6-9 mo post AQF and then recover. ±8-10% exhibit similar symptoms and do not reach immune/other homeostasis ≥1 yr	Systematic FU AQF patients needed, as 8-10% not recover ≥2 yrs post AQF	NA	NA

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)	
2002, M. Wildman [12]	UK, 2002. Duration study NA	PO, comment on [10]	No patients/controls. Characteristics and co-morbidity: NR	CFS study group's 1994 CDC definition; 3 fatigue levels with ↑ seve-rity: (i) fatigue, (ii) idiopathic CF, and (iii) CFS	NA	Findings in fatigue prevalence study [8] differs from 5-10% found by others [10]. However, prevalence of fatigue in UK's general practice population is 38% vs. 36.3% in controls [8]. Idiopathic CF: 18.3% general practice vs. 15% in [8]	Lack explicit measurement instruments make comparison fatigue between studies impossible. Increased fatigue scores in QF exposed cohort were measured with standardized and well-validated instru-ments, permitting replication. Fatigue measurement is essential and should be standardized to compare studies	NA	NA	
2003, T. Hatchette [13]	Canada, yr study NR, study period: 1999-2001	CoS	Post AQF (n=33), controls without AQF during same outbreak cohort (n=24). Characteristics and co-morbidity NR. To follow effect of AQF on quality of life of patients 3 and 27 mo post AQF	Questionnaires on nature and duration of symptoms, SF-36	NA	3 mo post AQF only General Health scores of *C.b.* infected were ↓ than controls (p=0.03). 27 mo post AQF scores 5/8 domains and physical/mental summary scales ↓ i.c.w. controls. 27 mo post AQF 52% *C.b.* infected still reported symptoms, incl. 7 with initially resolved symptoms 3 mo post AQF. Of 3 *C.b.* infected symptoms resolved at 27 mo, who initially had persistent symptoms. ↓ scores General Health, Mental Health, Vitality and physical summary scales in those with persistent symptoms i.c.w. no symptoms. No initial symptoms nor antibiotic treatment of AQF predictive for developing persistent symptoms post AQF	Post *C.b.* infection symptoms can persist >2 yrs with significant quality of life impact. Data reflect further evidence of QFFS. Differences may reflect socioeconomic, physiological/ psychological effects of being labelled with QF rather than true post-infectious sequelae	A	★ ★ ★ ★	★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)			
2004, H. Thomas [14]	UK, 1999. Study period March-July 1999	CC	Random sample farmers (n=425). Test seroprevalence *T. gondii* and *C.b.,* and association *T. gondii*, slow reaction and poor concentration, and between *C.b.* and persistent fatigue, and association organisms with depression/ depressive ideas. Duration infection unknown	CIS-R, venous blood	NA	15% relevant fatigue levels, 5% concentration problems, 5% depressive ideas, 4% depression, 6% general psychiatric morbidity. Seroprevalence: 45% *T. gondii,* ↑ with age, no gender differences; 31% *C.b.*, no association age/gender. 46 seropositive for both. Neither infection associated clinical relevant fatigue, concentration problems, depression, depressive ideas/overall psychiatric morbidity i.c.w. seronegative individuals, not associated ↑ risk psychiatric outcome after age and sex adjustment. ↑ % *C.b.* seronegative psychiatric symptoms i.c.w. seropositive	No evidence *T. gondii/C.b.* infections associated with neuropsychiatric morbidity, in particular poor concentration/ fatigue	NA	★ ★ ★		★ ★
2006, I. Hickie [15]	Australia, (sub study DIOS), yr study NR	CoS	N=253; 68 EBV (mean age 22, range 16-49, 57% ♀), 60 RRV (mean age 40, range 18-69, 45% ♀), 43 *C.b.* (mean age 40, range 16-73, 14% ♀), 82 not confirmed (mean age 38, range 16-77, 44% ♀). Control of fatigue: baseline, 3 and 6 wks, 3 and 12 mo post AI. Excluded; hypothyroidism/ primary sleep-/ psychiatric disorders. Controls (age and sex matched) recovered from AI at 6 mo	SPHERE, SOMA Laboratory and clinical examination	NA	Provisional PIFS case rate 35% at 6 wks, 27% at 3 mo, 12% at 6 mo, and 9% at 12 mo, regardless of the infective agent, age, gender or psychiatric disorders. Confirmed PIFS: 28 cases (14 ♂, 14 ♀, mean age 37, range 17-63); 5 EBV, 3 QF, 13 RRV, 8 unconfirmed infections. I.c.w. all participant, no difference in age/ sex. I.c.w. controls, comparable: premorbid psychiatric diagnosis, intercurrent psychiatric disorders. Confirmed PIFS: median score acute sickness factor rapidly ↓ to zero, for fatigue, musculoskeletal pain and neurocognitive disturbance remained ↑	Pro-inflammatory cytokines do not remain ↑ in PIFS. Key risk factor PIFS is severity acute illness; not demographic, psychological (premorbid/ intercurrent psychiatric disorders) factors	A	★ ★ ★ ★	★ ★	★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)		
2010, G. Limonard [16]	Netherlands, 2008	CC	54 post AQF patients (61.1% ♂, mean age 53.1, SD14.2, co-morbidity 40.7%, current smoker 44.4%). 23 seronegative neighbourhood controls (sex matched, age ±10 yrs) (42.3% ♂, mean age 53.6, SD9.7, co-morbidity 39.1%, current smoker 26.1). Asses health status 1 yr post AQF	NCSI	NA	*C.b.* cases scored 1 yr post AQF significantly worse for all subdomains of symptoms. 52% cases clinically significant fatigue vs. 26% controls. Abnormal fatigue score QF patients 74% vs. controls 48%. Severe levels resp. 52% vs. 26%. NCSI scores of 11 seropositive and 23 seronegative controls not different for 8 subdomains health status	Sustained ↓ in health status 1 yr post AQF. NCSI scores from seropositive controls without clinical QF history comparable with seronegative controls, suggesting that clinical expression of AQF is essential in subsequent sustained ↓ health status	A	★ ★ ★ ★	★ ★	★ ★
2010, G. Limonard [17]	Netherlands, yr study NR. Study period 2007-2008	CoS	85 AQF patients (62% ♂, mean age 49 (18-80)). No controls. Co-morbidity: n=26 (6 cardiovascular, 3 pulmonary, 1 neurological, 4 rheumatological, 1 haematological, 3 depression, 5 diabetes, 3 other). Hospitalisation: 24 AQF patients	Post AQF: history, physical examination (6, 12 mo), IFA, CFT (baseline, 3, 6, 12 mo). Single transthoracic echocar-diography	NA	Post AQF 59% persistent symptoms at 6 mo and 30% at 12 mo FU. Self-reported fatigue initially 69%, at 6 mo 52%, at 12 mo 26%. No CQF. 59% had cardiac valvulopathy. ↑ antibody titres up to 3 mo, and ↓ in the following 9 mo	Screening echocardiography is no longer standard post AQF. At 6 mo fatigue is the most common complaint. Further studies needed with a control group to assess health status	NA	★ ☆ ★ ★	☆ ☆	★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)		
2011, G. Morroy [18]	Netherlands, study period 2008-2011	CC	515 notified QF patients (2007 and 2008) with known 1st day of illness (mean age 50.4 and 51.8 yrs, 60% ♂, 57.2 % co-morbidity) vs. healthy individuals (n=65) and severe COPD patients (n=128) assessed 12-26 mo post AQF	NCSI	NA	Abnormal fatigue score 58.9% QF patients, of which 43.5% severe. Similar scores for participants older and younger than 50 yrs. I.c.w. healthy controls (12.3% fatigue) QF patients scored significantly worse but better than COPD controls for subdomain fatigue. Hospitalisation, heart and lung disease, arthritis and depression significantly influence degree of fatigue	Sustained ↓ in health status 12-26 mo post AQF regardless of age. Policy makers ought to take this into account when considering measures to curb the extensive outbreak. Hospitalisation and co-morbidity predictors ↓ health status. More attention needed prevention and treatment long-term consequences	A	★ ★ ★	★ ★	★ ★
2011, HC. van Woerden [19]	UK, yr study 2008	Nested-CC	32 post AQF 6 yrs post outbreak Newport Wales, 2002 (mean age 50.18, SD 9.85). 13 controls (mean age 53.57, SD 8.86). Assess if i) CF ii) depression, and iii) ↓ physical functioning were more common in AQF patients 2002 i.c.w. controls	*C.b.* IFA, PHQ-9, Chalder Fatigue scale, GHQ	NA	Chalder Fatigue scores cases significantly ↑ (P=0.047). PHQ-9 and GHQ scores equal i.c.w. controls. CS analysis relationship IgGII in 2008 and Chalder Fatigue scores (P=0.004) and PHQ-9 scores (0.049). Longitudinal association AQF and CF 6 yrs later. CS analysis relationship depression scores (PHQ-9) and positive QF serology	CF more common 6 yrs later in QF positive patients. Possible relationship ↑ *C.b.* IgGII, symptoms CF and depression. High antibody levels may indicate ↑ responder status rather than presence micro-organism. Points up the desirability trial antibiotic treatment in QFS	P/T	★ ★ ★ ★		★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2012, B. Strauss [20]	Germany, yr study NR	CC	84 post *C.b.* 2 yrs post Jena outbreak 2005 (mean age 48.4, SD15.2, ♀ 49%). 85 controls (mean age 49.3, SD16.8, ♀ 61% same general practitioner not controlled *C.b.)*. To investigate if fatigue/CF and/or CFS more frequent in *C.b.* infected vs. non-infected controls, and contrast QF patients with/without fatigue symptoms related to somatoform symptoms, hypochondrial worries/beliefs, psychosocial complaints and social support	MFI 20, SF-12, CDC-SI, SOMS, WI, OQ-45, F-Sozu K14, mini-DIPS	NA	Post *C.b.* more fatigue symptoms and CF i.c.w. controls (54.8 vs. 20%, 32.1 vs. 4.7%). Not more CFS criteria (1 patient each group). *C.b.* with fatigue symptoms had significantly ↑ scores SOMS, WI, ↑ psychosocial complaints with OQ-45. Health Related Quality of Life QF group ↓ than controls	Fatigue symptoms common among QF patients. No ↑ CFS prevalence among QF patients. Combination fatigue and other psychosocial symptoms support biopsychological aetiology. CBT might be optional for prolonged fatigue post QF for those with psychological distress	A, P/T	★ ★ ★ ★ ★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2012, G. Morroy [21]	Netherlands, yr study: 2008-2011, duration: 2007-2010	CoS	515 notified AQF, known 1st day of illness in 2007 or 2008 (mean age resp. 50.4 and 51.8; 60% ♂, 57.2% with co-morbidity) FU 12 or 26 mo post AQF. Quantification of sick leave post AQF and long-term symptoms	NCSI and open questions regarding work	NA	Post AQF 39.6% more 1 mo absent work. Hospitalisation during AQF, smoking and heart disease independent risk factors for long-term sick leave. At 12-26 mo post AQF 9.3% unable to function at pre QF levels due to fatigue and ↓ concentration. >30% not fully resumed daily activities; 80.8% due to fatigue, 4.9% due to respiratory problems. 12-26 mo post AQF 40% reported health complaints; fatigue 19.8%, difficulty concentrating 9.5%, muscle pain 9.0%, night sweats 7.9%, eye problems 3.8%	QF has considerable impact on productivity and perceived health status. Hospitalisation, indicator of AQF severity, was a predictor for long-term sick leave and fatigue	NA	★ ☆ ★ ☆ ☆ ★ ★
2012, Y. Arashima [22]	Japan, yr study NR	CR	♂ 46 yrs, general fatigue, slightly elevated body temperature, night sweats, noise in ears, taste disturbance, headache, cough. Result: depressed with thoughts of death. Disease period 3 mo earlier. Co-morbidity: high-level depression (SDS 65) after start symptoms. PS 6. IgMI, IgMII, and IgGI negative, IgGII 1:64, n-PCR serum positive	PS, SDS, n-PCR, IFA	Mino-cyc-line 1 mo 200 mg/d, swit-ched to 100 mg/d (total 3 mo). Anti-depres-sant p.o.	<1-2 weeks treatment, arthralgia and slightly elevated body temperature ↓, other symptoms improved. At completion, clinical symptoms almost resolved. IgMI, IgMII, IgGI, IgGII all negative, n-PCR negative. PS 1. SDS 47. 1 yr FU: no exacerbations	PQFS is associated with depression. Minocycline seems effective. Carefully monitor depression in PQFS	Diag, A, P/T	-/-, +, ++, ++, ++, NA, +/-, -/-

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2012, S. Yakubo [23]	Japan, yr study NR	CR	♂ 53 yrs, past QF infection, general fatigue, nausea, stomach pain, abnormal oral sensation, sore throat, trouble sleeping. Co-morbidity: depression	SDS	Antide-pres-sant, *C.b.* antibi-otic	Depression triggered by *C.b.* led to suicide	Treat *C.b.* with antibiotic. Check for depression, if present treat aggressive. Consider psychiatrist early. SDS is useful in these cases	Diag P/T	-/-, +/-, +/-, +/-, +/-, NA -, -/-
2013, M. van Asseldonk [24]	Netherlands, 2012. Study period: 2007-2011	Economic evalua-tion	No patients/controls. Co-morbidity and characteristics: NR. Assess economic impact QF outbreak in the Netherlands, clarify costs-benefits control campaign, quantify and compare costs livestock sector, human health costs and disease burden. ±25% post AQF who seek medical attention expected to have CFS. Recovery period CFS 5-10 yrs (calculated with 7.5 yrs, working 50% contract time). Disability rate/ weight factor: 0.14. 3.000 Euro/notified case	DALY (YLD, YLL). Deterministic socio-economic model	NA	Total disease burden 2462 DALY, of which CFS 1481 DALY, CQF 806 DALY. Income losses accumulate over time due to long duration paid sick leave. Treatment costs: <2% total human health costs. Using extreme upper and lower bounds; CFS 30% of cases, duration ≥10 yrs, disability weight 0.20, 18.167 Euro/DALY; CFS in 20% of cases, duration ≥5 yrs, disability weight 0.10; 87.602 Euro/DALY	Most long-term benefits implemented control programme reduced disease burden and human health costs. Majority short-term intervention costs in dairy goat sector. Estimated: total loss in public sector: 222 Million Euro; total loss 307 Million Euro. Estimated burden human health 2462 DALY's 2007-2011. CFS most prominent burden	NA	16/19 checklist items positive **

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2013, R. Brooke [25]	Netherlands, yr study NR. Study period Jan 2009-Apr 2010. Duration study NA	Burden of disease study	QF notifications Jan 2009-Dec 2009 (1407 ♂, 906 ♀) vs. influenza notifications Apr 2009-Apr 2010 (1219 ♂, 1508 ♀). Correction for underreporting QF (factor 12.6) and influenza (factor 4.4 to 5.6)	YLD, YLL (2009 Dutch life expectancy), DALYs, BCoDE comparison 2 infectious disease outbreaks	NA	QF: 5797 DALYs, 1771 from acute illness, 4027 from sequelae. PIFS 57% total burden, mainly 45-49 age group. Influenza: 24484 DALYs, 3033 from sequelae. Total no DALYs due to influenza higher than QF, but on per case basis QF more severe. QF is 8.28x worse than influenza regarding composite health measures due to long-term sequelae up to 10 yrs post AI	Intervention prioritization for QF should target immediate interventions for containment and support of long-term sequelae. Long-term sequelae contribute a high burden of disease	NA	NA
2013, Y. Arashima [26]	Japan, yr study NR	CR	♀ 31 yrs, general fatigue, cough, dyspnoea, slightly elevated body temperature, headache, dizziness, poor appetite, copious sweating, night sweating, nausea, vomiting, palpitations. QFS (IgMII 1:16, IgGII 1:128, n-PCR positive) 18 mo post URTI, no result antibiotic treatment. Bronchial asthma 1 mo post URTI, 3 mo steroid inhaler, no improvement. Co-morbidity: moderate/greater depression (SDS 54). Suicide attempt	PS, SDS, n-PCR, IFA	Steroid inhaler 3 mo. Mino-cycline 200 mg/d post diag-nosis QFS	At least 3 mo minocycline: improvement generalized symptoms and bronchial asthma. PS 1. n-PCR negative. IgMII 1:16, IgGII 1:16. FU 9 mo post treatment: bronchial asthma and fatigue disappeared. Depression alleviated	*C.b.* can cause bronchial asthma and should be considered when resistant to standard treatment accompanied by slightly elevated body temperature or general fatigue. Be aware of suicide attempts	Diag, P/T	-/-, ++, ++, ++, ++, NA, +/-, -/-

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2014, J. van Loen-hout [27]	Netherlands, yr study NR. Study period: 2011-2012, single measurement 12 mo post onset of illness	CC	QF patients (n=309, 53.7% ♂, mean age 49.9 (13.8), current smoker 28.8%, pre-existing health problems 40.6%, hospitalised 36.6%) vs. Legionella patients (n=190, 68.9% ♂, mean age 61.1 (11.5), current smoker 37.4%, pre-existing health problems 59.5%, hospitalised 61.1%), and QF group matched (age, gender) healthy controls (normal lung function, n=121, 55.4% ♂, mean age 51.4)). Assess and compare health status patients 1 yr post QF/Legionella	NCSI, SF-36	NA	Worse score QF vs. Legionella patients on subdomains fatigue (60.2% vs. 50.0%, i.c.w. 2.5% healthy controls), General Quality of Life (50.0% vs. 42.6%), Role Physical. Adjustment confounders: only Role Physical remained different. In both QF and Legionella: proportion severely affected patients ↑ i.c.w. controls	Certain infectious illnesses are followed by long term impaired health status, including PICF. QF and Legionella patients are affected on ≥1 aspects health status, especially fatigue, General Quality of Life, Role Physical. Impact QF seems higher than from Legionella. Health staff need to be aware of this impact in order to provide adequate care	NA	★ ★ ★ ★ ★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)		
2014, A. van Dam [28]	Netherlands, 2009-2011, inclusion 1st May-30th September 2009	CC	50 QF seropositive LRTI (mean age 48.1, SD14.3) vs. 32 QF seronegative LRTI patients (mean age 57.2, SD14.4); 18-75 yrs. Comparable gender (60% vs. 50% ♂), current smoking (40% vs. 30%), hospitalisation during LRTI (10% vs. 7%), co-morbidity (42% vs. 56%). QF positive: more often pneumonia i.c.w. QF negative. Assess if LRTI due to QF has higher health status impairment i.c.w. other LRTIs 15 mo post AI	NCSI (completion 10-19 mo post LRTI, mean 15 mo). QF positive tested with PCR, IFA or CFT	NA	QF positive LRTI: severely affected General Quality of Life (40%) and fatigue (40%), QF negative LRTI: fatigue (64%) and subjective pulmonary symptoms (35%). 40% QF positive and 56% QF negative severely affected on >1 subdomain. No difference health status scores QF positive and QF negative LRTI patients for all subdomains except subjective pulmonary symptoms	Large group LRTI patients affected >1 aspect of health status 15 mo post LRTI. Little difference in health status QF positive and QF negative LRTI patients. General practitioners ought to be aware of long-term health problems in LRTI patients in general	NA	★ ★ ★ ★	★ ★	★ ★
2015, J. van Loen-hout [29]	Netherlands, study period: 2010-2013, FU at 3, 6, 9, 12, 18, and 24 mo post AQF	CoS	336 post AQF patients (in 2010-2011, 54.8% ♂, mean age 48.5, SD13.9, co-morbidity 39.7%), comparison NCSI scores matched (age, gender) healthy controls. To assess health status progression of QF patients over 24-mo period, and identify influencing factors	NCSI (3, 12, 18, and 24 mo), SF-36, question-naires	NA	Significant linear improvement over time in 9/12 health status subdomains. Severely affected: fatigue 73.0% at 3 mo, 60.0% at 12 mo, 37.0% at 24 mo (vs. 2.5% healthy reference group), General Quality of Life 42.2% at 3 mo, 50.2% at 12 mo, 33.7% at 24 mo (vs. 19.8% healthy reference group). For 3 most severely affected subdomains (fatigue, General Quality of Life, Role Physical): females, young adults, pre-existing health problems, at baseline were associated with ↓ long-term health status	Despite linear improvement over time, >1/3 patients had ↓ health status at 24 mo. Results suggest that psychological distress is not an important factor in explaining ↑ fatigue levels	A	★ ★	★ ★	★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)
2015, J. van Loen-hout [30]	Netherlands, yr study 2011-2013, single measurement 4 yrs post AQF	CC	448 notified post AQF (2007-2008, 57.6% ♂, mean age 54.4, SD12.4, co-morbidity 51.1%) vs. 193 symptomatic non-notified post QF (2008-2009, 45.1% ♂, mean age 50.2, SD15.3, co-morbidity 52.6%), vs. healthy controls. To compare long-term health status notified and non-notified QF patients	NCSI	NA	Notified: more ♂, ↑ age vs. non-notified. Equal proportions followed additional treatment for long-lasting health effects of QF, but addition antibiotic treatment slightly ↑ in notified patients. In both groups: fatigue (notified 50.5% vs. non-notified 54.6%) and quality of life (notified 42.3% vs. non-notified 44.4%) most severely affected subdomains. No difference long-term health status notified vs. non-notified, patients scored worse all subdomains i.c.w. healthy controls	Long-term health status is not determined by symptoms during acute QF. Little improvement health status between 1 and 4 yrs post AQF. Implication 2007-2009 Dutch QF outbreak underestimated if only considering notified patients. True burden of disease due to QF outbreak is larger	A	★ ★ ★ ★ ★

S3 Table continued. Domain background/descriptive

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (CR or NOS)	
2015, J. van Loen-hout [31]	Netherlands, yr study NR. Study period: 2010-2012, FU 3, 6, 9 and 12 mo post AQF, 12 mo post AI Legionella	CoS, with partly CC	336 QF, 190 Legionella patients. Assess (progress of) work participation of QF patients up to 12 mo post AQF, identify associated factors, and compare work participation between QF and Legionella patients 12 mo post AI	Question-naire 3, 6, 9 and 12 mo post AQF, ADIQ at 12 mo both groups	NA	↓ Proportion QF patients with ↓ work participation, 45% at 3 mo to 19% at 12 mo (vs. 15% Legionella patients at 12 mo). Median proportion reduction hours worked stable over time. ↑ Proportion patients not reporting symptoms up to 12 mo. No symptoms at 12 mo: QF 44% vs. 57% Legionella. Most frequent symptoms at 12 mo QF: fatigue, concentration/ memory problems, headache (all 24%), and muscle pain 23%. Legionella: concentration/ memory problems (21%), fatigue, respiratory problems, joint pains (13%). Grieving process: QF ↑ score denial and resistance, ↓ acceptance i.c.w. Legionella. QF; associated factors ↓ work participation: symptoms, ↑ level sorrow, former smoker (i.c.w. never smoked), no alcohol consumption, following treatment for long-term health effects. Median time to full return to work in QF group <3 mo	Almost 1/5 QF patient and 1/6 Legionella patient ↓ work participation at 12 mo. Occupational and insurance physicians need to be aware of long-term impact of QF and Legionella on work participation. Suggestion; undergoing QF leads to grief process similar to progressive disease, underlining the severity of sequelae due to QF	NA	★ ★ ★ ★	★ ★

**** Definition of used study population in articles explained in a different table, including definitions of QFS and/or fatigue is applicable. Main information in this table is on background/descriptive. Some articles also contain relevant information on other domains: Diag= Diagnosis, A= Aetiology, P/T= Prevention/therapy.***

***** Quality assessment economic evaluation study was assessed using the 'Evers checklist' [32].***

Abbreviations: ADIQ= Acceptance of Disease and Impairments Questionnaire, to assess the different stages of the grieving process due to the infection that patients underwent, AI= Acute infection, AQF= Acute Q-fever, BCoDE= Burden of Communicable Diseases in Europe project, attributes DALYs of an infectious disease to the year the acute infection occurs. This allows for the attribution of long-term sequelae, which may generate a higher number of DALYs, to the causative infection rather than only the initial acute illness, *C.b.= Coxiella burnetii,* CBT= Cognitive behavioural therapy, CC= Case-control study, CDC= Centres for Disease Control and Prevention, CDC-SI= German version of the CDC-Symptom

Inventory. The inventory asks in detail for 11 symptoms that commonly accompany CFS. These symptoms have to be described with respect to their intensity and frequency related to the last months, CF= Chronic fatigue, CFS= Chronic fatigue syndrome, CFT= Complement fixation test, CIS-R=Revised Clinical Interview Schedule to assess the symptoms of neurotic psychopathology in the week prior to interview. The CIS-R is made up of 14 sections, each covering a particular area of neurotic symptoms. Summed scores from all 14 sections range from 0-57, the overall threshold for clinically significant psychiatric morbidity is 12, CNE= Culture negative endocarditis, CoS= Cohort study, CQF= Chronic Q-fever, CR= Case-report, CS= Cross-sectional, DALY= A composite health measure that represents one lost year of healthy life between the current health status and that of an ideal health situation. Calculated as the sum of YLD for incident cases and the YLL due to premature death, DIOS= Dubbo Infection Outcomes Study**,** cohort study of subjects ≥16 yrs followed from the onset of a confirmed and documented AI due to EBV; *C.b.*; or RRV ≤6 wks post AI until complete recovery, EBV= *Epstein-Barr virus*, ECG= Electrocardiography, F-Sozu K14= To assess social support, a 14-item questionnaire resulting in a total score describing the quality and quantity of a person's social support, FU= Follow-up, FUO= Fever of unknown origin, GHQ= General health questionnaire, 12-item questionnaire to detect current cases of psychiatric co-morbidity, I.c.w.= In comparison with, ICF= Idiopathic chronic fatigue, IFA= Immunofluorescence assay, IgGI= Anti-phase IgG I titre, IgGII= Anti-phase IgG II titre, IgMI= Anti-phase IgM I titre, IgMII= Anti-phase IgM II titre, LRTI= Lower respiratory tract infection, MFI 20= German version of the Multidimensional Fatigue Inventory, a commonly used 20-item questionnaire indicating different dimensions of fatigue, Mini-DIPS= Diagnostic interview, a short form of the diagnostic interview of psychological disorders, Mo= Month(s), MOS= Medical outcome study 20-item questionnaire, used to define functional impairment in the construction of the CFS definition, NA= Not applicable, NCSI= Nijmegen clinical screening instrument, originally developed to provide a detailed assessment of health status of COPD patients. It combines a number of existing health status questionnaires, NOS= Newcastle–Ottawa Scale: S= selection (maximum of 4 stars), C= comparability (maximum of 2 stars), O= outcome (maximum of 3 stars);★: star earned; ☆: item not applicable, N/No= Number (of), (n-)PCR= (nested-) Polymerase chain reaction, NR= Not reported, OQ-45= OQ-45, to measure psychological symptoms and general impairment. It is a common symptom inventory used in many psychotherapy studies to reflect total impairment, social as well as interpersonal distress and impairment of social role performance, PHQ-9= a self-administered subset of the PRIMA-MD diagnostic instrument for common mental disorders to assess symptoms severity of depression, PICF= Post-infectious chronic fatigue, PIF(S)= Post-infective fatigue (syndrome), P.o.= Oral, PO= Personal opinion, POB= Personal observation, PQCFS= Post-Q-fever chronic fatigue syndrome, PQDS= Post-Q-fever debility syndrome, PQFS= Post-(acute)Q-fever (fatigue) syndrome, Pros.= Prospective, PS= Performance status score (range 0-9), which reflects the grade of fatigue/malaise to assess the severity of CFS, QA-CR= Quality assessment; for CR no quality checklists are available. Therefore, the following eight criteria for quality assessment were determined; addressing an appropriate and clearly focused question, representative population, description of the survey method or data collection, outcome measures defined, outcome measures described, response rate reported and results valid and applicable to the patient group targeted. The articles scores on these items: -/-, -, +/-, +, or ++, based on the Coordination of Cancer Clinical Practice Guidelines in Europe criteria, QF= Q-fever, QF(F)S= Q-fever fatigue syndrome, Ref= Reference, RRV= *Ross River virus*, SD= Standard deviation, SDS= Self-rating depression scale, consisting of 20 questions, score per question: 1-4 points, SDQ= Somatic Discomfort Questionnaire, a checklist of 25 somatic symptoms, as somatic symptoms are important minor symptoms in the construction of CFS definition, SF-12= The Short Form (12) Health Survey, SF-36= The Short Form (36) Health Survey, a patient-reported survey of patient health to assess quality of life of patients, functional impairment and reduced health related quality of life, SOMA= Empirically derived subscale of the SPHERE, used to record PIFS or illness duration. This reliably predicts disability and reflects patients' and doctors' reports of reasons for presentation to primary care. Scores ≥3 represents a clinically-significant fatigue state. Provisional PIFS: SOMA scores ≥3 at all time points up ≤3 months. Confirmed PIFS: symptoms persisted >6

months, and alternative explanations for ongoing illness was excluded, SOMS= Screening for Somatoform Disorders, a 53-item questionnaire assessing symptoms common for somatoform and somatisation disorder leading to the calculation of different indices, SPHERE= Somatic and Psychological Health Report, to assess a wide range of physical and psychological symptoms, including severity and duration of symptoms, *T. gondii= Toxoplasma gondii,* UK= United Kingdom, URTI= Upper respiratory tract infection, VAS= Visual analogue score, 10cm scale to quantify symptom severity, Wks= Weeks, WI= Whiteley Index, to measure the patients' tendency for hypochondriacal worries and beliefs, YLD= Number of years lost due to disability: number of incident cases x average duration of the disease x weight factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (dead), YLL= Years of Life Lost due to premature death; number of deaths caused by the disease x standard life expectancy at the age at which death occurs, Yr(s)= Year(s).

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S4 Table. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)	
1998, B. Bennet [1]	Australia, yr study NR (sub study DIOS). Study period NR	Pros. CoS	17 EBV, 8 QF, 5 RRV (82% ♂, mean age 29 (15-77)). Explore longitudinal relationships between physical and psychological symptoms and immunological factors during peak illness (symptoms <4 wks before presentation) and recovery phase (2 and 4 wks after baseline) of AI (EBV, *C.b.*, and RRV)	Baseline: interview, POMS, GHQ, SOFA, CIDI, DTH skin response. At 2 wks: interview, POMS, GHQ, SOFA. At 4 wks: interview, POMS, GHQ, SOFA, DTH test	NA	Baseline: fatigue and malaise most common symptoms. Depressive and anxiety symptoms not prominent. 46% cases no DTH skin response, indicative of impaired cell-mediated immunity. Over 4 wk period, improvement somatic and psychological symptoms, but 63% remained fatigue. Most symptoms improved; somatic changes notable in fatigue and malaise, rather than psychological (anxiety and depression). Psychological changes due to changes in perception fatigue and vigour. ↓ reported fatigue correlated with ↑ DTH skin response (indicating relation between fatigue and cell-mediated immunity) and GHQ scores	Fatigue commonly remains a prominent complaint at 4 wks. Resolution of fatigue is associated with improvement in cell-mediated immunity, supporting an immunological basis for PIF	NA	★ ★ ★ ★	★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)		
1998, I. Penttila [2]	Australia, yr study NR. Single measure-ment study	CC	18 QFS patients (mean age 39 (33-45), symptom score >100, mean 145 (133-157)). 27 controls; 6 resolving QFS (symptom score <100, mean age 39 (31-48)), 5 past AQF (>6 mo) without QFS (symptom score 15 (1-35), mean age 39 (25-54)), 8 QF skin test - vaccinated (mean age 44 (31-56), symptom score 7 (1-13)), 8 QF - healthy controls (mean age 32 (23-40), symptom score 5 (1-9)). Mean age groups equal. Asses cytokine release patterns PBMC stimulated with QF (phase I and II), measles antigens and PHA, in 72-h culture	Interview; clinical history, presence, frequency, and intensity of 16 minor and major QFS symptoms scored on linear numerical scale 1-20. Analysis of: IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IFNy, TNFa, TNFb and TGFB	NA	Aberrant cytokine release patterns of PBMC QFS patients stimulated with QF antigens; ↑ IL-6 release (mean 502 pg/ml, i.c.w. 47-53 pg/ml in other groups, p=0.018). Mean PBMC response of QFS to PHA ↑ than controls, not significant. 72% QFS patients: IL-6 release values >100 pg/ml; 66pg/ml max value for 95% CI of control groups. QFS ↓ IL-2 responders with QF antigens (p=0.014), but equal IL-2 release post PHA stimulation. QFS: ↑ IFNy responders (p=0.0008); no difference median [IFNy] released (p=0.14). IL-1 ↑ on PHA stimulation PBMC from QFS than controls (p=0.03), no difference with QF antigens. >IL-5 responders among QFS with QF antigens. Correlation IL-6 in conditioned medium, total symptom score, and scores other key symptoms. In QFS: persistent IL-6 upregulation, no time-correlation with AQF	Hypothesis: cytokine deregulation due to chronic immune stimulation and modulation by persistent *C.b.*/antigens. Aberrant IL-6 response not claimed to explain QFS pathogenesis. IL-6 might contribute to QFS symptomatology. QFS development appears to require cellular immune response against *C.b.* antigens. A speculative unifying concept is that QFS and QIE represent different poles of dysfunctional cell-mediated immunity response to *C.b.* QFS patients have positive LMR to QF antigens, greatly ↑ IL-6 release patterns from PBMC, IFNy upregulated, but IL-2 downregulated. Fatigue can be intermittent (relapsing) or continuous in QFS	Diag, B/D	★ ★ ★	★	★ ★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)		
2000, R. Harris [3]	Australia, yr study NR. Study period NR. Mean period sampling 37 mo post AI (2 after 9 mo, remainder after ≥12)	CC	QFS (n=29); 18 from [2], 11 additional with QFS post AQF. PBMC (n=29), liver biopsy (n=14), BMA (n=20). Controls from [2]. PBMC: patients no QFS post AQF (n=5); post-vaccination (n=7), *C.b.* seronegative healthy controls without CFS (n=6). BMA (n=6) of patients with diseases other than QF. Positive PCR controls; QIE or recrudescent infection in pregnancy (n=10)	PCR (target IS1111a), several primer sets in conventional PCR and TaqMan PCR system	NA	*C.b.* detection in QFS: PBMC 5/29, liver biopsy 2/14, BMA 13/20. In PBMC: no QFS 0/5, vaccinated 0/7, seronegative 0/6. In BMA: other diseases 0/6. PCR positive in QIE/placentitis 10/10	*C.b.* DNA in bone marrow 0.75-5 yrs post AQF infection unveils new QF pathology state. *C.b.* live/dead/other bio-entities not defined. Pattern suggestive paucibaccillary infection presumably under immune control, but not eliminated. Supports previous reports relationship QFS, cytokine dysregulation and immunomodulation from *C.b.* persistence. Bone marrow could be focus cryptic infection which might seed other sides. Before drawing conclusions on QFS, investigate bone marrow in more patients with/without QFS/other sequelae	Diag	★ ★ ★ ★		★ ★
2002, J. Ayres [4]	UK, 1999. Study period 10 yrs post AQF	Nested CC	N=85 *C.b.*-exposed (85.6% ♂, mean age 54.7, SD12.0, co-morbidity 29.4%) vs. n=75 matched (sex & smoking) QF seronegative controls (86.7% ♂, mean age 55.3, SD11.4, co-morbidity 29.3%). Determine if persistent fatigue post AQF represents sub-clinical cardiomyopathy	Question-naires, 12-lead ECG, echo-cardiography, spirometry, shuttle walk distance, MUGA scan (only in subset)	NA	68.2% *C.b.* cases fatigue any duration, 42.4% fatigue excluding co-morbidity. 20% CDC-defined CFS vs. 5.3% controls, 8.2% excluding co-morbidity vs. 0% controls. Normal ECG's 76.5% cases, 69.3% controls, no differences. Echocardiography: controls ↓ fractional shortening. Fatigued vs. non-fatigued QF cases: comparable echocardiography, ECG, shuttle walk distances, pack years smoking. Normal MUGA scan 6 *C.b.* cases (CDC-defined CFS without co-morbidity)	Findings do not support the existence of a sub-clinical cardiomyopathy in patients with fatigue after AQF, therefore not explaining breathlessness and fatigue. Chronic heart disease following AQF is rare and limited to IE	B/D	★ ★ ★	★ ★	★ ★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
2002, D. Raoult [5]	France, 2002. Duration study NA	PO	No patients/controls. Characteristics and co-morbidity: NR	NA	NA	6 mo post AQF 5-10% residual asthenia, very few >1 yr. Subjective symptoms difficult to quantify. CF: difficult to define, with different prevalence. Unknown if CF psychological in origin/directly caused by bacterium. Might reflect observational bias, *C.b.* strain or cultural differences, or genetic susceptibility	Amplicon production PCR in peripheral blood CF patients needs confirmation. New tools might allow to examine aetiology incompletely understood diseases caused by intracellular bacteria	B/D	NA
2003, K. Helbig [6]	Australia, yr study NR. Single measurement study	CC	23 active/recovered QFS, 42 controls Red Cross blood donors, all Caucasians. To compare variability in phenotype distribution among range of cytokine and accessory immune response genes in PQFS and controls	Genotyping within NRAMP1 gene, HLA typing for HLA-DR and HLA-B; 25 polymorphic variants 14 genes analysed	NA	No significant variation frequency individual SNP patients and controls, but more variants differing from wild type in patients i.c.w. controls, p=0.025. Differences allelic frequencies HLA-DR, significant ↑ frequency HLA-DR11 in QFS, but not HLA-B. Phenotype frequencies SNP in genes not significantly different from controls. Variation allele distribution QFS and controls INFγ di-nucleotide repeat. IFNγ genes; ↑ prevalence homozygous state IFNγ allele 2 in intron 1 in QFS	Possible genetic role expression overt chronic manifestations, e.g. individual variation *C.b.* immune response. Given complexity of genetic control of immune system, a simple 1-to-1 relation between QFS expression/other chronic complication QF and a particular polymorphic variation in a cytokine or immune control gene is unlikely. Effects are more likely multigenic	Diag	★ ★ ★ ★ ★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)	
2003, K. Ikuta [7]	Japan, yr study NR. Single measurement study	CC	44 CFS (H1: 22 CFS, 14 ♂, 23-61 yrs; H2: 22 CFS, 17 ♂, 20-46 yrs), 38 healthy controls (20 ♂, 20-59 yrs). To investigate association viral infections with CFS and 2-5AS activity in PBMC in Japan in 2 hospitals (H1, H2) different areas	*C.b.,* IFA IgGII positive titre ≥1:64	NA	2-5AS activity: 19 (mean 2.23) in H1, 7 (mean 0.91) in H2, 4 in controls (mean 0.74). Differences H1 and H2, and H1 and controls ($p<0.01$). No difference H2 and controls. IFNa similar in few CFS patients and controls. No relationship 2-5AS and IFNa positivity. EBV anti-EA-IgG antibodies in 9% and 32%in H1 and H2. IgG *C.b.* positive 6/22 H1, 0/22 H2, 1/9 controls. No difference *C.b.* positive H1 and controls/patients H2 and controls. No correlation 2-5AS activity and *C.b.* titres ($p>0.05$)	2-5AS activity ↑ PBMC CFS patients. CFS may be associated EBV/*C.b.* ↑ 2-5AS suggests immunological dysfunctions with virus infections in CFS. No relation titres *C.b.* and 2-5AS activities. 2-5AS activity changed from positive to negative in 1 CFS patient when *C.b.* antibodies disappeared, suggests *C.b.* association 2-5AS activity some CFS patients. Imply 2-5AS in some CFS patients activated by other mechanisms, in addition to EBV and *C.b.*	NA	★ ★	★ ★
2005, B. Marmion [8]	Australia and UK, 2001, study period NR	CC (case follow-up study)	*C.b.* positive UK cases (n=92) 12 yr post AQF (Birmingham 1989, n=92 blood samples, n=91 PBMC, n=35 BMA), Australian cases (n=29) 9 mo-5 yrs post AQF (n=29 blood samples and PBMC, n=20 BMA, n=14 liver biopsy) with CFS (CDC-criteria). To compare prevalence infection markers between cohorts	I. *C.b.* PCR (directed against several targets in the genome) DNA detection PBMC and bone marrow, II. CFT, IFA Phase I & II, III. isolation *C.b.* cell cultures of mice - PCR positive	NA	Both groups remained seropositive irrespective clinical state. *C.b.* genomic DNA detected by PCR in 65% of BMA from Australian vs. 88% Birmingham patients. No *C.b.* isolated from PCR positive samples	Results indicate more complex interaction between host-regulated, persistent carriage of *C.b.* and disease. An additional variable factor of host regulation of cellular immune response must determine levels of persistence and symptomatic outcomes. Hypothesis: in QF without sequelae, process largely confined to bone marrow. In QFS, modulation by the patient's immunogenetic background causes ↑ levels of *C.b.* genomes in bone marrow and ↑ shedding into peripheral blood	Diag	★ ★ ★ ☆	★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)		
2005, K. Helbig [9]	Australia and UK, yr NR, study duration NR	CC (gene-tic asso-ciation)	31 QFS patients vs. uncomplicated recovery up to 12 yrs post AQF (n=22) vs. QIE (n=22, mean age 57, range 29-78, time lag infection-IE 8.8 yrs, SD12, range 2-40) i.c.w. standard control panels general population. To compare frequencies of allelic polymorphisms in immune response genes in different QF patient groups	Whole blood, DNA extraction, HLA typing, micro-satellite typing, SNP analysis	NA	Significant differences between 3 groups. QFS patients differed from QIE, the uncomplicated and controls in frequency of HLA-DRB1*11 and 2/2 genotype of IFNy intron 1 microsatellite. Carriage HLA DRB1*11 allele associated with ↓ IFNy and IL-2 responses from PBMC. QIE showed differences in IL-10 promoter microsatellites R and G, and ↑ frequency TNFa receptor II 196R polymorphism. QF patients with uncomplicated recovery, differed from those with QFS/QIE, but similar in allelic frequencies to control panels	Conclusions *C.b.*, parvovirus B19 infection and CFS studies suggest that 'idiopathic' CFS patients from the wider population, away from outbreaks/occupationally exposed groups, are unlikely to have laboratory evidence of infection with the same infective agent. A common immuno-genetically determined failure of cytokine homeostasis to infective agents with the capacity to persist long in hosts is more likely	Diag	★ ★ ★ ★	☆ ☆	★ ★
2007, U. Vollmer-Conna [10]	Australia, 1999 (sub study DIOS); 12 mo collection period. Appraisal 1, 2, 3, 6, 12 mo post AI	Pros. CoS	22 PIFS patients (11 EBV, 6 RRV, 5 *C.b.*) vs. 42 aged-matched controls who recovered <6 wks of EBV (n=17), RRV (n=14), and QF (n=11). Analysis influence PIFS status on symptom severity and cytokine production i.c.w. controls	SPHERE, BDQ. SOMA score ≥3 to record PIFS	NA	No group differences cytokine levels. Severity symptoms ↓ in time. ↑ Age associated with ↑ musculoskeletal pain and neurocognitive disturbance. PIFS stereotyped post different triggers, with equal acute-phase cytokine production. Psychological/microbial factors not predictive PIFS. PIFS: ↑ mean no. bed-days acute phase, and more days "out of role"	Ongoing production IL-1b, IL-2, IL-4, IL-6, IL-10, IL-12, TNFa and INFy have no role in PIFS. Evidence against hypothesis associating prolonged fatigue with altered cytokine levels. AI triggers, not drives symptoms. PIFS can persist wks to mo	B/D	★ ★ ★ ★	★ ★	★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
2009, B. Marmion [11]	Australia and UK, yr study NR	Labora-tory case study	10 Birmingham (1989) *C.b.* PCR positive and 1 IE. To retest PCR positive samples with more sensitive methods for viable *C.b.* and *C.b.* cell components antigen and specific LPS ≥12 yrs post AQF, and re-interpret previous results. Review literature for a concept of immuno-modulatory complex generated by current studies	3 SCID mice; spleen and liver examination by PCR (targets COM1 and IS1111a sequences), IFA	Inocu-lation patient samples in SCID mice for 60 d	All patients' specimens including heart valve with endocarditis were infection negative in SCID mice. Mice spleens and livers PCR negative. Spleen sections of all specimens showed Coxiella antigen LPS complex by IFA	Long-term persistence non-infective, biodegradable immunomodulatory complex traces genomic DNA. Immuno-modulatory complex survival >12 yrs, in 1 patient 70 yrs, implies repeated passage macrophages ↓ regulation biodegrading function. Systemic symptoms QFS may reflect wide distribution parasitized mononuclear phagocytes. QFS follows clinical overt infection, rarely subclinical infection	NA	NA

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)	
2009, L. Zhang [12]	United Kingdom, yr study and period NR. Single measurement study	CC	117 patients idiopathic CFS/ ME; 6 Q-CFS/ME. Controls: endogenous depression (n=14), blood donors (n=29). Attempt to reproduce genomic subtypes CFS/ME (with distinct: SF-36, clinical phenotypes, severity and geographical distribution), determine specificity signature CFS/ME, and test associations CFS/ME subtype and infection by determining expression levels 88 human genes	Chalder Fatigue Scale, SF-36, Somatic and Psychological Health Report, PSQ, McGill Pain Question-naire, PAXgene blood RNA kit, micro-spectro-photometry, qPCR	NA	In CFS/ME differential expression confirmed for all 88 genes. 8 genomic CFS/ME subtypes with marked differences global functioning, clinical symptoms, severity levels and geographical distribution. Q-CFS/ME similar patterns gene expression in peripheral blood to idiopathic CFS/ME, and markedly different from normal group. 5/6 Q-CFS/ ME patients clustered in subtype A, but no subtype-specific relationships found with *C.b.* antibodies. Evidence subtype-specific relationships EBV and enterovirus. Gene expression in endogenous depression similar to normal controls, except ↑ regulation 5 genes (APP, CREBBP, GNAS, PDCD2, and PDCD6). Q-CFS/ME patients ↑ McGill Pain Questionnaire scores i.c.w. other groups. SF-36 ↑, Mental and physical fatigue, and SPHERE scores ↓ i.c.w. all groups, except normal blood	Q-CFS/ME had similar patterns gene expression as idiopathic CFS/ME	Diag, B/D	★ ★ ★	★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
2010, Y. Kadota [13]	Australia, 1999 (sub study DIOS); single measurement	Pros. CC	23 PIFS patients (9 RRV, 7 EBV, 4 QF, 3 viral infection unknown origin) vs. 25 matched (age, sex, BMI, activity levels) healthy controls. Evaluation association of PIFS with bidirectional autonomic signalling disturbance	Pulse oximeter, pain test algometer, Stroop task, SPHERE, SOMA K10, BDQ, DS14	NA	PIFS patients: ↑ symptoms in general, fatigue related, or psychological distress, more days not fulfilling normal roles past mo, ↑ experience negative emotions, ↑ reporting functional impairment daily activities, ↑ resting heartbeat, ↑ sensitivity to physiological signals. Relation between heartbeat discrimination accuracy and pressure pain sensitivity. Different heart rate pattern in response to ongoing mental stressors	PIFS: ↑ interoceptive sensitivity (with strong symptoms correlation), distinct pattern cardiac response; evidence physiological hyper-vigilance and response inflexibility. ↑ Resting heart rate with ↓ heart rate variability: ↓ parasympathic drive. Autonomic dysfunction involves both disturbance processing incoming homeostatic information, and altered reactivity to stressors	B/D	★ ★ ★ ★ ★ ★ ★ ☆
2010, O. Sukoche-va [14]	Australia, yr study NR, duration NR	CC (labora-tory case study)	No patients/controls. Samples post AQF patients (Birmingham, 1989), 3 groups; recGr3: asymptomatic recovery post AQF. QFSGr5: QFS, no co-morbidity. QFSGr6: QFS fatigue associated co-morbidity. 12 yrs post outbreak, groups sampled *C.b.* antibody, blood leucocytes, PCR on BMA. PCR	Cell culture assay, PCR (target COM1 and IS1111a), CBA, skin granuloma test in guinea pigs, immunoche-mistry, histoche-mistry, image acquisition	Inocu-lation patient samples in NOD/ SCID mice, FU for infection evidence and presence DNA and specific antigens in spleen and liver	Culture samples 10 QF patients NOD/SCID mice, 12 yrs post AQF no viable *C.b.* No AI induced. Complexes material *C.b.* antigens found in mouse spleens, significantly higher amounts in samples QFS, also in bone marrow and liver in all cases. Immunomodulatory complex stimulate cytokine release in mice and THP-1 macrophages, and to provoke inflammatory reaction on intradermal injection into skin of QF hyperimmunized guinea pigs (with Qvax). QFSGr5 and 6: weight ↓ 1st	In QFS viable, infective *C.b.* are rarely, if ever, isolated from PBMC or bone marrow, but complex of antigen and Phase 1 LPS (immunomodulatory complex) is regularly present. This non-infective complex of *C.b.* antigens survives in host and provokes aberrant humoral and cell-mediated immunity responses – a possible pathogenic link between initial infec-tion and PQFFS. Different responses between endocarditis, asymptomatic/recovered	NA	NA

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
			positive samples (bone marrow, PBMC, or aortic valve specimens) 10 patients from subsets inoculated intraperitoneal NOD/ SCID mice. Control animals received blood PCR negative, seronegative controls. To isolate living *C.b.* to ascertain pathological effects, retest and determine nature residual *C.b.* cell components		macro-phages	week post inoculation, later recovered and steady weight gain consistent with absence infection. All mouse spleen specimens PCR negative (1:100 dilutions). Despite absence active infection, changes: moderate spleen enlargement QFSgr5 and 6 i.c.w. controls ($p<0.05$), no massive splenomegaly by live *C.b*. Sections mouse spleens with variable amounts aggregates stained to detect specific antigen, also in NOD/SCID mouse bone marrow and liver inoculated with QFS specimens. *C.b*. antigens no correlation low levels *C.b.*, suggests complexes to represent incompletely degraded cell material. *C.b.* antigens localized in spleen phagocytes, and *C.b.* immunomodulatory complex in lysosomes mouse splenocytes. L-6/IL-10 ratio and ↑ level IL-10 might signal important role in facilitating survival non-degraded bacterial material	and QFS patients considered due to immunogenetic differences in handling immunomodulatory complex and cytokine responses. Hypothetical pathogenetic sequence QFS; overt clinical QF and immunogenetic polymorphism --> defective antigen clearance (immune-modulatory complex persistence) --> persistent cell-mediated immunity and cytokine dysregulation --> cytokine-mediated somatic gene modulation --> QFS		

