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Original article

Risk of heart failure among individuals tested for *Borrelia burgdorferi* sensu lato antibodies, and serum *Borrelia burgdorferi* sensu lato seropositive individuals; a nationwide population-based, registry-based matched cohort study

Malte M. Tetens [a,*], Lars Haukali Omland [a], Ram B. Dessau [b,c], Svend Ellermann-Eriksen [d], Nanna S. Andersen [e,f], Charlotte Sværke Jørgensen [g], Christian Østergaard [h], Jacob Bodilsen [i,j], Kirstine K. Søgaard [j,k,l], Jette Bangsborg [m], Alex Christian Yde Nielsen [n], Jens Kjølseth Møller [c,o], Ming Chen [p], Jesper Hastrup Svendsen [q,r], Niels Obel [a,r], Anne-Mette Lebech [a,r]

[a] *Department of Infectious Diseases, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark*
[b] *Department of Clinical Microbiology, Zealand Hospital, Slagelse, Denmark*
[c] *Department of Regional Health Research, University of Southern Denmark, Odense, Denmark*
[d] *Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark*
[e] *Clinical Centre for Emerging and Vector-borne Infections, Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark*
[f] *Research Unit of Clinical Microbiology, University of Southern Denmark, Odense, Denmark*
[g] *Department of Virus & Microbiological Special Diagnostics, Statens Serum Institut, Copenhagen, Denmark*
[h] *Department of Clinical Microbiology, Copenhagen University Hospital - Hvidovre Hospital, Copenhagen, Denmark*
[i] *Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark*
[j] *Department of Clinical Medicine, Aalborg University, Aalborg, Denmark*
[k] *Department of Clinical Microbiology, Aalborg University Hospital, Aalborg, Denmark*
[l] *Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark*
[m] *Department of Clinical Microbiology, Copenhagen University Hospital - Herlev Hospital, Herlev, Denmark*
[n] *Department of Clinical Microbiology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark*
[o] *Department of Clinical Microbiology, Vejle Hospital, University Hospital of Southern Denmark, Vejle, Denmark*
[p] *Department of Clinical Microbiology, Sønderborg Hospital, Sønderborg, Denmark*
[q] *Department of Cardiology, Copenhagen University Hospital - Rigshospitalet, Copenhagen, Denmark*
[r] *Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*

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ABSTRACT

Background: Lyme borreliosis is a tick-borne disease caused by the bacterium Borrelia burgdorferi (Bb) sensu lato complex. Previous studies have suggested an association between Lyme borreliosis and heart failure, which have been suggested to be a possible manifestation of Lyme carditis. We aimed to investigate the risk of heart failure among individuals tested for serum Bb antibodies, and serum Bb seropositive individuals.

Methods: We performed a matched nationwide cohort study (Denmark, 1993–2020) and included 52,200 Bb seropositive individuals, and two age- and sex-matched comparison cohorts: 1) 104,400 Bb seronegative comparison cohort members, and 2) 261,000 population controls. We investigated the risk associated with 1) being tested for serum Bb antibodies, and 2) being Bb seropositive. Outcomes were: 1) a composite of heart failure, cardiomyopathy, and/or myocarditis diagnosis, and 2) redemption of cardiovascular medicine used for treatment of heart failure. We calculated short-term odds ratios (aOR) (within 1 month) and long-term hazard rates (aHR) (after 1 month) adjusted for age, sex, diabetes, pre-existing heart failure, and kidney disease.

Results: Compared with the population controls, individuals tested for Bb antibodies, regardless of the test result, had increased short-term risk of heart failure, cardiomyopathy, and myocarditis (aOR 8.3, 95 %CI: 6.7–10.2), and both increased short- and long-term risk of redemption of cardiovascular medicine (aOR 4.3, 95 %CI:

* Corresponding author: Malte Mose Tetens, Medical Doctor, Department of Infectious Diseases, Copenhagen University Hospital - Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark.
E-mail address: malte.mose.tetens.01@regionh.dk (M.M. Tetens).

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3.8–4.8, aHR 1.13, 95 % CI: 1.11–1.15). The Bb seropositive individuals had no increased short- or long-term risk of any outcome compared with Bb seronegative comparison cohort members.
Conclusions: In conclusion, Bb antibody tests seemed to be performed in the diagnostic work-up of heart failure, but Bb seropositivity was not associated with heart failure.

1. Introduction

Lyme borreliosis (LB) is a tick-borne disease caused by the bacterium *Borrelia burgdorferi* (*Bb*) sensu lato complex. During early disseminated infection, acute cardiac involvement, known as Lyme carditis (LC), may occur (Stanek et al., 4). Cardiovascular symptoms are reported in 1–10 % of Lyme borreliosis cases in the US, and in 0.3–4.0 % in Europe (Uzomah et al., 2021).

LC usually manifests within weeks to a few months after the tick bite, mainly as a fluctuating atrioventricular block (Stanek et al., 2012 Feb 4; Shen and McCarthy, 2022 Sep; van der Linde, 1991; Neville et al., 2021 Nov; Pinto, 2002 Mar; Tetens et al., 2024 Feb), and less frequently as other cardiac conduction abnormalities (Marcos et al., 2020 Dec). The diagnosis of LC is based on a combination of both clinical presentation in combination with and detection of specific serum *Bb* antibodies (Stanek et al., 2012 Feb 4; Lantos et al., 2021 Jan).

