

Letter to the Editor

Chloé Lamesa, Anne-Sophie Gauchez, Roseline Mazet, Luc Foroni, Daniel Fagret and Marie-Dominique Desruet*

Evaluation of a human anti-mouse antibody rapid test for patients requiring radio-immunodiagnostic

DOI 10.1515/cclm-2016-0866

Received September 28, 2016; accepted January 26, 2017

Keywords: HAMA; quick test; radiolabeled antibodies; radio-immunodiagnostic.

To the Editor,

Monoclonal antibodies (mAbs) play a significant part in the diagnostic and therapeutic arsenal. They are used in many fields of medicine, ranging from in vitro diagnosis to the treatment of multiple pathologies in such diverse areas as infectious diseases, immunology and oncology. The first mention of the use of radiolabeled antibodies dates from the 1950s. However, from the first tests, several problems with the use of radiolabeled antibodies appeared. One of these involved the use itself of the antibodies because they caused an immune response and the production of human anti-mouse antibodies (HAMAs) [1]. Complications related to this immunization are very rare and have a high inter-individual variability

[2–4]. It is widely accepted that the presence of these heterophilic antibodies could influence the effectiveness of the immunotherapy and immunoscintigraphy, and could also be considered as responsible for analytical interference in immunoassays [5, 6]. Indeed, HAMAs bind to the newly administered mAbs, leading to the formation of immune complexes (HAMA-mAb) that may decrease the therapeutic efficacy and diagnostic value of the new mAb conjugates. Similarly, allergic reactions could occur, motivating the search for HAMAs before any reintroduction of mAbs [7].

Current tests for HAMA detection do not allow one to quickly obtain the status of patients. To develop a simple, rapid and reliable test for monitoring the presence of HAMA in some patients, Milenia GmbH (Hamburg, Germany) has recently proposed a new qualitative test based on the principle of a rapid lateral flow test on a strip. We proposed to evaluate this Quicktest on our cohort of patients who had tested positive for HAMAs using the quantitative Medac HAMA-ELISA test.

Serum samples from patients positive for HAMA were obtained from a serum bank collected between 1992 and 1999 in the Nuclear Medicine Department of Grenoble University Hospital. The patients had received radio-immunotherapy and subsequently developed HAMAs demonstrated using the quantitative Medac test. Sera were estimated again in 2014 using the same test to verify the persistence of expression of HAMA. This test is a one-step enzyme immunoassay for the quantitative determination of HAMA in serum realized in 1 h. The test is calibrated against anti-mouse IgG antibodies. The measuring range is from 40 to 2000 ng/mL. Samples and peroxidase-labeled mouse IgG conjugate are added together to the plate that is pre-coated with mouse IgG (antigen). The HAMAs bind to the solid phase and to peroxidase-labeled mouse IgG. Then, there is an incubation step with a tetra-methyl benzidine substrate. The reaction is stopped by the addition of sulfuric acid, and the absorption is read photometrically at 450 nm. According to the manufacturer, the limit

*Corresponding author: Marie-Dominique Desruet, PhD, PharmD, Radiopharmacy/ Nuclear Medicine Department, Grenoble University Hospital, CS 10217 38043 GRENOBLE cedex 9, France, Phone: + (0)33 476 769 410, Fax: + (0)33 476 767 108, E-mail: mddesruet@chu-grenoble.fr; and Pharmacy Department, Grenoble University Hospital, Grenoble, France

Chloé Lamesa: Pharmacy Department, Grenoble University Hospital, Grenoble, France; and UMR-S INSERM 1039 Bioclinical Radiopharmaceutical Unit, Grenoble, France

Anne-Sophie Gauchez: UMR-S INSERM 1039 Bioclinical Radiopharmaceutical Unit, Grenoble, France; and Radioactivity Platform, Biology Department, Grenoble University Hospital, Grenoble, France

Roseline Mazet and Luc Foroni: Pharmacy Department, Grenoble University Hospital, Grenoble, France

Daniel Fagret: UMR-S INSERM 1039 Bioclinical Radiopharmaceutical Unit, Grenoble, France; and Nuclear Medicine Unit, Imaging Department, Grenoble University Hospital, Grenoble, France

of quantitation is 40 ng/mL, which is significantly above the zero range. The precision intra-assay, inter-assay and the dilution linearity are reported to be less than 10% and the mean recovery test is 99%.

