Polish Journal of Microbiology
2016, Vol. 65, No 1, 119–121

SHORT COMMUNICATION

IgG Avidity: an Important Serologic Marker for the Diagnosis of Tick-Borne Encephalitis Virus Infection

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Submitted 20 February 2015, revised 28 August 2015, accepted 28 August 2015

Abstract

A total of 52 serum samples from patients with symptoms suggestive of tick-borne encephalitis virus (TBEV) infection and positive IgM and/or IgG antibodies were tested for IgG avidity. Acute/recent TBEV infection was confirmed by low/borderline avidity index (AI) in 94.8% IgM positive/IgG positive samples, while in 5.2% high AI was found indicating persisting IgM antibodies. Majority of IgM negative/IgG positive samples (78.6%) showed high AI consistent with past TBEV infection. However, in 21.3% patients without measurable IgM antibodies current/recent infection was confirmed by AI. IgG avidity represents an additional serologic marker that improves diagnosis of TBEV, especially in cases of atypical antibody response.

Key words: IgG avidity, serology, tick-borne encephalitis virus

Tick-borne encephalitis virus (TBEV) is a small, enveloped virus that belongs to the family *Flaviviridae*, genus *Flavivirus*, tick-borne encephalitis serocomplex. There are three subtypes of TBEV: the European, the Far Eastern and the Siberian subtype which differ in geographical distribution, tick vector and clinical manifestation of disease (Lindenbach *et al.*, 2007). TBEV cause a wide spectrum of symptoms, from a subclinical course to aseptic meningitis, encephalitis, myelitis and radiculitis (Bogovic *et al.*, 2010). Infections caused by European type usually take a biphasic course (~75%). The first phase presents as a nonspecific influenza-like illness. After an afebrile and relative asymptomatic period, the second phase occurs with symptoms of central nervous system (CNS) (Mansfield *et al.*, 2009). Reverse-transcriptase polymerase chain reaction (RT-PCR) can be of diagnostic value in the first viremic phase of infection (Saksida *et al.*, 2005). As the patients usually seek medical assistance when neurologic symptoms develop, diagnosis of TBEV is most commonly performed by serological methods, usually enzyme-linked immunosorbent assay (ELISA) (Holzmann, 2003; Niedrig *et al.*, 2010; Donoso-Mantke *et al.*, 2011). IgM antibodies appear within six days of illness, and usually decline within few months. An early diagnosis of TBEV confirmed by detecting IgM is sometimes questionable, since IgM antibodies can persist for more than 9 months in some vaccinees or individuals who acquired the infection naturally (Stiasny *et al.*, 2012). This may lead to a misinterpretation of the serological results in case of another CNS disease within this time period (Holzmann, 2003; Kleiter *et al.*, 2007). In addition, in some patients with TBEV infection, the IgM response may be delayed or weak. IgG avidity determination could be helpful to prove or exclude a recent TBEV infection in these patients (Gassman and Bauer, 1997). The term avidity denotes the affinity of IgG antibodies to bind the antigen. IgG antibodies are of low specificity after primary antigenic challenge which results in low avidity, but increases progressively thereafter within few months (high avidity) (Fox *et al.*, 2006).

We analyzed the value of IgG avidity determination in diagnosis of TBEV infection.

From 2012–2014, a total of 52 serum samples from hospitalized patients with clinical symptoms suggestive of TBE (meningitis/meningoencefalitis) and

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serologically confirmed current or previous TBEV infection were tested for IgG avidity. Majority of them were from northwestern Croatia, an endemic area for TBE (Golubić and Dobler, 2012). TBEV IgM and IgG antibodies were detected using a commercial ELISA test (Euroimmun, Lübeck, Germany). IgG avidity was determined by ELISA test using urea as a denaturing agent (Euroimmun, Lübeck, Germany). The IgG avidity index (AI) was calculated and expressed as percentage by dividing the OD values with and without urea treatment and interpreted as follows: <40% low AI indicating acute primary infection; 40–60% borderline AI indicating recent infection; >60% high AI indicating past TBEV infection.

According to IgM/IgG results, samples were divided into two groups as follows: I – IgM positive/IgG positive samples indicating acute/recent TBEV infection (N=38; 73.1%), II group – IgM negative/IgG positive indicating past TBEV infection (N=14; 26.9%).

Using IgG avidity, 39 (75.0%) patients are classified as having current/recent TBEV infection while in 13 patients (25.0%) a previous TBEV infection was documented. Avidity indices for each patient are presented in the Figure 1.

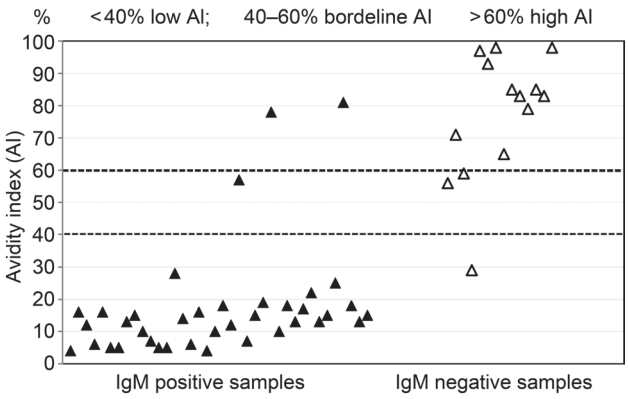

Fig. 1. Avidity indices in 52 patients with current/previous tick-borne encephalitis virus infection.

