Intrathecal antitreponemal antibody synthesis determination using the INNO-LIA™ Syphilis Score

V. Kotnik, K. Jordan, S. Stopinšek, S. Simčič, and M. Potočnik

ABSTRACT

Background: Laboratory detection of intrathecal synthesis of specific antitreponemal antibodies remains a challenge. Traditional syphilis serology is unable to provide a satisfactory result; therefore, several other diagnostic procedures were used to demonstrate central nervous system (CNS) involvement in this disease. The introduction of molecular methods makes today's laboratory testing easier.

Objective: Our study used a new commercially available test, the INNO-LIA™ Syphilis Score, intended for use on serum samples, to detect specific antitreponemal antibodies in the cerebrospinal fluid (CSF) of patients with the tertiary stage of syphilis.

Patients and methods: We tested 26 patients suspected of neurological complications of late syphilis with conventional immunological tests such as VDRL-RPR, TPHA, FTA-ABS IgG, FTA-ABS-IgM, and the molecular INNO-LIA™ Syphilis Score test for the presence of nontreponemal and treponemal antibodies. All tests were performed simultaneously in serum and CSF. The test results were evaluated with descriptive statistics and the probability was tested with an ANOVA test.

Results: All 26 samples of serum were LIA-S (line immune assay in serum) positive and presented anticardiolipin and antitreponemal antibodies in high titer. Seventeen samples of CSF were LIA-L (line immune assay in liquor) positive and nine were LIA-L negative. Anticardiolipin and antitreponemal antibodies were detected only in the group of LIA-L positive samples. Anticardiolipin antibodies were present in two cases, antitreponemal (TPHA) in five cases, specific IgG (FTA-ABS IgG) in six cases, and specific IgM (FTA-ABS IgM) in one case. Six patients with antitreponemal antibodies in CSF presented with pathologic albumin index, two with a milder form, and four with a severe form. Two had a pathological IgG index and four a pathological IgM index. Altogether, two of the patients had laboratory signs of neurosyphilis.

Conclusions: Detecting anticardiolipin and antitreponemal antibodies in CSF in patients with a late form of syphilis is laborious. Using the new INNO-LIA™ Syphilis Score molecular test, we were able to identify patients with silent neurosyphilis together with patients with active intrathecal synthesis of IgG antibodies. The development of a new generation of tests for the detection of specific antitreponemal antibodies in CSF offers a valuable tool for discovering possible CNS involvement in syphilis.

KEY WORDS

neurosyphilis, line immune assay, intrathecal, immunoglobulin

Introduction

Detection of specific cerebrospinal fluid (CSF) antibodies in patients with suspected neurosyphilis remains a challenge for immunological laboratories as well as for clinicians (1). Today, in a developed country with good medical education of the general population and functioning health care institutions, only a few cases of syphilis are detected per year, and this is therefore considered a disease of marginal concern (2). However, these few cases are of enormous importance for public health because of the possible uncontrolled spread of the disease and serious complications for patients if the disease is overlooked. One of the most important late complications of syphilis is neurosyphilis (3–6). If syphilis is not treated or is treated inappropriately, a chronic form of the disease may develop years after infection. Neurosyphilis is a consequence of inflammation caused by treponemas and their metabolic products passing the blood-CSF barrier. Inflammatory changes together with specific antitreponemal antibodies found in the CFS are important for diagnosing neurosyphilis (7). Because of the infrequency of neurosyphilis today, there are few data on the adequacy of modern methods for the detection of specific antitreponemal antibodies in CSF. In recent years, immunologic laboratories qualified for syphilis screening have developed a new molecular method (LIA, line immune assay) suitable for the detection of small quantities of specific antibodies against *Treponema pallidum* in serum or plasma (8, 9). We used this test to detect antitreponemal antibodies in the CSF of patients suspected of neurosyphilis.