The Milenia® QuickLine HAMA test (Milenia Biotec GmbH, Giessen, Germany) is a rapid lateral-flow immunoassay designed for the qualitative determination of HAMA in human serum. Fifty microliters of serum is pipetted in the sample application port of the test unit and the chase buffer is added immediately. The buffer forces the sample to migrate through the membrane of the test unit for 10 min at room temperature. HAMAs from the patient's sample bind first to mouse IgG antibodies coated on the test line of the nitrocellulose membrane and conjugated to gold nanoparticles. The result will be estimated compared with the level of color of the test line T: if the color is weaker, the result will be negative, whereas with identical or darker color, the result will be positive. In any case, control line C, used as functional control, has to appear to validate the test. The result must be interpreted immediately after incubation time. Sensitivity and specificity given by instructions for use were 97% and 91%, respectively.

For comparison, all quantitative results were converted to qualitative results according to the cut-off (40 ng/mL).

Pooled data were used to calculate sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-) and correctly classified percentage. The sets of results were compared using weighted kappa statistics to assess the agreement between the Medac test and the Milenia QuickLine test. We interpreted the kappa statistics using the following categories: poor agreement (≤ 0.20), fair agreement (0.21–0.40), moderate agreement (0.41–0.60), substantial agreement (0.61–0.80) and perfect agreement (0.81–1.00). All statistical analyses were performed using MedCalc Version 12.2.1.0-64-bit software (MedCalc Software, Ostend, Belgium). The performance of the test was calculated using the qualitative test as the reference. Confidence intervals were obtained using the Wilson score method.

One hundred and six samples were assayed using the Milenia Quickline test (Figure 1). Medac ELISA test gave 41 negative results and 65 positive results. The Milenia QuickLine HAMA test gave 39 negative results and 67 tested positive (Figure 2).

In the overall samples, six results did not agree with the Medac test: four false-positive (FP) results and two false-negative (FN) results. The Milenia® Quickline HAMA test exhibited high sensitivity and specificity 97% and 90%, respectively. LR+ was very high at 9.93 and LR- was low at 0.03, and the correctly classified percentage was 94.3%. The agreement between the Milenia® QuickLine

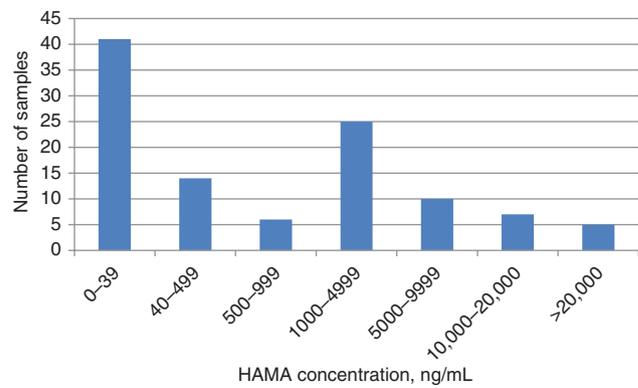


Figure 1: Distribution of samples according to HAMA concentrations determined by Medac HAMA-ELISA test.

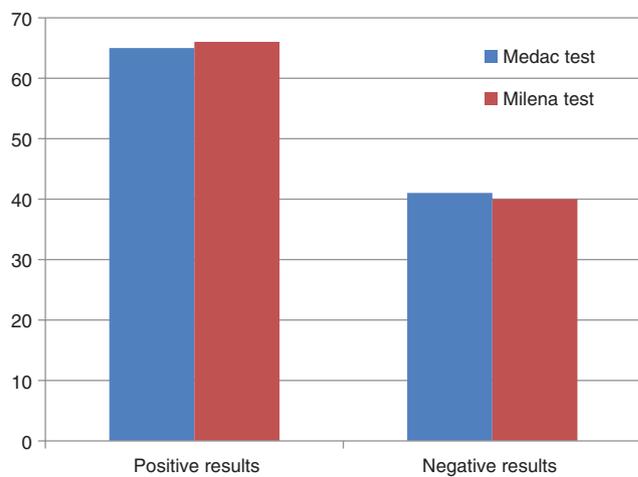


Figure 2: Qualitative results obtained with Medac test (in blue) and Milenia® test (in red).

