VALID SERODIAGNOSIS OF TBE

Background

The laboratory diagnosis of TBE is mostly done based on the detection of specific IgM and IgG sera and/or intrathecal antibodies. These antibodies appear in the second phase of infection, when CNS symptoms have manifested and TBE virus can no longer be detected by RT-PCR. High cross-reactivity among flaviviruses may cause problems when IgG ELISAs are used (see Snapshots week 5/2020, week 52/2019, week 22/2019, week 18/2019, week 12/2019). In contrast, IgM responses to flaviviruses generally are more virus-specific and are a reliable marker for an acute infection.

This newsletter discusses two new publications: one is dealing with a new commercially available IgM test and the other with the development of an ELISA for detection antibodies to the non-structural protein 1 (NS1).

Results

Detection of IgM

The new TBE IgM test (ReaScan) is a novel qualitative immunochromatographic lateral flow assay for the detection of acute TBE infections. Captured specific IgM is detected by a recombinant TBE virus antigen-colloidal gold complex. The amount of specific IgM is converted into a numerical value by the dedicated test reader. This new assay has been compared with a variety of serum samples collected from different European countries. The sensitivity of the new test was calculated to be 99.4%, and a specificity of 97.9% was found (based on 141 samples shown TBE virus IgM negative, using the routine method for each laboratory and other commercially available assays).

To evaluate the potential cross-reactivities with other flaviviruses, a total of 57 serum samples from individuals infected with Dengue-, Japanese Encephalitis-, West Nile- and Zika virus, and 15 sera from JEV or YFV vaccinated individuals, as well as ten serum samples from individuals infected with chikungunya virus (a togavirus). were analyzed. 81 of these 82 sera showed a negative result, giving a specificity of 98.8%. Further analyses with „serologically problematic“ sera from individuals infected with CMV, EBV, HSV, VZV and Anaplasma phagocytophilum showed a specificity of 96.4%.

However, to fulfill the EU criteria, detection of TBE virus IgM alone is not enough and has to be confirmed by detection of IgG antibodies and/or neutralization assay.

Detection of NS1

TBE vaccination is the only effective approach to prevent the disease. All licensed TBE vaccines are inactivated whole virus vaccines, of which the glycoprotein E is the main immunogen inducing neutralizing antibodies.

Despite the high effectiveness of TBE vaccination, breakthrough infections are reported. Unfortunately, ELISAs detecting TBE antibodies cannot discriminate between an immune response on an infection or on vaccination. A new approach uses antibodies directed to the nonstructural protein 1 (NS 1) as a diagnostic tool. The first assay developed to detect NS1 specific antibodies has been described by a Swedish team (see Newsletter February 2018). As NS 1 is not part of the virion, TBE vaccines have been thought to be free of NS 1. However, recently it has been shown that TBE vaccines may contain minor amounts of NS1 (see Newsletter November 2019), but no relevant NS 1 specific immune response can be observed in sera of vaccinees. Here, a new NS 1 specific ELISA (based on commercially available recombinant NS 1) is described, demonstrating its usefulness for discrimination between infection and vaccination induced immune response.

For determining the sensitivity of the new assay, 67 IgG positive sera from acutely ill patients were analyzed and except for three early-stage samples, all other sera reacted positive resulting in a sensitivity of 98.53%. Specificity was analyzed with 34 sera which were negative in IgM or IgG antibodies measured by IFA. Except for one serum, all other sera were negative in the NS 1 ELISA corresponding in a specificity of 97.06%.

Furthermore, 49 sera from vaccinated individuals were analyzed, and no serum reacted in the NS 1 assay (specificity 100%). Possible cross-reactions with other flaviviruses were analyzed with 9 sera from patients acutely ill with Dengue, one patient with WNF and 19 sera from patients vaccinated with YF vaccine. With one exception, all sera tested negative in the NS 1 ELISA indicating a specificity of 90.00%.

NS 1 antibodies may persist for at least several years after infection. This was shown by analyzing sera from patients with a history of TBE five to 28 years ago.

Discussion

Sensitive and specific tools are needed for diagnosing TBE virus infections. The new IgM assay fulfills these criteria and may be an appropriate means for detection of antibodies produced in an early stage of illness. A second test, later carried out, can then confirm TBE.

While commercially available IgM tests are generally valid, IgG ELISAs have certain imponderability due to cross-reactions with the structural proteins (mainly glycoprotein E) of other flaviviruses. IgG ELISAs cannot discriminate between an immune response to infection and vaccination because in both cases the main antibody response is directed to gE.

NS 1 is more specific for TBEV than structural components of the virus and thus is its antibody response. Among various flaviviruses tested, the greatest homology of NS 1 seems to be that of the YF virus - at least in individuals immunized with YV vaccines, an attenuated vaccine which replicates in vaccinees where NS 1 is expressed during the virus replication.

Future studies with more potentially cross-reacting samples will show the robustness and specificity of this new assay. The current knowledge about the persistence of NS 1 antibodies is yet incomplete and more detailed studies are necessary to draw conclusions about the long-term persistence of NS 1 antibodies.

Literature

Albinson et al.

Multi-laboratory evaluation of ReaScan TBE IgM rapid test, 2016-2017 *Euro Surveill*. 2020; 25(12): pii=1900427. doi.org/10.2807/1560-7917.ES.2020.12.1900427

Girl et al.

Tick borne encephalitis virus nonstructural protein 1 IgG enzyme-linked immunosorbent assay for differentiating infection versus vaccination antibody responses

J. Clin. Virol. 2020, 58e01783-19. doi.org/10.1128/JCM.01783-19

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