Materials and methods

Subjects

Samples of serum and CSF from twenty-one men (31 ± 11 years old) and five women (29 ± 9 years old) were examined in a special laboratory for advanced diagnosis of syphilis (Institute of Microbiology and Immunology, Medical faulty Ljubljana, University Ljubljana, Laboratory for cellular immunology, and diagnostics of complement system and syphilis). All 26 study subjects were patients at the Department of Dermatovenereology, University Medical Center diagnosed with latent syphilis and were admitted for medical survey and then routinely tested for the presence of anticardiolipin and antitreponemal antibodies in serum. Because neurological complications of the disease were suspected, lumbar puncture was performed and a CSF sample collected.

Laboratory tests used for screening CSF

VDRL-RPR (venereal disease research laboratory, rapid plasma regain) tests (RPR-nosticon®II, Ref. 280446, bioMerieux, France) were used to detect anticardiolipin antibodies relevant to treponemal infection. The principle of the tests is the precipitation of lipid (cardiolipin) antigen in the presence of appropriate antibodies in a sample. The qualitative method was used to screen for the presence of antibodies; the quantitative method was used to follow up the effects of antibiotic treatment.

Table 1. Descriptive statistics with ANOVA calculations on serum and CSF anticardiolipin and specific antitreponemal antibodies in samples from patients suspected of neurosyphilis.

			LIA positive		**LIA negative**		**ANOVA**
Sample	Test	Units	Mean	*SD*	Mean	*SD*	*p*
CSF and serum	Albumin Q	mg/l/mg/l	7.83	5.4	4.23	1.33	**0.0475**
	IgG i	mg/l/mg/l	0.64	0.75	0.44	0.06	0.4439
	IgM i	mg/l/mg/l	0.29	0.72	0.04	0.01	0.3143
CSF	VDRL-RPR	AU	0.12	0.33	0.00	0.00	0.3031
	TPHA	titer × 10^{-1}	689.41	2480.30	0.00	0.00	0.4170
	FTA-ABS IgG	titer × 10^{-1}	690.29	2480.04	0.00	0.00	0.4164
	FTA-ABS IgM	titer × 10^{-1}	0.47	1.94	0.00	0.00	0.4781
	LIA-L	AU	5.59	5.15	0.17	0.08	**0.0046**
Serum	VDRL-RPR	AU	0.76	0.44	0.56	0.53	0.2902
	TPHA	titer × 10^{-1}	7661.18	10231.54	466.66	793.98	**0.0478**
	FTA-ABS IgG	titer × 10^{-1}	6400.53	6428.28	848.89	1635.33	**0.0186**
	FTA-ABS IgM	titer × 10^{-1}	16.00	21.66	11.56	15.55	0.6238
	LIA-S	AU	12.38	3.00	8.39	3.10	**0.0039**

CSF = cerebrospinal fluid, SD *= standard deviation, LIA-L = line immune assay in CSF (liquor), LIA-S = line immune assay in serum, boldface = statistically significant, i = index, Q = Quotient*

TPHA: *Treponema pallidum* hemagglutination tests (BAG-TPHA-Test, Ref. 6040, Biologische Analysensystem GmbH, Germany) were used to detect specific antitreponemal antibodies. The principle of the test is the agglutination of erythrocytes coated with specific *Treponema pallidum* (Nichols strain) antigens with specific antitreponemal antibodies present in the sample. The test was performed as the quantitative variant.

FTA-ABS IgG: A fluorescent antitreponemal IgG antibody absorption test was used as one of the most sensitive tests available for the detection of specific antitreponemal antibodies in serum and CSF. The samples were treated with special absorbent containing *Treponema reiterii* antigens (ABS-FTA, Ref 75; 661, bioMerieux, France). In this way unspecific treponemal antibodies were removed. Then the absorbed sample was used in the assay. The principle of the test is the detection of antitreponemal antibodies bound to *Treponema pallidum* (Nichols strain) microorganisms adhered to glass slides (Trepo-Spot IF, Ref. 75; 681, bioMerieux, France). With antihuman IgG FITC labeled secondary antibodies, a specific human IgG bound to microorganisms was detected (Fluoline G, Ref. 75; 692, bioMerieux, France). The test was performed as a qualitative and quantitative variant.

