Transmission of Hepatitis B core Antibody and Galactomannan Enzyme Immunoassay positivity via immunoglobulin products: a comprehensive analysis

Isobel Ramsay[1,2], Rebecca L Gorton[3], Mauli Patel[4], Sarita Workman[5], Andrew Symes[5], Tanzina Haque[1], Dianne Irish[1], Suranjith L Seneviratne[5,6], Siobhan O Burns[5,6], Emmanuel Wey[7], David M Lowe[5,6]

[1] Department of Virology, Royal Free London NHS Foundation Trust, London, UK

[2] Department of Microbiology, Addenbrooke's Hospital NHS Foundation Trust, Cambridge, UK

[3] Department of Microbiology, Health Services Laboratory (HSL), Royal Free Hospital, London, UK

[4] Department of Virology, Health Services Laboratory (HSL), Royal Free Hospital, London, UK

[5] Department of Clinical Immunology, Royal Free London NHS Foundation Trust, London, UK

[6] Institute of Immunity and Transplantation, University College London, Royal Free Campus, London, UK

[7] Department of Microbiology, Royal Free London NHS Foundation Trust, London, UK

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Corresponding author: Dr David Lowe

Institute of Immunity and Transplantation, University College London, Royal Free Campus, London, NW3 2QG, UK

d.lowe@ucl.ac.uk

Summary: Therapeutic immunoglobulins can transmit clinically important antibodies, including those directed against Hepatitis B virus (HBV), and antigens producing false-positive results for galactomannan EIA. We demonstrate that immunoglobulin administration commonly leads to positive HBV core antibody and galactomannan but is product-dependent.

Abstract

Background: Therapeutic immunoglobulins are used as replacement or immunomodulatory therapy, but can transmit clinically important molecules. We investigated Hepatitis B virus (HBV) antibodies and galactomannan enzyme immunoassay (GM-EIA) positivity. Detection of HBV core antibody may prompt antiviral prophylaxis when commencing therapy such as rituximab; a positive GM-EIA result prompts investigation or treatment for invasive fungal disease.

Methods: Cross-sectional analysis of HBV serology in 80 patients established (>6 months) on immunoglobulin therapy; prospective analysis of HBV serology in 16 patients commencing intravenous immunoglobulin (IVIG); pre- and post-infusion analysis of GM-EIA in 37 patients receiving IVIG.

Results: Pre-IVIG, 9/80 patients tested positive for HBV surface antibody and 1/80 tested equivocal for HBV core antibody. On IVIG, 79/79 tested positive for surface antibody, 37/80 tested positive for core antibody and 10/80 tested equivocal for core antibody. There were significant differences by product, but among patients receiving products which appear to transmit core antibody, negative results correlated with lower surface antibody titres and longer time since infusion suggesting a simple concentration effect. There was a progressive increase with each infusion in the percentage of patients testing positive for HBV core antibody among patients newly commencing IVIG. Some patients 'sero-reverted' to negative during therapy. Certain IVIG products tested positive for GM-EIA and there were rises in index values in corresponding patient samples from pre- to post-infusion. Overall, 5/37 patient samples pre-infusion and 15/37 samples post-infusion tested positive for GM-EIA.

Conclusions: HBV antibodies and GM-EIA positivity are common in patients receiving IVIG and confound diagnostic results.

Introduction

Intravenous immunoglobulin (IVIG) and subcutaneous immunoglobulin (SCIG) are therapeutic antibody products derived from the pooled plasma of donors. Indications for use are as replacement therapy in antibody-deficiency syndromes (including 'primary' disorders such as Common Variable Immunodeficiency (CVID) and 'secondary' to other illnesses and medications, including haematological malignancy) or, in higher doses, as immunomodulatory treatment for autoimmune and autoinflammatory conditions (for example idiopathic thrombocytopenic purpura [1]).

