

SHORT COMMUNICATION

Borrelia burgdorferi sensu lato seroconversion after intravenous immunoglobulin treatment: A cohort study

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Abstract

Objective: Intravenous immunoglobulin (IVIg) consists of pooled donor immunoglobulins (IgG), possibly including anti-*Borrelia burgdorferi* (*Bbsl*) antibodies. Apparent IVIg-related *Bbsl* seroconversion could lead to incorrect diagnosis of Lyme borreliosis. This cohort study was designed to determine how often IVIg treatment leads to apparent *Bbsl* seroconversion and whether antibodies disappear post-treatment.

Methods: Sera from chronic inflammatory demyelinating polyneuropathy (CIDP) and myositis patients were analyzed, drawn pre-treatment and 6–12 weeks after the start of IVIg. In patients with apparent seroconversion, follow-up samples after treatment withdrawal were analyzed, if available. Patients treated with corticosteroids were included as controls. A two-tier protocol was used for serological testing consisting of the C6 Lyme ELISA (Oxford Immunotec) and confirmation by immunoglobulin M (IgM) and immunoglobulin G (IgG) immunoblot (Mikrogen®).

Results: We included 61 patients: 51 patients were treated with IVIg and 10 with dexamethasone. Of the patients treated with IVIg, 42 had CIDP (82%) and were treated with Nanogam® (Sanquin Plasma Products). Nine patients had myositis (18%) and were treated with Privigen® (CSL Behring). Anti-*Bbsl* IgG seroprevalence pre-treatment was 3% (2/61). Apparent seroconversion during IVIg treatment occurred in 39% (20/51) of patients, all treated with Nanogam. Post-treatment seroreversion occurred in 92% (12/13) of patients with available follow-up samples; in 78% (7/9) seroreversion was observed within 3 months.

Conclusions: Transient presence of anti-*Bbsl* IgG antibodies after IVIg is regularly observed. This effect appears to be dependent on the IVIg brand, probably reflecting variation in *Bbsl* exposure of plasma donors. Lyme borreliosis serological testing during, and weeks to months after, IVIg is therefore of limited utility.

KEYWORDS

apparent seroconversion, *Borrelia burgdorferi*, CIDP, intravenous immunoglobulins, IVIg, myositis

Ilse M. Lucke, Amber Vrijlandt, Joppe W. Hovius and Filip Eftimov contributed equally to this article.

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INTRODUCTION

Intravenous immunoglobulins (IVIg) are used in the treatment of inflammatory neuromuscular disorders, such as chronic inflammatory demyelinating polyneuropathy (CIDP) and myositis [1,2]. IVIg consist of pooled polyclonal immunoglobulin G (IgG) immunoglobulins from at least a thousand donors per batch, including antibodies directed towards different microorganisms that the donors have encountered such as *Borrelia burgdorferi* sensu lato (*Bbsl*) [3]. Because Lyme borreliosis is an important differential diagnosis in patients with CIDP, serological testing for *Bbsl* is frequently performed [4]. Lyme borreliosis is especially considered in CIDP patients without improvement or in deterioration of patients with Guillain Barre syndrome after start of IVIg treatment. Even though apparent seroconversion for anti-*Bbsl* antibodies after IVIg does not lead to any symptoms or illness in patients, it could lead to confusing results, delay in diagnosis or unnecessary antibiotic treatment.

The main objective of this study was to determine how often IVIg leads to the transient presence of anti-*Bbsl* antibodies in serum of CIDP and myositis patients.

METHODS

Patients and serum samples

In this cohort study we used consecutive serum samples collected from CIDP and myositis patients seen at our tertiary neuromuscular referral center. Samples were collected in the context of three studies: the Amsterdam UMC inflammatory neuromuscular diseases biobank, the International CIDP Outcome study, a prospective cohort study [5], and the IMMEDIATE study, a prospective cohort study investigating IVIg in myositis [2]. All studies were approved by the local ethics committee. All patients provided informed consent for storage and use of samples for future studies related to the disease and/or treatments. Patients were eligible if they were not treated with IVIg at baseline and were repeatedly treated with IVIg afterwards. CIDP controls were treated with dexamethasone only. After selection of patients, analysis was performed anonymously,

using leftover material from the above-mentioned biobank and study cohorts.

We analyzed samples from two different time points: pre-treatment and 6–12 weeks after initiation of treatment. In selected patients that showed apparent seroconversion, we analyzed, if available, follow-up samples up to 10 months post-treatment.