FTA-ABS IgM: The fluorescent antitreponemal IgM antibody absorption test was used as one of the most sensitive tests available for the detection of early specific antitreponemal antibodies in serum and CSF. The samples were first treated on an affinity column to remove the IgG fraction of serum or CSF immunoglobulines (Mini Rapi-Sep™-M, Panbio, Maryland, USA), and then treated with a special absorbent containing *Treponema reiterii* antigens (ABS-FTA, Ref 75; 661, bioMerieux, France). In this way unspecific treponemal antigens were removed. Then the absorbed sample was used in the assay. The principle of the test is the detection of antitreponemal antibodies bound to *Treponema pallidum* (Nichols strain) microorganisms adhered to glass slides (Trepo-Spot IF, Ref. 75; 681, bioMerieux, France). With antihuman IgM FITC labeled secondary antibodies, a specific human IgM bound to microorganisms was detected (Fluoline M, Ref. 75; 672, bioMerieux, France). The test was performed as qualitative and quantitative variant.

INNO-LIA™ Syphilis Score: The INNO-LIA™ Syphilis Score (Ref. 80542, Innogenetics N.V., Belgium) is a molecular technique designed for testing antibodies against several specific treponemal antigens. Three of these are recombinant proteins (TpN47, TpN17, and TpN15) and one is a synthetic peptide (TmpA). These antigens are lined up on a paper strip together with control lines. If the paper strip is soaked with serum, plasma, or liquor containing antibodies against the antigens bound, the resulting immune complexes are discovered through a specific enzymatic reaction.

Sample preparation, test procedure, reading of results, and validation

CSF samples were diluted 1:100 in test troughs and incubated at room temperature overnight on an orbital shaker, followed by three washing steps with a washing buffer before the addition of goat anti-human IgG conjugated to alkaline phosphatase. After a 30-minute incubation on the orbital shaker, three washing steps were performed again, followed by the addition of a substrate solution containing 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium. After a 30-minute incubation on the orbital shaker, color development was stopped with a stop solution containing 0.1 mol/l sulfuric acid. After a 10-minute incubation on the orbital shaker, the strips were removed from the test troughs and placed on absorbent paper to dry.

In a visual reading protocol, each line was compared to the control line, and the intensities were evaluated as follows: for no line or a line less intense than the cutoff line (score 0); for a line with an intensity of the cutoff line (score 1); for a line with an intensity higher than that of the cutoff line, but lower or equal to that of the control line (score 2); for a line with an intensity between that of the 1st control line and that of the 2nd control line (score 3); 2nd for a line equal to that of the 2nd control line (score 4); and for a line with an intensity greater than that of the 2nd control line (score 5).

IgG, IgM indexes, and albumin quotient

The intrathecal IgG and IgM production and blood-CSF barrier permeability were quantitated by CSF/serum IgG and IgM indexes, and albumin quotient. For this purpose, albumin, IgG, and IgM were measured by nephelometry using a BN ProSpec nephelometer (Dade Behring, Germany) in matched serum and CSF pairs using commercially available kits (Dade Behring, Germany). The IgG index was calculated according to the following formula: (CSF IgG (mg/l) × serum albumin (mg/l)) / (serum IgG (mg/l) × CSF albumin (mg/l)). An IgG index above 0.7 was indicative of an intrathecal IgG synthesis (10, 11). Similarly, the IgM index was calculated according to the following formula: (CSF IgM (mg/l) × serum albumin (mg/l)) / (serum IgM (mg/l) × CSF albumin (mg/l)). An IgM index above 0.1 was indicative of an intrathecal IgM synthesis (10, 12). The albumin quotient was calculated according to the following formula: (CSF albumin (mg/l)/ serum albumin (mg/l)) × 1000. An albumin quotient above 7.8 was indicative of a blood-CSF barrier dysfunction (13). In addition, the serum and CSF IgG, IgM, and albumin results were also interpreted by Protis (Results Interpretation Software, Dade Behring, Germany). The Protis CSF assessment supported us with a graphic results presentation using Reiber diagrams (7).