These products may contain clinically important antibodies which the recipient did not previously produce. Although IgG serology is not generally used to diagnose acute or active infection, there are situations where evidence of past infection significantly alters clinical management. Most importantly, antibodies against Hepatitis B virus (HBV) are measured when commencing certain immunosuppressive treatments (especially the anti-CD20 monoclonal antibody rituximab) due to the risk of HBV reactivation; a positive result in this context would prompt commencement of prophylactic antiviral medication. Two major anti-HBV antibodies are measured: surface antibody (sAb), which is generated in response to HBV vaccination, and core antibody (cAb), interpreted as a marker of current or past HBV infection [2].

It has been suggested that HBV antibodies are transmitted to recipients via IVIG [3-5]. However, these descriptions are limited to case reports plus one series of 11 patients [4] and the issue has never been systematically studied: in particular, the rate of transmission,

time to seroconversion, whether patients sero-revert to negative while receiving IVIG treatment, differences according to infused product and the overall prevalence of these antibodies in immunoglobulin-treated patients are unknown. Consequently, physicians remain unaware of this issue, with recent case reports describing the phenomenon as a novel finding [3,5].

Another diagnostic test which may be confounded by immunoglobulin treatment is the galactomannan (GM) antigen enzyme immunoassay (EIA). Galactomannan is a component of the cell wall of *Aspergillus* spp. and several other clinically important fungi. The GM-EIA is used largely as a screening test for early detection of invasive aspergillosis (IA) in at-risk patients, especially those with neutropenia or undergoing stem cell transplant [6]. It has been suggested in one abstract that IVIG products and their stabilisers may yield false-positive results using the GM-EIA [7]. However, this phenomenon has also not been systematically evaluated.

We therefore undertook studies to address these deficiencies in the literature: a cross-sectional study of HBV antibody prevalence in a cohort of patients receiving immunoglobulin and prospective studies to evaluate the kinetics of acquisition of both HBV antibodies and positive GM-EIA results.

Materials and Methods

Patients

For the cross-sectional study, patients were eligible if established on IVIG or SCIG treatment (>6 months), had baseline serum stored pre-IVIG and had not received IVIG within 6 months

before starting at the Royal Free Hospital, London, UK. For the prospective study of HBV antibody transmission, all patients newly commencing IVIG at our centre were eligible. For the prospective study of GM-EIA positivity, all patients established on IVIG were eligible. Most patients were receiving replacement doses of immunoglobulin for antibody deficiency syndromes; a minority were receiving higher doses, as detailed later.

Ethics

All patients provided written informed consent to use their blood samples for research (NHS Research Ethics Committee reference 04/Q0501/119).

Samples and data collection

Serum was derived from serum separation tubes (SST) or plain serum tubes (BD Vacutainer®). For the cross-sectional study, serum was collected at clinic visits, infusion visits or from submitted 'IgG trough' samples; these were analysed for HBV cAb, sAb and surface antigen (sAg) and for Hepatitis C virus (HCV) IgG. We recorded the current product being received and, for intravenous products, date of the last infusion. Patients on subcutaneous products were assumed to have infused within the last week.

For the prospective study of HBV antibody transmission, serum was collected before the first infusion and before each subsequent infusion up to a total of 5 or 6 infusions; samples were analysed for HBV and HCV antibodies and 'liver function tests' (bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase). Patients were asked about intercurrent jaundice or hepatitis at each visit. For the study of IVIG-associated GM-EIA

positivity, serum was collected before and after individual infusions. Immunoglobulin products were also analysed directly.

Laboratory tests

Viral serology and GM-EIA tests on serum were performed in NHS laboratories (full details available as Supplementary Methods). Immunoglobulin products were also assayed by GM-EIA, pan-fungal PCR and Aspergillus PCR by the Bristol Public Health England Mycology reference laboratory. 100µl aliquots were cultured on Sabouraud agar plates and incubated at 30^{o}C for three weeks.

Statistics

Two groups (non-parametric data) were compared with Mann-Whitney tests; three or more groups were compared with Kruskal-Wallis tests and post-hoc Dunn's correction.

