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Review Article

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The interference of laboratory tests by immunoglobulin infusion: Problems and solutions

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Abstract

Intravenous Immunoglobulin (IVIG) has been increasingly used in managing a variety of immune-mediated conditions. While patients benefit from this treatment, the infusion of IVIG has transmitted large variety of antibodies and biological agents from donors to patients, and transiently interfered a broad spectrum of clinical laboratory tests covering the fields of infectious and autoimmune serology, biochemistry, haematology and blood bank, delivering spurious results that could potentially cause extensive evaluation and inappropriate clinical interventions. The medical practitioners and laboratory professionals must improve the awareness of test interferences caused by IVIG infusion. Proactive actions and appropriate strategy for investigating and interpreting the affected laboratory tests must be taken to avoid adverse impact on patient's care.

Keywords: Intravenous immunoglobulin G; Interference; Laboratory; Serological test.

Abbreviations: IgG: Immunoglobulin G; IVIG: Intravenous immunoglobulin G; Anti-HBc: Anti–hepatitis B core antibody; SARS-COV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; RBC: Red blood cell; ELISA: Enzyme-linked immunosorbent assay; ISE: Ion-selective electrode.

Introduction

Therapeutic Immunoglobulin G (IgG) commonly called Intravenous Immunoglobulin G (IVIG) has been widely used in managing inflammatory and infectious diseases, autoimmune and immunodeficiency disorders [1]. The commercial blood products are prepared from a large number of human plasma donations (1000-100,000) containing antibody specificities (mainly IgG, trace amount of IgM and IgA) against a wide variety of infectious agents, self-antigens and other biological components related to many health conditions [2]. Although the screen for several infectious diseases such as HIV, hepatitis viruses and syphilis are mandatory, the presence of thousands of antibodies and other biological components in pooled plasma will inevitably contaminate the blood of recipients following IVIG administration, and subsequently affect a broad range of laboratory tests with antibodies related to IgG in particular. Depending on the manufacturers with different selection criteria and batches of blood products, the transmission of different IgG composition and other biological agents into recipient could vary. The serological results obtained from patients with IVIG treatment might be impacted by both passively transmitted and endog-

enously produced IgG. In addition, the matrix effect and non-specific reactivity introduced by IVIG products could also lead to spurious laboratory results, potentially mislead clinicians to make extensive investigation and inappropriate decisions. Herein we provide an overview of the interference in clinical laboratory tests caused by IVIG infusion and suggest possible solutions.

Review

- **IVIG infusion affects the tests for infectious disease serology**

It has been extensively reported that many positive screening and diagnostic serological results for infectious diseases could be introduced by passively transmitted antibodies from IVIG products, with most commonly found positive are anti-hepatitis B surface antibody and anti–hepatitis B core antibody (anti-HBc) [3-5]. IgG antibodies to rubella, cytomegalovirus, hepatitis A virus, hepatitis E virus, herpes simplex virus and varicella zoster virus in pooled plasma were also frequently detected [6]. In our unpublished retrospective study in investigating a small percentage of IVIG infused patients present with positive antibodies to Human T-Cell Lymphotropic Virus (HTLV) by a screening ELISA in an area of low HTLV prevalence, over 85% of screening positive cases could not be supported either by confirmatory testing or clinical history of HTLV infection. Syphilis serology, although showed negative in initial blood screening profile in donor populations, has been falsely reported as positive in several cases post IVIG treatment [7-9], without the support of clinical evidence, the false positivity is likely to result from a non-specific reactivity in immunoassays as opposed to the possible true positivity of other infectious disease serology from passively transmitted antibodies, such as anti-HBc.

A most recent report showed that the anti-SARS-COV-2 antibodies were detectable in several large plasma pools collected for IVIG products from healthy donors in European countries and USA [10]. This will become worldwide phenomenon given that we are currently facing global pandemic of COVID-19. The use of IVIG containing antibodies to SARS-COV-2, however, will cause some confusion when interpreting post COVID-19 infection and vaccine responses in patients received IVIG, which has similar problem with other exogenous antibodies against infectious diseases.

- **IVIG infusion affects the tests for autoimmune serology**

Since autoimmune diseases are not uncommon in general populations and the donors with these diseases are not excluded in blood collection for IVIG product, many antibodies against self-antigens could therefore present in pooled plasma. The most common autoantibodies transmitted to recipient's blood are anti-nuclear antibodies, anti-neutrophil cytoplasmic antibodies, followed by anti-cardiolipin IgG/IgM and anti-double stranded DNA antibodies [11,12]. Nevertheless, A recent study in a group of neurological patients before and after IVIG infusion revealed that the treatment generated marked reduction in sensitivity but maintain reliable specificity in testing of serum autoantibodies to neuronal antigens. In this study, the authors claimed that false low or negative neuronal antibodies were found in nearly 50% of patients following IVIG infusion as opposed to increased ANA and Ro52 titre in the same cohort of patients [13]. Another interesting report showed that the antibodies to glutamic acid decarboxylase and Aquaporin-4 in IVIG products could be detected by ELISA, but not by cell-based assay or tissue immunohistochemistry, suggesting that these exogenous autoantibodies might only react with linear rather than structural epitopes of corresponding antigens [14].