Results

Twenty-six patients with a history of syphilis and suspected of having neurological complications of the disease were tested for the presence of antitreponemal antibodies in CSF. The samples of CSF and serum were simultaneously tested with conventional tests such as VDRL-RPR, TPHA, FTA-ABS IgG, and FTA-ABS IgM together with the INNO-LIA™ Syphilis Score. The INNO-LIA™ Syphilis Score in CSF was expected to be the test able to detect minute amounts of antitreponemal antibodies in CSF, and therefore relevant for discriminating patients with neurosyphilis from patients without neurological complications. All patients were also tested for signs of inflammation in the cerebrospinal space or disturbance of the blood-CSF barrier. Albumin quotient, and IgG and IgM indexes were calculated from the data obtained from the determinations of serum albumin, CSF albumin, total serum and CSF IgG, and total serum and CSF IgM. For statistical evaluation, the patients were divided into two groups, one LIA-L-positive (L for liquor = CSF) and the other LIA-L-negative. Descriptive statistics were performed on each group of data and the statistical significance of differences was calculated using an ANOVA, with $p > 0.05$ taken as significant.

All 26 patients were positive for anticardiolipin and antitreponemal antibodies in serum, detected with all the tests listed. Nine of the 26 patients examined were LIA-L–negative and 17 were LIA-L–positive in the CSF. The group of LIA-L–negative patients had 0.17 ± 0.08 AU of antitreponemal antibodies in the CSF, and in the LIA-L–positive group 5.59 ± 5.15 AU of the same antibodies was determined. The groups tested were statistically different from each other ($p = 0.046$). Other characteristics of the samples examined are listed in Table 1.

We found that the LIA-L–positive and LIA-L–negative group of patients differ from each other in the amount of specific antitreponemal antibodies. Both groups had specific antitreponemal and antilipid antibodies present in the serum. The serum concentrations of all measured antibodies are much higher then the CSF concentrations. The most important difference between both groups seems to be that the antibodies in the CSF were detectable with conventional methods only in a few cases and, if they were detected, the meaning of their presence was irrelevant for the diagnosis of neurosyphilis. On the other hand, the LIA antibodies together with pathological albumin quotient (> 7.8) were detected in four patients in the LIA-L-positive group. Two of them had also a pathological IgM index and one IgG index. In conclusion, four patients in the LIA-L group presented a possible defect of the blood-CNS barrier with an albumin quotient higher than 7.8, three patients presented a higher IgM index (> 0.1), and one a higher IgG index (> 0.7). Thus,, only two patients in the LIA-L–positive group had the required laboratory signs of neurosyphilis.

Discussion

A group of 26 patients suspected of neurological complications of late syphilis were tested for the presence of intrathecal anticardiolipin and specific antitreponemal antibodies. A battery of laboratory tests (VDRL-RPR, TPHA, FTA-ABS IgG, FTA-ABS IgM, and LIA) was employed. All of the patients had antitreponemal antibodies in their serum and 17 of them in their CSF as well. Four LIA-positive patients had a possible blood-CNS barrier deficit, and two of them expressed laboratory signs of neurosyphilis. None of them had clinical signs of neurosyphilis. Our study recognized the need for simultaneous testing of serum or plasma and CSF if neurological complications of syphilis are anticipated. Demonstration of specific antitreponemal antibodies in CSF is indicative of a blood-CNS barrier defect or intrathecal antibody synthesis.

