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Original article

Exploring the dynamics of *Borrelia burgdorferi* sensu lato antibodies—a registry-based study on laboratory data from Sweden and Denmark

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ABSTRACT

Objectives: Lyme borreliosis (LB) is the most common tick-transmitted infection in the northern hemisphere and is caused by bacteria in the *Borrelia burgdorferi* sensu lato (*Bbsl*)-complex. The diagnosis is partially based on serology, and clinicians often take follow-up serum samples to look for seroconversion or an increase in IgG-antibody levels. In this registry-based study, we proposed a method for determining actual changes in IgG and examined antibody reactivity and decay.
Methods: Serological data from the departments of clinical microbiology at Karlstad Hospital, Sweden, and Slagelse Hospital, Denmark, were used to calculate a seroreactivity cut-off (SCOFF), above which changes between two samples from the patient cannot be explained by random variation. Increases in IgG reactivity as well as IgG and IgM decay were illustrated using time-to-event analysis and the SCOFF.
Results: A total of 44,861 serum samples from 34,157 patients were tested for *Bbsl*-antibodies. Of the 4301 patients with follow-up samples taken within 100 days, 201 (4.67%) were above the SCOFF of 1.42 with a median time to follow-up sample of 36 days (interquartile range: 21). IgG demonstrated longer median time for all antibody levels (indeterminate: 4.6 years, low: 7.0 years, moderate-high: 8.8 years) than IgM antibodies (indeterminate: 2.1 years, low: 3.9 years, moderate-high: 6.8 years) and higher initial antibody levels persisted significantly longer for both IgG and IgM antibodies ($p < 0.001$). Of the 7868 patients with follow-up samples, isolated IgM reactivity preceded an increase in IgG reactivity in 18 patients (0.23%).
Discussion: The SCOFF indicated little biological and random variation for *Bbsl*-specific IgG antibodies on the platforms used during the study. In most follow-up samples, both IgG and IgM antibodies persisted for years, with longer seropositivity associated with high initial antibody levels and IgG-type antibodies. The diagnostic value of isolated IgM reactivity was limited. **Marc Westerholt, Clin Microbiol Infect 2023;29:1561**

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Introduction

Lyme borreliosis (LB), the most common tick-transmitted infection in the Nordic countries, is caused by bacteria in the *Borrelia burgdorferi* sensu lato (*Bbsl*)-complex [1]. The diagnosis of LB is based on a combination of clinical assessment and detection of Borrelia-specific antibodies in the serum [2]. The presence of Borrelia-specific antibodies can strengthen an initial suspicion of LB

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and published guidelines sometimes recommend follow-up samples to detect changes in IgG or IgM antibodies. An increase in IgG antibodies on such follow-up samples suggests recent or on-going infection and a decline in or unaltered levels of IgG antibodies are suggestive of previous exposure. There are, however, no helpful guidelines for deciding whether a difference between two results reflects random variation or an actual antibody stimulation. Studies on the duration of *Bbsl*-specific IgM and IgG antibodies are also lacking and are based on small populations with a limited number of samples [3]. These observations demand further corroboration, as persisting IgM antibodies could be misinterpreted and result in unnecessary testing and possibly treatment [4].

Relative change (RC) is defined as the concentration of IgG antibodies in a follow-up sample divided by the concentration of IgG antibodies in the first sample and has mostly been used to evaluate antibody responses to vaccines [5]. Recently, RC has also been proposed as a tool to determine suitable threshold levels signifying actual changes in IgG reactivity [6]. In this registry-based study, we developed a method based on the concept of RC for the determination of a seroreactivity cut-off (SCOFF), above which changes in IgG cannot be explained by random variation and are thus indicative of actual changes on follow-up samples. Further, we examined the antibody reactivity and decay in recurrent samples from consecutive routine patients.