A few European studies have suggested an association between LC and chronic heart failure (Stanek et al., 1991; Stanek et al., 1990 Jan 25; Kubánek et al., 2012 Jun; Lardieri et al., 1993 Aug 21; Bartůněk et al., 2007; Palecek et al., 2010 May; Gasser et al., 1992 May 9; Beach et al., 2020 Feb; Shen et al., 2021 Jul). LC reportedly manifests as congestive heart failure, cardiomyopathy, or myocarditis in 2 %−4 % of hospitalized children with LC (Beach et al., 2020 Feb), and in 3 % of hospitalized adults with LC (Shen et al., 2021 Jul).

Only few studies describe short- and long-term risk of heart failure among patients with Lyme borreliosis. Furthermore, previous studies were cases or case-series (Stanek et al., 1990 Jan 25; Lardieri et al., 1993 Aug 21; Gasser et al., 1992 May 9; Shen et al., 2021 Jul), or limited by small sample size (Stanek et al., 1991; Kubánek et al., 2012 Jun), lack of a matched comparison cohort from the background population (Stanek et al., 1991; Bartůněk et al., 2007), and lack of a serum *Bb* seronegative comparison cohort (Stanek et al., 1991; Beach et al., 2020 Feb).

No studies have previously investigated the association between *Bb* seropositivity and heart failure in a large cohort with long-term follow-up. We aimed to investigate the risk of heart failure, cardiomyopathy, or myocarditis, and redemption of cardiovascular medicine used for treatment of heart failure among individuals tested for serum *Bb* antibodies, and among serum *Bb* seropositive individuals.

2. Material and methods

We performed a nationwide, population-based, matched cohort study to investigate the risk of heart failure, and redemption of cardiovascular medicine used for treatment of heart failure, among individuals tested for serum *Bb* antibodies compared with age- and sex-matched controls from the background population, and among serum *Bb* seropositive individuals compared with an age- and sex-matched *Bb* seronegative comparison cohort.

2.1. Setting

In the years of study inclusion, 1993–2020, Denmark had a population of 5.2 million to 5.8 million individuals (Population in Denmark [Internet] 2021). In Denmark, all residents have access to universal tax-supported health care free of charge (Schmidt et al., 2019). All Danish residents are assigned with a unique 10-digit personal identification number at birth or upon immigration, which is used to track individuals through the Danish health and administrative registries (Pedersen, 2011 Jul).

2.2. Data sources

We identified all serum *Bb* antibody tests that were assessed for the presence of both *Bb*-IgM and *Bb*-IgG antibodies from all Danish departments of clinical microbiology, during the period November 1, 1993, to July 1, 2020 (Supplementary Table 1). Serum samples were analyzed in a single-tiered test strategy for the presence of specific serum *Bb* IgM and IgG antibodies using either a capture IgM enzyme-linked immunosorbent assays (ELISA), an indirect IgG ELISA, or a chemiluminescence immunoassay (CLIA) (See Supplementary appendix 1). In Denmark, single-tiered test are used in all laboratories as routine testing, as two-tiered tests have not been shown to outperform single-tiered tests (Leeflang et al., 2016 Mar 25; Dessau et al., 2018 Feb). We defined a positive serum *Bb* antibody test as any test with detectable *Bb* specific IgM and/or IgG antibodies according to the manufacturers' definition. We defined a negative serum *Bb* antibody test as any test without detectable *Bb*-IgM and *Bb*-IgG antibodies.

We obtained data on sex, date of birth, immigration, emigration, and death from the Danish Civil Registration System, which was established in 1967 (Pedersen, 2011 Jul).

We obtained data on annual taxable income from the Income Statistics Register (Documentation of statistics: Income Statistics [Internet]., 2023).

We obtained data on diagnosis codes and dates of diagnoses from the Danish National Patient Registration System (Schmidt et al., 2015), which records data on all individuals discharged from non-psychiatric hospital since 1977, and all patients with outpatient or emergency hospital contact since 1994. Diagnoses were coded by the attending physician according to the International Statistical Classification of Diseases and Related Health Problems, 8th revision (ICD8) until 1994, and according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD10) thereafter.

We obtained data on redeemed medication and date of redemption from the Danish National Prescription Registry, which was established in 1995 (Pottegård et al., 2017 01). Medications were classified according to the Anatomical Therapeutic Chemical Classification System.

Preceding formal analyses, Statistics Denmark replaced the personal identification number of all individuals with a new unique identifier to pseudonymize the data.

2.3. Study population

Cohort of serum Borrelia burgdorferi seropositive individuals: All Danish residents with a first-time positive serum *Bb* antibody test between November 1, 1993, and July 1, 2020, were included and stratified as (Supplementary Figure 1):

- Only *Bb*-IgM seropositive cohort: All individuals with a first-time positive serum *Bb* antibody test that was positive for *Bb*-IgM antibodies and negative for *Bb*-IgG antibodies.
- Only *Bb*-IgG seropositive cohort: All individuals with a first-time positive serum *Bb* antibody test that was positive for *Bb*-IgG antibodies and negative for *Bb*-IgM antibodies.
- *Bb*-IgM-and-IgG seropositive cohort: All individuals with a first-time positive serum *Bb* antibody test that was positive for both *Bb*-IgM and *Bb*-IgG antibodies.

Date of study inclusion was defined as the first date of a positive serum *Bb* antibody test. The *Bb* seropositive individuals did not change

stratification group if they later had another serum *Bb* antibody test after study inclusion. We did not include 3145 *Bb* seropositive individuals tested for only either *Bb*-IgM antibodies or *Bb*-IgG antibodies.