As early as 1988, Walters et al. reported on the problem of intrathecal synthesis of anticardiolipin and specific antibodies in CSF in patients with suspected neurosyphilis (12). In their study, 203 syphilitic patients were tested for the presence of anticardiolipin antibodies (VDRL), raised CSF cell count and protein content, and specific antitreponemal antibody production (TPHA). To facilitate the evaluation of the results, they proposed that the calculation of immunoglobulin G and M index would be a helpful criterion for ruling in asymptomatic neurosyphilis. Moskophidids and Muller later reported that intrathecal synthesis of *Treponema pallidum*–specific IgG and IgM antibodies may be demonstrated in neurosyphilis (14). Due to atypical symptoms of central nervous system alterations during *T. pallidum* infection, the detection of specific antibodies is expected to be helpful. The sera and CSF of 302 syphilitic patients were tested with a modification of an enzyme immunoassay and specific antibodies quantitated as ELISA units per mg of total IgG or IgM. In 197 of 237 patients with neurosyphilis, an intrathecal synthesis of specific IgG and IgM antibodies could be demonstrated by a 3- to 450-fold higher antibody concentration in the CSF than in the corresponding serum. Hens et al. evaluated the occurrence and levels of intrathecal synthesis of IgG, IgM, IgA, and IgD. They examined eight patients with definite and four with suspected or possible neurosyphilis and calculated Ig indices according to the class. Four patients with active neurosyphilis displayed intrathecal synthesis of IgG, IgM, and IgA, and only two of them showed an elevated IgD index. The synthesis of IgD was connected with a possibility of severe CNS inflammation in response to diffuse treponemal damage. Penicillin therapy incited a transitory elevation of

one or more of the immunoglobulin index values in most studied patients that appeared to be a response to a massive treponemal lysis inside the CNS (15). Engohan et al. carried out a study in Gabon looking for the specific antitreponemal antibodies in the CSF of 13 children with active congenital syphilis. They used a VDRL test, TPHA test, FTA-ABS IgG, and IgM test. In 7 of the 13 children, they found positive IgG antibodies, but without correlation with the severity of the disease; the TPHA test was positive in four children and VDRL was always negative (16). Gendrel et al. reported on the same cases and concluded that a more sophisticated test would be needed, especially for the specific IgM antibodies (3). Drobocheff et al. reported the case of 63-year-old patient with tertiary cutaneous syphilis with neurological dysfunction. However, at that time tertiary tubercular syphilis was extremely rare. They were nevertheless successful in establishing a diagnosis. After treatment with penicillin, cutaneous lesions regressed, but neurological symptoms did not. In CSF, the VDRL test was negative, but FTA-ABS IgG and TPHA were positive. CSF protein was not increased, which was not in support of the assumption that the syphilitic process was active at that time (17). Gallo et al. examined the CSF of 70 patients with inflammatory and non-inflammatory neurological disease for the presence of anticardiolipin antibodies of class G and M. High levels of IgG and IgM anticardiolipin antibodies were synthesized intrathecally only in patients with neurosyphilis. Patients with other infectious or inflammatory neurological diseases very rarely showed detectable levels of anticardiolipin antibodies (5). These reports indicate how great the problem of laboratory diagnosis of neurosyphilis was at that time. Old valuable tests were insufficient for detecting minute amounts of antibodies, and there was a need for more sensitive methods that could detect specific antitreponemal antibodies in CSF.

