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The Value of Autoantibody and Viral Serologic Examinations in the Differential Diagnosis of Multiple Sclerosis and Stroke

Multipl Skleroz ve İnme Ayırıcı Tanısında Otoantikor ve Viral Serolojik İncelemelerin Katkısı

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Abstract

Objective: To evaluate the value of autoantibody and viral serologic examinations in the diagnosis of multiple sclerosis (MS) and stroke and their effect on the course of treatment.

Materials and Methods: Patients who were admitted to the neurology clinic between 2012 and 2016 were retrospectively evaluated. The patients were screened for autoantibodies including anti-nuclear antibody (ANA) and anti-dsDNA, and viral serology including Epstein-Barr virus, varicella-zoster virus, cytomegalovirus, herpes simplex virus type 1 and 2. The study cohort was grouped as the MS group, stroke group, and "other" diseases group (e.g. polyneuropathy, myasthenia gravis, dementia, headache, epilepsy). The data from all the groups were further analyzed to determine whether these tests provided an increase in diagnostic performance.

Results: Among the autoantibody and viral serologic tests, the most commonly used test was ANA (71 cases in the MS group, 160 cases in the stroke group, and 482 cases in the other diseases group). All test reports, based on positivity/negativity, did not lead to any change in the initial diagnosis of the disease and the treatment strategy in all groups.

Conclusion: Information obtained from autoantibody and viral serologic tests does not affect the diagnosis of MS and stroke. Performing these tests for routine screening is considered worthless unless there is an important finding regarding clinical disease.

Keywords: Multiple sclerosis, stroke, SLE, ANA, EBV, CMV

Öz

Amaç: Multipl skleroz (MS) ve inme tanısında başvurulan otoantikor ve viral serolojik incelemelerin tanıdaki değerini ve tedavi seyri üzerine etkisini değerlendirmektir.

Gereç ve Yöntem: Nöroloji kliniğine 2012-2016 yılları arasında başvuran hastalar retrospektif olarak incelendi. Otoantikor testleri [anti-nükleer antikor (ANA), anti-dsDNA] ve viral serolojik incelemeler (Epstein-Barr virüs, varisella-zoster virüs, sitomegalovirüs, herpes simpleks virüs tip 1 ve 2 IgG ve IgM) sorgulandı. Gruplar tanılarına göre MS, inme ve bu iki tanı dışında kalan tüm tanılar (polinöropati, myastenia gravis, demans, baş ağrısı, epilepsi, vb.) için "diğer" adı altında gruplandı. Tüm testlerden elde edilen veriler, bu testlerin tanısal performansta bir artış sağlayıp sağlamadığını belirlemek için analiz edildi.

Bulgular: Otoantikor ve viral serolojik testler arasında en sık kullanılan test ANA (71 MS olgusu, 160 inme olgusu ve 482 diğer olgu) idi. Pozitif/negatifliğe dayalı tüm test sonuçları, tüm gruplarda hastalığın ilk tanısında ve tedavi stratejisinde herhangi bir değişikliğe neden olmamıştır.

Sonuç: Otoantikor ve viral serolojik testlerden elde edilen bilgiler MS ve inme tanısını etkilemez. Rutin tarama için bu testlerin yapılması, klinik hastalıkla ilgili önemli bir bulgu olmadığı sürece faydasız bir işlem olarak görülmektedir.

Anahtar Kelimeler: Multipl skleroz, inme, SLE, ANA, EBV, CMV

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Introduction

Multiple sclerosis (MS) is a chronic disease with pathologic oligodendrocytes/axon damage or loss. It is more common among Caucasians, particularly those of Northern European descent and in women. Its etiopathogenesis is still unclear. Environmental factors (i.e. viral, bacterial, toxic) are thought to be influential in the pathogenesis of the disease on the basis of a genetic predisposition, and no definitive biomarker for the diagnosis of MS has yet been identified. Stroke, on the other hand, is a multi-etiologic disease, mostly due to many risk factors in Caucasians. In the presence of clinical and radiologic findings of MS and stroke, laboratory investigations are required to exclude other causes in MS and to determine the cause of stroke. Magnetic resonance imaging and computed tomography do not include the mechanism and causative agent by which the lesions are formed. Laboratory tests may include rheumatologic, viral, and bacterial serological tests. It is not often questioned as to what extent these frequently used tests affect the diagnosis-treatment course, and to what extent the test results are positive/negative. This study was designed and conducted retrospectively in order to determine to what extent the test results affect the course of diagnosis and treatment; in other words, to determine their necessity.