Treatment

Two different IVIg brands were used: Nanogam® (Sanquin Plasma Products, The Netherlands) and Privigen® (CSL Behring CSL Behring, King of Prussia, PA, USA). All patients received a loading dose of 2 g/kg followed by a maintenance dose of 1 g/kg every 3–4 weeks. Dexamethasone was given in a fixed scheme of 40 mg per day for 4 days every month, for 6 months [6].

Serological testing

Sera were analyzed according to a standard two-tier testing protocol, consisting of the C6 Lyme ELISA (Oxford Immunotec, Abingdon, Oxfordshire, UK) and, in the case of an equivocal or positive Lyme index, an IgM and IgG immunoblot (Mikrogen®). Cutoff values recommended by the manufacturers were used. Immunoblots were assessed by two independent researchers; in case of disagreement, a third opinion was decisive. Equivocal immunoblot results were considered negative and immunoblot results determined the final outcome. In addition, Nanogam and Privigen were tested by two-tier testing. Privigen was 1:2 diluted in dilution buffer, to equalize concentrations. Volumes used were those recommended for serum by the manufacturers.

Statistical methods

Statistical analysis was performed in SPSS (IBM SPSS Statistics 26) using a McNemar test. Statistical significance was determined as a *p* value <0.05.

TABLE 1 Two-tier *Borrelia burgdorferi* sensu lato testing of patients before and after intravenous immunoglobulin treatment

	IVIg n (%)						Dexamethasone n (%)	
	Total (n = 51)		Nanogam® (n = 42)		Privigen® (n = 9)		Total (n = 10)	
	C6 ELISA	Immunoblot	C6 ELISA	Immunoblot	C6 ELISA	Immunoblot	C6 ELISA	Immunoblot
	Seroprevalence ^a	4 (8)	2 (5)	3 (7)	2 (5)	1 (11)	0 (0)	0 (0)
Apparent seroconversion ^b	20 (39)	20 (39)	20 (48)	20 (48)	0 (0)	0 (0)	0 (0)	0 (0)

Abbreviations: *Bbsl*, *Borrelia burgdorferi* sensu lato; CIDP, chronic inflammatory demyelinating polyneuropathy; ELISA, enzyme-linked immunosorbent assay; IVIg, intravenous immunoglobulins.

^aPresence of anti-*Bbsl* antibodies before IVIg treatment.

^bPresence of anti-*Bbsl* antibodies 6–12 weeks after IVIg treatment.

RESULTS

A total of 61 patients were included: 52 CIDP and nine myositis patients. The median age was 64 (range 18–87) years and 38 (61%) patients were male. IVIg was administered to 51 patients: 42 patients had CIDP and received Nanogam, all myositis patients received Privigen. Dexamethasone was given to 10 CIDP patients. Patients were treated between 2015 and 2019.

Anti-*Bbsl* antibodies pre-treatment (seroprevalence) were found in 2/61 (3%) patients. Apparent seroconversion detected by the C6 enzyme-linked immunosorbent assay (ELISA) test occurred in 20 (39%) patients who were treated with IVIg. In all these patients seroconversion was confirmed by immunoblot (Table 1) ($p < 0.001$). All of these patients were treated with Nanogam, while apparent seroconversion occurred in none of the patients treated with Privigen. In addition, no apparent seroconversion was found in patients treated with dexamethasone.

The C6 Lyme index increased in 95% (40/42) of patients treated with Nanogam (Figure 1), reaching the cutoff (Lyme index 0.90) in 51% (20/39) of previously seronegative patients. In patients treated

with Privigen or dexamethasone, the C6 Lyme index did not increase or only marginally increased, with none reaching the cutoff. One Privigen-treated patient already had a positive C6 Lyme index before and during IVIg, without immunoblot confirmation, and was therefore considered seronegative.

One Nanogam-treated patient had also apparent seroconversion for IgM antibodies during IVIg. This patient had a negative C6 Lyme index pre-treatment; an additionally performed immunoblot was equivocal for IgM and negative for IgG. The follow-up sample of this patient was C6, IgM and IgG negative.