In 1992, Sanchez et al. reported a specific IgM response to *T. pallidum* antigens (47-kd antigen) in the CSF of some infants with clinical and laboratory evidence of congenital syphilis. They used a novel immunoblotting technique for the detection of antibodies against treponemal antigens with molecular weight of 72, 59, 47, 45, 42, 37, 34, 17, and 15 kd. All of these antigens elicited specific IgG antibody production, and the antigens with molecular weight of 47, 45, and 17 kd elicited production of IgM antibodies (18). In 2000, Ebel presented a report on a new test for the detection of antitreponemal antibodies. The test is a commercially available diagnostic test for syphilis. Up to that time, serologic techniques were also shown to be cross-reactive with antibodies directed to other treponemal species. False positive reactions were known in conditions such as Lyme borreliosis, autoimmune disease, and HIV infection. Instead of natural antigens, the INNO-LIA™ Syphilis Score test uses recombinant and synthetic peptides derived from *T. pallidum* (Nichols strain). Three immunodominant proteins (TpN47, TpN17, and TpN15) expressed in *E. coli* and synthetic peptide (TmpA) are used to manufacture a line immune assay strip with appropriate control bands (8). By measuring the sensitivity and specificity, a sensitivity of 100% and specificity of 99.3% were observed (9). The INNO-LIA Syphilis test is important for discriminating diseases caused by microorganisms with very similar antigenic determinants, such as Lyme borreliosis and syphilis (19). A new improved line immunoassay was recently introduced (the INNO-LIA™ Syphilis Score, Immunogenetics, Belgium) with 100% sensitivity and 98% specificity, and with 100% reproducibility of positive and negative results. It is recommended that this system has to be used in laboratories engaged in the expert diagnosis of syphilis (20).

In 1999, Paris-Hamelin et al. reported on immunoblotting as a useful tool for serodiagnosis of syphilis. They also found their test valuable for detecting specific antitreponemal antibodies in CSF. Because of the difficulties of performing the Nelson test in places other than specialized laboratories, they proposed that highly specific and sensitive immunoblotting could be used instead of the Nelson test (21). Neurosyphilis can develop during any stage of syphilis. Neurosyphilis can be grouped into two categories: early (meningeal and meningovascular neurosyphilis) and late (progressive paralysis and tabes dorsalis). The CSF changes in neurosyphilis include elevated cell count with lymphocytic-plasmocytic cell reaction, increased protein content, strongly positive IgG index, and positive blood and CSF serology. Serological tests are often difficult to interpret. CSF examination has played a major role in the diagnosis and treatment of all forms of neurosyphilis. CSF abnormalities usually improved with clinical improvement in early forms of neurosyphilis. Improvement in paresis and tabes are slow or nonexistent (22). During inflammation, the intrathecal synthesis of immunoglobulines takes place. It has been reported that the CSF of 50% of patients with parenchymal or meningovascular neurosyphilis shows the presence of IgA synthesis and, occasionally, a concomitant IgM synthesis (23). Therefore, intrathecal IgA synthesis does not necessarily contradict the diagnosis of neurosyphilis, as was proposed by Gschnait et al. (4). Not only immunoglobulines, also other products of immunity are found in CSF. Complement protein isoforms are possible biomarkers for neurodegenerative diseases and neurosyphilis (24).

Syphilis and HIV infection affect similar groups of patients and co-infection is common. Syphilis may present with atypical features in HIV-positive patients. There is often a high rate of asymptomatic primary syphilis and, proportionally, more HIV-positive patients present with secondary disease. Secondary infection may be more aggressive and there is an increased rate of early CNS and ophthalmic involvement (25). All HIV-

positive patients should be considered for evaluation of neurosyphilis. A lumbar puncture should be always performed when a serum RPR of 1/32 or more is present (6). Patients with latent syphilis or syphilis with unknown duration of the disease sometimes present with the tertiary disease or neurosyphilis. In these patients, a VDRL test is important for discrimination of CNS involvement. Not a single patient with neurosyphilis in the study by Wohrl et al. was VDRL-negative in serum and the median titer was significantly higher in patients with neurosyphilis than in those without. Hence, neurosyphilis is very unlikely in patients with a negative blood VDRL. Lumbar puncture is not recommended in these patients (26).

We can conclude that laboratory diagnosis of neurosyphilis remains laborious. Effective guidelines are welcome for making examination protocols clear and understandable. The MMWR recommendation reports are valuable sources of such information and should be followed, but also improved with relevant clinical and laboratory research data (1, 2, 27–30). The new INNO-LIA™ Syphilis Score molecular test is of great value in determining intrathecal synthesis of specific antitreponemal antibodies and provides new insight into the evolution of the specific CNS changes during late syphilis.