HAMA test and Medac test was almost perfect with a kappa score of 0.88.

Despite the presence of HAMAs, only 0.3% of allergic reactions have been reported in the literature [8], and no study has been able to establish a clear link between the presence of HAMAs and side effects [9]. However, testing for HAMAs is also required from a regulatory point of view. Thus, the European Medicine Agency has restricted the use of ^{99m}Tc -labeled besilesomab (Scintimun®), a murine anti-NCA-95 indicated in the diagnosis and localization of suspected infectious and non-infectious inflammatory lesions, to patients whose HAMA status is negative. As a result, most HAMA determinations are performed at the request of nuclear medicine centers before the injection of Scintimun®.

In this new regulatory context, the constraints of this pre-requisite, related to the low number of laboratories that can perform the test, make implementation of the

radio-immunotherapy long and tedious. In our study, the Milenia® QuickLine HAMA test had shown a very good sensitivity and specificity. The discrepancy results between the tests were around the Medac cut-off (40 ng/mL). This study had several limitations. Patients were recruited in hospital cohorts, 61% of them were positive. This population study did not reflect the targeted population for HAMA testing in which the prevalence of positive patients is unknown. The introduction of a rapid test that can be performed rapidly would be welcomed advantage. Subsequently, it could be extended to other treatments that use murine and chimeric antibodies.

This is the first study carried out with the Milenia® QuickLine HAMA test, which is available for clinical use. It has obtained EC label. The very good sensitivity and specificity results as well as a good LR+ and low LR- will allow this test to help both to improve the care of patients and to detect analytical interference. This is the first study evaluating a rapid and simple qualitative test, developed for clinical use, and of particular interest for nuclear medicine, oncology and rheumatology departments, as well as for medical biology laboratories.

Acknowledgments: We thank A. Foote (Grenoble Clinical Research Center) for translating the manuscript. We thank J.-L. Quesada (Grenoble Clinical Research Center) for statistical assistance.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: HAMA quick tests were kindly provided by Milenia.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Pressman D. The development and use of radiolabeled antitumor antibodies. *Cancer Res* 1980;40:2960–4
2. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;256:495–7
3. Reichert JM. Antibodies to watch in 2010. *mAbs* 2010;2:84–100.
4. Steenholdt C, Palarasah Y, Bendtzen K, Teisner A, Brynskov J, Teisner B, et al. Pre-existing IgG antibodies cross-reacting with the Fab region of infliximab predict efficacy and safety of infliximab therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2013;37:1172–83.
5. Robier C, Edler E, Klescher D, Neubauer M. False-positive D-dimer result in a latex enhanced immunoassay caused by interfering human anti-mouse antibodies. *Clin Chem Lab Med* 2014;52:e253–5.
6. Koper NP, Thomas CM, Massuger LF, Segers MF, Olthaar AJ, Verbeek AL. Quantitation of IgG and IgM human anti-mouse antibodies (HAMA) interference in CA 125 measurements using affinity chromatography. *Clin Chem Lab Med* 1998;36:23–8.
7. Navarro-Teulon I, Lozza C, Pèlegri A, Vivès E, Pouget J-P. General overview of radioimmunotherapy of solid tumors. *Immunother* 2013;5:467–87
8. Toft JC, Tromholt N. [Production of human antimurine antibody after in vivo use of radioisotope labelled murine antibodies]. *Ugeskr Laeger* 1991;153:2813–5.
9. Frödin JE, Lefvert AK, Mellstedt H. The clinical significance of HAMA in patients treated with mouse monoclonal antibodies. *Cell Biophys* 1992;21:153–65.