Methods

The study was mainly based on secondary use of data from the Department of Clinical Microbiology at Karlstad Hospital, Sweden. The laboratory is located in the city of Karlstad (population 95,000) and serves primary health care centres and three hospitals in Värmland, a sparsely populated, largely forested county with a population of 283,000, which borders Norway to the west [7]. In 2015, 12% and 3% of blood donors in the county of Värmland had detectable IgG and IgM antibodies against *Bbsl*, respectively. The following inquiries were examined:

1. How can a cross-laboratory SCOFF value indicative of actual increases in IgG reactivity on follow-up samples be determined?
2. What percentage of patients with follow-up samples demonstrate an actual increase in IgG antibodies?
3. How long do *Bbsl*-specific IgM and IgG antibodies persist?
4. How often does isolated IgM reactivity precede an actual increase in IgG antibodies on follow-up samples?

The development of statistical methods for examining these inquiries was one of the aims of the study. Data from the Department of Clinical Microbiology at Slagelse Hospital, Denmark, was used together with the Swedish data only to determine a suitable SCOFF. Swedish data were extracted from the Laboratory Information System Analytix (CompuGroup Medical, Austin, United States) and encompassed Borrelia-specific IgG and IgM results analysed from 7 November 2009 to 14 April 2023. Danish data were extracted from the Laboratory Information System MADS (Aarhus University Hospital, Denmark) and encompassed Borrelia-specific IgG results analysed from 8 May 2018 to 31 March 2023.

Laboratory analyses

The Liaison and Liaison XL platforms used during the study period were based on indirect chemiluminescence immunoassay technology from the same manufacturer (DiaSorin, Saluggia, Italy). Identical antigens were used in the form of variable major protein-like sequence expressed (VlsE) for the detection of IgG (assay number: 310880) and a combination of VlsE and outer surface protein C (OspC) for the detection of IgM (assay number: 310020).

The Liaison XL platform has been shown to give comparable results when the same samples are tested in different laboratories [8].

Statistical analyses

Statistical analysis and graphics were performed using R version 4.2.1 [9]. Several statistical methods from survival/time-to-event analysis were applied in this study. Seronegativity and actual increases in IgG were the events of interest in the analysis of antibody decay and actual IgG increases in follow-up samples, respectively. Patients with no further follow-up samples were censored. The 'survival' package version 2.38 [10] was used for the composition of Kaplan–Meier curves. 'Survminer' package version 0.4.9 [11] was used for the calculation of the Cox proportional hazards models and log-rank tests. Robust standard errors were used in the IgG decay model due to non-proportional hazards, the coefficients should then be interpreted as a time-weighted average of the hazard ratio as suggested [12]. Deviance residuals were negatively skewed in both models because of the copious amount of censoring/loss to follow-up in the study. For the statistical analyses of the Swedish data, results from the Liaison and Liaison XL were corrected based on correlations from prior validation reports to correct for any systematic biases and make results from the three instruments comparable.

Ethical considerations

The study was approved by the Swedish Ethical Review Authority (ref: 2023-03116-02) and the Danish Patient Safety Authority 2019 (ref: 3-3013-3165/1).

Results

In the Swedish cohort, a total of 44,861 serum samples from 34,157 patients were tested for antibodies against *Bbsl*. Of these, 25,506 samples (56.86%) were obtained from 19,365 females and 19,355 (43.14%) from 14,792 males. The median age was 54 (interquartile range [IQR]: 30, range: 0–99) and 18,572 (41.4%) were follow-up samples taken from 7868 patients. Patients <18 years of age constituted 422 (5.36%) of the patients with follow-up samples and had a median age of 12 (IQR: 8, range: 0–17). Of these, 287 (68%) had the first follow-up sample taken within 100 days of the first sample, corresponding to 4% of all patients with follow-up samples.

How can a cross-laboratory SCOFF value indicative of actual increases in IgG reactivity on follow-up samples be determined?