Results

A high proportion of patients on immunoglobulin therapy test positive for HBV core antibody

Characteristics of 80 patients included for cross-sectional analysis are presented in Table 1. Three patients were receiving high-dose (≥1 g/kg/month) immunoglobulin therapy; all others were receiving replacement doses (0.4–0.6 g/kg/month). Prescribed infusion frequency was every 3 or 4 weeks for intravenous and weekly for subcutaneous preparations. Most tests were performed immediately before infusions (i.e. at IgG trough).

Results of HBV serological testing are presented in Figure 1. No patients tested positive for HBV sAg or HCV IgG at either time point. At baseline, no patients tested positive for HBV cAb although one tested 'equivocal' (notably, this patient had received high-dose IVIG approximately 18 months before commencing maintenance doses) and 9/80 (11.3%) patients tested positive for sAb. When re-tested after at least 6 months of immunoglobulin replacement, 37/80 (46.3%) patients tested positive for cAb, 10/80 (12.5%) equivocal and 33/80 (41.3%) negative. 79/79 patients (100%; one not tested due to insufficient sample) tested positive for sAb.

Positive results for HBV cAb are predicted by product, concentration of HBV sAb and time since infusion for intravenous preparations

Differences in rates of cAb positivity existed according to product administered (Figure 2A). In particular, no patients on Intratect® (n=9) or Kiovig® (n=11) tested equivocal or positive for cAb. Patients on Subcuvia® also tested negative for cAb although we interpret this result cautiously in view of low numbers (n=2).

To explore why transmission of cAb was not universal, we analysed sAb titres and time from previous infusion. Patients on Kiovig® and Intratect® had similar or higher levels of sAb (Figure 2B) versus other products (significant difference between Intratect® (median [IQR] 393 [335–569] IU/ml) and Gammaplex® (median [IQR] 168 [118–209] IU/ml), $p<0.01$)).

However, restricting analysis to products which appear to transmit cAb, lower sAb titres were associated with negative cAb tests. As demonstrated for Privigen® (the most commonly represented product), sAb levels in samples testing negative for cAb were lower

than in those testing positive (median [IQR] 136 [94-143] IU/ml vs 260 [225-333] IU/ml, p<0.01) with a possible threshold for cAb positivity in the region of 200 IU/ml sAb titre (Figure 2C). Consistent with passive transfer, sAb titres correlated negatively with time since infusion (Pearson r -0.79, p=0.0001; Supplementary Figure S1). Correspondingly, all negative cAb results from patients receiving Flebogamma® DIF, Gammaplex®, Octagam® and Privigen® were taken at least 27 days since the last infusion (Figure 2D) and there was a significant difference in median days since infusion between patients testing positive (median [IQR] 21 [15–28] days) vs those testing equivocal (28 [27–34]), p<0.05) or negative (28 [28–33], p<0.001). There was no difference in median [IQR] days since infusion for patients on Kiovig® and Intratect® (28 [21.5–28]) vs patients on other products (27 [19–28], p=0.40). Patients on high-dose IVIG were excluded from these secondary analyses.

Acquisition of HBV core antibody occurs over several infusions and patient samples can revert to negative during treatment

We investigated the transmission of HBV antibodies prospectively in 16 patients commencing IVIG, taking samples immediately before each infusion. Table 2 details patient characteristics. During the study, two patients changed to subcutaneous therapy (one after a single infusion and one after three infusions). A further patient transferred hospital after three infusions and another discontinued therapy after 2 infusions; immunoglobulin products were changed in two patients (Supplementary Figure S2).

No patients tested positive for HBV sAg or HCV IgG at any time point or exhibited biochemical or clinical evidence of acute hepatitis. At baseline, no patients tested positive for HBV cAb and 4/16 (25%) tested positive for HBV sAb.

All patients became positive for sAb after a single infusion. Conversely, no patients demonstrated clear positive cAb after a single infusion (Figure 2E) and the percentage of equivocal or positive results increased with serial infusions. Some patients reverted to negative from positive or equivocal results (product changes excluded; Supplementary Figure S3). Two patients were tested in between infusions: one yielded a positive cAb and the other an equivocal result, but in both cases the preceding and subsequent 'IgG trough' results were negative. As before, no patients on Intratect® or Kiovig® tested positive for HBV cAb.

