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# New insights in *Coxiella burnetii* infection : diagnosis and therapeutic update

Cléa Melenotte[1,2], Matthieu Million[1,2], Didier Raoult[1,2*]

[1]Aix-Marseille Univ, IRD, APHM, MEPHI, Marseille, France

[2]IHU-Méditerranée Infection, Marseille, France

***Corresponding author :**

Didier Raoult

IHU - Méditerranée Infection

19-21 Boulevard Jean Moulin

13005 Marseille, France

Email : didier.raoult@gmail.com

**Abstract**

**Introduction:** *Coxiella burnetii* infection is still challenging physicians, mainly because no international coordination has been stated to standardize the therapeutic strategy and improve the clinical outcomes.

**Areas covered :** Based on the recent knowledge on Q fever, we review here the clinical practices from Q fever diagnosis to therapy. We searched Pubmed and Google Scholar to perform the qualitative synthesis.

**Expert opinion:** Four major critical points are highlighted in this review. The first point is that Q fever diagnosis has been reviewed in the light of the new diagnosis tools, including molecular biology, transthoracic echocardiography and 18F-FDG-PET/CT -scan imaging. Q fever diagnosis results from the presence of a microbiological criterion in addition to a lesional criterion. Second, the identification of the anticardiolipin antibodies as a novel biological predictive marker for acute Q fever complications (haemophagocytic syndrome, acute Q fever endocarditis, alithiasic cholecystitis, hepatitis, and meningitis). Third, the observation of a coincidence between Q fever and NHL that has made persistent *C. burnetii* infection a risk of non-Hodgkin's lymphoma. Finally, we expose here the close follow-up we proposed from the French National Reference Center for patients with Q fever infection to detect relapse and complications.

**Key words:** Q fever, *Coxiella burnetii*, diagnosis, anticardiolipins, therapy, imaging tools,

**Article highlights**

- Q fever diagnosis is based on the combination of a microbiological criterion in addition to a lesional criterion.
- The anticardiolipin antibodies emerged as a useful biological predictive marker for acute Q fever complications.
- Persistent *C. burnetii* infection is a risk of non-Hodgkin's lymphoma.

## 1. Introduction

Q fever is a severe worldwide disease caused by *Coxiella burnetii* that can lead to major epidemics outbreaks, as reported in the Netherlands with more than 4000 cases in 4 years and nearly 118 deaths, among which 86 were Q fever related [1][2]. *C. burnetii* persistent focalized infection is still challenging physicians, first, because diagnosis of persistent infection can be difficult without symptoms, but mainly because no recent international recommendations have been stated to standardize the therapeutic strategy and improve clinical outcomes. The last two decades have seen new diagnostic tools that have revolutionized clinical practice, polymerase chain reaction and the 18F-FDG-PET/CT scan imaging [3–7]. This has allowed us to identify new *C. burnetii* infectious foci and not to miss persistent infections, even if serological levels are low (15).

Historically, “chronic Q fever” definition was proposed by Peacock in 1983, in patients with endocarditis and granulomatous hepatitis, without specifying the chronological evolutivity of the disease [8]. Peacock observed that patients with “chronic Q fever” had high levels of IgG phase I and therefore proposed: “Acute and chronic Q fever clinical entities may be recognized by serological profile of phase I and II antibodies” [8]. Then, Tissot-Dupont *et al.,* showed that IgG phase I >800 was predictive for “chronic Q fever” [9]. Subsequently, Frankel published that the higher the IgG Phase I, the higher the positive predictive value of IgG Phase I for the diagnosis of "chronic Q fever" [10]. Nevertheless, in recent decades, three specific observations have challenged this old paradigm in which "chronic" infection is defined according to the serological threshold. The first is that of a high and persistent IgG phase I titer in the acute phase of the disease, especially in the French Guiana, where a specific hypervirulent strain is endemic, the Cb175 strain, MST 17[11,12]. The exacerbated strain-dependent serological response was replicated in a mouse model [13]. The second point is the recent detection of persistent *C. burnetii* infectious focus with low serological titers

(endocarditis, osteoarticular infection and vascular infection) [3,14]. In fact, in the French Q fever National reference center (NRC) database, from 1991 to 2016, we identified that 5% of patients with persistent focused infection had a low IgG serological titer that never reached 1/800.

Third, the definition of "chronic Q fever fatigue syndrome", in which a positive Q fever serology is associated with subjective symptoms without identifying an infectious outbreak, is confusing [15]. Thus, as the question that is regularly asked to us is "how to treat these patients with high serological titers with no obvious focus of *C. burnetii*, and, conversely, how to treat patients with low serological titer and a suspected infectious focus?", we were prompted to redefine Q fever diagnosis with new criteria. We propose here a narrative review that aims to highlight new advances in the diagnosis of Q fever and therapeutic strategies that have improved the health of patients with Q fever.

## 2. Material and methods

This review is based on clinical reports and results published in the scientific literature and is not the result of an international expert consensus of opinions. We searched Pubmed and Google Scholar, and a total of 84 references were included in the final qualitative synthesis. Key words were: Q fever, *Coxiella burnetii*, doxycycline, hydroxychloroquine, treatment, transthoracic echocardiography, 18F-FDG-PET/CT -scan, anticardiolipins, acute, persistent, pregnancy, children, immunosuppression.

Figure 1 is from a previous work published in the JAMA Network Open as a supplementary figure 13, and is published in agreement with the journal's editorial board[4]. Based on the two previous works on *C. burnetii* associated NHL, we have performed a manual mini meta-analysis. [16,17]

## 3. New criteria: One microbiology criterion and one organic/lesional criterion

Acute Q fever is a primary infection defined by clinical symptoms secondary to the entry of the bacteria in the organism. It may be accompanied by systemic manifestations. Clinical presentation includes transaminitis, pneumonia, encephalitis, meningitis, pericarditis, endocarditis, haemophagocytic syndrome, alithiasic cholecystitis, and isolated fever and/or flu like syndrome [4]. Acute symptoms could be associated with systemic autoimmune reaction (see next chapter). On 18F-FDG-PET/CT scan imaging, bone marrow, liver, and spleen hypermetabolism can be observed [5]. Acute Q fever is defined as a clinical manifestation of less than 3 months. The infection can resolve spontaneously, but the bacteria can also persist with or without symptoms in a replicative niche, such as lymph node, placenta, heart valve, bone marrow, and in sanctuary cells such as adipocytes and macrophages [18]. By escaping the immune system and replicating silently, *C. burnetii* can cause persistent focalized infection, defined by the identification of an infectious focus in addition to the microbiological criteria. To confirm the presence of a persistent infection, isolated fever is not sufficient, a microbiological criterion in addition to a clinical or organic lesional criterion is required [15,19,20]. The diagnostic criteria used (one microbiological and one lesional) for acute Q fever and persistent *C. burnetii* infection are summarized in Tables 1 & 2.



## 4. Microbiological tools

### 4.1 Serology

Serology remains the main tool to diagnose *C. burnetii* infection. Seroconversion is usually detected within the 6 weeks after the onset of symptoms and is estimated to be positive in 90% of the cases 3 weeks after initial symptoms. Indirect immunofluorescence antibody test (immunofluorescence assay, IFA), complement fixation, ELISA and microagglutination are

the techniques the most used to diagnose Q fever, the first three being commercially available [21]. IFA remains the reference method because of its simplicity and accuracy [22][23]. As most reference laboratories have developed their own immunofluorescence test and the serological tests used are sometimes variable in the event of an outbreak, the comparison of serological levels and the exploitation of the cutoff remain difficult. Cross reactivity have been described with *Legionella micdadei* (34%) and *Bartonella* (50%)[24][25].

The presence of Phase II IgM ≥ 50 is part of the microbiological criteria for acute Q fever. Nevertheless, according to the NRC for Q fever, 5% of patients with primary infection with *C. burnetii* had only positive Phase I and Phase II IgG antibodies without IgM. In these cases, the distinction between acute Q fever and past infection remains difficult to assess, especially because acute Q fever clinical presentation is multifaceted and could be similar to other infectious diseases. Based on the avidity test used in case of cytomegalovirus and toxoplasmosis infection, we performed an avidity test for *C. burnetii* infection [26]. Phase I and II protein denaturation with urea was observed in case of recent infection, whereas a strong avidity was observed in patients with persistent infection >6 months [26]. These results were confirmed using Western blot [26]. This new serological test helps to exclude a recent infection when the avidity is high, and could be especially beneficial in pregnancy, since women are mostly asymptomatic. Then, if the avidity test is low and the diagnosis of acute Q fever is retained, a transthoracic echocardiogram in addition to anticardiolipin antibodies should be proposed to detect any potential complications (see next chapter). In case of high avidity, persistent focalized infection should be sought (see next chapter).