Comparison cohort of serum Borrelia burgdorferi seronegative individuals: We identified 355,689 Danish individuals who had a first-time negative serum *Bb* antibody test with no previous positive serum *Bb* antibody test between November 1, 1993, and July 1, 2020, (Supplementary Figure 1). From this population, we randomly extracted 2 controls for each *Bb* seropositive individual matched on age at serum *Bb* antibody test (+/- 5 years), date of serum *Bb* antibody test (+/- 5 years), and sex. Study inclusion was defined as the first date of a negative serum *Bb* antibody test. If the *Bb*-seronegative comparison cohort member had a positive serum *Bb* antibody test after study inclusion, they were censored on the date of first positive serum *Bb* antibody test.

Cohort of individuals tested for serum Borrelia burgdorferi antibodies: To form a cohort of individuals who were tested for serum *Bb* antibodies, we included all the serum *Bb* seropositive individuals, and controls from the *Bb* seronegative comparison cohort (described above).

Comparison cohort of population controls: To form a comparison cohort from the Danish background population, we randomly extracted individuals without a positive serum *Bb* antibody test matched on month of birth and sex from the Danish Civil Registration System (Supplementary Figure 1). Controls in the population cohort were assigned the same date of study inclusion as the corresponding *Bb* seropositive individual.

2.4. Exposures

We investigated 1) the risk associated with having had a *Bb* antibody test (cohort of individuals tested for serum *Bb* antibodies compared with the background population control cohort members), and 2) the added risk associated with having a positive test for serum *Bb* antibodies (*Bb* seropositive individuals compared with *Bb* seronegative comparison cohort members).

2.5. Outcomes measures

We investigated 1) a composite outcome of first-time diagnosis of either heart failure, cardiomyopathy, or myocarditis according to ICD8 and ICD10 codes, and 2) first-time redemption of cardiovascular medicine used for treatment of heart failure (beta blocking agents, agents acting on the renin-angiotensin system, loop-diuretics, aldosterone antagonists, and other diuretics and antihypertensives (Løgstrup et al., 2023 Jun 9)) according to ATC codes (Supplementary Table 3).

2.6. Confounding factors

We identified comorbidity status according to Charlson Comorbidity Index (CCI) > 1, and diagnoses of diabetes, heart failure, and kidney disease according to ICD8 and ICD10 codes (See Supplementary Table 3) prior to study inclusion (Quan et al., 2011 Mar 15). To ascertain differences in healthcare-seeking behaviour, we ascertained the proportion of individuals with 1 or more hospital contacts per year from 10 years preceding study inclusion. Further we ascertained the proportion of individuals with 1 or more hospital contacts per year up to 20 years after study inclusion. Finally, we obtained the annual taxable income of all individuals older than 18 years as measure of socioeconomic status.

2.7. Statistical analysis

2.7.1. Short-term risk

We used descriptive statistics to calculate the absolute risk of outcomes in the period between 1 month before and 1 month after study inclusion, and a logistic regression model to calculate the odds ratio (OR) and corresponding 95 % confidence intervals (CIs) of outcomes associated with the exposures. We adjusted the logistic regression model for age, sex, prior diabetes, prior heart failure, and prior kidney disease at study inclusion.

2.7.2. Long-term risk

We calculated the time from 1 month after study inclusion until event of interest, date of death, date of emigration, loss to follow-up, 20 years after study inclusion or September 1, 2021, whichever occurred first. In these analyses, we excluded all individuals who had an event of interest prior to 1 month after study inclusion. We calculated incidence rates of outcomes per 10,000 person-years and cumulative incidence risks accounting for competing risk of death. We used a Cox regression model to calculate cause-specific hazard ratios (HR) and corresponding 95 % CIs for the outcomes associated with the exposures. We adjusted the Cox-regression model for age, sex, diabetes, heart failure, and kidney disease.

We stratified all the above analyses on whether the *Bb*-test was positive for only *Bb*-IgM antibodies, only *Bb*-IgG antibodies, or *Bb*-IgM-and-IgG antibodies.

3. Results

The *Bb* seropositive cohort included 52,200 individuals, while the *Bb* seronegative comparison cohort and the population control cohorts included 104,400, and 261,000 controls, respectively. Thus, the cohort of individuals tested for serum *Bb* antibodies included a total of 156,600 individuals. The total observation time was 4396,395 person-years (Table 1). We observed no substantial differences in baseline characteristics between the cohorts, except that the individuals tested for serum *Bb* antibodies had more hospital contacts prior to study inclusion and higher annual incomes (Table 1 & Fig. 1).

Of the *Bb* seropositive individuals, 26,103 (50 %) were only *Bb*-IgM seropositive, 18,698 (36 %) only *Bb*-IgG seropositive, and 7399 (14 %) *Bb*-IgM-and-IgG seropositive. Generally, the *Bb* seropositive individuals had higher annual incomes and fewer hospital contacts prior to study inclusion than the corresponding *Bb* seronegative comparison cohort members. In the *Bb* seropositive cohort, the only *Bb*-IgM seropositive individuals were the youngest and the *Bb*-IgG seropositive individuals the oldest (Table 1). Presumably due to differences in age, the only *Bb*-IgM seropositive individuals had fewer comorbidities and lower incomes, while the *Bb*-IgG seropositive individuals had more comorbidities and higher incomes (Table 1).

3.1. Short-term risk

Compared with the population controls, individuals tested for *Bb* antibodies had an increased short-term risk of diagnosis of heart failure, cardiomyopathy, or myocarditis (OR: 8.3, 95 % CI: 6.7–10.2), and redemption of cardiovascular medicine used for treatment of heart failure (OR: 4.3, 95 % CI: 3.8–4.7) (Table 2 & Supplementary Table 4). The increased risk was not associated with the test result, as the *Bb* seropositive individuals had no increased short-term risk of any of these outcomes compared with the *Bb* seronegative comparison cohort members (Table 2 & Supplementary Table 4).

