

Unexpected reactions of the anti-IgA antibody particle gel immunoassay[†]

E. Strobel¹ & A. von Meyer²

¹Department Mikrobiologie, Medizet, Städtisches Klinikum München GmbH, München, Germany, and ²Department Klinische Chemie, Medizet, Städtisches Klinikum München GmbH, München, Germany

Received 2 June 2013; accepted for publication 15 November 2013

SUMMARY

Background: The cause of allergic transfusion reactions remains often unknown, but in rare cases anti-immunoglobulin A (IgA) antibodies in patients with IgA-deficiency can be found. We report on the use of the DiaMed particle gel immunoassay (PaGIA) for detection of anti-IgA antibodies in patients with allergic transfusion reactions.

Methods: The examination of the suspected adverse reactions included an anti-IgA antibody test (ID-PaGIA Anti-IgA antibody test; DiaMed GmbH, Cressier, Switzerland) and measurement of IgA concentration in the patient's plasma. In the case of a discrepancy IgA subclasses were examined and neutralization of the anti-IgA antibodies by pure IgA was performed.

Results: Of 142 patients tested for IgA concentration and anti-IgA antibodies, 8 gave positive results for the anti-IgA antibody test. In seven of these cases (4.9% of the patients tested) IgA levels were found to be normal, and in four of five so tested, the positive result could not be neutralized with purified IgA. Only one patient had confirmed IgA deficiency with anti-IgA antibodies that were neutralized by addition of purified IgA.

Conclusion: Cause and clinical relevance of a positive reaction of the anti-IgA antibody test in patients with normal total IgA and normal IgA subclasses remains unknown. Because of the high false positive rate we do not recommend this test as a screening test for anti-IgA antibodies when evaluating allergic transfusion reactions, but instead recommend measurement of total IgA in patient's plasma or serum as a primary screen for IgA deficiency with antibodies as a cause of allergic transfusion reaction.

Key words: allergy, anti-IgA antibody, IgA-deficiency, particle gel immunoassay, transfusion reaction.

Mild allergic reactions are a frequent complication of blood transfusion, but most times their cause remains unknown. In rare cases antibodies of a patient with a congenital plasma protein deficiency [e.g. of immunoglobulin A (IgA) or haptoglobin] can cause a severe transfusion reaction when plasma-containing blood products are administered (Sandler *et al.*, 1995; Shimada *et al.*, 2002). On the other side, transmission of anti-IgA antibodies by donor plasma seems not to be correlated with a higher rate of adverse transfusion reactions (Winters *et al.*, 2004).

In patients with IgA-deficiency and anti-IgA antibodies washed red blood cell concentrates (RBCCs) or platelet concentrates (PCs) and IgA-deficient fresh frozen plasma should be given to prevent further adverse reactions (German Medical Association – Scientific Advisory Board, 2009; Mazzei *et al.*, 2011). Therefore the evaluation of a suspected severe allergic transfusion reaction should include looking for a congenital IgA-deficiency and for anti-IgA antibodies (German Medical Association – Scientific Advisory Board, 2009). For this purpose a commercially available test system on the basis of a particle gel immunoassay (PaGIA) (ID-PaGIA Anti-IgA antibody test; DiaMed GmbH, Cressier, Switzerland) is now available (Salama *et al.*, 2001).

In the following we report on our experience with this test in the evaluation of allergic transfusion reactions.

MATERIALS AND METHODS

In five well-examined cases in which the DiaMed anti-IgA antibody PaGIA showed a positive result, a mild to moderate suspected adverse transfusion reaction was reported to our laboratory. All patients were male and their age ranged from 10 months to 61 years. The proportion of males to females tested in our study was 90:52. Therefore we suggest that it was by chance that all five well-examined patients were male. Four patients had a malignancy, one a florid infection. In four cases a leucocyte-depleted RBCC was transfused, in one case

Correspondence: Priv.-Doz. Dr med. Dr phil. Erwin Strobel, Department Mikrobiologie, Medizet, Städtisches Klinikum München GmbH, Kölner Platz 1, D-80804 München, Germany.
Tel.: +49 89 3068 3360; fax: +49 89 3068 3835; e-mail: erwin.strobel@klinikum-muenchen.de

[†]Parts of this work have been presented as a poster contribution at the 45. Jahreskongress der Deutschen Gesellschaft für Transfusionsmedizin und Immunhämatologie, 11. – 14.09.2012 in Graz, Austria.

an apheresis-PC, leucocyte-reduced by filtration. While four patients showed an increase of body temperature, only one of them also suffered from urticaria and pruritus. One patient only showed shivering and tachycardia. All patients recovered from the suspected adverse reaction.