### 4.2 Polymerase chain reaction

Polymerase chain reaction (PCR) has been used to detect *C. burnetii* DNA or RNA on different samples, i.e. blood, serum and fresh tissues. Two specific repeated sequences for *C.*

*burnetii* are used, IS*1111* and IS*30*a (IS for Insertion Sequence). Recent advances have demonstrated that lyophylisation improves the sensibility of *C. burnetii* PCR by concentrating bacterial DNA [27]. Lyophylisation has two main indications: first, at the acute stage of the disease, in negative serum, especially in the context of a Q fever outbreaks and the second, in patients with suspected *C. burnetii* persistent infection as endocarditis, vascular infection and osteoarticular infection, because positive PCR is part of microbiological criteria [27][19]. Positive PCR without evidence of an organic lesion is not sufficient to diagnose *C. burnetii* infection.

### 4.3 Culture

*C. burnetii* is a highly fastidious intracellular bacterium that requires a parasitophorous acid *Coxiella* containing vacuole to grow. Therefore, L929 cells culture media is broadly used to study *C. burnetii in vitro* [28]. Axenic culture has been developed to facilitate the manipulation of the bacterium [29].

### 4.4 Immunohistochemistry

Immunohistochemistry is a specific reaction based on antigen-antibody reaction with a marked antibody affinity against *C. burnetii*. It is used for the *in situ* detection of *C. burnetii* and has a low sensitivity as a major limitation [30].

### ` 4.5 Fluorescence *in situ* hybridization

Recently, fluorescent *in situ* hybridization has been proposed to detect specific *C.*

*burnetii* DNA or RNA *in situ* with a higher sensitivity than immunohistochemistry[6]. This technique was used with clinical revealance in animals and humans. First in placenta of ruminant abortion, second in the lymphoma tissues from patients with persistent *C. burnetii* infection, and then in the cardiovascular tissues of patients with cardiovascular *C. burnetii* infection. [6,31–33] Recently, the systematic use of PCR in the valvular tissue of a patient operated on for aortic stenosis led us to identify valvular *C. burnetii* infection by PCR confirmed by FISH [34] The high sensitivity of FISH allows the visualization of *C. burnetii in situ* and positive 16S RNA helps to determinate the viable stage of the bacterium.

### 4.6 Diagnostic tools as markers of the disease progression and remission

Finally, in our center, we use *C. burnetii* PCR and serology for the diagnosis, but also as markers of evolution. We consider a favorable biological evolution when 3 criteria are met: i. a dilution loss of 2 of IgG phase I at 12 months ii. the absence of IgM phase II at 12 months and iii. a negative blood or serum PCR at 12 months.

## 5. Clinical and radiological tools

### 5.1 Clinical exam

The first step to diagnose infectious focus is the clinical examination. Pneumonia, hepatomegalia, heart murmur and lymphadenopathy are the four main clinical foci to be investigated in the first clinical evaluation. The presence of a lymphadenitis should guide the realization of a 18F-FDG-PET/CT scan to seek other deep lymphadenitis and to follow this lymphatic focus, considered as a critical step to lymphomagenesis (see next chapter).

### 5.2 Transthoracic echocardiography (TTE)

Transthoracic echocardiography is the key tool for the management of acute and persistent *C. burnetii* infection and has transformed *C. burnetii* endocarditis from a chronic fatal illness to a curable and preventable disease. While *C. burnetii* endocarditis concerns fewer than 5% of patients with Q fever, it was initially considered as a slowly evolving disease, affecting men with underlying valvulopathy, and characterized by marked clinical and biological manifestations. The common use of TTE first led to the identification of an underlying valvulopathy as a risk factor for *C. burnetii* endocarditis [35,36]. TTE was then systematically recommended within the three months of acute Q fever diagnosis to identify predisposing factors for persistent infection and to initiate antibiotic prophylaxis. This strategy has significantly reduced the morbidity induced by *C. burnetii* infection since in patients with acute Q fever and underlying valvulopathy, the estimated risk of developing endocarditis is 40% (See next chapter) [36–38]. The systematic use of TTE at increasingly early stage (within the 3 months) led to the identification of a new clinical entity, acute Q fever endocarditis [39,40][41]. Therefore, TTE plays a crucial role in the management of acute Q fever. This diagnosis tool is recommended to detect acute Q fever endocarditis, to identify unknown underlying valvulopathies and to confirm a known valvulopathy [36,42]. This diagnostic strategy has been discussed in the Netherlands, as De Lange *et al*. did not identify a statistically significant difference between patients with acute Q fever with and without valvulopathy detected by screening echocardiography [43]. Nevertheless, whether in France, Australia, Spain or Israel, 95%, 92%, 83% and 100% of patients with persistent *C. burnetii* endocarditis have underlying valvulopathy respectively. In the Netherlands, it affects 19% of patients with “chronic” Q fever [44,45][46][47]. Interestingly in the Netherlands, as new cases of *C. burnetii* endocarditis were observed 7 years after the outbreaks, De Lange *et al.* proposed to screen patient with underlying valvulopathy to prevent complications, which constitutes a first step.[48]

### 5.3 Transoesophageal echocardiography (TOE)

With regard to transoesophageal echocardiography, in the case of acute Q fever, we have recently identified four factors that determine the indication for this imaging examination: being a male, over 40 years of age, with aCL>60 GPLU and a negative or inconclusive TTE. In these cases, when the 4 factors are fulfilled, TOE may help to detect acute Q fever endocarditis and underlying valvulopathy [41]. In case of persistent *C. burnetii* infection, TOE is recommended when TTE is inconclusive.

### 5.4 18F-FDG-PET/CT scan

18F-FDG-PET/CT scan imaging has become a new tool for the detection of *C. burnetii* deep focalized infectious focus, because classical morphological tools often fail to detect slight anatomical changes. In fact, *C. burnetii* persistent focalized endocarditis is characterized by valvular remodeling associated with calcification, whereas typical vegetation is detected only in 30% of the cases and valvular insufficiency is detected in only 70 % of the cases [44,49]. With CT scan, only vascular aneurysm and vascular fistula can be detected. As systemic disease, Q fever can affect several organs at once, so that whole body 18F-FDG-PET/CT scan imaging represents a revolutionary tool for identifying all *C. burnetii* infectious foci. In a study performed by the NRC for Q fever, in 167 patients with Q fever, 18F-FDG-PET/CT scanner altered the diagnosis in 62.6% of cases, and 42% of patients had 2 or more hypermetabolic foci [5]. 18F-FDG-PET/CT scan imaging has been shown to be useful in the diagnosis of prosthetic valve endocarditis since 2/3 of patients with valvular hypermetabolic focus had a prosthetic valve and 44% of patients with newly diagnosed vascular foci had a vascular prosthesis [5]. Nonetheless, the sensitivity of the18F-FDG-PET/CT is much more higher for the diagnosis of prosthetic valve endocarditis than native valve endocarditis.

[50][51]. In a study conducted in the Netherlands involving 273 patients with possible, probable or definite chronic Q fever, according to their definition criteria, 18F-FDG-PET/CT scan contributed and led to the modification of the diagnosis in 13.5% of the cases [51][52]. In a recent report of 2,434 cases from the French NRC for Q fever, 18F-FDG-PET/CT scan allowed the identification of 43% of deep lymphadenitis [40].

Consequently, we recommend 18F-FDG-PET/CT scanner imaging to assess deep infectious foci in patients with high IgG phase I, in patients with positive *C. burnetii* PCR on blood or serum and in patients with persistent symptoms.

## 6. Anticardiolipin, a new marker for acute Q fever complications

In the 1980s, a first description of the circulating anticoagulant was reported in cases of acute Q fever [53][54]. Then, anti-nuclear antibodies, inhibitor of blood coagulation factor IX, cold agglutinins, anti-ß-2microglobuline, mitochondrial antibodies and smooth muscle antibodies were reported during *C. burnetii* infection without significant clinical impact [55][56][57]. Clinical relevance of positive anticardiolipin antibodies (aCL) has recently been demonstrated.

### 6.1 Anticardiolipin antibodies and acute Q fever complications

Anticardiolipin antibodies are reported to be elevated in 47 to 81 % of acute Q fever [57]. Elevated IgG anticardiolipins in patients with underlying valvulopathy were first identified as predictive for evolution to *C. burnetii* persistent infection and then described as associated with transient valvular lesion in the acute phase of the disease [40][39]. In another cohort study performed by the French NRC for Q fever, in cases of elevated anticardiolipin antibodies, 1.9% of patients were diagnosed with thrombosis [58].