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)			
2011, S. Galbraith [15]	Australia, yr study NR (sub study DIOS). Study period: baseline measure (T1 0<6 wks), T2 6<12 wks, T3 3<9 mo or >9 mo, T4 >12 mo, FU after 2 and 4 wks	Longi-tudinal, nested CC	Caucasians with PIFS (n=18; EBV, RRV, *C.b.*) (mean age: 40, SD18 years). Matched (age, sex & infection type) controls (n=18) who recovered promptly (mean age: 39, SD16). 11 ♂ per group. 127 samples analysed, 3-4 time points/subject. In longitudinally collected samples peripheral blood transcriptomes studied for gene expression patterns in PIFS patients and controls. Differential expression sought between early illness and late recovery (within-subject comparison), PIFS cases and recovered controls (between subjects comparison), and genes correlated with end phenotypes derived by principal components analysis (between-cohorts)	Microarray and confirmatory qPCR. SPHERE, SOMA	NA	23 genes with modest differential expression (0.6-2.3-fold change) in within-subject comparisons of early, symptomatic time points with late, recovered time points. Modest differences 63 genes, in CS comparison cases-controls 6 mo post AI in regression model. 223 genes correlated with individual symptom domains. qPCR confirmed 33/45 genes, none consistent across cohorts. Within subject comparison: 12 subjects (5 with QF) T1 SOMA scores ≥3, T4 SOMA scores <3. No genes with adjusted significance <0.05. Relative lack variance gene expression levels over ≥12 mo. Between subject comparisons: 17 cases (6 QF), 11 controls (2 QF). No genes with adjusted significance <0.05. QF subjects predominantly ♂ and older. 13 genes adjusted significance <0.05, 1 (CYBA) associated with fatigue in 2 of 3 infective cohorts (EBV, QF). Analysis identified illness severity, fatigue and neurocognitive disturbance, correlated for EBV and QF cohorts. Correlation test: 96 genes unadjusted significant at 5% for EBV and QF for severity, 93 for fatigue symptom domain, 106 for neurocognitive disturbance. Repeated correlation analysis: no genes correlated for EBV and QF in association with severity, fatigue, neurocognitive disturbance	Several infections trigger PIFS, which share key illness characteristics with each other and CFS. Previous CS CC studies of CFS suggested unique gene expression signature in peripheral blood samples. Although illness characteristics of PIFS patients have more similarities than differences, no reliable peripheral blood gene expression correlate is evident. No genes consistently associated with illness. CFS incidence closely comparable between EBV, RRV, *C.b.* Lack of coherent set of gene expression correlates across cohorts argues against validity of previously proposed signatures for PIFS or CFS. PIFS likely to be truly post-infective, un-associated with ongoing active replication of triggering agent	NA	★ ★ ★ ★	★ ★	★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)
2012, B. Piraino [16]	Australia, yr study NR (sub study DIOS). Study period NR. Baseline, FU 2-3wks, 4-6wks, 3-mo interval until 12 mo post AI	CoS	Caucasians (mean age 34.2, 49% ♀), <6 wks post AI (n=296), EBV, RRV, QF. Principal components analysis acute phase, self-report symptom data to empirically derived indices fatigue, pain, neurocognitive difficulties, mood disturbance, overall illness severity. Apply endophenotype concept to clinical dataset describing symptom domains of acute sickness response post viral/ non-viral pathogens, and validation by showing association with SNP in cytokine genes (IL-6, TNFa, IFNy, IL-10)	SPHERE (and SOMA), PSC, BDQ, principal component analysis, NanoDropR ND-1000 (DNA quantification), Sequenom MassARRAY® (genotyping of SNP)	NA	Individual symptom indices correlated with overall severity and functional status. Domain scores stable over time within subjects, but varied between subjects with same infection, and across infection sub-cohorts. Overall illness severity may have been comparable in some subjects, relative contributions from individual symptom domains making up the illness complex varied between these subjects. T allele IFNy+874T/A SNP best predictor of ↑ fatigue. ♀ more likely grouped in ↑ fatigue extreme. C allele of IL-10-592C/A SNP exerted protective effect on neurocognitive difficulties. A allele IL-10-592 SNP and G allele IL-6-174G/C SNP associated ↑ mood disturbance	Acute illness response has discrete symptoms including fatigue with unique genetic associations. Study offers new pathophysiological inside fatigue states. Illness severity phenotype not dependent on age/sex/ infection subtype. Robust correlation between illness severity and reported disability in AI. ♀ over represented in high severity group fatigue, mood disturbance, neurocognitive difficulties	NA	★ ★ ★ ★ ★

S4 Table continued. Domain aetiology

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Inter-ven-tion	Outcome	Conclusions/ recommendations	Other do-main	QA (NOS)	
2012, H. Hussain-Yusuf [17]	UK, 2008	CC	Cohort 211 UK factory workers *C.b.*-exposed 2002. FU 6 yrs post outbreak, comparison QF serology, presence viable *C.b.*, its DNA and fatigue in post AQF cases (n=38, 3 uncertain serology 2002) vs. seronegative, same outbreak (n=14). Assess if *C.b.* antigens (immunomodulatory complex) remain undegraded in some post AQF, with abnormal cytokine profile causing ongoing fatigue	Chalder Fatigue Scale, qPCR (com1 gene) on PBMC and VERO cultures (detect *C.b.* DNA), IFA, SCID mice inocula-tion (detect viable *C.b.*)	NA	18% became seronegative, remainder 10 phase I, 21 phase I en II antibodies. 29% controls became seropositive. No patient/ control PBMC contained viable *C.b.*/DNA. No viable *C.b.* in PMBC tested in cell culture and SCID mice inoculation. Chalder Fatigue Scale score after 6 yrs (n=11): 4 significant fatigue, 4 some, 3 not fatigued. No relationship between fatigue levels and serology, nor with presence of viable *C.b.*/DNA	6 yrs post AQF, some patients became seronegative but none contained viable *C.b.*/DNA in their PBMC. Correlation PQFF and persistent DNA could not be examined. A more sensitive DNA assays or more invasive sampling needed to test hypothesis. IgGII most useful to test past QF exposure	B/D	★ ★ ★ ★	★ ★
2014, M. Kremers [18]	Netherlands, yr study 2013-2014. Study period: April-August 2009, FU 4 yrs post AQF	CoS	102 seronegative PCR positive, symptomatic, AQF patients (64.7% ♂, mean age 48, SD16, range 17-85); 24 hospitalised. 93 FU 3, 6 or 12 mo for IFA IgGI and II. NCSI 4 yrs post AQF (n=58). Assess if ↑ CRP AQF coincides with ↑ IL-6 and if levels correlate with *C.b.* DNA load and disease severity, expressed by hospital admission and fatigue development	NCSI, PCR (Ct value), IFA, CRP, IL-6	NA	92 patients ↑ IL-6, 101 ↑ CRP during AQF. Significant weak negative correlation *C.b.* DNA loads, IL-6 and CRP, significant moderate-strong positive correlation IL-6 and CRP. Hospitalised patients: ↑ IL-6 and CRP than the non-hospitalised, *C.b.* DNA load equal. NCSI: 58 respondents, 34 abnormal outcome (58.6%) mild and severe fatigue. No difference in Ct values, CRP and IL-6 in AQF between patients with normal outcome and abnormal outcome subdomain fatigue	Correlation IL-6 and CRP in AQF points to immune activation pathway in which IL-6 induces CRP. Differences IL-6 and CRP between hospitalised vs. the non-hospitalised despite identical DNA load suggest an important role for host factors. ↑ IL-6 and CRP seems predictive of more severe disease. No support that IL-6 or CRP levels during AQF are prognostic for fatigue development	NA	★ ☆ ★	★ ★

**** Definition of used study population in articles explained in a different table, including definitions of QFS and/or fatigue is applicable. Main information is on aetiology. Some articles also contain relevant information on other domains: Diag= Diagnosis, B/D= Background/descriptive, P/T= Prevention/therapy.***

Abbreviations: 2-5AS= 2',5'-oligoadenylate synthetase, AI= Acute infection, AQF= Acute Q-fever, BDQ= Brief Disability Questionnaire, assessment of the impact of illness on functional capacity, and days out of role quantified the days over the past months the respondent was unable to carry out usual daily activities fully, BMA= Bone marrow aspirate, BMI= Body Mass Index, *C.b.= Coxiella burnetii,* CBA= Cytometric bead array, uses the sensitivity of amplified fluorescence detection by flow cytometry to measure soluble analytes (e.g. interleukins) in a particle-based immunoassay, CC= Case-control study, CDC= Centres for Disease Control and Prevention, CF= Chronic fatigue, CFS(/ME)= Chronic fatigue syndrome (/myeloencephalitis), CFT= Complement fixation test, CIDI= Composite international diagnostic interview to screen for any history of depression, anxiety or somatisation disorder. This computerised program formulates ICD-10 and DSM-III-R diagnoses and records current as well as pre-existing psychiatric morbidity, CoS= Cohort study, CRP= C-reactive protein, CS= Cross-sectional, DIOS= Dubbo Infection Outcomes Study**,** cohort study of subjects ≥16 yrs followed from the onset of a confirmed and documented AI due to EBV; *C.b.*; or RRV ≤6 wks post AI until complete recovery, DS14= Distressed personality scale, assessment of negative affectivity (an enduring tendency to experience negative emotions) and trait social inhibition (the tendency to feel inhibited, tense, and insecure when with others), DTH= Delayed-type hypersensitivity, to assess cell-mediated immune function in vivo, EBV= *Epstein-Barr virus*, ECG= Electrocardiography, FU= Follow-up, GHQ= General health questionnaire, 12-item questionnaire to detect current cases of psychiatric co-morbidity, I.c.w.= In comparison with, IFA= Immunofluorescence assay, IFN= Interferon, IgG= Anti-phase IgG, IgGI= Anti-phase IgG I titre, IgGII= Anti-phase IgG II titre, IL= Interleukin, IS= Insertion sequence, K10= Kessler 10, to assess current psychological distress, LMR= Lymphocyte mitogenic responses, LPS= Lipopolysaccharide, Mo= Month(s), MUGA scan= Multi Gated Acquisition Scan (gated cardiac radio-nuclide scans), a time-proven nuclear medicine test to evaluate the function of the right and left ventricles of the heart, allowing informed diagnostic intervention in heart failure, NA= Not applicable, NCSI= Nijmegen clinical screening instrument, originally developed to provide a detailed assessment of health status of COPD patients. It combines a number of existing health status questionnaires, NOS= Newcastle–Ottawa Scale: S= selection (maximum of 4 stars), C= comparability (maximum of 2 stars), O= outcome (maximum of 3 stars); ★: star earned; ☆: item not applicable, N/No= Number (of), (n-)PCR= (nested-) Polymerase chain reaction, NR= Not reported, Pain test algometer= For pressure pain threshold test to measure pain sensitivity, PBMC= Peripheral blood mononuclear cells, PHA= phytohaemagglutinin, PIF(S)= Post-infective fatigue (syndrome), PO= Personal opinion, POMS= Profile of Mood States to assess current mood status. This instrument includes 7 subscales: 'fatigue', 'depression', 'anxiety', 'vigour', 'anger', 'friendliness', and 'confusion', PQFF= Post-Q-fever fatigue, PQF(F)S= Post-(acute)Q-fever (fatigue) syndrome, Pros.= Prospective, PSC= Physical Symptoms Checklist, consisting of 51 symptom items, PSQ= Pittsburgh Sleep Questionnaire, to assess sleep abnormalities, QA = Quality assessment, Q-CFS(/ME)= Q-fever induced chronic fatigue syndrome (/myeloencephalitis), QF= Q-fever, QF(F)S= Q-fever fatigue syndrome, (Q)IE= (Q-fever induced) Infective endocarditis, Ref= Reference, RRV= *Ross River virus*, SCID= Severe combined immunodeficiency, SD= Standard deviation, SF-36= The Short Form (36) Health Survey, a patient-reported survey of patient health to assess quality of life of patients, functional impairment and reduced health related quality of life, SNP= Single nucleotide polymorphism, SOFA= Schedule of Fatigue and Anergy to identify cases of chronic fatigue syndrome. The subject rates 10 items on a 4-point scale. Subjects who score ≥3 items as 'a good part of the time' or 'most of the time' are classified as cases of 'fatigue/neurasthenia', SOMA= Empirically derived subscale of the SPHERE, used to record PIFS or illness duration. This reliably predicts disability and reflects patients' and doctors' reports of reasons for presentation to primary care. Scores ≥3 represents a clinically-significant fatigue state. Provisional PIFS: SOMA scores ≥3 at all time points up ≤3 months. Confirmed PIFS: symptoms persisted >6 months, and alternative explanations for ongoing illness was excluded.

SPHERE= Somatic and Psychological Health Report, to assess a wide range of physical and psychological symptoms, including severity and duration of symptoms, Stroop task= To assess cardiac response, TGFB= Transforming growth factor beta, TNF= Tumor necrosis factor, UK= United Kingdom, Wks= Weeks, Yr(s)= Year(s).

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S5 Table. Domain prevention/therapy

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Intervention	Outcome	Conclusions/ recommen-dations	Other do-main	QA (CR or NOS)		
2004, Y. Arashima [1]	Japan, yr study NR. Period: Jul-Nov 2001, baseline, 4, 8 and 12 wks post start treatment	CoS	20 QFS patients (3 ♂, mean age 34.6±5.7) with subjective symptoms (duration 20.8±3.3 mo, range 3 mo-4 yrs): fatigue (20/20), slightly elevated body temperature (17/20), arthralgia or myalgia (10/20), headache (12/20), cough or sore throat (16/20), ↑ sweating (10/20), and gastrointestinal symptoms (13/20). To address presence post QFS in Japan, and evaluation of minocycline for post QFS in changes in subjective symptoms, *C.b.* antibody titres and *C.b.* DNA. No controls	Question-naires (assess severity of subjective symptoms), PS score, IFA, n-PCR. Antibiotic side effects evaluated by interview and laboratory examination results	3 mo: minocycline 100mg/d (n=18)/ erythromycin 400mg/d (n=1)/ levofloxacin 200mg/d (n=1)	No leucocytosis or ↑ ESR. Slightly ↑ CRP 5 patients. All 7 who had been DNA positive, became negative with improvement subjective symptoms. IgM and IgG antibodies became negative post treatment. Clinical picture all patients improved: general fatigue (20/20), ↓ body temperature (12/17), gastrointestinal symptoms (10/13) and headache (9/12). PS score related to fatigue unchanged in 2 mo, but finally ↓, PS scores ↑	Minocycline administration useful for improving chronic nonspecific symptoms considered to be post QFS, and should be first-line drug for QFS. Observations may reflect existence of live *C.b.* in QFS patients	Diag, B/D	☆ ★ ☆	☆ ☆	★ ★ ★

S5 Table continued. Domain prevention/therapy

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Intervention	Outcome	Conclusions/ recommen-dations	Other do-main	QA (CR or NOS)	
2005, E. Iwakami [2]	Japan, May 2001-March 2003. Period: baseline, 3 mo treatment	CoS	4/8 CFS patients (2 ♂; 1 with IgGII 1:128; 3 with *C.b.* DNA positive); mean age 29, SD4, range 23-33, duration complaints: 52.0 mo, SD55.3, range 8 mo-11 yrs. Fatigue (PS score 7±1.2), slightly elevated body temperature, headache, arthralgia/myalgia (100%), cough/sore throat (75%). 54 QFS patients (10 ♂) positive *C.b.* DNA (n=34), IgMII ≥1:32 (n=15)/IgGII ≥1:128 (n=34); mean age 38, SD16, range 11-77, duration complaints: 21.1 mo, SD24.3, range 1 mo-10 yrs. Fatigue (PS score 5.3±2.4), slightly elevated body temperature (100%), headache (63%). To explore *C.b.* in CFS by antibiotic treatment, monitor symptom changes, PCR and *C.b.* antibodies	n-PCR, *C.b.* antibodies initial examination and 3 mo after start treatment. Questionnaire survey, PS	3 mo: minocycline 100mg/d (n=29)/ doxycycline 100mg/d (n=26)/ levofloxacin 200mg/d (n=3)	All 58 patients tested *C.b.* after treatment; all n-PCR positives became negative. CFS group (n=4): no improvement PS (p=0.422), no difference pre- and post-treatment temperatures (p=0.07) or headache (p=0.39) scores. QFS group: PS scores improved (p<0.001), temperature (p<0.001) and headache scores ↓ (p<0.001) post treatment	Possibility direct involvement *C.b.* pathological state CFS low. Different response to tetracycline suggest direct *C.b.* involvement pathological state QFS. Latent *C.b.* infection not involved either onset CFS or appearance symptoms	Diag, B/D	★ ★	★ ★

S5 Table continued. Domain prevention/therapy

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Intervention	Outcome	Conclusions/ recommen-dations	Other do-main	QA (CR or NOS)
2007, D. Ledina [3]	Croatia, yr study NR, study period: 2000-2004	Case-series	N=3 post AQF with PQFS. 2 ♂ (34 and 30 yrs), 1 ♀ (30 yrs). Initial treatment AQF: erythromycin and gentamycine 2 wks (n=1), doxycycline 2 wks (n=2). Emphasize existence and incidence CFS post AQF according to CDC CFS criteria, and show effects antibiotic treatment in QFS	Question-naires before and after treatment for subjective symptoms. Noted in 4 degrees absent-severe	Case 1: 9 mo doxycycline 200mg/d + ciprofloxacin 1000mg/d. Case 2: ciprofloxacin 1000mg/d 2 mo, then doxycycline 200mg/d 4 mo. Case 3: 1 mo cortico-steroids, then 3 mo doxycycline	Case 1: still fatigue after physical activity (disappears after 30 min rest) and low intensity headache. Muscle pain and slightly elevated body temperature disappeared. No criteria CFS post-treatment. Case 2: regression symptoms, except minor headache. No criteria CFS post-treatment. Case 3: still fatigued, disrupted sleep, headache, muscle and joint pains, still fulfils CFS criteria post treatment	Results prolonged antibiotic treatment CFS inconsistent. Diagnostic criteria and therapeutic recommen-dations for PQFS require further investigation	Diag, B/D	9/18 criteria **
2013, S. Keijmel [4]	Netherlands, yr study: 2011-2015	RCT proto-col	Objective: include 180 QFS patients, ♂ and ♀. Evaluation of efficacy of long-term doxycycline and CBT in QFS-patients	CIS; SIP total score, total score SCL-90, *C.b.* PCR and serology	24 wks of: placebo, doxycycline 200 mg/d, or CBT	Still treating patients	NA	Diag	NA
2013, S. Yakubo [5]	Japan, yr study NR	CR	♀ 71 yrs, 6 yrs post AI with general malaise, spasm left hand, slightly elevated body temperature. Co-morbidity: NR. Negative n-PCR for *C.b.*, IgMI and IgMII <1:16, IgGI <1:16, IgGII 1:32	n-PCR, IFA	Kampo formula Tsumura Shakuyaku-Kanzo-To granules (7,5g/d) 3 mo	Alleviation of stiffness in hand and arm after 2 days treatment, symptom disappeared completely. 6 mo after start treatment reappearance stiffness and IgGI 1:128	QFS may feature intermittent muscle spasms, ameliorated by Shakuyaku-Kanzo-To granules, warrants further research	NA	-/-, -, +, +, +, NA, -/-, -/-

S5 Table continued. Domain prevention/therapy

Ref	Country, yr study, period and duration	Study type	Patients, controls, characteristics, co-morbidity*	Tool	Intervention	Outcome	Conclusions/ recommen-dations	Other do-main	QA (CR or NOS)
2013, S. Yakubo [6]	Japan, yr study NR	CR	♂ 13 yrs, fatigue and severe malaise, slightly elevated body temperature, arthralgia, myalgia, lassitude, disease period 2 mo earlier. Extended period no school attendance. Co-morbidity: NR. IFA IgMII and IgGII negative, n-PCR positive	n-PCR, IFA	Kampo formula Tsumura Hochu-ekki-To granules (7,5mg/d) 1 mo, then erythromycin 800mg/d 1 mo, then doxycycline 200mg/d 1 mo, then erythromycin 800mg/d at least 6 mo	Slight improvement 1 mo post erythromycin, none post doxycycline, fever stopped after long-term erythromycin, general malaise continued. Improvement after continued treatment	Consider *C.b.* as possible cause in cases of long-term school absence due to severe malaise similar to that caused by CFS	Diag, B/D	-/-, +, -/-, +/-, +/-, NA, +/-, -/-

**** Definition of used study population in articles explained in a different table, including definitions of QFS and/or fatigue is applicable. Main information is on prevention/therapy. Some articles also contain relevant information on other domains: Diag= Diagnosis, B/D= Background/descriptive, A= Aetiology.***

***** Quality assessment for case-series was performed with a quality appraisal tool making use of 18 criteria with a considered acceptable quality if at least 14 criteria were scored (≥70%) [7].***

Abbreviations: AI= Acute infection, AQF= Acute Q-fever, *C.b.= Coxiella burnetii,* CBT= Cognitive behavioural therapy, CDC= Centre of Disease Control, CFS= Chronic fatigue syndrome, CIS= subscale fatigue of the Checklist Individual Strength, to indicate the level of fatigue experienced in the previous two weeks, measured with eight items on a seven-point Likert-scale (range 8–56), CoS= Cohort study, CRP= C-reactive protein, CR= Case-report, ESR= Erythrocyte sedimentation rate, IFA= Immunofluorescence assay, IgG= Anti-phase IgG, IgGII= Anti-phase IgG II titre, IgM= Anti-phase IgM, IgMII= Anti-phase IgM II titre, Mo= Month(s), NA= Not applicable, NOS= Newcastle–Ottawa Scale: S= selection (maximum of 4 stars), C= comparability (maximum of 2 stars), O= outcome (maximum of 3 stars); ★: star earned; ☆: item not applicable, N/No= Number (of), (n-)PCR= (nested-) Polymerase chain reaction, NR= Not reported, PQFS= Post-(acute)Q-fever (fatigue) syndrome, PS= Performance status score (range 0-9), which reflects the grade of fatigue/malaise to assess the severity of CFS, QA-CR= Quality assessment; for CR no quality checklists are available. Therefore, the following eight criteria for quality assessment were determined; addressing an appropriate and clearly focused question, representative population, description of the survey method or data collection, outcome measures defined, outcome measures described, response rate reported and results valid and applicable to the patient group targeted. The articles scores on these items: -/-, -, +/-, +, or ++, based on the Coordination of Cancer Clinical Practice Guidelines in Europe criteria, RCT= Randomised controlled trial, QF(F)S= Q-fever fatigue syndrome, Ref= Reference, SCL-90= Symptom Checklist 90, to measure the level of psychological distress, consisting of 90 items scored on a five-point Likert-scale (range 90-450), SD= Standard deviation, SIP= Sickness Impact Profile, to measure the level of functional impairment. A total score is derived out of the scores on the subscales: sleep-rest, household, mobility, social interactions, walking, alertness and intellectual functioning, work, and recreation, Wks= Weeks, Yr(s)= Year(s).

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S6 Table. Grey literature

Ref	Country, yr study, period and duration	Document	Patients, controls, characteristics, co-morbidity	Tool	Outcome/advice	Conclusions/ recommendations	Do-mains	QA
1992, M. Shannon [1]	Australia, yr study NR, study period NR	Thesis	Abattoir workers (n=117), immune status assessed 1981-1986. Group of clinical history AQF and serology CFT Phase I and II, and IFA (n=39). All either ↑ CFT antibody titre and/raised IFA IgM as indication current QF. Unexposed comparison cohort (n=39): vaccinated and non-vaccinated (seropositives without clinical history AQF). Occurrence infection not noted	*C.b.* CFT, IFA, questionnaires	Definition QFS; laboratory proven, clinically manifest QF, commences within 12 mo of illness, duration ≥6 mo. 5 major symptoms; 1. fatigue of 2-≥7dys, ≥6x/yr continuously with some absence from work, 2. malaise – as above except work, 3. muscle twitches/ fasciculations, 4. nausea ≥6x/yr, 5. abnormal sweating ≥10x/yr, might be accompanied by other symptoms. Most subjects healthy before AQF regarding depression. Mental problems; depression, lack of concentration, impairment short memory, mood lability, altered sleep pattern following AQF. Some general practitioners stated that tricyclic antidepressants were beneficial. 30-40 cases/1000 abattoir workers/yr, each costs 2-88.000 in medical care and loss of wages, endocarditis 50-10.000/yr, QFS 20-50.000/yr. Duration QFS 6 mo-20 yrs	Approximately 23% develops QFS post overt AQF. No grounds to dismiss QFS as a psychiatric depressive illness. Aetiology is unclear, might be due to immune stimulation and a disordered function of the lymphocyte-macrophage interaction. Same pathways to mood change may be involved in depression and QFS and altered by chemotherapy	**B/D**, P/T	NA
2009, B. Marmion [2]	Australia and UK, yr study NR, study period NR	Book (chapter)	No patients/controls. Characteristics and co-morbidity: NR. Experience from several studies	NA	Start often 6 mo-1 yr post AQF. Symptoms complex not limited to fatigue, also nausea, headache, night sweats, myalgia, arthralgia, fasciculations, painful lymph nodes, disturbed sleep pattern, anger, ↓ concentration, mental acuity ↓. Duration: >1 yr, often 5-10 yrs. Antigens in samples SCID mice, cellular immune response heightened, cytokine dysregulation: IL-6 ↑, IL-10, IL-2 ↓, low fever. Pathogenesis; no consensus. Bacteraemia restricted by humoral and cell-mediated immunity, by product clearing *C.b.* DNA containing components with an immunomodulatory effect. Cell-mediated immunity and dendritic cells causing dysregulation, cytokines and other immune mediators give rise to symptoms	In Australia QFS is the most common chronic sequel of AQF affecting 10-15% of patients. It usually follows AQF and rarely if ever subclinical infection	**B/D**, A	NA

S6 Table continued. Grey literature

Ref	Country, yr study, period and duration	Document	Patients, controls, characteristics, co-morbidity	Tool	Outcome/advice	Conclusions/ recommendations	Do-mains	QA
2011, C. Tempel-man [3]	Netherlands, yr study 2011	Report on economic evaluation	Economic costs – human and veterinary Dutch QF outbreak 2007-2010 assessed with 4024 notification AQF. Assumptions: 25% (n=503) AQF get QFS duration 5-10 yrs. Results: quality of life ↓, assumed period sick leave 5-10 yrs, productivity 50% ↓. Assumption 60% of those with QFS were gainfully employed	Interviews, public data outbreak	QFS duration 5-10 yrs costs ↓ quality of life 55.6-104.7 million euros. Costs of sick-leave due to QFS are not separately presented but together with CQF and therefore not mentioned	Economic costs due to QF outbreak are considerable as the course of disease especially due to QFS is protracted and reflected in ↓ quality of life, ↓ productivity, and ↓ income	**B/D**	NA
2012 Guideline working group on QFS [4]	Netherlands, yr study 2011-2012	Guideline	Achieve uniformity diagnosis and treatment QFS	QFS and CFS literature and multidisciplina-ry consensus	QFS definition: severe fatigue causing significant disabilities daily life ≥6 mo, reference to lab confirmed AQF, not caused by somatic/psychiatric co-morbidity, fatigue absent before AQF/significantly ↑ since. Diagnosis on history, physical and laboratory examination excluding other causes of fatigue (including ESR, CRP, CK, TSH, leukocytes with differentiation, creatinine, alkaline phosphatase, ALT, glucose, ferritin, urinary sediment). Cave diagnosis in case of morbid obesity (BMI>40) or substance abuse. Impossible to diagnose QFS in case of: depression/depression preceded current symptoms, schizophrenia, psychoses, any type dementia, eating disorders, unless resolved ≥5 yrs	Advice patients ≤6 mo post AQF: i) stay mentally/physically active, adjust pace if necessary; ii) alternate activities, also within activities; iii) keep fulfilling daily role; iv) keep steady sleep-wake pattern; v) avoid focussing on fatigue; vi) focus on feasible activities, appreciate accomplishments. Advice CBT/GET after QFS diagnosis. GET might be an additional treatment strategy	**Diag**, B/D, A, P/T	NA

These documents contain relevant information for the domains: Diag= Diagnosis, B/D= Background/descriptive, A= Aetiology, P/T= Prevention/therapy. Main domain indicated in bolt.

Abbreviations: ALT= Alanin aminotransferase, AQF= Acute Q-fever, BMI= body mass index, *C.b.= Coxiella burnetii,* CBT= Cognitive behavioural therapy, CFS= Chronic fatigue syndrome, CFT= complement fixation test, CK= creatine kinase, CRP= C-reactive protein, CQF= chronic Q-fever, ESR= Erythrocyte sedimentation rate, IFA= Immunofluorescence assay, IL= Interleukin, Mo= Month(s), NA= Not applicable, NR= Not reported, QF= Q-fever, QF(F)S= Q-fever fatigue syndrome, Ref= Reference, TSH= thyroid stimulating hormone, Yr(s)= Year(s).

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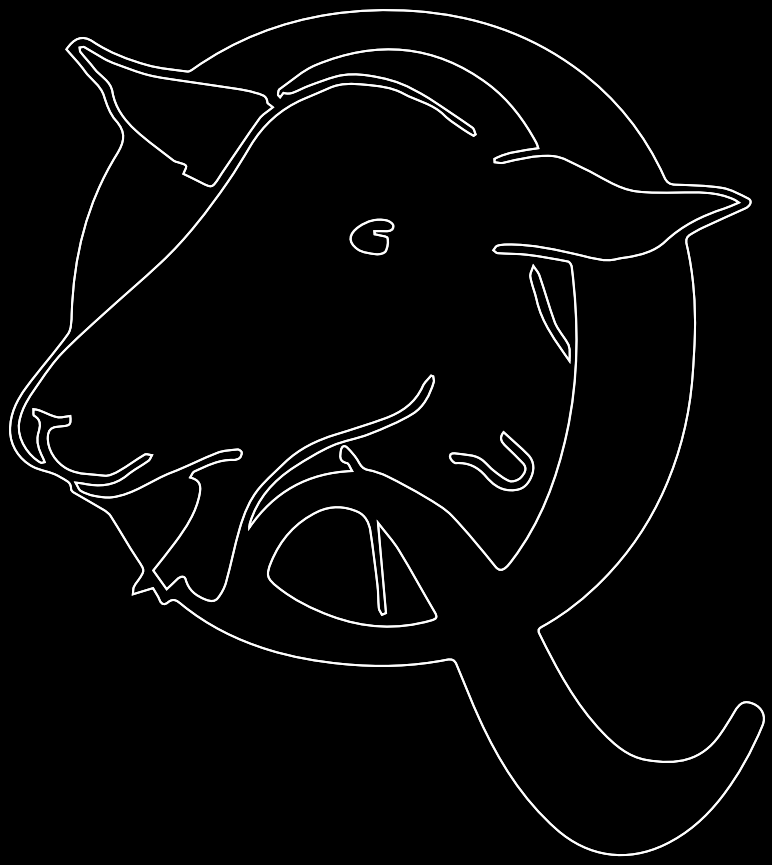

CHAPTER 3

A COMPARISON OF PATIENTS WITH Q FEVER FATIGUE SYNDROME AND PATIENTS WITH CHRONIC FATIGUE SYNDROME WITH A FOCUS ON INFLAMMATORY MARKERS AND POSSIBLE FATIGUE PERPETUATING COGNITIONS AND BEHAVIOUR

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ABSTRACT

Objective: Comparison of Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS) patients, with a focus on markers of inflammation and fatigue-related cognitive-behavioural variables.

Methods: Data from two independent prospective studies on QFS (n=117) and CFS (n=173), respectively, were pooled and analyzed.

Results: QFS patients were less often female, had a higher BMI, and had less often received treatment for depression before the onset of symptoms. After controlling for symptom duration and correcting for differences in diagnostic criteria for QFS and CFS with respect to the level of impairment and the presence of additional symptoms, differences in the proportion of females and BMI remained significant. After correction, QFS patients were also significantly older. In all analyses QFS patients were as fatigued and distressed as CFS patients, but reported less additional symptoms. QFS patients had stronger somatic attributions, and higher levels of physical activity. No differences were found with regard to inflammatory markers and in other fatigue-related cognitive-behavioural variables. The relationship between cognitive-behavioural variables and fatigue, previously established in CFS, could not be confirmed in QFS patients with the exception of the negative relationship between physical activity and fatigue.

Conclusion: Differences and similarities between QFS and CFS patients were found. Although the relationship between perpetuating factors and fatigue previously established in CFS could not be confirmed in QFS patients, the considerable overlap in fatigue-related cognitive-behavioural variables and the relationship found between physical activity and fatigue may suggest that behavioural interventions could reduce fatigue severity in QFS patients.

INTRODUCTION

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii,* occurring all over the world [1]. From 2007 until 2011, over 4000 cases of symptomatic acute Q fever were reported in the Netherlands [2], and over 32,000 people were infected during this outbreak [3, 4]. Chronic Q fever, characterized by the persistence of *C. burnetii*, occurs in 1-5% of cases [5]. In addition, around 20% of the known symptomatic acute Q fever patients remain chronically fatigued, and this condition has been named Q fever fatigue syndrome (QFS) [6-8]. QFS appeared to be one of the major causes of the Q fever-related economical sequelae during the Dutch outbreak, leading to loss of quality of life and health-related absenteeism [9]. With an increasing number of patients with QFS in the aftermath of the outbreak, and the societal need for uniform criteria for the syndrome, a national guideline on QFS was formulated and published in 2012 [10]. This consensus guideline was partly based on the diagnosis and treatment of chronic fatigue syndrome (CFS), as QFS and CFS at least partly overlap in symptoms [11]. In this guideline QFS is defined as a severe fatigue causing significant disabilities in daily life with a duration of at least six months, with a reference to an acute Q fever infection, and not being caused by somatic or psychiatric co-morbidity. In addition, the fatigue should be absent before the acute Q fever infection or significantly increased since the acute Q fever infection. No study has been published so far comparing the clinical characteristics of QFS and CFS patients. One study determined the prevalence of CFS in patients with Q fever compared to a healthy control group. In both groups only one patient met these criteria, although a substantial proportion of the patients with Q fever was chronically fatigued [12].

Little is known about the aetiology of QFS. It has been hypothesized that persistence of *C. burnetii* or its antigens could result in inflammation [13]. Ferritin, a cellular storage protein for iron that is important in iron absorption control, orchestrates cellular defence against oxidative stress and inflammation and is an acute phase reactant. It is induced by cytokines such as interleukin (IL)-6 and IL-18, and has been found to be significantly higher in acute Q fever patients than in controls [14]. Furthermore, elevated ferritin concentrations were observed in QFS patients, whereas in medically unexplained fatigue, such inflammatory markers are normally not present. To explore the presence of an inflammatory component in the pathogenesis of QFS, inflammatory markers of QFS patients were compared with those of CFS patients.

Previous research in CFS patients has shown that cognitive-behavioural variables, such as a reduced level of activity and fatigue-related dysfunctional beliefs, play an important role in the perpetuation of fatigue and disabilities. According to the model of perpetuating factors of CFS developed by Vercoulen et al. [15], fatigue is maintained by a low self-efficacy with respect to fatigue, a tendency to focus on fatigue and a lower level of activity. These fatigue-maintaining factors are addressed in behavioural interventions, leading to significant reductions of fatigue and disability in CFS [16, 17]. Somatic attribution of symptoms has an indirect influence on fatigue and disability in CFS by further lowering the level of physical activity [15]. In other studies it was found that the tendency to catastrophize in response to fatigue and depressive mood could also play a role in the perpetuation of symptoms and disability in CFS patients [18, 19]. A depressive mood may also directly produce fatigue,

which can result in lower levels of physical activity because of inactivity. However, several studies showed that mood disorder is not an essential factor in the perpetuation of fatigue in CFS [20, 21]. It is unclear to what extent this is also true for QFS as the role of cognitive-behavioural variables in the perpetuation of fatigue has not been investigated so far in QFS. The main objective of this study was to explore both differences and similarities between QFS and CFS with a focus on inflammatory markers and cognitive-behavioural factors thought to perpetuate chronic fatigue. In an exploratory analysis we investigated whether there was a significant relationship between these cognitive-behavioural variables and fatigue in QFS patients.

METHOD

Study populations

The study population consisted of patients from two independently conducted prospective studies, one in QFS [22], and one in CFS [23]. All included patients were severely fatigued, defined by a score ≥35 on the subscale fatigue severity of the Checklist Individual Strength (CIS) [24]; all patients were ≥18 years. The fatigue lasted at least 6 months, in accordance with diagnostic criteria of both QFS and CFS (see below). In addition, all QFS patients met the criteria for QFS as formulated in the Dutch algorithm on QFS [14], with a sudden onset of fatigue related to a symptomatic acute Q fever infection. Fatigue was to be either absent before, or significantly increased after the acute Q fever infection. In all QFS patients, the fatigue resulted in significant functional impairment, defined as a score ≥450 on the Sickness Impact Profile (SIP8). Chronic Q fever and other causes of fatigue, somatic or psychiatric, were excluded. All QFS patients had suffered from laboratory-proven acute Q fever and/or a positive serology compatible with past *C. burnetii* infection [25]. All QFS patients (n=117), were assessed at the Radboud Expertise Centre for Q fever of the Radboud university medical center (Radboudumc) between 2011 and 2013.

The cohort of CFS patients was referred to the Expert Centre for Chronic Fatigue of the Radboudumc for cognitive-behavioural therapy (CBT) between 2008 and 2010. All CFS patients met the Centers for Disease Control and Prevention (CDC) criteria for CFS [26, 27], and were functionally impaired, operationalized as scoring ≥700 on the SIP8 and reporting ≥4 additional symptoms. These criteria were met by 183 patients; however, it was unclear whether Q fever was considered as a possible origin of complaints. Therefore, as QFS is characterized by a sudden onset of fatigue, all CFS patients with a sudden or unknown onset of fatigue after 2007 (the start of the Q fever outbreak) were excluded (n=10). Both studies were approved by the medical ethical board of the Radboudumc, and all patients gave written informed consent.

Measures

Demographics and premorbid psychiatric treatment

Age, body mass index (BMI), gender, educational level, and marital status were recorded. Patients were asked if they had received treatment for an eating disorder, substance abuse, anxiety disorder, or depressive disorder in the past [16]. Previous treatment for these

psychiatric disorders was assumed to reflect prevalence of premorbid psychiatric illness.

Symptoms

Fatigue

Fatigue was assessed with the subscale fatigue severity of the CIS [24], indicating the level of fatigue experienced in the previous two weeks, measured with eight items on a seven-point Likert-scale (range 8–56). It is a reliable and validated instrument (Cronbach's alpha .83–.92) [15, 28, 29]. Duration of fatigue was measured in months.

Functional impairment

The level of functional impairment was measured with the SIP8 total score [30, 31], a reliable instrument which shows good correlations with other health status and functional status measures (Cronbach's alpha of the Dutch version is .91) [32]. A total score is derived out of the scores on the subscales: sleep-rest, household, mobility, social interactions, walking, alertness and intellectual functioning, work, and recreation.

Additional somatic symptoms

To determine the frequency of additional symptoms according to the CDC criteria for CFS, patients filled out a questionnaire with a 4-point scale to report prevalence of the following eight symptoms during the last six months: post-exertional malaise, unrefreshing sleep, memory or concentration impairment, muscle pain, joint pain, headaches, tender lymph nodes, and a sore throat.

Psychological distress and depression

The level of psychological distress was measured with the Symptom Checklist 90 (SCL90), consisting of 90 items scored on a five-point Likert-scale (range 90–450). Higher scores reflect more psychological distress. The SCL-90 is a reliable and validated instrument (Cronbach's alpha of the subscales is .73–.89) [33, 34]. Depressive symptoms were measured with the Beck Depression Inventory-Primary Care questionnaire (BDI, Cronbach's alpha .86) [35], with a score ≥4 indicative for a clinical depression.

Laboratory tests

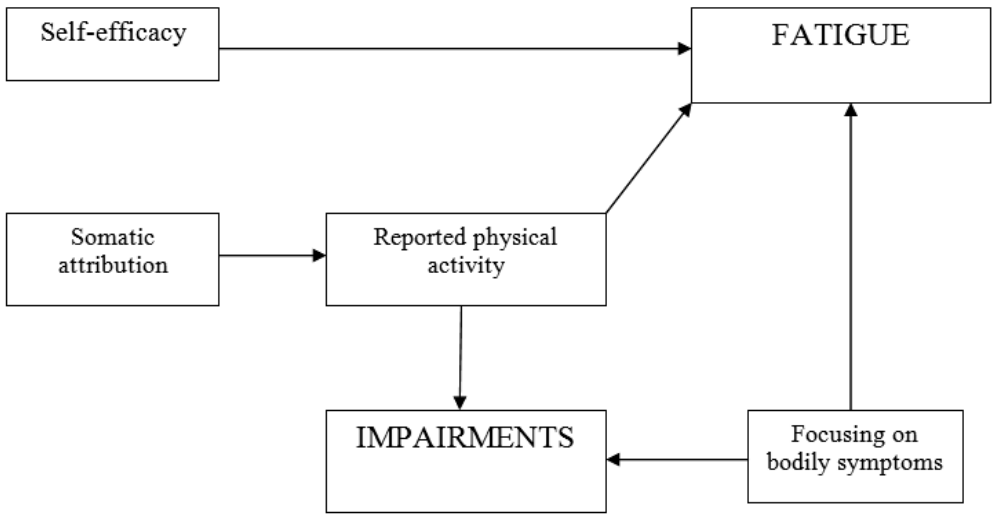
For CFS patients the laboratory values had to be determined <1 year before assessment, and were derived from medical records. Laboratory values for QFS patients were derived from the assessment at the Radboud Expertise Centre for Q fever. Analyzed were: the inflammatory markers ferritin, leukocyte count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), both indicators of the physical response to inflammation (acute phase response), and creatine kinase (CK), an enzyme used in the evaluation of patients presenting with muscle weakness or myalgias.

Cognitive-behavioural variables

Previous research revealed that cognition and behaviour perpetuate fatigue and disability in CFS [15]. A lowered self-efficacy with respect to fatigue, lowered levels of (self reported

and actual) physical activity, and focusing on bodily symptoms were perpetuating factors. Somatic attributions of symptoms indirectly influenced fatigue due to their negative effect on the level of physical activity. A model of perpetuating factors developed by Vercoulen et al [36] is depicted in *figure 1*. To explore if the same cognitive-behavioural variables also perpetuate fatigue in QFS the following variables were assessed: lowered self-efficacy with respect to fatigue, lowered levels of (self reported and actual) physical activity, and focusing on bodily symptoms.

Figure 1: Model of perpetuating factors for chronic fatigue syndrome (CFS)



Structural equation model for CFS patients [15, 36]. Reprinted from [15] with permission.
Abbreviations: *CFS* = Chronic fatigue syndrome.

Fatigue-related beliefs

The Self-Efficacy Scale (SES) was used to assess the patients' sense of control over their symptoms (Cronbach's alpha .68–.77) [15, 23, 37]. Seven items were scored on a 4-point Likert-scale, with higher scores indicating a higher sense of control over fatigue.

Physical activity

The level of physical activity was objectified using an actometer, a motion sensing device that registers and quantifies physical activity [38], worn during a period of 12 days around the ankle. A mean activity level was calculated and two activity patterns were discerned; a persistent low-active pattern and a fluctuating active pattern [38]. A fluctuating active pattern is characterized by fluctuating bursts of activity followed by a period of inactivity. Low active patients are characterized by consistent low levels of physical activity. The actometer is a reliable and valid instrument for the assessment of physical activity in CFS [38]. Self-reported activity was measured by the SIP8 mobility subscale.

Focusing on bodily symptoms
Focusing on symptoms was measured with the shortened subscale 'focusing on symptoms' of the Illness Management Questionnaire (IMQ, Cronbach's alpha .88) [39] [40], consisting of 9 items measured on a 6-point Likert-scale (ranging from 'never' to 'always'). With the Jacobsen Fatigue Catastrophizing Scale (JFCS) [41], catastrophizing thoughts with respect to fatigue were assessed. The JFCS consists of 10 items, rated on a 5-point scale, and is a reliable instrument (Cronbach's alpha .86) [40]. Higher scores reflect a stronger tendency to catastrophize in response to fatigue.

Attributions of symptoms
Somatic attributions regarding symptoms were measured with the Causal Attributions List (CAL, Cronbach's alpha .71–.77) [42], which consists of five questions about the causes of fatigue measured on a 4-point Likert-scale (range 5–20). Higher scores indicate a stronger tendency to attribute symptoms to a certain cause.

Statistical analysis
All data were analyzed using SPSS (Version 20.0, SPSS, Inc.). The significance level was set at p=0.05. To correct for multiple testing, Bonferroni correction was used dividing 0.05 by the total number of comparisons for baseline characteristics and symptoms, inflammatory markers, and cognitive-behavioural factors separately.
For assessment of demographic variables, data on premorbid psychiatric treatment, and symptoms and disability, descriptive statistics were used including means and standard deviations for continuous variables, and tested with the independent t-test. Categorical variables were described with percentages, and tested with the χ^2 test. The p-value after Bonferroni correction was $p<0.002$ for baseline characteristics and symptoms. For assessment of laboratory diagnostics, independent t-tests were used when comparing both groups. Bonferroni correction resulted in a p-value of $p<0.006$. Analyses were performed only if data of 20 or more patients were available in each group. For cognitive-behavioural factors, the χ^2test was used for categorical variables (level of activity), and an independent t-test was used for continuous variables. After correction for multiple testing a value was found significant if $p<0.007$.
Different inclusion criteria were used for QFS and CFS with respect to the level of impairment assessed with the SIP8 total score and the number of additional symptoms that had to be reported. Furthermore, CFS patients with a sudden or unknown onset of fatigue after 2007 (the start of the Q fever outbreak) were excluded. In addition, because included QFS patients could have experienced symptoms for a maximum of 4 to 6 years, compared to CFS patients who could have had symptoms long before 2007, difference in duration of illness between both groups exists. This leads to a priori differences between the total group of QFS patients and the CFS group that are not the focus of this study. Therefore, we analysed the differences between QFS and CFS patients in two steps. First, we compared the total group of QFS patients with the CFS group. Second, we compared a subgroup of QFS patients with CFS patients, by excluding all QFS patients with a SIP8 score ≤700 and <4 CDC symptoms and compared the remaining patients with the CFS patients. We used ANCOVA with duration of

symptoms as covariate to correct for differences in this variable.
Using the method "enter" in a multiple regression analysis in the total group of QFS patients, with potential perpetuating factors as predictors and fatigue severity as dependent variable, it was explored whether the perpetuating factors in CFS also predict fatigue severity in QFS.

RESULTS

Demographics and premorbid psychiatric treatment

The total group of QFS patients were less often female (52% vs. 75%, p<0.001), had a higher BMI (mean 26 vs. 24, p<0.001), and were less often treated for depression (17% vs. 35%, p=0.001) (*Table 1*). The number of patients who had received treatment for other psychiatric disorders than depression did not differ between CFS and QFS. Age and marital status also did not differ between the groups (*Table 1*). After excluding all QFS patients with <4 additional symptoms (n=14) and a SIP8 total score <700 (n=18), a total of 88/117 (75.2%) QFS patients met the criteria as applied for CFS. We compared this subgroup of QFS patients with CFS patients in an ANCOVA with symptom duration as covariate. The subgroup of QFS patients were still less often female (p=0.001), still had a higher BMI (p=0.001), but were also significantly older (p=0.001). Difference in previous treatment for depression was just as large as when all patients were compared (35% vs. 16%); however, the strength of the evidence for this difference was borderline (p=0.002), given the Bonferroni correction.

Table 1: Characteristics of Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS) patients, and the subgroup of QFS patients meeting the CFS criteria

		QFS N=117	CFS N=173	Subgroup QFS N=88	QFS vs. CFS	Subgroup QFS vs. CFS
		Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	P-value	P-value
Age in years [Range]		43 (13) [19-64]	39 (11) [19-63]	43 (13) [19-64]	0.003[a]	0.001[b*]
BMI (kg/m^2)		26 (5)[1]	24 (4)[2]	26 (5)[3]	<0.001[a*]	0.001[4,5,b*]
Gender	woman	61 (52%)	129 (75%)	47 (53%)	<0.001[c*]	0.001[c*]
	man	56 (48%)	44 (25%)	41 (47%)		
Marital status	married/living together	84 (72%)	108 (62%)	64 (73%)	0.121[c]	0.158[c]
	living on their own	20 (18%)	48 (28%)	13 (15%)		
	living with parents	13 (11%)	17 (10%)	11 (13%)		
Previously treated eating disorder		0 (0%)	7 (4%)[6]	0 (0%)	0.027[c]	0.054[c]
Previously treated alcohol disorder		2 (2%)	2 (1%)[6]	1 (1%)	0.701[c]	0.981[c]
Previously treated depression		20 (17%)	59 (35%)[6]	14 (16%)	0.001[c*]	0.002[c]
Previously treated anxiety disorder		13 (11%)	31 (18%)[6]	10 (11%)	0.104[c]	0.158[c]

Abbreviations: *QFS* = Q fever fatigue syndrome, *CFS* = Chronic fatigue syndrome, *Subgroup QFS* = excluding all QFS patients with <4 additional symptoms and a SIP8 total score <700, *SD* = Standard Deviation, *BMI* = Body mass index.
[1] From a total of 115 patients. [2] From a total of 168 patients. [3] From a total of 87 patients. [4] From a total of 83 QFS patients. [5] From a total of 155 CFS patients. [6] From a total of 171 patients.
* Significant result after Bonferroni correction.
[a] Calculated using student t-test with significance level at p<0.002.
[b] Calculated using ANCOVA with duration of symptoms as covariate with significance level at p<0.002.
[c] Calculated using Pearson Chi-square test with significance level at p<0.002.

Symptoms

There was no difference in fatigue severity with a mean CIS fatigue of 50 (SD=5) in both groups (p=0.306, *table 2*). As expected, the total group of QFS patients showed less functional impairment (mean 1317±550 vs. 1547±530, p<0.001) and had fewer additional symptoms (mean 5.6±1.8 vs. 6.6±1.3, p<0.001). No significant differences between QFS and CFS patients were observed in psychological distress (SCL90 total score 155±33 vs. 163±34, respectively) and depressive symptoms (BDI score ≥4 in 26% vs. 31%, respectively). After correction for duration of symptoms and for differences in inclusion criteria the subgroup of QFS patients still reported fewer additional symptoms (p=0.001).

Table 2: Comparison of symptoms of Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS) patients, and the subgroup of QFS patients meeting the CFS criteria

		QFS N=117	CFS N=173	Subgroup QFS N=88	QFS vs. CFS	Subgroup QFS vs. CFS
		Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	P-value	P-value
CIS fatigue		50 (5)	50 (5)	51 (5)	0.306[a]	0.247[b]
Length symptoms (in months)		35 (18)[1]	88 (81)[2]	35 (15)[3]	<0.001[a*]	NA
CDC number of symptoms		5.6 (1.8)[4]	6.6 (1.3)	6.2 (1.3)	<0.001[a*]	0.001[b*]
CDC forgetfulness		92 (80%)[4]	163 (94%)	81 (92%)	<0.001[c*]	0.501[c]
CDC concentration problems		100 (87%)[4]	168 (97%)	84 (95%)	0.001[c*]	0.488[c]
CDC throat pain		45 (39%)[4]	98 (57%)	41 (47%)	0.004[c]	0.124[c]
CDC sore neck- or axillar glands		28 (24%)[4]	94 (54%)	25 (28%)	<0.001[c*]	<0.001[c*]
CDC sore muscles		84 (73%)[4]	152 (88%)	74 (84%)	0.001[c*]	0.398[c]
CDC painful joints		71 (62%)[4]	138 (80%)	62 (70%)	0.001[c*]	0.093[c]
CDC headache		96 (83%)[4]	148 (86%)	79 (90%)	0.632[c]	0.338[c]
CDC waking up not well rested		107 (93%)[4]	172 (99%)	86 (98%)	0.002[c*]	0.225[c]
CDC increase in symptoms after physical activity		107 (93%)[4]	164 (95%)	87 (99%)	0.536[c]	0.106[c]
SCL90 total score		155 (33)	163 (34)	161 (32)	0.030[a]	0.667[b]
BDI score	<4	86 (74%)	118 (69%)[5]	65 (74%)	0.370[c]	0.379[c]
	≥4	31 (26%)	54 (31%)[5]	23 (26%)		
SIP8 total score		1317 (550)	1547 (530)	1470 (500)	<0.001[a*]	0.133[b]

Abbreviations: *QFS* = Q fever fatigue syndrome, *CFS* = Chronic fatigue syndrome, *Subgroup QFS* = excluding all QFS patients with <4 additional symptoms and a SIP8 total score <700, *SD* = Standard Deviation, *CIS* = Checklist Individual Strength, *NA* = Not applicable, *CDC* = Centers for Disease Control and Prevention questionnaire, *SCL90* = Symptom Checklist 90, *BDI* = Beck Depression Inventory-Primary Care (score ≥4 indicating clinical significant level of depressive symptoms), *SIP8* = Sickness Impact Profile.