- **IVIG infusion affects the tests in blood bank**

In addition to a broad spectrum of antibodies against viral, bacterial, and other microorganisms, the pooled plasma will unavoidably introduce antibodies against red blood cell (RBC) antigens to recipients from healthy donors, these antibodies may contribute to false-positive results in blood cross-matching tests and complicate the transfusion service [15]. The most frequently identified RBC alloantibodies were anti-A or anti-B, followed by anti-D and anti-K. Indirect anti-human globulin and isoagglutinins may also be seen [16-19]. Despite the regulation from WHO for controlling the titre of anti-A and anti-B and special requirements for irregular antibodies in blood products [20], the positivity of above RBC alloantibodies in IVIG could still lead to ABO blood group discrepancies and incompatible cross-matches [21].

- **IVIG infusion affects the tests in haematology and chemistry**

Compared to major focus on the test interference in IgG antibodies serology, the reports for IVIG infusion affecting haematology and chemistry testing profiles are relatively fewer, however, the influence to the laboratory tests of blood cells and chemical compounds does not seem to be any less significant. Koffman and colleagues had conducted a comprehensive study for investigating the impact of IVIG administration to haematological and biochemical testing profiles in blood collected within 1-24 hours before and after infusion in patients with neuromuscular diseases. By comparing the change of test value in a group infused with IVIG to another group infused with equal volume of placebo (dextrose), they reported that the following test values were significantly different in patients with IVIG vs. controlled placebo: (i) Circulating white cells including neutrophil, monocyte and lymphocytes were decreased; (ii) Up to 34% decrease was observed in lymphocytes with a selective reduction of the T cells, but not the B or interleukin 2 receptor-positive cells; (iii) Erythrocyte sedimentation rate was transiently increased (up to 275%); (iv) In chemistry profiles, the levels of sodium, calcium, magnesium, cholesterol and enzymes (i.e. alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatine kinase and aldolase) were significantly reduced. In contrast, analytes such as triglycerides, serum protein, viscosity and calculated osmolality were considerably elevated [22]. Among electrolytes, cases of pseudohyponatremia were frequently reported by this and subsequent studies [22-24]. The transient interference to the tests of haematology and chemistry may vary depending on the physiological and pathological conditions of individuals, the dose of IVIG, and the timing of blood collection post infusion. The phenomenon could partly be attributed to the increased serum viscosity and altered serum matrix effect introduced by the infusion of significant amount of immunoglobulins.

Discussion

The use of IVIG has increased rapidly over the past decades. While the treatment provides significant benefits to patients, it has potential pitfalls in confounding the laboratory diagnosis for infectious and autoimmune diseases. In fact, any antibodies present in donor's populations would be transferred to patient's blood causing a difficulty in determining the source of antibodies. The interference of a broad spectrum of serum chemical and hematological tests could be attributed to IVIG product itself (i.e. hypergammaglobulinemia), hemodilution and subsequent immunomodulatory and immunoregulatory effects from exogenous immunoglobulins.

As discussed above, a wide range of laboratory testing can be significantly influenced by the use of IVIG. The extent of interference may be far beyond clinician and laboratorian's recognition. Given that a large variety of antibodies present in the general population, any laboratory assays based on immunological reactions could potentially be disrupted by the infusion of IVIG. In fact, pooled immunoglobulin product is one of most "notorious" medication affecting clinical laboratory tests. For this reason, IgG related testing should be avoided especially in patients receiving high dose of IVIG therapy. A good practice is to collect blood for infectious and autoimmune serological testing prior to IVIG treatment. Nonetheless, this simple practice may not happen as forgetfulness often occurs in busy clinical settings.

The following strategies may be helpful in interpreting suspicious laboratory results for patients infused with IVIG:

1. Check the datasheet for blood product, it may provide valuable information in determining whether the antibodies are produced endogenously.
2. Investigate the clinical history and communicate with the pathologist and laboratory staff for result interpretation.
3. Check the timing of blood collection, the influence of IVIG to haematology and biochemistry tests are significantly less after 96 hours post-infusion but the influence to serological testing could last up to 4 months.
4. Check the level of positivity. Unlike passive transmitted antibodies, the titres of antibodies produced by recipients from recent infections are usually higher and uncorrelated to the dose of IVIG.
5. Repeat testing at a later date, the antibodies from blood product may show significantly decreased positivity with the time approaching to its half-life (approximate 4 weeks) and disappear after 2-4 months from the cease of IVIG.
6. Request for testing by other methodologies that are not affected by transmitted antibodies, such as molecular assays, or methodologies for testing antibodies against conformational epitopes of detecting antigens as the processed IgG in IVIG products might only bind to linear epitopes of antigens while endogenous IgG could bind to both linear and conformational epitopes [14]. For electrolytes, they can be measured on a separate analyser using different technologies that are less susceptible to the interference of IVIG to obtain a more accurate result, such as direct Ion-Selective Electrode (ISE) instead of indirect ISE [25].
7. Choose assays for detecting IgM or IgA antibodies if applicable, the trace amount of IgM and IgA in pooled blood product should not affect the serological testing of patients.
8. Test sample with higher dilution or use an additional neutralisation procedure for antibodies to rule out possible non-specific reaction in immunoassays. The false positivity attributed to non-specific binding is usually at lower titre and likely to disappear in a small scale of dilution, also, it would not be neutralized due to low specificity.
9. Retrospectively test the samples collected prior to infusion of blood product if there is one available.

Conclusion

In summary, the IVIG infusion affecting laboratory tests must be considered at all time when interpreting the results in clinical and laboratory settings. Clinician and laboratory professionals should be more sensitized on this issue and take proactive and correct measures to combat this challenge to avoid inappropriate clinical decisions.

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