Several Immunoglobulin products test positive by GM-EIA and patients can convert from negative to positive GM-EIA results after a single IVIG infusion

Aliquots of immunoglobulin products were tested by GM-EIA yielding positive results for all except Octagam® and Privigen® (Table 3). Cultures on Sabouraud agar plates did not reveal any fungal growth after 21 days incubation; PCR for *Aspergillus spp*. and pan-fungal DNA were negative.

To investigate the impact of immunoglobulin administration on serum GM-EIA results, we recruited further patients (on maintenance doses of IVIG) and tested serum pre and post infusion. Results are shown in Table 3 and Figure 3. IVIG products which tested negative by GM-EIA (index value <0.5: Privigen®, Octagam®) did not significantly affect serum values from pre to post infusion (median [IQR] GM-EIA index 0.26 [0.21-0.42] vs 0.29 [0.21-0.41], p=1.0; Figure 3B) and almost all samples tested negative (except one borderline-positive, index =0.503). For products testing positive by GM-EIA, there was an increase in patient serum results from pre to post infusion (median [IQR] GM-EIA index 0.22 [0.17-0.38] vs 0.52

[0.26-1.05], p=0.002). However, although Gammaplex® IVIG tested positive, GM-EIA index 1.27, samples from patients receiving this product were not positive and a significant increase in levels was not observed post infusion (p=0.55, Table 3). The highest index values were observed in patients receiving Kiovig® and Intratect®, consistent with the high GM-EIA results observed in these products (Table 3).

Across all products, 5/37 (13.5%) of serum samples tested positive pre-infusion and 15/37 (40.5%) tested positive post-infusion; the increase in GM-EIA index values was significant (p=0.006).

Discussion

This study has revealed important results which affect the interpretation of diagnostic tests in patients on immunoglobulin treatment. Specifically, we found that 46.3% of patients receiving immunoglobulins (generally at replacement doses) test positive for HBV cAb when sampled cross-sectionally and that immediately after infusion 40.5% of patients test 'positive' for galactomannan.

Detection of HBV cAb can lead to patients being told that they have evidence of past infection with a sexually transmitted virus, prompting anxiety and testing of sexual partners. Furthermore, these patient populations have a high probability of requiring treatment with rituximab (and probably newer similar monoclonal antibodies). Rituximab shares many indications with IVIG, including autoimmune and autoinflammatory conditions [8,9], while patients with primary antibody deficiency syndromes frequently suffer autoimmune

cytopenias requiring rituximab treatment; they are also at high risk of lymphoma, for which rituximab is a common therapy [10].

Since rituximab therapy can reactivate even apparently 'cleared' infections with HBV, patients with serological evidence of previous infection (i.e. positive cAb) are recommended to receive antiviral prophylaxis during and after treatment, with monitoring of HBV DNA [11-13]. Patients with 'false-positive' cAb results from receiving IVIG might therefore receive unnecessary antiviral prophylaxis (which can potentially confer harmful side effects) and needless monitoring.

Acquisition of HBV sAb was universal, albeit with significant variation in titre. This variation is potentially informative, since the presence of cAb correlated with higher sAb titre and correspondingly shorter time since infusion. Overall this suggests concentration- or dose-dependent cAb transmission. This may also help to explain differences between products. For example, neat Kiovig® has been reported to test positive for cAb [5] but no patients in our cohort receiving Kiovig® yielded positive results. We hypothesise that this reflects lower cAb concentration in this product and thus a higher dose threshold for transmission. Importantly, it may still be possible to acquire false-positive cAb results from Kiovig® (and perhaps Intratect®) if receiving high-dose IVIG and tested soon after infusion.