Materials and Methods

Ethical consent was obtained from Adnan Menderes University Faculty of Medicine Non-Interventional Ethics Committee (protocol no: 2017/1134). The anti-nuclear antibody (ANA), anti-dsDNA, Epstein-Barr virus (EBV), varicella-zoster virus (VZV), cytomegalovirus (CMV), herpes simplex virus type 1 and 2 (HSV1-2), immunoglobulin (Ig) G and IgM values of patients who were hospitalized in the neurology clinic between January 2012 and December 2016, were extracted from the hospital electronic database retrospectively. No pediatric cases were included because only patients aged over 18 years were followed up. The diagnostic groups were as follows: MS, stroke, and other diseases (e.g. headache, polyneuropathy, vertigo, radiculopathy, dementia, epilepsy, conversion disorder, ataxia, myasthenia gravis, Parkinson's disease, syncope, chorea). All patients with test results were included in the study, and only one admission record was evaluated if the same patient had similar examinations at different times. All participants completed and signed a consent form.

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics 17.0 program and descriptive analyses (numbers and frequencies) are shown in the tables. The difference between the groups was investigated using chi-square analysis and the p values <0.05 were accepted as significant.

Results

The distribution characteristics of the study cohort according to age and sex are given in Table 1. Patients in the MS group were observed to be younger than those in the other two groups (ANOVA, $p<0.05$). It was found that there were more women in the MS group, and more men in the stroke group ($\chi^2=9.38$, $p<0.05$).

The most commonly used test in the diagnostic process was the ANA test. The results of 745 cases were reached and 713 cases were included after repeated studies were removed (Table 2). According to the chi-square analysis, there was no difference between the groups in terms of ANA values ($\chi^2=0.56$, $p>0.05$). The ANA positivity rate was 14-15% and the cut-off rate was 6-8%.

Anti-dsDNA tests were studied in 673 cases and were found as negative in almost all groups (Table 2). There was no difference between the groups ($\chi^2=0.99$, $p>0.05$).

The EBV IgG test was studied in 501 cases and was found to be positive in almost all groups (Table 3). There was no difference between the groups ($\chi^2=5.48$, $p>0.05$). The EBV IgG positivity rate was 91-97% and the cut-off rate was 1-5%.

EBV IgM antibodies were studied in 486 cases and were found to be negative in almost all groups (Table 3). There was no difference between the groups ($\chi^2=0.56$, $p>0.05$, $\chi^2=4.17$, $p>0.05$). The EBV IgM positivity rate was 1% and the cut-off rate was 1-2%.

VZV IgG antibodies were studied in 378 cases (Table 3) and there was no difference between the groups ($\chi^2=0.56$, $p>0.05$, $\chi^2=2.16$, $p>0.05$). It was positive in almost all groups. The VZV IgG positivity rate was 96-100% and the cut-off rate was 1-2%.

Table 1. Distribution characteristics of the study group according to age and sex

Diagnosis	Age n (mean±SD)		
	Female	Male	Total
MS	49** (38.6±10.5)	22 (39.5±14.3)	71 (38.9±11.7)*
Stroke	78 (46.3±12.6)	82** (51.5±13.4)	160 (48.9±13.2)
Other diseases	285 (44.7±14.6)	197 (49.3±16)	482 (46.6±15.4)

n: Number, SD: Standard deviation, MS: Multiple sclerosis

*In terms of age, it is seen that patients with MS were younger than those in the other two groups (ANOVA, $p<0.05$)

**In terms of sex, there were more women in the MS group and more men in the stroke group ($\chi^2=9.38$, $p<0.05$)

Table 2. The numbers and rates of anti-nuclear antibody and anti-ds DNA antibodies according to diagnostic groups

Diagnosis	Antibody titers n (%)				
	ANA			Anti-dsDNA	
	Negative	Positive	Cut-off	Negative	Positive
MS	55 (77.5)	10 (14.1)	6 (8.5)	67 (77.5)	0
Stroke	125 (78.1)	25 (15.6)	10 (6.3)	148 (99.3)	1 (0.7)
Other diseases	375 (77.8)	70 (14.5)	37 (7.7)	456 (99.8)	1 (0.2)

n: Number, %: Percent, MS: Multiple sclerosis, ANA: Anti-nuclear antibody

VZV IgM antibodies were studied in 397 cases and were found as negative in almost all groups (Table 3). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=3.72, p>0.05). The VZV IgM positivity rate was 4-6% and the cut-off rate was 1-8%.