Very recently, we analyzed 2,434 patients, including 1,328 patients who were tested for anticardiolipin antibodies. Positive aCL (n=498) has been associated with 6 rare complications of acute Q fever, heamophagocytic syndrome, alithiasic cholecystitis, meningitis, thrombosis, acute Q fever endocarditis and hepatitis (table 3 ROC analysis) [4]. Interestingly, pneumonia was not associated with the elevation of aCL. Strikingly, positive aCL were reported as significantly higher in men than in women, and men were found to be more affected than women by Q fever complications [59].

Elevation of aCL in acute Q fever could therefore be associated with vascular lesions, ranging from large vessels causing deep arterial and venous thrombosis to small vessels, causing capillary thrombi and small perivascular hemorrhages as described in *C. burnetii* meningo-encephalitis [60][61][62]. The aCL tropism for vascular endothelium is confirmed by the observation of acute Q fever valvular injury [41].

### 6.2 Exploration of patients with increased anticardiolipin antibodies

In case of positive aCL, some imaging could be recommended to search for complications. TTE should be performed, whatever the height of the anticardiolipin levels. TOE should be performed in male, aged over 40 years, with aCL>60 IgG phospholipid-binding unit, (GPLU) and negative or inconclusive TTE [41]. If the patient presents abdominal pain, abdominal CT scan or ultrasonography must be performed to look for alithiasic cholecystitis. In case of meningitis, magnetic resonance imaging should look for encephalitis such as intracerebral sign of vasculitis. Cytopenia should be carefully investigated, haemophagocytic should be seriously considered and bone marrow aspirate should be performed in case of haemophagocytic syndrome suspicion. As the haemophagocytic syndrome has been shown to be significantly associated with a risk of non-Hodgkin lymphoma (OR=19.1, 95% CI [3.4-

108.6] p<.001), 18F-FDG-PET/CT scan must also be performed to detect deep hypermetabolism, such as lymphadenitis [4].

Finally, in case of elevated aCL without identified focus, TTE ± TOE must be performed to detect valvular injury. CT-scan could be proposed in the first instance if no valvular lesions are detected on echocardiography to look for the deep infection site and visceral complications associated with aCL (thrombosis, cholecystitis). Then, if CT-scan is negative, 18F-FDG-PET/CT scan could be proposed to search deep infectious and aCL-associated complications.

**7. Acute Q fever treatment**

Acute Q fever treatment could be separated in 3 clinical entities, acute Q fever without complicated focus, acute Q fever with positive antiphospholipid antibodies (IgG aCL and IgM aCL) with or without complication and acute Q fever with underlying valvulopathy (Table 4). If the patient is spontaneously apyretic, no treatment is proposed. In case of acute Q fever without positive aCL or without acute Q fever complications, doxycycline alone for 2 to 3 weeks is recommended without evidence. [63] Doxycycline antibiotics for 3 days after apyrexia could be proposed as well, although the exact and optimal duration of doxycycline treatment for acute Q fever is unknown due to the lack of scientific data on this topic. In case of positive aCL, hydroxychloroquine is added until normalization of the antiphospholipid and for a minimum period of 3 weeks [58].

In case of acute Q fever endocarditis, doxycycline associated with hydroxychloroquine for a period of 18 months was proposed by analogy with persistent *C. burnetii* endocarditis on native valve, as acute Q fever endocarditis was associated with an increased risk of development of persistent *C. burnetii* infection.

In case of acute Q fever with underlying valvulopathy, which is a risk factor for evolution toward persistent *C. burnetii* endocarditis, antibiotic prophylaxis has been shown to be effective. Indeed, in a study involving 72 patients, antibiotics prophylactic treatment was effective for 31 patients with a valvulopathy (HR, 0.002; 95% CI, .00–.77; P = .04) [38]. In patients with acute Q fever and valvulopathy, the estimated risk of developing endocarditis is 40% [36]. The evolution from acute Q fever to endocarditis can be prevented by antibiotic prophylaxis [38]. Nonetheless, this therapeutic strategy has been criticized in the scientific literature and deemed not cost-effective [43].

## 8. Persistent *C. burnetii* infection

### 8.1 Persistent focalized *C. burnetii* infection

Persistent focalized *C. burnetii* infection is defined by the presence of clinical symptoms more than 3 months after the onset of symptoms and is associated with a microbiological criteria in addition to a lesional criteria. Persistent *C. burnetii* infection is a rare complication of acute Q fever with less than 5% of the cases. The French NRC identified *C. burnetii* persistent endocarditis as the main presentation of persistent *C. burnetii* focalized infection representing 76% of the foci, followed by persistent *C. burnetii* vascular infection accounting for 19% and osteoarticular infection in 7% [4]. We identified *C. burnetii* persistent lymphadenitis as a new clinical entity of persistent infection [4]. Some risk factors were predictive for evolution to persistent *C. burnetii* infection including, underlying valvulopathy, being a male, the presence of a *C. burnetii* lymphadenitis, thrombosis, acute Q fever endocarditis and the presence of high serological titers phase I IgG >800. In addition, positive aCL have already been reported as predictive factors for evolution to persistent *C. burnetii* infection [40]. In a recent Australian report on Q fever endocarditis, 92% of the patients had underlying valvulopathy and Armstrong *et al.* underlines the fact that 2 patients with persistent *C. burnetii* endocarditis

had low serological titers [45]. *C. burnetii* cardiovascular infection leads to death in the absence of treatment and constitutes is the most serious complication of Q fever. The use adequate antibiotic therapy for *C. burnetii* cardiovascular infection has allowed patients to survive. (See next chapter: "Treatment of persistent focalized infection")

### 8.2 *C. burnetii* persistent infection with low serological titers

From the NRC, 13% of patients with cardiovascular infection and osteoarticular infection had phase I IgG titers <800 and we have recently identified 28 patients with definite cardiovascular infection and low serological titers [14]. We even observed a case of *C. burnetii* seronegative endocarditis in a patient receiving an immunomodulatory treatment for a multiple sclerosis (unpublished data), thus highlighting the importance of new molecular tools such as PCR and FISH in the diagnosis of persistent focalized *C. burnetii* infection. Based only on the serological cutoff diagnostic criteria, all these patients with persistent *C. burnetii* infection would have been diagnosed with a serological past infection since phase I IgG was <800. Consequently, *C. burnetii* serology should be cautiously considered and interpreted according to the clinical context, the cardiovascular predisposition and the immune status of the host (immunosuppressive and immunomodulatory drugs, chemotherapy, lymphopenia). It is already known that *C. burnetii* serological response is strain dependent, since Phase I IgG were higher with the Guiana strain than with the Nine Mile strain in human and in mice [12,13]. The microbial properties of strains are involved in the humoral response of *C. burnetii*. On the other hand, primary or acquired immunodeficiency may influence the humoral response and be linked with low antibodies titers. Nonetheless, precise immune mechanisms that led to high or low humoral response have not been yet elucidated. Immunosuppression, such as hypogammaglobulinemia could be responsible for low IgG production. Advanced age is a condition characterized by the lack of clonotypic immune

response to new extracellular pathogens. Thus, increased B cell memory and decreased naïve B cells may represent the hallmark of immunosenescence, and were characterized by higher IgG and IgA with age [64]. In case of *Clostridium difficile* infection, the decreased of naïve B-cells has been reported to be associated with a low humoral response as it was observed in cases of vancomycin treatment as well as in mice infected with *C. difficile*, while low IgG antibodies to TcdA and TcdB toxins were associated with recurrences [65][66].

On the other hand, we strikingly observed that patients with non-Hodgkin lymphoma and *C. burnetii* endocarditis had low serological titer (IgGI ≤ 1:400, 4/18) in a higher proportion than patients without non-Hodgkin lymphoma (unpublished data). Rituximab, an anti-CD20 therapy used in non-Hodgkin lymphoma treatment results in impaired T-cell independent antibody response [67]. This could explain the low serological response observed in patients with non-Hodgkin lymphoma. In addition, we demonstrated that patients with *C. burnetii* persistent infection overexpressed interleukine-10 (IL-10), an immunomodulatory molecule involved in lymphomagenesis [17]. IL-10 also induces activated B cells to secrete large amounts of IgG, IgA, and IgM [68].