We selected the first and second sample from patients who were sampled within the same winter season between the months of November to March, and had their samples analysed for the presence of IgG antibodies (SWE: $n = 1542$, DK: $n = 597$). Negligible tick exposure was assumed during this period. Patients without seroreactivity (<10 AU/mL) in all samples and patients with a truncated antibody level in any sample (<5 AU/mL or >240 AU/mL) were excluded (SWE: $n = 747$ DK: $n = 507$), since no methodological variation can be calculated from these. A total of 885 patients (SWE: $n = 795$, DK: $n = 90$; Liaison XL: $n = 297$, Liaison: $n = 588$) remained for the determination of a cross-laboratory SCOFF. Statistical outliers (n = 49) from both countries were identified in the data using Tukey's fences (Fig. 1). The upper fence

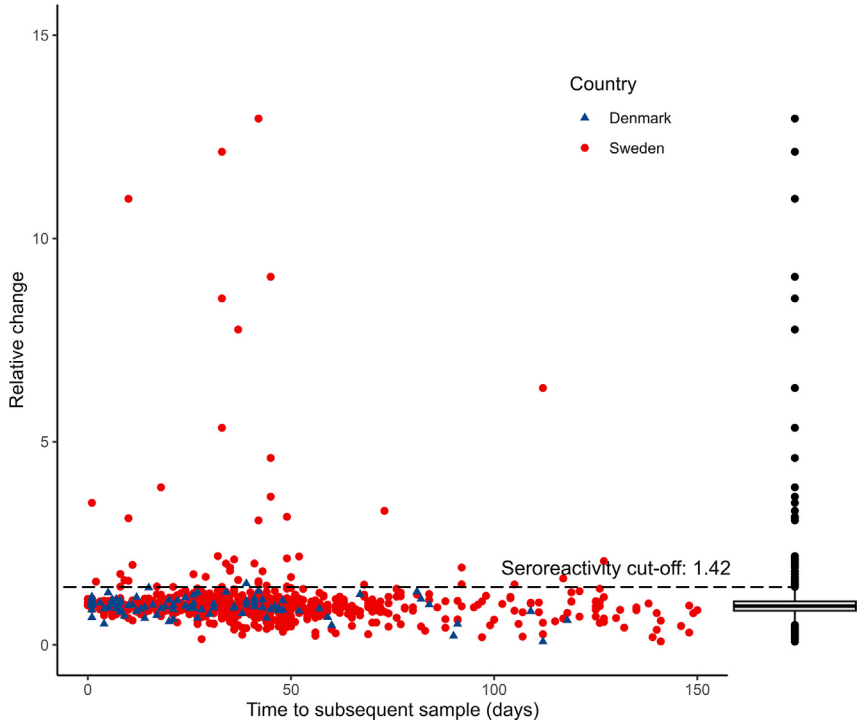

Fig. 1. Scatterplot showing country of origin and relative change values with a marginal boxplot illustrating Tukey's upper fence used for establishing the seroreactivity cut-off (SCOFF).

distinguished the maximum RC value smaller than the upper quartile (75th percentile) + 1.5 x IQR to determine a SCOFF of 1.42.

What percentage of patients with follow-up samples demonstrate an actual increase in IgG antibodies?

We chose to look at patients with follow-up samples taken within 100 days of the first sample (n = 4301). The follow-up period of 100 days was chosen as most antibodies directed against microbial agents usually appear within the first three months of exposure and most follow-up samples are taken within this time period. An actual increase in IgG antibodies was defined as a RC equal to or greater than the SCOFF. For patients with multiple follow-up samples, the first follow-up sample above the SCOFF was used for patients with actual IgG increases, to show the earliest timepoint at which an increase was detectable. The last follow-up sample was used for patients without any actual increases, to avoid missing samples with a late IgG increase.

A total of 201 (4.67%) patients demonstrated an actual increase in IgG antibodies using the SCOFF on follow-up samples. The median time to the first follow-up sample showing an actual increase in IgG and to the last sample without any actual increase in IgG was 36 days (IQR: 21) and 36 days (IQR: 26), respectively. A Kaplan–Meier curve showing the development of actual increases in IgG in these patients within the first 100 days (n = 201) is presented in Fig. 2.