3.2. Long-term risk

Compared with the population controls, individuals tested for *Bb* antibodies had no increased long-term risk of heart failure, cardiomyopathy, or myocarditis, but had an increased long-term risk of redemption of cardiovascular medicine used for treatment of heart failure (HR: 1.13, 95 % CI: 1.11–1.15) (Table 3, Fig. 2, & Supplementary Table 5). The *Bb* seropositive individuals had no increased long-term risk of any of the examined outcomes compared with the *Bb* seronegative comparison cohort members.

Table 1
Characteristic at study inclusion and observation time of the individuals tested for *Borrelia burgdorferi* (*Bb*) antibodies (Tested), the population controls (Controls), the *Bb* seropositive cohorts (Seropositive), and the *Bb* seronegative comparison cohort (Seronegative). Values are numbers (percentages) unless stated otherwise.

	Cohorts							
	Individuals tested for *Bb* antibodies		Only *Bb*-IgM		Only *Bb*-IgG		*Bb*-IgM-and-IgG	
	Tested	Controls	Seropositive[1]	Seronegative[2]	Seropositive[3]	Seronegative[2]	Seropositive[4]	Seronegative[2]
BASELINE CHARACTERISTICS								
Total number	156,600	261,000	26,103	52,206	18,698	37,396	7399	14,798
Age at study inclusion, years[5]	52 (36–65)	52 (36–65)	45 (29–58)	45 (29–58)	60 (47–70)	60 (47–70)	55 (41–66)	55 (41–66)
Age < 16 years at study inclusion (%)	16,644 (11)	27,957 (11)	3642 (14)	7180 (14)	1180 (6)	2341 (6)	768 (10)	1533 (10)
Female sex (%)	81,465 (52)	135,775 (52)	15,132 (58)	30,264 (58)	8225 (44)	16.450 (44)	3798 (51)	7202 (51)
Charlson comorbidity index score > 1	17,790 (11)	33,590 (11)	2348 (9)	4184 (8)	2964 (16)	5255 (14)	881 (14)	1821 (12)
Diabetes mellitus (%)	5115 (3)	9072 (3)	1423 (3)	429 (2)	620 (3)	1858 (5)	140 (2)	645 (4)
Heart failure (%)	2109 (1)	3760 (1)	168 (1)	481 (1)	280 (1)	534 (1)	56 (1)	240 (2)
Chronic kidney disease (%)	1786 (1)	2666 (1)	193 (1)	480 (1)	205 (1)	450 (1)	61 (1)	201 (1)
Had 1 or more hospital contacts (%)								
10 years prior to study inclusion	45,411 (32)	65,209 (28)	6876 (30)	14,341 (31)	5386 (31)	12,186 (35)	1923 (29)	4699 (35)
5 years prior to study inclusion	45,411 (32)	65,209 (28)	9611 (38)	20,230 (40)	7498 (41)	16,360 (45)	2750 (39)	6414 (45)
4 years prior to study inclusion	62,863 (42)	89,635 (36)	10,129 (40)	21,278 (41)	7754 (42)	18,322 (47)	2838 (39)	6642 (46)
3 years prior to study inclusion	65,670 (43)	93,355 (37)	10,633 (42)	51,196 (44)	8172 (44)	18,297 (50)	2984 (41)	7070 (49)
2 years prior to study inclusion	69,488 (45)	97,802 (38)	11,128 (43)	23,810 (46)	8708 (47)	18,915 (51)	3178 (43)	7485 (51)
1 years prior to study inclusion	73,224 (47)	101,590 (39)	11,980 (46)	26,355 (51)	9440 (51)	20,901 (56)	3265 (44)	8106 (55)
Annual taxable income, 1000DKK[5]	226 (128–347)	217 (123–334)	223 (106–342)	213 (140–344)	246 (145–379)	230 (144–354)	254 (138–395)	229 (135–356)
OBSERVATION TIME								
Observation time, years[5]	9 (5–15)	9 (5–15)	12 (7–18)	12 (6–17)	8 (5–13)	8 (5–13)	8 (5–13)	8 (5–13)
Total observation time, years	2753,974	1642,421	319,514	613,853	172,267	345,764	71,435	136,907

[1] *Bb*-IgM seropositive individuals: All individuals with a first-time *Bb* antibody test, positive for *Bb*-IgM antibodies and negative for *Bb*-IgG antibodies.
[2] *Bb* seronegative comparison cohort: Individuals with a first-time negative *Bb* antibody test, negative for both *Bb*-IgM and IgG antibodies.
[3] *Bb*-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for *Bb*-IgG antibodies and negative for *Bb*-IgM antibodies.
[4] *Bb*-IgM and *Bb*-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for both *Bb*-IgM and IgG antibodies.
[5] Median (interquartile range).

4. Discussion

In this Danish nationwide, population-based, matched cohort study, we found an increased short-time risk of a composite outcome of first-time heart failure, cardiomyopathy, or myocarditis, as well as a composite outcome of first-time redemption of cardiovascular medicine used for treatment of heart failure among individuals tested for serum *Bb* antibodies, compared with population controls extracted from the background population. Furthermore, we found an increased long-term risk of redemption of cardiovascular medicine used for treatment of heart failure among individuals tested for serum *Bb* antibodies compared with age- and sex-matched controls from the background population. Individuals who tested positive for serum *Bb* antibodies had no increased short- or long-term risk of any outcome compared with age- and sex matched serum *Bb* seronegative comparison cohort members. Our data suggest that being tested for serum *Bb* antibodies, and not the result of the test, was associated with the increased risks e.g., as part of the diagnostic work-up for cardiovascular symptoms.