Follow-up samples post-treatment were available for 13 patients with apparent seroconversion. Median treatment duration was 4 (range 4–12) months. Twelve patients (92%) showed seroreversion. An early follow-up sample (1–3 months) after withdrawal of IVIg was available in nine patients; seven patients (78%) had seroreversion and two patients had an equivocal C6 Lyme index. Of these two patients, one had negative IgM and IgG immunoblots and the C6 Lyme index 8 months after withdrawal was negative. In the second patient the IgG immunoblot was positive and no further follow-up samples were available. In later follow-up samples

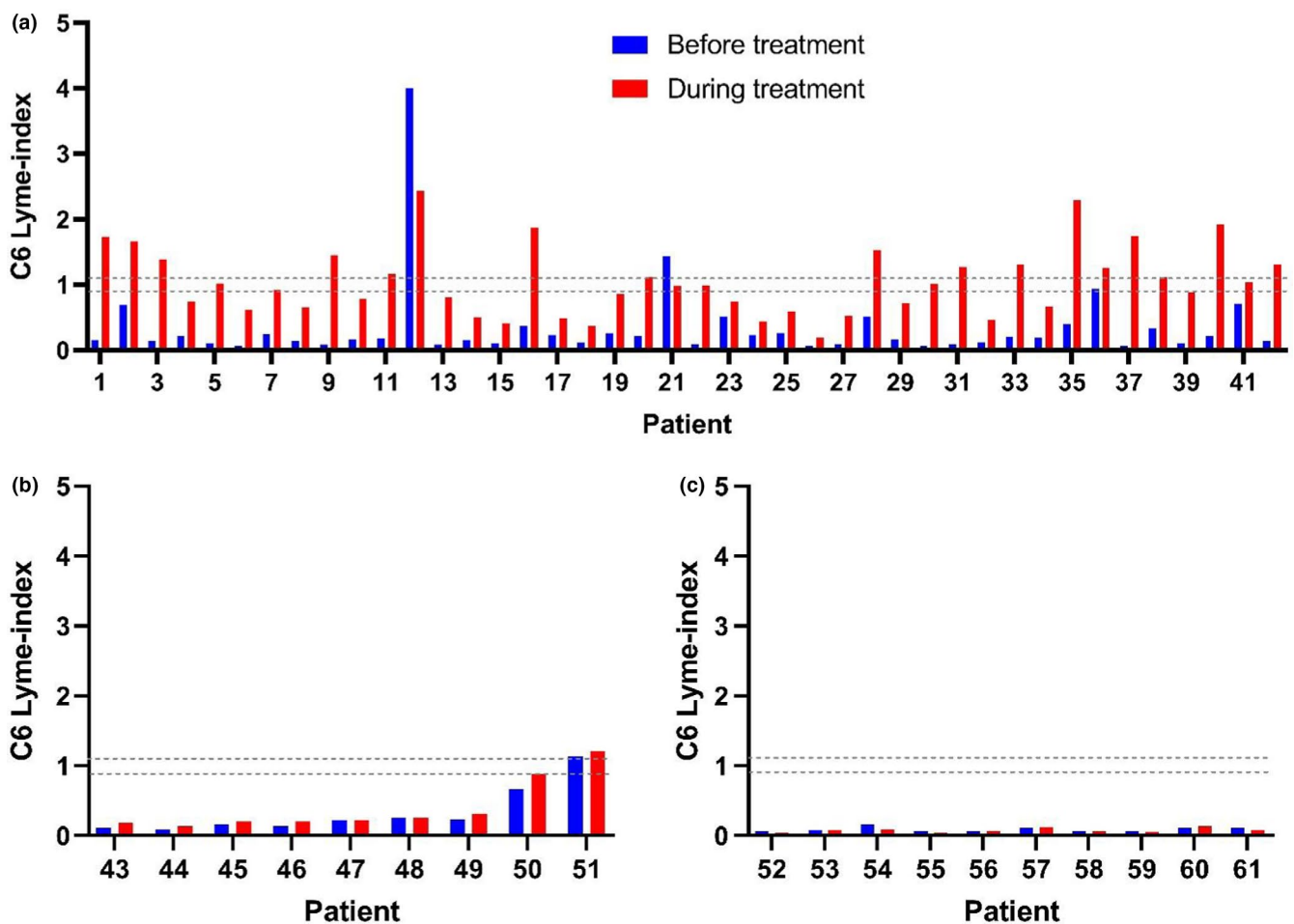


FIGURE 1 Quantitative C6 Lyme index in patients treated with intravenous immunoglobulin (IVIg) or dexamethasone. (a) Patients treated with Nanogam®. (b) Patients treated with Privigen®. (c) Patients treated with dexamethasone. Cutoff values recommended by the manufacturer are depicted by the dotted lines; C6 Lyme index ≤ 0.90 is considered a negative result, 0.90–1.09 an equivocal result and ≥ 1.10 a positive result [Colour figure can be viewed at wileyonlinelibrary.com]

no equivocal or positive C6 Lyme indexes were found. The C6 Lyme index in these patients pre-, during and post-IVIg are shown in Figure S1 ,