How long do Bbsl-specific IgM and IgG antibodies persist?

To illustrate the persistence of antibodies against *Bbsl*, Kaplan–Meier curves were created to show the percentage of patients with detectable antibodies over time at different initial antibody levels. Separate Kaplan–Meier curves were created for IgM (Fig. 3) and IgG (Fig. 4) antibodies using patients with detectable IgM or IgG in the first sample and one or more follow-up samples (2598 patients for IgG and 938 for IgM). A higher median time to seronegativity in IgG (indeterminate: 4.6 years, low: 7.0 years, moderate-high: 8.8 years) compared with IgM (indeterminate: 2.1 years, low: 3.9 years, moderate-high: 6.8 years) was observed for all initial antibody levels. Cox regression (Table 1) showed that patients with high initial levels of IgG and IgM had a significantly longer time to seronegativity compared with patients who had indeterminate initial antibody levels ($p < 0.001$). For IgG antibodies, a significant difference was also observed between groups with moderate-high and low initial antibody levels ($p < 0.001$). The significant differences in the covariates sex and age should be interpreted with caution due to non-proportional hazards.

How often does isolated IgM reactivity precede an actual increase in IgG antibodies on follow-up samples?

The prevalence of IgM in the first sample was 5.63% (148/2629) for patients <18 years and 8% (2521/31,528) for patients ≥18 years. To determine how often isolated IgM reactivity preceded an increase in IgG, patients with two or more samples and initial isolated IgM reactivity were selected (n = 507). Of these, 18 patients (3.55%) demonstrated an actual increase in IgG antibodies, corresponding to 0.23% of the 7868 patients with follow-up samples. The clinical indications for sampling the patients, as stated in the laboratory referrals, were as follows: skin manifestations (n = 6), neurological symptoms (n = 3) and suspicion of LB in patient with extended duration between initial and follow-up sample (n = 5, median: 1070 days, range: 295–3360 days). Three of the patients had an extended duration between samples and concomitant neurological symptoms (n = 2) or persistent IgM in multiple samples prior to an actual increase in IgG (n = 1). One patient had no available clinical information in the laboratory referral. The median time to follow-up sample for all 507 patients with initial isolated IgM was 57 days (IQR: 397, range 0–3360 days).

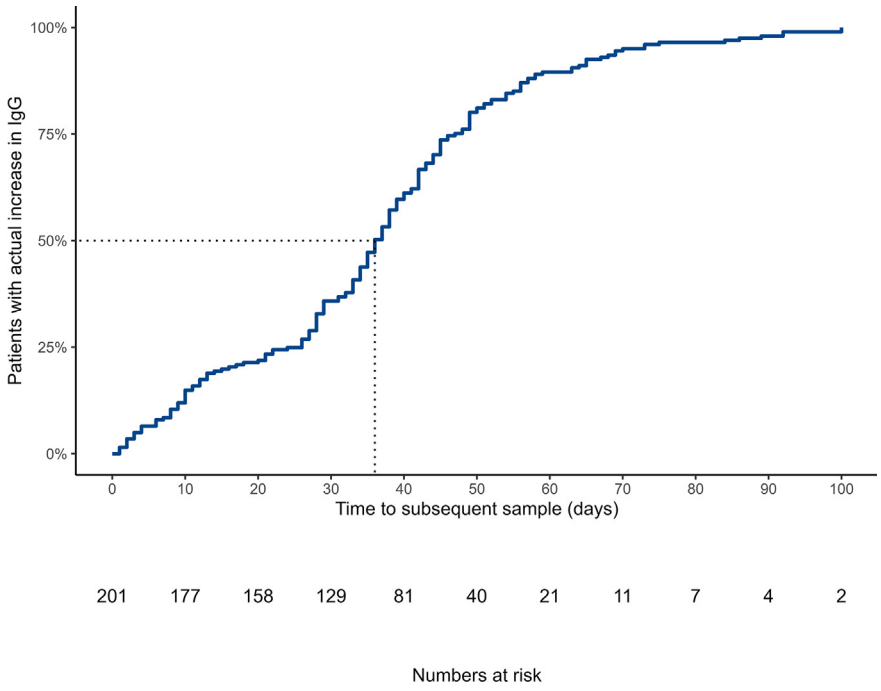

Fig. 2. Kaplan-Meier curve showing the development of actual increases in IgG over time and corresponding median of 36 days in patients who demonstrated such increases within 100 days of the initial sample.