### 8.3 *C. burnetii* persistent infection and Non Hodgkin lymphoma

We have recently identified the co-incidence of *C. burnetii* persistent infection and non-Hodgkin lymphoma (NHL). We showed that *C. burnetii* persistent infection was associated with 9-fold increased risk of non-Hodgkin lymphoma [16,17]. In the Netherlands, persistent *C. burnetti* infection was described as associated with a 5-fold increased risk of non-Hodgkin lymphoma after the Q fever epidemic outbreaks [16]. Fourty-five cases have been reported in the literature to date. *C. burnetii* is associated with a wide range of non-Hodgkin lymphoma subtypes (follicular lymphoma, diffuse and large B-cell lymphoma, marginal lymphoma, lymphoplasmacytic lymphoma, mantle lymphoma and chronic lymphocytic leukaemia, B-

acute lymphocytic leukaemia, MALT lymphoma) (Figure 3) [16,17,69,70]. According to the French report, *C. burnetii* was identified in 5 of the 11 non-Hodgkin lymphoma biopsy samples, in the tumour microenvironment, within macrophages and plasmacytoid dendritic cells, but not in B-lymphocytes [17]. Interleukin 10 was described as an immunomodulatory cytokine favouring B-cell proliferation [17]. In the Netherlands, *C. burnetii* was identified in 5 of 13 biopsies samples analysed among 18 patients with *C. burnetii* and non-Hodgkin lymphoma [17,70]. Finally, *C. burnetii* was identified *in situ* in 41% of cases analysed (10/24).

In addition, *C. burnetii* lymphadenitis was considered a critical step, first, because the bacterium was identified within the lymph node biopsy, secondly, because in patients with *C. burnetii* lymphadenitis we observed an upregulation of the anti-apoptotic genes involved in B-cell lymphoproliferative diseases (REL, Bcl2, SP100, ETS, MIR17HG) [71]. Since then, we have proposed that special attention be paid to monitoring *C. burnetii* lymphadenitis with 18F-FDG-PET/CT scan to early detect an increase in lymph node size and, if it is increased, to perform a biopsy and detect the development of non-Hodgkin's lymphoma [4,17][71].

*C. burnetii* persistent diagnostic criteria are described in table 4.

Numerous evidences support the hypothesis of a link between Q fever and non-Hodgkin lymphoma, and van Roeden *et al.* refused to conclude to an association between the two diseases. Nevertheless, both studies identified that *C. burnetii* persistent infection was associated with a significant increased risk of non-Hodgkin lymphoma, in both studies the delay between Q fever and non-Hodgkin lymphoma was 8 months and *C. burnetii* was identified *in situ* in the non-Hodgkin lymphoma biopsies samples [16],[72]. In addition and with regards to the Bradford-Hill criteria, a dose gradiant was reported in the Dutch study since a significant association with non-Hodgkin lymphoma was noted in 2009 for areas with high endemicity of Q fever compared with low endemic area. Causality is a vivid

fundamental question in medical science and that has been extensively revisited since the Koch postulate. It is no longer true that a disease must result from a single cause.[73] Lymphomagenesis is a multifactorial disease involving various risk factors, some of which are common to all non-Hodgkin lymphoma (B-cell activating diseases).[74] Thus, it is clear that *C. burnetii* is not the only risk factor for non-Hodgkin lymphoma. Nonetheless, the scientific evidences provide strength, consistency, temporality, coherence plausibility, biological gradiant and analogy, *i.e.* 8 of the 9 Bradford Hill criteria. [75] As a conclusion, we that a greater attention be paid to the detection of non-Hodgkin lymphoma after Q fever, and more specifically when lymphadenitis is observed in this context.

**9. Treatment of persistent focalized *C. burnetii* infection**

Doxycycline plus hydroxychloroquine are the gold standard treatments for *C. burnetii* persistent focalized infection. This association is recommended for an 18 months period in case of native valve or vascular *C. burnetii* infection without prosthetic material, and for 24 months in presence of prosthetic material. Regarding osteoarticular infection, there is no evidence to determine the duration of doxycycline and hydroxychloroquine therapy. We proposed a 18F-FDG-PET/CT scan imaging follow-up. In case of *C. burnetii* lymphadenitis, the treatment must be continued until the disappearance of hypermetabolism on the 18F-FDG-PET/CT scanner, or until the disappearance of lymphadenitis on CT-scan. Repeated 18F-FDG-PET/CT scanner in patients with *C. burnetii* vascular infection could also be proposed before stopping the treatment. [51]

As a therapeutic and follow-up strategy, we proposed serological and antibiotic (doxycycline plus hydroxychloroquine) blood levels every month to ensure the therapeutic observance [44][76]. This strict monthly monitoring is justified at least until the serological decrease and therapeutic dosages are repeatedly in therapeutic range (doxycycline between 5-10 mg/l and

hydroxychloroquine 0.8-1.2 mg/l). Then, a 3-month period for monitoring seems adequate. A four time decrease in the phase I IgA and IgG titre and a complete desappearance of phase II IgM at one year were reported as a favourable outcome for persistent *C. burnetii* endocarditis.[44] In patients with *C. burnetii* endocarditis, 5-year serological monitoring is required [44]. Duration therapy could be prolonged in case of reascension of the serological titers, positive PCR, or new lesional focus on CT scan or 18F-FDG-PET/CT -scan and in case of antibiotics blood dosage under therapeutic threshold.

## 10. Peculiar presentation

Special focus should be placed on three specific presentations of Q fever. First, the treatment of Q fever in children, second, the treatment in case of pregnancy and third the treatment of Q fever in case of immunosuppression.

### 10.1 Children

Children can have acute or persistent *C. burnetii* infection. In the recent Q fever cohort conducted by the French NRC, we identified 58 children with Q fever, including lymphadenitis and osteomyelitis as the two main clinical manifestations followed by endocarditis. Mean age was 10±5 [4]. Interestingly, Q fever during childhood has been characterized to be less symptomatic [77]. Doxycycline for 2 weeks is recommended for acute Q fever, even before the age of 8 years, because few or no effects of doxycycline on dental discoloration or dental enamel hypoplasia have been reported in recent studies [63,78]. In case of persistent *C. burnetii* focalized infection, doxycycline in association with hydroxychloroquine can be used, but the therapeutic duration is still unclear [63,78]. As alternative therapy, cotrimoxazole and quinolones can be considered [63].

### 10.2 Pregnancy

There are two clinical peculiarities in Q fever during pregnancy. First, acute Q fever is paucy-symptomatic and may be unnoticed. Second, pregnant women are at risk of developing persistent *C. burnetii* infection and fetal complications due to the immunosuppression stage and *C. burnetii'*s ability to use placental trophoblasts as a replicative niche [73]. Complications can be severe for the fetus, involved in almost 60% of the series of cases reported inducing intrauterine growth retardation, intrauterine fetal death and spontaneous abortion [4,79,80]. Nonetheless, obstetrical morbidity has been reported as relative to different geographical area, since no obstetrical complication outbreaks have been reported after the Dutch Q fever outbreaks.[81] This could be related to strain specificity since *C. burnetii* strains harboring the QpVD plasmid were reported as frequently associated with abortion [82]. Otherwise, this could be related to a failure of public health authorities to detect early miscarriages [82].

Doxycycline is contraindicated during pregnancy, so the treatment of choice is cotrimoxazole twice daily until the end of the 8$^{th}$ month of pregnancy. After delivery, *C. burnetii* evaluation of the mother must be performed, and the treatment should be restart according to the clinical focus identified as previously exposed for adults. Treatment should not be instituted only based on positive anti-phase 1 $\geq$800 for IgG. Breastfeeding should be avoided in developed countries where the risk of *C. burnetii* infection is higher than the benefit of breastfeeding for children. Nonetheless, in developed countries where access to drinking water is difficult the beneficial/risk balance may be in favor of breastfeeding.

### 10.3 Immunosuppresion

Immunosuppression has been considered a risk factor for persistent *C. burnetii* infection [36,83]. In the recent study published by our center, *C. burnetii* was not significantly associated with an increased risk for persistent infection. Nonetheless, we chose to include patients with severe immunosuppression, *i.e.* patients receiving immunosuppressive drugs, chemotherapy or who were splenectomized [4]. There is no recommendation because no study is available to determine how to treat immunocompromised patients with Q fever. In our center, we treat Q fever with doxycycline throughout the duration of immunosuppression. (Table 4).

## 11. Conclusion

Studies carried out on Q fever over the past two decades have identified new and rare clinical manifestations. With easy access to medical imaging and new imaging tools such as the 18F-FDG-PET/CT scanner, the definition of Q fever has been reviewed. During the acute phase of the disease, positive anticardiolipin antibodies are useful for detecting complications and for treatment since hydroxychloroquine in addition to doxycycline is proposed until negativisation of these antibodies. TTE is determinant to detect underlying valvulopathy, acute Q fever endocarditis and to guide therapeutics. The identification of an organic lesion is now decisive for concluding that there is a persistent infection with *C. burnetii* while the serological threshold alone becomes obsolete.

