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Diagnosis of tick-borne encephalitis

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Abstract

The actual diagnosis of a tick-borne encephalitis (TBE) must be established in the laboratory because of the non-specific clinical features it presents. The method of choice is the demonstration of specific IgM- and IgG-serum antibodies by enzyme-linked immuno-sorbent assay (ELISA), since these antibodies are detectable in practically every case at the time of hospitalization. Early after onset of disease in the cerebrospinal fluid specific antibodies can only be found in 50% of the patients, but by the 10th day of illness they almost invariably become detectable. If an infection occurs after and despite the post-exposure administration of a specific immunoglobulin the seroconversion can be delayed and may cause diagnostic problems. Virusisolation from the blood, or the detection of specific nucleic acid in the blood or the cerebrospinal fluid by reverse-transcriptase polymerase chain reaction (RT-PCR) usually is only successful during the first viremic phase of the disease before seroconversion. In fatal cases, the virus can be isolated or detected by RT-PCR from the brain and other organs. For testing immunity after a TBE virus infection or after vaccination, most often the IgG ELISA is used. However, in cases of other flavivirus contacts (e.g. vaccinations against yellow fever or Japanese encephalitis; dengue virus infections), the performance of a neutralization assay is necessary for assessing immunity due to the interference of flavivirus cross-reactive antibodies in ELISA and hemagglutination inhibition (HI) test.

Keywords: Laboratory diagnosis; Flaviviruses; Tick-borne encephalitis

1. Introduction:

Tick-borne encephalitis (TBE) typically takes a biphasic course (Fig. 1). After a short incubation period, the first phase presents as an uncharacteristic influenza-like illness, which may be followed by an asymptomatic interval. In the second phase, CNS symptoms occur. This is most often the point in time, when a patient—with high fever and severe headache—goes to see a physician or is admitted to the hospital, and the diagnostic procedure to identify a tick-borne encephalitis starts.

At the beginning, a detailed anamnesis helps to include TBE into the differential diagnosis. Important questions are therefore whether the patient has visited a TBE virus endemic area within the last 4 weeks, and whether he/she remembers a tick bite 1–3 weeks before the onset of the clinical symptoms. Concerning the last question, it has to be kept in mind that only 50–60% of the TBE patients have recognized the tick bite. Since the virus can also be transmitted via the milk of infected animals, also milk-borne TBE is possible after the consumption of unpasteurized milk or milk-products from animals, and especially goats. This means of transmission has to be considered particularly in cases of local epidemics. Additionally, for a complete anamnesis (and for a correct interpretation of the test results that follow), the questions have to be included whether and when the patient has been vaccinated against TBE or other flaviviruses such as yellow fever or Japanese encephalitis virus. This is important, because TBE virus is antigenically closely related to other flaviviruses, and cross-reactive antibodies can interfere in certain test systems (see below).

2. Diagnosis of TBE

2.1. Clinical and laboratory findings

Clinically, the patient can present with signs of a meningitis, meningoencephalitis, or meningoencephalomyelitis/radiculitis, clinical pictures which are associated with a number of different neurological disorders including herpes encephalitis. Cerebospinal fluid (CSF) findings, which support, but do not prove the diagnosis TBE are a moderate pleocytosis (100–300 cells/μl) with a possible predominance

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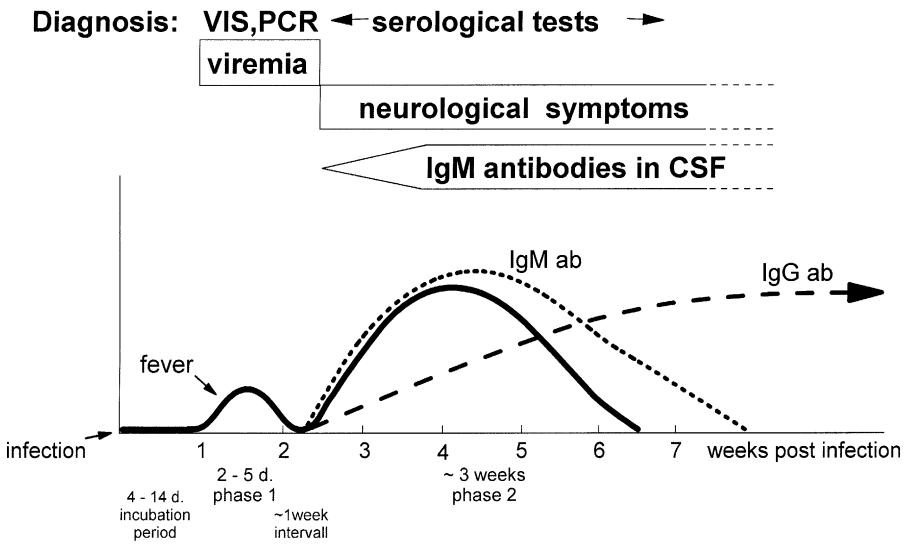