Discussion

We observed only minor fluctuations in IgG-antibody reactivity in individual patients during months of negligible tick activity with a SCOFF of 1.42. This threshold encompasses biological variation, assay variation and interlaboratory variation and can be used to determine if a difference in IgG is indicative of an actual increase in antibody levels. Further, using this threshold to indicate actual increases in IgG levels and time-to-event analysis, we illustrated the IgG and IgM antibody reactivity and decay, and made the following observations:

1) A low percentage (4.67%) of patients developed an actual increase in IgG levels within 100 days of the initial sample, with a median time to increase of 36 days.

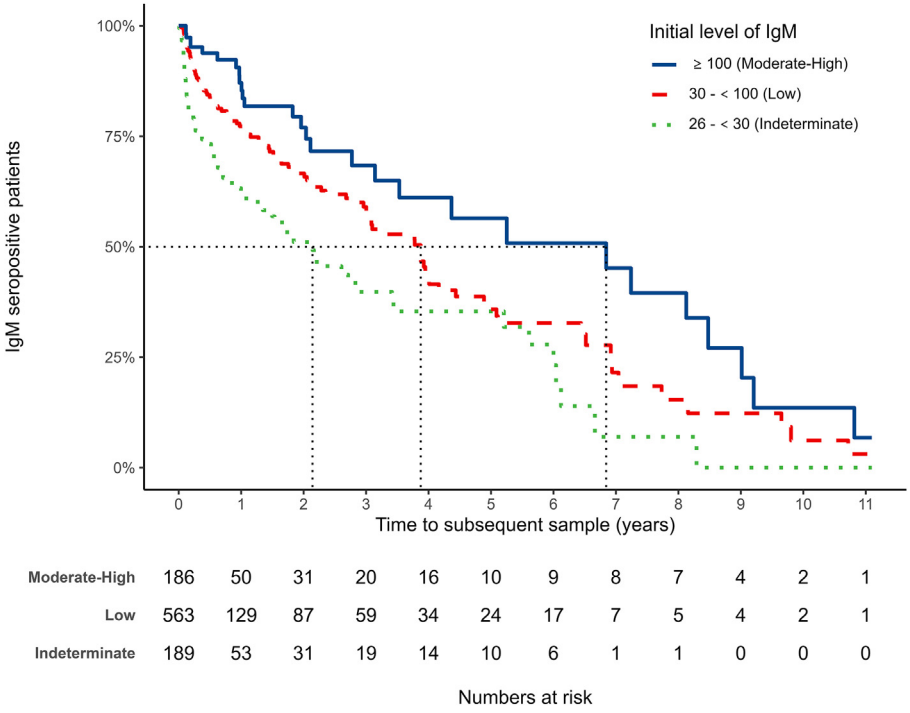

Fig. 3. Kaplan-Meier curve showing the percentage of patients with detectable IgM antibodies over time at different initial antibody levels and corresponding medians (indeterminate: 2.1 years, low: 3.9 years, moderate-high: 6.8 years).