A comprehensive battery of tests was undertaken to identify the cause of the transfusion reactions. These included the following tests to identify IgA deficiency and the presence of anti-IgA antibodies:

- 1 Anti-IgA antibodies in the patient's pretransfusion plasma using a particle gel immunoassay technique (ID-PaGIA Anti-IgA antibody test; DiaMed GmbH), that was performed according to the manufacturer's instructions.
- 2 Total IgA concentration in the patient's plasma to look for a complete IgA-deficiency (measured by a turbidimetric technique; Immunoglobulin A, Architect™ c System, Abbott, Chicago, IL, USA).
- 3 Total IgA concentration in the patient's plasma was controlled by a nephelometric technique (Immunoglobulin A, BN Prospec™, Siemens Healthcare Diagnostic Products, Marburg, Germany).
- 4 Anti-IgA antibodies were tested by the PaGIA in a patient's posttransfusion serum (in four cases) to show that the result did not depend on the kind of the blood sample. In one case the positive reaction of the patient's posttransfusion plasma was confirmed by using another lot of particles and gelcards for the PaGIA.
- 5 IgA subclasses in patient's plasma or serum were tested by an external laboratory.
- 6 Neutralization of the positive reaction of the anti-IgA antibody test by adding one part human serum pool (from 10 persons), or one part pure IgA solution (from human colostrum; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) to either one part patient plasma (two cases), one part patient serum (two cases) or three parts patient plasma (one case), incubating at room temperature for 10 min and then performing the anti-IgA antibody PaGIA. As a control test 0.9% NaCl solution was added to the patient's plasma or serum in the same proportion. Furthermore the serum pool respectively the pure IgA were also added to the positive control serum and the negative control serum of the test kit (one part to one part) before performing the internal controls to check the neutralization test.
- 7 Because of the suspicion of an unspecific reaction of the PaGIA technique, another PaGIA (for heparin/PF4 antibodies; ID-PaGIA heparin/PF4 antibody test; DiaMed GmbH) was performed from the patient's serum in three of four cases without IgA-deficiency (Meyer *et al.*, 1999). Heparin/PF4 antibodies were controlled by an immunoglobulin class-specific enzyme immunoassay (EIA) in the patient's serum (Zymutest HIA IgG, IgA, IgM specific isotyping assay; Hyphen BioMed, Neuville sur Oise, France).

RESULTS

In 2010–2012, 170 cases of suspected adverse transfusion reactions were reported to our immunohaematological laboratory. In 143 cases both, total IgA measurement and anti-IgA antibody PaGIA were performed, but in one of these cases the report cannot be evaluated retrospectively. In 134 of 142 cases the anti-IgA antibody PaGIA was negative and there was no hint for a complete IgA-deficiency (94.4%). In seven cases the anti-IgA antibody PaGIA was positive, but there was no hint for a complete IgA-deficiency (4.9%). In three of these cases the further examination was not completely performed. In two of them only total IgA was measured and was not decreased. In the third case total IgA and the IgA subclasses were measured and were normal. But in all these three patients no neutralization of the positive anti-IgA antibody PaGIA by pure IgA was done. In one case (0.7%) the anti-IgA antibody PaGIA was positive and the total IgA level was under the limit of detection. Therefore our series reported here includes only five well-examined cases (four with a false positive and one with a right positive result of the anti-IgA antibody PaGIA), but for estimation of the rate of unexpected results of the anti-IgA PaGIA all seven cases with a false positive result are included.

All five well-examined patients showed a positive reaction in the anti-IgA antibody PaGIA. In four cases the test was repeated with patient's serum, where the reaction of the patient's plasma was confirmed. In four patients a complete IgA-deficiency could be excluded by an inconspicuous total IgA concentration in patient's plasma measured by two different methods. Also the IgA subclass concentrations were in the normal range. Only in one case a complete IgA deficiency was found.

To examine the specificity of the positive reaction of the anti-IgA antibody PaGIA, neutralization tests were performed, first with a serum pool, later with pure IgA solution. While the positive reactions could be neutralized in all cases with the serum pool, in those patients with normal IgA levels the positive reaction could not be neutralized with pure IgA. In the patient with complete IgA-deficiency the positive reaction of the anti-IgA antibody PaGIA could be neutralized with pure IgA solution confirming the specificity of the antibody.