## 12. Expert opinion

Both acute Q fever and persistent focalized *C. burnetii* infection remained an underestimated and under-diagnosed disease with fatal complications if not treated. From the French NRC for Q fever, we experienced Q fever diagnosis, management and treatment that led us to propose new standardized diagnostic criteria and new therapeutic protocols. With these advances, it should be possible to detect acute complications early and to prevent and control persistent complications. As a result, the preventive measures implemented have improved the survival of patients with Q fever. Nonetheless, two critical points, which are part of the recent discoveries on Q fever would deserve to be further explored in the coming 5 years.

The first one is the increased risk to develop non-Hodgkin lymphoma in case of *C. burnetii* persistent infection. This has now been evidenced by two different medical scientific teams from two different parts of Europe, France and the Netherlands, two endemic areas for Q fever.[16,17] The next step is to accurately identify the target population at risk of developing lymphoma. We need to determine the characteristics of this population, including medical history and clinical presentation and to search for biological markers predictive of lymphoma. To date, *C. burnetii* lymphadenitis has been identified as a critical step towards the development of lymphoma, and *C. burnetii* persistent infection as a risk for lymphoma. As a consequence, we have proposed a diagnostic strategy using 18F-FDG-PET/CT scanner for the early detection and follow-up of persistent lymphadenitis. Nonetheless, no specific strategy has been proposed to identify, among patient with *C. burnetii* persistent infection, without persistent lymphadenitis, those who are at risk of non-Hodgkin lymphoma. We must therefore, on the basis of future epidemiological data, identify the characteristics of this population. As biological marker, interleukine-10 cytokine was expected to play an immunosuppressive role favoring persistence of the bacteria and B-cell proliferation in patients with persistent *C. burnetii* infections. [17] On the other hand, we have identified a

transcriptional signature of *C. burnetii* associated non-Hodgkin lymphoma and *C. burnetii* lymphadenitis, in which the genes involved in anti-apoptotic process are up-regulated, REL, Bcl2, SP100, ETS and MIR17HG.[71] To support the role of these molecules as biological markers of lymphoma progression, an international cohort study testing interleukine-10 cytokine in sera, and the expression of REL, SP100 and MIR17HG genes in the blood of patients with *C. burnetii* lymphadenitis and persistent infection must be conducted. This will help physicians decide whether to perform a surgical or radiological biopsy in case of positive biomarker in addition to 18F-FDG-PET/CT scan hypermetabolism. This will also allow us to propose a standardized monitoring of the at-risk population. In addition, as *C. burnetii* persistent endocarditis is a risk factor for non-Hodgkin lymphoma, and as *C. burnetii* persistent endocarditis has also been associated with an overproduction of PD-1 (mediated by IL-10 production), future perspectives on anti PD-1 treatment should be explored in case of *C. burnetii* associated lymphoma [84].

The second point to be developed is the interest of adding hydroxychloroquine to doxycycline in case of acute Q fever with positive anticardiolipins. We have shown that the production of aCL antibodies is predictive of acute Q fever complications and to the evolution toward persistent *C. burnetii* infection.[4] We therefore proposed to treat these patients with hydroxychloroquine in addition to doxycycyline until these antibodies are negative for a minimum period of 3 weeks. This therapeutic strategy has been implemented in the French NRC for Q fever since 2016. Nonetheless, scientific evidences are required to confirm the advantage of the early addition of hydroxychloroquine to doxycycline in case of elevated aCL antibodies. To this end, we intend to conduct a study comparing acute Q fever complications associated with aCL antibodies before and after 2016. Patients with acute Q fever before 2016, *i.e.* before the use of hydroxychloroquine plus doxycycline in case of positive aCL antibodies, are going to be compared to patients with acute Q fever after 2016, *i.e.* after the

use of hydroxychloroquine plus doxycycline in case of positive aCL antibodies. This will allow us to assess the impact of hydroxychloroquine and doxycycline versus doxycycline alone in the incidence of clinical complications associated with positive aCL antibodies. We will also evaluate the kinetics of the decrease in serology and aCL antibodies under the influence of each of these two therapeutics options, before and after 2016. Finally, in the future, we will propose systematic treatment with doxycycline plus hydroxychloroquine for 2 to 3 weeks in case of acute Q fever.

Finally, future perspective to improve *C. burnetii* infection outcome lies in the early detection of lymphatic and cardiovascular infectious focus to provide close monitoring, prevent the evolution to lymphoma and provide standardized evidence-based therapy.

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**References**

Papers of special note have been highlighted as:

* of interest, and ** of considerable interest


[1] Dijkstra F, van der Hoek W, Wijers N, et al. The 2007–2010 Q fever epidemic in The Netherlands: characteristics of notified acute Q fever patients and the association with dairy goat farming. FEMS Immunol. Med. Microbiol. 2012;64:3–12.

[2] van Roeden SE, Wever PC, Kampschreur LM, et al. Chronic Q fever-related complications and mortality: data from a nationwide cohort. Clin. Microbiol. Infect. 2018;

[3] Edouard S, Million M, Lepidi H, et al. Persistence of DNA in a cured patient and positive culture in cases with low antibody levels bring into question diagnosis of Q fever endocarditis. J. Clin. Microbiol. 2013;51:3012–3017.

[4] Melenotte C. Clinical Features and Complications of Coxiella burnetii Infections From the French National Reference Center for Q Fever. JAMA Network Open. 2018 Aug 24;1–15.

[5] Eldin C, Melenotte C, Million M, et al. 18F-FDG PET/CT as a central tool in the shift from chronic Q fever to Coxiella burnetii persistent focalized infection: A consecutive case series. Medicine (Baltimore). 2016;95:e4287.

[6] Prudent E, Lepidi H, Angelakis E, et al. Fluorescence In Situ Hybridization (FISH) and Peptide Nucleic Acid Probe-Based FISH for Diagnosis of Q Fever Endocarditis and Vascular Infections. J. Clin. Microbiol. 2018;56.

[7] Prudent E, Le Guenno G, Jonckheere S, et al. Fluorescent in situ hybridization can be used as a complementary assay for the diagnosis of Tropheryma whipplei infection. Infection. 2018;

[8] Peacock MG, Philip RN, Williams JC, et al. Serological evaluation of O fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infect. Immun. 1983;41:1089–1098.

[9] Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin. Diagn. Lab. Immunol. 1994;1:189–196.

[10] Frankel D, Richet H, Renvoisé A, et al. Q fever in France, 1985-2009. Emerging Infect. Dis. 2011;17:350–356.

[11] Mahamat A, Edouard S, Demar M, et al. Unique clone of Coxiella burnetii causing severe Q fever, French Guiana. Emerging Infect. Dis. 2013;19:1102–1104.

[12] Edouard S, Mahamat A, Demar M, et al. Comparison between emerging Q fever in French Guiana and endemic Q fever in Marseille, France. Am. J. Trop. Med. Hyg. 2014;90:915–919.

[13] Melenotte C. The hypervirulent Coxiella burnetii Guiana strain compared in silico, in vitro and in vivo to the Nine Mile and the German strain. 2018;

[14] Melenotte C, Bart G, Kraeber-Bodere F, et al. Isolation of Coxiella burnetii from an acromioclavicular infection with low serological titers. Int. J. Infect. Dis. 2018;

[15] Raoult D. Q Fever: Confusion Between Chronic Infection and Chronic Fatigue. Clin. Infect. Dis. 2017;65:1054–1055.