[1] From a total of 111 patients. [2] From a total of 160 patients. [3] From a total of 84 patients. [4] From a total of 115 patients. [5] From a total of 172 patients.

* Significant result after Bonferroni correction.

a Calculated using student t-test with significance level at p<0.002.

[b] Calculated using ANCOVA with duration of symptoms as covariate with significance level at p<0.002.

[c] Calculated using Pearson Chi-square test with significance level at p<0.002.

Of the eight CDC additional symptoms, QFS patient reported significantly less often sore glands (p<0.001). After correction QFS and CFS patients did not differ with respect to fatigue severity (p=0.247), functional impairment (p=0.133), and psychological distress (p=0.667).

Inflammatory markers

The total group of QFS patients had a lower ESR (mean 5±4 vs. 8±7, p=0.001), and higher serum ferritin concentrations (mean 118±117 vs. 61±45, p<0.001; *table 3*). After excluding the two QFS patients and the six CFS patients with an elevated ESR (>20 mm/h in women and >15 mm/h in men), no significant differences in ESR between both groups remained (p=0.013). Nine out of 117 QFS and none of the CFS patients had an elevated ferritin serum concentration (>190 ng/mL in women and >280 ng/mL in men). The illness haemochromatosis, a condition of accumulation of iron resulting in systemic iron overload and end-organ damage, which could be a possible explanation for both fatigue and elevated ferritin concentrations, was excluded in these QFS patients. After excluding patients with an elevated serum concentration, the serum ferritin concentrations still differed significantly (mean 95±65 vs. 61±45, p=0.001). However, correcting ferritin concentrations for gender resulted in no significant differences between both men (mean 180±140 vs. 133±55, p=0.387) and women (mean 62±43 vs. 50±33, p=0.118; *table 3, figure 2)*. No difference was found in CRP, leukocyte count, and CK. The pattern of results was not different when laboratory values of the subgroup of QFS patients were compared with those of CFS patients.

Cognitive-behavioural variables

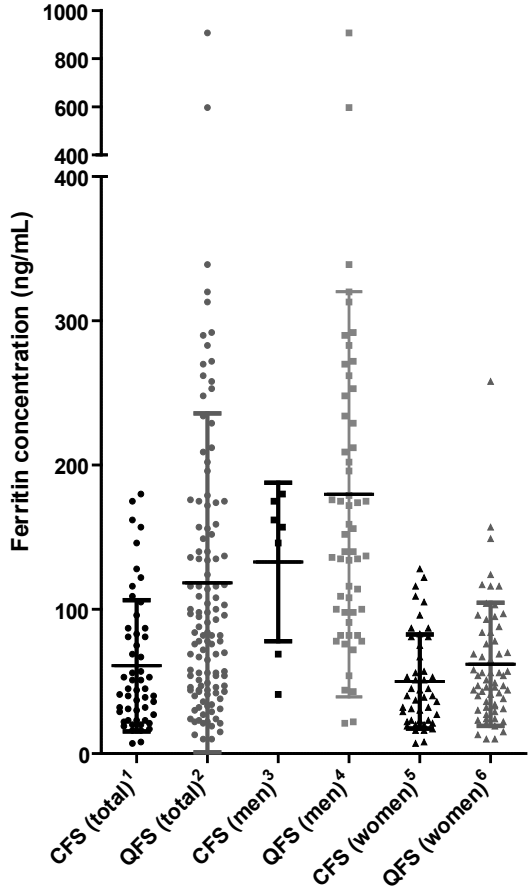
Results of the actometer showed that QFS patients were physically more active than CFS patients (75±18 vs. 67±19, p=0.001), with more fluctuating active patients (93% vs. 79%, p=0.001, *table 4*). No difference was found in self-efficacy with respect to fatigue, focusing on symptoms, and catastrophizing thoughts in response to fatigue. Compared to CFS patients, QFS patients attributed their symptoms more strongly to physical causes (14±3 vs. 12±3, p<0.001). The strength of the somatic attribution was not related to the degree of physical activity (Pearson's correlation of 0.02). The pattern of results remained the same comparing the subgroup of QFS patients with CFS patients. QFS patients were still physically more active (p=0.004), more often fluctuating active (p=0.001), and attributed their symptoms more strongly to physical causes (p<0.001).

In a multiple regression analysis with CIS fatigue as dependent variable and presumed perpetuating factors as predictors, the adjusted R^2 was 0.047, which was not significant (F=2.148, p=0.065). A significant negative correlation was observed between actual (measured with the actometer) physical activity and CIS fatigue (p=0.034), and a significant positive correlation between self-reported limitation in physical activity (measured with the SIP8 subscale mobility) and CIS fatigue severity (p=0.039; *table 5*). In an exploratory analysis catastrophizing (JFCS) and ferritin levels were added to the multiple regression analysis as predictors, but this did not improve the model (data not shown). The same multiple regression analysis was performed in the CFS group, with CIS fatigue as dependent variable and the previously found perpetuating factors as predictors. The model significantly predicted fatigue (F=5.406, p<0.001). A significant direct relationship was found between

self-efficacy and CIS fatigue (p=0.005), and self-reported physical activity and CIS fatigue (p=0.021), and a near significant relationship between focusing on bodily symptoms and CIS fatigue (p=0.082). Finally, adding catastrophizing and ferritin levels as predictors did not improve the model, as in QFS.

Figure 2: Ferritin concentrations

Scatter dot plot showing ferritin concentration (in ng/mL) for both CFS and QFS patients.



Abbreviations: *CFS* = Chronic fatigue syndrome, *QFS* = Q fever fatigue syndrome.

Normal ferritin values: ≤280 ng/mL in men, and ≤190 ng/mL in women.

[1] From a total of 53 patients. [2] From a total of 117 patients. [3] From a total of 7 patients. [4] From a total of 56 patients. [5] From a total of 46 patients. [6] From a total of 61 patients.

Table 3: Laboratory values Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS) patients, and the subgroup of QFS patients meeting the CFS criteria

	QFS N=117	CFS N=variable	Subgroup QFS N=88	QFS vs. CFS	Subgroup QFS vs. CFS
	Mean (SD)	Mean (SD)	Mean (SD)	P-value	P-value
ESR	5 (4)	8 (7)[1]	6 (4)	0.001[a*]	0.003[b,2*]
CRP	6 (2)	6 (3)[3]	6 (2)	0.963[a]	0.591[b,4]
Leukocyte count	7 (2)	8 (2)[5]	7 (2)	0.162[a]	0.236[b,6]
CK	106 (59)	96 (39)[7]	104 (62)	0.370[a]	0.599[b,8]
Ferritin concentration	118 (117)	61 (45)[9]	112 (112)	<0.001[a*]	0.003[b,10*]
Ferritin concentration excluding outliers	95 (65)[11]	61 (45)[9]	96 (64)[12]	0.001[a*]	0.001[b,13*]
Ferritin concentration in *men* only	180 (140)[14]	133 (55)[15]	173 (137)[16]	0.387[a]	0.630[b,17]
Ferritin concentration in *women* only	62 (43)[18]	50 (33)[19]	59 (33)[20]	0.118[a]	0.163[b,21]

Abbreviations: *QFS* = Q fever fatigue syndrome, *CFS* = Chronic fatigue syndrome, *Subgroup QFS* = excluding all QFS patients with <4 additional symptoms and a SIP8 total score <700, *SD* = Standard Deviation, *ESR* = Erythrocyte sedimentation rate, *CRP* = C reactive protein, *CK* = Creatine kinase. Ferritin elevated values: >190 ng/mL in women, and >280 ng/mL in men.

[1] From a total of 66 patients. [2] From a total of 84 QFS and 64 CFS patients. [3] From a total of 50 patients. [4] From a total of 84 QFS and 49 CFS patients. [5] From a total of 72 patients. [6] From a total of 84 QFS and 69 CFS patients. [7] From a total of 30 patients. [8] From a total of 84 QFS and 29 CFS patients. [9] From a total of 53 patients. [10] From a total of 84 QFS and 52 CFS patients. [11] From a total of 108 patients. [12] From a total of 84 patients. [13] From a total of 80 QFS and 52 CFS patients. [14] From a total of 56 patients. [15] From a total of 7 patients. [16] From a total of 41 patients. [17] From a total of 39 QFS and 6 CFS patients. [18] From a total of 61 patients. [19] From a total of 46 patients. [20] From a total of 47 patients. [21] From a total of 45 QFS and 46 CFS patients.

* Significant result after Bonferroni correction.

[a] Calculated using student t-test with significance level at p<0.006.

[b] Calculated using ANCOVA with duration of symptoms as covariate with significance level at p<0.006.

Table 4: Possible cognitive-behavioural perpetuating factors of fatigue in Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS) patients, and the subgroup of QFS patients meeting the CFS criteria

		QFS N=117	CFS N=173	Subgroup QFS N=88	QFS vs. CFS	Subgroup QFS vs. CFS
		Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	Mean (SD) or proportion (%)	P-value	P-value
Sense of control over fatigue (SES28)		17 (3)	18 (3)	17 (3)	0.127[a]	0.296[b,1]
Actometer (Daily observed mean score)		75 (18)	67 (19)	74 (17)	0.001[a*]	0.004[b,1*]
Level of activity	fluctuating active	109 (93%)	137 (79%)	83 (94%)	0.001[c*]	0.001[c*]
	low-active	8 (7%)	36 (21%)	6 (7%)		
Self reported physical activity (SIP8 – mobility)		49 (68)	70 (83)	53 (71)	0.020[a]	0.058[b,1]
Focusing on symptoms (IMQ focusing)		30 (10)	32 (9)	30 (10)	0.024[a]	0.179[b,1]
Catastrophizing thoughts with respect to fatigue (JFCS)		22 (7)	22 (6)	22 (7)	0.370[a]	0.885[b,1]
Somatic attributions regarding symptoms (CAL physical total score)		14 (3)	12 (3)[2]	14 (2)	<0.001[a*]	<0.001[b,3*]

Abbreviations: *QFS* = Q fever fatigue syndrome, *CFS* = Chronic fatigue syndrome, *Subgroup QFS* = excluding all QFS patients with <4 additional symptoms and a SIP8 total score <700, *SD* = Standard Deviation, *SES28* = Self Efficacy Scale, *SIP8 – mobility* = Sickness Impact Profile – Self reported physical activity, *IMQ focusing* = Symptom focusing of the illness Management Questionnaire, *JFCS* = Jacobson Fatigue Catastrophizing Scale, *CAL* = Causal attribution list.

[1] From a total of 84 QFS patients and 160 CFS patients. [2] From a total of 172 patients. [3] From a total of 84 QFS and 159 CFS patients.

* Significant result after Bonferroni correction.

[a] Calculated using student t-test with significance level at p<0.007.

[b] Calculated using ANCOVA with duration of symptoms as covariate with significance level at p<0.007.

[c] Calculated using Pearson Chi-square test with significance level at p<0.007.

Table 5: Multiple regression analysis of perpetuating factors for Q fever fatigue syndrome (QFS) with CIS fatigue as dependent variable

Predictors[a]	Unstandardized Coefficients	Standardized Coefficients	t	P-value
	B	β		
(Constant)	54.294		11.578	<.001
Self-efficacy with respect to fatigue[1]	-.080	-.053	-.521	.603
Somatic attribution of symptoms[2]	-.016	-.008	-.088	.930
Level of physical activity[3]	-.055	-.195	-2.146	.034
Level of self-reported physical activitiy[4]	.014	.190	2.088	.039
Focusing on bodily symptoms[5]	.035	.069	.670	.504

a. Multiple regression, method enter. Dependent Variable: CIS fatigue. N = 117 QFS patients.
Abbreviations: *QFS* = Q fever fatigue syndrome, *CIS* = Checklist Individual Strength.
[1] Measured with the Self-Efficacy Scale (SES28).
[2] Measured with the Causal Attribution List (CAL).
[3] Measured with the DOM score of the actometer.
[4] Measured with the subscale "mobility" of the Sickness Impact Profile (SIP8).
[5] Measured with the subscale "focusing on symptoms" of the Illness Management Questionnaire (IMQ).

DISCUSSION

To our knowledge, this is the first study directly comparing QFS and CFS patients. We found that QFS and CFS patients differed on several aspects. These differences could partly be explained by the fact that different criteria were used with respect to level of functional impairment and the number of additional symptoms to diagnose both syndromes, and difference in duration of symptoms. Differences in duration of illness between both groups can be explained by the fact that the Q fever outbreak in the Netherlands started in 2007, compared to CFS patients who could have had symptoms long before 2007. Included QFS patients could have experienced symptoms for a maximum of 4 to 6 years. However, comparing the subgroup of QFS patients with CFS patients whilst taking into account the different diagnostic criteria used and duration of symptoms still showed differences.

In all analyses, QFS patients had a higher BMI, a known risk factor for chronic fatigue, and were less often female. Consistent with previous research [43], 75% of our CFS patients were female, which has been shown to be a predisposing factor for CFS [44]. In contrast, only half of the QFS patients were female. Even though male gender predominates in notified acute Q fever patients [45], no significant difference in gender was found in a seroprevalence study [3], and no difference in gender was found between non-notified and notified acute Q fever cases, with equally severely affected health status 4 years after infection [46]. Based on the absence of gender difference in seroprevalence, and the fact that the QFS cohort also included non-notified acute Q fever cases, female gender does not seem to be a predisposing factor for QFS. Finally, the total group of QFS patients less often had received treatment for depression, assumed to reflect lower prevalence of premorbid psychiatric illness. In the subgroup analysis with QFS patients who met the CFS criteria, there was a tendency towards less often having received treatment for depression in QFS patients. This could be caused by the relatively small group of QFS patients meeting CFS criteria, which reduces the power

to detect differences. Even though the strength of this evidence was borderline significant after Bonferroni correction, the difference in relation to previous treatment for depression still may be clinically important. Analysis in a larger group of QFS patient might show that previous depressive disorders, a predisposing factor of CFS, are less prevalent. However, there was no difference in current psychological distress or depressive symptoms, indicating that premorbid psychiatric illness in CFS might not be related to current complaints, but only plays a predisposing role, and that current psychological problems are secondary to the chronic fatigue itself and its consequences.

Compared to CFS patients, the group of QFS patients reported fewer additional symptoms, also when differences in diagnostic criteria and duration of symptoms were taken into account. This suggests the presence of a true difference in number of additional symptoms. However, only symptoms were registered as mentioned in the CDC consensus definition of CFS, whereas QFS patients frequently report other complaints such as blurred vision, alcohol intolerance, increased sweating, night sweats, and dyspnoea [7, 10].

A comparison of QFS and CFS patients with regard to inflammatory markers showed that the ESR was significantly higher in CFS patients, which can be explained by a selection bias with only 66 (38%) CFS patients with a known ESR level and relatively more CFS patients with ESR levels above the upper limit of normal compared to QFS patients (9.1% vs. 1.7%, respectively). The mean serum ferritin concentration in QFS patients was approximately twice as high as in CFS patients. However, after correction for gender, no difference in ferritin concentrations between both groups remained. But, as groups sizes were small (only seven male CFS patients) and mean values of ferritin concentration for both men and women were higher in QFS patients, it cannot be ruled out that a lack of power made that the differences failed to reach significance. It should be noted that ferritin concentrations were in the abnormal range in nine QFS patients and in none of the CFS patients. It is known that in diseases with elevated ferritin levels such as haemochromatosis, fatigue is one of the most common symptoms [47, 48]. More research is necessary to find out whether there is a significant ferritin response in QFS patients and how it is driven.

In this paper we explored whether the perpetuating factors found in CFS [15], also predicted fatigue severity in QFS patients. In fact they did not, even though no significant difference was found in fatigue severity between QFS and CFS patients. QFS patients had a significantly higher somatic attribution regarding symptoms, but also significantly higher levels of physical activity. Both were unrelated in QFS patients. This is in contrast to findings in CFS patients, in which stronger attributions of complaints to a somatic cause are associated with lower levels of physical activity [15]. Higher somatic attribution could perhaps be explained by the fact that QFS patients had a known exposure for their complaints, whereas often in CFS such a marker is not present. Because the relationship between somatic attributions and physical activity levels are mediated by patients' interpretations regarding the meaning of symptoms, this might explain the different relationship found in QFS.

The relationship previously found between perpetuating factors and fatigue in CFS could not be confirmed in QFS patients. As expected, the model significantly predicted fatigue in CFS patients, with CIS fatigue being significantly related to both self-efficacy and self-reported physical activity. The relationship between CIS fatigue and focusing on bodily symptoms was

nearly significant, which perhaps can be explained by the relatively small sample size. In QFS patients, a significant negative correlation was found between objectively assessed physical activity and CIS fatigue. Also, self-reported limitations in physical activity were related to fatigue severity. Both may suggest that higher activity levels are associated with reduced fatigue. This has also been found in CFS and other conditions like rheumatoid arthritis [49]. The fact that other cognitive-behavioural variables were not related to fatigue in QFS may indicate that the processes involved in the perpetuation of fatigue in QFS are different from the processes related to fatigue in CFS. On the other hand, the small sample size might be an alternative explanation of bad fit of the model of perpetuating cognitions and behaviour. As the pathophysiological mechanism of QFS still needs to be clarified, treatment based on aetiological insight is hampered. However, CBT aimed at fatigue-related beliefs and behaviour, has already proved to be effective in other forms of chronic fatigue [50, 51]. CBT is a complex intervention in which several fatigue-related beliefs and therefore several (potential) perpetuating factors are influenced. Because factors related to cognition and behaviour overlap substantially between QFS and CFS patients, and gradually increasing physical activity is a key component of CBT, QFS patients might benefit from treatment directed at these factors. Furthermore, the inverse relation between physical activity and fatigue severity suggests that aside from CBT, graded exercise therapy might also be beneficial [52].

CONCLUSION

We conclude that there are differences but also similarities between QFS and CFS patients. With respect to fatigue severity, both groups are similar, but differences in demographics, number of symptoms, and fatigue-related cognitive-behavioural variables were found. Differences in gender and BMI – both known predisposing factors for chronic fatigue – suggest that there are different predisposing factors for developing QFS. More research is necessary to find out whether there is a significant ferritin response in QFS patients and how it is driven, as elevated serum ferritin concentrations were not found at all in CFS patients. Although the relationship between perpetuating factors and fatigue in CFS could not be confirmed in QFS patients, with the exception of the relation between fatigue and lowered levels of activity, the considerable overlap in fatigue-related cognitive-behavioural variables between both groups may imply that behavioural interventions could reduce fatigue severity in QFS patients.

AUTHORS' CONTRIBUTIONS

SK and CB planned and designed the research study, and have been involved in the analysis and interpretation of data, as well as drafting and critical revision of the manuscript. JS has been involved in the design and acquisition of data, has done the analysis and interpretation of data, and drafted the manuscript. HK and SN participated in interpretation and analysis of results, as well as drafting the manuscript and providing critical revisions. JvdM, GB, and MN participated in interpretation of results and writing of the manuscript. All authors read and approved the final manuscript.

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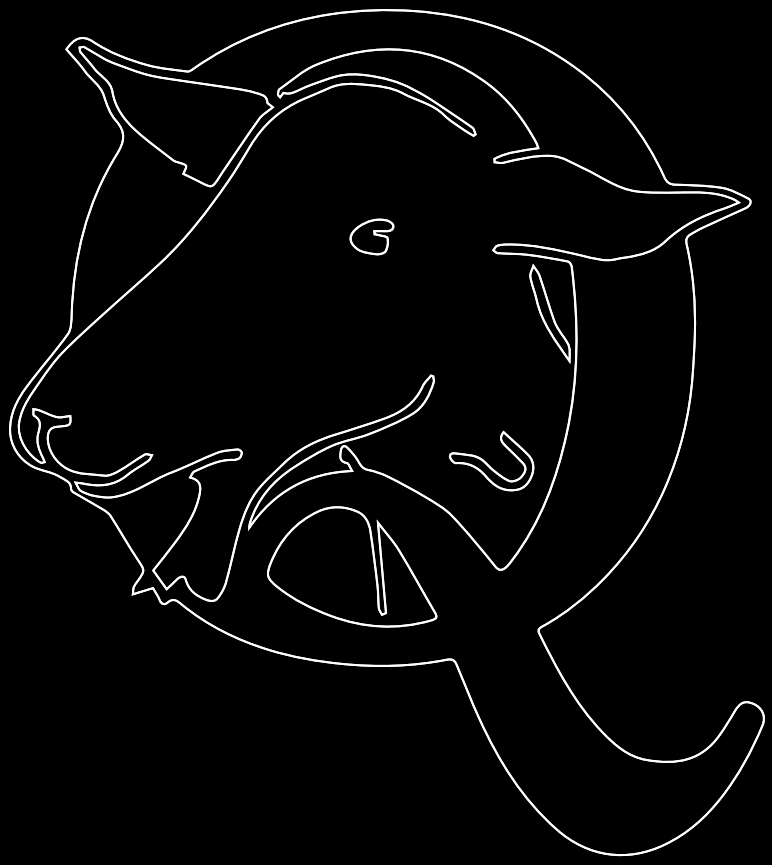

CHAPTER 4

ALTERED INTERFERON-γ RESPONSE IN PATIENTS WITH Q-FEVER FATIGUE SYNDROME

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ABSTRACT

Objectives: Whether immunological mechanisms underlie Q fever fatigue syndrome (QFS) remains unclear. For acute Q fever, the antigen-specific interferon-γ (IFNγ) response may be a useful tool for diagnosis, and the IFNγ/interleukin(IL)-2 production ratio may be a marker for chronic Q fever and treatment monitoring. Here we explored the specific IFNγ production and IFNγ/IL-2 ratio in QFS patients.

Methods: IFNγ and IL-2 production were tested in *ex-vivo* stimulated whole blood of QFS patients (n=20), and compared to those previously determined in seropositive controls (n=135), and chronic Q fever patients (n=28). Also, the correlation between patient characteristics and IFNγ, IL-2, and IFNγ/IL-2 ratio was determined.

Results: QFS patients were younger ($p<0.001$), but gender distribution was similar to seropositive controls and chronic Q fever patients. *Coxiella burnetii* Nine Mile stimulation revealed a higher IFNγ production in QFS (median 319.5 pg/ml) than in seropositive controls (120 pg/ml, $p<0.01$), but comparable to chronic Q fever (2846 pg/ml). The IFNγ/IL-2 ratio was similar to that in seropositive controls, but lower than in chronic Q fever patients ($p<0.01$). Symptom duration was positively correlated with IL-2 production, and negatively correlated with the IFNγ/IL-2 ratio.

Conclusions: These results point to an altered cell-mediated immunity in QFS, and suggest a different immune response than in chronic Q fever.

HIGHLIGHTS

- We explored the specific IFNγ production, and the IFNγ/IL-2 ratio in QFS patients.
- QFS patients have a significant higher IFNγ production than seropositive controls.
- The IFNγ/IL-2 ratio is significantly lower in QFS than in chronic Q fever patients.
- These results point to an altered cell-mediated immunity in QFS.

INTRODUCTION

At present, the Netherlands is faced with the aftermath of the largest Q fever outbreak worldwide lasting from 2007 to 2011 [1]. During this period, over 4000 patients with symptomatic acute Q fever were reported, and it was estimated that over 40,000 individuals experienced a latent infection [2, 3]. Although most patients with symptomatic acute Q fever recover completely with only a serological scar left, infection with *Coxiella burnetii* is notorious for causing long-term sequelae, i.e., chronic Q fever and Q fever fatigue syndrome (QFS). Chronic Q fever, characterized by the persistence of viable *C. burnetii*, may develop in 1-5% of both symptomatic and asymptomatic cases of acute Q fever. Chronic Q fever presents mainly as vascular infection [4], including mycotic aneurysms and infections of vascular prosthesis, and endocarditis [5]. QFS, a debilitating fatigue syndrome following acute Q fever, may become manifest in approximately 20% of patients [6-10]. Lasting up to 10 years after the acute illness [11], QFS is considered to be the major cause of the Q fever-related economical burden following the Dutch outbreak [12]. The pathophysiological mechanisms underlying QFS remain to be elucidated. Interpretations range from compensation-driven and psychogenic perpetuation of the original symptoms [7], to attribution of the syndrome to cytokine dysregulation due to chronic immune stimulation [7]. The latter might be caused by persisting *C. burnetii*, or by persisting non-infectious *C. burnetii* antigens [13-18]. White blood cells from QFS patients exposed to Q fever antigens were found to exhibit a marked interleukin-6 (IL-6) production [13], and the IL-6 production was similar in both chronic Q fever patients and seropositive controls, which was significantly higher than in seronegative controls [19]. In addition, the group of QFS patients contained significantly more interferon-γ (IFNγ) responders than a group of controls, whilst the proportion of IL-2 responders was lower among QFS patients [13]. IFNγ is a cytokine that plays an important role in the host defence against intracellular bacteria such as *C. burnetii* [20-23]. To date, no diagnostic test is available to diagnose QFS directly and diagnosis partly relies on measurement of *C. burnetii*-specific antibodies, e.g. serology, reflecting humoral immunity. Recently our group developed a *C. burnetii*-specific whole–blood IFNγ production assay, which is a promising diagnostic tool for *C. burnetii* infection [24], with similar performance and practical advantages over serology [25]. In addition, a high IFNγ/IL-2 ratio appeared to be indicative of chronic Q fever, and may be a useful diagnostic marker for chronic Q fever and treatment monitoring [19, 26]. In addition, as suggested in animal experiments, antigen-specific IFNγ production could also be a useful tool for diagnosis of acute Q fever [27].

In the present study, we addressed the question whether there is an aberrant antigen-specific IFNγ production and IFNγ/IL-2 ratio in QFS patients. If so, this might provide additional insight in the potential pathophysiological mechanisms underlying this debilitating long-term complication and might contribute, as immunological markers, to the diagnostic workup of QFS.

MATERIALS AND METHODS

Study population

The study population consisted of QFS patients (n=20), Q fever seropositive controls (n=135), and patients with proven chronic Q fever (n=28). All QFS patients were diagnosed with QFS at the Radboud Expertise Centre for Q fever, Nijmegen, the Netherlands, after a uniform work-up according to the Dutch guideline on QFS [28]. All QFS patients met the following diagnostic criteria: i. fatigue lasted ≥6 months; ii. sudden onset of severe fatigue (defined as a score ≥35 on the subscale fatigue severity of the Checklist Individual Strength (CIS)), or significant increase in fatigue related to a symptomatic acute Q fever infection; iii. chronic Q fever and other causes of fatigue, somatic or psychiatric, were excluded; and iv. fatigue resulted in significant functional impairment (defined as a total score ≥450 on the Sickness Impact Profile (SIP)). Blood samples were collected during regular patient care between May 2011 and February 2012. The seropositive controls were anonymously derived from the Dutch Q fever vaccination campaign, which was organized from January to April 2011 [29]; data on their antigen-specific IFNy production has been published previously [25]. All controls had pre-existing risk factors for development of Q fever endocarditis or vascular infection, and were Q fever seropositive ≥1 year after the Q fever epidemic (IgG phase I or II ≥1:32, but IgG phase I ≤1:512), without clues for persistent Q fever infection. Chronic Q fever patients were diagnosed at participating hospitals [19], and blood samples were collected between December 2010 and March 2012. At the time of sampling, all patients were diagnosed with either Q fever endocarditis (n=9) or vascular (prosthesis) infection (n=18), according to the Dutch guideline on chronic Q fever [30]; patient characteristics and data on the cytokine production of these patients also have been published before [19, 25].

Serological measurements and detection of C. burnetii DNA

IgM and IgG antibodies against *C. burnetii* phase I and phase II antigens were measured by a commercially available immunofluorescence assay (IFA; Focus Diagnostics, Cypress, CA, USA). The PCR assay used to detect DNA of *C. burnetii* in serum was an in-house real time PCR directed against the insertion sequence IS1111a.

In-vitro whole blood stimulation

Whole blood stimulation, followed by measurement of IFNy and IL-2 production, was done as previously described [25]. In brief, venous blood was drawn into 5mL endotoxin-free lithium-heparin tubes (Vacutainer, BD Bioscience) and samples were processed within 12h. Incubation of samples was done as previously described [25]. *C. burnetii* Nine Mile (NM) RSA 493 phase I, heat-inactivated, was used [25, 31], and the mitogen phytohemagglutinin (PHA) (Sigma–Aldrich, St Louis, MO, USA) as a positive control. As a negative control, incubation with only Roswell Park Memorial Institute medium (RPMI, 1640 Dutch modification, Life Technologies/Invitrogen, Breda, the Netherlands) was performed. After incubation, blood samples were centrifuged at 4656 g for 10 min and supernatants were stored at -20^{o}C until cytokine measurement.

Cytokine measurements

The IFNγ production was measured by enzyme-linked immunosorbent assay (ELISA; Pelikine compact, Sanquin, Amsterdam, the Netherlands), in undiluted whole blood incubated for 24h either with PHA, or *C. burnetii*–NM in all patients, as described [24, 25]. IL-2 was measured using a multiplex beads assay (Merck Millipore, Billerica, MA, USA) according to the manufacturer's instructions.

Ethical statement

This study was exempt from ethical approval by the local ethics committee, as there was no additional burden for patients. Samples were obtained during regular patient care after obtaining oral and written informed consent, and, in case of individuals from the Dutch Q fever vaccination campaign, individuals signed written informed consent to use drawn blood for research purposes.

Statistical analysis

Data were analyzed using Graphpad Prism (Graphpad Software Inc., version 5.03) and SPSS (Version 22.0, SPSS, Inc). The Kruskall–Wallis test was used as non-parametric ANOVA to determine differences between groups. Statistical significance was attained if $p<0.05$. In case of significance, by post-hoc analysis using Dunn's multiple comparison test was performed to look at pair wise comparisons between the groups, taking into account the number of comparisons made. The correlation between patient characteristics and IFNγ and IL-2 production, and the IFNγ/IL-2 ratio was determined with the non-parametric Spearman's rank correlation coefficient.

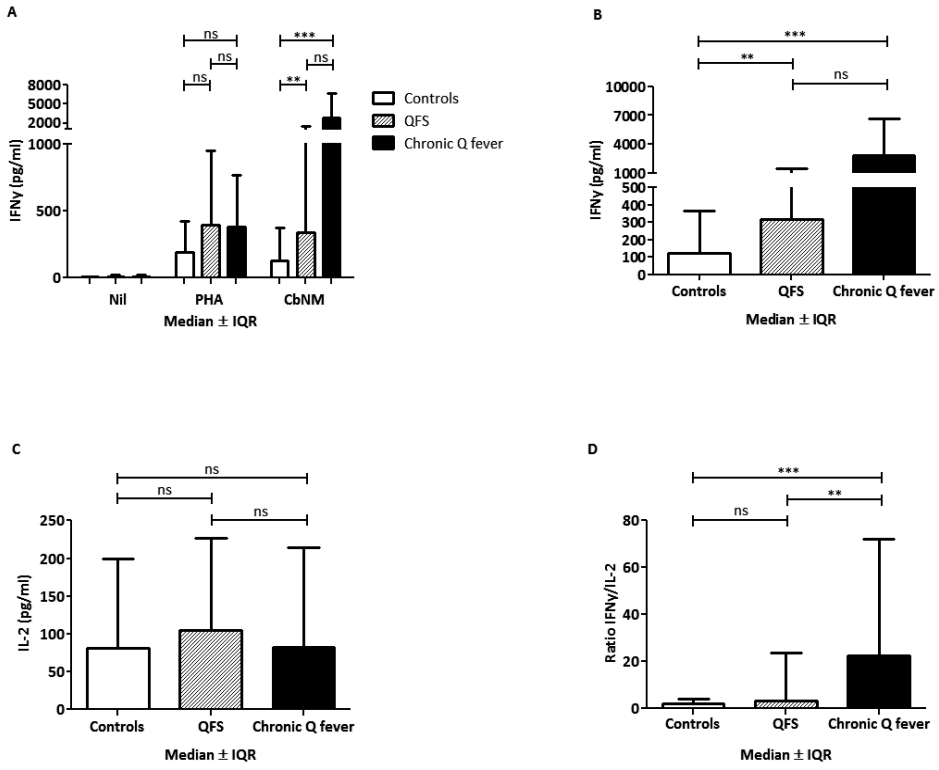
RESULTS

Patients and controls

At the time of blood collection, QFS was already diagnosed but treatment had yet to be started (*Table 1*). The symptom duration of QFS patients, defined as the time of symptom onset until blood sampling, varied between 12 and 51 months (*Table 1*). All seropositive controls had IgG phase I or phase II titres ≥1:32, but IgG phase I ≤1:512, and none of them showed serological signs of an acute or recent Q fever infection, reflected by IgM antibodies in absence of IgG antibodies. The mean age of QFS patients was 50.2 yrs (SD 9.3), which was significantly younger ($p<0.001$) than 60.8 years (SD 15.1) and 66.2 years (SD 11.8) for the seropositive controls and chronic Q fever group, respectively. There was no correlation between age and IFNγ production (Spearman's rank correlation coefficient -0.71, $p=0.341$), between age and IL-2 production (Spearman's rank correlation coefficient -0.002, $p=0.978$), and between age and IFNγ/IL-2 ratio (Spearman's rank correlation coefficient 0.060 ($p=0.466$)). All groups had a predominant male distribution, with 70% being male in the QFS group, 78% in the seropositive control group, and 79% in the chronic Q fever group (*not significant*).

Figure 1: IFNy and IL-2 production in Q fever fatigue syndrome (QFS) patients, chronic Q fever patients and Q fever seropositive controls

(A) Comparable aspecific PHA-induced IFNy production between QFS patients, seropositive controls and chronic Q fever patients after 24h incubation of whole blood. There is no significant difference in specific *Cb*NM-induced IFNy production between QFS and chronic Q fever patients. (B) *Cb*NM-induced IFNy production (stimulated minus unstimulated) after 24h incubation of whole blood, showing a significant difference in IFNy production between seropositive controls and QFS and chronic Q fever patients, with an increasing trend of IFNy production towards chronic Q fever patients. (C) *Cb*NM-induced IL-2 production (stimulated) between seropositive controls, QFS patients and chronic Q fever patients after 24h incubation of whole blood. (D) IFNy/IL-2 ratio, showing a significant difference between chronic Q fever patients and both seropositive controls and QFS patients. A trend towards a higher IFNy/IL-2 ratio is observed towards chronic Q fever patients. Median ± IQR are shown. The Kruskall–Wallis test was used, and, in case of significance, post-hoc analysis using the Dunn's multiple comparison test was performed to look at pair wise comparisons between the groups, taking into account the number of comparisons made.



Abbreviations: *IFNy* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q fever fatigue syndrome; *PHA* = Phytohemagglutinin; *CbNM* = *Coxiella burnetii* Nine Mile; *ns* = not significant; *IQR* = Interquartile range; *controls* = seropositive controls.

** p-value <0.01.*** p-value <0.001.

IFNγ and IL-2 production and IFNγ/IL-2 ratio

Aspecific PHA-induced IFNγ production was similar in QFS patients, seropositive controls, and chronic Q fever patients (*Table 2, Figure 1A*). Specific stimulation with *C. burnetii* NM for 24h in QFS patients showed a median IFNγ production of 319.5 pg/ml, which was significantly higher ($p<0.01$) than in seropositive controls (median 120 pg/ml), but not significantly different from chronic Q fever patients (median 2846 pg/ml) ($p=0.110$) (*Figure 1B*). No significant difference was observed in IL-2 production between QFS patients (median 104.5 pg/ml), seropositive controls (median 81 pg/ml), and chronic Q fever patients (median 82.5 pg/ml) (*Figure 1C*). The IFNγ/IL-2 ratio was calculated for each individual. The IFNγ/IL-2 ratio in QFS patients was not significantly different from seropositive controls, but significantly lower than the ratio found in chronic Q fever patients ($p<0.01$) (*Figure 1D*).

Correlations between patient characteristics and cytokine measurements

Correlations between the most important characteristics of QFS patients (*Table 1*) and the measured cytokine productions were assessed (*Table 3*). The duration of symptoms did not significantly correlate with IFNγ production, but did so with IL-2 production ($p=0.032$); it negatively correlated with the IFNγ/IL-2 ratio ($p=0.025$). No correlation was found between the level of fatigue and IFNγ or IL-2 production, as well as the IFNγ/IL-2 ratio. A positive correlation was found between the level of perceived disabilities, reflected by the SIP total score, and IL-2 production ($p=0.047$), but no correlation was found with either IFNγ production or the IFNγ/IL-2 ratio. Finally, no correlation was found between the IgG phase I titres and either IFNγ or IL-2 production, or the IFNγ/IL-2 ratio.

DISCUSSION

In this study we assessed the antigen-specific IFNγ production and IFNγ/IL-2 ratio in *C. burnetii*-stimulated whole blood of QFS patients. We found that the IFNγ production of QFS and chronic Q fever patients was not significantly different, but for both significantly increased compared to seropositive controls. In addition, the IFNγ/IL-2 ratio in QFS patients was similar to that in seropositive controls, but lower than in chronic Q fever patients. Of note, no differences in IL-2 production between the three groups were found. These results suggest that *C. burnetii*-induced IFNγ production and IFNγ/IL-2 ratio may discriminate seropositive controls from QFS and chronic Q fever patients.

At present, the measurement of the specific humoral immune response, i.e. serology, has a central position in the diagnosis of Q fever, but it is increasingly accepted that cell-mediate immune responses are also relevant to describe the anti-*C. burnetii* host response. However, the precise relationship between T-cell function and protective immunity remains unknown. Memory T lymphocytes can be broadly divided in central memory T-cells, which lack immediate effector function and mainly secrete IL-2, and effector memory T-cells, displaying immediate effector function, e.g. IFNγ and IL-2 secretion [32]. IFNγ plays a pivotal role in protective immunity against many intracellular bacteria, but is also a marker of infection, immunity, and the extent of immune-mediated pathology [20].

It has been proposed that full activation of the macrophage by IFNy is required to eliminate *C. burnetii*, and that the phase 1 antigen can promote downregulation of IFNy by lymphocytes, perhaps by modulating IL-2 production [33]. This is however difficult to reconcile with the finding that chronic Q fever patients exhibit a very high specific IFNy production. It has been postulated that distinct IFNy/IL-2 functional profiles correlate with different models of infection [20]. This concept is supported by previous findings, showing a high IL-2 production in seropositive controls, assumed to have cleared the infection successfully, and high IFNy and low IL-2 production in chronic Q fever patients [19]. Interestingly, our study revealed that QFS patients had a markedly higher *C. burnetii*-specific IFNy production than seropositive controls. In addition, the IFNy production in QFS patients and chronic Q fever patients did not significantly differ, although there was a trend that QFS patients had lower IFNy production than chronic Q fever patients, and it can be expected that with larger numbers of patients these differences would become significant. In that case, it is tempting to hypothesize that QFS represents an altered cell-mediated immunity in the spectrum of Q fever related syndromes, i.e. an inactive state without viable *C. burnetii* in contrast to chronic Q fever. The combined use of IFNy production and IL-2 production allows a better distinction between QFS patients, seropositive controls, and chronic Q fever patients [19]. Also, a positive correlation between IL-2 production and both symptom duration and level of perceived disabilities was found, suggesting that QFS patients slowly attain an inactive state of infection, with a subsequent negative correlation between symptom duration and IFNy/IL-2 ratio. Similarly, resolution of fatigue in the acute sickness response appeared to be associated with improvement of cell-mediated immunity [34]. The IFNy/IL-2 ratio was proposed as an additional diagnostic marker for chronic Q fever [19], and our results indicate that the IFNy/IL-2 ratio also discriminates between QFS and chronic Q fever patients, but not between QFS patients and seropositive controls. Our data are supported by another study in the literature, showing IFNy upregulation and IL-2 downregulation in QFS patients compared to control groups [13]. All these results point to an altered cell-mediated immune response in those who do not recover completely, implicating that both antigen-specific IFNy production and IFNy/IL-2 ratio might be used as immunological marker in the diagnostic workup of QFS. Although the results are strikingly similar, both our study and that of Penttila et al [13] deal with low numbers of patients. Thus further confirmation is needed.

Other limitations of our study are that the cytokine studies in the seropositive controls and chronic Q fever patients were performed earlier and derived from published studies of our group [19, 25]. Ideally, these studies should have been done completely in parallel to avoid laboratory artefacts. However, the determination of IFNy production is a standard procedure and therefore inter- and intra-individual variation is limited. In addition, the best control group for comparison with QFS patients would be patients with a previous Q fever infection with asymptomatic recovery, i.e., without QFS or other co-morbidity. In contrast, the seropositive controls were anonymously derived from a vaccination campaign; these subjects had an indication for vaccination but were not vaccinated because of positive Q fever serology. We cannot exclude that some of these patients suffered from fatigue. Finally, IL-6 production was not measured though it has been found that the IL-6 production was accentuated in QFS patients, with a significant correlation with total symptom scores

[13], and also higher in chronic Q fever patients and seropositive controls compared to seronegative controls [19].
Thus, it is too early to advice the usage of the immunological assays described here in a routine clinical setting. To overcome the mentioned limitations, and to investigate whether the IFNγ production assay or IFNγ/IL-2 ratio, and other cytokines such as IL-6, would be useful in clinical practice for diagnosing QFS, i.e. regardless of the time-point of sampling, a case–control study with comparison of QFS patients, CFS patients, seropositive controls without co-morbidity, and healthy controls will be performed in the near future.

CONCLUSION

In conclusion, the IFNγ production in QFS patients is significantly higher than in seropositive controls, and the IFNγ/IL-2 ratio is significantly lower than in chronic Q fever patients. Further investigation in larger cohorts of QFS patients is warranted, as these results point to an altered cell-mediated immunity in QFS, and hence opens up avenues for better understanding the pathogenesis of this enigmatic complication of Q fever and of other fatigue syndromes.

AUTHORS' CONTRIBUTION

SK, CB, TS, and MvD planned and designed the study, and have been involved in the analysis and interpretation of data. SK and RR drafted the manuscript. RR was also involved in the analysis and interpretation of data. SK, TS and CB collected samples of patients at the outpatient clinic. TS performed the experiments, and was involved in data collection, as well as in drafting and critical revision of the manuscript. JvdM, MN, CB, and MvD participated in interpretation of results. Furthermore, they provided critical revisions to the first drafts of the manuscript. All authors read and approved the final manuscript.

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Table 1: Baseline characteristics of 20 patients with Q fever fatigue syndrome (QFS)

Gender & age (yr)	Symptom duration[a] (months)	CIS fatigue	SIP total score	PCR serum	IFA IgM phase I	IFA IgM phase II	IFA IgG phase I	IFA IgG phase II	ELISA	CFA
W, 45	32	54	587	Negative	Negative	Negative	1:64	1:128	Negative	Negative
M, 55	35	51	1726	Negative	1:32	1:256	1:512	1:512	Positive	40
M, 57	18	49	1037	Negative	Negative	Negative	1:128	1:128	Negative	Negative
M, 64	37	47	2376	Negative	Negative	1:128	1:128	1:512	Dubious	40
M, 58	35	56	1583	Negative	1:64	Negative	1:32	1:128	Negative	Negative
W, 58	36	56	1205	Negative	Negative	1:128	Negative	1:32	Positive	Negative
M, 44	49	55	888	Negative	1:256	1:256	1:128	1:1024	Positive	80
M, 49	20	55	1374	Negative	1:16	1:32	Negative	1:16	Negative	Negative
M, 57	24	49	1792	Negative	Negative	Negative	1:128	1:128	Negative	Negative
M, 47	12	41	641	Negative	Negative	Negative	1:32	1:32	Negative	Negative
W, 48	16	41	1115	Negative	1:128	1:512	1:256	1:512	Positive	40
M, 46	17	50	546	Negative	Negative	Negative	1:256	1:512	Negative	10
M, 56	30	54	1408	Negative	1:64	1:128	1:512	1:512	Positive	40
M, 42	27	56	578	Negative	1:128	1:32	1:128	1:256	Negative	Negative
M, 59	28	45	1801	Negative	Negative	1:32	1:512	1:512	Dubious	40
M, 38	30	56	634	Negative	1:16	Negative	1:512	1:1024	Negative	80
W, 49	21	45	953	Negative	1:32	1:64	1:64	1:256	Dubious	20
W, 51	29	44	527	Negative	Negative	Negative	1:128	1:256	Dubious	20
M, 57	51	46	1389	Negative	1:16	Negative	1:128	1:256	Negative	80
W, 23	23	56	1194	Negative	1:16	Negative	Negative	1:16	Positive	Negative

Abbreviations: *QFS* = Q fever fatigue syndrome; *CIS* = Checklist Individual Strength, subscale fatigue; *SIP* = Sickness Impact Profile; *PCR* = Polymerase chain reaction, in-house real time PCR directed against the insertion sequence IS1111a; *IFA* = Immunofluorescence assay (Focus Diagnostics, California, U.S.A), detecting IgM and IgG antibodies against phase I- and phase II-antigens; *ELISA* = Enzyme-linked immunosorbent assay (Panbio®, Australia, Coxiella burnetii (Q Fever) IgM ELISA, a screenings test directed against IgM phase II; *CFA* = Complement fixation assay (CFA) (Virion-Serion, Würzburg, Germany) directed against *C. burnetii* phase II antigens; *M* = Man; *W* = Woman.
[a] Symptom duration: time onset of symptoms until blood sampling.

Table 2: IFNy and IL-2 production in 20 patients with Q fever fatigue syndrome (QFS)

Patients	IFNy production (pg/ml)				IL-2 production (pg/ml)	Ratio IFNy/IL-2
Gender & age	RPMI	PHA [10µg/ml]	C. burnetii NM [10^7/ml]	C. burnetii NM [10^7/ml] - RPMI	C. burnetii NM [10^7/ml]	C. burnetii NM [10^7/ml]
W, 45	8	551	231	223	103	2.2
M, 55	22	477	356	334	107	3.1
M, 57	8	933	5347	5339	170	31.4
M, 64	10	935	5142	5132	820	6.3
M, 58	17	80	234	217	299	0.7
W, 58	29	5600	915	886	141	6.3
M, 44	8	5000	389	381	287	1.3
M, 49	8	236	266	258	59	4.4
M, 57	8	148	192	184	96	1.9
M, 47	8	125	500	492	16	30.8
W, 48	8	248	270	262	114	2.3
M, 46	12	953	4545	4533	78	58.1
M, 56	21	538	1754	1733	47	36.9
M, 42	39	311	2683	2644	106	24.9
M, 59	8	83	135	127	39	3.3
M, 38	8	146	643	635	362	1.8
W, 49	20	23	25	5	22	0.2
W, 51	19	2320	146	127	245	0.5
M, 57	20	81	325	305	16	19.1
W, 23	23	956	102	79	100	0.8

Net IFNy production is shown after 24h incubation of whole blood with PHA or *C. burnetii* NM. Furthermore, net IL-2 production is shown after 24h incubation of whole blood with *C. burnetii* NM.
Abbreviations: *IFNy* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q fever fatigue syndrome; *PHA* = Phytohemagglutinin; *C. burnetii* = *Coxiella burnetii; NM* = Nine Mile strain; *RPMI* = Roswell Park Memorial Institute medium, (1640 Dutch modification, Life Technologies/Invitrogen, Breda, the Netherlands); *M* = Man; W = Woman.

Table 3: Correlations between patient characteristics and IFNy and IL-2 production in Q fever fatigue syndrome (QFS) patients

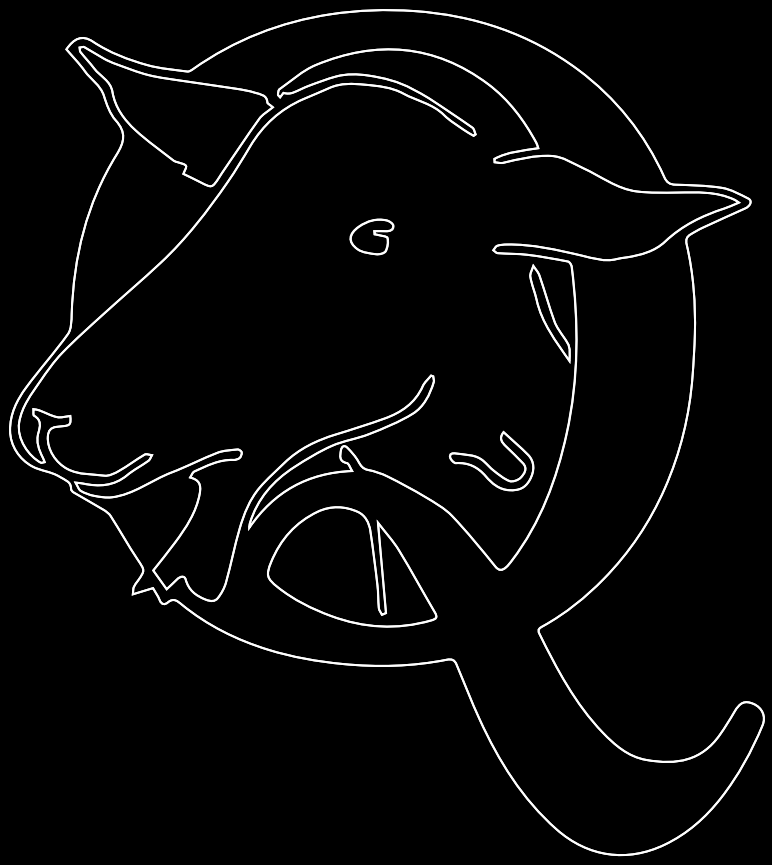
Patient characteristics	Duration of symptoms (months)[a]		CIS fatigue score		SIP score		IFA IgG phase 1 titres	
Cytokine production	Correlation (ρ)[b]	p-value	Correlation (ρ)[b]	p-value	Correlation (ρ)[b]	p-value	Correlation (ρ)[b]	p-value
IFNy (pg/ml)	-0.235	0.320	-0.077	0.748	0.028	0.907	0.600	0.242
IL-2 (pg/ml)	0.480	0.032[c]	-0.106	0.657	0.449	0.047[c]	-0.086	0.919
IFNy/IL-2 ratio	-0.498	0.025[c]	0.112	0.637	-0.147	0.535	0.314	0.564

Abbreviations: *IFNy* = Interferon-gamma; *IL* = Interleukin; *QFS* = Q fever fatigue syndrome; *CIS* = Checklist Individual Strength, subscale fatigue; *SIP* = Sickness Impact Profile; *IFA* = Immunofluorescence assay (Focus Diagnostics, California, U.S.A), detecting IgM and IgG antibodies against phase I- and phase II-antigens.

[a] Symptom duration: time onset of symptoms until blood sampling.

[b] Calculated using Spearman's rank correlation coefficient (ρ).

[c] Significant correlation of $p \leq 0.05$.



CHAPTER 5

THE QURE STUDY: Q FEVER FATIGUE SYNDROME – RESPONSE TO TREATMENT; A RANDOMIZED PLACEBO-CONTROLLED TRIAL (STUDY PROTOCOL)

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ABSTRACT

Background: Q fever is a zoonosis that is present in many countries. Q fever fatigue syndrome (QFS) is one of the most frequent sequelae after an acute Q fever infection. QFS is characterized by persistent fatigue following an acute Q fever infection, leading to substantial morbidity and a high socio-economic burden. The occurrence of QFS is well-documented, and has been described in many countries over the past decades. However, a treatment with proven efficacy is not available. Only a few uncontrolled studies have tested the efficacy of treatment with antibiotics on QFS. These studies suggest a positive effect of long-term treatment with a tetracycline on performance state; however, no randomized controlled trials have been performed. Cognitive behavioral therapy (CBT) has been proven to be an effective treatment modality for chronic fatigue in other diseases, but has not yet been tested in QFS. Therefore, we designed a trial to assess the efficacy of long-term treatment with the tetracycline doxycycline and CBT in patients with QFS.