4.1. Discussion of our own results

Individuals tested for serum *Bb* antibodies had an increased short-term risk of both heart failure and redemption of cardiovascular medicine, regardless of the test result. The lack of an association between serum *Bb* seropositivity and heart failure suggests that the increased risk among the individuals tested for *Bb* antibodies was not due to a causal association between *Bb* infection and heart failure. The association could have other explanations 1*) That Bb antibody tests were performed in the diagnostic work-up of heart failure:* An increased test activity in the diagnostic work-up of patients with heart failure may explain an immediate increased short-term risk. However, as being tested for *Bb* antibodies was not associated with an increased long-term risk of heart failure, early diagnostic work-up for heart failure is unlikely to explain the long-term increased risk of redemption of cardiovascular medicine. 2*) An overall increased disease burden in individuals who had a Bb antibody test*: Individuals tested for *Bb* antibodies did not differ from the background population comparison cohort with regards to CCI, diabetes, heart failure, or kidney disease at study inclusion, which does not support an increased disease burden. 3*)Healthcare-seeking behaviour:* We found that individuals tested for serum *Bb* antibodies had different healthcare-seeking behaviour, with more hospital contacts than the background population comparison cohort members both before and after study inclusion. This may partially explain why the individuals tested for serum *Bb* antibodies more frequently received hospital-assigned diagnoses or prescriptions for medicine. Therefore, the short-term increased risk among individuals tested for *Bb* antibodies was most likely caused by an increased test activity as part of the diagnostic work-up, or differences in health seeking behaviour among individuals who had a *Bb* antibody test. This suggests that some *Bb* antibody tests were performed despite a lack of clear clinical indication. Conversely, the increased long-term risk of redemption of cardiovascular medicine, was likely explained by differences in healthcare-seeking behaviour, and not an increased disease burden due to heart failure. Notably, the serum *Bb* seropositive individuals exhibited less healthcare-seeking behaviour than the serum *Bb* seronegative comparison cohort members both preceding, during, and after the year of study inclusion. The decreased healthcare-seeking behaviour may partly explain the decreased risk of heart failure and redemption of cardiovascular medicine used for treatment of heart failure among the serum *Bb* seropositive individuals compared with the serum *Bb* seronegative individuals. It has been suggested that such differences in healthcare-seeking behaviour induce bias in studies with test negative design, which supports this explanation (Vandenbroucke and Pearce, 2019 Nov; Sullivan et al., 2016 Sep 1).

