False interpretation of diagnostic serology tests for patients treated with pooled human immunoglobulin G infusions: a trap for the unwary

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ABSTRACT

Therapeutic immunoglobulin G (IgG) products are produced from numerous plasma donations, and are infused in many medical conditions. The serological testing of patients who have received IgG infusions may well produce falsely positive and misleading results from this infused IgG, rather than endogenously produced IgG. We present two example cases of clinical situations where this could cause concern. We tested multiple IgG products with a range of serological tests performed in infective or autoimmune conditions, including hepatitis B, syphilis, human immunodeficiency virus, human T-lymphotropic virus, anti-neutrophil cytoplasmic antibody (ANCA), anti-nuclear antibody (ANA), anti-cardiolipin antibodies and anti-double stranded DNA (dsDNA) antibody. We found positivity within these products for hepatitis B surface and core antibody, syphilis, ANCA, ANA, anti-cardiolipin IgG and dsDNA antibody, which may result from specific or non-specific reactivity. The serological testing of patients who have received IgG treatment detects the administered IgG in addition to IgG produced by the patient.

KEYWORDS: Serology, antibody, immunoglobulin, false positive

Introduction

Therapeutic immunoglobulin G (IgG) infusions are used in a wide variety of inflammatory, infective and immunodeficiency disorders, and contain only trace amounts of IgA and IgM. Large doses (eg 2 g/kg) are used in the context of immunomodulatory therapy and the half-life of IgG is several weeks. IgG products are prepared from a large number of human plasma donations (eg 100–60,000 donations). Many routine laboratory tests for infective or autoimmune conditions are reliant on serological methods of testing for binding of specific IgG to a particular antigen. Serology results obtained from patients treated with pooled IgG will reflect both infused IgG antibodies contained in the administered IgG as well as endogenously produced IgG from the patient. IgG antibody tests on such patients within six weeks of IgG administration are generally unhelpful and sometimes dangerously misleading. We present two example cases below.

Example cases

Case 1

A 34 year-old female patient was referred to gastroenterology by rheumatology with an unexplained raised alanine aminotransferase (ALT) of 200 U/l (normal range 5–40 U/l). This patient had antibody deficiency secondary to gold therapy and multiple other disease modifying immunosuppressive agents for rheumatoid arthritis. Increased bacterial infections justified anti-infective prophylaxis including intravenous IgG (Privigen 20 g every 3 weeks). Her IgG on this treatment was usually within the normal range, but her IgM and IgA were very low, indicating that she was unlikely to be producing much endogenous antibody. Investigations for the cause of this hepatitis included serology for infectious hepatitis (Roche Cobas e 411 method) and revealed:

> IgG hepatitis B surface antibody strongly positive >100 mIU/ml
> IgG/IgM hepatitis B core antibody positive
> hepatitis B surface antigen negative.

The virology laboratory report read: 'Antibody to hepatitis B due to naturally acquired past infection. At risk of reactivation if immunocompromised. Consider discussion with viral hepatitis service.'

In this immunocompromised context this raised concern, including the possibility of reactivation of hepatitis B disease in the future. Hepatitis B virus (HBV) nucleic acid testing by polymerase chain reaction was negative. A liver biopsy

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showed non-diagnostic inflammatory changes in the portal tracts and parenchyma, associated with mild steatosis and a modest periportal fibrosis. The histopathologist could not exclude hepatitis B as a cause of these changes. Subsequent multidisciplinary discussions involving the immunology team led to the conclusion that active hepatitis B was very unlikely. Pooled normal human IgG will contain hepatitis B surface antibody. The positive core antibody test may well be the result of previously cleared hepatitis B infection in donors.

Case 2

A 32-year-old female patient on treatment for antibody deficiency with replacement intravenous IgG (Vigam 30 g every 2 weeks) became pregnant. As part of the routine screening for her pregnancy she was tested for syphilis, yielding the following profile:

> syphilis rapid plasma reagin (RPR) negative
> syphilis treponema pallidum particle aggulutination (TPPA) negative
> syphilis IgM enzyme immunoassay (EIA) negative
> syphilis total antibody EIA positive
> syphilis IgG immunoblot indeterminate.

Syphilis serology was repeated on a further sample and confirmed the original findings. The obstetric team were understandably concerned by the results, which were relayed to the patient. On discussion between the treating immunologist and virologist, the results were thought to represent false positives secondary to the IgG replacement therapy. Treatment was considered and discussed at length with the patient, and it was agreed that further antibiotics were not necessary over and above those previously received to treat confirmed infections associated with the antibody deficiency.

We suspected that the positive serological results in the example cases were the result of reactivity from administered IgG. Plasma donors for pooled human IgG are tested for antibodies to HIV, syphilis and hepatitis C virus (HCV); HBV surface antigen; and the viral nucleic acids of HIV, HBV, HCV, hepatitis A and parvovirus B19. Individual plasma units, minipools and the final plasma product are also variously tested for viral antibodies, HBV surface antigen and viral nucleic acids. The companies that produce these IgG products do not test syphilis serology or hepatitis B core antibodies in the finished IgG product. Donors who have previously been exposed to hepatitis B and cleared it, and therefore have positive hepatitis B surface and core antibodies with negative hepatitis B surface antigen, are therefore eligible to donate. We therefore tested a number of commercial IgG products with a variety of different serological laboratory assays, including some HBV markers.