Methods/design: A randomized placebo-controlled trial will be conducted. One-hundred-eighty adult patients diagnosed with QFS will be recruited and randomized between one of three groups: CBT, long-term doxycycline or placebo. First, participants will be randomized between CBT and medication (ratio 1:2). A second double-blinded randomization between doxycycline and placebo (ratio 1:1) will be performed in the medication condition. Each group will be treated for six months. Outcome measures will be assessed at baseline and post intervention. The primary outcome measure is fatigue severity. Secondary outcome measures are functional impairment, level of psychological distress, and *Coxiella burnetii* PCR and serology.

Discussion: The Qure study is the first randomized placebo-controlled trial, which evaluates the efficacy of long-term doxycycline and of cognitive behavioral therapy in patients with QFS. The results of this study will provide knowledge about evidence-based treatment options for adult patients with QFS.

Trial registration: ClinicalTrials.gov: NCT01318356, and Netherlands Trial Register: NTR2797.

INTRODUCTION

Q fever, a zoonosis caused by *Coxiella burnetii*, has been present all over the world for many years [1]. Between 2007 and 2010, the south-eastern part of the Netherlands has faced the largest outbreak of Q fever ever reported. To date, more than 4000 people have developed symptomatic disease [2], and at least up to 44,000 are estimated to have been infected [3, 4]. In recent years, several studies have described the sequelae of Q fever. Acute Q fever is followed by a chronic infection in 1-5% of cases [5-7]. In addition, following acute Q fever, patients frequently report long-lasting fatigue, which often persists for more than six months [8-10]. After an outbreak of Q fever in the UK, 10 years of follow-up revealed a high percentage of persisting fatigue, with almost 20% of patients fulfilling the Centre for Disease Control (CDC) criteria of chronic fatigue syndrome, compared to 4% in healthy controls [11]. A study among abattoir employees in Australia showed that 28% of patients with proven acute Q fever fulfilled the CDC criteria of chronic fatigue syndrome five years after the infection compared to none of the seronegative controls [10]. A recent study carried out in the Netherlands among 85 patients with acute Q fever found that 59% of patients had persistent symptoms at six months after disease onset, with fatigue being the most prevalent complaint in 52% of patients. Furthermore, over 25% still had complaints after one year [12]. Another recent survey in the Netherlands among 515 patients with Q fever found that 20% had severe fatigue and an impaired health status at 12–26 months of follow-up [13]. This fatigue following acute Q fever, sometimes accompanied by several other complaints, has been designated Q fever fatigue syndrome (QFS) [14-16]. According to the recently published Dutch algorithm on QFS [14], the diagnosis of QFS can be made after a uniform diagnostic work-up. There has to be a severe fatigue, which lasts for at least six months and has a reference to an acute Q fever infection. There must be an absence of fatigue before the episode of acute Q fever or a significant increase in fatigue since the acute Q fever infection. Furthermore, it is causing significant disabilities in daily practice. Finally, chronic Q fever and other causes of fatigue, somatic or psychiatric, need to be excluded.

In the Netherlands, QFS resulted in a large incurred loss due to loss of quality of life and health-related absenteeism in the past few years [17]. Currently, extrapolating the present data, at least 800 patients suffer from QFS in the Netherlands. It is expected that Q fever will remain an endemic disease, leading to a further increase in patients with QFS, stressing the need for further research into treatment regimens for QFS.

Both acute and chronic Q fever have been extensively studied in recent years; however, less attention has been given to QFS. Although QFS is a well-documented finding and has already been described in 1996 [8, 10], at present there is no consensus on the pathogenetic process underlying QFS [15, 18, 19]. In QFS, as in chronic fatigue syndrome, persistence of live microbes has been suggested [19]. Furthermore, it is still unclear whether effective treatment for QFS is possible. So far, few studies on the effect of treatment with antibiotics on fatigue after Q fever have been done. The available studies suggest a positive effect of long-term treatment with a tetracycline on performance status [20-22]; however, these studies suffer from several limitations. So far, no controlled trials have been performed and the above long-term treatment is currently not often used in clinical care of patients with QFS. Previously, it has been shown in patients with chronic fatigue syndrome (CFS) that

fatigue-related cognitions and behavior can maintain chronic fatigue [23-26]. CBT for chronic fatigue is aimed at these fatigue-related cognitions and behavior thought to perpetuate the symptoms. Several systematic reviews and meta-analyses demonstrated that CBT for CFS is able to reduce symptoms and to improve function in patients with CFS [26-28]. To date, the efficacy of CBT has not been studied in patients with QFS. However, our recent clinical experience with this treatment modality in a small cohort of QFS patients shows promising results.

The primary aim of our study is to determine the effect of different treatment modalities which have been suggested to be effective for patients with QFS. In this paper we describe the protocol to assess the efficacy of two treatment strategies for QFS: long-term treatment with either doxycycline or CBT.

METHODS/DESIGN

Study design

A randomized placebo-controlled trial (RCT), the Qure study, will be performed to determine whether long-term treatment with doxycycline or CBT will lead to a reduction of fatigue and disabilities in patients with QFS. Both treatment modalities will be compared to a placebo group. This study will be performed in the Radboud University Nijmegen Medical Centre in the Q fever outpatient clinic of the department of Internal Medicine, and in the Expert Centre for Chronic Fatigue (ECCF). QFS will be diagnosed at the Q fever outpatient clinic after a uniform diagnostic work-up according to the Dutch algorithm on QFS. Once the diagnosis is established, study eligibility will be assessed by the first author (SPK) according to specific inclusion and exclusion criteria (*Tables 1 and 2*). Eligible patients will be asked to participate in the Qure study after receiving verbal and written information about the study. If patients are willing to participate, written informed consent will be obtained. After inclusion, an individual study code is allocated to the participants. Results from the clinical assessment before inclusion will be used as baseline measures as well. If patients decide not to participate in this study, an attempt will be made to elucidate the reason for this, but patients are not obligated to motivate their refusal.

Table 1: Inclusion criteria

Inclusion criteria*	
(1)	Males or non-pregnant, non-lactating females who are 18 years or older
(2)	Laboratory-proven acute Q fever since the year 2007 and/or positive serology fitting a past infection with *Coxiella burnetii*
(3)	AND being severely fatigued, defined by scoring ≥ 35 on the subscale fatigue severity of the CIS
(4)	AND being fatigued for at least 6 months
(5)	AND being disabled because of the fatigue, defined by scoring 450 or higher on the SIP
(6)	Subjects must sign a written informed consent form

* All participants have to meet the criteria for QFS according to the recently published Dutch algorithm on QFS [14]. In addition to the mentioned inclusion criteria and according to the Dutch algorithm on QFS, there has to be a severe fatigue with a reference to an acute Q fever infection. Furthermore, there must be an absence of fatigue before the episode of acute Q fever or a significant increase in fatigue since the acute Q fever infection.
Abbreviations: *CIS* = Checklist Individual Strength questionnaire, *SIP* = Sickness Impact Profile questionnaire.

Study population

It is intended to include 180 patients diagnosed with QFS, equally randomized between three different treatment modalities, namely long-term doxycycline (n=60), CBT (n=60) or placebo (n=60). All eligible patients directly referred to Radboud University Nijmegen Medical Centre will be asked to participate in this study. Patients with a suspicion of QFS presenting to other hospitals in the area will be referred to the Q fever outpatient clinic of the Radboud University Nijmegen Medical Centre for screening and enrollment in the study. In addition, all physicians working at specific Q fever outpatient clinics in other hospitals will be informed about the study. Patients connected to Q-uestion, a foundation for patients with Q fever, will be informed about the Qure study by newsletters, and a brief description will be available at the website of Q-uestion. Furthermore, patients who participated in previous studies on Q fever in the past few years (Q-Quest II study, ZonMw dossier number: 204004003, and The PrediQt study, ZonMw dossier number: 205520003, NL36477.091.11), will be informed about the Qure study by letter. Finally, all general practitioners in the endemic Q fever region will be informed about this study by letter.

Ethical approval

According to the Dutch law, this study has been reviewed and approved by the Medical Ethical Review Committee of the Radboud University Nijmegen Medical Centre (registration number 2011/069, NL35755.091.11). This study will be conducted according to the principles of the Declaration of Helsinki. The inclusion of patients started in May 2011.

Table 2: Exclusion criteria

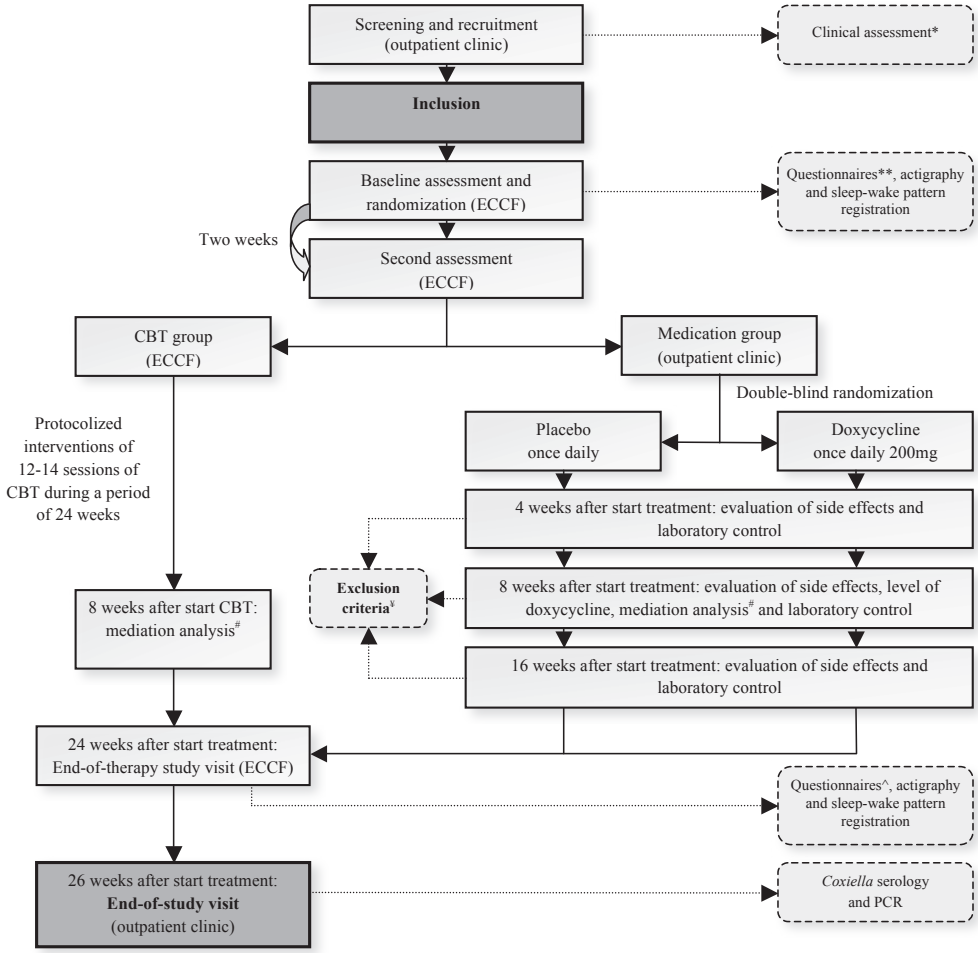
	Exclusion criteria
(1)	Fulfilling criteria for chronic Q fever*
(2)	Acute Q fever in the setting of a prosthetic cardiac valve or aneurysm surgery or stenting, necessitating prophylactic use of doxycycline
(3)	Pregnancy or unwillingness to use effective contraceptives during the entire study period
(4)	Imminent death
(5)	Inability to give informed consent
(6)	Allergy or intolerance to doxycycline
(7)	Somatic or psychiatric illness that could explain the chronic fatigue
(8)	Subjects who are currently enrolled in other investigational drug trials or receiving investigational agents
(9)	Receiving or having received antibiotics for > 4 weeks, potentially active against *Coxiella burnetii*, for any other reason since Q fever diagnosis
(10)	Subjects who are receiving and cannot discontinue barbiturates, phenytoin, or carbamazepine**
(11)	Moderate or severe liver disease (AF, ALT, AST > 3 times the upper limit of normal)
(12)	Current engagement in a legal procedure concerning financial benefits#

* According to the guideline chronic Q fever from the *Dutch Q fever consensus group* [29].
** These drugs may increase the metabolism of doxycycline; consequently, reducing the half-life of doxycycline.
# Temporary exclusion criterion, while current involvement interferes with the effectivity of cognitive behavioral therapy [30]. Once the appeal procedure ends, subjects can be included.
Abbreviations: *AF* = alkaline phosphatase, *ALT* = alanine aminotransferase, *AST* = aspartate aminotransferase.

Baseline assessment

After inclusion, participants will first visit the ECCF for the baseline assessment, including questionnaires and measurement with an actometer (*see figure 1*). An actometer is a motion-sensing device worn at the ankle that registers and quantifies physical activity. The actometer has a piezoelectric sensor that is sensitive in three directions. Accelerations of the built-in sensor larger than a predefined threshold are considered as activity and are stored in an internal memory every 5 minutes. It is worn day and night during a period of twelve consecutive days [31]. A general physical activity score that expressed the mean activity level over this period in the mean number of accelerations per 5 minute interval will be calculated. During the period of twelve days participants rate fatigue, pain, and activity levels on a pre-scheduled Self-Observation List four times daily on a scale of 0 (not at all) to 4 (very much).

Figure 1: Flowchart of trial design.



* According to the Dutch guideline Q fever fatigue syndrome [14]. Including questionnaires: general questionnaire, CIS, SIP total score.

** General questionnaire, PARS, SES28, IMQ, CBRSQ, JFCS, CAL, and SCL90.

Questionnaires used for mediation analysis: PARS, SES28, IMQ, CBRSQ, and CIS.

¥ Exclusion criteria: pregnancy; serious adverse events; AST/ALT >5 times normal value; AF >3 times normal value; >10 days use of quinolon, co-trimoxazol, macroliden or tetracycline; or discontinuation of study medication >7 consecutive days.

^ CIS, PARS, IMQ, JFCS, SIP, SES28, CBRSQ, and SCL90.

Abbreviations: *CIS* = Checklist Individual Strength, *SIP* = Sickness Impact Profile, *ECCF* = Expert Centre for Chronic Fatigue, *PARS* = Physical Activity Rating, *SES28* = Self Efficacy Scale, *IMQ* = Symptom focusing of the illness Management Questionnaire, *CBRSQ* = Cognitive and Behavioral Responses to Symptoms Questionnaire, *JFCS* = Jacobson Fatigue Catastrophising Scale, *CAL* = Causal Attribution List, *SCL90* = Symptom Checklist 90, *CBT* = cognitive behavioral therapy, *AST* = aspartate aminotransferase, *ALT* = alanine aminotransferase, *AF* = alkaline phosphatase.

Randomization procedure and blinding

The randomization order is created by an independent biostatistician using block-randomization. An administrative assistant with no affiliation to the project group made envelopes for individual study codes ranging from 1–180, according to the *Figure 1:* randomization list. At the end of the first visit to the ECCF, participants receive their envelope (which contains a corresponding number coherent to the individual study code) from the psychological assistant, to see to which treatment they are randomized. First, participants will be randomized between CBT and medication (ratio 1:2). Secondly, double-blinded randomization between doxycycline treatment or placebo (ratio 1:1) will be performed within the medication condition by the study pharmacist (department of Clinical Pharmacy, Radboud University Nijmegen Medical Centre). The double-blinded randomization assignment will be known to the study pharmacist only, and is available in a sealed envelope stored at the pharmacist's office for emergency use. If the code is broken, it will render the participant not eligible. The first randomization list and second double-blinded randomization list will be made available respectively by the independent biostatistician and the study pharmacist to the principal investigator when the entire study is completed. Obviously, allocation to the CBT intervention cannot be blinded.

Interventions

Study medication

Preparation and labeling of doxycycline and placebo will be performed by the Clinical Trials Unit department of the Clinical Pharmacy of the Radboud University Nijmegen Medical Centre, and will be done according to the relevant Good Manufacturing Practice (GMP) guidelines. Study medication will be prepared as capsules with identical appearance. Participants allocated to study medication will be treated at the Q fever outpatient clinic. Participants will receive either doxycycline (200 mg once daily) or placebo (once daily), both orally administered, for a period of 24 weeks. Study medication will be provided by the first author (SPK). For safety considerations all participants in the medication condition will visit the Q fever outpatient clinic 4, 8, and 16 weeks after start of the treatment (*see figure 1*). Furthermore, liver enzymes will be checked, and drug utilization will be recorded. Therefore, patients are required to bring the study medication to all visits. In addition, blood samples drawn 8 weeks after start of treatment will be stored by the study pharmacist, who performed the double-blinded randomization. Eventually, doxycycline levels will only be determined in participants receiving doxycycline, and results will be kept secret until the entire study is completed. After completion, it is known whether doxycycline levels were sufficient to sort out effect [32]. Participants will be excluded in case of: serious side effects; aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels more than 5 times the upper limit of normal; alkaline phosphatase (AF) levels more than 3 times the upper limit of normal; more than 10 days use of antibiotics potentially active against *C. burnetii* (co-trimoxazol, quinolon, macrolides or tetracyclines); or discontinuation of study medication for more than 7 consecutive days.

Cognitive behavioral therapy

CBT for QFS is aimed at changing the beliefs and behaviors assumed to maintain fatigue. On average, CBT consists of 12–14 sessions over a period of 24 weeks, and is individually delivered by trained cognitive-behavioral therapists from the ECCF, according to a written treatment manual. The treatment is based on CBT for CFS [33]. First, the model of fatigue perpetuating beliefs and behaviors is explained to patients. At the start of the therapy patients formulate their goals in behavioral terms. These goals usually include the resumption of work, hobbies, and other activities that imply that the patient is no longer severely fatigued and disabled, which is the goal of CBT for QFS. Patients regulate their bedtimes and stop sleeping during the day in order to stop possible disruption of the circadian rhythm. During the sessions, the therapist elicits and challenges patients' non-accepting and catastrophising beliefs with respect to fatigue. Additionally, patients are taught how to distract their attention from their fatigue. Two groups of patients are discerned: relatively active patients, who are characterized by bursts of activity followed by periods of relative inactivity, and low active patients, who have extremely low activity levels on most days [31]. Relatively active patients first learn how to divide their activities more evenly across the day. Low active patients start with a graded activity program immediately after the initial cognitive interventions. This activity program consists of daily walking or cycling, which is gradually increased. The increase in activity is not determined by the level of symptoms, but is time contingent. When patients succeed in increasing their physical activity, they also start to increase their social and mental activities. In the last phase of therapy, patients work systematically towards reaching their goals, which are formulated at the start of the therapy. Following this, they are encouraged to perceive feelings of fatigue as a normal part of an active and healthy life.

Post intervention

Twenty-four weeks after start of treatment, all participants visit the ECCF for the end-of-therapy study visit, including assessment of the outcome measures (*see figure 1*). Twenty-six weeks after start of treatment, participants visit the Q fever outpatient clinic for the end-of-study visit. During this end-of-study visit, *C. burnetii* serology and PCR will be determined.

Outcome measures

The primary outcome measure is the fatigue severity measured by the subscale *fatigue severity* (8 items, 7-point Likert Scale) of the Checklist Individual Strength (CIS questionnaire) [34] with a severity range from 8–56. High scores indicate a high level of fatigue. Patients with a cut-off score of ≥35 are classified as severely fatigued. This questionnaire has excellent psychometric properties, including good reliability and discriminative validity [35, 36].

Secondary outcome measures are:

(1) Level of functional impairment measured with the Sickness Impact Profile (SIP) [37, 38]. The SIP is an instrument that is used to gauge sickness-related dysfunction. The weighted total score on eight sub-scales of the SIP8 (SIP8 total score) will be used to assess functional disability in all domains of functioning. This instrument is reliable with sufficient content validity, and it shows good correlations with other health status and functional status measures [39].

(2) Level of psychological distress measured with the total score of the Symptom Checklist 90 (SCL90). The SCL90 consist of 90 items scored on a five-point scale. Scores range from 90–450. A low total score reflects psychological well-being. The SCL-90 is a reliable and valid instrument [40].
(3) *C. burnetii* serology (immunofluorescence assay; Focus Diagnostics, Inc., Cypress, CA, USA) and serum PCR.

Other study parameters will be: demographic data; data on symptoms, diagnosis and treatment of acute Q fever; previous history; serology results performed before inclusion in the study; use of medication, smoking, and the use of alcohol or drugs; and data on self reported symptoms, disabilities, and behavioral factors.

Mediation analysis

Testing mediation is a strategy to identify variables that intervene in the relationship between treatment and outcome. Mediation analysis can help to better understand how treatment works [41]. To assess a change in variables that might affect fatigue severity, possible mediators and fatigue severity will be assessed at baseline, eight weeks after start of treatment, and at end of therapy in all treatment modalities (*see figure 1*). The proposed mediators are fatigue related cognitions and behaviors. Four instruments will be used to assess the mediators: 1) Subscales 'resting/avoidance', 'all-or-nothing' behavior, and 'catastrophising' of the Cognitive Behavioral Responses to Symptoms Questionnaire (CBRSQ) [42], 2) Subscale focusing on symptoms of the Illness Management Questionnaire (IMQ) [43, 44], 3) Total score on the Physical Activity Rating Scale (PARS, measuring the level of confidence and expectation on fatigue performing 16 different activities, rated on a five-point scale), and 4) Total score on the Self Efficacy Scale (SES28) [45].

Withdrawal of individual participants

Participants are informed that they can stop participating in the study at any time, without consequences. Although participants will be asked for the reason for discontinuation, giving a reason for withdrawal is not obligatory. The investigator can decide to withdraw a participant from the study in case of medical urgency. In addition, study medication will be stopped in case of pregnancy, and the participant will be withdrawn. According to the Intention To Treat (ITT) principle the analysis will be based on the initial treatment intent. Therefore, in case of discontinuation, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation in accordance to the study protocol end-of-therapy study visit will be performed if the withdrawn participant agrees. Because of absence of an evidence-based treatment for QFS, other treatment options for QFS in regular health care for withdrawn participants in the CBT group are not available. Long-term doxycycline treatment is not offered, because of possible (serious) side-effects and a lack of evidence so far.

Adverse events

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the experimental treatment. All adverse

events in the medication condition will be recorded during the pre-scheduled controls at the outpatient clinic, and, if applicable, during the trial if spontaneously reported by the participant. The most frequent side-effects of doxycycline include gastrointestinal complaints, like nausea and diarrhea, and photo-sensibilisation. Other side-effects are rare. The drug should not be given to children and to pregnant women. This RCT involves a non-critical indication for the use of doxycycline, and the drug under investigation is well characterised and commonly used in daily practice. Even though the delivery of CBT to adults is considered safe [46, 47], all adverse events reported spontaneously by the participant or observed by the therapist will be recorded by the psychological assistant at pre-scheduled time-points during the therapy (8 weeks after the start of therapy, and 24 weeks after start of therapy). All adverse events will be followed until they have abated, or until a stable situation has been reached. If applicable, serious adverse events in both groups will be reported according to the principles of Good Clinical Practice (GCP).

Statistical analysis

The primary analysis will be the comparison between the experimental groups (CBT or doxycycline) and the placebo group. ITT will be the basis for all analysis. The primary analysis will be done on the data of completers. Completers are all participants who completed the post intervention measurements. When statistical significant differences are found, a sensitivity analysis will be performed on the basis of different assumptions about the values of missing data. To determine if there is a significant difference between the intervention arm and placebo condition, ANCOVA will be used with the outcome measure on the second assessment as dependent measure, the baseline score as covariate, and condition as fixed factor. A priori contrasts will be defined for the factor condition comparing CBT versus placebo, and comparing doxycycline versus placebo. For the secondary outcome measures, namely psychological distress and functional limitations, the same analysis will be repeated, but with the secondary outcome measures at the second assessment as dependent variable, and the scores at baseline as covariate. In this kind of trials ANCOVA yields greater power than other statistical methods [48]. Statistical significance will be assumed at $p<0.05$ in all analysis. Data will be presented as quantitative results.

Power calculation

The power calculation is based on the estimated maximal number of eligible patients who will be available for the study. In the Netherlands there has been only one major outbreak of Q fever. Since then, the number of new cases is limited. Furthermore, following the outbreak several studies investigating the symptoms following Q fever are ongoing which limits the number of eligible patients that will be available to enter the present study. The maximal number of available patients is estimated to be 180, 60 patients for each arm of the study. We assumed a drop-out rate of 20 percent, leaving a sample size for the power calculation of 50 participants per arm. Compared to a *t*-test, using ANCOVA increases statistical power. The sample size of 50 can be divided by a design factor of 0.884 ($1–0.34^2$), with 0.34 being the correlation between the CIS *fatigue severity* at baseline and second assessment [49]. The required effect size was estimated using G-Power 3.1.5. based on a sample size of

56, a power of 0.80 and an alpha of 0.05. The analysis showed that we need to assume a moderate controlled effect size of 0.53 to obtain a power of 0.8 for demonstrating a significant difference between the results in the treatment groups and in the placebo group.

DISCUSSION

The Qure study will be the first randomized placebo-controlled clinical trial to assess the efficacy of long-term treatment with doxycycline and CBT in adult patients with QFS. A limited amount of previous uncontrolled studies suggest a positive effect of long-term treatment with a tetracycline on performance state. The result of one study shows improvement in symptoms, including fatigue, in all patients after 3 months of treatment. However, not all patients met the current criteria for QFS, whereas 7 patients were PCR positive, meeting the current criteria for chronic Q fever [20]. Furthermore, patients were included with complaints lasting for only 3 months, whereas chances for spontaneous recovery are high in the first 6 months after the initial infection. The other study, primarily focussing on the role of *C. burnetii* in CFS, reports improvement in performance status, a decreased mean headache score, and a decrease in mean weekly temperature after treatment [21]. However, of the 54 patients included, 34 patients were PCR positive at baseline, suggesting chronic Q fever. Furthermore, patients were included with complaints lasting for only 1 month. Therefore, these results cannot be extrapolated, and this long-term treatment is currently not often used in clinical care of patients with QFS. Furthermore, the efficacy of CBT in patients with QFS has not been evaluated in a randomized design. Currently, the decision whether or not to treat is made arbitrarily, as evidence-based strategies are lacking. The Dutch outbreak offers us a great and maybe the only opportunity to conduct research on the best treatment of QFS.

In conclusion, the Qure study will provide greater insight into effectiveness of treatment options for adult patients with QFS. If an effective treatment modality for QFS will be found, significant benefit can be achieved in quality of life, efficiency in treatment and cost-effectiveness. Furthermore, this study will possibly contribute to the establishment of evidence-based guidelines for the treatment of QFS.

AUTHORS' CONTRIBUTIONS

SPK participated in the design of the study and is responsible for data collection and analysis, and for drafting the manuscript. CED participated in the design of the study as an expert on infectious diseases, and will supervise the study and data collection. TS participated in the design of the study as an expert on infectious diseases. GB participated in the design of the study as an expert on chronic fatigue, helped to coordinate and supervise the study, and will be responsible for the logistics surrounding cognitive behavioral therapy. JvdM participated in the design of the study as an expert of infectious diseases and chronic fatigue, and helped to coordinate and supervise the study. HK participated in the design of the study as an expert on chronic fatigue, and will be responsible for the logistics surrounding cognitive behavioral therapy. CPBR initiated and participated in the design of the study as an expert on infectious diseases, obtained funding for the study, and will coordinate and supervise the study and data collection. All authors revised the draft manuscript and approved the final manuscript.

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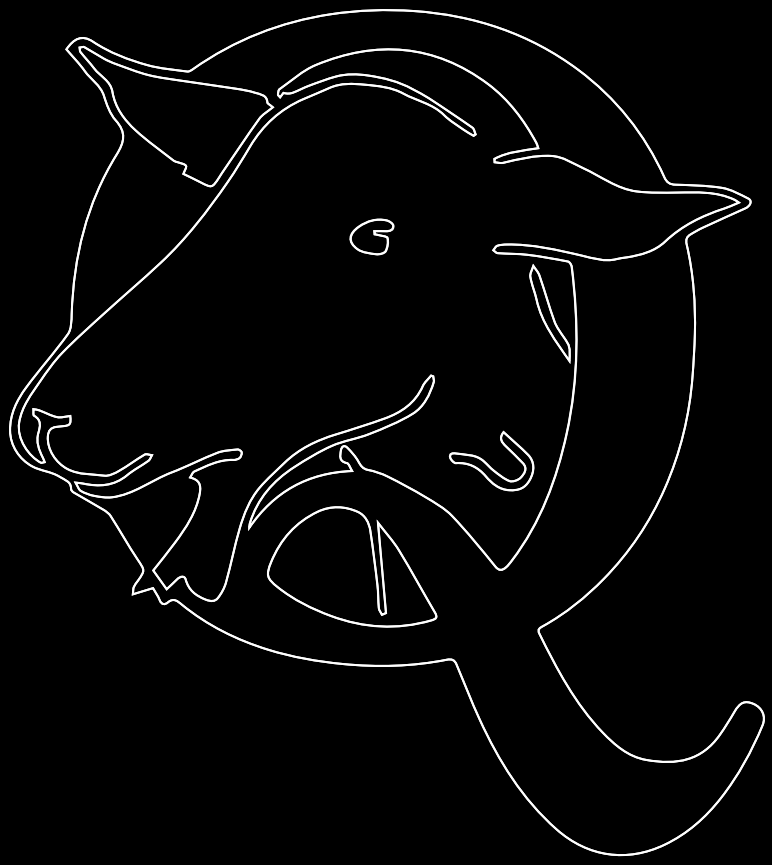

CHAPTER 6

EFFECTIVENESS OF LONG-TERM DOXYCYCLINE TREATMENT AND COGNITIVE BEHAVIORAL THERAPY ON FATIGUE SEVERITY IN PATIENTS WITH Q FEVER FATIGUE SYNDROME (QURE STUDY); A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

Background: Approximately 20% of patients with acute Q fever will develop chronic fatigue, referred to as Q fever fatigue syndrome (QFS). The objective of this randomized controlled clinical trial was to assess the efficacy of either long-term treatment with doxycycline or cognitive-behavioral therapy (CBT) in reducing fatigue severity in patients with QFS.

Methods: Adult patients were included who met the QFS criteria according to the Dutch guideline: a new onset of severe fatigue lasting ≥6 months with significant disabilities, related to an acute Q fever infection, without other somatic or psychiatric comorbidity explaining the fatigue. Using block randomization, patients were randomized between oral study medication and CBT (2:1) for 24 weeks. Second, a double-blind randomization between doxycycline (200 mg/day, once daily) and placebo was performed in the medication group. Primary outcome was fatigue severity at end of treatment (EOT; week 26), assessed with the Checklist Individual Strength subscale Fatigue Severity.

Results: Of 155 patients randomized, 154 were included in the intention-to-treat analysis (doxycycline, 52; placebo, 52; CBT, 50). At EOT, fatigue severity was similar between doxycycline (40.8 [95% confidence interval {CI}, 37.3–44.3]) and placebo (37.8 [95% CI, 34.3–41.2]; difference, doxycycline vs placebo, −3.0 [97.5% CI, −8.7 to 2.6]; P = .45). Fatigue severity was significantly lower after CBT (31.6 [95% CI, 28.0–35.1]) than after placebo (difference, CBT vs placebo, 6.2 [97.5% CI, .5–11.9]; P = .03).

Conclusions: CBT is effective in reducing fatigue severity in QFS patients. Long-term treatment with doxycycline does not reduce fatigue severity in QFS patients compared to placebo.

Clinical Trials Registration: NCT01318356, and Netherlands Trial Register: NTR2797.

INTRODUCTION

Q fever, caused by the gram-negative intracellular coccobacillus *Coxiella burnetii*, is notorious for long-term sequelae. Besides chronic Q fever (ie, persistent *C. burnetii* infection), which occurs in 1%–5% of cases [1], a debilitating fatigue syndrome has been described [2–11]. This Q fever fatigue syndrome (QFS) persists for years in approximately 20% of cases following acute Q fever [2–6, 9–11]. Many QFS patients fulfill the case definition of chronic fatigue syndrome (CFS) [2, 8, 10, 12]. QFS has major health impacts with severe fatigue, substantial disabilities, and reduced quality of life [8, 11, 13–15]. Following the largest Q fever outbreak ever reported [1], which occurred in the Netherlands with >4000 notified patients, the need for an evidence-based treatment regimen increased. The large number of QFS patients had major economical consequences [16]. The pathophysiology of QFS remains to be elucidated, hampering treatment based on etiology.

Long-term treatment with tetracyclines has been reported to improve performance status and reduce fatigue in QFS [4, 17], but subsequent reports have been conflicting [5, 18]. No randomized controlled trials (RCTs) have been performed, and available studies all have major limitations, precluding extrapolation of these results. Cognitive-behavioral therapy (CBT), aimed at fatigue-related cognitions and behavior thought to perpetuate symptoms, can reduce symptoms and improve functioning in CFS [19]. A considerable overlap in fatigue-perpetuating factors between QFS and CFS implies that CBT might also reduce fatigue severity in QFS [12].

We performed an RCT (the Qure study) to assess the efficacy of long-term treatment with either doxycycline or CBT in patients with QFS.

METHODS

Study Design, Setting, and Participants

The trial was approved by the Medical Ethical Review Committee region Arnhem-Nijmegen (2011/069, NL35755.091.11) and conducted in compliance with the most recent provisions of the Declaration of Helsinki, the International Conference on Harmonisation guidelines on Good Clinical Practice, and appropriate regulatory requirements. The trial was performed at 2 sites of the Radboud university medical center (Radboudumc): the Radboud Expertise Center for Q fever and the Expert Center for Chronic Fatigue (ECCF). The study protocol has been published [20]. This trial was overseen by an independent monitor.

All men and nonpregnant, nonlactating women, aged ≥18 years suspected of Q fever-related fatigue were screened for QFS, using standard clinical and laboratory protocols. Eligibility was assessed according to previously described inclusion and exclusion criteria (*Supplementary Table 1*) [20]. QFS was defined as severe fatigue (score ≥35 on the Checklist Individual Strength [CIS] subscale Fatigue Severity) for ≥6 months, causing significant disabilities (score ≥450 on the Sickness Impact Profile [SIP8]) in daily functioning, not being caused by chronic Q fever or other somatic or psychiatric morbidity, directly related to an acute Q fever infection, and the fatigue should have been either absent before or have significantly increased since the acute Q fever infection. Chronic Q fever was excluded based on negative serum polymerase chain reaction (PCR), Q fever serology (immunoglobulin G phase I titers <1:1024), and absence of signs of endocarditis or vascular infection.All enrolled patients provided written informed consent.

Randomization and Blinding

Patients were randomly assigned to receive either study medication or CBT (2:1 ratio). Second, a double-blind randomization was performed within the medication group, allocating patients to doxycycline or placebo (1:1 ratio). The randomization sequence was computer-generated using block randomization, performed by an independent biostatistician. Allocation concealment was achieved by sealed opaque envelopes with individual codes according to the randomization list, made by an administrative assistant with no affiliation to the project group. The double-blind randomization within the medication condition was performed by the pharmacist. The first randomization list and the double-blind randomization list were made available by the independent biostatistician and the study pharmacist, respectively, to the principal investigator after completion of the study. All trial-related personnel, except the study pharmacist, and participants were masked with regard to the medication group. Allocation to CBT was not blinded.

Interventions

Patients in the medication group were treated with doxycycline 200 mg or placebo, both orally administered once daily, for 24 weeks. Study medication was prepared and labeled by the Clinical Trials Unit department of the Clinical Pharmacy of Radboudumc, according to Good Manufacturing Practice guidelines. Doxycycline was reencapsulated and placebo was prepared as capsules with identical appearance. Study visits were at 4, 8, and 16 weeks after start of treatment, including medical history, physical examination, and laboratory investigation. Patients were excluded if they met the exclusion criteria during treatment with medication (*Supplementary Table 2*) [20]. Compliance was verified by pill counting. Patients allocated to CBT received approximately 24 weeks of individual CBT, based on the manual of CBT for CFS [20, 21], by trained and supervised cognitive-behavioral therapists [20]. Treatment frequency was determined on individual basis, with intended sessions once every 2 weeks. Details of the assessments per visit have been published [20].

Outcomes

Outcomes were assessed by self-completed questionnaires and laboratory investigation at baseline, 26 weeks (end of treatment period [EOT]), and 28 weeks (end of study [EOS]). The primary outcome measure was fatigue severity at EOT, measured by the CIS subscale Fatigue Severity [22], with a cutoff score of ≥35 as classification for severe fatigue. Clinical meaningful improvement, taking into account whether the magnitude of change on the CIS subscale Fatigue Severity is clinically relevant, was defined as a reliable change index (RCI) × 1.96 plus a CIS Fatigue Severity score of <35 [23]. The RCI was calculated based on the standard deviation of the baseline CIS fatigue score with 0.88 as reliability factor [22]. Secondary outcomes were level of functional impairment at EOT, measured with weighted total score on 8 subscales of the SIP8 with a cutoff score of ≥450 indicating significant disabilities [24], the level of psychological distress at EOT, measured with the total score of the Symptom Checklist 90 (SCL-90) with a low total score reflecting psychological well-being [25], and *C. burnetii* serology (immunofluorescence assay; Focus Diagnostics, Cypress, California) and serum PCR (in-house, real-time PCR directed against insertion sequence IS1111a) at EOS.

Adverse Events

Safety was assessed by monitoring adverse events (AEs) and concomitant drug use. AEs in the medication condition were recorded during the prescheduled study visits, and, if applicable, during the trial when reported by the patient. For patients allocated to CBT, AEs were monitored at 8 weeks after start of therapy and at EOT.

Statistical Analysis

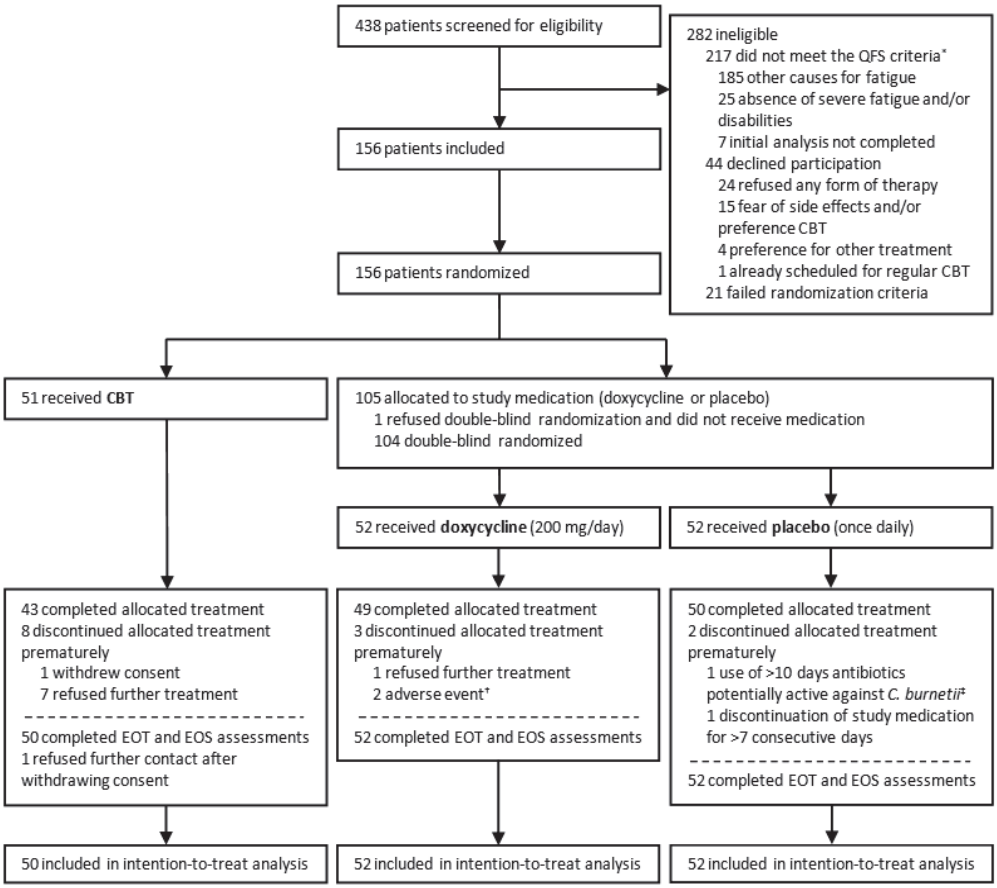
Following the Dutch Q fever outbreak, the number of new cases decreased drastically and several studies concurrently investigated health-related aspects following acute Q fever, limiting the number of eligible patients. Because there were only a limited number of patients available for participation, a traditional power analysis was not possible. Instead, we performed an analysis to estimate the effect size that has to be assumed for a power of 80%. The maximum number of available patients was estimated as 180 (60 patients per arm). Assuming a 20% dropout rate, this left a sample size of 50 patients per arm. This sample size was divided by a design factor of 0.884 (1–0.342), with 0.34 being the correlation between fatigue severity at baseline and EOT [26], leaving a sample size of 56. Using G*Power software (version 3.1.5) based on a sample size of 56, a power of 0.80, and an α of .05, a moderate effect size of 0.53 needed to be assumed to obtain a power of 0.8 for demonstrating a significant difference.

Primary analyses were performed on the data of all participants who completed the postintervention measurements, irrespective of whether or not they completed the treatment: intention-to-treat was the basis for all analyses. In the primary analysis, each of the experimental groups (doxycycline and CBT) was compared to the placebo group at EOT using analysis of covariance with the EOT CIS fatigue score as dependent measure, baseline CIS fatigue score as covariate, and the condition as fixed factor. For the secondary outcome measures, the same analysis was repeated but with the EOT secondary outcome measures as dependent variable and scores at baseline as covariate. No interim analyses were undertaken. Two-sided 5% significance levels were used. Because primary and secondary analyses entailed 2 separate hypotheses, Bonferroni correction was used, which means that reported P values are twice the P values found in the analyses. Also, when reporting estimated effects, 97.5% confidence intervals (CIs) were used. Statistical analyses were performed blinded for group allocation, using SPSS version 22 and SAS version 9.2 software.

RESULTS

Figure 1 shows the trial profile. In total, 438 patients with suspected QFS were screened for eligibility. The most prevalent reason for ineligibility was another cause for the fatigue. Of the 221 patients meeting the QFS criteria, 21 were not eligible for study participation and 44 refused participation (22%). Between May 2011 and January 2015, 156 patients signed informed consent and were randomized; of these, 155 started treatment, either doxycycline (n = 52), placebo (n = 52), or CBT (n = 51). One patient refused double-blind randomization after allocation to the medication group, and received no treatment. There were no significant baseline differences between the treatment groups (*Table 1*; *Supplementary Table 3*). The intention-to-treat analysis included 154 patients. There was a median of 1.0 pill left at EOT in

Figure 1. Trial profile. Primary analyses were based on intention-to-treat and included the data of all patients who completed the end of treatment (EOT) and end of study (EOS) assessments. *As described in the study protocol [20], including a cutoff score of ≥35 on the Checklist Individual Strength subscale Fatigue Severity, and a cutoff score of ≥450 on the Sickness Impact Profile 8 total score to classify severe fatigue and substantial fatigue-related disabilities. †Leading to discontinuation of study medication for >7 consecutive days. ‡Use of ciprofloxacin of 14 days because of prostatitis.



Abbreviations: CBT, cognitive-behavioral therapy; EOS, end of study; EOT, end of treatment; QFS, Q fever fatigue syndrome; SIP8, Sickness Impact Profile.

both the doxycycline and placebo groups. In the CBT group, patients received a median of 9 sessions (interquartile range, 7.50–11.25). Treatment was completed by 142 patients (92%): doxycycline, 49 (94%); placebo, 50 (96%); and CBT, 43 (84%). During CBT, 1 patient withdrew informed consent, and the other 7 patients discontinued treatment because they could not adhere to the therapy for various reasons.

Table 1. Baseline Characteristics of All Included Patients with Q Fever Fatigue Syndrome[a]

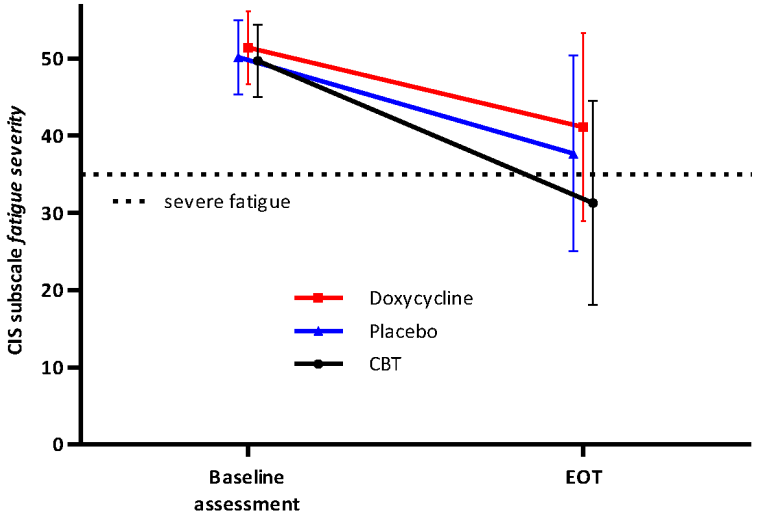
Characteristic	Doxycycline (n=52)	Placebo (n=52)	CBT (n=51)
Female sex, No. (%)	29 (56)	20 (38)	25 (49)
Age, y, mean ± SD	43.6 ± 10.2	44.6 ± 12.3	43.3 ± 13.7
Duration of symptoms, mo			
Median	36.00	37.50	40.00
Interquartile range	24.25 – 57.00	25.50 – 50.75	22.00 – 59.00
CIS subscale Fatigue Severity, mean ± SD	51.4 ± 4.7	50.2 ± 4.8	49.7 ± 4.7
SIP8 total score, mean ± SD	1304.9 ± 537.7	1295.1 ± 593.7	1369.4 ± 646.7
SCL-90 total score, mean ± SD	152.2 ± 31.4	159.1 ± 41.0	156.4 ± 35.0
IFA, No. (%)			
IgM phase I	24 (46)	28 (54)	25 (49)
IgM phase II	30 (58)	32 (62)	32 (63)
IgG phase I	45 (87)	42 (81)	40 (78)
IgG phase II	52 (100)	50 (96)	49 (96)
Negative *Coxiella burnetii* PCR, No. (%)	52 (100)	52 (100)	51 (100)

[a]Between-group differences in primary and secondary outcome characteristics at baseline were analyzed with analysis of variance for continuous variables.
Abbreviations: *CBT*, cognitive-behavioral therapy; *CIS*, Checklist Individual Strength; *IFA*, immunofluorescence assay; *IgG*, immunoglobulin G; *IgM*, immunoglobulin M; *PCR*, polymerase chain reaction; *SCL-90*, Symptom Checklist 90; *SD*, standard deviation; *SIP8*, Sickness Impact Profile.

Primary Endpoint

The primary endpoint in the intention-to-treat analysis, fatigue severity at EOT adjusted for baseline fatigue severity, did not significantly differ between doxycycline (40.8 [95% CI, 37.3– 44.3]) and placebo (37.8 [95% CI, 34.3–41.2]; difference, doxycycline vs placebo, −3.0 [97.5% CI, −8.7 to 2.6]; P = .45), and was significantly lower after CBT (31.6 [95% CI, 28.0–35.1]) than after placebo (difference, CBT vs placebo, 6.2 [97.5% CI, .5–11.9]; P = .03) (*Table 2*; *Figure 2*). Clinically meaningful improvement, that is, a reduction of 9 points on the CIS subscale Fatigue Severity plus a score of <35, was reached by 44% of patients: doxycycline, 31%; placebo, 46%; CBT, 56% (P = .04; *Supplementary Table 4*).

Figure 2. Mean fatigue severity and standard deviation per treatment group at baseline and at end of treatment (EOT), 26 weeks, measured with the Checklist Individual Strength subscale Fatigue Severity with a severity range from 8 to 56. Higher scores indicate a higher level of fatigue. Patients with a cutoff score of ≥35 are classified as severely fatigued.



Abbreviations: CBT, cognitive-behavioral therapy; CIS, Checklist Individual Strength; EOT, end of treatment.

Secondary Endpoints

At EOT, the mean SIP8 total score did not differ significantly between either doxycycline and placebo (difference, doxycycline vs placebo, −137.7 [97.5% CI, −409.9 to 134.6]; P = .51) or CBT and placebo (difference, CBT vs placebo, 177.0 [97.5% CI, −98.3 to 452.3]; P = .30). Doxycycline yielded no difference in SCL-90 total score compared with placebo (difference, doxycycline vs placebo, −6.5 [97.5% CI, −18.7 to 5.7]; P = .45), whereas the SCL-90 total score significantly improved after CBT compared with placebo (difference, CBT vs placebo, 15.6 [97.5% CI, 3.3–27.8]; P = .010). At EOS, the majority of patients had stable or declining antibody titers compared to baseline, and the number of patients with declining titers was similar in all groups (*Supplementary Tables 3 and 5*). *Coxiella burnetii* PCR remained negative in all patients.

Adverse Events

Overall, 138 (90%) patients reported at least 1 AE, and 2 (1%) AEs of gastrointestinal origin led to study discontinuation, both in the doxycycline group. In the doxycycline group, both the total number of AEs and the median number of AEs per patients were highest, and fewer patients reported no AEs (*Supplementary Table 6*). No serious adverse events (SAEs) occurred during treatment with doxycycline. Two SAEs were reported in the placebo group. One patient who had not yet started treatment was admitted to hospital with urosepsis. The other patient was admitted for clinical evaluation of preexisting cardiological symptoms,

Table 2. Treatment Effect on Primary and Secondary Endpoints for Patients Included in the Intention-to-Treat Analysis[a]

Outcome	Doxycycline (n=52), Mean (95% CI)	Placebo (n=52), Mean (95% CI)	CBT (n=50), Mean (95% CI)	Dox vs Placebo, P value[b]	Dox vs Placebo, Difference (97.5% CI)	Dox vs Placebo, Standardized Effect Size[c]	CBT vs Placebo, P value[b]	CBT vs Placebo, Difference (97.5% CI)	CBT vs Placebo, Standardized Effect Size[c]
Primary endpoint									
CIS subscale *fatigue severity*	40.8 (37.3 - 44.3)	37.8 (34.3 - 41.2)	31.6 (28.0 - 35.1)	.45	-3.0 (-8.7 - 2.6)	.24	.03	6.2 (0.5 - 11.9)	.49
Secondary endpoints: questionnaires									
SIP8 total score	1101.5 (933.5 - 1269.6)	963.8 (795.8 - 1131.9)	786.8 (615.3 - 958.3)	.51	-137.7 (-409.9 - 134.6)	.20	.30	177.0 (-98.3 - 452.3)	.26
SCL-90 total score	149.2 (141.6 - 156.7)	142.6 (135.1 - 150.1)	127.1 (119.4 - 134.7)	.45	-6.5 (-18.7 - 5.7)	.18	.01	15.6 (3.3 - 27.8)	.43
Secondary endpoints: serology and PCR, No. (%)									
IFA									
IgM phase I	24 (46)	28 (54)	20 (40)	.68	NA	NA	.36	NA	NA
IgM phase II	27 (52)	32 (62)	29 (58)	1.0	NA	NA	1.0	NA	NA
IgG phase I	43 (83)	39 (75)	37 (74)	.87	NA	NA	1.0	NA	NA
IgG phase II	51 (98)	50 (96)	46 (92)	.33	NA	NA	.36	NA	NA
Negative *C. burnetii* PCR	52 (100)	52 (100)	50 (100)	NA	NA	NA	NA	NA	NA

[a]*P* values were based on analysis of covariance. All scores are adjusted for baseline.
[b]Pairwise comparisons between treatment arms with Bonferroni correction.
[c]Standardized effect sizes are computed as difference scores divided by the pooled standard deviation of the postmeasurements.
Abbreviations: *CBT*, cognitive-behavioral therapy; *C. burnetii, Coxiella burnetii*; *CI*, confidence interval; *CIS*, Checklist Individual Strength; *Dox*, doxycycline; *IFA*, immunofluorescence assay; *IgG*, immunoglobulin G; *IgM*, immunoglobulin M; *NA*, not applicable; *PCR*, polymerase chain reaction; *SCL-90*, Symptom Checklist 90; *SIP8*, Sickness Impact Profile.

which yielded no diagnosis. In the CBT group, 42 (84%) patients reported at least 1 AE. No SAE occurred during CBT treatment.