Fig. 1. Biphasic course of a TBE virus infection: detection of the virus or viral nucleic acids and development of specific antibodies (ab) in serum and cerebrospinal fluid (CSF). VIS, virus isolation; PCR, polymerase chain reaction [7].

of segmented granulocytes (60–70%) over lymphocytes (30–40%), a moderate impairment of the blood–CSF barrier (increased CSF/serum albumin ratio), and intrathecal synthesis of immunoglobulins, predominantly of IgM [13–15]. Magnetic resonance imaging (MRI), and regional cerebral blood flow investigations using single photon emission computed tomography (SPECT) are of limited value only [6,13].

2.2. Specific laboratory diagnosis

As the clinical features of TBE are non-specific, the actual diagnosis must be established in the laboratory. In principle, the virus can be isolated from the blood during the first, viremic phase of the disease, or virus-specific nucleic acid can be detected by reverse-transcriptase-polymerase chain reaction (RT-PCR) within this period. Also, in the fortunately rare fatal cases, the virus can be identified in CNS by electron microscopy [17], or isolated or detected by RT-PCR from the brain and other organs. However, in practice these techniques are of minor importance since admission to the hospital usually takes place in the second phase of the disease, when the neurological symptoms become manifest. At this time point, the virus has already been cleared from the blood (and the cerebrospinal fluid), and a humoral immune response has started. Attempts to use the very sensitive RT-PCR method to trace TBE virus RNA during the acute phase from CSF or serum have, to a large extent, been unsuccessful with only a few samples having been found positive in patients before seroconversion, or in the IgM-positive, but IgG-negative phase, i.e. in cases which are very rare [5,18]. This means in practice, in contrast to many other viral infections including those with other flaviviruses, the PCR method is not very useful for the laboratory diagnosis of TBE. Therefore, the diagnosis of TBE is based on the demonstration of specific antibodies, which are usually detectable at the beginning of the second phase, and which rapidly rise to high titers (see Fig. 1). Prior to the 1980s, paired sera were tested for a rise in titer in complement fixation [19], and hemagglutination inhibition (HI) tests [1,16]. Specific, acute phase IgM antibodies for the rapid diagnosis of TBE could first be detected using the 2-mercaptoethanol (2-ME) reduction method in HI tests [16]. This test was based on the fact that 2-ME is capable of inactivating antibodies of the IgM-type. In the early acute phase sera tested in the HI test prior to and after treatment with 0.05 M 2-ME, demonstrated a drop in HI titer. Nowadays enzyme-linked immuno-sorbent assays (ELISAs) are the method of choice for the rapid detection of TBE-specific IgM- and IgG-antibodies in serum and CSF. For the IgM ELISA a format has to be chosen that avoids non-specific positive results, e.g. due to the interference of rheumatoid factor or heterophile antibodies, as it is the case when the μ-capture technique is used [5,8,10].

In the majority of patients who come down with manifest disease, TBE-specific IgM- and IgG-antibodies can be detected in the first serum sample. In the cerebrospinal fluid, however, specific antibodies can only be found in 50% of these patients, but within 10 days after onset of illness they almost invariably become detectable. In the very rare case that only IgM-antibodies can be detected in the first serum sample, this result has to be confirmed in a second one, because the demonstration of IgM-antibodies alone does not suffice to establish a diagnosis. IgM-antibodies may be detectable in serum for several months after infection, whereas IgG-antibodies persist for the whole life and mediate an immunity that prevents reinfection (Table 1).