Results

To ensure that the positive results for hepatitis B core antibody were not a result of one particular routine serological laboratory method or one particular IgG commercial product we tested seven undiluted IgG products using kits from different companies (Table 1).

We then tested five IgG products for antibodies to hepatitis B, syphilis, HIV and human T-lymphotropic virus (HTLV). To cover a limited number of common autoimmune serological

Table 1. Testing of undiluted IgG products for HBV core Ab by different company kits.

Products	Roche (Cobas system)	Ortho-clinical diagnostics (Vitros system)	Biomerieux (VIDAS system)
Octagam	Pos	Pos	Pos
Privigen	Pos	Pos	Not done
Flebogamma DIF	Pos	Pos	Pos
Kiovig	Pos	Pos	Pos
Vigam	Pos	Not done	Pos
Gammaplex	Pos	Not done	Pos
Subcuvia	Pos	Not done	Pos

Ab = antibody; HBV = hepatitis B virus; IgG = immunoglobulin G; Pos = positive.

investigations we tested the products for anti-neutrophil cytoplasmic antibodies (ANCA), anti-nuclear antibodies (ANA), anti-cardiolipin IgG and IgM (positive in the antiphospholipid syndrome) antibodies and anti-double stranded DNA (dsDNA) antibodies (Table 2). Results are given for the neat product and for diluted product to a physiological concentration of 8 g/l by dilution in distilled water. 8 g/l was chosen as it represents a reasonable target trough concentration of IgG in blood in patients on replacement immunoglobulin products.

Discussion on hepatitis B core antibody testing

Positive HBV core antibody was found for all IgG products tested at neat concentration by all three different methods tested. At physiological concentrations, all IgG products tested were positive for HBV core antibody apart from Kiovig (where the two results were discrepant – see Table 2). HBV surface antibody was strongly positive for all IgG products tested at both neat and physiological concentrations, and HBV surface antigen was uniformly negative. There are published examples of similar cases to case 1, such as a patient on IgG with a positive HBV core antibody who was negative on repeat testing 4 weeks later once the IgG was stopped,[1] and a spuriously positive HBV core antibody and HBV surface antigen for a patient on IgG treatment.[2]

Occult HBV infection detected by HBV DNA testing has been shown in 0.1–2.4% (median 1%) of HBV surface antigen-negative, HBV core antibody-positive blood donors in Western countries such as the United States.[3] HBV transmission by blood components negative for HBV surface antigen has occurred historically,[4–6] but is highly unlikely with current manufacturing methods. Risk of transmission of hepatitis B from plasma products appears to be negligible when concurrent HBV surface antibody is present in the blood above a certain level (100–200 mIU/ml), and it has been observed that blood containing detectable HBV surface antibody carries no increased risk of transmitting hepatitis B when compared with blood that lacks this antibody.[7] Screening and prevention of blood donations from those donors positive for HBV core antibody is only feasible in areas of low endemic disease. Since commercial companies do not screen donors for HBV core antibody but do screen for HBV DNA and HBV surface antigen,

Table 2. Testing of IgG products at neat and physiological concentrations for a multitude of laboratory tests.

Product	HBV surface Ab (mIU/ml)	HBV surface Ag	HBV core Ab	Syphilis EIA total Ab	Syphilis EIA IgM Ab	Syphilis RPR	Syphilis TPPA	HIV Ab/Ag	HTLV Ab	ANCA Ab	Hep2 ANA	Anti-cardiolipin IgG	Anti-cardiolipin IgM	Anti-dsDNA
Octagam neat (IV)	>999	Neg	Pos	Pos	Neg	Neg	Pos 1/640	Neg	Neg	Atypical C-ANCA	Weak pos	Pos	Neg	Pos
Octagam 8g/l (IV)	344	Neg	Pos	Pos	Neg	Neg	Pos 1/80	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Flebo-gamma DIF neat (IV)	>999	Neg	Pos	Pos	Neg	Neg	Pos 1/80	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Flebo-gamma DIF 8 g/l (IV)	319	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Hizentra neat (SC)	Too viscous	Too viscous	Too viscous	Pos	Neg	Neg	Pos 1/80	Too viscous	Too viscous	P-ANCA	Weak pos	Neg	Neg	Borderline
Hizentra 8 g/l (SC)	505	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Weak P-ANCA	Neg	Neg	Neg	Neg
Privigen neat (IV)	>999	Neg	Pos	Pos	Neg	Neg	Pos 1/320	Neg	Neg	Atypical ANCA	Pos	Pos	Neg	Pos
Privigen 8g/l (IV)	591	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	Atypical ANCA	Neg	Neg	Neg	Neg
Kiovig neat (IV)	>999	Neg	Pos	Pos	Neg	Neg	Pos 1/160	Neg	Neg	Atyptical C-ANCA	Weak pos	Neg	Neg	Pos
Kiovig 8 g/l (IV)	987	Neg	Pos/neg[a]	Pos	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Virology/syphilis tests were performed twice and the only discrepant result is indicated[a]. An average result was produced for HBV surface antibody. For methods of serological testing see Appendix 1. Ab = antibody; Ag = antigen; ANA = anti-nuclear antibody; ANCA = anti-neutrophil cytoplasmic antibody (C = cytoplasmic; P = perinuclear); dsDNA = double stranded DNA; EIA = enzyme immunoassay; HBV = hepatitis B virus; HTLV = human T-lymphotropic virus; IV = intravenous; Neg = negative; Pos = positive; RPR = rapid plasma reagin; SC = subcutaneous; TPPA = treponema pallidum particle agglutination.