DISCUSSION

In this RCT in QFS patients, long-term treatment with doxycycline was associated with a reduction in fatigue severity compared to baseline, but no more than with placebo, whereas CBT proved to be effective in reducing fatigue severity and the level of psychological distress compared to placebo. None of the treatment regimens showed a significant effect on functional impairment. Significantly more QFS patients showed a clinically meaningful improvement in fatigue following CBT.

This study is the first RCT evaluating both long-term treatment with doxycycline and CBT in QFS patients. The finding that long-term treatment with doxycycline was no more effective than placebo was contrary to previously published results [4, 17]. Both Arashima et al [4] and Iwakami et al [17] reported clinical improvement in QFS patients who received tetracycline treatment for 3 months. In the former uncontrolled open-label study [4], 20 patients were treated with minocycline 200 mg/ day (n = 18), levofloxacin 200 mg/day, or erythromycin 400 mg/ day. In the latter pilot study [17], 58 patients (54 with assumed QFS) received minocycline 100 mg/day (n = 29), doxycycline 100 mg/day (n = 26), or levofloxacin 200 mg/ day (n = 3). However, both studies lacked a clear description of the criteria for QFS, and included patients who were *C. burnetii* PCR positive at baseline, indicating chronic Q fever; such patients might benefit from antibiotic treatment because of persistent infection. In our study, patients with a possible persistent (chronic) Q fever infection—based on clinical signs, serology, and PCR results—were not included. Furthermore, both previous studies included patients with a symptom duration of 1–4 months, whereas it is known that the percentage of patients experiencing severe fatigue decreases in the first months following acute Q fever while only a subset of patients will experience persistent fatigue [9, 11]. In contrast to these positive studies, in a case series of QFS patients [5] and in a case report [18], long-term treatment with a tetracycline showed inconsistent results. This study with a longer duration of antibiotic administration does not support long-term treatment with doxycycline for QFS, and such treatment should not be advised. These results will hopefully prevent discussions on the value of long-term antibiotic treatment for QFS and prevent patients from unnecessary prolonged antimicrobial therapy. This has already been seen in the treatment of prolonged symptoms attributed to Lyme disease, which eventually also proved ineffective [27]. In addition, most AEs occurred in the doxycycline group, including the highest median number of AEs per patient. In contrast to doxycycline, 2 SAEs were noticed in the placebo group; none of these were drug related. In this study, the observed placebo effect is remarkably high. This can be explained by the regular follow-up visits during the treatment course, which included standard advice on how to manage chronic fatigue (eg, regulation of bedtimes, quitting sleeping during the day, and maintaining mental and physical activities as much as possible). For several years no standard care was available for QFS patients, and this study, the initiation of which was partly patient-driven, provided support for patients.

CBT had significantly better results than placebo in all but 1 of the secondary outcomes.

In addition, the positive effect of CBT on fatigue severity was also clinically relevant. CBT is effective in reducing symptoms and improving functioning in CFS patients [19] and in chronic fatigue in chronic illnesses [28–30]. CBT is a complex intervention, encompassing a stepwise increase in physical activity and challenging dysfunctional fatigue-related beliefs. A change in beliefs about fatigue and the ability to become active seems to mediate the positive effects in CBT for CFS [31]. Previously, an overlap in fatigue-related and cognitive-behavioral variables between QFS and CFS was found, but the relationship between perpetuating factors and fatigue as is found in CFS could not be confirmed in QFS [12]. Although CBT proved effective in reducing fatigue and psychological distress in QFS patients as well, it remains unclear whether the process of change during CBT in QFS is similar to that in CFS [31]. Different processes involved in the perpetuation of disabilities might explain the absence of effect of CBT on functional impairment, for which CBT for CFS has proven efficacy [32–34]. However, this might also be due to the inclusion of patients with moderate levels of overall impairment (SIP8 total score ≥450) [32–34] and, thus, less opportunity for improvement. The mean number of AEs per patient was lowest in the CBT group, and no SAE occurred in this group. Therefore, patients need not be concerned about safety if CBT is performed by qualified and trained therapists [35].

The effectiveness of CBT does not imply that the cause of QFS is psychological. Several hypotheses regarding the etiology of QFS exist, varying from a biopsychological etiology with *C. burnetii* acting as trigger for fatigue development [6] and the determination of symptoms by host and genetic factors [36], to cytokine dysregulation, supported by low levels of *C. burnetii* DNA found in bone marrow aspirates, thin-needle liver biopsies, and blood mononuclear cells [37–39]. In addition, it should be noted that prevalence of chronic fatigue differs between studies in different countries [40]. Although this could be due to a real difference in prevalence, this could also be explained by different research methods. Nevertheless, further research into the etiology is necessary.

The present findings are strengthened by the high therapy compliance in all groups and low number of dropouts and missing data. This study also has limitations. It was not designed to compare doxycycline and CBT directly, due to the limited number of available patients. However, as the EOT scores in the doxycycline group were similar to placebo, with even higher mean scores, the results imply a favorable effect of CBT. As masking for CBT was not possible, this trial was partly blinded. CBT was directly compared to placebo plus usual care, which might explain some of the differences observed as patients in the CBT group clearly know they are being treated. Due to the maximum number of available patients, it was not possible to include a control group without any form of treatment. Finally, it is unclear whether the detected effects will be sustained over time. To evaluate the long-term beneficial effects of CBT, as has been shown for CBT for CFS [41], patients are currently surveyed by poststudy questionnaires 12–15 months posttreatment. Furthermore, a mediation analysis is planned to identify cognitive and behavioral variables that mediate the positive effect of CBT on fatigue in QFS.

In conclusion, CBT is effective in reducing fatigue severity and the level of psychological distress in QFS patients. Longterm treatment with doxycycline does not significantly reduce fatigue severity in QFS patients and should not be advised.

AUTHORS' CONTRIBUTIONS

C. P. B.-R. initiated the study and obtained funding in collaboration with C. E. D., G. B., H. K., and T. S. S. P. K., C. E. D., G. B., J. W. M. vdM., H. K., and C. P. B.-R. designed the study, in collaboration with T. S. and M. H. N.-F. G. B., J. W. M. vdM., H. K., C. E. D., and C. P. B.-R. helped to coordinate and supervise the study. C. E. D. and C. P. B.-R. were responsible for the daily supervision. M. L., L. M. K., and M. vdB. provided their intellectual contribution during the conduct of the study, increased the awareness of the study, and subsequently increased referral and enrollment of patients. G. B. and H. K. were responsible for the logistics surrounding CBT. M. H. N.-F. was responsible for the microbiological assessments. R. T. D. performed the statistical analyses. S. P. K. was responsible for the study conduct, data collection, and analysis. S. P. K. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This report was mainly written by S. P. K., and was critically reviewed and subsequently approved by all authors.

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SUPPLEMENTARY DATA

Effectiveness of long-term doxycycline treatment and cognitive behavioral therapy on fatigue severity in patients with Q fever fatigue syndrome (Qure study); a randomized controlled trial

Table of Contents

Supplementary Table 1. Inclusion and Exclusion Criteria

Inclusion criteria*	
1.	Males or non-pregnant, non-lactating females who are ≥18 years
2.	Laboratory-proven acute Q fever since the year 2007 and/or positive serology fitting a past infection with *C. burnetii*
3.	AND being severely fatigued, defined by scoring ≥35 on the CIS subscale *fatigue severity*
4.	AND being fatigued for ≥6 months
5.	AND being disabled because of the fatigue, defined by scoring ≥450 on the SIP8
6.	Subjects must sign a written informed consent form
Exclusion criteria	
1.	Fulfilling criteria for chronic Q fever†
2.	Acute Q fever in the setting of a prosthetic cardiac valve or aneurysm surgery or stenting, necessitating prophylactic use of doxycycline
3.	Pregnancy or unwillingness to use effective contraceptives during the entire study period
4.	Imminent death
5.	Inability to give informed consent
6.	Allergy or intolerance to doxycycline
7.	Somatic or psychiatric illness that could explain the chronic fatigue
8.	Subjects who are currently enrolled in other investigational drug trials or receiving investigational agents
9.	Receiving or having received antibiotics for >4 weeks, potentially active against *C. burnetii*, for any other reason since Q fever diagnosis
10.	Subjects who are receiving and cannot discontinue barbiturates, phenytoin, or carbamazepine‡
11.	Moderate or severe liver disease (ALP, ALT, AST >3 times the upper limit of normal)
12.	Current engagement in a legal procedure concerning financial benefits§

*In addition to the inclusion criteria, the fatigue needed to be directly related to an acute Q fever infection, and should be either absent before or significantly increased since the acute Q fever infection.

†Chronic Q fever was excluded with a negative serum PCR, or an IgG phase I <1:1024, in combination with the absence of clinical signs of endocarditis or vascular infection (including both vascular prosthesis and mycotic aneurysms).

‡These drugs may increase the metabolism of doxycycline; consequently, reducing the half-life of doxycycline.

§Temporary exclusion criterion, as current involvement interferes with the effectiveness of cognitive-behavioral therapy [1]. Once the appeal procedure ends, patients can be included.

Abbreviations: *CIS*, Checklist Individual Strength; *SIP8*, Sickness Impact Profile; *ALP*, alkaline phosphatase; *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase.

Supplementary Table 2. Key Exclusion Criteria During Treatment with Medication

Exclusion criteria
1. Pregnancy
2. Serious side effects
3. >10 days use of other antibiotics potentially active against *C. burnetii*[*]
4. Discontinuation of study medication for >7 consecutive days
5. Moderate or severe liver disease, defined as ALT or AST >5 times, and ALP >3 times the upper limit of normal

[*]Quinolon, co-trimoxazol, macrolide or tetracycline.
Abbreviations: *ALT*, alanine aminotransferase; *AST*, aspartate aminotransferase; *ALP*, alkaline phosphatase.

Supplementary Table 3. IFA at Baseline of Patients Included in the Intention-To-Treat Analysis[*]

Characteristic	**Total (n=154)**	**Doxycycline (n=52)**	**Placebo (n=52)**	**CBT (n=50)**
IFA, No. (%)				
IgM phase I	77 (50)	24 (46)	28 (54)	25 (50)
1:16	23 (15)	8 (15)	8 (15)	7 (14)
1:32	23 (15)	7 (13)	8 (15)	8 (16)
1:64	18 (12)	7 (13)	3 (6)	8 (16)
1:128	8 (5)	1 (2)	5 (10)	2 (4)
1:256	4 (3)	1 (2)	3 (6)	0 (0)
1:512	1 (1)	0 (0)	1 (2)	0 (0)
IgM phase II	93 (60)	30 (58)	32 (62)	31 (62)
1:16	21 (14)	11 (21)	7 (13)	3 (6)
1:32	24 (16)	10 (19)	8 (15)	6 (12)
1:64	18 (12)	6 (12)	3 (6)	9 (18)
1:128	12 (8)	1 (2)	6 (12)	5 (10)
1:256	12 (8)	1 (2)	6 (12)	5 (10)
1:512	4 (3)	0 (0)	2 (4)	2 (4)
1:1024	2 (1)	1 (2)	0 (0)	1 (2)
IgG phase I	126 (82)	45 (87)	42 (81)	39 (78)
1:16	18 (12)	10 (19)	2 (4)	6 (12)
1:32	25 (16)	11 (21)	9 (17)	5 (10)
1:64	30 (19)	9 (17)	10 (19)	11 (22)
1:128	28 (18)	6 (12)	13 (25)	9 (18)
1:256	18 (12)	7 (13)	6 (12)	5 (10)
1:512	7 (5)	2 (4)	2 (4)	3 (6)
IgG phase II	150 (97)	52 (100)	50 (96)	48 (96)
1:16	9 (6)	2 (4)	3 (6)	4 (8)
1:32	10 (6)	4 (8)	2 (4)	4 (8)
1:64	23 (15)	9 (17)	10 (19)	4 (8)
1:128	30 (19)	16 (31)	5 (10)	9 (18)
1:256	34 (22)	10 (19)	12 (23)	12 (24)
1:512	34 (22)	8 (15)	13 (25)	13 (26)
1:1024	7 (5)	2 (4)	3 (6)	2 (4)
1:2048	3 (2)	1 (2)	2 (4)	0 (0)

[*]Focus Diagnostics, Inc., Cypress, CA, USA, detecting IgM and IgG antibodies against phase I- and phase II-antigens, with a titer of >1:16 being considered positive.
Abbreviations: *CBT*, cognitive-behavioral therapy; *IFA*, immunofluorescence assay.

Supplementary Table 4. Clinical Meaningful Improvement at End Of Treatment of Patients Included in the Intention-To-Treat Analysis

	Doxycycline (n=52)	Placebo (n=52)	CBT (n=50)	*P* value*
Clinical meaningful improvement				
CIS subscale Fatigue Severity <35	16 (31%)	24 (46%)	29 (58%)	0.02
CIS subscale Fatigue Severity <35 and a minimal drop of nine points†	16 (31%)	24 (46%)	28 (56%)	0.03

**P* values were based on the Chi-square test for comparison of the three groups.
†Taking into account whether the magnitude of change is clinically relevant, defined as: reliable change index (RCI) * 1.96 surplus a CIS fatigue severity score of <35 [2]. The mean SD baseline CIS fatigue was 4.87, and with 0.88 as reliability factor [3], the RCI was 4.28. This score is multiplied with 1.96 (= 8.40), and means a minimal drop of nine points on the CIS subscale Fatigue Severity.
Abbreviations: *CBT*, cognitive-behavioral therapy; *CIS*, Checklist Individual Strength questionnaire; *RCI*, reliable change index.

Supplementary Table 5. IFA at End Of Study of Patients Included in the Intention-To-Treat Analysis*

Characteristic	Total (n=154)	Doxycycline (n=52)	Placebo (n=52)	CBT (n=50)
IFA, No. (%)				
IgM phase I	72 (47)	24 (46)	28 (54)	20 (40)
1:16	36 (23)	15 (29)	12 (23)	9 (18)
1:32	15 (10)	5 (10)	5 (10)	5 (10)
1:64	15 (10)	3 (6)	7 (13)	5 (10)
1:128	4 (3)	0 (0)	3 (6)	1 (2)
1:256	1 (1)	1 (2)	0 (0)	0 (0)
1:512	1 (1)	0 (0)	1 (2)	0 (0)
IgM phase II	88 (57)	27 (52)	32 (62)	29 (58)
1:16	28 (18)	11 (21)	10 (19)	7 (14)
1:32	20 (13)	6 (12)	8 (15)	6 (12)
1:64	17 (11)	8 (15)	5 (10)	4 (8)
1:128	12 (8)	1 (2)	6 (12)	5 (10)
1:256	7 (5)	0 (0)	1 (2)	6 (12)
1:512	2 (1)	0 (0)	2 (4)	0 (0)
1:1024	1 (1)	0 (0)	0 (0)	1 (2)
1:2048	1 (1)	1 (2)	0 (0)	0 (0)
IgG phase I	119 (77)	43 (83)	39 (75)	37 (74)
1:16	34 (22)	17 (33)	8 (15)	9 (18)
1:32	26 (17)	7 (13)	8 (15)	11 (22)
1:64	26 (17)	9 (17)	12 (23)	5 (10)
1:128	23 (15)	6 (12)	7 (13)	10 (20)
1:256	8 (5)	2 (4)	4 (8)	2 (4)
1:512	2 (1)	2 (4)	0 (0)	0 (0)
IgG phase II	147 (95)	51 (98)	50 (96)	46 (92)
1:16	6 (4)	1 (2)	3 (6)	2 (4)
1:32	11 (7)	6 (12)	2 (4)	3 (6)
1:64	25 (16)	12 (23)	7 (13)	6 (12)
1:128	43 (28)	15 (29)	11 (21)	17 (34)
1:256	33 (21)	10 (19)	13 (25)	10 (20)
1:512	22 (14)	5 (10)	11 (21)	6 (12)
1:1024	6 (4)	2 (4)	3 (6)	1 (2)
1:2048	1 (1)	0 (0)	0 (0)	1 (2)

*Focus Diagnostics, Inc., Cypress, CA, USA, detecting IgM and IgG antibodies against phase I- and phase II-antigens, with a titer of >1:16 being considered positive.
Abbreviations: *CBT*, cognitive-behavioral therapy; *IFA*, immunofluorescence assay.

Supplementary Table 6. Adverse Events of Patients Included in the Intention-To-Treat Analysis*

Type of event	Total (n=154)	Doxycycline (n=52)	Placebo (n=52)	CBT (n=50)
Any AE, No. (%)	138 (90)	51 (98)	45 (87)	42 (84)
Discontinued treatment due to AE, No. (%)	2 (1)	2 (4)	0 (0)	0 (0)
Any SAE, No. (%)	2 (1)	0 (0)	2 (4)	0 (0)
No. AE – patients, No. (%)				
0	16 (10)	1 (2)	7 (13)	8 (16)
1	27 (18)	8 (15)	6 (12)	13 (26)
2	33 (21)	9 (17)	12 (23)	12 (24)
3	24 (16)	7 (13)	10 (19)	7 (14)
4	19 (12)	9 (17)	7 (13)	3 (6)
5	18 (12)	8 (15)	8 (15)	2 (4)
6	9 (6)	4 (8)	1 (2)	4 (8)
7	5 (3)	4 (8)	1 (2)	0 (0)
8	2 (1)	1 (2)	0 (0)	1 (2)
9	1 (1)	1 (2)	0 (0)	0 (0)
Median no. AE†	3.0	4.0	3.0	2.0
Total no. AE	445	192	141	112
Type of AE – patients, No. (%)				
Infection	77 (50)	22 (42)	26 (50)	29 (58)
Gastrointestinal	63 (41)	31 (60)	27 (52)	5 (10)
Musculoskeletal	53 (34)	22 (42)	17 (33)	14 (28)
Skin	35 (23)	20 (38)	10 (19)	5 (10)
Neurological	29 (19)	13 (25)	10 (19)	6 (12)
Bone and teeth	6 (4)	3 (6)	2 (4)	1 (2)
Allergic reaction	0 (0)	0 (0)	0 (0)	0 (0)
Other§	55 (36)	24 (46)	13 (25)	18 (36)
Laboratorial	21 (20)**	14 (27)	7 (13)	NA
Total no. AE per type, No. (%)				
Infection	133 (30)	33 (17)	46 (33)	54 (48)
Gastrointestinal	89 (20)	51 (27)	33 (23)	5 (4)
Musculoskeletal	68 (15)	28 (15)	22 (16)	18 (16)
Skin	46 (10)	29 (15)	12 (9)	5 (4)
Neurological	32 (7)	13 (7)	11 (8)	8 (7)
Bone and teeth	7 (2)	4 (2)	2 (1)	1 (1)
Allergic reaction	0 (0)	0 (0)	0 (0)	0 (0)
Other§	70 (16)	34 (18)	15 (11)	21 (19)
Laboratorial	24 (7)**	16 (8)	8 (6)	NA

*Laboratorial AE were excluded, as laboratory investigations for safety were only performed in the doxycycline and placebo group.

†The median number of AE per patient per group was significantly different based on a Kruskall-Wallis nonparametric test (p=0.001).

§Includes respiratory, gynecological, urological, and endocrinological complaints, cardiological symptoms, ocular symptoms, onycholysis, operations, wounds, weight loss/gain, insomnia, and an increase in depressive thoughts, forgetfulness, fatigue, or sweating.

**Only based on patients from the doxycycline and placebo group.

Abbreviations: *CBT*, cognitive-behavioral therapy; *AE*, adverse event; *SAE*, serious adverse event; *NA*, not applicable.

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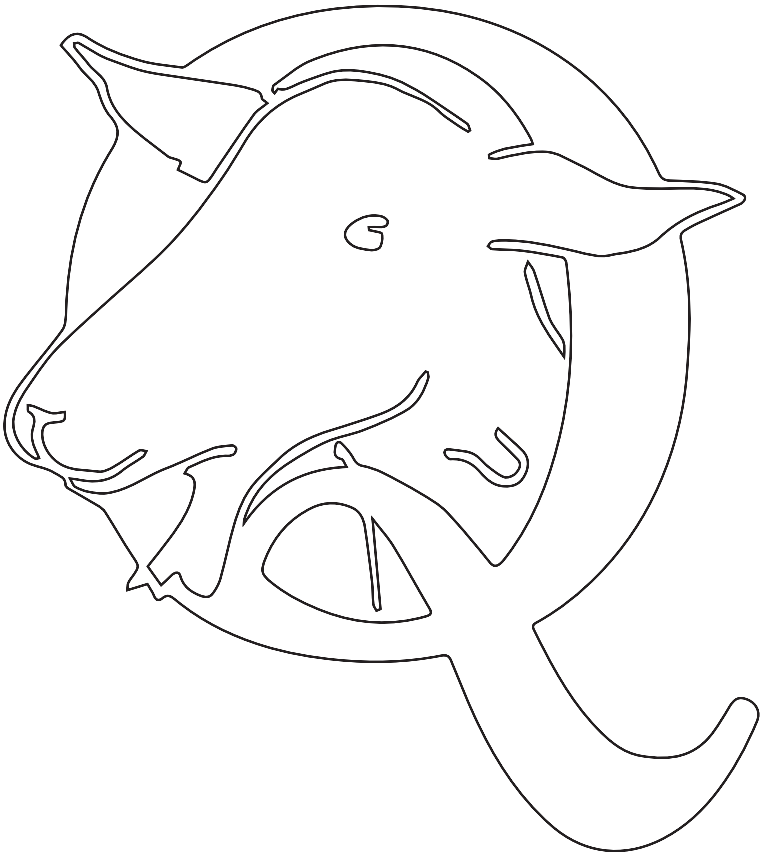
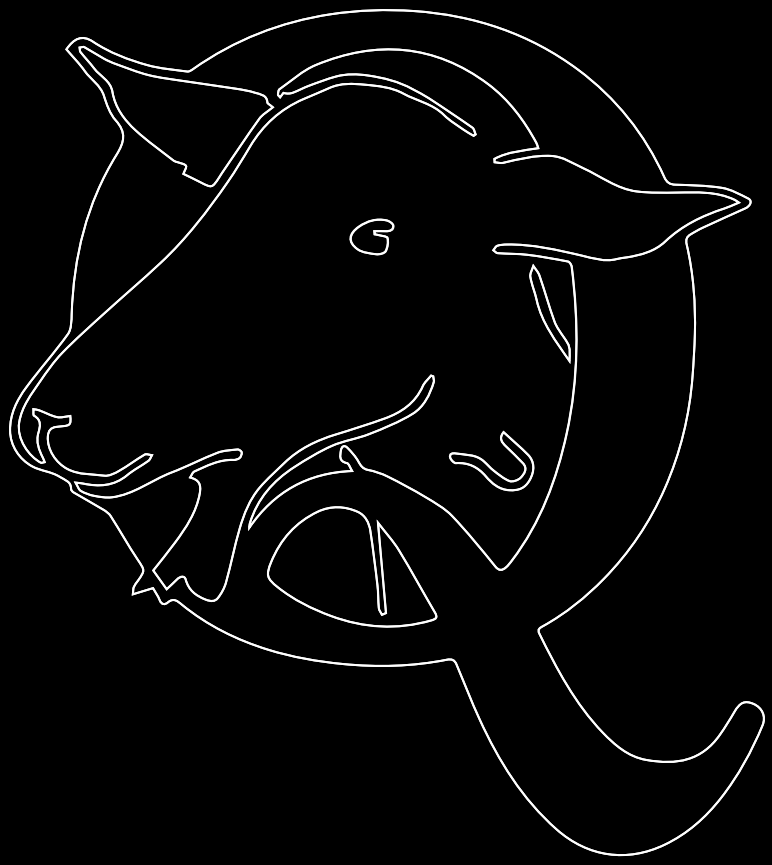


PART II

CHALLENGES IN DIAGNOSIS AND TREATMENT OF ACUTE AND CHRONIC Q FEVER



CHAPTER 7

DIFFERENTIATION OF ACUTE Q FEVER FROM OTHER INFECTIONS IN PATIENTS PRESENTING TO HOSPITALS, THE NETHERLANDS

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ABSTRACT

Differentiating acute Q fever from infections caused by other pathogens is essential. We conducted a retrospective case–control study to evaluate differences in clinical signs, symptoms, and outcomes for 82 patients with acute Q fever and 52 control patients who had pneumonia, fever and lower respiratory tract symptoms, or fever and hepatitis, but had negative serologic results for Q fever. Patients with acute Q fever were younger and had higher C-reactive protein levels but lower leukocyte counts. However, a large overlap was found. In patients with an indication for prophylaxis, chronic Q fever did not develop after patients received prophylaxis but did develop in 50% of patients who did not receive prophylaxis. Differentiating acute Q fever from other respiratory infections, fever, or hepatitis is not possible without serologic testing or PCR. If risk factors for chronic Q fever are present, prophylactic treatment is advised.

INTRODUCTION

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. During 2007–2010, the southern part of the Netherlands had the largest outbreak of Q fever ever reported [1, 2]. Infection with *C. burnetii* is symptomatic in ≈40% of all patients [3]. Clinical signs range from a mild influenza-like illness to pneumonia or a hepatitis-like syndrome and can differ by region [4, 5]. After initial infection, chronic Q fever will develop in 1%–5% of patients [1, 3]. Furthermore, long-lasting fatigue will develop in ≈20% of all patients with symptomatic acute Q fever [6-8] without development of chronic Q fever [9].

Treatment for acute infection decreases the duration of fever, increases recovery from pneumonia [10], and might lead to a lower percentage of patients in whom chronic Q fever will develop [10-13]. In addition, several reports indicate that, in acute Q fever patients at risk for development of chronic Q fever, prophylactic treatment might prevent persistent infection [12, 14]. Therefore, recognizing Q fever in an early stage is a useful strategy.

The only available data on symptoms of acute Q fever in the Netherlands were obtained from a retrospective study that collected data several months after onset of disease by sending questionnaires to patients with acute Q fever [15]. However, this method for obtaining data is limited by a high risk for recall bias. To help physicians differentiate acute Q fever from other diseases, a clear description of signs and symptoms compatible with *C. burnetii* infection is desirable. The purpose of this case–control study was to evaluate differences in clinical signs and symptoms between patients with acute Q fever referred to a hospital and a control group of patients with signs and symptoms that led to addition of Q fever in the differential diagnosis. Furthermore, outcome of patients hospitalized with acute Q fever were evaluated, and the effect of prophylactic treatment for those patients with an indication to prevent development of chronic Q fever was analyzed.

MATERIALS AND METHODS

Patients

The study group consisted of adult patients who came to the Radboud university medical center or Canisius Wilhelmina Hospital in Nijmegen, the Netherlands, during January 2007–March 2011 with pneumonia, fever and lower respiratory tract symptoms, or fever and hepatitis, and who were given a diagnosis of acute Q fever. Symptoms had to be present for <3 weeks before presentation. Exclusion criteria were chronic Q fever and a known previous acute Q fever episode. The same clinical criteria were used for the control group, but Q fever serologic results and, if available, PCR results had to remain negative. A standardized case report form was completed for every patient. According to national law, this study was exempt from approval by an ethics committee because of the retrospective characteristics of the study and the anonymous storage of data.

PCR and serologic analysis

During January 2007–March 2011, several laboratory techniques were used to diagnose acute Q fever. Because both hospitals collaborate extensively, the same microbiological laboratory techniques were used in both hospitals. The PCR used to detect DNA of *C. burnetii*

in serum was an in-house, real-time PCR directed against insertion sequence IS1111a.
Serologic analysis was performed for blood samples by using the *Coxiella burnetii* (Q Fever) IgM ELISA (PanBio Pty Ltd., Windsor, Queensland, Australia), which detects IgM against phase II antigens and has a cutoff index of 1.1; a complement fixation assay (CFA) (Virion-Serion, Würzburg, Germany), which detects *C. burnetii* phase II antigens and shows a positive result if the titer is >1:10; and a Q fever immunofluorescent assay (IFA) for IgG and IgM (Focus Diagnostics Inc., Cypress, CA, USA), which detects IgM and IgG against phase I and phase II antigens and shows a positive result if the titer is >1:16.

Definition of acute Q fever
On the basis of the algorithm published by the Dutch working group on diagnostics of acute Q fever [16], the following definition of acute Q fever was used for all included patients: pneumonia, lower respiratory tract symptoms and fever, or hepatitis-like symptoms and fever, all <3 weeks before presentation; and 1) a positive serum PCR result <21 days of onset of disease; or 2) a negative serum PCR result, but a positive ELISA result for IgM against phase II antigens of *C. burnetii* and a positive CFA result for immunoglobulins against *C. burnetii*; or 3) a negative serum PCR result but a positive ELISA result and a positive IFA result for IgM and IgG against phase I and phase II antigens of *C. burnetii*; or 4) two serum samples tested by CFA or IFA during an interval of >2 weeks that showed seroconversion or a 4-fold increase in titer.
A blood sample for Q fever serologic analysis obtained >2 weeks after the first day of illness was required because it was not possible to rule out acute Q fever if serologic samples are taken only at an earlier point, even if PCR results were negative during that period [16]. Patients were selected only if an appropriate diagnostic procedure for Q fever was performed.

Treatment
Adequate treatment for acute Q fever was defined as antimicrobial drug therapy with doxycycline (200 mg/d), moxifloxacin (400 mg 1×/d), or ciprofloxacin (500 mg 2×/d) for >14 days [17, 18]. Indications for prophylactic treatment to prevent development of chronic Q fever were patients who met the criteria for endocarditis prophylaxis according to the international guidelines of the American Heart Association [19]; patients with a structural aortic valve defect or mitral valve defect [12]; patients with a known aneurysm of the aorta or other large vessels; and patients with a vascular prosthesis. Adequate prophylactic treatment was defined as doxycycline (200 mg/d) and hydroxychloroquine (200 mg 3×/d) for >6 months.

Statistical methods
All data were analyzed by using SPSS version 20.0 (IBM, Armonk, NY, USA). For analysis of qualitative data, the Pearson's χ^2 test was used. To evaluate the effect of prophylactic treatment, the Barnard exact test was used because this test is more powerful than the Fisher exact test for instances of smaller sample sizes [20]. For quantitative data, the Student t-test was used. A p-value <0.05 was considered significant.

RESULTS

General characteristics

A total of 82 patients with acute Q fever who fulfilled inclusion criteria for the study group and 52 patients who fulfilled criteria for the control group were included in the study (*Table 1*). Patients with acute Q fever were younger (mean ± SD age 52 ± 16 years vs. 59 ± 16 years; p=0.03); had less often a history of lung disease (p=0.001); and were immunocompromised less often (p=0.002). Patients with acute Q fever had more history of smoking (p=0.01) and a higher frequency of a sore throat (p=0.008) (*Table 2*). Production of sputum was reported less frequently by patients with acute Q fever (p=0.049).

Table 1: Characteristics for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group	Control group	p-value
No. patients	82	52	NS†
Male sex, no. (%)	53 (65)	38 (73)	NS‡
Mean ± SD age, y (range)	52±16 (23-91)	59±16 (19-85)	0.027†
Mean no. days between first day of sickness and presentation	5.5	5.4	NS†
History of lung disease	8/78 (10)	18/51 (35)	0.001‡
Immunocompromised§	5/81 (6)	13/51 (25)	0.002‡
Valvular dysfunction	8/81 (10)	3/52 (6)	NS‡
Valve prosthesis	3/82 (4)	0/52 (0)	NS‡
Aneurysm	2/82 (2)	3/52 (6)	NS‡
Vascular prosthesis	3/82 (4)	3/52 (6)	NS‡
Liver disease	1/82 (1)	1/52 (2)	NS‡
Malignancy	2/82 (2)	9/52 (17)	0.002‡
Diabetes	9/82 (11)	7/52 (13)	NS‡
Contact with cattle	29/47 (62)	8/20 (40)	NS‡
History of smoking	58/74 (78)	25/44 (57)	0.013‡
Alcohol use	17/44 (39)	12/27 (44)	NS‡
Illicit drugs	4/35 (11)	0/18 (0)	NS‡
Proton pump inhibitors¶	13/82 (16)	22/52 (42)	0.001‡
Corticosteroids¶	5/82 (6)	10/51 (20)	0.017‡

* Values are no. positive/no. tested (%) unless otherwise indicated. NS, not significant.
† By Student *t*-test.
‡ By χ^2 test.
§ Also includes patients using corticosteroids.
¶ Only medications that differed significantly between groups is shown.

Table 2: Signs and symptoms for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n=82, no. positive/no. tested (%)	Control group, n=52, no. positive/no. tested (%)	p-value†
Fever	64/75 (85)	37/49 (76)	NS
Chills	31/42 (74)	16/28 (57)	NS
Myalgia	22/24 (92)	11/14 (79)	NS
Night sweats	12/19 (63)	9/17 (53)	NS
Weight loss	11/26 (42)	7/14 (50)	NS
Chest pain	11/55 (20)	13/38 (34)	NS
Dyspnea	37/65 (57)	31/43 (72)	NS
Rhinorrhea	1/12 (8)	7/14 (50)	NS
Sore throat	12/22 (55)	1/12 (8)	0.008
Cough	49/76 (64)	38/48 (79)	NS
Sputum production	18/73 (25)	20/48 (42)	0.049
Nausea	14/48 (29)	12/37 (32)	NS
Vomiting	17/47 (36)	10/39 (26)	NS
Abdominal pain	9/51 (18)	6/33 (18)	NS
Diarrhea	9/50 (18)	4/36 (11)	NS
Headache	38/54 (70)	21/27 (78)	NS
Weakness	9/21 (43)	1/9 (11)	NS
Painful joints	7/20 (35)	2/16 (13)	NS
Arthritis	0/17 (0)	1/16 (6)	NS

* NS, not significant.
† By χ^2 test.

Physical examination

Of patients with acute Q fever, 18% had shortness of breath (*Table 3*) compared with 44% in the control group (p=0.03). A total of 4% of patients with acute Q fever had rhonchi at pulmonary examination compared with 22% in the control group (p=0.005). Oxygen saturation was significantly higher in patients with acute Q fever (p=0.02).

Laboratory values

Patients with acute Q fever had a higher levels of C-reactive protein (mean 167 mg/L vs. 117 mg/L; p=0.02) (*Table 4*) and lower leukocyte counts (mean 9.0 × 109 cells/L vs. 11.5 × 109 cells/L; p=0.006). Leukocyte counts remained significantly lower in the first 3 days after presentation (p=0.006–0.043). At admission to the hospital, no differences were found between the groups for levels of alkaline phosphatase and y-glutamyl transpeptidase. However, from day 1 onward, levels of alkaline phosphatase and y-glutamyl transpeptidase were significantly higher in patients with acute Q fever (p=0.01–0.047 and p=0.007–0.05, respectively).

PCR and serologic analysis

Serum PCR for DNA of *C. burnetii* was performed for 41 patients in the study group (*Table 5*). Blood samples were obtained at day 8 ± 7 (mean ± SD) of illness. The sensitivity of this PCR was 56%. For 4 patients, a second blood sample was obtained at day 12 ± 5 of illness. The sensitivity of this PCR was 25%.

Table 3: Physical examination results for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n=82	Control group, n=52	p-value
Dyspnea	13/73 (18)	18/41 (44)	0.03†
Abnormal heart sounds	1/80 (1)	0/51 (0)	NS†
Cardiac murmur	11/80 (14)	4/50 (8)	NS†
Decreased breath sounds	6/78 (8)	7/46 (15)	NS†
Bronchial breath sounds	9/64 (14)	5/37 (14)	NS†
Crackles	36/76 (47)	19/43 (44)	NS†
Rhonchi	3/68 (4)	9/41 (22)	0.005†
Palpable liver	1/69 (1)	1/39 (3)	NS†
Palpable spleen	0/68 (0)	0/36 (0)	NS†
Exanthema	2/9 (22)	0/6 (0)	NS†
Lymphadenopathy	2/27 (7)	2/21 (10)	NS†
Temperature, ˚C (no. patients)	38.4 (67)	38.3 (48)	NS‡
Heart rate, beats/min (no. patients)	93 (73)	91 (50)	NS‡
Systolic blood pressure, mmHg (no. patients)	134 (73)	138 (49)	NS‡
Respiratory rate, breaths/min (no. patients)	25 (24)	25 (21)	NS‡
Saturation, % oxygenation (no. patients)§	97 (57)	95 (34)	0.022‡

* Values are no. positive/no. tested (%) unless otherwise indicated. NS, not significant.
† By χ^2 test.
‡ By Student *t*-test.
§ Saturation without oxygen.

ELISA was performed on samples from 33 patients with acute Q fever and 18 patients in the control group. Blood samples were obtained from the study group at day 10 ± 8 of illness and from the control group at day 7 ± 6 of illness. Sensitivity of this ELISA was 61%.
CFA, which was performed for 81 patients in the study group at day 9 ± 19 of illness and for 52 patients in the control group at day 8 ± 6 of illness, showed a sensitivity of 22% (*Table 5*). A total of 57 patients were hospitalized, of whom 36 were given a diagnosis of acute Q fever during their hospitalization.

Imaging studies
A total of 78% of chest radiographs for patients with acute Q fever showed signs of pneumonia. A total of 54% of chest radiographs for patients in the control group showed signs of pneumonia (p=0.003) (*Table 5*).

Treatment
Treatment was started before a diagnosis was made. Significantly more patients with acute Q fever started treatment with doxycycline than patients in the control group (35% vs.

Table 4: Laboratory values for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Laboratory value		Study group, n=82		Control group, n=52		p-value‡
	Day†	Mean	No. tested	Mean	No. tested	
Hemoglobin, mmol/L; reference range: men 8.1-10.7 mmol/L, women 7.3-9.7 mmol/L	0	8.3	77	8.0	51	NS
	1	7.4	28	7.3	34	NS
	2-3	7.7	27	7.0	29	0.036
	4-6	7.6	27	7.0	29	NS
Leukocytes, x 10^9 cells/L; reference range 3.5–11.0 x 10^9 cells/L	0	9.0	80	11.5	50	0.006
	1	8.5	40	10.8	28	0.043
	2-3	8.0	34	11.1	33	0.021
	4-6	10.9	28	9.2	31	NS
Platelets, x 10^9/L; reference range 20–350 x 10^9/L	0	239	78	208	50	NS
	1	242	23	178	29	0.038
	2-3	229	19	172	26	0.042
	4-6	298	24	208	27	0.011
Total bilirubin, µmol/L; reference value <17 µmol/L	0	14	26	16	20	NS
	1	12	14	14	8	NS
	2-3	9	12	28	6	0.017
	4-6	8	12	9	6	NS
AP, U/L; reference value <120 U/L	0	104	75	85	50	NS
	1	127	19	75	12	0.047
	2-3	126	26	66	12	0.010
	4-6	145	23	95	15	0.036
ALT, U/L; reference value <45 U/L	0	45	76	37	49	NS
	1	64	22	58	16	NS
	2-3	66	30	40	13	0.050
	4-6	81	22	84	18	NS
γ-GT, U/L; reference value: men <50 U/L, women <35	0	74	68	65	49	NS
	1	117	21	53	12	0.030
	2-3	106	27	42	9	0.007
	4-6	112	22	66	14	0.050
CRP, mg/L; reference value <10 mg/L	0	167	79	117	50	0.015
	1	184	44	150	37	NS
	2-3	132	46	147	32	NS
	4-6	76	41	98	27	NS
Urea, mmol/L; reference value 2.5–7 mmol/L	0	6.4	79	8.6	51	0.039
	1	6.4	33	7.9	35	NS
	2-3	5.4	38	8.7	35	0.014
	4-6	5.8	34	9.3	30	0.018
Creatinine, µmol/L; reference value: men <110 µmol/L, women <90 µmol/L	0	86	80	105	52	0.042
	1	84	38	103	38	NS
	2-3	79	37	103	37	NS
	4-6	81	36	136	31	NS

* NS, not significant; AP, alkaline phosphatase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transpeptidase; CRP, C-reactive protein.
† Day 0 is the day of coming to the hospital.
‡ By Student *t*-test.

Table 5: PCR and serologic results for patients in study group with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n=82	Control group, n=52	Day of illness for study group, mean ± SD	Day of illness for control group, mean ± SD	Sensitivity, %
PCR					
First sample	23/41	0/15	8 ± 7	8 ± 7	56
Second sample	1/4	0/1	12 ± 5	30 ± 0	25
ELISA					
First sample	20/33	0/18	10 ± 8	7 ± 6	61
Second sample	15/18	0/2	20 ± 11	25 ± 8	83
CFA					
First sample	18/81	0/52	9 ± 19	8 ± 6	22
Second sample	27/34	0/28	18 ± 9	20 ± 12	79
Third sample	5/5	0/3	21 ± 6	26 ± 5	100
Culture					
Blood†	0/42 (0)	0/40 (0)	NA	NA	NA
Urine†	0/30 (0)	0/37 (0)	NA	NA	NA
Sputum‡	1/15 (7)	3/22 (14)	NA	NA	NA
Chest radiograph§	62/79 (78)	28/52 (54)	NA	NA	¶

* Values are no. positive/no. tested (%) unless otherwise indicated. CFA, complement fixation assay; NA, not applicable.
† Includes only results for first cultures obtained after coming to the hospital.
‡ Includes only results for first cultures obtained after coming to the hospital. In the study group, 1 patient was positive for parainfluenza virus. In the control group, 1 patient was positive for *Moraxella catarrhalis,* 1 patient was positive for *Legionella pneumophila*, and 1 patient was positive for *Streptococcus pneumonia* and *Staphylococcus aureus*.
§ Includes only first chest radiographs after coming to the hospital. Values are no. abnormal/no. tested (%).
¶ p=0.003, by χ^2 test.

15%; p=0.001) (*Table 6*). For 8 patients in the study group, the duration of antimicrobial drug treatment was unknown. Of the remaining 74 patients with acute Q fever, 34 (46%) patients were given adequate treatment. The mean ± SD follow-up time for patients given adequate treatment was 11.7 ± 5 months compared with 13.3 ± 9 months for patients given inadequate treatment.

Table 6: Initial treatment for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Initial treatment	Study group, n=82, no. positive/no. tested (%)	Control group, n=52, no. positive/no. tested (%)	p-value†
Doxycycline	29/82 (35)	8/52 (15)	0.001
Moxifloxacin	5/82 (6)	2/52 (4)	NS
Ciprofloxacin	7/82 (9)	6/52 (12)	NS
Penicillin	7/82 (9)	1/52 (2)	0.049
Amoxicillin	13/82 (16)	5/52 (10)	NS
Amoxicillin/clavulanic acid	3/82 (4)	4/52 (8)	NS
Piperacillin/tazobactam	1/82 (1)	5/52 (10)	NS
Cephalosporin	14/82 (17)	17/52 (33)	NS
Co-trimoxazole	0/82 (0)	1/52 (2)	NS
Flucloxacillin	2/82 (2)	0/52 (0)	NS
Clarithromycin	0/82 (0)	1/52 (2)	NS
No treatment	1/82 (1)	1/52 (2)	NS
Unknown	0/82 (0)	1/52 (2)	NS
Patients with adequate treatment‡	34/74 (46)	NA	NA

* NS, not significant; NA, not applicable.
† By χ^2 test.
‡ Defined as use of doxycycline (200 mg/d), moxifloxacin (400 mg 1x/d), or ciprofloxacin (500 mg 2x/d) for ≥2 wk.

Outcomes

Hospitalization (70% vs. 94%; p=0.001), admission to an intensive care unit (4% vs. 18%; p=0.002), and need for respiratory support (2% vs. 16%; p=0.001) were less common for the study group than for the control group (*Table 7*). Also, duration of hospital stay was shorter for patients with acute Q fever (9 ± 7 days vs. 17 ± 15 days; p=0.001). Accurate follow-up data were available for 59 of 82 patients with acute Q fever who had a mean ± SD follow-up of 12.8 ± 8.2 months. Chronic Q fever developed in 6 (10%) patients in the Q fever group.

Sixteen patients with acute Q fever met the criteria for prophylactic treatment to prevent development of chronic Q fever (*Table 8*). Indications were valvular dysfunction (n=8); cardiac valve prosthesis (n=3); aneurysm (n=1); vascular prosthesis (n=3, of whom 1 patient also had a cardiac valve prosthesis); and a new cardiac murmur (n=2). Eight (50%) of these patients received prophylactic treatment. Proper follow-up data for development of chronic Q fever were available for 14 patients with an indication for prophylaxis. Chronic Q fever did not develop in any of the 8 patients who received prophylaxis. The other 6 patients with an indication for prophylaxis for whom follow-up serum samples were available did not receive prophylaxis because the indication for prophylaxis was not recognized by the treating physician. Chronic Q fever developed in 3 (50%) of these 6 patients (p=0.02). In the group without an indication for prophylaxis, chronic Q fever developed in 3 (6%) patients. Six (11%) of 56 patients in the study group for whom these data were available reported long-lasting fatigue.

The mortality rate during a 12-month follow-up period was 6% for the study group

compared with 19% for the control group (p=0.02). None of the patients in the study group died during the episode of acute Q fever. Four patients in the study group died because of reasons unrelated to Q fever. One patient died of consequences of an infected vascular prosthesis caused by chronic Q fever, although adequate treatment was started after the diagnosis. In contrast, 2 patients in the control group died during initial hospitalization, 1 of a *Mycoplasma* sp. infection and 1 of pneumonia without a known causative agent. Eight patients in the control group died during follow-up. One of them died of a non-Hodgkin lymphoma and 1 of consequences of an *Aspergillus* sp. infection. For the other 6 patients who died, no detailed information was available.

A total of 49 control patients were given a diagnosis of pneumonia; for 38 of these patients, no causative agent was found. For the remaining 11 patients, causative agents were *Pneumocystis jiroveci*, *Moraxella catarrhalis*, *Legionnella pneumophila*, *Chlamydia* sp., *Haemophilus influenzae* (2 patients), *Mycoplasma* sp. (3 patients), influenza virus and *Mycoplasma* sp., and *Staphylococcus aureus* and *Streptococcus pneumonia*. The remaining 3 patients were given diagnoses of acute myeloid leukemia, non-Hodgkin lymphoma, and restrictive pericarditis.

Table 7: Outcome, follow-up, and prophylaxis for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group	Control group	p-value
Outcome			
Hospitalized	57/82 (70)	49/52 (94)	0.001†
Need for ICU	2/57 (4)	9/49 (18)	0.002†
Need for respiratory support	1/57 (2)	8/49 (16)	0.001†
Mean ± SD duration of hospitalization, d	9 ± 7	17 ± 15	0.001‡
Mean ± SD duration of time in ICU, d	5 ± 1	14 ± 10	0.266‡
Follow-up			
Development of chronic Q fever	6/59 (10)	NA	NA
Development of long-lasting fatigue§	6/56 (11)	NA	NA
Death	5/82 (6)	10/52 (19)	0.019†
Q fever-related death	1/82 (1)¶	NA	NA
Indication for prophylaxis	16/82 (20)	NA	NA
Development of chronic Q fever			
Prophylactic treatment	0/8 (0)	NA	NA
No prophylactic treatment	3/6 (50)	NA	0.018#

* Values are no. positive/no. tested (%) unless otherwise indicated. ICU, intensive care unit; NA, not applicable.

† By χ^2 test.

‡ By Student *t*-test.

§ Defined as persisting fatigue for >6 months after acute Q fever in the absence of chronic Q fever.

¶ This patient died of consequences of an infected vascular prosthesis caused by chronic Q fever.

By unilateral Barnard exact test.

Table 8: Characteristics of 16 patients with acute Q fever with an indication for prophylaxis, the Netherlands*

Patient no.	Age, y/sex	Hospitalized	Indication at presentation for prophylactic treatment	Prophylactic treatment and duration, mo	Chronic Q fever	Died
1	42/M	Yes	Valvular dysfunction (AS)	D + H, 12	No	No
2	49/M	Yes	Cardiac bioprosthesis and vascular prosthesis	D + H, 12	No	No
3	51/M	Yes	Cardiac bioprosthesis and TOF	D 12 + H 4 (added after 8)	No	No
4	54/M	Yes	Aneurysm common iliac artery	D + H, 9	No	No
5	43/M	Yes	Valvular dysfunction (TI) and TGV	D + H, 7	No	No
6	78/F	Yes	Cardiac bioprosthesis	D + H, 1, switched to Mox, 3	No	Yes†
7	26/M	No	Vascular prosthesis	D + H, 2.5	No	No
8	81/F	Yes	Valvular dysfunction (MI)	D + H, 12	No	Yes‡
9	65/M	Yes	Valvular dysfunction (MI)	No	No	No
10	80/M	Yes	Valvular dysfunction (MI)	No	No	No
11	78/F	No	Valvular dysfunction (MI)	No	No	No
12	64/F	Yes	Vascular prosthesis	No	Yes	Yes§
13	75/F	Yes	New cardiac murmur	No	Yes	No
14	75/M	No	New cardiac murmur	No	Yes	No
15	57/F	No	Valvular dysfunction (AS)	No	Unknown¶	No
16	58/M	Yes	Valvular dysfunction (MI)	No	Unknown¶	No

* AS, aortic valve sclerosis; D, doxycycline 100 mg 2x/d; H, hydroxychloroquine 200 mg 3x/d; TOF, tetralogy of Fallot; TI, tricuspid insufficiency; TGV, transposition of the great vessels; Mox, moxifloxacine 400 mg 1x/d; MI, mitral insufficiency; CFA, complement fixation assay; IFA, immunofluorescence assay.

† This patient was rehospitalized shortly after the acute Q fever episode and died because of a reason unrelated to Q fever. The last available serologic follow-up showed no signs of chronic Q fever (negative PCR result; CFA titer 1:10, IFA IgG phase I negative result; IgG phase II titer 1:256; IgM phase I negative result; and IgM phase II titer 1:64).

‡ This patient eventually died because of a reason unrelated to Q fever. The last available serologic follow-up 1 year after the acute Q fever episode showed no signs of chronic Q fever (negative PCR result; CFA titer 1:10; IFA IgG phase I titer 1:64; IgG phase II titer 1:512; IgM phase I titer 1:16, and IgM phase II titer 1:16).

§ This patient was hospitalized and admitted to the intensive care unit for 5 d. She was treated with several antimicrobial drugs (penicillin, ciprofloxacin, cefuroxim, metronidazol, ceftazidim, and teicoplanin) before given a diagnosis of an infected vascular prosthesis caused by chronic Q fever. Although doxycycline and hydroxychloroquine were given after the diagnosis was made, this patient eventually died from consequences of an infected vascular prosthesis caused by chronic Q fever.

¶ No follow-up with reference to Q fever was performed for this patient.

DISCUSSION

This retrospective case–control study evaluated differences in clinical signs and symptoms between patients with acute Q fever referred to a hospital and a control group. Because patients in the control group had Q fever included in the differential diagnosis, a selection bias is possible. However, differences were found between the 2 groups. In addition, because of the Q fever outbreak during that time, *C. burnetii* was considered a possible etiologic agent in many patients who came to a hospital. The higher number of patients in the study group can be explained by strict implementation of inclusion criteria for the control group.

Consistent with findings of earlier studies [1, 21], we found that patients with acute Q fever more often had a history of smoking. However, a history of lung disease was found less often. A lower mean age in the study group than in the control group might explain this finding. Previous studies suggest typical signs and symptoms of acute Q fever: fever, headache, and cough [1, 3, 22]. However, no difference was observed in the occurrence of fever. It has been postulated that headache is rather specific for acute Q fever [5, 23]. However, in our study, headache was less common in patients with acute Q fever than in the control group. Although cough was a relatively common sign in both groups, sputum production was reported less often in patients with acute Q fever. In addition, a sore throat was reported more often in the study group, which has not been previously reported.