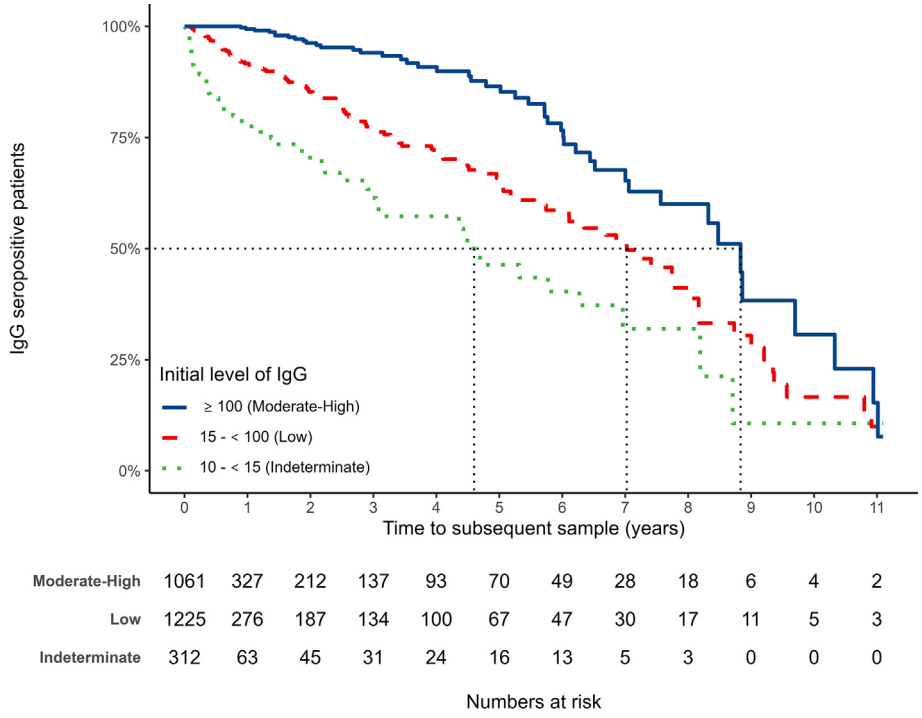

Fig. 4. Kaplan-Meier curve showing the percentage of patients with detectable IgG antibodies over time at different initial antibody levels and corresponding medians (indeterminate: 4.6 years, low: 7.0 years, moderate-high: 8.8 years).

2) A prolonged years long gradual decay of both IgM and IgG antibodies followed presumed exposure with longer durations for higher initial antibody levels and for IgG antibodies.
3) Isolated IgM reactivity rarely preceded an actual IgG increase.

The calculated SCOFF of 1.42 suggests a relatively low level of biological variation for *Bbsl*-specific IgG antibodies in line with the reported within-subject biological variation for total IgG (CV%: 3.5) and a low random variation for this analysis on the platforms used [13]. In comparison, one study demonstrated that a 1.8 and 2.7-fold increase in IgG antibodies could be indicative of actual change in antibody concentration for laboratories using a flagellar or C6 IgG assay, respectively [6]. Apart from differences in antigen and instruments used, the authors calculated the threshold for actual change using a different method which could explain the deviating results.

It is common practice in Sweden to recommend clinicians to take follow-up samples to detect changes in specific IgG or IgM antibodies in patients with suspected LB other than erythema migrans and with symptom duration of less than two months. Only 4.67% of the 4301 patients who had a follow-up sample taken within 100 days of the first sample demonstrated an actual increase in IgG antibodies. However, as the symptom duration prior to the healthcare visit is unknown, it is possible that some patients with LB already had an actual increase in IgG prior to the initial healthcare visit.

A gradual, years long fall in the number of seropositive individuals for both IgM and IgG can be observed in the Kaplan–Meier curves (Figs. 3 and 4). Of note, antibodies in samples with higher initial antibody levels of both IgM and IgG persisted significantly ($p < 0.001$) longer than antibodies with initially low levels (Table 1). A prior study showed persisting IgM and IgG for up to 10 to 20 years after active infection [3]. However, this study was based on a small sample ($n = 79$) of individuals who had two samples taken with a 10 to 20-year interval and even though none of the included persons reported any clinical signs of reinfection in the time between samples, re-exposure could not be ruled out.

