REPLY

Reply to the letter to the editor by Johannes Ring and Rudi Valenta on the article "Assessment of dextran antigenicity of intravenous iron products by an immunodiffusion assay"

Susann Neiser, Katrin Schwarz, Maria Wilhelm, Felix Funk, Peter Geisser, Susanna Burckhardt

Vifor (International) Inc. St. Gallen, Switzerland

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In response to the letter by Johannes Ring and Rudi Valenta, we want to clarify that, in our original paper[1], we did not "postulate that antibodies [...] can be used to predict the risk of clinical allergic reactions in patients". We agree with Ring and Valenta that in vitro tests of possible antigens with monoclonal antibodies can generally neither assess the risk of anaphylaxis in an individual patient, nor can they predict the numerical risk of such anaphylaxis in the clinical setting. We did, however, find a correlation between our results and clinical findings with the tested intravenous iron preparations and thus concluded that "immunoassay data agree well with clinical observations and thus represent a *possible approach* for the *evaluation* of the risk of dextran-induced anaphylactic reaction (DIAR)".

Iron sucrose (Venofer®) was introduced in the 1950s by Laboratorien Hausmann AG, the predecessor of Vifor (International) Inc., as the first dextran-free intravenous iron preparation in Europe. In connection with the registration of Venofer® in the US, the reverse single radial immunodiffusion assay used in our study[1] was developed in 1998 by Dr. H. Hedin (Pharmacia & Upjohn AB, Uppsala, Sweden) for Vifor to fully exclude the presence of dextran, which might occur as an impurity of sucrose. Although no such test is required by the regulatory authorities, it is still used today as an additional safety measure in Vifor's routine quality control analyses. Over the years, we have tested with this assay not only various types of dextrans but also different intravenous iron dextran preparations, which all showed a positive result. In contrast, carbohydrates and intravenous iron products that do not contain dextran always gave a negative result. When the two new dextran-based iron preparations Feraheme® and MonoFer® came on the market, we tested them in this assay. Ferumoxytol (Feraheme®, Rienso®) contains a carboxymethylated dextran[2] and was marketed as non-immunogenic, but caused an anaphylactic reaction after application to a patient with a known history of adverse reaction to iron dextran[3]. Iron isomaltoside 1000 (MonoFer®) contains reduced Dextran 1 as a ligand[4], which does not react in the immunoassay and probably acts as a hapten like Dextran 1[5]. Thus, both intravenous iron preparations were expected to give a negative result. Surprisingly, we found that Feraheme® and MonoFer® gave a positive reaction in the immunodiffusion assay.

Because we were running out of the antibody used for quality control, we recently outsourced the development of a new antidextran antibody and of an enzyme-linked immune-sorbent assay (ELISA) based on this new antibody to an independent, external laboratory (GenScript, Piscalaway, NJ, USA). The antibody was developed by immunisation of BALB/c mice with dextran 50'000–KLH conjugate. Monoclonal antibodies (mouse IgG1-isotype) were produced in hybridoma cells.

Reverse single radial immunodiffusion assays with this new antidextran antibody confirmed the published results[1], i.e. the positive reactions for Feraheme®, MonoFer®, and Dextran 5, as well as the negative result for the reduced Dextran 1 isolated from MonoFer®. Moreover, ELISAs were performed by GenScript on blinded

samples, and the results were fully aligned with the previously reported immunodiffusion assay data[1]: positive reactions were observed with CosmoFer®, Feraheme®, MonoFer®, and Dextran 5, and negative results with Ferinject®, Venofer®, Ferrlecit®, the reduced Dextran 1 isolated from MonoFer®, and Dextran 1 (manuscript in preparation). Taken together, these additional data confirm our published results[1] and address the criticisms of insufficient characterisation of the antibody as well as that of the test method used.

Although this additional evidence strongly corroborates our previous data, we would like to address a few of the other criticisms raised by Ring and Valenta. The area of the precipitate in immunodiffusion assays depends on the relative concentrations of antigen and antibody. For the inverse technique we used, the size of the precipitate area has even been shown to be inversely proportional to the concentration of a given antigen[6]. The quantification of the antigens was beyond the scope of our work, and no conclusion can be drawn in this regard. However, the observed precipitates definitely reflect positive reactions of the antidextran antibodies with the respective antigen.

As highlighted by Ring and Valenta, the mechanism of severe DIAR is an immune complex anaphylaxis. This mechanism obviously requires the presence of specific antibodies, which have been suggested to play a causal role in the development of DIAR[7]. The antibody titre, especially that of IgG, has been correlated to the severity of DIAR in several publications[8-11]. The statement cited by Ring and Valenta, that the antibodies per se are of no pathogenic importance, is misleading. Correctly, Richter and Hedin (1982)[12] state that “all patients with severe reactions have high titers”. However, “[…] only a small proportion of those with high titers develop anaphylactic reactions.”

In conclusion, since monoclonal as well as human antidextran antibodies react to the repetitive structure of dextran, a positive in vitro reaction between an intravenous iron preparation and a monoclonal antidextran antibody suggests that a similar reaction can occur in vivo – even if its likelihood to provoke a DIAR and thus its clinical relevance cannot be assessed. Working with human antisera would yield little additional value since it also would not allow for the assessment of the numerical risk of DIAR in a clinical setting.

Conflict of interest statement.

The authors are employed by Vifor (International) Inc. St. Gallen, Switzerland.

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Correspondence to:
Dr Susanna Burckhardt
Head of Chemical and Preclinical Research & Development
Vifor Pharma · Vifor (International) Ltd.
Rechenstrasse 37 · P.O. Box · CH-9001 St. Gallen, Switzerland.
E-mail:susanna.burckhardt@viforpharma.com