A limitation of these results is the retrospective nature of the study because physicians probably did not include all signs and symptoms in patient charts. In general, patients with lung disease often use corticosteroids, which might explain why fewer patients in the study group were classified as immunocompromised. In contrast to medical and physical examination results, more patients with acute Q fever showed signs of an infiltrate on chest radiographs when they came to the hospital. Although acute Q fever usually is a relatively mild influenza-like disease, it has been reported that chest radiographs often shows signs of an infiltrate [24]. Compared with our control group, fewer patients in the study group needed hospitalization, and duration of hospitalization was shorter. These findings might be explained by the lower mean age of patients with acute Q fever, assuming that they were in a more healthy condition. Furthermore, *C. burnetii* is known for its self-limiting character, in contrast to those of other pathogens found in the control group.

In the Netherlands, a Q fever hospitalization rate of 50% in 2007 was registered, which stabilized at ≈20% in later years [25]. This rate is higher than that previously reported (2%–5%)[5]. However, large variations in hospitalization rates for acute Q fever patients have been reported [26]. In this study, 70% of patients with acute Q fever were hospitalized. Most patients with acute Q fever are asymptomatic or have only a mild influenza-like illness. Thus, a selection bias caused by the study design is likely. We found that 78% of patients in the study group had an abnormal result on a chest radiograph, which might indicate that only patients with severe symptoms were hospitalized.

Although C-reactive protein levels and leukocyte counts differed between the study group and the control group, this finding did not contribute to differentiation between *C. burnetii* and other pathogens at hospitalization because differences were small and showed much overlap. In addition, although leukocyte counts were usually within the reference range, patients with acute Q fever more often had a lower leukocyte count, which is consistent with

results of other studies [3, 4]. In contrast to these studies, which found thrombocytopenia in patients with acute Q fever, we found slightly higher levels of platelets, all within the reference range, in the study group than in the control group. Increased levels of liver enzymes have been reported in patients with acute Q fever [3, 5, 22]. However, we found no differences in these levels between both groups at hospitalization. Furthermore, creatinine levels were not increased, in contrast to results reported in a previous study [3].

Although antimicrobial drug treatment was inadequate in an unexplainably high percentage of patients with acute Q fever, more patients in the study group than in the control group were initially treated with doxycycline, the treatment of choice for patients with acute Q fever. The choice of antimicrobial drug treatment in patients with community-acquired pneumonia (CAP) of unknown origin in the Netherlands depends on the Confusion, Urea nitrogen level in blood, Respiratory rate, Blood pressure, age ≥65 years (CURB-65) score [27]. In addition, although CURB-65 scores could not be calculated for all patients, fewer patients in the study group were hospitalized, needed admission to an intensive care unit, and needed respiratory support, which suggests lower CURB-65 scores in the study group than in the control group.

Although changes were made in the national guidelines for treating CAP issued by the Dutch Working Party on Antibiotic Policy in 2011 [28], until 2011, doxycycline was the first choice for patients with a low CURB-65 score [29]. In addition, more patients in the study group were given a diagnosis of having an infiltrate, which suggested that initial treatment in the study group was also aimed at atypical microorganisms. Presumably, patients in the control group were treated with broader spectrum antimicrobial drugs because of higher CURB-65 scores. Also, more patients in the control group were immunocompromised, which also could have influenced the choice of treatment.

Long-term prophylactic treatment with doxycycline and hydroxychloroquine has been suggested for patients with risk factors for development of chronic Q fever [12, 14]. Although controversy still exists (e.g., with regard to treatment duration and patient selection), prophylactic treatment of high-risk patients after an episode of acute Q fever can be beneficial and is widely advised [30-32]. In our study, not all patients who had an indication according to our definition received prophylaxis. Chronic Q fever developed in 3 of 6 patients who did not receive prophylaxis, in contrast to none of the patients who received prophylaxis, which is a difference that clearly supports findings of other studies in which prophylactic treatment was suggested to prevent development of chronic Q fever in patients with risk factors for this disease [12, 14]. On the basis of these results, prophylactic treatment is advised if risk factors for developing chronic Q fever exist, but potential side effects must be taken into consideration [33].

For 48 of 67 patients without indication for prophylactic treatment, follow-up data were available on development of chronic Q fever. Chronic Q fever developed in 3 (6%) of these patients, which is slightly higher than expected [1, 34]. This finding might be explained by the fact that we included only patients who were referred to a hospital, and therefore selected patients most affected by *C. burnetii infection*. It is possible that more severely acute Q fever predisposes for development of chronic Q fever [13].

After having acute Q fever, patients often report long-lasting fatigue, which frequently

persists for >6 months. This symptom after acute Q fever has been designated Q fever fatigue syndrome. Our data suggest a prevalence of 11%, which is lower than expected; other studies reported a prevalence of ≈20% worldwide and a higher prevalence in the Netherlands [6, 35, 36]. The prevalence found in this study is presumably an underestimation because proper analysis was not performed for most patients.

Although we found some differences in clinical manifestations for patients with acute Q fever coming to a hospital compared with controls, considerable overlap between both groups hamper the use of these variables for clinical differentiation. Differentiating *C. burnetii* from other pathogens is not possible without Q fever serologic analysis and PCR in patients coming to a hospital. In disease-endemic areas or in instances in which patients have risk factors for Q fever, suspicion should remain high, and the threshold for performing Q fever serologic analysis and PCR should remain low. Because only 46% of patients received adequate treatment acute Q fever in our study, treatment for acute Q fever should be improved. Furthermore, our findings underline the recommendation that prophylactic treatment should be given to patients with risk factors for developing chronic Q fever. However, more studies are needed to develop uniform guidelines with regard to optimal prophylactic treatment.

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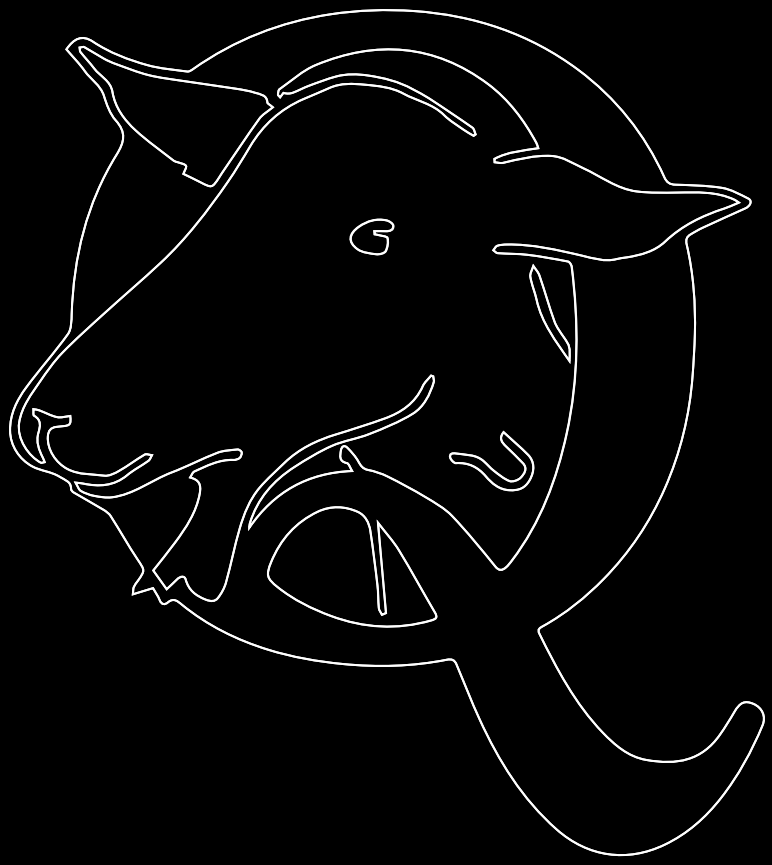

CHAPTER 8

LOCALIZING CHRONIC Q FEVER: A CHALLENGING QUERY

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ABSTRACT

Background: Chronic Q fever usually presents as endocarditis or endovascular infection. We investigated whether ^{18}F-FDG PET/CT and echocardiography were able to detect the localization of infection. Also, the utility of the modified Duke criteria was assessed.

Methods: Fifty-two patients, who had an IgG titre of ≥1024 against *C. burnetii* phase I ≥3 months after primary infection or a positive PCR ≥1 month after primary infection, were retrospectively included. Data on serology, the results of all imaging studies, possible risk factors for developing proven chronic Q fever and clinical outcome were recorded.

Results: According to the *Dutch consensus on Q fever diagnostics*, 18 patients had proven chronic Q fever, 14 probable chronic Q fever, and 20 possible chronic Q fever. Of the patients with proven chronic Q fever, 22% were diagnosed with endocarditis, 17% with an infected vascular prosthesis, and 39% with a mycotic aneurysm. 56% of patients with proven chronic Q fever did not recall an episode of acute Q fever. Ten out of 13 ^{18}F-FDG PET/CT-scans in patients with proven chronic Q fever localized the infection. TTE and TEE were helpful in only 6% and 50% of patients, respectively.

Conclusions: If chronic Q fever is diagnosed, ^{18}F-FDG PET/CT is a helpful imaging technique for localization of vascular infections due to chronic Q fever. Patients with proven chronic Q fever were diagnosed significantly more often with mycotic aneurysms than in previous case series. Definite endocarditis due to chronic Q fever was less frequently diagnosed in the current study. Chronic Q fever often occurs in patients without a known episode of acute Q fever, so clinical suspicion should remain high, especially in endemic regions.

BACKGROUND

Q fever is a zoonosis caused by *Coxiella burnetii* [1, 2]. The acute form of Q fever is asymptomatic in 60% of patients. Patients with symptomatic disease usually present with mild flu-like symptoms, pneumonia or hepatitis [1, 3]. Following primary infection, 1-5% of patients develop chronic Q fever [1, 4-6]. In the literature, the most described localization of chronic Q fever is endocarditis, accounting for 60-80% of cases [1, 2, 7, 8]. Less frequently reported manifestations of chronic Q fever include infections of aneurysms or vascular prostheses (9% of cases), chronic infections during pregnancy (5%) and other persistent infections (8%), such as osteomyelitis and chronic hepatitis [8, 9]. However, following the recent Q fever epidemic in the Netherlands [10-12], substantially more patients have been diagnosed with an infected aneurysm or vascular prosthesis [4, 13].

The diagnosis of chronic Q fever is challenging. Persistent infection usually develops insidiously and most patients present with non-specific symptoms such as low-grade fever, night sweats, weight loss, hepatosplenomegaly, and a persistently raised erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) [1, 3, 8, 14, 15]. Both serology and PCR aid the laboratory diagnosis of chronic Q fever [16, 17]. High levels of antibodies to phase I more than 3 months after primary infection are found in chronic Q fever, whereas antibodies to phase II predominate after convalescence from acute Q fever without signs of chronic infection [5, 16, 18]. Localization of infectious foci is important, because, in addition to prolonged antimicrobial therapy, adjuvant therapeutic measures such as surgical drainage or graft replacement are often necessary [9, 19]. This demonstrates the need for reliable imaging methods. Infected aneurysms or vascular prostheses can be identified by using computed tomography (CT) or ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET/CT) [20-23]. In case of Q fever endocarditis, however, the diagnosis is usually more complex and vegetations are rarely seen by echocardiography [18, 24, 25]. This commonly delays the diagnosis with several months [26].

From 2007 until 2010, the southern part of the Netherlands faced the largest outbreak of Q fever ever reported [4, 10]. Physicians were confronted with an increasing number of patients with suspected chronic infection. The *Dutch Q fever consensus group* provided a new guideline on the diagnosis of chronic Q fever discriminating 3 categories: possible, probable and proven chronic Q fever [15]. We investigated whether FDG-PET/CT and echocardiography were able to detect the localization of infection in all patients with chronic Q fever treated at 2 hospitals specialized in Q fever in the Netherlands. In addition, the utility of the modified Duke criteria was assessed.

METHODS

Study design and patients

All patients referred to Radboud University Nijmegen Medical Centre and Canisius Wilhelmina Hospital in Nijmegen, the Netherlands between August 2008 and March 2011 were retrospectively included if they fulfilled the following criteria: detection of *C. burnetii* DNA in serum or tissue by PCR ≥1 month after primary infection or an anti-phase I IgG titre of ≥1024 against *C. burnetii* phase I ≥3 months following acute Q fever. Patients without symptomatic acute infection were included if anti-phase I IgG remained >1024 over the

course of >3 months, or if there was positive serum PCR over the course of >1 month. The exclusion criterion was age <18 years. For each patient a standardized case report form was completed. According to the Dutch law, this study was exempt from approval by an ethics committee, because of the retrospective character of this study and the anonymous storage of data.

Diagnostic work-up

Serology and molecular detection

In 1994, the French National Centre for Rickettsial Diseases proposed a cut-off value for anti-phase I IgG of 1:800 for the diagnosis of chronic Q fever, using an in-house immunofluorescence assay (IFA) [16]. This cut-off value was adopted by the modified Duke criteria [27] and is considered as diagnostic for chronic Q fever in most literature. However, it is recently recognized that the results of Q fever IFA vary according to the centre in which they are carried out and the methods used (commercially available immunofluorescence kits) [28, 29]. This also applies to the Dutch situation, where much higher anti-phase I IgG titres were measured, especially during the first months after acute infection [4]. The *Dutch Q fever consensus group* proposed a cut-off value for anti-phase I IgG of 1:1024 (immunofluorescence assay; Focus Diagnostics, Inc., Cypress, CA, USA), measured at least 3 months after acute infection, for the diagnosis of chronic Q fever in the Netherlands. In our study, sera were also tested for *C. burnetii* antibodies using a complement fixation test (CFT) (Institute Virion/Serion, GmbH, Würzburg, Germany), testing only anti-phase II antibodies.

Dutch consensus on chronic Q fever

The guideline on the classification of chronic Q fever [15], that has been developed by the *Dutch Q fever consensus group*, was used for diagnosis and classification of chronic Q fever in this study. This guideline uses a combined approach based on risk factors, symptoms, microbiological findings and imaging studies to discriminate 3 groups of chronic Q fever:

Proven chronic Q fever Chronic Q fever is considered proven in case of (1) a positive *C. burnetii* PCR on blood or tissue without evidence for acute Q fever OR (2) IFA anti-phase I IgG ≥1024 is present >3 months after acute infection AND definite endocarditis according to the modified Duke criteria OR (3) IFA ≥1024 for anti-phase I IgG AND proven vascular infection by abdominal ultrasound (AUS), CT, or FDG-PET/CT.

Probable chronic Q fever Chronic Q fever is classified as probable when IFA anti-phase I IgG ≥1024 is present >3 months after acute infection in combination with (1) valvular defects not meeting the modified Duke criteria OR (2) a known aneurysm and/or vascular or cardiac valve prosthesis without signs of infection by means of echocardiography, FDG-PET/CT, CT or AUS OR (3) suspected osteomyelitis or hepatitis as manifestation of chronic Q fever OR (4) pregnancy OR (5) symptoms of chronic infection OR (6) granulomatous tissue inflammation, histologically proven OR (7) being immunocompromised.

Possible chronic Q fever Possible chronic Q fever is diagnosed when IFA anti-phase I IgG

≥1024 is present >3 months after acute infection without manifestations meeting the criteria for proven or probable chronic Q fever.

Modified Duke criteria
The modified Duke criteria for infective endocarditis (IE) [27] were applied to all patients who underwent echocardiography. As a result, patients were stratified into 3 different groups: definite, possible and rejected IE. Besides the well-adopted modified Duke criteria by Li and colleagues [27], we also assessed 2 adjusted versions of these criteria that have been used previously in studies on Q fever endocarditis. In the first adjustment, the molecular (serum PCR) diagnosis of *C. burnetii* was considered as an additional major criterion [17, 30]. In the second adjustment, the echocardiographic minor criteria that were eliminated by the modified Duke criteria in 2000 were reintroduced [27, 31]. Echocardiographic minor criteria include nodular valvular thickening, nonoscillating targets, and new valvular fenestrations [31].

Imaging studies
Data on the following imaging studies were recorded: AUS, CT, FDG-PET/CT, transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE). FDG-PET/CT-scans were performed according to international guidelines [32], using integrated PET/CT-scanners (Biograph™; Siemens, Knoxville, TN, USA or Gemini™, Philips, Eindhoven, the Netherlands). All FDG-PET/CT-scans were performed in regular patient care and therefore reviewed by specialized nuclear radiologists from the department of Nuclear Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, as well as the department of Nuclear Medicine, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands. Higher metabolic activity than physiological uptake in surrounding tissue in tissues with normally low physiological uptake was considered to be indicative of infection. In addition, irregular/localized FDG-uptake in tissues with normally homogenous uptake was considered indicative of infection. Each original report was used to score for relevant abnormal findings. If these findings enabled localization of infection, they were considered helpful. Abnormal results that gave rise to further analysis, i.e. suspected malignancy, but were not caused by chronic Q fever, were labelled as unexpected findings.

Clinical data and outcome
Acute Q fever infection was regarded symptomatic if patients were diagnosed with Q fever pneumonia or if they could recall an episode of fever and pneumonia and/or headache, that was not caused by other known pathogens and that preceded the first positive Q fever serology or positive serum PCR. Patients were regarded to have pre-existing valvular disease if they were previously known with a valvulopathy ≥grade II (stenosis or insufficiency, including congenital heart disease), or if they had a medical history of valve replacement. Valvular dysfunction was defined as the aggravation of pre-existing valvulopathies to ≥grade 2, the occurrence of new valvulopathies of ≥grade 2 or signs of artificial valve dysfunction, or evidence of increasing heart failure or the need for acute cardiac valve replacement. Data on other possible risk factors for chronic Q fever were collected (age, smoking, known

aneurysm, presence of a vascular prosthesis, immunosuppression or –deficiency, other co-morbidity, and symptomatic acute Q fever). The diagnostic work-up was considered complete if both echocardiography and screening for abdominal infection were completed. Patients were considered to be cured if their anti-phase I IgG antibody titre at least showed a fourfold decrease or had declined to <1024 during subsequent serological testing, serum PCR had become and/or remained negative, and diagnostic imaging during follow-up showed no signs of active infection.

Statistical methods

All data were analyzed using SPSS (version 16.0, SPSS, Inc.). Two-tailed Pearson's chi-square tests or Fischer's exact tests were used to compare qualitative data, whereas mean values were analyzed by Student's t-tests. Differences were considered to be statistically significant at a p-value less than 0.05.

RESULTS

All 52 patients fulfilling the inclusion criteria were included in the study (*Tables 1, 2, 3*).

Table 1: Population characteristics of 52 patients with possible, probable and proven chronic Q fever**[*]

	Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
	Number of patients (% or range)	Number of patients (% or range)	Number of patients (% or range)
General			
Number of patients	18	14	20
Male sex	17 (94)	8 (57)	11 (55)
Age at diagnosis	61 ± 16 yrs (26-88)	63 ± 12 yrs (43-84)	54 ± 15 yrs (26 – 81)
Mean BMI	25 ± 3 kg/m^2 (18-30)	25 ± 4 kg/m^2 (18-30)	25 ± 7 kg/m^2 (19-41)
History of smoking	14 (78)	9 (64)	10 (50)
Symptomatic acute infection	8 (44)	12 (86)	13 (65)
Symptomatic chronic infection	14 (78)	2 (14)	0
Mean interval acute Q fever to analysis	12 ± 9 months (1-27)	16 ± 11 months (1-41)	7 ± 5 months (1-15)
Antibiotic therapy for chronic Q fever	18 (100)	7 (50)	3 (15)
Localization of infection	13 (72)	2 (14)	0
Definite endocarditis	4 (22)[†‡]	2 (14)[§]	0
Vascular prosthesis	3 (17)[‡]	0	0
Mycotic aneurysm	7 (39)	0	0
Focus unknown	5 (28)	12 (86)	20 (100)

* Adapted from Wegdam-Blans et al. [15].

† Definite endocarditis according to the modified Duke criteria.

‡ One patient had a definite endocarditis according to the modified Duke criteria and an infected vascular prosthesis.

§ Possible endocarditis according to the modified Duke criteria.

Abbreviation: *BMI* = body mass index.

Table 2: Risk factors for developing chronic Q fever in 52 patients with possible, probable and proven chronic Q fever*

	Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
	Number of patients (%)	Number of patients (%)	Number of patients (%)
Number of patients	18	14	20
Pre-existing valvular disease†‡	5 (28)	4 (29)	0
Mitral regurgitation	0	2 (14)	0
Tricuspid regurgitation	0	1 (7)	0
Bicuspid aortic valve	0	1 (7)	0
Congenital (not bicuspid aortic valve)	1 (6)	1 (7)	0
Rheumatic fever	1 (6)	0	0
Cardiac valve prosthesis†	4 (22)	0	0
Biological aortic prosthesis	*3 (17)*	*0*	*0*
Biological mitral prosthesis	*1 (6)*	*0*	*0*
Mechanical aortic prosthesis	*1 (6)*	*0*	*0*
Known aneurysm	8 (44)	1 (7)	0
Abdominal aortic aneurysm	7 (39)	0	0
Dilated aortic root	1 (6)	0	0
Cerebral aneurysm	0	1 (7)	0
Vascular prosthesis	11 (61)	4 (29)	0
Abdominal aortic graft	7 (39)	1 (7)	0
Thoracic aortic graft	2 (11)	0	0
PTA, iliacal or renal arteries	1 (6)	2 (14)	0
Goretex vascular shunt	1 (6)	0	0
Coiling of cerebral aneurysm	0	1 (7)	0
Immunocompromised	1 (6)	6 (43)	0
Immunosuppressive therapy	1 (6)	4 (29)	0
Myelodysplastic syndrome	0	2 (14)	0
Co-morbidity†	18 (100)	14 (100)	8 (40)
Chronic renal insufficiency	6 (33)	4 (29)	1 (5)
Diabetes	3 (17)	2 (14)	4 (20)
Active malignancy	1 (6)	4 (29)	1 (5)
Systemic sclerosis	1 (6)	2 (14)	0
COPD	2 (11)	3 (21)	5 (25)
Other§	5 (28)	3 (21)	3 (15)

* Adapted from Wegdam-Blans et al. [15].
† Multiple predisposing conditions are possible for a patient.
‡ Including cardiac valve prosthesis; valvulopathies were considered clinically significant if ≥grade II.
§ Including severe peripheral arterial disease, coronary artery bypass graft, congestive heart failure and liver cirrhosis.
Abbreviations: *PTA* = Percutaneous transluminal angioplasty, *COPD* = Chronic obstructive pulmonary disease.

Table 3: Diagnostics, treatment and outcomes in 52 patients with possible, probable and proven chronic Q fever*

	Proven chronic Q fever	Probable chronic Q fever	Possible chronic Q fever
	Number of patients (% or range)	Number of patients (% or range)	Number of patients (% or range)
Number of patients	18	14	20
Serum PCR	12 (67)	0	0
Tissue PCR	6 (33)	0	0
Anti-phase I IgG at diagnosis	4096 (256-65536)	2048 (1024-32768)	2048 (1024-16384)
CFT at diagnosis	1280 (0-20480)	320 (80-5120)	320 (40-2560)
Time to anti-phase I IgG <1024 (months)	23.3 ± 7.9 [n=4]	12.6 ± 3.9 [n=5]	7.5 ± 5.1 [n=8]
Time to negative serum PCR	3.6 ± 3.0 [n=7]	NA	NA
Complete diagnostic work-up	16 (89)	9 (64)	8 (40)
Abdominal ultrasound	8 (44)	6 (43)	8 (40)
Fluid collection	3	0	0
Increased diameter of aneurysm	1	0	0
Helpfulness	4/8 (50)	0	0
Screening abdominal CT	2 (11%)	1 (7)	-
Aneurysm	2	0	-
Suggestive of infected aneurysm or prosthesis	1	0	-
Helpfulness	2/2 (100)	0	-
CT on account of PET/CT	3 (17)	0	0
Aneurysm	2	-	-
Suggestive of infected aneurysm or prosthesis	3	-	-
Helpfulness	3/3 (100)	-	-
FDG-PET/CT	13 (72)	8 (57)	9 (45)
Focal uptake aneurysm	7	0	0
Focal uptake vascular prosthesis	3	0	0
Soft tissue inflammation	4	0	0
Para-aortal lymfadenopathy	1	0	0
Mediastinal lymfadenopathy	1	3	0
Unexpected findings	4	4	2
Helpfulness	10/13 (77)	0	0
TTE	16 (89)	13 (93)	12 (60)
Echocardiographic major criteria	0	0	0
Echocardiographic minor criteria	12	8	4
Helpfulness	1/16 (6)	1/13 (8)†	0
TEE	6 (33)	3 (21)	4 (20)
Echocardiographic major criteria	2	0	0
Echocardiographic minor criteria	6	1	3
Helpfulness	3/6 (50)	1/3 (33)†	0
Antibiotic therapy	18 (100)	7 (50)	3 (15)
Mortality during treatment	3	0	0
Ongoing treatment	13	7	2
Treatment completed successfully	2	0	1
Mean duration of treatment (months)	21.5 ± 6.4 [n=2]	-	2 [n=1]
Surgery	6 (33)	0	0
Aortic graft surgery‡	4 (22)	-	-
Cardiac valve surgery	2 (11)	-	-
Mortality	3 (17)	0	0

* Adapted from Wegdam-Blans et al. [15].

† TTE and TEE were considered helpful in 2 patients where pre-existing valvulopathies aggravated.

‡ One patient had surgery twice.

Abbreviations: *PCR* = Polymerase chain reaction, *CFT* = Complement fixation test, *NA* = Not applicable, *CT* = Computed tomography, *FDG-PET/CT* = ^{18}F-fluorodeoxyglucose positron emission tomography combined with CT, *TTE* = Transthoracic echocardiography, *TEE* = Transesophageal echocardiography.

Proven chronic Q fever

Proven chronic Q fever was diagnosed in 18 patients (*Table 1*). One patient developed systemic sclerosis during treatment. Only 8 patients (44%) recalled an episode of acute Q fever. Fourteen patients (78%) had symptomatic chronic infection: fever (9/14), abdominal pain (4/14), fatigue (3/14), weight loss (3/14), valvular dysfunction (3/14), night sweats (2/14) or lumbar pain (2/14). In two out of five patients with a pre-existing valvulopathy, valvular dysfunction occurred (left ventricular function deterioration due to Q fever endocarditis, and a new dysfunction of an artificial cardiac valve, as a consequence of Q fever endocarditis). One patient with valvular dysfunction was not familiar with a previous valvulopathy. The mean interval between symptomatic acute Q fever and the diagnosis of chronic Q fever was 12 ± 9 months (range: 1-27). Definite endocarditis was diagnosed in 4 patients (22%), an infected vascular prosthesis in 3 patients (17%), and an infected aneurysm in 7 patients (39%). One of these patients had both a definite endocarditis and an infected vascular prosthesis. In 5 patients (28%), no definite focus was identified. According to the modified Duke criteria, 4 of these patients had possible endocarditis and the fifth patient declined further diagnostic tests due to his age and underlying medical condition.

The median anti-phase I IgG titre at diagnosis was 4096 (range: 256-65536), and the median height of CFT was 1280 (range: 0-20480) (*Figures 1 and 2*). One patient had an anti-phase I IgG titre of only 256 and a negative CFT at diagnosis, but was considered to have proven chronic Q fever because serum PCR tested positive >1 month following primary infection. In 4 patients, the anti-phase I IgG titre decreased to <1024 after a mean duration of treatment of 23.3 ± 7.9 months. By PCR, *C. burnetii* DNA was successfully isolated from tissue samples (cardiac valve, vascular prosthesis) in 5 out of 6 patients who underwent surgery (1 patient underwent surgery twice). There was 1 positive PCR on fluid spontaneously draining from a fistula between an abscess around a vascular prosthesis and the skin. Four out of the 6 patients with positive fluid/tissue PCR were analyzed by FDG-PET/CT, all of which showed FDG-positive lesions. The other 2 were already found to have definite IE according to the modified Duke criteria and no FDG-PET/CT was performed. In these 2 patients, PCR was positive on infected cardiac valves that were replaced by surgery. In 7 of 12 patients with a positive serum PCR, PCR became negative after an average of 3.6 ± 3.0 months. Two patients died when PCR had not become negative yet, 1 patient was lost to follow-up, and 2 patients still had a positive serum PCR after 4 and 6 weeks of treatment, respectively.

A complete diagnostic work-up for chronic Q fever was performed in 16 patients (89%) (*Table 3*). In 2 patients, this work-up was incomplete: 1 patient refused further analysis, and in 1 patient only echocardiography was done. In 13 patients (72%), FDG-PET/CT was performed, which was helpful in identifying the site of infection in 10 of 13 investigations (77%). All 7 patients with an aneurysm as identified site of Q fever infection showed focal FDG-uptake of the aneurysm. Furthermore, all 3 patients with a vascular prosthesis as identified site of Q fever infection showed focal uptake around the vascular prosthesis (*Figure 3*). In 4 out of the 13 above mentioned FDG-PET/CT-scans, FDG-positive lesions were confirmed by positive *C. burnetii* PCR on tissue. In all of these 4 patients, FDG-PET/CT was conducted prior to PCR. In the remaining patients, surgery was not indicated and the lesions were very difficult to reach so tissue PCR could not be performed. Unexpected findings were observed

in 4 patients (31%). As a result, 2 patients required a biopsy because of focal FDG-uptake in the lungs, leading to the diagnosis of lung carcinoma in 1 patient and fibrosis in the other. In 1 patient, massive mediastinal lymphadenopathy was seen, eventually leading to the diagnosis of systemic sclerosis. CT was performed in 5 patients. Two of these investigations were done initially ('screening abdominal CT'), and the remaining 3 were conducted on account of a preceding abnormal FDG-PET/CT-scan (one chest CT and two CT-scans of both chest and abdomen). Both screening CT-scans enabled localization of infection and were considered helpful. The 3 CT-scans that were performed on the basis of pathology on FDG-PET/CT all confirmed the abnormal FDG-PET/CT findings. TTE was performed in 16 patients (89%); none of these examinations showed a major criterion, whereas echocardiographic minor criteria were seen in 12 patients (75%). Nevertheless, TTE was regarded helpful in 1 patient where nodular valvular thickening of an aortic bioprosthesis was seen. TEE was performed in 6 patients (33%), 4 following a prior TTE. In 2 patients, an echocardiographic major criterion was observed, whereas echocardiographic minor criteria were recorded in all of the performed TEEs. In 3 patients (50%), TEE was considered helpful: 2 because of echocardiographic major criteria and 1 as a result of aggravated pre-existing valvular disease. In 4 out of 5 patients with no definite localization and possible IE there were minor echocardiographic criteria. In all 5 patients TTE was performed. Two out of 5 TTE's showed minor criteria. In 4 patients TEE was performed, 3 of which showed minor criteria.
Long-term antibiotic treatment (doxycycline 200 mg/day and hydroxychloroquine 600 mg/day) was given to all patients. Thirteen patients (72%) are still under treatment, 3 of whom are being treated for more than 18 months. Three patients (17%) died during the course of therapy as a consequence of chronic Q fever infection. Death from chronic Q fever was defined as death as a result of active chronic infection. One patient died at 11 months following cardiac valve replacement due to progressive heart failure, probably as a result of artificial valve dysfunction due to chronic Q fever. PCR on valve tissue was positive. The second patient died in the perioperative period (in the first month) due to bleeding following acute aneurysm repair for a symptomatic aneurysm. PCR on aneurysm tissue was positive for Q fever. The third patient died in the perioperative period (in the first month) due to SIRS following acute cardiac valve replacement for severe Q fever endocarditis, with tissue PCR being positive. In 2 patients (11%), treatment was completed successfully after a treatment duration of 18 and 26 months with a follow-up after completion of treatment of 16 and 4 months, respectively. Six patients (33%) underwent surgery: abdominal aortic graft surgery with open repair was performed in 1 patient, endovascular aneurysm repair (EVAR) in 2 patients, first EVAR later followed by abscess drainage, excision of infected tissue and lavage with omentum plasty in 1 patient, and cardiac valve replacement in 2 patients.

Probable chronic Q fever

Probable chronic Q fever was diagnosed in 14 patients (*Table 1*). In this group, 6 patients (43%) were immunocompromised (*Table 2*). Twelve patients (86%) experienced symptomatic acute infection in the past. Two patients (14%) experienced symptoms of chronic infection: fever and night sweats (n=1), and weight loss and fatigue (n=1). The mean interval between acute Q fever and analysis for chronic infection was 16 ± 11 months (range: 1-41 months).

Figure 1: Titres of anti-phase I IgG at the time of chronic Q fever diagnosis

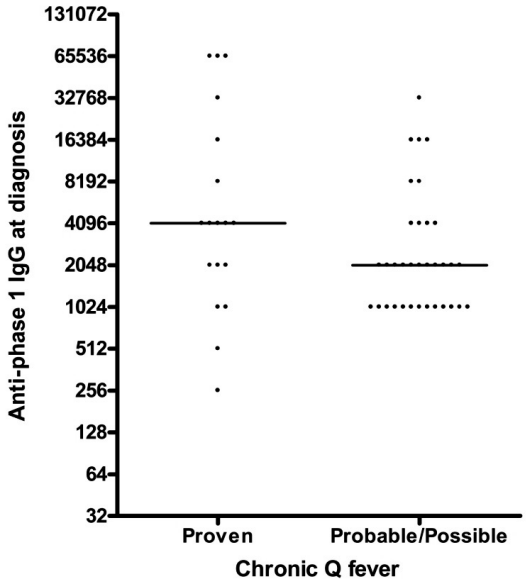
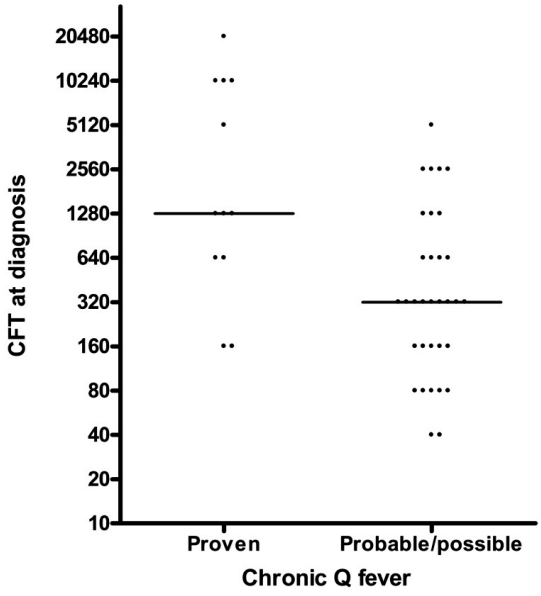


Figure 2: Titres of complement fixation test at the time of chronic Q fever diagnosis

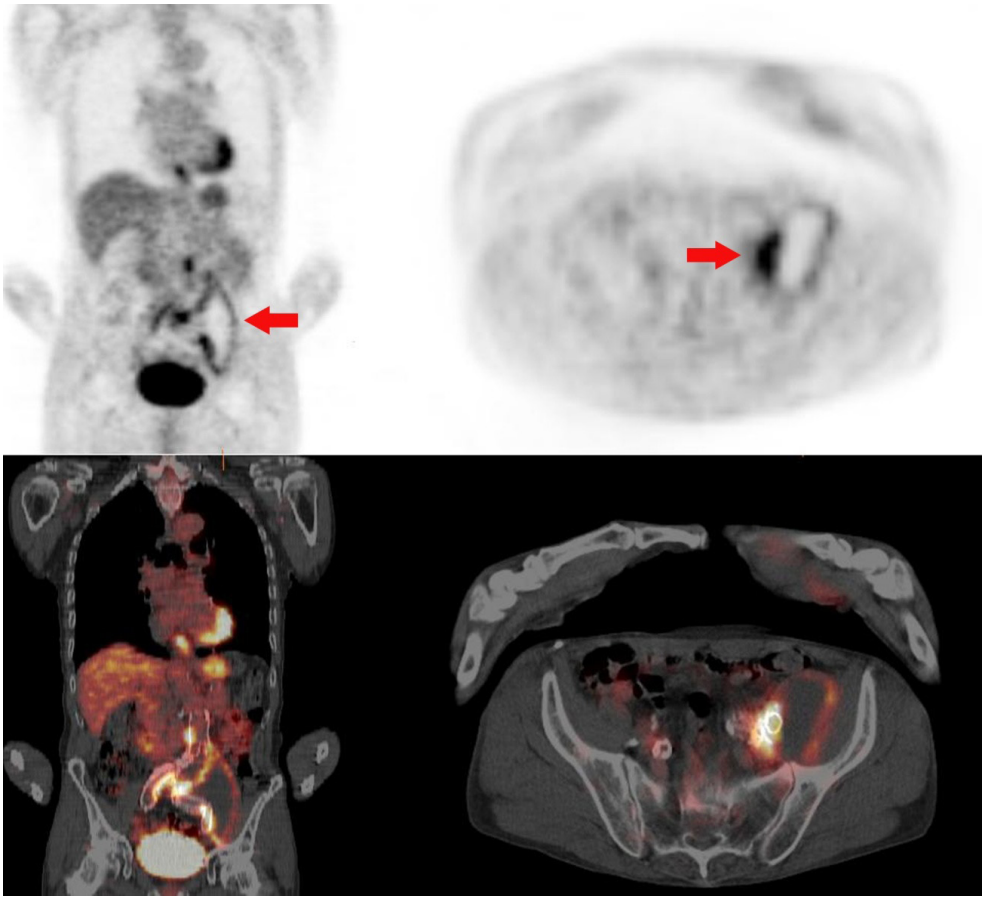


Abbreviation: *CFT* = Complement fixation test.

Figure 3. ^{18}F-FDG-PET/CT image demonstrating a mycotic aneurysm

^{18}F-FDG-PET/CT images (left column coronal sections, right column transverse sections, upper row PET images, lower row PET/CT fusion images) of a patient with proven chronic Q fever demonstrating a mycotic aneurysm and associated abscess adjacent to the left common iliac artery (arrows).

Abbreviations: *^{18}FDG-PET/CT* = ^{18}F-fluorodeoxyglucose positron emission tomography combined with CT.



While in 12 patients (86%) no focus was localized, endocarditis (possible endocarditis according to the modified Duke criteria) was regarded as the most probable site of infection in 2 cases.
The median anti-phase I IgG titre at first analysis was 2048 (range: 1024-32768) and the median CFT-value was 320 (range: 80-5120). In 5 patients the anti-phase I IgG titre decreased to <1024. Of these 5 patients, 2 received treatment for chronic Q fever and their anti-phase I IgG titre decreased to <1024 in 12 and 18 months, respectively. In the 3 patients without treatment, anti-phase I IgG titre decreased to <1024 after 4, 10, and 12 months, respectively.
A complete diagnostic work-up was performed in 9 patients (64%) (*Table 3*). Two patients were asymptomatic and considered low-risk, 1 patient refused further analysis because of co-morbidity, and in 2 patients FDG-PET/ CT was postponed (because of recent surgery and a concomitant severe pneumonia, respectively). FDG-PET/CT was performed in 8 patients (57%). None of these investigations localized infection (otherwise the patient would have proven chronic Q fever). Four of the performed FDG-PET/CT-scans (50%) revealed unexpected findings. These include mediastinal lymphadenopathy (eventually leading to the diagnosis of systemic sclerosis) in 1 patient, and focal FDG-uptake in the dental region in another patient. In 1 patient with multiple enlarged mediastinal lymph nodes, subsequent broncheoalveolar lavage (BAL) did not lead to a definitive diagnosis. In 1 patient, multiple unexpected findings were observed (focal uptake in the left thyroid gland followed by hemithyroidectomy leading to a diagnosis of adenoma and multiple foci in the prostate and iliac bone, leading to the diagnosis of prostate carcinoma). TTE was performed in 13 patients (93%) and was considered helpful once (8%), because progression of pre-existing valvular disease was observed. In 8 patients (62%), echocardiographic minor criteria were recorded. TEE was performed in 3 patients (21%), which was helpful in 1 patient. Echocardiographic minor criteria were seen in 1 patient (33%).
Seven patients (50%) received long-term treatment with antibiotics (doxycycline 200 mg/ day and hydroxychloroquine 600 mg/day); none of these patients completed treatment yet. Of the remaining 7 patients, a decision on treatment was pending in 2 patients, 3 were not treated because of severe co-morbidity, and 3 were asymptomatic and considered low-risk. All patients that were not on antibiotic treatment were followed closely.

Possible chronic Q fever
Twenty patients were diagnosed with possible chronic Q fever (*Table 1*). Thirteen (65%) patients could recall a symptomatic episode of acute Q fever and none of the patients experienced symptoms of chronic infection. The mean interval between acute infection and analysis for chronic Q fever was 7 ± 5 months (range: 1-15 months). In 77% of the patients with possible chronic Q fever and a previously known episode of acute Q fever, routine serological follow-up at 3, 6, 9 and 12 months was performed.
The median anti-phase I IgG titre at first analysis was 2048 (range: 1024-16384), and the median CFT-value was 320 (range: 40-2560). In 8 patients, the anti-phase I IgG titre decreased to <1024, with an average of 7.5 ± 5.1 months. Of these patients, 1 patient was treated and the anti-phase I IgG titre decreased to <1024 within 7 months.
A complete diagnostic work-up was performed in 8 out of 20 patients (40%) (*Table 3*).

Six patients were asymptomatic and considered low-risk, 1 patient suffered from severe co-morbidity, 3 patients were lost to follow-up, and 2 patients were not yet completely analyzed. FDG-PET/CT was performed in 9 patients (45%). None of these investigations were helpful. Two FDG-PET/CT-scans (22%) resulted in an unexpected finding: 1 patient with FDG-uptake in the colon, followed by colonoscopy diagnosing a non-neoplastic polyp, and 1 patient with FDG-uptake in the left clavicle, followed by CT that was normal. TTE was performed in 12 patients (60%) and was considered helpful in none of the investigations. In 4 patients (33%), echocardiographic minor criteria were recorded. TEE was performed in 4 patients (20%), being helpful in none of the patients. Echocardiographic minor criteria were seen in 3 patients (75%).
Long-term antibiotic treatment was prescribed to three patients (15%) because of debilitating symptoms (severe fatigue and muscle ache). One of these patients initially started treatment because of suspected chronic Q fever, but stopped after 2 months because anti-phase I IgG titres were rapidly decreasing. The 2 other patients had not completed treatment yet. Of the remaining 17 patients, 11 were considered low-risk, in 5 a decision on treatment was pending, and 1 patient had severe co-morbidity.

Comparison between patients with proven chronic Q fever and patients with probable and possible chronic Q fever

In order to evaluate potential differences between patients with proven chronic Q fever and those with possible or probable chronic Q fever, data were compared by univariate analysis (*Table 4*). Age at diagnosis, history of smoking, and mean interval from acute infection to analysis for chronic Q fever did not differ significantly between the groups. Male sex ($p=0.04$) and symptomatic chronic infection ($p<0.01$) were significantly more present in patients with proven chronic Q fever. Concerning risk factors, which were found previously in other studies, the presence of pre-existing valvular disease, indication for endocarditis prophylaxis, and immunodeficiency did not differ significantly between the groups in our study. In contrast, cardiac valve prostheses ($p=0.01$), known aneurysms ($p<0.01$), and vascular prostheses ($p<0.01$) were significantly associated with proven chronic Q fever.
Anti-phase I IgG ($p=0.01$) and CFT-values ($p<0.01$) were significantly higher in patients with proven chronic Q fever when compared to the groups of probable and possible Q fever combined (*Figures 1 and 2*). Also, the mean time to anti-phase I IgG <1024 was significantly longer in this group ($p<0.01$). In contrast to AUS and FDG-PET/CT, the helpfulness of CT, TTE and TEE showed no significant differences between the groups. Both antibiotic treatment ($p<0.01$) and surgery ($p<0.01$) were used more often in patients with proven chronic Q fever. Most importantly, a clear association was seen between proven chronic Q fever and mortality rates ($p=0.03$).

Table 4: Significant differences between patients with proven chronic Q fever and patients with probable and possible chronic Q fever (univariate analysis)*

	Proven chronic Q fever	Probable and possible chronic Q fever	Significance
	Number of patients (% or range)	Number of patients (% or range)	(p-value)
Patient characteristics			
Number of patients	18	34	
Male sex	17 (94)	19 (56)	0.04
Symptomatic chronic infection	14 (78)	2 (6)	<0.0001
Cardiac valve prosthesis	4 (22)	0	0.01
Known aneurysm	8 (44)	1 (3)	0.0004
Abdominal aortic aneurysm, infrarenal	7 (39)	0	0.0003
Vascular prosthesis	11 (61)	4 (12)	0.004
Co-morbidities	18 (100)	22 (65)	0.021
Diagnostic work-up	16 (89)	17 (50)	0.04
Positive serum PCR	12 (67)	0	<0.0001
Positive tissue PCR	6 (33)	0	0.011
Anti-phase I IgG at diagnosis	4096 (256-65536)	2048 (1024-32768)	0.013
CFT at diagnosis	1280 (0-20480)	320 (40-5120)	0.001
Months to anti-phase I IgG <1024	23.3 ± 7.9 [n=4]	9.5 ± 5.2 [n=13]	0.001
Helpfulness of abdominal ultrasound	4/8 (50)	0/14 (0)	0.01
Helpfulness of FDG-PET/CT	10/13 (77)	0	<0.0001
Antibiotic therapy	18 (100)	10 (29)	<0.0001
Mortality during treatment	3/18 (17)	0	0.037
Ongoing treatment	13/18 (72)	9/10 (90)	0.008
Surgery	6 (33)	0	0.001
Aortic graft surgery†	4 (22)	0	0.011
Cardiac valve surgery	2 (11)	0	NS
Mortality	3 (17)	0	0.033

* Adapted from Wegdam-Blans et al. [15].

† One patient had surgery twice.

Abbreviations: *PCR* = polymerase chain reaction; *CFT* = complement fixation test; *FDG-PET/CT* = ^{18}F-fluorodeoxyglucose positron emission tomography; *NS* = not significant.

Analysis after adjustments to the modified Duke criteria

The modified Duke criteria and the 2 aforementioned adjustments to these criteria were applied to all patients (*Table 5*). Applying the modified Duke criteria, 4 cases of definite IE were diagnosed, and 20 cases of possible IE. Of 20 patients with possible IE (all groups), 11 out of 19 patients who underwent TTE had minor criteria by TTE, and 4 out of 7 patients who underwent TEE had minor criteria. When echocardiographic minor criteria were included (first adjustment), 8 cases were considered definite IE and 28 cases possible IE. Including a positive serum PCR for *C. burnetii* as a major criterion (second adjustment), 12 patients scored definite IE and 14 possible IE. The modified Duke criteria were compared with the modified Duke criteria including our first and second adjustments, respectively, by a 2-tailed Wilcoxon test, which showed significant differences (p=0.046 and p<0.01, respectively).

Table 5: Comparison of (adjustments to) modified Duke criteria: complete case series

	Modified Duke criteria [27]	Modified Duke criteria, including echocardiographic minor criteria [31]	Significance† (comparison with modified Duke criteria) (p-value)‡	Modified Duke criteria, including PCR as a major criterion [30]	Significance† (comparison with modified Duke criteria) (p-value)§
Definite IE (%)	4 (9)	8 (19)	0.046	12 (28)	0.005
Possible IE (%)	20 (47)	28 (65)	0.046	14 (33)	0.034
Rejected IE (%)	19 (44)	7 (16)	0.001	17 (40)	0.157
Total	43*	43*	-	43*	-

† Wilcoxon test, 2-tailed.
‡ Modified Duke criteria compared to 'modified Duke criteria, including echocardiographic minor criteria'.
§ Modified Duke criteria compared to 'modified Duke criteria, including PCR as a major criterion'.
* Nine patients were not examined by echocardiography; the modified Duke criteria could therefore not be calculated.
Abbreviations: *PCR* = polymerase chain reaction; *IE* = infective endocarditis.

DISCUSSION

In this study, the diagnostic work-up of 52 patients with chronic Q fever according to the *Dutch consensus on Q fever diagnostics* was evaluated. We demonstrated that FDG-PET/CT might be a valuable tool for localization of vascular infection with *C. burnetii*. It was shown that infected aneurysms or vascular prostheses are the most common manifestation of proven chronic Q fever in our population.

The mean age of patients was similar to previously reported case series of chronic Q fever [6, 8, 17]. The overall male predominance has been shown before as well, but the portion of male patients with proven chronic Q fever (94%) was distinct. This possibly results from a higher incidence of aneurysms and cardiovascular disease in male subjects, which are clear risk factors for developing chronic Q fever [3, 8, 33]. A history of smoking was established as a risk factor for chronic Q fever, especially in those patients with proven chronic Q fever. Smoking was not included in the possible risk factors for developing chronic Q fever in the recently published Dutch study by Kampschreur et al. [33]. Furthermore, patients with proven chronic Q fever more often had a cardiac valve prosthesis, a known aneurysm, or a vascular prosthesis as was also found by Kampschreur et al. [33]. Although reported in some previous studies, pre-existing valvular disease other than valve prosthesis did not appear to be an important risk factor in this study [6, 14, 33]. A similar observation was done by another Dutch group [34, 35], that found a low risk of progression to Q fever endocarditis in the presence of degenerative valvular disease.

Only 44% of patients with proven chronic Q fever could recall an episode of acute Q fever, compared to 74% of those with possible/probable Q fever. Symptomatic acute infection most often results in antibiotic treatment, which might reduce the chance of developing proven chronic Q fever. In addition, in patients with acute Q fever, serological follow-up is performed while this was of course not the case in patients without symptomatic (and thus usually unknown) infection. It is possible that elevating titres of IgG anti-phase I found during follow-up led to earlier diagnosis and treatment, possibly preventing progression from possible and probable chronic Q fever to proven chronic Q fever.

A large retrospective study from France identified endocarditis as the predominant manifestation of chronic Q fever (73% of cases) [8]. In contrast, only 22% of our patients with proven chronic Q fever have been diagnosed with Q fever endocarditis. Infected aneurysms and infected vascular prostheses were found in 39% and 17% of patients, respectively. It has been suggested that mycotic aneurysms may be caused by non-diagnosed endocarditis in patients with chronic Q fever. However, applying the modified Duke criteria to all patients with proven chronic Q fever, only 1 patient had an infected vascular prosthesis and definite IE at the same time. One patient with an infected vascular prosthesis and 1 patient with an infected aneurysm had rejected IE according to the modified Duke criteria. The last patient with an infected vascular prosthesis and all other patients with an infected aneurysm had possible IE according to the modified Duke criteria. The cause of this striking difference in predominant manifestation of chronic Q fever remains largely unclear, and probably results from a combination of factors. First, most patients in other series were evaluated because of endocarditis, whereas in our case series, also other complaints (fever, night sweats, presence of aneurysm) and routine serological follow-up after acute Q fever led to evaluation for Q fever because of the current epidemic. In addition, not all patients underwent echocardiography, possibly leading to an underestimation of endocarditis in our group. Furthermore, it is possible that in those patients without a full diagnostic work-up only one site of infection was notified, whereas it is possible that patients had 2 sites of infection. Second, pre-existing valvular disease was seen less often in this case series than in those patients reported in literature. This could be influenced by the fact that screening echocardiography is not performed in patients with acute Q fever in the Netherlands. Although our study did not found pre-existing valvular disease to be a significant risk factor for proven chronic Q fever, this contrasts with previous studies [6, 14], but is in accordance with the other Dutch study on risk factors for developing proven chronic Q fever [33]. Third, the Dutch *C. burnetii* strain is possibly more likely to cause endovascular infection other than endocarditis. Even though it is possible that more vascular infections were found because FDG-PET/CT was performed more often, it is unlikely that vascular infections would go unnoticed in other chronic Q fever series, in which hardly any vascular infection was seen. If these patients would have had unidentified vascular infection in addition to endocarditis, more complications would be expected because of the high mortality rate of vascular chronic Q fever, even in case of optimal (surgical) treatment. Finally, it is not clear if other research groups applied the modified Duke criteria in the same strict manner as we did for this study. In 1994, Durack et al. [31] introduced a new set of diagnostic criteria for IE that subsequently came to be known as the Duke criteria. Li and colleagues [27] proposed modifications to the Duke criteria in 2000, adding a positive serology for *C. burnetii* as a major criterion, which had already been proposed earlier by Fournier et al. [18]. In addition, the modifications included the elimination of echocardiographic minor criteria, because a widespread use of TEE was assumed. It is well-recognized that the sensitivity of these criteria is diminished in Q fever endocarditis, since it is known for its subtle valve abnormalities and absence of vegetations [4, 18, 24]. Nonetheless, we strictly applied the modified Duke criteria to this case series, resulting in only 4 patients (9%) with definite IE and 20 patients (47%) with possible IE. Even if we merely reflect on patients with proven chronic Q fever, the percentage of definite IE

was only 22%. There were another 5 patients (28%) with an unidentified focus, 4 of whom had possible IE according to the modified Duke criteria. TEE was performed in a minority of patients (25%), while the elimination of echocardiographic minor criteria was based on the widespread use of TEE [27]. We cannot rule out the possibility that in some patients vegetations were missed because TTE was conducted exclusively.