[16] van Roeden SE, van Houwelingen F, Donkers CMJ, et al. Exposure to Coxiella burnetii and risk of non-Hodgkin lymphoma: a retrospective population-based analysis in the Netherlands. Lancet Haematol. 2018;

[17] Melenotte C, Million M, Audoly G, et al. B-cell non-Hodgkin lymphoma linked to

Coxiella burnetii. Blood. 2016;127:113–121.
[18] Melenotte C, Drancourt M, Gorvel JP, et al. Post-bacterial infection chronic fatigue syndrome is not a latent infection. Med Mal Infect. 2019;49:140–149.
[19] Raoult D. Chronic Q fever: expert opinion versus literature analysis and consensus. J. Infect. 2012;65:102–108.
[20] Eldin C, Mélenotte C, Mediannikov O, et al. From Q Fever to Coxiella burnetii Infection: a Paradigm Change. Clin Microbiol Rev. 2017;30:115–190.
[21] Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. J. Clin. Microbiol. 1998;36:1823–1834.
[22] Miller HK, Binder AM, Peterson A, et al. Trends in Q fever serologic testing by immunofluorescence from four large reference laboratories in the United States, 2012-2016. Sci Rep. 2018;8:16670.
[23] Murphy AM, Field PR. The persistence of complement-fixing antibodies to Q-fever (Coxiella burneti) after infection. Med. J. Aust. 1970;1:1148–1150.
[24] Musso D, Raoult D. Serological cross-reactions between Coxiella burnetii and Legionella micdadei. Clin. Diagn. Lab. Immunol. 1997;4:208–212.
[25] La Scola B, Raoult D. Serological cross-reactions between Bartonella quintana, Bartonella henselae, and Coxiella burnetii. J. Clin. Microbiol. 1996;34:2270–2274.
[26] LUCIANI L, L'OLLIVIER C, MILLION M, et al. Introduction to the mesurement of the avidity of anti-coxiella burnetii IgG in the diagnosis of Q fever. Journal of Clinical Microbiology. 2019;
[27] Edouard S, Raoult D. Lyophilization to improve the sensitivity of qPCR for bacterial DNA detection in serum: the Q fever paradigm. J. Med. Microbiol. 2016;65:462–467.
[28] Raoult D, Yeaman MR, Baca OG. Susceptibility of Coxiella burnetii to pefloxacin and ofloxacin in ovo and in persistently infected L929 cells. Antimicrob. Agents Chemother. 1989;33:621–623.
[29] Sanchez SE, Vallejo-Esquerra E, Omsland A. Use of Axenic Culture Tools to Study Coxiella burnetii. Curr Protoc Microbiol. 2018;50:e52.
[30] Lepidi H, Gouriet F, Raoult D. Immunohistochemical detection of Coxiella burnetii in chronic Q fever hepatitis. Clin. Microbiol. Infect. 2009;15 Suppl 2:169–170.
[31] Jensen TK, Montgomery DL, Jaeger PT, et al. Application of fluorescent in situ hybridisation for demonstration of Coxiella burnetii in placentas from ruminant abortions. APMIS. 2007;115:347–353.
[32] Hansen MS, Rodolakis A, Cochonneau D, et al. Coxiella burnetii associated placental lesions and infection level in parturient cows. Vet. J. 2011;190:e135-139.
[33] Agerholm JS, Jensen TK, Agger JF, et al. Presence of Coxiella burnetii DNA in inflamed bovine cardiac valves. BMC Vet. Res. 2017;13:69.
[34] Melenotte C, Loukil A, Rico A, et al. Blood culture negative cardiovascular infection in a patient with multiple sclerosis. Open Forum Infectious Diseases. 2019;
[35] Houpikian P, Habib G, Mesana T, et al. Changing clinical presentation of Q fever endocarditis. Clin. Infect. Dis. 2002;34:E28-31.
[36] Fenollar F, Fournier PE, Carrieri MP, et al. Risks factors and prevention of Q fever endocarditis. Clin. Infect. Dis. 2001;33:312–316.
[37] Robson AO, Shimmin CD. Chronic Q fever. I. Clinical aspects of a patient with endocarditis. Br Med J. 1959;2:980–983.
[38] Million M, Walter G, Thuny F, et al. Evolution from acute Q fever to endocarditis is associated with underlying valvulopathy and age and can be prevented by prolonged antibiotic treatment. Clin. Infect. Dis. 2013;57:836–844.

[39] Million M, Thuny F, Bardin N, et al. Antiphospholipid Antibody Syndrome With Valvular Vegetations in Acute Q Fever. Clin. Infect. Dis. 2015;
[40] Million M, Walter G, Bardin N, et al. Immunoglobulin G anticardiolipin antibodies and progression to Q fever endocarditis. Clin. Infect. Dis. 2013;57:57–64.
[41] Melenotte C. Acute Q fever endocarditis: a paradigm shift following the systematic use of transthoracic echocardiography during acute Q fever. 2018;
[42] Fenollar F, Thuny F, Xeridat B, et al. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin. Infect. Dis. 2006;42:818–821.
[43] de Lange MMA, Gijsen LEV, Wielders CCH, et al. Should acute Q-fever patients be screened for valvulopathy to prevent endocarditis? Clin. Infect. Dis. 2018;
[44] Million M, Thuny F, Richet H, et al. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis. 2010;10:527–535.
[45] Armstrong MR, McCarthy KL, Horvath RL. A contemporary 16-year review of Coxiella burnetii infective endocarditis in a tertiary cardiac center in Queensland, Australia. Infect Dis (Lond). 2018;50:531–538.
[46] Mogollón MV, Anguita MP, Aguado JM, et al. Q fever endocarditis in Spain. Clinical characteristics and outcome. Enferm. Infecc. Microbiol. Clin. 2011;29:109–116.
[47] Siegman-Igra Y, Kaufman O, Keysary A, et al. Q fever endocarditis in Israel and a worldwide review. Scand. J. Infect. Dis. 1997;29:41–49.
[48] de Lange MMA, Scheepmaker A, van der Hoek W, et al. Risk of chronic Q fever in patients with cardiac valvulopathy, seven years after a large epidemic in the Netherlands. PLoS ONE. 2019;14:e0221247.
[49] Lepidi H, Houpikian P, Liang Z, et al. Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. J. Infect. Dis. 2003;187:1097–1106.
[50] Swart LE, Gomes A, Scholtens AM, et al. Improving the Diagnostic Performance of 18F-Fluorodeoxyglucose Positron-Emission Tomography/Computed Tomography in Prosthetic Heart Valve Endocarditis. Circulation. 2018;138:1412–1427.
[51] Kouijzer IJE, Kampschreur LM, Wever PC, et al. The Value of 18F-FDG PET/CT in Diagnosis and During Follow-up in 273 Patients with Chronic Q Fever. J. Nucl. Med. 2018;59:127–133.
[52] Kampschreur LM, Wegdam-Blans MCA, Wever PC, et al. Chronic Q Fever Diagnosis— Consensus Guideline versus Expert Opinion. Emerging Infect. Dis. 2015;21:1183–1188.
[53] Arlet P, Botreau Y, Duffaut M, et al. [Circulating anticoagulant during primosecondary syphilis and Q fever]. Nouv Presse Med. 1981;10:2909.
[54] Besancenot JF, Cannard JF, Janin-Manificat L, et al. [Q fever and circulating anticoagulant]. Presse Med. 1983;12:2188.
[55] Levy P, Raoult D, Razongles JJ. Q-fever and autoimmunity. Eur. J. Epidemiol. 1989;5:447–453.
[56] Ordi-Ros J, Selva-O'Callaghan A, Monegal-Ferran F, et al. Prevalence, Significance, and Specificity of Antibodies to Phospholipids in Q Fever. Clinical Infectious Diseases. 1994;18:213–218.
[57] Jansen AFM, Raijmakers RPH, Keijmel SP, et al. Autoimmunity and B-cell dyscrasia in acute and chronic Q fever: A review of the literature. Eur. J. Intern. Med. 2018;54:6–12.
[58] Million M, Bardin N, Bessis S, et al. Thrombosis and antiphospholipid antibody syndrome during acute Q fever: A cross-sectional study. Medicine (Baltimore). 2017;96:e7578.