Dose-dependent acquisition of cAb was also supported by our prospective data, since patients required several infusions before a positive result was detected. We cannot exclude an additional 'batch effect' in the transmission of cAb. Indeed, we noted reversion of seropositivity (in itself strong evidence for passive antibody transfer) and that the relationship between sAb titre and cAb positivity was less clear for Flebogamma® DIF. However, overall the evidence for a batch effect is weaker than for a simple dose effect. In

particular, universal acquisition of HBV sAb after a single infusion suggests that heterogeneity of IgG content in the products is limited.

A false-positive GM-EIA result is also potentially significant for patients. In appropriate circumstances, such as prolonged neutropenia, a positive GM-EIA result is interpreted as an early biomarker of invasive aspergillosis or other fungal infection [6]. Patients may therefore undergo investigations including computed tomography (CT) scans or bronchoscopy; they may even be commenced on antifungal therapy. As a stand-alone test the specificity and positive predictive value of the GM-EIA are sub-optimal [14], and other products including piperacillin-tazobactam can lead to 'false-positives' [6]. However, clinicians must also recognise IVIG as a potential confounder.

Serum GM-EIA index values demonstrated distinct rises from pre to post infusion in patients receiving relevant products. This often converted the result from 'negative' to 'positive' when applying the manufacturers' threshold of >0.5 to define a positive result. As with transmission of HBV cAb, there were clear relationships with IVIG product. However, this did not seem to reflect solely a dose effect since two neat products (Octagam® and Privigen®) tested negative and no patients receiving these products acquired false-positive GM-EIA results.

The cause of the GM-EIA positivity is unclear. There was no evidence of fungal growth or PCR positivity in the products, excluding gross fungal contamination. The stabilisers used in products testing positive are not known to confound the assay, and we did not recapitulate the previous finding that only sorbitol-stabilised products were positive [7]. Glycine was a common excipient, but this was used as a buffer (and negative control) supplied with the original BioRad *Pastorex* latex agglutination galactomannan assay: it thus should not be the

source of positive GM-EIA results. We note that the results for Flebogamma® DIF 10% were proportionally higher than for Flebogamma® DIF 5%, suggesting that the strength of GM-EIA positivity correlates directly with the immunoglobulin component. This observation was not so clear in Intratect® 5% and 10% preparations, probably because the level was so high it saturated the assay's upper detection limit. We hypothesise that a manufacturing process leads to positive GM-EIA results, either via true galactomannan antigen or a cross-reacting molecule; further analyses are underway.

Our study has limitations. Numbers of patients on some subcutaneous products were small. We cannot definitively prove passive HBV cAb acquisition in the cross-sectional study, but with an annual incidence of HBV infection of 2 per 100,000 in London [15], true infection seems extremely unlikely. In the prospective study, absence of surface antigen and documented sero-reversion confirms passive antibody transfer. We have not tested other clinically important markers which can be transmitted via IVIG such as syphilis antibodies [16] or (1,3)-β-D-glucan [17].

In conclusion, we have demonstrated significant transmission of HBV cAb and induction of GM-EIA positivity from immunoglobulin preparations. We recommend measuring baseline HBV cAb when commencing immunoglobulin therapy. If negative, then in the absence of intercurrent hepatitis or risk factors any future positive results in the context of ongoing immunoglobulin therapy should be interpreted as 'false-positives' and antiviral treatment should not be instituted, even if rituximab therapy is contemplated. HBV sAg or HBV DNA could be checked if disturbance of liver function tests occurs. More caution should be applied in patients receiving Intratect® or Kiovig®, where any positive results should be investigated further with HBV DNA, liver function tests and repeat serology at nadir

immunoglobulin levels as long as possible after the last infusion. In order to retain use of the GM-EIA assay in patients receiving immunoglobulin products, a baseline serum level should be performed before the infusion, an aliquot of the IVIG should be submitted for GM-EIA analysis and any positive serum results post-infusion should be interpreted in light of the pre-infusion and neat product results.