In the past, several adjustments have been proposed to further improve the sensitivity of the modified Duke criteria. One of these adjustments was the use of PCR techniques as a major criterion [30], which is not implemented in international guidelines. However, in a recent study on Q fever endocarditis [6], a positive serum PCR served as major criterion. It is not clear whether PCR was an additional major criterion or served as substitute for serology. The theoretical addition of a positive serum PCR as major criterion to the modified Duke criteria appeared most useful. From our experience, we suggest that a positive serum PCR for *C. burnetii* in patients with chronic Q fever without an identified site of infection should be treated as Q fever endocarditis. Furthermore, the presence of echocardiographic minor criteria should raise the clinician's suspicion of endocarditis, and TEE should be performed in all patients with chronic Q fever with an unknown focus. It is essential to bear in mind that the Duke criteria are useful for the classification of IE, but that they were designed for research purposes and thus should not replace clinical judgment in clinical practice.

CT was performed initially in only 2 patients with proven chronic Q fever, making it impossible to estimate the helpfulness of this technique. In contrast, FDG-PET/CT, localized infection in 77% of patients with proven chronic Q fever, which suggests that FDG-PET/CT is a valuable tool for the localization of vascular Q fever infection. FDG-PET/CT is also very well suited for diagnosing osteomyelitis, which is another possible focus of chronic Q fever. A well-recognized disadvantage of FDG-PET/CT is its specificity, as it does not differentiate between inflammation, infection, and malignancy. As such, unexpected findings were observed in 9 patients (30%), including the detection of previously unknown malignancies in 2 patients and newly diagnosed systemic sclerosis in another 2 patients. Five patients underwent invasive diagnostic procedures as a result of suspected malignancies, but pathological examination remained negative. The number of unexpected findings is higher than found in previous studies on the use of FDG-PET in other infections and fever of unknown origin (FUO) [36, 37], which might be explained by the higher age of the patients and the male predominance in combination with a higher than average percentage of smokers, increasing the risk of associated malignancy when compared to patients with FUO. A limitation of our study is of course its retrospective character. Unfortunately, not all patients underwent a complete diagnostic work-up. Therefore, it is important to bear in mind that some patients might have had two sites of infection, which might have been missed. This emphasizes the need for a full diagnostic work-up in patients with chronic Q fever. Also, the time point of diagnostic imaging in the course of infection differed between the patients, which might have influenced the helpfulness.

CONCLUSIONS

In conclusion, if chronic Q fever is diagnosed, FDG-PET/CT is a helpful imaging technique for localization of vascular infection. Patients with proven chronic Q fever were diagnosed

significantly more often with mycotic aneurysms than in previous case series. Theoretical adjustment of the modified Duke criteria by adding serum PCR as a major criterion results in more diagnoses of Q fever endocarditis. We recommend treating patients with chronic Q fever with a positive serum PCR for *C. burnetii* without an identified site of infection as Q fever endocarditis. To increase sensitivity after previous exclusion of echocardiographic minor criteria from the modified Duke criteria, TEE is recommended in patients with chronic Q fever. A minority of all patients with proven chronic Q fever recalls a previous episode of acute Q fever, so clinical suspicion should remain high, especially in endemic regions.

AUTHORS' CONTRIBUTIONS

CB and CD planned and designed the research study, and have been involved in the analysis and interpretation of data, as well as critical revision of the manuscript. DB has been involved in the design and acquisition of data, has done the analysis and interpretation of data and drafted the manuscript. SK has been involved in the design of the study, and participated in interpretation of results and critical revision of the manuscript. JT and WO participated in interpretation of results and revision of the manuscript. TS and MN provided microbiological expertise and patient data. All authors read and approved the final manuscript.

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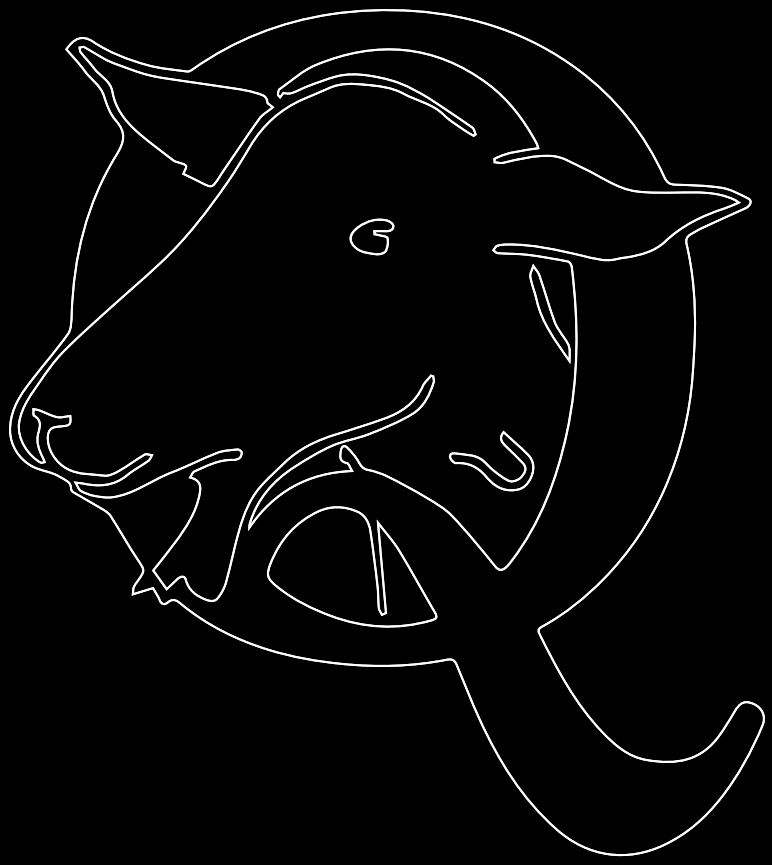

CHAPTER 9

CUTANEOUS HYPERPIGMENTATION INDUCED BY DOXYCYCLINE: A CASE SERIES

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ABSTRACT

Cutaneous hyperpigmentation is a well-known side effect of tetracyclines, but doxycycline-induced cutaneous hyperpigmentation has only been described in one patient with a therapeutic dosage of doxycycline, and in one patient using suprapharmacological doses. We describe four patients with cutaneous hyperpigmentation in previously unaffected skin, and speculate that this was due to treatment with doxycycline in therapeutic doses. After cessation of therapy, the hyperpigmentation diminished in all four patients, illustrating the need for recognition and timely cessation of therapy.

What was known on this topic?

Cutaneous hyperpigmentation induced by doxycycline is a very uncommon side effect.

What does this add?

Cutaneous hyperpigmentation is a potential side effect of doxycycline. Awareness and recognition of this reversible or partially reversible side effect of this widespread prescribed antibiotic is necessary in order to discontinue therapy in time.

INTRODUCTION

Well-known side effects of doxycycline are photosensitivity, teeth discolouration, nausea, vomiting, and diarrhoea. Cutaneous hyperpigmentation is a common side effect of minocycline and, to a lesser extent, of other tetracyclines, with only one report of a patient with progressive, symmetric blue-grey periocular discolouration due to three years of treatment with therapeutic doses of doxycycline [1]. Furthermore, hyperpigmentation has been described in one patient with self-induced intoxication by doxycycline (1 gm/day) for 12 years [2]. Both brown discolouration of the fingernails and discolouration of acne scars have been described after a short course of doxycycline [3, 4]. We report four patients who received long-term treatment with doxycycline and hydroxychloroquine because of either chronic Q fever or Whipple's disease. They showed extensive cutaneous hyperpigmentation in previously unaffected skin, probably induced by doxycycline.

CASE DESCRIPTIONS

Case 1

A 75-year-old man with an abdominal aneurysm, immunosuppressive therapy because of rheumatoid arthritis and a known valvulopathy was diagnosed with chronic Q fever. Doxycycline 200 mg/day was initiated, in addition to hydroxychloroquine 400 mg/day, which he had already been taking for more than five years because of rheumatoid arthritis. After four months, doxycycline 300 mg/day was introduced because of persistently low doxycycline levels. Eight months after the start of therapy, progressive bluish-purple to black cutaneous hyperpigmentation of his lower arms, back of his hands, and interdigital areas (*Figure 1A*) developed since increasing the doxycycline dose (serum concentrations of 5.8 mg/ml). The doxycycline was stopped and hydroxychloroquine was continued. The hyperpigmentation slowly diminished, but 12 months later dark bluish-grey macules were still visible on the back of his hands and his lower arms (*Figure 1B*).

Case 2

A 72-year-old man, diagnosed with relapse of Whipple's disease, was treated with ceftriaxone for four weeks, followed by doxycycline 200 mg/day and hydroxychloroquine 600 mg/day. Eight months later, increasing black discolouration on the back of both hands was seen (doxycycline serum concentrations of 5.7 mg/ml) (*Figure 2A*). Therapy was stopped, and co-trimoxazole was reintroduced. Ten months later his cutaneous hyperpigmentation was slowly fading (*Figure 2B*).

Case 3

A 71-year-old man with an endovascular aneurysm repair (EVAR) and a femoral-popliteal bypass was referred because of aortitis due to chronic Q fever, and started on doxycycline 200 mg/day and hydroxychloroquine 600 mg/day. After 48 months of therapy, he reported increasing pretibial bluish-brown-black discolouration on both legs, and the dorsal side of his feet (*Figure 3*). In retrospect, the discolouration started 11 months before, but he had never reported it. Doxycycline and hydroxychloroquine were substituted by moxifloxacin and rifampicin. Six months later, the discolouration diminished.

Case 4

A 72-year-old man with an infected EVAR with retroperitoneal abscesses due to chronic Q fever was referred for surgery. He had already received six months of doxycycline 300 mg/day and hydroxychloroquine 600 mg/day (doxycycline serum concentration: 6.2 mg/ml), which was continued post-surgery. For six months, he received doxycycline 200 mg/day because of side effects. However, because of a low doxycycline serum concentration (2.8 mg/ml), doxycycline 300 mg/day was reintroduced, leading to a near-therapeutic concentration (4.7 mg/ml). Eight months post-surgery, he presented with increasing black discolouration around the surgical scars on both legs. Doxycycline and hydroxychloroquine were substituted by moxifloxacin. Two months later, the black discolouration diminished.

DISCUSSION

We describe four patients with hyperpigmentation of previously healthy skin after prolonged use of doxycycline. This has been described before in only one patient with therapeutic doses of doxycycline [1], and in a patient with self-induced doxycycline intoxication (1 g/day during 12 years leading to doxycycline serum concentrations of 34 mg/ml, normal therapeutic range: 1-5 mg/ml, for chronic Q fever: 5-10 mg/ml)[2, 5]. In our cases, patients received relatively high doses with serum concentrations in the therapeutic range, and developed marked cutaneous hyperpigmentation. However, compared with other indications for which doxycycline is given, chronic Q fever and Whipple's disease require prolonged treatment with a higher therapeutic range. Because tetracyclines produce autofluorescence, with positive *in-vivo* conjunctival autofluorescence of palpebral conjunctival minocycline deposits [6], the hyperpigmentation of the first two cases was investigated with Wood's light (extinction 365 nm). However, no fluorescent signal was obtained (*Figures 1C and 2C*). This may have been due to the long time that elapsed between the cessation of doxycycline and this investigation (12 and 10 months, respectively). As the dorsal side of the hands of the first patient still showed clear pigmentations (*Figure 1B*), the pigment might not represent the doxycycline itself. Previously, biopsies of doxycycline-induced hyperpigmentation revealed increased melanisation in the basal layers of the epidermal keratinocytes [4, 5], suggesting activation of melanocytes either by the tetracycline derivative itself or by another co-stimulus. Also, indications were found for the presence of melanin or melanin-like pigment in the histiocytes of the upper dermis. In contrast, in histiocytes of the lower dermis and subcutaneous fat, pigment was stored with increased amounts of iron and calcium, and no melanosomes were detected, suggesting a different nature of the pigment. Furthermore, data suggested that doxycycline, possibly chelated with iron and/or calcium, was directly deposited in the lesional skin [5]. The role of hydroxychloroquine and its interaction with doxycycline in these cases cannot be completely ruled out, as cutaneous hyperpigmentation induced by hydroxychloroquine has been described in 13% of treated patients, mainly as a bluish-grey pigmentation [7], mostly localised at the hard palate, gums, face, and pretibial area [8]. To our knowledge, no literature exists describing an increased risk of hyperpigmentation using doxycycline and hydroxychloroquine concomitantly. As both medications can cause cutaneous hyperpigmentation a synergistic effect on the development of hyperpigmentation might exist. However, based on the localisation of

hyperpigmentation, without mucosal involvement [9-11], doxycycline is still thought to be the main aetiological agent in our cases. Furthermore, in the first patient, hyperpigmentation developed after introduction of doxycycline 300 mg/day, and significantly diminished after stopping doxycycline, while hydroxychloroquine was continued. And, as seen in our fourth patient, discolouration restricted to scars has been reported with doxycycline [4]. Most described cases of cutaneous hyperpigmentation during tetracycline treatment are induced by minocycline [12], which is frequently prescribed for long periods. However, indications for prolonged therapy with doxycycline also exist, with an increasing number of chronic Q fever patients [13]. It should be advised to discontinue therapy. As in our patients, partial to complete resolution of cutaneous hyperpigmentation has been described eight months after cessation of prolonged doxycycline therapy [1]. Furthermore, in the case with doxycycline intoxication, the pretibial hyperpigmentation had faded significantly one year after doxycycline cessation [2]. Finally, almost complete disappearance of methacycline-induced hyperpigmentation was reported five years after onset, except for two patients who were substituted with doxycycline [14]. Complete disappearance of hyperpigmentation after cessation of therapy is possible; however, recovery may take up to several years [14].
In conclusion, cutaneous hyperpigmentation is a potential side effect of doxycycline therapy within the therapeutic dose range, and the chance to evoke this adverse effect might be increased with the concomitant use of hydroxychloroquine. Given the widespread use of doxycycline, in both short and prolonged regimens, it is important to recognise this reversible or partially reversible side effect in order to discontinue therapy. Especially its use in chronic Q fever, when prolonged relatively high doses are given nowadays in combination with hydroxychloroquine, prescribers and patients should be aware of this side effect.

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Figure 1

A 75-year-old man with chronic Q fever, with a progressive bluish-purple to black cutaneous hyperpigmentation of his lower arms, back of his hands, and interdigital area, during therapy with doxycycline (A). Twelve months after stopping doxycycline, the cutaneous hyperpigmentation had diminished. However, dark bluish-grey macules were still visible (B). No fluorescent signal of the hyperpigmentation was obtained using Wood's light, 12 months after cessation of therapy (C).

Figure 2

A 72-year old man, with Whipple's disease, presented with black discolouration on the back of his hands during therapy with doxycycline and hydroxychloroquine (A). Ten months after discontinuation of therapy, the cutaneous hyperpigmentation was significantly reduced, but confluating grey-brown-bluish macules were still visible (B). Wood's light investigation showed no fluorescent signal, ten months after cessation of therapy (C).

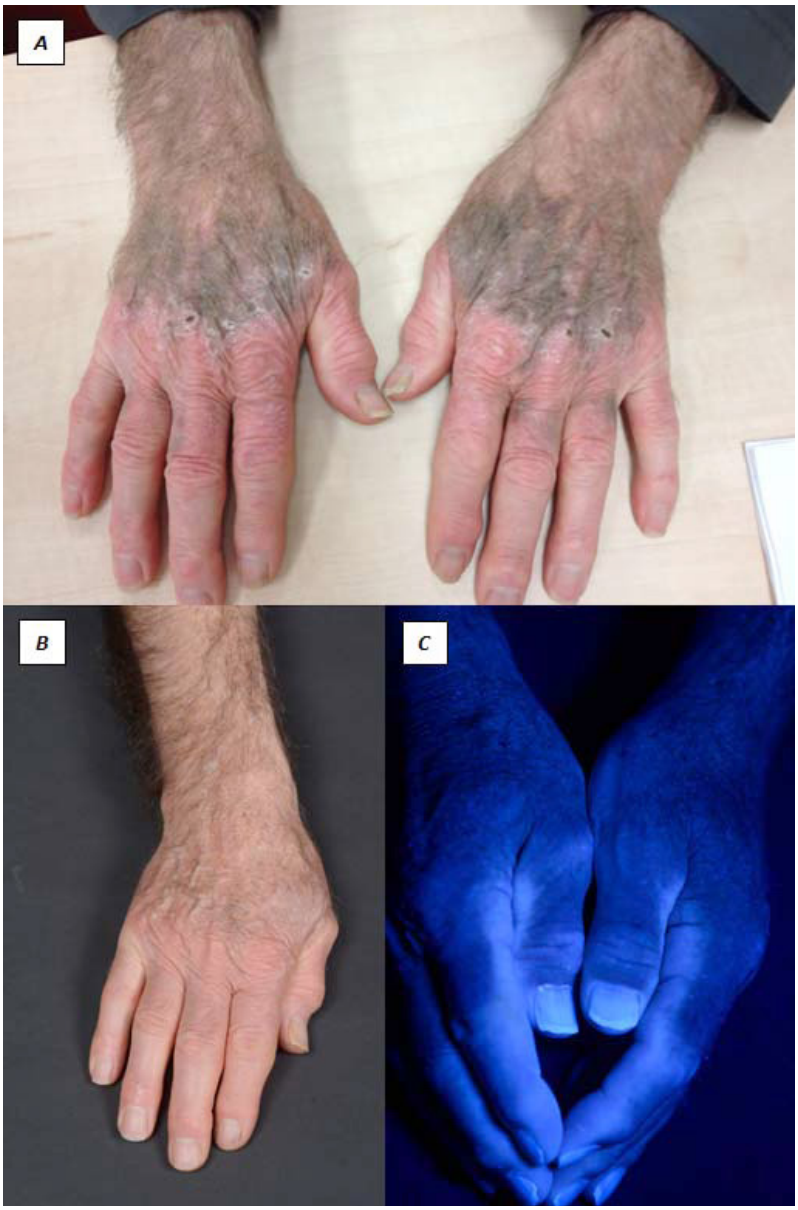
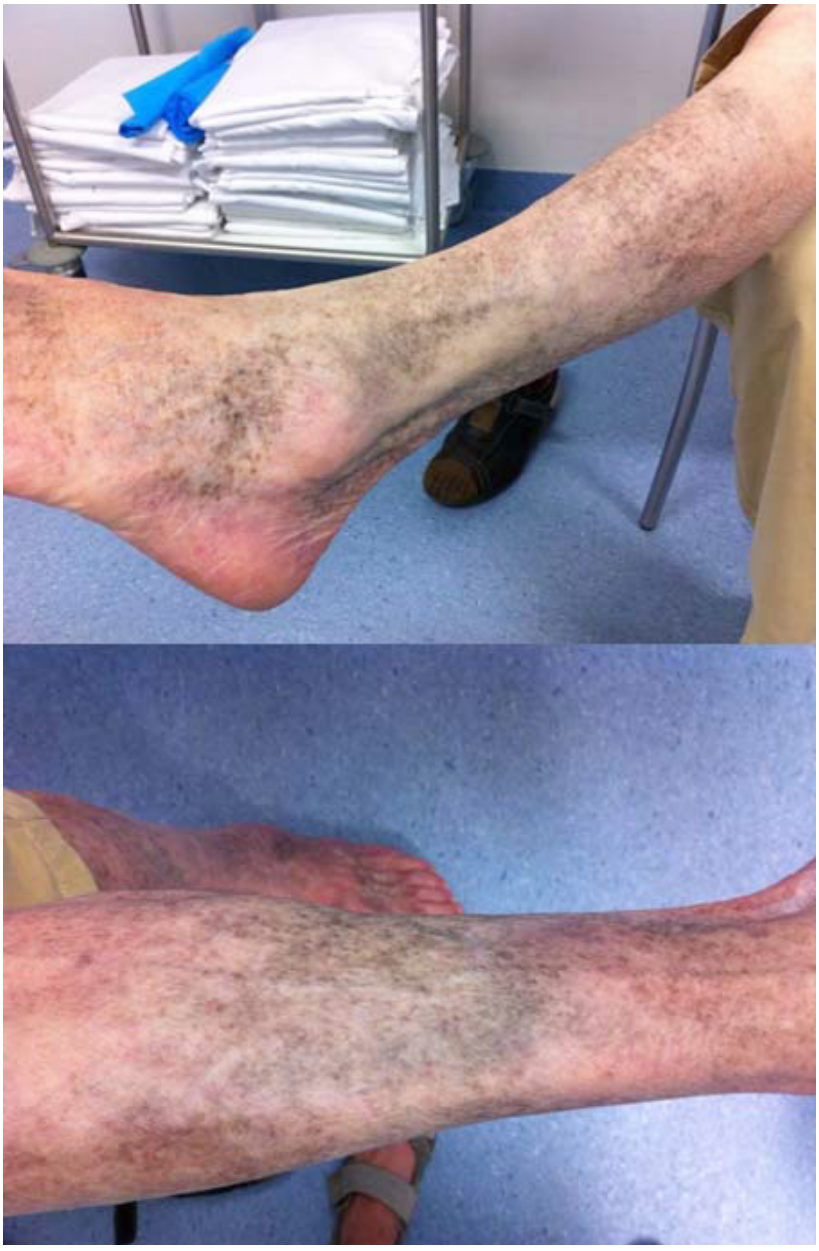
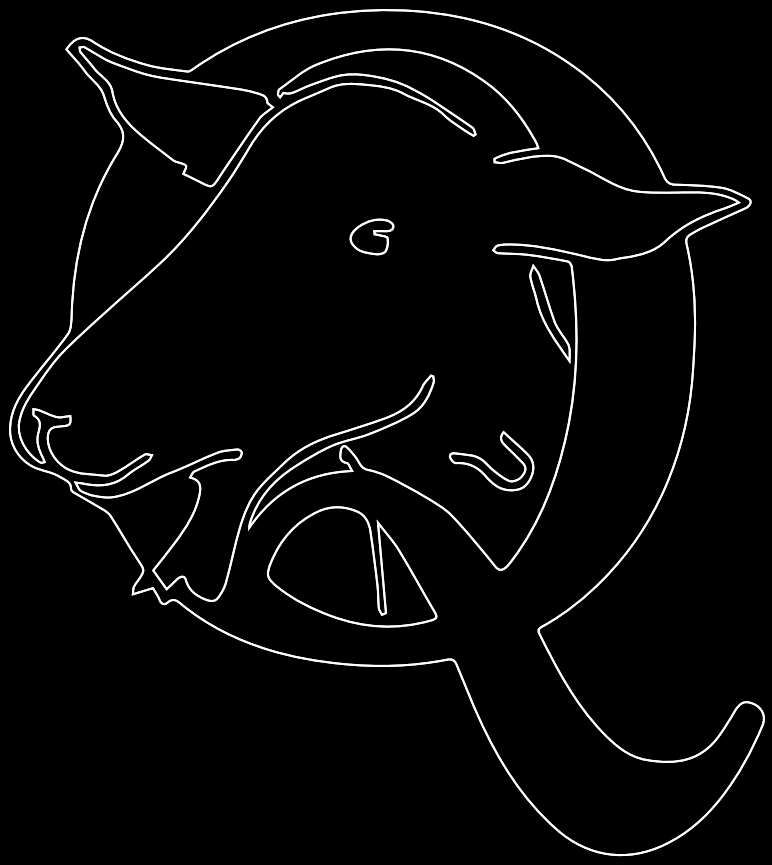


Figure 3

A 71-year-old man with chronic Q fever developed an increasing bluish-brown-black pretibial discolouration on both legs, and the dorsal side of his feet, during therapy with doxycycline and hydroxychloroquine. Six months after stopping therapy, the cutaneous discolouration had clearly diminished.





CHAPTER 10

A FATAL CASE OF DISSEMINATED CHRONIC Q FEVER: A CASE REPORT AND BRIEF REVIEW OF THE LITERATURE

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ABSTRACT

Background: Chronic Q fever is a rare infection, which mainly manifests as endocarditis, infection of vascular prostheses or aortic aneurysms. We present the case of a 74-year-old immunocompromised man with a haematologically disseminated *Coxiella burnetii* infection, which has never been reported before.

Case report: He was diagnosed with a chronic Q fever infection of an aneurysm with an endovascular prosthesis in 2015, but he died despite optimal treatment. Autopsy revealed a disseminated *C. burnetii* infection, confirmed by a positive PCR on samples from several organs. Retrospectively, he already had complaints and signs of inflammation since 2012, for which he had already been admitted in February 2014. At that time, Q fever diagnostics using PCR, complement fixation assay, and enzyme-linked immunosorbent assay on serum were all negative. In retrospect however, retesting available samples from February 2014 using immunofluorescence assay (IFA) already revealed serology compatible with chronic Q fever.

Conclusion: Clinicians should be aware of this silent killer, especially in case of risk factors, and perform an appropriate diagnostic work-up for Q fever including IFA serology and PCR.

INTRODUCTION

Following primary infection with *Coxiella burnetii*, an intracellular Gram-negative coccobacillus, 1-5% of patients develop chronic Q fever, which is characterized by the persistence of *C. burnetii*. Chronic Q fever mainly manifests as endocarditis, infection of vascular prostheses or aortic aneurysms, or both [1]. Increasingly, other manifestations are reported, such as osteomyelitis, pericarditis, hepatitis, pseudotumor(s) of the lung, chronic pulmonary fibrosis, cerebral venous thrombosis, and musculoskeletal infections [2, 3]. However, there are no reports describing a disseminated chronic Q fever infection with both locoregional and haematogenous seeding of *C. burnetii*. We report a fatal case of a disseminated chronic Q fever infection, confirmed by positive PCR for *C. burnetii* on lung tissue, an endovascular aneurysm repair (EVAR) specimen, a psoas abscess specimen, and ascites from the abdominal right lower quadrant.

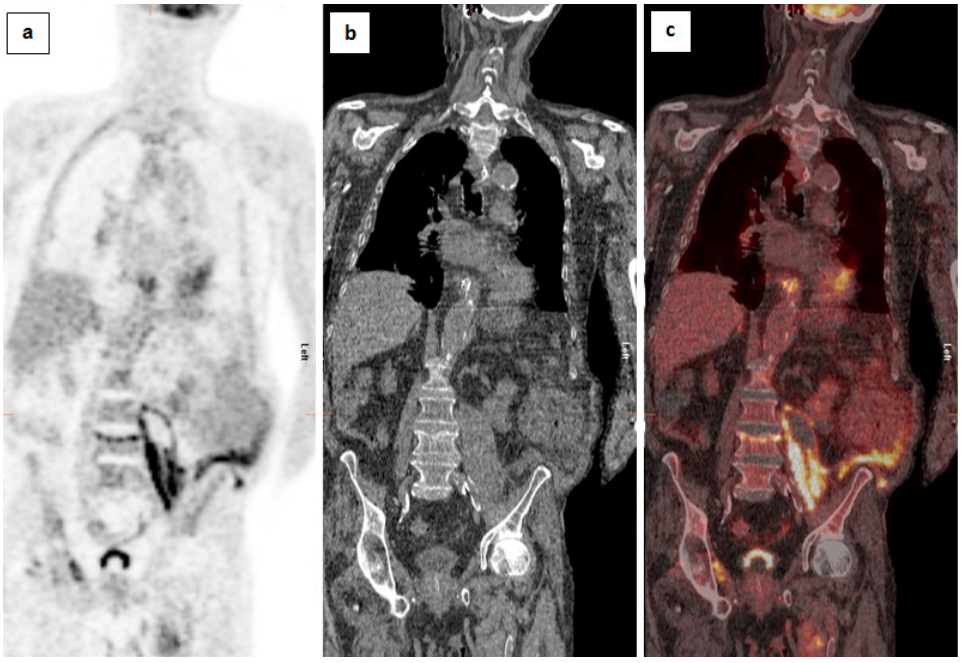
CASE REPORT

A 74-year-old man was admitted to our department in January 2015 with general malaise, weight loss, dyspnoea, abdominal pain and back pain. His history revealed active rheumatoid factor positive rheumatoid arthritis (RA) since 1972, treated with prednisone since January 2000 and abatacept since August 2014, deep venous thrombosis, emphysema, and hypertension. In 2008, an infrarenal abdominal aortic aneurysm (AAA) was diagnosed and treated with an endovascular aneurysm repair (EVAR) in February 2012 after symptomatic presentation. In October 2012, transthoracic echocardiography (TTE) revealed aneurysms of the aortic sinus (44 mm) and ascending aorta (42 mm), without valve abnormalities. In February 2014, increasing back pain and left-sided abdominal pain, without fever, night sweats or weight loss, resulted in admission to the department of Surgery. CT angiography (CTA) showed right renal artery occlusion, and an expanded AAA connecting with a fluid collection around the left iliopsoas muscle. The infectious diseases specialist advised to perform Q fever diagnostics. The PCR (in-house real-time PCR targeting IS1111a), enzyme-linked immunosorbent assay (ELISA, PanBio Pty Ltd., Windsor, QLD, Australia), and complement fixation assay (CFA; Virion-Serion, Würzburg, Germany) on serum were negative. Repetitive TTE in 2014 depicted a stable cardiac condition. On physical examination at presentation in January 2015, he was afebrile with a blood pressure of 184/97 mmHg, with 96% saturation. Cardiac examination was normal, endocarditis stigmata were absent, as was lymphadenopathy. Pulmonary examination revealed left-sided rales and right-sided crackles. He reported tenderness on palpation of the thoracic spine. Besides a C-reactive protein (CRP) of 67 mg/l (normal range, <5 mg/l) and hemoglobin level of 7.3 mmol/l (normal range, 8.4–10.8 mmol/l), laboratory results were normal. Chest X-ray revealed a recent thoracic spinal fracture, and abdominal ultrasound showed hepatomegaly and a psoas hematoma. CTA showed no leakage of the aortic graft. ^{18}F-fluorodeoxyglucose positron emission tomography/low-dose CT (^{18}FDG-PET/CT) 3 days later showed a normal FDG distribution in the patients' head, neck, and brain parenchyma, but a high pulmonary FDG-uptake suggestive for pneumonia, and signs of an infected AAA expanding to the left psoas muscle. CT-guided puncture of the psoas abscess revealed pus, which was PCR positive for *C. burnetii*. Immunofluorescence assay (IFA; Focus Diagnostics Inc., Cypress,

CA, USA) showed high anti-*C. burnetii* antibody titres: IgG phase I 1:4096, phase II 1:2048, IgM phase I and II negative. Serum PCR remained negative. Chronic Q fever was diagnosed and treatment with doxycycline 200 mg/day and hydroxychloroquine 600 mg/day was initiated. Prednisone (5 mg/day) was continued, but abatacept was stopped and the abscess was drained percutaneously. Shortly after being discharged, he was readmitted because of collapse, confusion, and increasing back pain. CT showed a new thoracic aortic aneurysm (52 mm) and an expanded multiloculated psoas abscess, which again was drained percutaneously. In the absence of a clinical response, moxifloxacin 400 mg/day was added, but had to be stopped due to a markedly prolonged QTc-interval. Despite several drains in the multiloculated abscess, CRP increased to 261 mg/l and he developed a fever. His hospital stay was complicated by two episodes of presumed hospital-acquired pneumonia (for which he received piperacillin/tazobactam), acute decompensated heart failure, respiratory failure presumably due to an aspiration pneumonia, and sepsis, for which he was temporarily transferred to the intensive care unit twice. Furthermore, he developed a gastroparesis, acute progressive renal insufficiency and a delirium. A new ^{18}FDG-PET/CT (Figs. 1, 2) showed increased FDG-uptake extending into the vertebrae and high FDG-uptake in his spleen

Figure 1

^{18}F-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) (**a**), low-dose CT (**b**), and integrated ^{18}FDG-PET/CT (**c**) images, demonstrating increased FDG-uptake in the abscess formation in the left iliopsoas muscle, extending into the intervertebral space cranially of L4 and into the adipose tissue reaching the left abdominal wall. The ^{18}FDG-PET could not be assessed for disseminated lesions in the brain due to a motion artifact of the head during the procedure.



suggestive for satellite infection. Despite treatment with adequate doxycycline levels, the patient died 4 months after presentation. Autopsy was performed, macroscopically showing inflamed tissue around the EVAR (Fig. 3) with fistulas to the iliopsoas muscle in continuation with the spine with softened vertebrae. Microscopy yielded a chronic granulomatous necrotizing inflammation of the aortic vascular wall around the EVAR, fully necrotic iliopsoas muscle and surrounding area, and a hypertrophic cardiomyopathy. Necrotizing granulomas were found in both lungs, being PCR positive for *C. burnetii*, as were EVAR specimens, pus from the psoas abscess and ascites from the abdominal right lower quadrant around the appendix. Cultures for *C. burnetii* remained negative. Post-mortem examination of the brain was not performed. Retrospectively, IFA was performed on stored serum from February 2014, already showing an IgG phase I 1:4096, IgG phase II 1:2048, with negative IgM phase I and phase II, suggestive for chronic Q fever. Retesting the stored serum with CFA and ELISA confirmed the previously found negative results.

Figure 2

Transversal integrated ^{18}F-fluorodeoxyglucose positron emission tomography/low-dose CT (^{18}FDG-PET/CT) images, from cranial to caudal, demonstrating: (**a**) increased FDG-uptake in the left iliopsoas muscle dorsally extending through the musculature of the back, and increased FDG-uptake in the wall of the aortic aneurysm adjacent to the endovascular aneurysm repair (*EVAR*). (**b**) a per continuitatem infection arising from the abdominal aortic aneurysm (*AAA*), thrombosis of aortic aneurysm and low activity in the cavity of the EVAR resulting from blood flow. The infection extends to the abscess and left iliopsoas muscle. (**c**) percutanous drain *in situ* in the abscess, increased FDG-uptake in the cranial portion of the vertebra, and increased FDG-uptake in adipose tissue of the left abdominal wall in continuitatem with the abscess (not visible at the level of this transversal slice). (**d**) increased FDG-uptake in the aortic wall adjacent to the caudal part of the EVAR, and increased FDG-uptake extending into adipose tissue of the left abdominal wall.

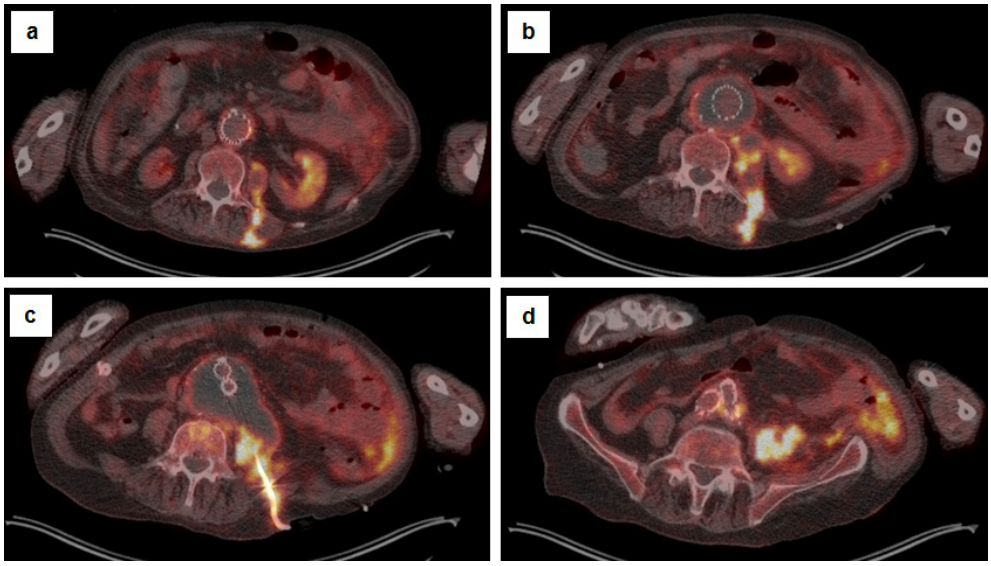
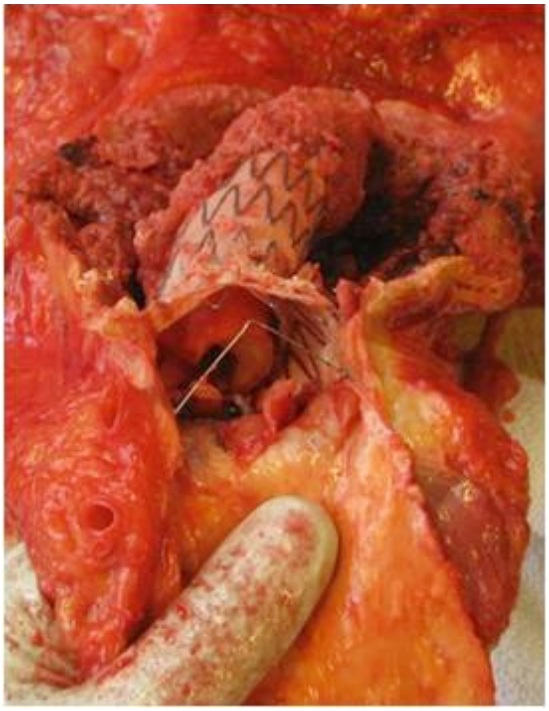


Figure 3

Cranial view, during autopsy, of the abdominal aorta with the endovascular aneurysm repair (*EVAR*) stent-graft. The lumen of the celiac trunk and superior mesenteric artery are visible. Around the EVAR the aneurysmatic plaque inside the dilated vascular wall is still in situ, the material was PCR positive for *C. burnetii*. A fistula from the abdominal aortic aneurysm (*AAA*) to the psoas abscess was present (not visible on picture). Inside the EVAR an intra-prosthetic deposition of amorphous material is visible.



DISCUSSION

We describe an immunocompromised patient with a widely disseminated chronic Q fever infection with infectious foci in the EVAR and surrounding AAA, both lungs, iliopsoas muscle, spine, spleen, and in ascites from the abdominal right lower quadrant. To our knowledge, such an extensive *C. burnetii* infection has not been described before. Rare complications, e.g., osteomyelitis [2], periaortic adenopathy, aortaduodenal fistula, psoas abscesses [4, 5], and fistula to the groin [6], have been described as part of locoregional spreading of *C. burnetii*. Such locoregional expansion is probably the result of a contiguous infected vascular aneurysm. In our patient, however, besides locoregional spreading, haematogenous seeding of *C. burnetii* is likely because of signs of metastatic infection in the spleen and the presence of *C. burnetii* DNA and granulomatous inflammation in lung tissue. Haematogenous spread can also result in hepatic abscesses, described in one patient with both splenic and hepatic abscesses [3]. However, this occurred during an acute *C. burnetii* infection, instead of chronic Q fever as in our case, with complete resolution of symptoms and abscesses after 21 days of doxycycline.

Probably the immunocompromised state of the patient (due to the use of abatacept and prednisone) contributed to the widespread infection. A disseminated Q fever infection with acute endocarditis in experimentally infected immunocompromised mice 10 days after intraperitoneal inoculation of *C. burnetii* has been described, showing microabscesses, granulomas, and microthrombi in spleen, liver, myocardium and bone marrow [7]. Such a disseminated infection was also found in immunocompetent mice [8]. However, these self-limiting systemic infections were found after intraperitoneally induced acute infection, with characteristic histopathological changes only in the acute setting, whereas persistent infection was found only in the kidneys of a single immunocompromised animal [7]. Abatacept treatment, so far, has not been complicated by many opportunistic or serious infections, in contrast to anti-TNF treatment [9]. However, based on a small number of RA patients, the use of TNF blockers was not associated with increased risk of chronic Q fever, in contrast to corticosteroid use [10], which our patient also used. In addition, it was suggested that RA and its treatment, either with or without anti-TNF, may be considered as a risk factor for chronic Q fever development, and it was advised to monitor RA patients carefully in case of *C. burnetii* infection [10]. The role of abatacept in the dissemination of *C. burnetii* in our patient remains unresolved. Abatacept, inhibiting T cell activation by preventing co-stimulatory interaction between CD80/CD86 and CD28, did not prevent formation of *C. burnetii*-positive granulomata, corresponding with previous findings in *C. burnetii*-infected CD28-deficient mice, in which granuloma formation was also not affected [11]. Interestingly, in these CD28-deficient mice, the *C. burnetii* burden in infected tissue was decreased, suggesting that costimulation of CD28 increases *C. burnetii* replication, implicating a favourable effect of abatacept. Although abatacept was stopped, prednisone was continued during the course of disease because of the long-term use with subsequent hypothalamic–pituitary–adrenal axis suppression. In addition, the patient needed steroid stress dosing due to several complications. However, despite the continuation of prednisone in this specific case, physicians should always consider stopping immunosuppressive therapy while treating chronic Q fever. Another explanation for the widespread infection might

be *C. burnetii* resistance to doxycycline, as doxycycline resistant isolates do exist [12, 13]. However, this does not appear to be a common occurrence [14], and it is more likely that the patient died due to an already widely disseminated Q fever infection at the time doxycycline and hydroxychloroquine were initiated, while the immunosuppressive therapy favoured the expansion of the infection.

Diagnosing chronic Q fever is challenging, and often delayed because of the lack of recognition by physicians, mainly due to non-specific symptoms and unfamiliarity with chronic Q fever. However, early diagnosis has major implications, as chronic Q fever causes high morbidity and mortality [1]. Eventually, the indication to test for Q fever was recognized in this case, but retrospectively the patient already reported general malaise for years, chronic chest pain and left flank pain ever since the EVAR procedure. Furthermore, he already had an elevated CRP whilst consulting the cardiologist, pulmonologist and rheumatologist in the years before presentation, who related this to his active RA and intercurrent problems. Our patient lived in an area in the Netherlands with the highest incidence of Q fever during the large Q fever outbreak from 2007 until 2010 [15, 16], and inhalation of contaminated aerosols was probably the route of initial infection [17]. In Q fever endemic areas or in the years after outbreaks, physicians should stay alert on signs and symptoms suggestive for chronic Q fever, especially in case of risk factors, also in the absence of a known acute Q fever episode. Well-known risk factors for developing chronic Q fever include vascular grafts and aneurysms, cardiac valve prosthesis or valvulopathy, and immunosuppresion [18]. Despite the fact that EVAR specimens appeared to be PCR positive for *C. burnetii*, the EVAR could not be revised in this case. The main reason for the decision to abstain from surgical intervention was the already expanded infection, and the patients' deteriorating physical condition. However, in case of a chronic Q fever infection of a vascular prosthesis, surgical interventions can lead to a better outcome and should always be considered [2, 19]. Our case further emphasizes the need for using IFA to screen for chronic Q fever, as CFA and ELISA have limited sensitivity. Also, this case illustrates that PCR alone is insufficient to rule out chronic Q fever due to the low sensitivity in blood specimens [1].

CONCLUSION

In conclusion, we report a fatal case of an immunocompromised patient with a confirmed disseminated chronic Q fever infection, underlining the severity of this disease and the diversity of signs and symptoms that may occur, and highlighting the need for increased awareness and recognition by physicians especially in case of risk factors. Furthermore, we advocate performing an adequate diagnostic work-up using at least IFA serology and PCR for screening for chronic Q fever.

AUTHORS' CONTRIBUTIONS

SPK and RPHR drafted the initial manuscript. TS was involved in drafting and critical revise of the manuscript. MCWS performed the autopsy, interpreted the autopsy results, and provided the image of the EVAR and its description. CPBR was the treating physician of the patient, initiated this case report and provided critical revisions to the manuscript. All authors read and approved the final manuscript.

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CONSENT
Written informed consent for publication of the clinical details and images was obtained from the patient's spouse.

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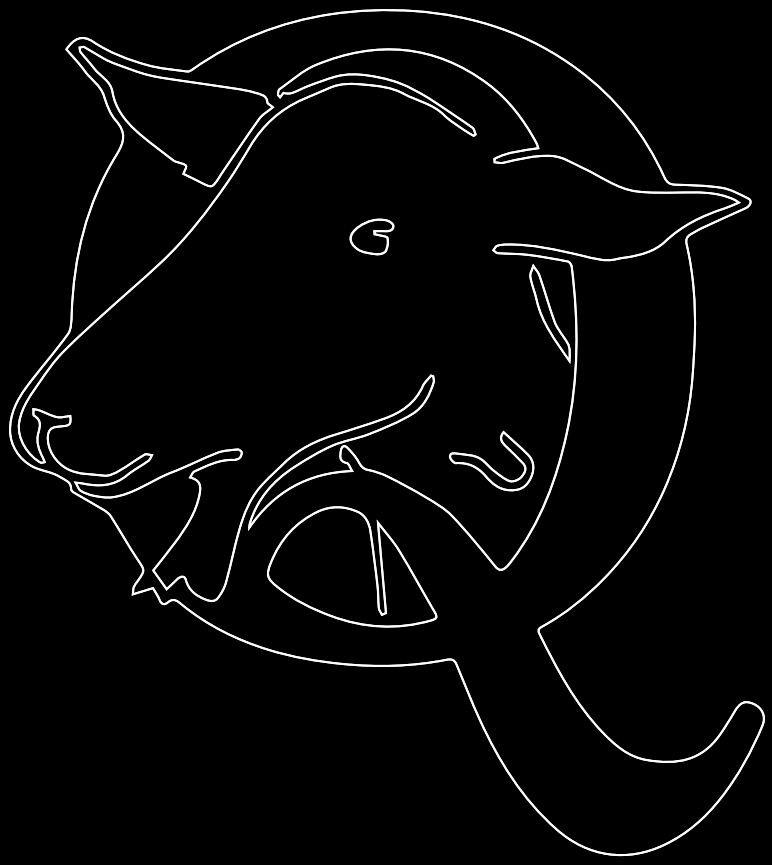

CHAPTER 11

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

GENERAL DISCUSSION

In this thesis, the findings of several retrospective and prospective studies in patients with Q fever fatigue syndrome (QFS) were described. Also, challenges in diagnosis and treatment of acute and chronic Q fever were addressed. This thesis underscores that Q fever is a complex disease with diverse manifestations and still many queries.

Awareness and recognition of fatigue following acute Q fever

Although fatigue following acute Q fever has been recognised for years worldwide [1-6], the systematic review presented in **chapter 2** illustrates that information on aetiology, prevention, treatment, and prognosis of QFS is scarce in the international literature. Several names have been used to indicate the presence of persistent fatigue following acute Q fever, but it was concluded that QFS is the preferred international term to aid comparison between studies.

Definition and diagnosis of QFS

Although QFS is the preferred international term, in **chapter 2** it was concluded that the main limitations in the international literature with regard to QFS are the lack of a uniform definition and the absence of a standardized diagnostic tool. In order to facilitate comparison of findings, and as platform for future studies, a uniform definition and diagnostic work-up and uniform measurement tools for QFS are necessary. This will also provide an aid for physicians and recognition for patients. A detailed description of QFS has been published in the Dutch guideline on QFS [7] and in an Australian thesis [8]. The latter is, however, based on a retrospective comparative-cohort study and is not available online, which limits its usefulness for international comparisons. Although the Dutch guideline on QFS was originally written in Dutch, the definition has been translated and includes a detailed description of QFS, and might be used as international uniform definition to achieve uniformity in diagnosis, treatment, and comparison of research results [7, 9, 10]. In brief, QFS is defined as severe fatigue causing significant disabilities in daily life, present for at least 6 months, with a temporal relationship with acute Q fever, and not caused by co-morbidity. Fatigue should be absent before the onset of acute Q fever or should have significantly increased since the infection. In addition, it is essential to use validated screening instruments for measuring fatigue severity and disabilities, e.g., the Checklist Individual Strength [11, 12] and Sickness Impact Profile [13-15], respectively. Guidelines with regard to the examination of chronic fatigue should be followed to rule out other diseases that can cause chronic fatigue. In addition, QFS should not be confused with chronic Q fever [16]. QFS is accompanied by high morbidity, but in contrast to chronic Q fever, does not account for Q fever-related mortality. The definition of QFS clearly excludes chronic Q fever based on a negative serum PCR, Q fever serology (IgG phase I titer <1:1024), and the absence of signs of endocarditis and vascular infection. Therefore, there is neither controversy nor confusion between QFS and chronic Q fever [17].

Which symptoms should be part of the case definition of QFS?

Many nonspecific symptoms accompanying fatigue in QFS have been described, but these have not been systematically registered in patients. QFS patients frequently report symptoms like myalgia, arthralgia, neurocognitive problems, sleeping problems, headache, blurred vision, mood disorders, and increased (night) perspiration. Although these symptoms should all be taken seriously, they should not yet be included in the QFS case definition. Until prospective follow-up studies become available of well-defined QFS populations, one should refrain from attributing additional symptoms to QFS. There should be reluctance to diagnose QFS solely based on a list of symptoms for which the causal relationship with previous *Coxiella burnetii* infection is unknown.

Differences between QFS and chronic fatigue syndrome

Until more research on QFS has been performed, it is prudent to identify QFS and CFS as separate entities, as there are several differences. In CFS the precipitating factor is usually unknown, while in QFS a *C. burnetii* infection can be identified as such. Furthermore, in QFS there is a sudden onset of fatigue, while in CFS this is not always the case. In addition, in a study of two independently conducted prospective studies, presented in **chapter 3**, the direct comparison of QFS and CFS patients revealed several differences in demographics (including gender), number of symptoms, and fatigue-related cognitive-behavioural variables. The relationship between perpetuating factors and fatigue in CFS - as found in a previous study [18] - could not be confirmed in QFS patients. This suggests that the mechanisms involved in the perpetuation of fatigue in QFS are different from those related to fatigue in CFS, despite the considerable overlap in fatigue-related cognitive behavioural variables. Finally, there is still a lack of knowledge with regard to the pathogenetic process underlying QFS, but might be precipitated by *C. burnetii* as trigger [19-21], and therefore this might not be identical to CFS. It could be debated whether QFS represents a subset of CFS patients, i.e., those with post-infectious fatigue syndrome. Whether this is the case or not, until now it is wise to differentiate QFS from CFS. The differences found between QFS and CFS as well as the importance of the attribution for patients still justify the use of the term QFS.