An actual increase in IgG was rarely (18/507, 3.55%) preceded by isolated IgM reactivity indicating that isolated IgM reactivity is seldom indicative of exposure to *Bbsl*. Further, the presence of IgM did not aid in the diagnosis of LB in any of these patients. In 11 of them, the clinical indication for sampling was dubious with 6 of them being tested due to skin manifestations, despite erythema migrans being a clinical diagnosis [2], and 5 due to neurological symptoms, despite the diagnosis of neuroborreliosis requiring lumbar puncture according to European criteria [14]. Almost all remaining patients had either persistent IgM on multiple occasions prior to the increase in IgG or had a prolonged time interval between the initial and follow-up sample, rendering any comparisons between the two samples of limited clinical value. These results are in line with several recent studies showing limited additional diagnostic utility of IgM in combination with IgG compared with IgG alone [4,8].

The development of actual increases in IgG over time was influenced by the general recommendation of the clinical microbiology laboratory in Karlstad to acquire follow-up samples within 4 to 6 weeks of the initial sample. This recommendation has clustered many of the follow-up samples around that time point and risks introducing a time delay bias in patients with early actual increases in IgG (Fig. 2). Further, the stratification of IgG and IgM levels for the Swedish cohort was based on local categorization used at the department of clinical microbiology at Karlstad Hospital. Despite no ubiquitously accepted categorisation of antibody levels, this seemingly arbitrary classification remains a weakness of the study. Another limitation was the lack of data regarding clinical indication for testing available on the laboratory referrals. A previous Danish study showed that 38% of patients were tested

Table 1
Cox proportional hazards models and log-rank tests

Covariate	N	Hazard ratio (95% CI)	p	Log-rank p	N events
IgG decay					
	2598			1.014e-19	214
Initial IgG level					
≥100 (Moderate-High)	312	0.18 (0.12–0.27)	<0.001		
15-<100 (Low)	1225	0.42 (0.29–0.61)	<0.001		
10-<15 (Indeterminate)	1061	Reference			
Age					
≤65 years	1398	Reference			
>65 years	1200	0.70 (0.52–0.93)	0.013		
Sex					
Female	1221	Reference			
Male	1377	0.64 (0.48–0.86)	0.003		
IgM decay					
	938			2.479e-05	215
Initial IgM level					
≥100 (Moderate-High)	186	0.34 (0.22–0.53)	<0.001		
30-<100 (Low)	563	0.56 (0.41–0.75)	<0.001		
26-<30 (Indeterminate)	189	Reference			
Age					
≤65 years	644	Reference			
>65 years	294	1.02 (0.77–1.37)	0.877		
Sex					
Female	611	Reference			
Male	327	0.92 (0.69–1.22)	0.554		

because of an erythema or rash and the rest due to various mostly neurological or musculoskeletal complaints [15]. This pattern of serological testing is probably similar in Sweden and is unlikely to have changed much since the data were collected.

In conclusion, the SCOFF can be a useful tool in assessing differences in IgG on follow-up samples. Our results imply a low biological variation for IgG antibodies against *Bbsl* suggesting that relatively small increases in IgG on follow-up samples could be indicative of actual *Bbsl* exposure. Despite this, few patients demonstrated actual IgG increases why clinicians should exhibit a restrained approach when taking follow-up samples and rely more heavily on the clinical manifestations when investigating patients suspected of LB. Clinicians must also be aware that not only IgG but also IgM antibodies can persist for years following *Bbsl* exposure and that higher initial antibody levels result in longer periods of seropositivity. Further, as isolated IgM reactivity is only rarely followed by IgG seroconversion, it is a poor early marker of LB and must be interpreted with caution in a clinical setting.

Author contributions

Conception and design: LFO, MW, RBD and KAK. Data collection: MW and RBD. Data analysis and interpretation: MW, LFO and RBD. Writing – Original draft: LFO and MW. Revision of the manuscript: MW, LFO, RBD, and KAK. All authors approved the final version of the manuscript.

Transparency declaration

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