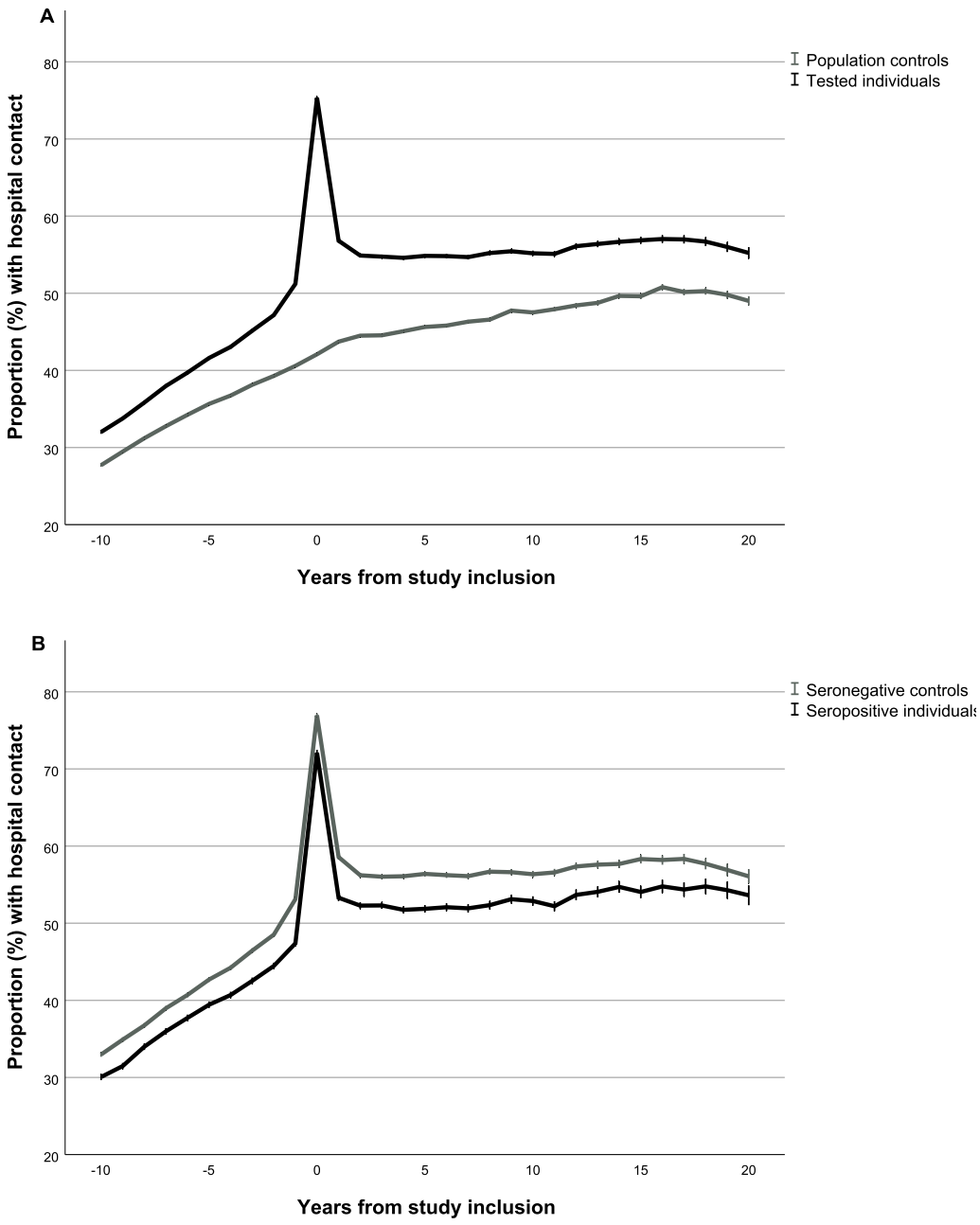

Fig. 1. Hospital contact

Fig. 1: Yearly proportion (percentage) with corresponding 95 % confidence intervals of 1 or more hospital contacts among individuals tested for serum Borrelia burgdorferi (Bb) antibodies (Tested individuals), and population controls (1.A), and serum Bb seropositive individuals, and serum Bb seronegative comparison cohort members (1.B).

4.2. Comparison with other studies

We have previously validated that our study design could identify individuals with Lyme carditis, i.e., diagnosis code for atrioventricular block combined with the presence of both *Bb*-IgM-and-IgG antibodies (Tetens et al., 2024 Feb). If Lyme carditis could manifest as heart failure, we would likely have been able to demonstrate a similar association.

The specificity of serum *Bb* IgM antibodies for Lyme borreliosis is lower than that of *Bb* IgG antibodies (Tetens et al., 2022 Nov; Hillerdal and Henningsson, 2021 Jun). This is partly caused by false positive *Bb* IgM antibody tests due to oligoclonal stimulation and cross-reactivity with other pathogens such as Epstein-Barr virus and cytomegalovirus (Hillerdal and Henningsson, 2021 Jun). This likely explains why we identified more serum *Bb* IgM seropositive individuals than serum *Bb* IgG seropositive individuals.

We found no increased risk of heart failure or redemption of cardiovascular medicine among *Bb* seropositive individuals compared with *Bb* seronegative comparison cohort members. Previous studies have revealed an association between LC and heart failure (Stanek et al., 1991; Stanek et al., 1990 Jan 25; Kubánek et al., 2012 Jun; Lardieri et al., 1993 Aug 21; Bartůněk et al., 2007; Palecek et al., 2010 May; Gasser et al., 1992 May 9; Beach et al., 2020 Feb; Shen et al., 2021 Jul). Patients with LC reportedly had congestive heart failure, cardiomyopathy, or myocarditis in 2 %–4 % of cases. Patients with chronic heart failure were more frequently *Bb* seropositive than healthy blood donors (Stanek et al., 1991). These case series on the relationship between heart failure and Lyme borreliosis lack causality. However, in selected studies, *Bb* has been cultured or identified by PCR or electron microscopy from endomyocardial biopsies in 38 patients with heart failure (Stanek et al., 1990 Jan 25; Kubánek et al., 2012 Jun; Lardieri et al., 1993 Aug 21;

Table 2
Short-term risk (between 1 month before and after study inclusion) of cardiac outcomes among individuals tested for *Borrelia burgdorferi* sensu lato (*Bb*) antibodies.

Outcome	Number of events (Absolute risk, ‰)		Short-term adjusted* OR (95 % CI)
Heart failure, cardiomyopathy, or myocarditis			
Risk among individuals tested for Bb antibodies compared with the population controls			
	Tested	Population controls	
Individuals tested for *Bb* antibodies	536 (3.5 ‰)	108 (0.4 ‰)	8.3 (6.7–10.2)
Risk among Bb seropositive individuals compared with Bb seronegative comparison cohort members			
	Bb seropositive	*Bb* seronegative[1]	
Bb-IgM seropositive[2]	60 (2.3 ‰)	177 (3.5 ‰)	0.7 (0.5–0.9)
Bb-IgG seropositive[3]	63 (3.4 ‰)	173 (4.8 ‰)	0.7 (0.5–1.0)
Bb-IgM-and-IgG seropositive[4]	12 (1.6 ‰)	51 (3.4 ‰)	0.5 (0.3–0.9)
Redemption of cardiovascular medicine used for treatment of heart failure			
Risk among individuals tested for Bb antibodies compared with the population controls			
	Tested	Population controls	
Individuals tested for *Bb* antibodies	1526 (16.5 ‰)	522 (3.6 ‰)	4.3 (3.8–4.8)
Risk among Bb seropositive individuals compared with Bb seronegative comparison cohort members			
	Bb seropositive	*Bb* seronegative[1]	
Bb-IgM seropositive[2]	256 (12.8 ‰)	484 (15.2 ‰)	0.7 (0.6–0.8)
Bb-IgG seropositive[3]	269 (22.7 ‰)	354 (22.6 ‰)	0.8 (0.7–1.0)
Bb-IgM-and-IgG seropositive[4]	54 (10.1 ‰)	109 (14.6 ‰)	0.5 (0.4–0.8)

*Adjusted for age, sex, diabetes, heart failure, and kidney disease.
[1] *Bb* seronegative comparison cohort: Individuals with a first-time negative *Bb* antibody test, negative for both *Bb*-IgM and IgG antibodies.
[2] *Bb*-IgM seropositive individuals: All individuals with a first-time positive *Bb* antibody test, for *Bb*-IgM antibodies and negative for *Bb*-IgG antibodies.
[3] *Bb*-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for *Bb*-IgG antibodies and negative for *Bb*-IgM antibodies.
[4] *Bb*-IgM-and-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for both *Bb*-IgM and *Bb*-IgG antibodies.