Aetiology and the use of immunological assays in QFS

Several hypotheses regarding the underlying pathophysiological mechanism of QFS have been proposed, but no conclusive answers have been identified yet. At present, it is tempting to hypothesize that QFS represents a state of altered cell-mediated immunity against *C. burnetii* in the spectrum of Q fever-related syndromes. To date, no diagnostic test is available to diagnose QFS. Ever since the discovery of C. *burnetii*, the specific humoral immune response played a central role in the diagnosis of Q fever. Increasingly, the cell-mediated immune responses appear also relevant in the anti-*C. burnetii* host response. Interferon-γ (IFNγ) and other cytokines such as interleukin-2 (IL-2) already proved to play a pivotal role in the host defence against intracellular bacteria such as *C. burnetii* [22-25]. The antigen-specific IFNγ production was developed for the diagnosis of acute Q fever [26], and the IFNγ production assay already proved to be a useful diagnostic tool for *C. burnetii* infection [27, 28]. Q fever seropositive controls showed a high IL-2 production,

whilst a high IFNy/IL-2 ratio appeared indicative for chronic Q fever. Subsequently, the IFNy/IL-2 ratio was proposed as additional diagnostic marker for chronic Q fever and treatment monitoring [29, 30]. In QFS, however, the added value of immunological assays was unclear. IFNy upregulation and IL-2 downregulation in QFS patients compared to control groups was found, but this study only included a small number of QFS patients [21]. In **chapter 4** it was shown that the IFNy production in QFS patients is significantly higher than in seropositive controls, and that the IFNy/IL-2 ratio is significantly lower than in chronic Q fever patients. As such, both the antigen-specific IFNy production and IFNy/IL-2 ratio may become a tool in the diagnostic workup of QFS, as the combined use of IFNy and IL-2 production might allow a better distinction between QFS patients, seropositive controls, and chronic Q fever patients. However, widespread use of immunological assays in QFS patients cannot be recommended in clinical practice before these results are confirmed and compared with other control groups in larger cohorts of patients. In addition, it should be evaluated whether these results only holds true on group level, or whether individual patients can be classified into QFS, seropositive control, or chronic Q fever, solely on the basis of immunological assays.

Treatment of QFS

From the randomised, partly double-blind, placebo-controlled trial described in **chapters 5 and 6**, it can be concluded that cognitive behavioural therapy (CBT) is effective in reducing fatigue severity and the level of psychological distress in QFS patients. The sensitivity analysis revealed a consistently positive effect, and the positive effect of CBT on fatigue severity was also clinically relevant. In addition, the mean number of adverse events per patient was lowest in this group and no serious adverse events occurred. Therefore, CBT for QFS is a safe therapy if performed by qualified and trained therapists, as has been reported before for CBT in CFS [31]. CBT already proved effective in reducing symptoms and improving functioning in CFS patients [32, 33], and in chronic fatigue in several chronic diseases [34-36], and this study proved its efficacy in QFS patients. CBT should therefore be recommended to QFS patients following diagnosis. However, no data are available with regard to the effect of the patients' attitude on treatment engagement and outcome. In CFS, the attitude of the patient towards the treatment model appeared to be an important contributor to treatment engagement, and therefore possibly outcome, in a cognitive behavioural intervention [37]. It is likely that QFS patients with a negative attitude towards CBT and its underlying treatment model will probably not accept referral for CBT or will drop-out in an early stage. Motivating interventions by the referring physician could be valuable to optimise treatment expectancies and subsequently treatment engagement and outcome even before starting CBT. This already starts with the communication towards patients before referral, as for many physicians it is tempting to regard QFS as either a somatic disorder or a psychological disorder, in a Cartesian fashion. However, QFS should be seen as a syndrome in which somatic, psychological, social, and behavioural factors all play an important role. Explaining this to patients is difficult, but increases patients' insight in their complaints and subsequently increases treatment motivation. Solely regarding QFS as somatic disorder will increase the somatic attribution of patients, which influences the motivation and treatment engagement negatively. Although CBT proved an effective

treatment for fatigue in QFS patients, the underlying mechanisms by which CBT has a positive effect on fatigue are unknown. Identifying cognitive and behavioural variables that intervene in the relation between treatment and outcome is of major importance to individualize and optimize therapy, which can lead to an even better outcome. A mediation analysis is therefore planned. Furthermore, to evaluate the long-term beneficial effects of CBT, patients are currently surveyed by questionnaires 12–15 months post-treatment.

It can be concluded that long-term treatment with doxycycline does not significantly reduce fatigue severity in QFS patients. This is the first randomised controlled trial ever performed in QFS patients, and results with regard to the effect of long-term doxycycline clearly contradict those previously described [4, 38]. As described, all previously published studies had major limitations, precluding the extrapolation of the described results [3, 4, 38, 39]. All the limitations in these studies were addressed in this randomised controlled trial, and the period of antibiotic administration was even longer. Strengthened by the low number of dropouts and missing data, our results do not support a positive effect of long-term treatment with doxycycline for QFS. In addition, the mean number of adverse events per patient was highest among patients who received doxycycline. Hence, prescription of prolonged antimicrobial therapy in case of QFS is useless, and such treatment should not be prescribed. This advice also holds true for the alternative therapies described in literature, which were both case reports [39, 40].

One of the limitations of this study is that it was not designed to compare doxycycline and CBT directly, due to the limited number of eligible patients available and the impossibility to blind for the treatment modality. However, the scores in the doxycycline group at end of treatment were similar to placebo with even worse mean scores. The results therefore imply a favourable effect of CBT, but it should be noted that this was not formally investigated. Furthermore, it can be debated whether the level of evidence originating from this randomized controlled trial should be supported by confirmation studies as basis for guideline recommendations. Evidence-based practice usually relies on a broad, diverse base of evidence, which is obviously not available for the treatment of QFS. In a scientific view, these results should be verified in other randomized controlled trials. However, based on a practical view, it is very unlikely that a study of this size can be repeated to confirm our findings because the recruitment of sufficient QFS patients will be extremely difficult. Although it is likely that new Q fever outbreaks will occur, an exceptionally large Q fever outbreak as occurred in the Netherlands is rare, and may not happen again in the near future. Until an outbreak occurs that facilitates the confirmation of these results, this study provides the strongest level of evidence so far.

Diagnosing acute Q fever

Both acute and chronic Q fever are often underdiagnosed due to poor recognition among clinicians [41, 42]. Previous studies suggest typical signs and symptoms of acute Q fever: fever, headache, and cough [43-45], and headache has been postulated to be rather specific for acute Q fever [46, 47]. However, results from the retrospective case-control study

presented in **chapter 7**, contradict a typical presentation of acute Q fever. Although some differences in clinical manifestations between acute Q fever patients coming to a hospital and controls were found, the considerable overlap between both groups hamper the use of these variables for clinical differentiation. Although others previously observed remarkable differences in clinical presentation between hospitalized *C. burnetii* pneumonia patients and patients hospitalized for pneumonia with a different aetiology [48], it can be concluded that differentiating *C. burnetii* from other pathogens is not possible without Q fever serological analysis and PCR in patients coming to a hospital. The cornerstone in diagnosing acute Q fever is therefore the awareness among physicians to consider *C. burnetii* as possible aetiological agent and requesting appropriate diagnostic tests.

Prophylactic treatment of high-risk patients

Long-term prophylactic treatment with doxycycline and hydroxychloroquine has been suggested for acute Q fever in patients with risk factors for development of chronic Q fever [49, 50]. As demonstrated in the retrospective study described in **chapter 7**, of the patients with an indication for prophylaxis, none of the patients who received prophylaxis developed chronic Q fever, in contrast to 50% of patients who did not receive prophylaxis despite the indication. These findings clearly support the recommendation that prophylactic treatment is beneficial and should be given to patients with risk factors for developing chronic Q fever [49-53], but potential side effects must be taken into consideration [54].

Diagnosis and clinical manifestations of chronic Q fever

The diagnosis of chronic Q fever is also challenging, and relies on a combination of symptoms, risk factors, microbiological findings, and imaging techniques [55]. The diagnosis is often delayed, and hampered by the fact that many known chronic Q fever patients do not recall an acute Q fever episode [56], which is supported by findings presented in **chapter 8**. However, early diagnosis has major implications [56], and as illustrated in **chapter 10**, a diagnostic delay can lead to a fatal outcome. Clinicians should be aware of this silent killer, especially in disease-endemic areas or when patients have risk factors for the development of chronic Q fever. The case presented in **chapter 10** also illustrates the need for an appropriate diagnostic work-up for Q fever including at least IFA serology and PCR. Other diagnostic tests, for example complement fixation assay and enzyme-linked immunosorbent assay, or performing PCR alone proved insufficient to rule out chronic Q fever. Besides microbiological findings, imaging methods play an important role in the diagnosis of chronic Q fever. Localisation of infectious foci is important, because surgical interventions can lead to a better outcome and should always be considered in chronic Q fever patients [57, 58]. The results described in **chapter 8** further demonstrated that ^{18}F-fluorodeoxyglucose positron emission tomography/CT (^{18}F-FDG PET/CT) is a valuable tool for localisation of vascular infection with *C. burnetii*. It is therefore recommended to perform ^{18}F-FDG PET/CT in all patients with a suspicion of chronic Q fever, especially because it has already been shown that infected aneurysms or vascular prostheses are present more commonly in the Netherlands compared to other countries [43, 59-61], which is also illustrated in **chapter 8**. Furthermore, the data emphasise the need for performing transesophageal echocardiography (TEE) instead of

transthoracic echocardiography (TTE) in patients with a suspicion of Q fever endocarditis. Q fever endocarditis is known for its subtle valve abnormalities that are easily missed using only TTE in the absence of vegetations [43, 62, 63]. Chronic Q fever mainly manifests as endocarditis or vascular infection, but the clinical features are, like in acute Q fever, diverse. There are many reports describing rare complications as a result of locoregional expansion of *C. burnetii* [58, 64-66]. In **chapter 10,** however, it was demonstrated for the first time that besides locoregional spreading, haematogenous seeding beyond the vascular tree of *C. burnetii* is possible in chronic Q fever. This finding is important as it increases our knowledge on the pathophysiology and treatment of chronic Q fever.

Treatment of chronic Q fever

Following a diagnosis of chronic Q fever, treatment is the next challenge. If left untreated, a high mortality rate is observed, but also in case of adequate treatment, chronic Q fever remains an unpredictable disease with a high mortality rate, as illustrated in **chapter 10**. No single drug has been shown to be bactericidal against *C. burnetii* as monotherapy [44]. Consequently, treatment preferably consists of an antibiotic combination regime, i.e. doxycycline and hydroxychloroquine, for a prolonged period, which proved to be effective in patients with Q fever endocarditis [67-70]. Although the regimen for vascular chronic Q fever has not been investigated as thoroughly as in Q fever endocarditis, the antibiotic regimes for Q fever endocarditis have been applied to this disease entity as well. Pursuing the optimal treatment in patients normally favours the outcome, but many chronic Q fever patients who use doxycycline and hydroxychloroquine experience side effects, including severe photosensitivity, nausea, vomiting, diarrhoea, and cutaneous hyperpigmentation [69]. The latter is demonstrated in the case series presented in **chapter 9**, describing cutaneous hyperpigmentation that occurred during doxycycline therapy within the therapeutic dose range due to the prolonged treatment regimen for chronic Q fever. Side effects can have a major effect on the quality of life [71], and are an important reason for discontinuation of therapy. Therefore, both prescribers and patients should be aware of potential side effects. In case of unacceptable side effects or in case of treatment failure using doxycycline and hydroxychloroquine, physicians should consider to switch to other antibiotic regimens.

FUTURE PERSPECTIVES

The Q fever outbreak in the Netherlands provided the opportunity to gain knowledge about different aspects of this relatively rare but serious infectious disease. As the number of notified acute Q fever cases in the Netherlands significantly decreased since 2010, the research focus changed from the acute illness to its long-term consequences, i.e. QFS and chronic Q fever. Several questions regarding the long-term consequences are still unanswered and the results presented in this thesis also open up avenues for future research by producing new questions. It is now known that the long-term consequences of Q fever have major impact on public health. For example, the majority of patients return to work within the first 12 months after acute Q-fever, but up to 20% reported reduced work participation [72]. In addition, it is observed that many patients still report decreased psychosocial functioning years after the primary Q fever infection. However, information

with regard to the long-term (>5 years) impact on work and psychosocial functioning in both QFS and chronic Q fever patients is lacking. By comparing the functioning of patients to references groups, it will be possible to determine which part of the impact or reduced psychosocial functioning can be attributed to QFS and chronic Q fever.

Still little is known about the pathogenesis of QFS, and one of the main questions is why patients remain fatigued. Research into the pathophysiological mechanism of QFS is therefore necessary. For example, it can be hypothesized that *C. burnetii* elicits epigenetic changes in monocytes, macrophages and perhaps microglial cells, ultimately resulting in a changed cytokine profile that might result in state of prolonged fatigue (QFS). Ideally, this should be investigated in a cohort of acute Q fever patients with a follow-up period long enough to investigate the role of epigenetic changes in the development of QFS.

Furthermore, it is important to try to find an objective method to diagnose QFS to optimise individual patient care. Although it is too early to use immunological assays in a routine clinical setting, these assays seem promising for diagnosing QFS and warrants further investigation, in which at least the positive and negative predictive values should be known. Revealing the pathophysiological mechanism of QFS might also result in additional treatment options for QFS patients, and might also contribute to prevention of this debilitating syndrome. By defining early predictors for the development of QFS, new therapeutic modalities may be developed. This might lead to earlier treatment regimens or, even more preferably, interventions to reduce or prevent the development of QFS.

Although CBT is an effective treatment modality to reduce fatigue severity, many patients experience CBT as time-consuming, intense, and strenuous. In addition, the treatment capacity is limited. Providing web-based CBT and tailoring the amount of contact with the therapist to the individual needs of the patient may overcome these issues [73-76]. Another possibility might be graded exercise therapy, which has also proved effective for CFS [77, 78], but has not yet been investigated for QFS. Finally, the long-term beneficial effects of CBT for QFS are currently under investigation.

Despite the advances in knowledge on chronic Q fever in recent years, diagnosis and treatment of chronic Q fever remains challenging. Early case-finding, by targeted screening and increased awareness among physicians, will improve prognosis. Furthermore, it is necessary to gain more insight into the immunological mechanisms leading to chronic Q fever. It is still not entirely understood why *C. burnetii* is cleared ineffectively after the initial infection in those individuals who develop chronic Q fever. It is also unknown why persistent *C. burnetii* infection predominantly manifests as endocarditis or vascular infection, instead of primarily targeting other organs. Little is known about the auto-immune phenomena that are increasingly recognised in chronic Q fever patients, and still many questions exist with regard to the best treatment strategies. It is therefore essential to gain knowledge on the IFNγ pathway in the primary and late defence mechanism, identifying a *C. burnetii*-specific immune response (immunological footprint), and to identify genetic factors that increase

the likelihood of developing chronic Q fever. Although prophylactic antibiotic treatment should be given to high-risk patients after an episode of acute Q fever, controversy still exist with regard to treatment duration, dosage, and patient selection. Therefore, more studies are needed to develop uniform guidelines with regard to optimal prophylactic treatment. Furthermore, the first choice antibiotic regime in case of chronic Q fever, i.e. doxycycline and hydroxychloroquine, accounts for many side effects and the efficacy is not entirely clear. The latter also holds true for alternative antibiotic treatment regimens used for chronic Q fever in daily practice. A randomised controlled trial with regard to treatment of chronic Q fever in the future is desirable in case a second epidemic with similar expanse would take place. Because acute Q-fever is no longer a common disease in the Netherlands (only 12 reported new acute Q fever cases in 2016 [79]), international collaboration is mandatory to obtain sufficient patients for these studies.

So far, most Q fever-related research has been descriptive and retrospective in nature. The Dutch epidemic provided opportunities to do prospective studies, but since the epidemic is over the possibility for new prospective studies is limited. As *C. burnetii* has caused numerous outbreaks all over the world since its discovery in 1935, it is likely that new outbreaks will occur in the future. When such outbreaks occur, funding should be made available without delay, to perform prospective studies on the questions that remain unanswered regarding Q fever and its long-term consequences.

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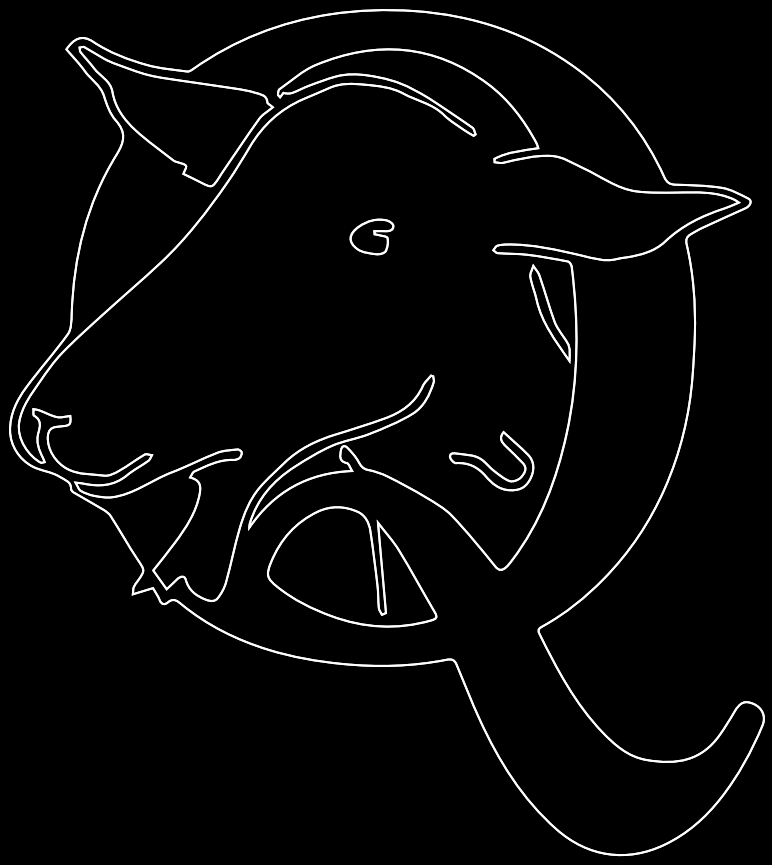


CHAPTER 12

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Query (Q) fever, the original name related to the consequences of a *Coxiella burnetii* infection because of the unfamiliarity with the causative pathogen, still seems an appropriate name, reflecting all queries with regard to the different clinical manifestations of this disease. The Q fever outbreak in the Netherlands has been the largest Q fever outbreak reported to date, and offered the opportunity to gain new insight with respect to Q fever. In this thesis, some challenging questions with regard to Q fever were investigated with an emphasis on Q fever fatigue syndrome (QFS). The primary aims of this thesis were increasing the recognition of QFS, revealing new insights in the pathophysiology of QFS, and evaluating the efficacy of treatment with long-term doxycycline and cognitive behavioural therapy (CBT) in QFS patients. A secondary aim was to investigate diagnostic and treatment challenges in both acute and chronic Q fever. Following a general introduction and outline of the thesis in **chapter 1**, this thesis is divided in two main themes: recognition and treatment of QFS (*part I*) and challenges in diagnosis and treatment of acute and chronic Q fever *(part II)*.

PART I: Recognition and treatment of QFS

In **chapter 2**, a systematic review is provided to describe the literature, and identify knowledge gaps regarding the definition, diagnosis, background, description, aetiology, prevention, therapy, and prognosis, of fatigue following acute Q fever. Although most patients recover from fatigue within 6-12 months after acute Q fever, approximately 20% remain chronically fatigued. It is concluded that the occurrence and long-term persistence of fatigue following acute Q fever, generally referred to as QFS, has major health-related consequences. However, still several questions with regard to QFS exist, as information on aetiology, prevention, treatment, and prognosis of QFS is underrepresented in the international literature. In order to facilitate comparison of findings and as a platform for future studies, an international uniform definition is desirable. It is therefore proposed to use the definition and diagnostic work-up for QFS according to the Dutch QFS guideline.

In **chapter 3**, differences and similarities between QFS and chronic fatigue syndrome (CFS) patients were investigated, with a focus on inflammatory markers and fatigue-related cognitive-behavioural factors. In an exploratory analysis, the relationship between these cognitive-behavioural variables and fatigue in QFS patients was investigated. Data from two independent prospective studies on QFS (n=117) and CFS (n=173), respectively, were pooled and analysed. QFS patients were less often female, had a higher body-mass index (BMI), and had less often received treatment for depression before the onset of symptoms. After controlling for symptom duration and correcting for differences in diagnostic criteria for QFS and CFS, differences in the proportion of females and BMI remained significant, and QFS patients appeared to be older. QFS patients were as fatigued and distressed as CFS patients, but reported less additional symptoms. QFS patients had stronger somatic attributions, and higher levels of physical activity. No differences were found with regard to inflammatory markers or other fatigue-related cognitive-behavioural variables. Differences in known predisposing factors for chronic fatigue suggest other predisposing factors for developing QFS. Although the relationship between cognitive-behavioural variables

and fatigue previously established in CFS could not be confirmed in QFS patients, the considerable overlap in fatigue-related cognitive-behavioural variables and the relationship found between physical activity and fatigue suggest that behavioural interventions could reduce fatigue severity in QFS patients.

In **chapter 4**, the specific interferon-γ (IFNγ) production and IFNγ/Interleukin(IL)-2 ratio in 20 QFS patients was explored and compared to those previously determined in seropositive controls (n=135), and chronic Q fever patients (n=28). Also, the correlation between patient characteristics and IFNγ and IL-2 production, and IFNγ/IL-2 ratio was determined. QFS patients were younger, but gender distribution was similar to seropositive controls and chronic Q fever patients. The IFNγ production in QFS patients was significantly higher than in seropositive controls, and the IFNγ/IL-2 ratio was significantly lower than in chronic Q fever patients. Symptom duration was positively correlated with IL-2 production, and negatively correlated with the IFNγ/IL-2 ratio. It is concluded that these results point to an altered cell-mediated immunity in QFS, and suggest an immune response different from that in chronic Q fever.

In **chapter 5**, the study protocol of a prospective randomised, partly double-blind, placebo-controlled trial (the Qure study) is provided, which evaluates the efficacy of long-term doxycycline and CBT in QFS patients compared to placebo. In **chapter 6**, the results of this trial are described. Of the 155 patients randomised to CBT (n=51), doxycycline (n=52), or placebo (n=52), 154 patients were included in the intention-to-treat analysis. Fatigue severity following treatment, corrected for baseline fatigue severity, did not significantly differ between doxycycline and placebo, and was significantly lower after CBT than after placebo. The level of functional impairment did not differ significantly between both doxycycline and placebo and CBT and placebo. Doxycycline yielded no difference in the level of psychological distress compared to placebo, whereas the level of psychological distress significantly improved after CBT compared to placebo. Most patients had stable or declining antibody titres compared to baseline, and the number of patients with declining antibody titres was similar in all groups. It is concluded that CBT is effective in reducing fatigue severity and the level of psychological distress in QFS patients. Long-term treatment with doxycycline does not significantly reduce fatigue severity in QFS patients, and should not be advised.

PART II: Challenges in diagnosis and treatment of acute and chronic Q fever

In **chapter 7**, it was investigated whether acute Q fever could be differentiated from infections caused by other pathogens in patients presenting to hospitals, and whether prophylactic antibiotic treatment was effective to prevent the development of chronic Q fever in acute Q fever patients with risk factors. A retrospective case–control study was performed, evaluating differences in clinical signs, symptoms, and outcomes for 82 acute Q fever patients and 52 control patients who had pneumonia, fever and lower respiratory tract symptoms, or fever and hepatitis, but had negative serologic results for Q fever. Acute Q fever patients were younger and had higher C-reactive protein levels but lower leukocyte

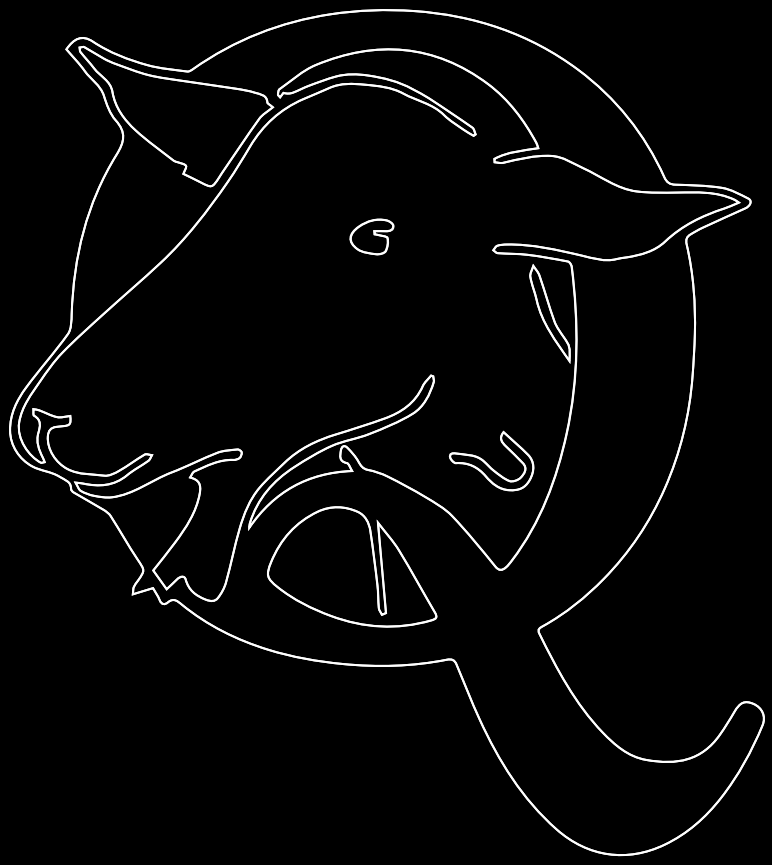
counts. However, a large overlap was found. It is concluded that differentiating acute Q fever from other respiratory infections, fever, or hepatitis is not possible without serologic testing or PCR. Furthermore, the data showed that in patients with an indication for antibiotic prophylaxis, chronic Q fever did not develop in patients who received such prophylaxis, but did develop in 50% of patients who did not receive prophylaxis. This underlines the recommendation that prophylactic treatment should be given to patients with risk factors for developing chronic Q fever.

In **chapter 8**, it was retrospectively evaluated whether ^{18}F-fluorodeoxyglucose positron emission tomography/CT (^{18}F-FDG PET/CT) and echocardiography were able to detect the localisation of infection in 52 chronic Q fever patients (18 proven, 14 probable, and 20 possible chronic Q fever patients according to the *Dutch Q fever consensus group*). Data on serology, the results of all imaging studies, possible risk factors for developing proven chronic Q fever and clinical outcome were recorded. Of those with proven chronic Q fever, 22% had endocarditis, 17% had an infected vascular prosthesis, and 39% had a mycotic aneurysm. Ten out of 13 ^{18}F-FDG PET/CT-scans in patients with proven chronic Q fever demonstrated the localisation of the infection. Transthoracic echocardiography and transesophageal echocardiography were helpful in only 6% and 50% of patients, respectively. Furthermore, 56% of these patients did not recall an acute Q fever episode. Our data show that if chronic Q fever is diagnosed, ^{18}F-FDG PET/CT is a helpful imaging technique for localisation of vascular infections due to chronic Q fever. Patients with proven chronic Q fever were diagnosed significantly more often with mycotic aneurysms than in previous case series. Furthermore, chronic Q fever often occurs in patients without a known episode of acute Q fever, so clinical suspicion should remain high, especially in endemic regions.

In **chapter 9**, a case series of four patients with treatment-induced cutaneous hyperpigmentation in previously unaffected skin is described. This relatively rare phenomenon diminished in all four patients after cessation of therapy, illustrating the need for recognition and timely cessation of therapy. It was not possible to determine the nature of the pigment deposited in the skin. It is concluded that cutaneous hyperpigmentation is a potential side effect of doxycycline therapy within the therapeutic dose range, and that the chance to evoke this adverse effect might be increased with the concomitant use of hydroxychloroquine. This is especially of importance in chronic Q fever, for which prolonged relatively high doses are given in combination with hydroxychloroquine.

In **chapter 10**, a fatal case of an immunocompromised patient with a confirmed unusual haematogeneously disseminated chronic Q fever infection is reported. This underlines the severity of this disease and the diversity of signs and symptoms that may occur, and highlights the need for increased awareness and recognition by physicians especially in case of risk factors. Also, a brief review of the literature with regard to the diverse clinical presentation of chronic Q fever is provided. It is concluded that an adequate diagnostic work-up using at least IFA serology and PCR for screening for chronic Q fever should be performed.

Chapter 11 contains a general discussion of the results presented in this thesis and their possible future implications.



CHAPTER 13

SAMENVATTING EN CONCLUSIES

SAMENVATTING EN CONCLUSIES

Query (Q) fever, de naam die oorspronkelijk werd verbonden aan een *Coxiella burnetii* infectie vanwege onbekendheid met het veroorzakende pathogeen, lijkt nog steeds een toepasselijke naam. Tot op heden is de Nederlandse Q-koortsuitbraak de grootste die ooit beschreven werd. Deze uitbraak heeft de mogelijkheid geboden nieuwe inzichten te verkrijgen in diverse vraagstukken op het gebied van Q-koorts. In dit proefschrift wordt een aantal uitdagende vragen op het gebied van Q-koorts onderzocht, waarbij de nadruk ligt op het Q-koortsvermoeidheidssyndroom (QVS). De primaire doelstellingen van dit proefschrift waren het vergroten van de (h)erkenning van QVS, het verkrijgen van nieuwe inzichten in de pathofysiologie van QVS en het evalueren van het effect van behandeling met langdurig doxycycline en cognitieve gedragstherapie (CGT) in QVS-patiënten. Het tweede doel was het onderzoeken van diagnostiek en behandeling van acute en chronische Q-koorts. **Hoofdstuk 1** bevat een algemene inleiding over Q-koorts en de diverse klinische manifestaties van deze ziekte. Tevens wordt hierin een overzicht gegeven van de inhoud en de doelen van dit proefschrift. Hierna wordt het proefschrift onderverdeeld in twee hoofdthema's: herkenning en behandeling van QVS (*deel I*) en uitdagingen in de diagnostiek en behandeling van acute en chronische Q-koorts (*deel II*).

DEEL I: Herkenning en behandeling van QVS

In **hoofdstuk 2** wordt aan de hand van een 'systematic review' een overzicht gegeven van de literatuur over vermoeidheid na een acute Q-koortsinfectie. Deze studie identificeert lacunes in de huidige kennis over vermoeidheid na een acute Q-koortsinfectie met betrekking tot de definitie, diagnose, achtergrond, beschrijving, etiologie, preventie, therapie en prognose. Ondanks dat de meeste patiënten binnen 6-12 maanden na een acute Q-koortsinfectie herstellen, blijft ongeveer 20% last houden van chronische vermoeidheid. Vermoeidheid na acute Q-koorts wordt over het algemeen aangeduid als QVS. Er wordt geconcludeerd dat het bestaan en langdurig aanwezig blijven van vermoeidheid na een acute Q-koortsinfectie een grote impact heeft. Er bestaan echter nog steeds diverse vragen over QVS, aangezien informatie over de etiologie, preventie, behandeling en prognose van QVS ondervertegenwoordigd is in de internationale literatuur. Een internationale definitie is wenselijk in toekomstige studies om bevindingen te kunnen vergelijken. Daarom wordt het voorstel gedaan om de definitie en het diagnostische algoritme van de Nederlandse 'Multidisciplinaire LCI-richtlijn Q-koortsvermoeidheidssyndroom (QVS)' internationaal te gebruiken.

Hoofdstuk 3 beschrijft een onderzoek naar verschillen en overeenkomsten tussen QVS-patiënten en patiënten met chronisch vermoeidheidssyndroom (CVS), waarbij de nadruk ligt op ontstekingswaarden en vermoeidheidsgerelateerde cognitieve gedragsfactoren. Tevens werd in een exploratieve analyse de relatie tussen deze cognitieve gedragsfactoren en vermoeidheid in QVS-patiënten onderzocht. Hiervoor werden de gegevens van twee onafhankelijke prospectieve studies op het gebied van QVS (n=117 patiënten) en CVS (n=173 patiënten) samengevoegd en geanalyseerd. QVS-patiënten bleken minder vaak vrouw te zijn, hadden een hogere body-mass index (BMI) en hadden voordat hun klachten begonnen

minder vaak een behandeling ondergaan voor een depressie. Na het corrigeren voor klachtenduur en diagnostische criteria voor QVS en CVS bleek dat het verschil in geslacht en BMI nog steeds significant was. Ook bleek dat QVS-patiënten ouder waren. De ernst van zowel vermoeidheid als psychische klachten bleek bij QVS-patiënten niet te verschillen van CVS-patiënten, maar QVS-patiënten rapporteerden minder additionele symptomen. Verder hadden QVS-patiënten een sterkere somatische attributie en een hogere fysieke activiteit. Er werden geen verschillen gevonden op het gebied van ontstekingswaarden en in andere vermoeidheidsgerelateerde cognitieve gedragsfactoren. De gevonden verschillen in bekende predisponerende factoren voor chronische vermoeidheid suggereren dat andere predisponerende factoren een rol spelen bij het ontstaan van QVS. De relatie tussen cognitieve gedragsfactoren en vermoeidheid zoals eerder vastgesteld in CVS kon niet worden bevestigd bij QVS-patiënten. Desondanks is er wel een aanzienlijke overlap in vermoeidheidsgerelateerde cognitieve gedragsfactoren. Samen met de gevonden relatie tussen fysieke activiteit en vermoeidheid suggereert dit dat gedragsinterventies zouden kunnen leiden tot een afname van de ernst van vermoeidheid in QVS-patiënten.

In **hoofdstuk 4** worden de resultaten beschreven van de specifieke interferon-γ (IFNγ) productie en de IFNγ/Interleukine(IL)-2 ratio in 20 QVS-patiënten. Deze resultaten werden vergeleken met eerdere resultaten bij seropositieve controles (n=135) en chronische Q-koortspatiënten (n=28). Daarnaast werd gekeken naar de correlatie tussen karakteristieken van QVS-patiënten en de IFNγ- en IL-2-productie en de IFNγ/IL-2-ratio. QVS-patiënten waren jonger, maar de geslachtsverdeling was identiek aan die van seropositieve controles en chronische Q-koortspatiënten. QVS-patiënten hadden een significant hogere IFNγ-productie dan seropositieve controles. Bij QVS-patiënten bleek de IFNγ/IL-2-ratio significant lager te zijn dan die in chronische Q-koortspatiënten. Daarnaast bleek de klachtenduur positief te zijn gecorreleerd met de IL-2-productie en negatief te zijn gecorreleerd met de IFNγ/IL-2-ratio. Er wordt geconcludeerd dat deze resultaten wijzen op een veranderde celgemedieerde immuniteit in QVS-patiënten. Daarnaast lijkt er sprake van een andere immuunrespons dan in chronische Q-koorts.

In **hoofdstuk 5** wordt het studieprotocol gepresenteerd van een prospectieve, gerandomiseerde, deels geblindeerde, placebo gecontroleerde studie (de Qure-studie). Het doel van deze studie was het evalueren van de effectiviteit van langdurig doxycycline en CGT in QVS-patiënten in vergelijking met placebo. In **hoofdstuk 6** worden de resultaten van deze studie weergegeven. Van de 155 patiënten die zijn gerandomiseerd tussen CGT (n=51), doxycycline (n=52) en placebo (n=52), zijn 154 patiënten geincludeerd in de intention-to-treat analyse. Er bleek geen significant verschil in ernst van de vermoeidheid na behandeling met doxycycline in vergelijking met placebo. De ernst van de vermoeidheid was significant lager na CGT in vergelijking met placebo. Deze resultaten zijn gecorrigeerd voor de ernst van de vermoeidheid bij aanvang van de studie. De ernst van de dagelijkse beperkingen bleek na behandeling met zowel doxycycline als CGT niet significant te verschillen in vergelijking met placebo. Behandeling met doxycycline verschilde in effect op de ernst van psychische klachten niet van placebo, terwijl na behandeling met CGT daarentegen de ernst van de psychische

klachten significant afnam in vergelijking met placebo. In vergelijking met de meting bij aanvang van de studie hadden de meeste patiënten stabiele of gedaalde antistoftiters na behandeling. Het aantal patiënten waarbij de antistoftiter was gedaald gedurende de behandeling was niet verschillend tussen alle groepen. Er wordt geconcludeerd dat CGT effectief is in het reduceren van de ernst van de vermoeidheid en de ernst van psychische klachten in QVS-patiënten. Langdurige behandeling met doxycycline zorgt echter niet voor een significante daling van de ernst van vermoeidheid en wordt niet geadviseerd.

DEEL II: Uitdagingen in de diagnostiek en behandeling van acute en chronische Q-koorts
In **hoofdstuk 7** worden de resultaten weergegeven van een retrospectief patiënt controle-onderzoek waarin onderzocht werd of acute Q-koorts kan worden onderscheiden van infecties veroorzaakt door andere pathogenen bij patiënten die zich presenteerden in het ziekenhuis. Ook werd onderzocht of profylactische behandeling met antibiotica bij acute Q-koortspatiënten met risicofactoren effectief is om het ontstaan van chronische Q-koorts te voorkomen. Gegevens over klinische symptomen, klachten en het beloop werden verzameld van 82 patiënten met acute Q-koorts. Deze gegevens werden vergeleken met die van 52 controle-patiënten die zich presenteerden met een pneumonie, of met koorts en lage luchtwegklachten, of met koorts en hepatitis, maar waarbij acute Q-koorts uiteindelijk kon worden uitgesloten. Patiënten met acute Q-koorts waren jonger, hadden een hoger C-reactief proteïne, maar een lager leukocytenaantal. Desondanks werd een grote overlap gevonden tussen patiënten met acute Q-koorts en controles. Geconcludeerd wordt dat het onderscheiden van acute Q-koorts ten opzichte van andere respiratoire infecties, koorts, of hepatitis, niet mogelijk is zonder serologische analyse of PCR. Verder bleek dat bij acute Q-koortspatiënten met een indicatie voor profylactische behandeling met antibiotica er geen chronische Q-koorts ontwikkelde indien deze patiënten daadwerkelijk profylaxe ontvingen, terwijl 50% van de patiënten die geen profylaxe ontvingen wel chronische Q-koorts ontwikkelde. Dit bevestigt de aanbeveling om acute Q-koortspatiënten met risicofactoren voor het ontwikkelen van chronische Q-koorts profylactisch te behandelen met antibiotica.

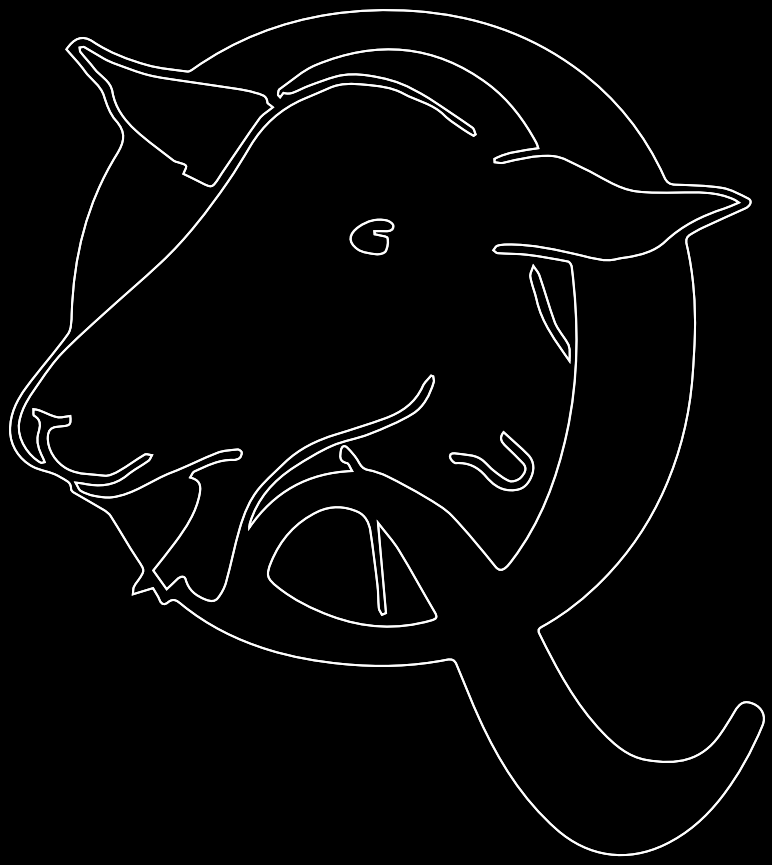
Hoofdstuk 8 beschrijft een retrospectieve studie naar de waarde van ^{18}F-fluorodeoxyglucose positron emissie tomografie/CT (^{18}F-FDG PET/CT) en echocardiografie in het detecteren van de lokalisatie van de infectie in 52 chronische Q-koortspatiënten (onderverdeeld in 18 bewezen, 14 waarschijnlijke en 20 mogelijke chronische Q-koortspatiënten volgens de *Nederlandse consensusgroep diagnostiek Q-koorts*). De serologische resultaten, resultaten van beeldvormende onderzoeken, mogelijke risicofactoren voor het ontwikkelen van een bewezen chronische Q-koortsinfectie en gegevens over het verdere klinische beloop werden verzameld. Van de patiënten met een bewezen chronische Q-koortsinfectie bleek 22% een endocarditis te hebben, 17% had een geïnfecteerde vaatprothese en 39% een mycotisch aneurysma. Tien van de 13 ^{18}F-FDG PET/CT-scans die werden verricht bij patiënten met een bewezen chronische Q-koortsinfectie toonden de lokalisatie van de infectie aan. Transthoracale echocardiografie en transoesofageale echocardiografie waren respectievelijk maar in 6% en 50% van deze patiënten behulpzaam in het lokaliseren van de infectie. Verder bleek dat 56% van de patiënten met een bewezen chronische Q-koortsinfectie zich geen

acute Q-koortsepisode kon herinneren. Geconcludeerd wordt dat als chronische Q-koorts is gediagnosticeerd, ^{18}F-FDG PET/CT een waardevolle beeldvormende techniek is voor het lokaliseren van vaatinfecties veroorzaakt door chronische Q-koorts. In deze studie werden patiënten met een bewezen chronische Q-koortsinfectie significant vaker gediagnosticeerd met een mycotisch aneurysma dan in eerdere case series. Verder komt chronische Q-koorts vaak voor zonder dat patiënten weten dat ze een (acute) Q-koortsinfectie hebben doorgemaakt. Daarom moet de klinische verdenking op chronische Q-koorts hoog blijven, voornamelijk in gebieden waar Q-koorts endemisch is.

In **hoofdstuk 9** wordt een reeks casussen beschreven van vier patiënten met cutane hyperpigmentatie die door behandeling met doxycycline werd geïnduceerd. Voorafgaand aan deze behandeling was er op de plaatsen met hyperpigmentatie sprake van een normale, gezonde huid. De uitgebreidheid van dit relatief zeldzame fenomeen nam af na het stoppen van de therapie in alle beschreven patiënten. Dit illustreert het belang van tijdige herkenning en het tijdig stoppen van de behandeling. Het was niet mogelijk om de aard van het pigment in de huid te bepalen. Er wordt geconcludeerd dat cutane hyperpigmentatie een potentiële bijwerking is van behandeling met doxycycline binnen de therapeutische marge en dat de kans op deze bijwerking mogelijk wordt vergroot door het gelijktijdig gebruik van hydroxychloroquine. Dit is vooral van belang in de behandeling van chronische Q-koorts, waarvoor gedurende een lange periode een relatief hoge dosering doxycycline wordt voorgeschreven in combinatie met hydroxychloroquine.

In **hoofdstuk 10** wordt een casus met fatale afloop beschreven van een immuungecompromitteerde patiënt met een zeldzame hematologisch gedissemineerde chronische Q-koortsinfectie. Deze casus onderstreept de ernst van deze ziekte en de diversiteit aan symptomen die kunnen optreden bij chronische Q-koorts. Daarnaast illustreert deze casus het belang van verhoogde waakzaamheid bij artsen voor en herkenning van chronische Q-koorts, vooral indien risicofactoren aanwezig zijn. Verder wordt een kort overzicht gegeven van de beschikbare literatuur over de diverse klinische presentatievormen van chronische Q-koorts. Er wordt geconcludeerd dat adequate diagnostiek naar chronische Q-koorts moet worden verricht, waarbij tenminste gebruik gemaakt moet worden van IFA serologie en PCR.

Hoofdstuk 11 bevat een algemene discussie aangaande de belangrijkste bevindingen uit dit proefschrift. Tevens wordt het mogelijke vervolg van de onderzoeken in dit proefschrift toegelicht.



CHAPTER 14

DANKWOORD
LIST OF PUBLICATIONS
CURRICULUM VITAE

DANKWOORD

"Quand il n'y a pas de solution, il n'y a pas de problème", was het gezegde dat ik in mijn achterhoofd had toen ik mijn promotie-onderzoek startte. Met oplossingsgericht denken kan men veel bereiken, maar zonder samenwerking en, zowel directe als indirecte, hulp van anderen, zou dit proefschrift niet tot stand zijn gekomen. Dit dankwoord is aan hen gericht.

Wellicht niet standaard en eigenwijs (of op mijn eigen manier, zoals ik ook mijn promotietraject heb doorlopen), wil ik mijn dankwoord in eerste instantie richten aan alle deelnemende Q-koortspatiënten die mijn proefschrift mogelijk hebben gemaakt. Zij hebben een centrale positie in de zorg en in mijn onderzoeken en daarmee ook in mijn dankwoord. Het positieve beeld omtrent het onderzoek is grotendeels te danken aan een actieve patiëntenvereniging (**Q-uestion**, Stichting voor mensen met Q-koorts). Dank voor het meedenken, en het beschikbaar stellen van jullie kwaliteiten en mogelijkheden om het onderzoek op de kaart te zetten en uit te voeren. In het bijzonder noem ik hier **Michel van den Berg**, voormalig voorzitter en één van de drijvende krachten achter deze stichting. Dank voor je onuitputtelijke inzet voor alle Q-koortspatienten en daarmee ook voor mijn onderzoek. Ook **Q-support** heeft door subsidiëring en blijvende aandacht voor o.a. de Qure-studie een groot aandeel in het afronden van dit proefschrift gehad.

Mijn promotores en co-promotor verdienen elk eigenlijk een apart boekwerk als dankwoord. Helaas kreeg ik een restrictie opgelegd om het aantal pagina's beperkt te houden (dezelfde restrictie kreeg ik overigens voor mijn poliklinische correspondentie over patiënten…).

Prof. dr. van der Meer, beste **Jos**, veel promovendi dromen er van onder jou te mogen promoveren, een eer die voor mij is weggelegd. Je onuitputtelijke kennis over o.a. chronische vermoeidheid, infectieziekten en de onderliggende relatie tilden de onderzoeken naar een hoger niveau. Op elk vraagstuk heb je een gefundeerd antwoord. Ook ben jij diegene die mij heeft aangenomen voor de opleiding tot internist. Ik ben er trots op dat je één van mijn promotoren wilt zijn.

Prof. dr. Bleijenberg, beste **Gijs**, van begin tot eind ben ik onder de indruk geweest van je gave een wetenschappelijke blik te combineren met patiëntgerichtheid. Je bent een expert op het gebied van chronische vermoeidheid en met hart en ziel betrokken bij zowel patiëntenzorg als promovendi. Overleg was altijd mogelijk, manuscripten kwamen snel retour voorzien van zorgvuldig commentaar vanuit diverse invalshoeken. De communicatietraining die ik van jou kreeg opende nieuwe deuren voor patiëntenzorg en het verrichten van wetenschappelijk onderzoek. Nog steeds pluk ik hier dagelijks de vruchten van. Dank voor je vertrouwen en de investering die je in mij hebt gestopt.

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Leden van de manuscriptcommissie, prof. dr. Speckens, prof. dr. Van Dissel en prof. dr. Wertheim: hartelijk dank voor het lezen en beoordelen van mijn manuscript.

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CURRICULUM VITAE

Stephan Patrick Keijmel werd geboren op 9 september 1986 in Deventer en groeide op in de Hanzestad Zutphen. Hij voltooide in 2004 het VWO aan het Isendoorn College te Warnsveld, waar hij een gecombineerd profiel deed van Natuur & Techniek en Natuur & Gezondheid.
In datzelfde jaar startte hij de opleiding Geneeskunde aan de Radboud Universiteit Nijmegen, waarvoor hij een jaar later zijn propedeuse behaalde. In december 2010 rondde hij de opleiding Geneeskunde af. Sinds het tweede jaar van zijn opleiding tot op heden woont hij in Nijmegen. Zijn interesse voor infectieziekten is sinds zijn studie geneeskunde alleen maar toegenomen. Na deelname als proefpersoon aan de AMA-1 studie, een vaccinatie-studie tegen malaria, hielp hij met het rekruteren van proefpersonen in opvolgende malariastudies (EHMI-8 studie, PfLSA-3-rec studie). Ook nam hij van 2007 tot 2008 als student deel aan de dataverwerking van een grootschalig onderzoek naar de lange termijn uitkomst van prematuren (ELBW studie). Voor de verplichte onderzoeksstage in de opleiding Geneeskunde verbleef hij van januari 2008 t/m juni 2008 in Sumba, Indonesië, waar hij onderzoek deed naar malaria (hepcidine concentratie in asymptomatisch dragerschap van *P. falciparum* en *P. vivax* malaria). Daarna werkte hij tot en met januari 2011 naast zijn opleiding bij de Thuiszorg in Nijmegen. Hij deed zijn seniorcoschap op de afdeling Infectieziekten in het Radboudumc en werd daar gevraagd als kandidaat voor dit promotietraject.
In februari 2011 begon hij aan zijn promotietraject met het hoofdonderwerp "*Q-koortsvermoeidheidssyndroom (QVS*)", onder begeleiding van prof. dr. van der Meer, prof. dr. Bleijenberg, prof. dr. Knoop en dr. Bleeker-Rovers. Diverse onderzoeken werden gedaan, met als grootste de *Qure-studie*. Hij zag meer dan 500 nieuwe patiënten met klachten na Q-koorts op de polikliniek van het Radboud Q-koorts Expertisecentrum. Stephan nam intensief deel aan de ontwikkeling van de landelijke LCI-richtlijn QVS, die in februari 2012 werd gepubliceerd, en aan de Vlaams-Nederlandse Onderzoekersgroep-Chronische Vermoeidheid (VNO-CHROVER). Hij spande zich succesvol in om de wachtlijstproblematiek voor cognitieve gedragstherapie voor QVS-patiënten te beperken middels een gehonoreerde aanvraag voor financiële ondersteuning vanuit het ministerie van Volksgezondheid, Welzijn en Sport. Vanaf juni 2013 tot en met december 2015 was hij naast zijn onderzoekswerk in het Nijmeegs Kenniscentrum Chronische Vermoeidheid (NKCV), betrokken bij de screening van patiënten voor de behandeling van chronische vermoeidheid. Hij gaf onderwijs aan studenten Geneeskunde en begeleidde vier studenten in hun verplichte wetenschappelijke stage voor de opleiding Geneeskunde. Ook was hij intensief betrokken bij de aanvraag van inmiddels gehonoreerde subsidies ("*De rol van het immuunsysteem hij QVS*", "*De impact van Q-koorts op arbeid en psychosociaal functioneren van patiënten met chronische Q-koorts of QVS*" en *"De Nederlandse Q-koortsepidemie in kaart gebracht: een meta-analyse van de impact op korte en lange termijn"*, allen gesubsidieerd vanuit Q-support) en verzorgde hij een deel van de begeleiding van de nieuw aangestelde promovendus op het eerstgenoemde project in 2015. Daarnaast heeft hij zich vanaf 2011 belangeloos ingezet voor vele informatieavonden van Q-uestion, Stichting voor mensen met Q-koorts, en stichting Q-support.
Hij is inmiddels per 1 april 2016 gestart met de opleiding tot internist, waarvoor hij reeds werd aangenomen tijdens zijn promotietraject, met als uiteindelijke differentiatie infectieziekten.