CMV IgG antibodies were studied in 484 cases and were found as positive in almost all groups (Table 3). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=2.96, p>0.05). The CMV IgG positivity rate was 97-100% and the cut-off rate was 2-6%.

CMV IgM antibodies were studied in 464 cases and were found as negative in almost all groups (Table 3). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=0.56, p>0.05, χ^2=0.42, p>0.05). The CMV IgM positivity rate was 1-2% and the cut-off rate was 1-2%.

HSV1 IgG antibodies were studied in 407 cases and were found as positive in almost all groups (Table 4). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=7.91, p>0.05). The HSV1 IgG positivity rate was 91-98% and the cut-off rate was 1-2%.

HSV1 IgM antibodies were studied in 406 cases and were found as negative in almost all groups (Table 4). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=6.96, p>0.05). The HSV1 IgM positivity rate was 4-7% and the cut-off rate was 1-7%.

HSV2 IgG antibodies were studied in 260 cases and were found as negative in almost all groups (Table 4). There was no difference between the groups (χ^2=0.56, p>0.05, χ^2=2.18, p>0.05). The HSV2 IgG positivity rate was 0-2% and the cut-off rate was 13-18%.

HSV2 IgM antibodies were studied in 259 cases and were not positive in any groups (Table 4).

Regarding sex, there was no difference in the MS group in any of the tests. In the stroke group, only HSV IgG was higher in men than in women (χ^2=6.2, p<0.05). In the other diseases group, ANA positivity was higher in women (χ^2=7.92, p<0.05).

The distribution of patients in other diseases group is shown in Table 5.

Discussion

In the diagnosis of MS and stroke, rheumatologic and serologic laboratory examinations are frequently used to exclude other diseases and to determine the etiology. However, the usefulness of these tests to differentiate MS and stroke from other neurologic diseases is unknown. In this study, this subject was investigated by using retrospective methods and the differences between other neurologic diseases (Table 5) and MS-stroke were revealed.

While the prevalence of MS is 100-200/100000 (1), the prevalence of systemic lupus erythematosus (SLE) associated with the most screened serologic examination, which is likely to interfere with MS, is 12-50/100,000 (2). Although there is no definitive biomarker for the diagnosis of MS, disease biomarkers identified for the diagnosis of SLE are ANA and dsDNA, and they have a sensitivity of 95% and 70%, and a specificity of 60% and

Table 3. Serologic results of Epstein-Barr virus, varicella-zoster virus, cytomegalovirus and their distribution according to groups

Group	Antibody titers n (%)																	
	EBV IgG			EBV IgM			VZV IgG			VZV IgM			CMV IgG			CMV IgM		
	N	P	C	N	P	C	N	P	C	N	P	C	N	P	C	N	P	C
MS	1 (1.7)	55 (93.2)	3 (5.1)	53 (98.1)	0	1 (1.9)	0	42 (100)	0	35 (87.5)	2 (5)	3 (7.5)	0	58 (100)	0	52 (96.3)	1 (1.9)	1 (1.9)
Stroke	2 (1.8)	110 (97.3)	1 (0.9)	109 (98.2)	2 (1.8)	0	1 (1.2)	83 (97.6)	1 (1.2)	81 (93.1)	5 (5.7)	1 (1.1)	2 (1.8)	106 (96.4)	2 (1.8)	101 (97.1)	2 (1.9)	1 (1)
Other diseases	11 (3.3)	300 (91.2)	18 (5.5)	315 (98.1)	5 (1.6)	1 (0.3)	4 (1.6)	241 (96)	6 (2.4)	249 (92.2)	12 (4.4)	9 (3.3)	5 (1.6)	309 (97.8)	2 (0.6)	296 (96.7)	7 (2.3)	3 (1)

n: Number, %: Percent, EBV: Epstein-Barr virus, VZV: Varicella-zoster virus, CMV: Cytomegalovirus, Ig: Immunoglobulin, N: Negative, P: Positive, C: Cut-off value, MS: Multiple sclerosis

Table 4. Serologic results of HSV1 and HSV2 viruses and their distribution according to groups