Because of the suspicion of unspecific reactions of the PaGIA technique, in three of four patients without IgA-deficiency another PaGIA (for heparin/PF4 antibodies) was performed with patient's serum. They also showed a positive result, but none of them could be confirmed by the heparin/PF4 antibody EIA.

DISCUSSION

Earlier methods for detection of anti-IgA antibodies, e.g. the passive haemagglutination assay, were difficult and could only be performed in specialized laboratories (Sandler *et al.*, 1995). Now, by means of a commercially available PaGIA, this examination can be performed in a simple and rapid way, bringing advantages particularly in urgent cases (Yuan & Goldfinger, 2008).

In four cases of our series of five well-examined cases we found a positive reaction of the anti-IgA antibody PaGIA and

a normal total IgA level. There are several reasons to assume an unspecific reaction of the anti-IgA antibody PaGIA in these cases:

- 1 All four patients had no complete or subclass specific IgA deficiency.
- 2 In all four patients the positive reactions of the anti-IgA antibody test could not be neutralized with pure human IgA.
- 3 In three patients tested with another PaGIA (for heparin/PF4 antibodies), this PaGIA also showed false positive results.
- 4 In three of four patients the clinical symptoms were not typical for an allergic transfusion reaction and in the remaining patient the onset of symptoms had a delay of 15 h, which is also not typical for an allergic transfusion reaction.

In contrast to these four cases, the fifth patient had a complete IgA deficiency and showed a positive reaction of the anti-IgA antibody PaGIA that could be neutralized with pure IgA.

The reason for the unexpected positive result of the anti-IgA antibody PaGIA in the above reported four cases remains unknown. As all reactions could be neutralized with a human plasma pool, there might be another specificity of the antibody in the plasma of these patients. Also allotype-specific

antibodies directed against IgA2m(1) or IgA2m(2) could not be differentiated with the PaGIA. On the other hand, it can be speculated that the sera of the four patients showed an unspecific reaction with the beads making this assay and others, using a similar particle substrate, false positive. As there are no unloaded particles provided in test kit for a negative control this suggestion could not be further examined.

In conclusion, the examination of an allergic transfusion reaction should primarily include the measurement of IgA in the patient's plasma. In all cases of an IgA deficiency in the patient's plasma, an anti-IgA antibody test can be performed and its specificity has to be confirmed by a neutralization test of the anti-IgA antibodies with pure IgA as the false positive to the true positive ratio of the anti-IgA antibody PaGIA was 7:1 in our series of patients.

ACKNOWLEDGMENTS

E. S. and A. v. M. performed the research. E. S. designed the research study and wrote the paper. All authors approved the final version of the manuscript submitted for publication.

CONFLICT OF INTERESTS

The authors have no competing interests.

REFERENCES

- German Medical Association - Scientific Advisory Board (2009) Cross-sectional guidelines for therapy with blood components and plasma derivatives (4th revised edition, 2008). *Transfusion Medicine and Hemotherapy*, **36**, 468.
- Mazzei, C.A., Popovsky, M.A. & Kopko, P.M. (2011) Noninfectious complications of blood transfusion. In: *Technical Manual* (eds Roback, J.D., Grossman, B.J., Harris, T. & Hillyer, C.D.), **17**, 745. American Association of Blood Banks, Bethesda.
- Meyer, O., Salama, A., Pittet, N. & Schwind, P. (1999) Rapid detection of heparin-induced platelet antibodies with particle gel immunoassay (ID-HPF4). *Lancet*, **354**, 1525–1526.
- Salama, A., Schwind, P., Schönhage, K. *et al.* (2001) Rapid detection of antibodies to immunoglobulin A molecules by using the particle gel immunoassay. *Vox Sanguinis*, **81**, 45–48.
- Sandler, S.G., Mallroy, D., Malamut, D. & Eckrich, R. (1995) IgA anaphylactic transfusion reaction. *Transfusion Medicine Reviews*, **9**, 1–8.
- Shimada, E., Tadokoro, K., Watanabe, Y. *et al.* (2002) Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies. *Transfusion*, **42**, 766–773.
- Winters, J.L., Moore, S.B., Sandness, C. & Miller, D.V. (2004) Transfusion of apheresis PLTs from IgA-deficient donors with anti-IgA is not associated with an increase in transfusion reactions. *Transfusion*, **44**, 382–385.
- Yuan, S. & Goldfinger, D. (2008) A readily available assay for anti-immunoglobulin A: is this what we have been waiting for? *Transfusion*, **48**, 2048–2050.