Acknowledgement

We would like to thank Maja Mrgole, Jelka Markelj, and Petra Simić for their expert technical assistance and Marija Šimenc for conscientious collection of the clinical samples. Part of this study was funded by the Slovenian Research Agency (Grant no. P3-0083/381-029).

REFERENCES

1. Lautenschlager S. Diagnosis of syphilis: clinical and laboratory problems. J Dtsch Dermatol Ges. 2006;4:1058–75.

2. Klavs I, Bergant N,Kustec T, Kastelic Z. Sexually transmitted infections in Slovenia in the year 2006 - annual report. From http://www.ivz.si/javne_datoteke/datoteke/930-porocilo_1.pdf

3. Gendrel D, Mefane C, Nardou M, Moreno JL, Engohan E, Moussavou A, Nguembymbina C. Serological tests in cerebrospinal fluid for congenital syphilis in central Africa. Ann Trop Paediatr. 1992;12:273–6.

4. Gschnait F, Schmidt BL, Luger A. Cerebrospinal fluid immunoglobulins in neurosyphilis. Br J Vener Dis. 1981;57:238–40.

5. Gallo P, Sivieri S, Ferrarini AM, Giometto B, Ruffatti A, Ritter E, Chizzolini C, Tavolato B. Cerebrovascular and neurological disorders associated with antiphospholipid antibodies in CSF and serum. J Neurol Sci. 1994;122:97–101.

6. Libois A, De Wit S, Poll B, Garcia F, Florence E, Del Rio A, Sanchez P, Negredo E, Vandenbruaene M, Gatell JM, Clumeck N. HIV and syphilis: when to perform a lumbar puncture. Sex Transm Dis. 2007;34:141–4.

7. Reiber H, Peter JB. Cerebrospinal fluid analysis: disease-related data patterns and evaluation programs. J Neurol Sci. 2001;184:101–22.

8. Ebel A, Vanneste L, Cardinaels M, Sablon E, Samson I, De Bosschere K, Hulstaert F, Zrein M. Validation of the INNO-LIA syphilis kit as a confirmatory assay for Treponema pallidum antibodies. J Clin Microbiol. 2000;38:215–9.

9. Hagedorn HJ, Kraminer-Hagedorn A, De Bosschere K, Hulstaert F, Pottel H, Zrein M. Evaluation of INNO-LIA syphilis assay as a confirmatory test for syphilis. J Clin Microbiol. 2002;40:973–8.

10. Luger AF, Schmidt BL, Kaulich M. Significance of laboratory findings for the diagnosis of neurosyphilis. Int J STD AIDS. 2000;11:224–34.

11. van Eijk RV, Wolters EC, Tutuarima JA, Hische EA, Bos JD, van Trotsenburg L, de Koning GA, van der Helm HJ. Effect of early and late syphilis on central nervous system: cerebrospinal fluid changes and neurological deficit. Genitourin Med. 1987;63:77–82.

12. Wolters EC, Hische EA, Tutuarima JA, van Trotsenburg L, van Eijk RV, Bos JD, Starink TM, Emsbroek LJ, van der Helm HJ. Central nervous system involvement in early and late syphilis: the problem of asymptomatic neurosyphilis. J Neurol Sci. 1988;88:229–39.

13. Goh BT, van Voorst Vader PC. European guideline for the management of syphilis. Int J STD AIDS. 2001;12 Suppl 3:14–26.

14. Moskophidis M, Muller F. Immunology of neurosyphilis: intrathecal synthesis of Treponema pallidum–specific IgG and IgM antibodies. Immun Infekt. 1985;13:91–8.

15. Hens MJ, Lolli F, Martin-Moro M, Gimenez-Roldan S, Link H. High-dose intravenous-penicillin in neurosyphilis: effect on intrathecal synthesis of IgG, IgM, IgA and IgD. Acta Neurol Scand. 1990;82:381–5.

16. Engohan E, Mefane C, Nardou M, Moreno JL, Toure R, Moussavou A, Benoni D, Nguembymbina C, Gendrel D. Serological tests in cerebrospinal fluid in congenital syphilis in central Africa. Bull Soc Pathol Exot. 1991;84:603–9.