[59] Melenotte C. The sexual dimorphism of anticardiolipin auto-antibodies in acute Q fever patients. Clinical Microbiology and Infection. 2019;
[60] Thorn G, Adams R, Braunwald E, et al. The rickettioses. Principles of Internal Medicine. 1977;953–957.
[61] Lumio J, Penttinen K, Pettersson T. Q fever in Finland: clinical, immunological and epidemiological findings. Scand. J. Infect. Dis. 1981;13:17–21.
[62] Sawaishi Y, Takahashi I, Hirayama Y, et al. Acute cerebellitis caused by Coxiella burnetii. Ann. Neurol. 1999;45:124–127.
[63] Anderson A, Bijlmer H, Fournier P-E, et al. Diagnosis and management of Q fever--United States, 2013: recommendations from CDC and the Q Fever Working Group. MMWR Recomm Rep. 2013;62:1–30.
[64] Listì F, Candore G, Modica MA, et al. A study of serum immunoglobulin levels in elderly persons that provides new insights into B cell immunosenescence. Ann. N. Y. Acad. Sci. 2006;1089:487–495.
[65] van Opstal E, Kolling GL, Moore JH, et al. Vancomycin Treatment Alters Humoral Immunity and Intestinal Microbiota in an Aged Mouse Model of Clostridium difficile Infection. J. Infect. Dis. 2016;214:130–139.
[66] Bauer MP, Nibbering PH, Poxton IR, et al. Humoral immune response as predictor of recurrence in Clostridium difficile infection. Clin. Microbiol. Infect. 2014;20:1323–1328.
[67] Kuijpers TW, Bende RJ, Baars PA, et al. CD20 deficiency in humans results in impaired T cell-independent antibody responses. J. Clin. Invest. 2010;120:214–222.
[68] Rousset F, Garcia E, Defrance T, et al. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc. Natl. Acad. Sci. U.S.A. 1992;89:1890–1893.
[69] van Roeden SE, Melenotte C, Hermans MHA, et al. Case report: Coxiella burnetii vascular infection and lymphoma in the Netherlands. Infection. 2018;46:131–134.
[70] van Roeden SE, Hermans MHA, Nooijen PTGA, et al. Coxiella burnetii in non-Hodgkin lymphoma tissue samples: Innocent until proven otherwise? Immunobiology. 2018;
[71] Melenotte C, Mezouar S, Ben Amara A, et al. A transcriptional signature associated with non-Hodgkin lymphoma in the blood of patients with Q fever. PLoS ONE. 2019;14:e0217542.
[72] Melenotte C, Million M, Audoly G, et al. B-cell non-Hodgkin lymphoma linked to Coxiella burnetii. Blood. 2015;
[73] Evans AS. Causation and Disease A chronological Journey. Kluwer Academic / Plenum Publishers (avril 1993). 1993.
[74] Morton LM, Slager SL, Cerhan JR, et al. Etiologic heterogeneity among non-Hodgkin lymphoma subtypes: the InterLymph Non-Hodgkin Lymphoma Subtypes Project. J. Natl. Cancer Inst. Monographs. 2014;2014:130–144.
[75] Hill AB. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proc. R. Soc. Med. 1965;58:295–300.
[76] Rolain J-M, Boulos A, Mallet M-N, et al. Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. Antimicrob. Agents Chemother. 2005;49:2673–2676.
[77] Maltezou HC, Raoult D. Q fever in children. Lancet Infect Dis. 2002;2:686–691.
[78] Gaillard T, Briolant S, Madamet M, et al. The end of a dogma: the safety of doxycycline use in young children for malaria treatment. Malar. J. 2017;16:148.
[79] Million M, Roblot F, Carles D, et al. Reevaluation of the risk of fetal death and

malformation after Q Fever. Clin. Infect. Dis. 2014;59:256–260.
[80] Coste Mazeau P, Hantz S, Eyraud J-L, et al. Q fever and pregnancy: experience from the Limoges Regional University Hospital. Arch. Gynecol. Obstet. 2016;294:233–238.
[81] de Lange MMA, Hukkelhoven CWPM, Munster JM, et al. Nationwide registry-based ecological analysis of Q fever incidence and pregnancy outcome during an outbreak in the Netherlands. BMJ Open. 2015;5:e006821.
[82] Angelakis E, Million M, D'Amato F, et al. Q fever and pregnancy: disease, prevention, and strain specificity. Eur. J. Clin. Microbiol. Infect. Dis. 2013;32:361–368.
[83] Raoult D. Host factors in the severity of Q fever. Ann. N. Y. Acad. Sci. 1990;590:33–38.
[84] Ka MB, Gondois-Rey F, Capo C, et al. Imbalance of circulating monocyte subsets and PD-1 dysregulation in Q fever endocarditis: the role of IL-10 in PD-1 modulation. PLoS ONE. 2014;9:e107533.

**[4] Melenotte C. Clinical Features and Complications of Coxiella burnetii Infections From the French National Reference Center for Q Fever. JAMA Network Open. 2018 Aug 24;1–15.
This is the most important reference because it is the largest Q fever cohort never reported with 2434 patients included from the NRC for Q fever. Diagnosis is standardized and this work includes a large broad of clinical microbiological and radiological data. This large observational cohort study has allowed us to identify rare manifestations and complication of Q fever.

** [5] Eldin C, Melenotte C, Million M, et al. 18F-FDG PET/CT as a central tool in the shift from chronic Q fever to Coxiella burnetii persistent focalized infection: A consecutive case series. Medicine (Baltimore). 2016;95:e4287.
This is an important review updating the microbiological feature on Q fever. This review predates the work published in the JAMA network Open.

*[6] Prudent E, Lepidi H, Angelakis E, et al. Fluorescence In Situ Hybridization (FISH) and Peptide Nucleic Acid Probe-Based FISH for Diagnosis of Q Fever Endocarditis and Vascular Infections. J. Clin. Microbiol. 2018;56.
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*[7] Prudent E, Le Guenno G, Jonckheere S, et al. Fluorescent in situ hybridization can be used as a complementary assay for the diagnosis of Tropheryma whipplei infection. Infection. 2018;
This article is important because it shows the use of a new biomolecular technique to detect Q fever in vardiovascular tissues samples the FISH.

**[8] Peacock MG, Philip RN, Williams JC, et al. Serological evaluation of O fever in humans: enhanced phase I titers of immunoglobulins G and A are diagnostic for Q fever endocarditis. Infect. Immun. 1983;41:1089–1098.
This is an important and seminal article in diagnosis.

**Table 1. Acute Q fever diagnosis criteria**

| | | |
|---|---|---|
| **ACUTE Q FEVER focus definition (**Symptoms < 3 months) | | |
| **Microbiological criteria:** | | |
| - Phase II IgG≥ 200 and phase II IgM ≥ 50 and/or<br>- Phase I IgG ≥ 200 with low avidity and/or<br>- Séroconversion and/or<br>- Positive PCR positive on blood or serum without cardio-vascular infection | | |
| **Organic criteria** | | |
| **Acute Q fever denomination** | **Infectious focus** | **Evidence of the organic lesion** |
| Acute Q fever | Isolated fever<br>Hepatitis<br>Pneumonia | No clinical focus or identified by imaging<br>Elevated transaminitis<br>Clinical or radiological lung opacity or interstitial image<br>Possible hypermetabolic bone marrow and spleen on 18F-FDG-PET/CT |
| Acute Q fever complications | Alithiasic cholecystitis<br>Meningitis<br>Encephalitis<br>Haemophagocytic syndrome<br>Thrombosis | Abdominal echocardiography or CT scan<br>>5 cells in CSF<br>Clinical encephalitis, imaging cerebral lesions<br>Proved on bone spin aspirate<br>Proved on doppler ultrasonography or CT scan |
| | Acute Q fever endocarditis | Definite<br>vegetation,<br>a valvular nodular thickening (irregular round nodule from >3mm),<br>valvular chorda tendinea rupture<br>Possible<br>Valve thickeness (diastolic thickening > 5 mm)<br>chorda tendinea thickeness |

| | | |
|---|---|---|
| Acute Q fever with underlying valvulopathy | Whatever the clinical presentation | Aortic bicuspidy<br>Mitral insufficiency<br>Aortic stenosis<br>Rheumatic valve<br>Prosthetic valve<br>Valve regurgitation or stenosis ≥ grade II |

18F-FDG-PET/CT: positron emission tomography; CT: computed tomography; CSF: cerebro-spinal fluid