Bartůněk et al., 2007; Palecek et al., 2010 May). Among 175 patients referred for possible cardiac transplantation due to heart failure, the proportion who were serum *Bb* seropositive was larger than among healthy blood donors, but comparable to patients with ischemic heart disease (Sonnesyn et al., 1995 Jul 1). However, none of these previous studies included a matched *Bb* seronegative comparison cohort, which was a main asset of our study. If we had compared the *Bb* seropositive individuals with controls from the general population, rather than the *Bb* seronegative comparison cohort, we would have wrongly concluded that *Bb* seropositive individuals were at an increased short-term risk of heart failure and even long-term risk of redemption of cardiovascular medicine. Only the inclusion of the *Bb* seronegative comparison cohort allowed us to separately investigate the risk associated with having had a *Bb* antibody test, and of being *Bb* seropositive.

In agreement with our results, a British study found no increased prevalence of *Bb* serum IgG antibodies among patients with dilated cardiomyopathy compared with a matched comparison cohort (Rees et al., 1994 May). Furthermore, no *Bb* DNA was detected in myocardial tissue from 68 patients who had undergone heart transplantation due to heart failure (Suedkamp et al., 1999 Aug).

The Infectious Diseases Society of America recommends against routine testing for Lyme borreliosis among patients with dilated cardiomyopathy, though this is only a weak recommendation as it is based on low-quality evidence (Lantos et al., 2021 Jan). The results of the current study support this statement, and the recommendation can now be based on higher-quality evidence.

Table 3
Long-term risk (after 1 month after study inclusion) of cardiac outcomes among individuals tested for *Borrelia burgdorferi* sensu lato (*Bb*) antibodies.

Outcome	Incidence rate per 10,000 person-years		Long-term adjusted* HR (95 % CI)
Heart failure, cardiomyopathy, or myocarditis			
Risk among individuals tested for Bb antibodies compared with the population controls			
	Tested	Population controls	
Individuals tested for *Bb* antibodies	31.4	31.3	0.99 (0.96–1.03)
Risk among Bb seropositive individuals compared with Bb seronegative comparison cohort members			
	Bb seropositive	*Bb* seronegative[1]	
Bb-IgM seropositive[2]	20.8	24.5	0.82 (0.75–0.90)
Bb-IgG seropositive[3]	42.3	49.3	0.83 (0.75–0.90)
Bb-IgM-and-IgG seropositive[4]	26.1	34.2	0.74 (0.62–0.89)
Redemption of cardiovascular medicine used for treatment of heart failure			
Risk among individuals tested for Bb antibodies compared with the population controls			
	Tested	Population controls	
Individuals tested for *Bb* antibodies	281.0	250.6	1.13 (1.11–1.15)
Risk among Bb seropositive individuals compared with Bb seronegative comparison cohort members			
	Bb seropositive	*Bb* seronegative[1]	
Bb-IgM seropositive[2]	236.5	255.4	0.88 (0.85–0.92)
Bb-IgG seropositive[3]	337.2	358.1	0.97 (0.84–0.91)
Bb-IgM-and-IgG seropositive[4]	253.8	311.1	0.75 (0.70–0.81)

*Adjusted for age, sex, diabetes, chronic heart failure, and kidney disease.
[1] *Bb* seronegative comparison cohort members: Individuals with a first-time negative *Bb* antibody test, negative for both *Bb*-IgM and IgG antibodies.
[2] *Bb*-IgM seropositive individuals: All individuals with a first-time positive *Bb* antibody test, for *Bb*-IgM antibodies and negative for *Bb*-IgG antibodies.
[3] *Bb*-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for *Bb*-IgG antibodies and negative for *Bb*-IgM antibodies.
[4] *Bb*-IgM-and-IgG seropositive individuals: All individuals with a first-time positive *Bb* antibody test, positive for both *Bb*-IgM and *Bb*-IgG antibodies.

4.3. Study strengths and limitations

The strengths of this study include the large sample size, nationwide design, and high-quality data accessible at an individual level in the Danish national health and administrative registries. This combination of large cohorts with long-term and almost complete follow-up is unlikely to be possible in most other settings. The unique asset of our study is the inclusion of both an age- and sex matched comparison cohort from the general population, and a *Bb* seronegative comparison cohort. This enabled us to investigate the effect of being tested for serum *Bb* antibodies, and serum *Bb* seropositivity individually.

The study does have some limitations. We did not have access to data on the indication for the serum *Bb* antibody test. Additionally, the study was restricted to hospital diagnoses and did not include data from primary care. This may lead to an underestimation of risk in patients with less severe heart failure. However, this was partly mitigated by inclusion of data on redeemed cardiovascular medication.

5. Conclusions

We found no evidence of an association between heart failure and *Bb* seropositivity, neither during short-term nor long-term follow-up.

Individuals tested for the presence of serum *Bb* antibodies were at an increased long-term risk of redemption of cardiovascular medicine, regardless of the result of the test, which suggests differences in health seeking behaviour.