Potential conflicts of interest: AS is on an advisory board for Baxalta and has received grant funding from CSL Behring. SW has received support to attend a conference and an honorarium for speaking from LFB pharmaceuticals, plus support to attend conferences from Baxalta, CSL Behring, Grifols, BPL, Octapharma and Biotest (UK). SOB has received an honorarium for speaking from CSL Behring, and has received support to attend conferences from Immunodeficiency Canada/IAACI, CSL Behring and Baxalta US Inc. DML has received support to attend a conference and has participated in an advisory board for Biotest (UK). All other authors declare no conflicts of interest.

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Table 1. Details of patient cohort recruited for cross-sectional study of Hepatitis B serology. CVID = Common Variable Immunodeficiency; Ig = Immunoglobulin; SPAD = Specific Antibody

Age (years)	Mean	57
	Range	21 – 91
Sex (n, %)	Male	19 (23.8)
	Female	61 (76.3)
Product / manufacturer (n, %)	Flebogamma® DIF / Grifols	13 (16.3)
	Gammanorm® / Octapharma	3 (3.8)
	Gammaplex® / BPL	11 (13.8)
	Hizentra® / CSL Behring	3 (3.8)
	Intratect® / Biotest	9 (11.3)
	Kiovig® / Baxalta	11 (13.8)
	Octagam® / Octapharma	8 (10.0)
	Privigen® / CSL Behring	18 (22.5)
	Subcuvia® / Baxalta	2 (2.5)
	Subgam® / BPL	2 (2.5)
Underlying diagnosis (n, %)	CVID	29 (36.3)
	Probable CVID	3 (3.8)
	IgA deficiency + SPAD	6 (7.5)
	Low IgG +SPAD	9 (11.3)
	IgG1 subclass deficiency	5 (6.3)
	Lymphoma (+/- RTX)	6 (7.5)
	Myeloma or MGUS	4 (5.0)
	CLL / Monoclonal B lymphocytosis	2 (2.5)
	Rheumatoid or vasculitis (+/- RTX)	7 (8.8)
	Other	9 (11.3)
Duration of immunoglobulin replacement since commencement (months)	Median	24.3
	Range	6.5 – 132
Interval between last infusion and study sample (IV products only; days)	Median	27
	Range	1 – 56

Deficiency; RTX = Rituximab; MGUS = Monoclonal gammopathy of uncertain significance; CLL = Chronic lymphocytic leukaemia; IV = intravenous.

Table 2. Details of patients recruited for prospective study of Hepatitis B antibody transmission via intravenous immunoglobulin.

Age (years)	Mean	52
	Range	19 – 80
Sex (n, %)	Male	3 (18.8%)
	Female	13 (81.3%)
Initial product (n, %)	Flebogamma®	3 (18.8%)
	Intratect®	2 (12.5%)
	Kiovig®	3 (18.8%)
	Octagam®	3 (18.8%)
	Privigen®	5 (31.3%)
Underlying diagnosis (n, %)	CVID	3 (18.8%)
	Probable CVID	2 (12.5%)
	Lymphoma (+/- RTX)	3 (18.8%)
	CLL / Monoclonal B lymphocytosis	2 (12.5%)
	Rheumatoid or vasculitis (+/- RTX)	4 (25%)
	Other	2 (12.5%)
History of Hepatitis B infection	Yes	0 (0%)
	Unsure	1 (6.3%)
	No	15 (93.8%)
History of Hepatitis B vaccination	Yes	3 (18.8%)
	Unsure	6 (37.5%)
	No	7 (43.8%)
History of Hepatitis C infection	Yes	0 (0%)
	Unsure	1 (6.3%)
	No	15 (93.8%)
History of jaundice	Yes	2 (12.5%)
	Unsure	0 (0%)
	No	14 (87.5%)

CVID = Common Variable Immunodeficiency; RTX = Rituximab; CLL = Chronic lymphocytic leukaemia; IV = intravenous.

Table 3. Galactomannan EIA in IVIG products and patient samples pre- and post-infusion.