Table 1
Virological interpretations of serological test results in case of a clinically suspected TBE

Serological test result	Interpretation
Specific IgM- and IgG-positive	Proven TBE virus infection, provided that long-persisting IgM-antibodies due to a 1st or 2nd vaccination within the previous months can be excluded
Specific IgM- and IgG-negative	Testing of a control sample necessary
Specific IgM-positive, IgG-negative	Suspected TBE, testing of a control sample necessary
Specific IgM-negative, IgG-positive	Immunity/successful vaccination; Exceptions: passive immunization, vaccination failure, cross-reactive antibodies

2.3. Special diagnostic problems and pitfalls

2.3.1. Infection despite hyperimmunoglobulin administration

If an infection occurs after and despite the post-exposure administration of a specific immunoglobulin, the immune response (seroconversion) can be delayed. In this case, it is possible that at the beginning of the neurological disease no TBE-specific antibodies can be found in the first serum sample, or they are only weakly positive, two results which make the testing of additional serum samples (controls) necessary.

2.3.2. Vaccination failure

TBE vaccination failures are fortunately extremely rare, but if they do occur, the serodiagnosis may be difficult. In some of these patients the serological pattern is similar to the non-vaccinated patients. However, sometimes only serum IgG-antibodies are detected first, and they increase rapidly, followed by a delayed formation of IgM-antibodies. Therefore, if a TBE is clinically suspected in a patient with a vaccination anamnesis, who has high titers of specific IgG-antibodies in the first serum sample, a second one should be taken after about 10 days and tested for the presence of IgM-antibodies in order to exclude a vaccination failure. Other tools for the verification of a vaccination failure are the detection of an intrathecal antibody response (discriminated from serum antibodies due to a compromised blood–brain barrier), or avidity measurements of TBE IgG activity [4,5,9,10].

2.3.3. Long-persisting IgM-antibodies

It also has to be considered that after a TBE infection as well as after the first two vaccinations, TBE-specific IgM-antibodies may be detectable for several months [8]. This may lead to a wrong interpretation of the serological results in case of another CNS disease or infection within this time period (in the summer months, e.g. enterovirus infections are most common). Therefore, like in the cases of a suspected vaccination failure, in the CSF a differentiation has to be made between locally produced IgM-antibodies, and a leakage of the blood–brain barrier. And again, in some of these cases IgG avidity tests are helpful to prove or exclude a recent TBE virus infection [12].

2.3.4. Determination of immunity

For testing immunity after a TBE virus infection or for monitoring the immune response after TBE vaccination, the IgG ELISA is normally used, due to its simplicity and ease of automation. This test can be rapidly performed in a quantitative format using purified virus as an antigen [8]. It has to be kept in mind that with this method, TBE virus-binding antibodies are detected, but this is not necessarily predictive for the presence of protective, virus-neutralizing antibodies. As TBE virus, a member of the genus flavivirus, shares common antigenic sites within its E protein with several other flaviviruses that infect humans, the interference of cross-reactive, but non-neutralizing antibodies have to be considered. However, it has been demonstrated that there is an excellent and highly significant correlation between ELISA IgG units and the antibody titers obtained by HI or neutralization test (NT), provided that there was no other exposure to flavivirus antigens except TBE virus/vaccination [11]. Therefore, the level of IgG-antibodies determined by ELISA is a good marker for predicting the presence of neutralizing antibodies against TBE virus, but only in persons without a history of other flavivirus vaccinations or infections (Fig. 2). Nevertheless, laboratories have to be cautious about confirming a protective immunity when the ELISA results are only weakly positive.

2.3.5. Flavivirus cross-reactive antibodies

A major obstacle for using the level of ELISA antibodies as a surrogate marker for neutralizing antibodies are cross-reactive antibodies induced by other flavivirus infections/vaccinations [11]. These are able to interfere in the IgG ELISA as well as in the HI test, but they do not mediate protection. This is an increasing problem in Europe, because the popularity of travel to tropical and subtropical countries where other flaviviruses are endemic has resulted in a growing number of individuals being vaccinated against yellow fever or Japanese encephalitis and in the increased likelihood of travellers acquiring other virus infections such as dengue or West Nile encephalitis. Additionally, there is the problem of the “original antigenic sin phenomenon” with an increase of heterologous antibodies before the development of a homologous TBE antibody response in vaccinees with a history of previous flavivirus contacts [2,3,11]. Therefore, in cases of other flavivirus contacts, the performance of a