**Table 2. Diagnostic criteria of *C. burnetii* persistent focalized infection***

| **C. *burnetii* endocarditis** | ***C. burnetii* vascular infection** [3] | ***C. burnetii* prosthetic joint arthritis** | ***C. burnetii* osteoarticular infection (without prosthesis).** | ***C. burnetii* lymphadenitis** | ***C. burnetii Interstitial lung disease*** |
|---|---|---|---|---|---|
| **I. Definite criterion**<br>Positive culture, PCR, immunochemistry, or FISH of a cardiac valve | **I. Definite criterion**<br>Positive culture, PCR, immunochemistry or FISH of an arterial sample (prosthesis or aneurism) or a periarterial abscess or a spondylodiscitis linked to aorta. | **I. Definite criterion**<br>Positive culture, PCR, or immunochemistry or FISH of a periprosthetic biopsy or joint aspirate | **I. Definite criterion**<br>Positive culture, PCR, immunochemistry or FISH of bone or synovial biopsy, joint aspirate. | **I. Definite criterion**<br>Positive culture, PCR, immunohistochemistry or FISH of lymphadenitis. | **I. Definite criterion**<br>Positive culture, PCR, immunochemistry or FISH of lung fibrotic biopsy |
| **Major criteria**<br>***A. Microbiology***:<br>positive culture or PCR of the blood, an emboli or serology with IgG1 antibody titer ≥6400<br><br>***B. Evidence of endocardial involvement or high risk valvulopathy:***<br>-Echocardiogram positive for IE: oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets or on implanted material in the absence of an alternative anatomic explanation; or abscess; or new partial dehiscence of a prosthetic valve; or new valvular regurgitation (worsening or changing of preexisting murmur is not sufficient).<br>-18F-FDG-PET/CT scan displaying a specific valve fixation and mycotic aneurism.<br>-surgical valvulopathy<br>- valvular prosthesis<br>- paravalvular lesions on CT-scan | **Major criteria**<br>***A. Microbiology***:<br>Positive culture, PCR of the blood or emboli, or serology with IgGI antibodies ≥6400<br><br>***B. Evidence of vascular involvement or vascular disease with high risk:***<br>-CT-scan: aneurism or vascular prosthesis + periarterial abscess, fistula, or spondylodiscitis.<br>-18F-FDG-PET/CT scan specific fixation on an aneurism or vascular prosthesis. | **Major criteria**<br>***A. Microbiology:***<br>Positive culture or polymerase chain reaction of the blood<br>-Positive Coxiella burnetii serology with IgGI antibodies ≥ 6400<br><br>***B. Evidence of prosthetic involvement:***<br>-Computed tomography scan or MRI positive for prosthetic infection: collection or pseudo-tumor of the prosthesis<br>-Positron emission tomography scan or indium leukocyte scan showing a specific prosthetic hypermetabolism consistent with infection† | **Major criteria**<br>***A. Microbiology***:<br>-Positive culture or positive PCR of the blood<br>-Positive serology with IgGI antibodies ≥ 800<br><br>***B. Evidence of bone or joint involvement:***<br>-Clinical arthritis, osteitis or tenosynovitis<br>-CT-scan or ultrasonography (for joint) or MRI: osteoarticular destruction, joint effusion, intra-articular collection, spondylodiscitis, synovitis, acromio-clavicular localization.<br>-18F-FDG-PET/CT scan or indium leukocyte scan showing a specific osteoarticular uptake. | **Major criteria**<br>***A. Microbiology***:<br>-Positive culture or positive PCR of the blood<br>-Positive serology with IgGI antibodies ≥ 100<br><br>***B. Evidence of lymph node involvement:***<br>-Clinical lymphadenitis<br>-CT-scan or ultrasonography (for joint) or MRI: lymphadenitis > 1cm.<br>-18F-FDG-PET/CT scan showing specific lymph node uptake. | **Major criteria**<br>***A. Microbiology***:<br>-Positive culture or positive PCR of the blood<br>-Positive serology with IgGI antibodies ≥ 400<br><br>***B. Evidence of lung involvement:***<br>-thoracic CT-scan imaging of ILD |
| **Minor criteria**<br>a-Predisposing heart condition (known or found on ultrasound)<br><br>b-Vascular phenomena, major arterial emboli, septic pulmonary infarcts, mycotic aneurysm | **Minor criteria**<br>a-Serological **IgGI ≥ 100** < 6400<br><br>b-Emboli<br><br>c-Underlying vascular predisposition (aneurism or vascular prosthesis) | **Minor criteria**<br><br>a-Joint pain at the prosthetic site<br><br>b-Serologic evidence: positive *C. burnetii* serology with IgGI antibodies ≥ 100 and <6400 mg/dL | **Minor criteria**<br>a- Mono- or polyarthralgia<br><br>b- Serological IgGI ≥ 100 < 800 mg/dL | | |

| | | | | | |
|---|---|---|---|---|---|
| (observed during PET scan), intracranial hemorrhage, conjunctival hemorrhages and Janeway lesions.<br><br>c-Immunologic phenomena: glomerulonephritis, Osler's nodes, Roth spots, or rheumatoid factor.<br><br>d-Serological evidence: IgG1 antibody **titers ≥ 100** < 6400 | | | | | |
| ***Definite diagnosis***<br>1) I<br>2) A+B<br><br>***Possible diagnosis***<br>3) A+ a or b or c in a patient with valvulopathy<br>4) B +d (whatever b and c) | ***Definite diagnosis***<br>1) I<br>2) A+B<br><br>***Possible diagnosis***<br>3) A+ b or c<br>4) B+ a (whatever b and c) | ***Definite diagnosis***<br>1) I<br>2) A+B<br><br>***Possible diagnosis***<br>3) A+a<br>4) B +cb | ***Definite diagnosis***<br>1) I<br>2) A+B<br><br>***Possible diagnosis***<br>1) A+a<br>2) B+b | ***Definite diagnosis***<br>1) I<br><br>***Possible diagnosis***<br>1) A+B | ***Diagnosis definite***<br>1) I<br><br>***Possible diagnosis***<br>1) A+B |

*Fever is a symptom leading to search for an infection (typically, fever of unknown origin is an indication for a 18F-FDG-PET/CT -scan) but is not anymore a diagnostic criteria for *Coxiella burnetii* persistent infections since it has a very low sensitivity and specificity.
PCR: polymerase chain reaction; FISH: Fluorescent in situ hybridization; ILD: interstitial lung diseases
MRI: Magnetic resonance imaging; CT: computed tomography; 18F-FDG-PET/CT: positron emission tomograph

**Table 3.** Acute Q fever complications associated with elevated anticardiolipin antibodies (Reproduced with the agreement of the JAMA Network Open Journal from supplementary Table 13 [4])

| Variable | AUC | 95% CI | | P |
|---|---|---|---|---|
| Acute Q fever endocarditis | .67 | .58 | .76 | .0001 |
| Haemophagocytic syndrome | .78 | .67 | .89 | .003 |
| Meningitis | .68 | .56 | .79 | .01 |
| Thrombosis | .72 | .6 | .85 | .002 |
| Alithiasic cholecystitis | .75 | .6 | .9 | .05 |

AUC : area under the curve, CI : confidence interval

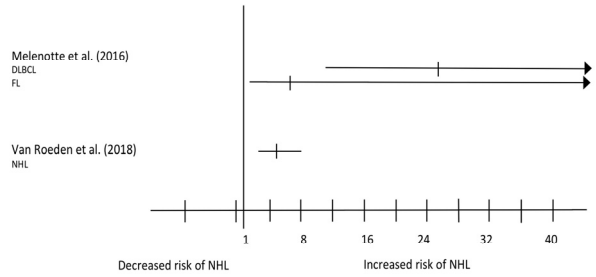
**Table 4. Q fever treatment according to the infectious focus**

| | **Infectious focus** | **Treatment** |
|---|---|---|
| **ACUTE Q FEVER** | | |
| Acute Q fever | Spontaneous recovery<br><br>Isolated fever<br>Hepatitis<br>Pneumonia | No treatment<br><br>D 3 we |
| Acute Q fever<br>+<br>Antiphospholipid antibodies | Isolated fever<br>Hepatitis<br>Pneumonia<br>Alithiasic cholecystitis<br>Meningitis<br>Haemophagocytic syndrome<br>Thrombosis<br><br>Acute Q fever endocarditis | DH until normalization of antiphospholipid antibodies (IgG, IgM, Beta2 GP1) and normalization of aPTT AND cure of any antiphospholipid antibodies related complications<br><br>DH (18 mo)* |
| Acute Q fever with underlying valvulopathy | Whatever the clinical presentation | DH (12 mo) |
| **PERSISTENT COXIELLA BURNETII INFECTION** | | |
| **Infectious focus** | | **Treatment** |
| **Endocarditis (possible or definite)**<br><br>Native valve | | DH 18 mo<br>If a surgery is necessary, wait 3 weeks of treatment |

| | |
|---|---|
| Intra-cardiac prosthetic material | DH 24 months<br>If a surgery is necessary, wait 3 weeks of treatment |
| **Vascular infection (possible or definite)**<br><br>Native vessel<br><br><br><br>Prosthetic material | <br><br>DH 18 months<br>Systematic remove of infected material after one month of treatment. Before surgery if possible, wait 4 weeks of treatment<br><br>DH 24 months<br>Systematic remove of infected material after one month of treatment<br>Before surgery if possible, wait 4 weeks of treatment |
| Lymphadenitis | DH till TEP cure<br>TEP-scan every 3 months |
| Osteoarticular infection<br><br>On native bone<br><br>On bone with material (prosthesis,…) | Call National Reference Center for experts advice |
| For all the other cases | Call National Reference Center for experts advice |

We: week, mo: months, D : doxycycline, H : hydroxychloroquine, *May be shortened to 12 months in the next future (ongoing study). PET: positron emission tomography; CT: computed tomography

**Figure 1.** Mini meta analysis *Coxiella burnetii* and the risk of NHL




Foot note: the risk of NHL in cas of Q fever was compared to the risk of NHL in the general population in both studies
[a]SIR for DLBCL (Standardized Incidence ratio) (95% CI) was 25,4 [11.4-56.4] and SIR for FL was 6.7 [0.9-47.9]
[b]RR (Relative risk) was 4.99 [2.07-11.98] p=0.003
DLBCL: diffuse and large B-cell lymphoma, FL: follicular lymphoma, NHL: Non Hodgkin lymphoma