Figure 2 shows AI according to the IgM/IgG results. Current/recent TBEV infection was documented by low AI in 35/38 (92.1%) patients with positive IgM antibodies and 1/14 (7.1%) patients with negative IgM antibodies. In addition, one patient with positive IgM (2.6%) and two patients with negative IgM (14.2%) showed borderline AI (57%, 56%, and 59% respectively) consistent with recent TBEV infection. In 2/38 patients (5.2%) with positive IgM antibodies and 11/14 patients (78.6%) with negative IgM antibodies, high AI was found consistent with past TBEV infection.

Detection of IgM antibodies is usually considered as a marker of acute/recent infection. However, virus serology based on IgM detection alone may lead to misdiagnosing of acute infection since IgM antibodies may exhibit irregular patterns (Holzmann, 2003). IgM antibodies may be long-persisting for several months after primary TBEV infection which could result in a false-positive diagnosis of acute infection. Since cross-reactions are common in flavivirus infections, especially among viruses within the same serocomplex, IgM antibodies may be cross-reactive, induced by other flaviviruses (Stiasny *et al*., 2013). In addition, IgM antibodies may be detectable for a very short period or at low titers and thus lead to a false exclusion of current TBEV infection (Gasmann and Bauer, 1997; Stiasny *et al*., 2012). The monitoring of an increase of IgG titers in paired serum sample two weeks later is rarely carried out, since for many patients only one sample is available for testing. Avidity assays have been used for the diagnosis of some flaviviruses such as dengue virus (Prince *et al*., 2011) and West Nile virus (Levett *et al*., 2005; Fox *et al*., 2006). To our knowledge, there is only one published study in 1997 which analyzed the IgG avidity in diagnosis of TBEV infection (Gassmann and Bauer, 1997).

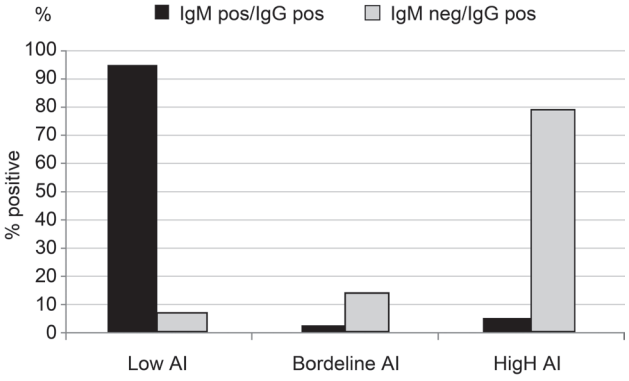

Fig. 2. IgG avidity index according to the IgM/IgG results.

In this study, a very high proportion of IgM positive samples (94.8%) showed low/borderline AI indicating current/recent infection. Small proportion IgM positive patients (5.2%) showed high AI indicating persisting TBEV IgM antibodies unrelated to a current infection. In addition, some other clinical situations can be related to the presence of IgM antibodies such as polyclonal stimulation or cross-reactive IgM due to other antigens which could be recognized by IgG avidity (Holzmann, 2003).

Majority of IgM negative patients (78.6%) showed high AI consistent with past TBEV infection. However, serological testing for IgM/IgG fails to detect current/recent infection in 21.3% patients with no measurable IgM antibodies. This percentage is even higher than that reported from Gassman and Bauer (1997) who detected acute TBEV infection in 11.1% IgM negative patients. In these cases, current or recent infection could be recognized only by demonstration of low/bor-

derline AI. These findings highlight the importance of IgG avidity testing not only in IgM positive/IgG positive but also in IgM negative/IgG positive patients.

The results of this study showed that 9.6% of patients with atypical serologic response were probably incorrectly classified based on the routine serology (TBEV IgM/IgG result). Incorrect classification was confirmed in both IgM positive and IgM negative group. Using IgG avidity, 21.3% IgM negative patients were diagnosed as having current/recent TBEV infection and 5.2% of IgM positive patients as having past TBEV infection. Since clinical symptoms of TBE resemble some other CNS diseases which may require treatment, rapid and accurate TBEV diagnosis is of a particular importance.

In conclusion, presented results confirmed IgG avidity as an additional serologic marker that improves diagnosis of TBEV, especially in cases of atypical antibody response. Combination of IgM and IgG avidity seems to be highly helpful for reliable diagnosis of TBEV infection in both IgM positive and IgM negative patients. Our results highlight the need for IgG avidity testing in the routine TBEV serology.

Acknowledgments

Authors thank Snjezana Artl and Ljiljana Milasincic for technical assistance.

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