Our results strengthen current guidelines that recommend against

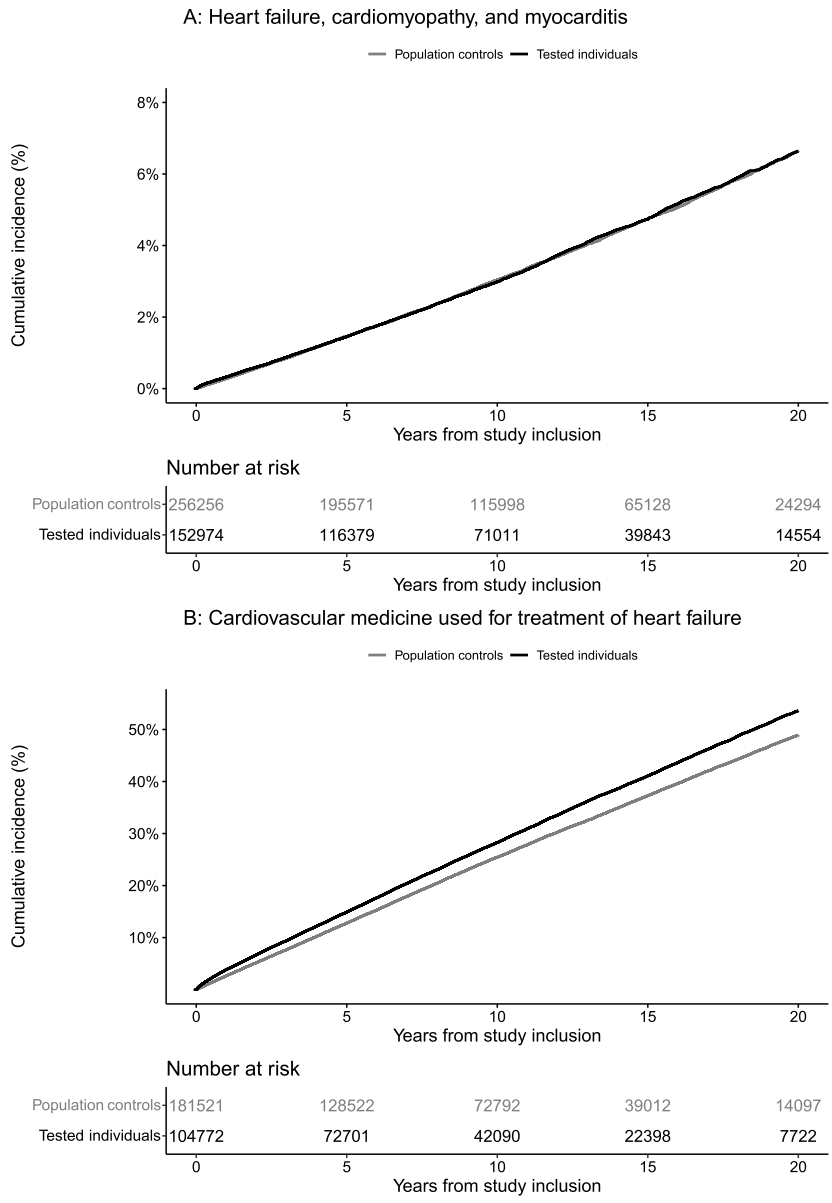

Fig. 2. Long-term risk of cardiac outcomes
Fig. 2: Long-term (after 1 month after study inclusion) risk of cardiac outcomes among tested individuals (individuals tested for serum Borrelia burgdorferi (Bb) antibodies) and population controls.

routine testing for *Bb* antibodies in patients with heart failure.

CRediT authorship contribution statement

Malte M. Tetens: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft. **Lars Haukali Omland:** Conceptualization, Funding acquisition, Supervision, Project administration, Writing – review & editing. **Ram B. Dessau:** Investigation, Writing – review & editing. **Svend Ellermann-Eriksen:** Investigation, Writing – review & editing. **Nanna S. Andersen:** Investigation, Writing – review & editing. **Charlotte Sværke Jørgensen:** Investigation, Writing – review & editing. **Christian Østergaard:** Investigation, Writing – review & editing. **Jacob Bodilsen:** Investigation, Writing – review & editing. **Kirstine K. Søgaard:** Investigation, Writing – review & editing. **Jette Bangsborg:** Investigation, Writing – review & editing. **Alex Christian Yde Nielsen:** Investigation, Writing – review & editing. **Jens Kjølseth Møller:** Investigation, Writing – review & editing. **Ming Chen:** Investigation, Writing – review & editing. **Jesper Hastrup Svendsen:** Conceptualization, Funding acquisition, Validation, Writing – review & editing. **Niels Obel:** Supervision, Conceptualization, Project administration, Methodology, Funding acquisition, Writing – review & editing. **Anne-Mette Lebech:** Supervision, Conceptualization, Project administration, Funding acquisition, Validation, Writing – review & editing.

Conflict of interest

MMT has received travel grants outside this work from GlaxoSmithKline Pharma A/S. RBD has advisory board activity with Pfizer. JHS reports institutional research grant from Medtronic (outside this study), speakers' honorarium from Medtronic and membership of an advisory board in Medtronic. AML reports speakers' honorarium/travel grants/advisory board activity and unrestricted grant from Gilead, speakers honorarium /travel grants from GSK, speaker's honorarium/ advisory board activity from Pfizer outside this work. None of the authors report any conflict of interests in connection with this article.

Data availability

The authors do not have permission to share data.

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Ethics approval

The study was approved by the Danish Data Protection Agency and the National Board of Health. Studies based on data from Danish national registries do not require informed consent from study participants according to Danish law.

Data availability statement

The ethical approval of this study from the Danish Data Protection Agency states the data that has been used in this article cannot be shared publicly. Upon a reasonable request to the corresponding author the data can be shared and assessed at our institution.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2024.102345.

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