it is possible that the positivity in commercial IgG reflects specific antibody from donors with cleared HBV infection.

A particular clinical scenario that could significantly impact patient care would be in a patient being prepared for immunosuppression, such as in the context of idiopathic thrombocytopaenic purpura (ITP). These patients are frequently treated with IgG and/or rituximab. Rituximab may cause reactivation of HBV even in patients with remote historical infection,[8–12] so testing for HBV serology prior to rituximab treatment is advised. In the context of IgG treatment, falsely positive HBV core antibody serology is likely and would complicate the decision to use rituximab therapy. Arnold *et al*[13] published a case series on this clinical situation which indicated 11 ITP cases were seropositive for hepatitis B core antibody, of which 10 had received IgG. Seven of these 10 patients subsequently reverted to negative hepatitis B core antibody on repeat testing having discontinued IgG.

Discussion on syphilis serological testing

The syphilis serological results of commercial IgG products showed uniform negativity for syphilis enzyme immunoassay IgM, which is unsurprising given there is a negligible amount of IgM in IgG products (which are over 99% IgG). However, syphilis testing of products was uniformly positive at neat and physiological concentrations for the syphilis enzyme immunoassay for total (IgG and IgM) reactivity. The IgG TPPA results showed some disparity, with most neat solutions producing a positive result and most physiological products producing a negative result. It has previously been reported that there have been false positive serological results for syphilis in patients on IgG treatment. Constable *et al*[14] reported a patient on IgG treatment for Guillain–Barre syndrome with positive syphilis serology (EIA and TPPA, but negative RPR) 24 hours after infusion, with subsequent testing at 8 and 13 weeks following infusion being negative. Similarly, Rossi *et al*[15] described a pregnant patient who tested positive for syphilis from IgG therapy for neonatal alloimmune thrombocytopenia. This patient had negative serology testing prior to and 6 weeks following her last treatment, but she received antibiotic treatment during pregnancy for syphilis anyway.

In view of the screening questions, medical examination and serological screening for syphilis in donor populations, it is likely that the above positive syphilis serological results are the result of non-specific reactivity, as opposed to the possible true positivity for HBV core antibody. It is unlikely that there is a significant burden of syphilis in the donor populations as donors are usually regularly tested for syphilis. There remains a question as to whether treatment of syphilis in these patients during pregnancy is warranted in view of the likelihood of it relating to the infused IgG. The treatment for syphilis is safe and effective, and the outcomes of active syphilis for the fetus are potentially severe (stillbirth or severe disability). This decision should be made on an individual basis for each patient after taking a thorough sexual history.

Discussion on other microbiological serological tests

Serological testing of products for HIV and HTLV antibody were uniformly negative. False positive tests for toxoplasma serology have been found in patients on IgG treatment

previously,[16] and would be expected from a normal population of donors not screened for this infection.

Discussion on rheumatological serological testing

At physiological concentrations, most of the autoantibody tests were negative. Weak positive ANCA results were seen for two of the products. At neat (non-physiological) concentrations, ANCA, ANA, anti-cardiolipin IgG and anti-dsDNA variably tested positive. This is suggestive of non-specific binding in these assays, but could reflect low level positivity of these antibodies within the IgG products. Since there are only trace amounts of IgM in IgG products, the uniform negativity of testing products for IgM anti-cardiolipin antibody is expected.

Conclusion

The serological testing of patients on IgG treatment tests for the presence of specific antibody in the IgG product, in addition to the endogenous specific antibody of the patient. False positivity on serological testing may also result from non-specific reactivity from donor IgG. Results should therefore be interpreted with care, especially when positive, as they may well be falsely so. Knowledge of the manufacturing and testing processes and the likely seropositivity of large donor pools of IgG allow for some interpretation of results, but we suggest that in some situations, serological testing should simply be avoided in patients on IgG (eg HBV core antibody), especially those receiving high dose therapy. Delayed repeat serological testing weeks after IgG administration may show low or absent positivity on a given serological test, consistent with a passively acquired effect.

Summary points

> IgG is detectable for some weeks after administration, and serology tests during this time will identify the administered IgG in addition to endogenously produced IgG.
> This may result in false positivity to a range of serological tests utilised in infective or autoimmune conditions, such as hepatitis B serology, syphilis serology and ANCA. ∎

Competing interests

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