17. Drobacheff C, Moulin T, Van Landuyt H, Merle C, Vigan M, Laurent R. Cutaneous tertiary syphilis with neurological symptoms. Ann Dermatol Venereol. 1994;121:34–6.

18. Sanchez PJ, Wendel GD, Norgard MV. IgM antibody to Treponema pallidum in cerebrospinal fluid of infants with congenital syphilis. Am J Dis Child. (1960) 1992;146:1171–5.

19. Kotnik V, Ružiš-Sabljiš E, Potočnik M. Inno-lia syphilis and bag-borrelia-blot test may be helpful for discrimination of diagnostic dilemmas in syphilis positive and syphilis negative patients. Acta Dermatovenerol Alp Pannonica Adriat. 2002;11:143–4.

20. Frigo NV, Rotanov SV, Nesterenko VG, Lovenetskii AN, Dudareva LA. Results of study of the diagnostic efficiency of the new linear enzyme immunoassay test system "INNO-Lia Syphilis Score". Klin Lab Diagn. 2006;36–41.

21. Paris-Hamelin A, Debruyne M, Fustec-Isarboure S. Immunoblotting for the serodiagnosis of syphilis. A candidate to replace the Nelson-Mayer test. Ann Pharm Fr. 1999;57:68–75.

22. Caudie C, Garel F, Bancel J, Lombard C, Vandenberghe N. Diagnosis and biological monitoring of 6 neurosyphilis cases: value of cerebrospinal fluid analysis. Ann Biol Clin. 2003;61:563–9.

23. Ebinger M, Grauer MT, Uhr M. Intrathecal IgA synthesis in neurosyphilis. J Neurol Sci. 2005;228:21–5.

24. Finehout EJ, Franck Z, Lee KH. Complement protein isoforms in CSF as possible biomarkers for neurodegenerative disease. Dis Markers. 2005;21:93–101.

25. Karumudi UR, Augenbraun M. Syphilis and HIV: a dangerous duo. Expert Rev Anti Infect Ther. 2005;3:825–31.

26. Wohrl S, Geusau A. Neurosyphilis is unlikely in patients with late latent syphilis and a negative blood VDRL-test. Acta Derm Venereol. 2006;86:335–9.

27. Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. MMWR Recomm Rep. 2006;55:1–94.

28. Schofer H, Brockmeyer NH, Hagedorn HJ, Hamouda O, Handrick W, Krause W, Marcus U, Munstermann D, Petry KU, Prange H, Potthoff A, Gross G. Syphilis. Guideline of the German Sexually Transmitted Diseases Society for diagnosis and therapy of syphilis. J Dtsch Dermatol Ges. 2006;4:160–77.

29. Niermeijer JM, Hettinga YM, Wokke JH, Rothova A, Hart W. Clinical reasoning and decision-making in practice. A patient with loss of vision and painful legs. Ned Tijdschr Geneeskd. 2006;150:1173–8.

30. Paraskevas GP, Kapaki E, Kararizou E, Mitsonis C, Sfagos C, Vassilopoulos D. Cerebrospinal fluid tau protein is increased in neurosyphilis: a discrimination from syphilis without nervous system involvement? Sex Transm Dis. 2006;34(4):220–3.

AUTHORS' ADDRESSES

Vladimir Kotnik, MD, PhD, Professor, Head of the Microbiology and Immunology Chair, Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Korytkova 2, 1000 Ljubljana, Slovenia, corresponding author, E-mail: vladimir.kotnik@mf.uni-lj.si
Katarina Jordan, BS (biology), same address
Sanja Stopinšek, BS (biology), same address
Saša Simčič, PhD, specialist consultant, same address
Marko Potočnik, MD, DMD, PhD, Senior Consulting Dermatologist, Department of Dermatovenereology, University Medical Center, Zaloška 4, 1000 Ljubljana, Slovenia