IVIG Product	Excipients	Galactomannan (GM) EIA index (neat product)	Median (range) GM EIA index in patient samples immediately pre-infusion	Median (range) GM EIA index in patient samples immediately pre-infusion	Number of patients tested
Flebogamma®	D-Sorbitol	1.17 (5% product)	0.16 (0.11 – 0.62)	0.25 (0.11 – 0.75)	5
	Water	2.53 (10% product)	0.24 (0.20 – 0.32)	0.49 (0.32 – 0.60)	5
Gammaplex®	D-Sorbitol Glycine Sodium Chloride Acetate Polysorbate 80	1.27	0.20 (0.15 – 0.36)	0.18 (0.13 – 0.26)	5
Intratect®	Glycine	4.43 (5% product)	0.49 (0.19 – 1.05)	1.47 (0.29 – 3.43)	4
	Water	5.53 (10% product)	0.20 (0.11 – 0.22)	1.27 (0.97 – 2.06)	4
Kiovig®	Glycine Water	5.25	0.41 (0.09 – 0.55)	0.93 (0.35 – 1.59)	5
Octagam®	Maltose Water	<0.4 (10% product)	0.25 (0.21 – 0.44)	0.29 (0.18 – 0.41)	5
Privigen®	L-proline Water	<0.4	0.30 (0.15 – 0.47)	0.28 (0.12 – 0.50)	4

EIA = Enzyme immunoassay; GM = Galactomannan

Figure Legends

Figure 1. Transmission of Hepatitis B antibodies via immunoglobulin is common. Results (n, %) for Hepatitis B serology are presented from 80 patients established on IVIG/SCIG treatment for at least 6 months, (A) HBV surface antibody (sAb) pre-IVIG/SCIG treatment, (B) HBV sAb on IVIG/SCIG treatment, (C) HBV core antibody (cAb) pre-IVIG/SCIG treatment, (B) HBV cAb on IVIG/SCIG treatment. White = negative, dark grey = equivocal, black = positive, light grey = unknown.

Figure 2. Positive results for Hepatitis B core antibody are predicted by product, concentration of sAb, time since infusion and number of infusions for intravenous preparations. A. Results (n) are presented for Hepatitis B cAb from 80 patients established on IVIG/SCIG treatment for at least 6 months according to product infused. White = negative, grey = equivocal, black = positive. B. Hepatitis B sAb titres are presented according to product infused for 77 patients established on IVIG/SCIG treatment (patients on high-dose treatment excluded) Lines represent medians. ** $p<0.01$, Kruskal-Wallis test with Dunn's correction. C. Hepatitis B sAb titres are presented according to Hepatitis B cAb result for patients established on replacement-dose Privigen® treatment (n=17). ** $p<0.01$, Kruskal-Wallis test with Dunn's correction. D. Days since infusion are plotted according to Hepatitis B cAb result (n=77). Left of dotted line: Flebogamma® DIF, Gammaplex®, Octagam®, Privigen®; right of dotted line: Intratect®, Kiovig®. * $p<0.05$, *** $p<0.001$, Kruskal-Wallis test with Dunn's correction. E. Results (%) are presented for Hepatitis B cAb from 16 patients commencing IVIG treatment, according to infusion number (0 = pre-IVIG). White = negative, grey = equivocal, black = positive. N per infusion number: 0 = 16, 1 = 15, 2 = 15, 3 = 13, 4 = 12, 5 = 12.

Figure 3. Change in galactomannan titre with intravenous immunoglobulin infusions. A. Serum from 28 patients receiving IVIG products whose neat contents tested positive (> 0.5) for GM EIA (Flebogamma® DIF, Gammaplex®, Intratect®, Kiovig®) was tested immediately before and after infusion using the BioRad Galactomannan Immunoassay. Lines represent medians. The dotted line represents the threshold for a patient's result to be declared 'positive'. ** $p<0.01$, Mann-Whitney test. B. Serum from 9 patients receiving IVIG products whose neat contents tested negative (> 0.5) for GM EIA (Octagam®, Privigen®) was tested immediately before and after infusion using the BioRad Galactomannan Immunoassay. Lines represent medians. The dotted line represents the threshold for a patient's result to be declared 'positive'.