Direct testing of IVIg showed a positive C6 Lyme index (3.25) for Nanogam and a negative C6-index (0.50) for Privigen. The immunoblot performed on Nanogam was highly positive for anti-*Bbsl* IgG antibodies and negative for IgM.

DISCUSSION

In this study we observed apparent seroconversion for anti-*Bbsl* antibodies in 48% of patients treated with Nanogam. None of the patients treated with Privigen or dexamethasone showed apparent seroconversion. Interestingly, antibodies disappeared in 92% of patients during follow-up after IVIg withdrawal.

We found an anti-*Bbsl* antibody seroprevalence of 3%, comparable with the Dutch population (4%–8%) [7]. However, the prevalence of anti-*Bbsl* antibodies depends on geographical region [8], possibly explaining the difference in apparent seroconversion between patients receiving Nanogam, a Dutch product, and Privigen, produced in either the USA or Germany. The finding that the C6 Lyme index was positive in Nanogam and negative in Privigen, is in accordance with previous findings [3].

Interestingly, one patient experienced apparent seroconversion for both IgM and IgG antibodies during IVIg treatment (Nanogam). IVIg consists of at least 95% of IgG antibodies, and a very small proportion of IgA antibodies (CSL Behring prescribing information on Privigen; Sanquin product information on Nanogam). We cannot exclude the presence of a minimal fraction of IgM, although anti-*Bbsl* IgM antibodies were not demonstrated in the Nanogam batch we tested. Alternatively, the finding in this particular patient that both IgM and IgG were negative during follow-up, without antibiotic treatment, makes Lyme borreliosis unlikely.

All but one patient with 'IVIg-induced apparent seroconversion' returned to a seronegative state within a few months post-treatment. In this patient, anti-*Bbsl* IgG antibodies were still detectable by immunoblot 8 weeks post-treatment. This could most likely be explained by the finding that IVIg metabolism differs greatly between patients, with a half-life ranging between 18 and 32 days [9]. Unfortunately, no later follow-up samples were available for this patient. Alternatively, albeit less likely, this could have reflected interim exposure to *Bbsl*.

The strengths of this study include the relatively large sample size. Samples from at least two time points were available for all patients and two different types of IVIg were used. A limitation is that not all follow-up samples were taken at the same time point. Therefore, we are not able to calculate when serological testing for Lyme borreliosis would be reliable again after IVIg treatment. Unfortunately, we did not test different batches of Nanogam to see if apparent seroconversion was batch-related. The batch numbers of the Nanogam the patients were treated with were not available. Patients were treated over a period of 5 years, meaning that

different batches were used. We assume it is not exceptional for a batch of Nanogam to contain anti-*Bbsl* antibodies, given the high seroprevalence of anti-*Bbsl* antibodies in the Dutch population and the high number of different donor immunoglobulins used in one batch of IVIg.

In conclusion, we show that IVIg can lead to transient presence of anti-*Bbsl* antibodies. Therefore, when patients received IVIg produced in a Lyme borreliosis endemic region, clinicians should be careful interpreting the results of *Bbsl* serological assays. When Lyme borreliosis is part of the differential diagnosis, it would be highly recommendable to test for antibodies either before or several months after IVIg administration.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Ilse M. Lucke: Conceptualization (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Amber Vrijlandt:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing-original draft (equal); Writing-review & editing (equal). **Johan Lim:** Data curation (equal); Writing-review & editing (equal). **Anneke J. van der Kooi:** Supervision (equal); Writing-review & editing (equal). **Ivo N. van Schaik:** Supervision (equal); Writing-review & editing (equal). **Hans L Zaaier:** Conceptualization (equal); Writing-review & editing (equal). **Joppe W. Hovius:** Conceptualization (equal); Methodology (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal). **Filip Eftimov:** Conceptualization (equal); Investigation (equal); Methodology

(equal); Resources (equal); Supervision (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

All data obtained by this study are published in this article and are available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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