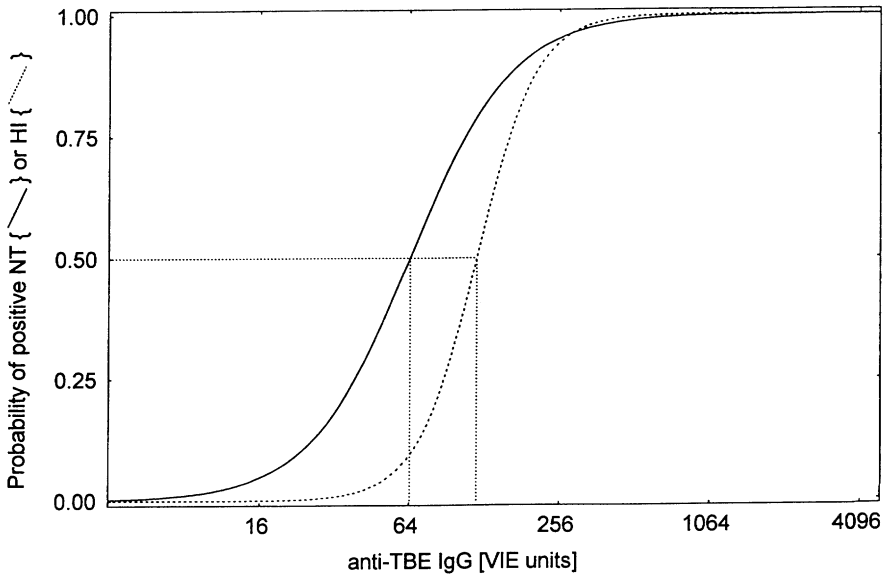

Fig. 2. Probability of positive neutralization- and hemagglutination inhibition test results in relation to ELISA IgG units [11].

neutralization assay, the most type specific serological test, is necessary to assess immunity. The NT is also required to confirm positive ELISA results obtained in serological surveys conducted in areas where TBE virus has not been previously shown to be endemic. Due to the use of infectious virus, the NT on cell cultures can only be performed in special laboratories, and it is a technically difficult and expensive test. Different formats have been described, e.g. antigen detection in the cell supernatant using a four-layer ELISA [11], a plaque reduction neutralisation test (PRNT), or a rapid fluorescent focus inhibition test (RFFIT) [20].

Although commercially available, the CF test should no longer be used for the diagnosis of an acute TBE virus infection due to the low sensitivity, and it is of no value for the determination of an existing immunity.

Unfortunately, no international standards for the TBE diagnosis exist.

3. Summary and conclusions

The actual diagnosis of a TBE virus infection must be established in the laboratory because of the uncharacteristic clinical picture it presents. In principle, it is possible to isolate the virus from the blood or to detect the viral nucleic acid using RT-PCR during the first viremic phase. However, in practice this is of minor importance since admission to the hospital usually takes place in the second phase of the disease, when the neurological symptoms become manifest. At this time point, the virus has already been cleared from the blood (and the cerebrospinal fluid), and specific IgM- and IgG-antibodies are formed, which rapidly rise to high titers. Since these antibodies are detectable in practically every case at the time of hospitalization the demonstration of specific IgM- and IgG-serum antibodies by ELISA is the method of choice for the specific diagnosis of TBE. IgM-antibodies may be detectable for several months after infection, whereas IgG-antibodies persist for the whole life and mediate an immunity that prevents reinfection. In the cerebrospinal fluid, shortly after onset of the disease, specific antibodies can only be found in 50% of the patients, but by the 10th day of illness they almost invariably become detectable. If an infection occurs after and despite the post-exposure administration of a specific immunoglobulin the seroconversion can be delayed and may cause diagnostic problems. In fatal cases, the virus can be isolated or detected by RT-PCR from the brain and other organs.

Successful vaccination can be easily monitored by the demonstration of specific IgG-antibodies in the serum by ELISA. However, there is a potential for misleading results, because all flaviviruses are serologically related and infections or vaccinations with one flavivirus will give rise to antibodies that also react with all other flaviviruses in ELISAs. Vaccinations against yellow fever, Japanese encephalitis or infections with dengue viruses will thus result in cross-reactive but non-neutralizing antibodies yielding a positive result in TBE ELISAs. In such cases, the specific immunity against TBE virus can only be assessed in a virus neutralization assay.

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