Group	Antibody titers n (%)											
	HSV1 IgG			HSV1 IgM			HSV2 IgG			HSV2 IgM		
	N	P	C	N	P	C	N	P	C	N	P	C
MS	3 (6.7)	41 (91.1)	1 (2.2)	39 (86.7)	3 (6.7)	3 (6.7)	21 (87.5)	0	3 (12.5)	24 (100)	0	0
Stroke	3 (3.2)	92 (96.8)	0	86 (91.5)	4 (4.3)	4 (4.3)	52 (82.5)	0	11 (17.5)	63 (100)	0	0
Other diseases	4 (1.5)	262 (98.1)	1 (0.4)	250 (93.6)	14 (5.2)	3 (1.1)	147 (85)	3 (1.7)	23 (13.3)	172 (100)	0	0

n: Number, %: Percent, HSV: Herpes simplex virus, Ig: Immunoglobulin, N: Negative, P: Positive, C: Cut-off value, MS: Multiple sclerosis

95%, respectively (3). However, these antibodies may also be found in Sjögren's syndrome, rheumatoid arthritis, mixed connective tissue disease, scleroderma and polymyocyte/dermatomyositis, as well as in SLE. They are even found in non-rheumatic diseases such as Hashimoto's thyroiditis, Graves' disease, autoimmune hepatitis, primary autoimmune cholangitis, primary pulmonary hypertension, infection or malignancy, and also in the elderly (3). Despite the expression "ANA titration value is distinctive", the cut-off value (1/40) was found in 20-30% of healthy adults and above cut-off values (1/80 titer and above) were found in 12% (4,5). Titers of 1/640 have been reported in the pediatric age group, even

Table 5. Distribution of cases in the other diseases group according to diagnosis and sex

Diagnosis	Female	Male	Total
Headache	121	60	181
Polyneuropathy	53	46	99
Diabetes mellitus	17	21	38
Vertigo	17	15	32
Radiculopathy	18	6	24
Alzheimer's disease	7	8	15
Conversion disorder	4	7	11
Epilepsy	8	3	11
Ataxia	4	4	8
Diplopia	1	6	7
Myasthenia gravis	4	3	7
Abdominal pain	4	2	6
Parkinson's disease	2	3	5
Mononeuropathy	3	2	5
Syncope	5	0	5
Myopathy	2	2	4
Anxiety	3	1	4
Korea	3	0	3
Motor neuron disease	3	0	3
Hypertension	1	1	2
Tremor	1	1	2
6. Cranial nerve paralysis	0	1	1
Dysphagia	0	1	1
Joint pain	0	1	1
Hyperlipidemia	0	1	1
Lymphoma	0	1	1
Neuralgia	0	1	1
Anisocoria	1	0	1
Dystonia	1	0	1
Dysarthria	1	0	1
Pituitary tumor	1	0	1

without disease (6). It was found as 22.5-26% in patients with MS and was considered to be a reflection of ongoing immune activation during disease activity (7,8). It was emphasized that a positive test without clinical findings had no diagnostic or prognostic value (4) and that it would be appropriate to ask for a test only in the presence of clinically compatible findings (5). In this study, 55 patients with MS, 125 with stroke and 375 patients with different diseases showed similar results, and the positive cut-off rate was 22%, similar to previous studies. ANA positivity was observed in 1/100 and 1/320 titers in seven cases, whereas dsDNA, which is more specific than the ANA test, was not positive in almost any case (Table 2), and the diagnosis did not change in any patients. When the 'biomarker' value of antibody tests in predicting disease before the emergence of clinical signs was questioned in patients with SLE, anti-dsDNA showed a higher odds value, and greater ability to predict disease, than ANA (18.3-11.5) (9). Therefore, it may be more rational to screen anti-dsDNA instead of ANA to show the absence of disease. Although the presence of ANA was associated with ongoing immune activation in patients with MS, it was observed in a similar rate in the other diseases group, and the association with activation was not supported.

Although MS is a disease in which T cell-mediated autoimmune processes are active and goes through the processes of neuroinflammation and neurodegeneration, the etiology is still unknown. Hypotheses related to viral agents have been proposed, blaming endogenous or exogenous causes, but no conclusive evidence has been presented (10,11,12,13). According to the 'hygiene hypothesis', encountering the agent in early childhood provides protection against MS in the later period and encountering the causative agent in the late period causes the disease. The 'prevalence hypothesis' is based on the fact that the prevalence of infections and MS in geographic areas is very similar (14).

The possible molecular mechanisms of viral agents in MS pathogenesis are as follows:

1) Self auto-reactivation of T cells by antigen-presenting cells stimulated by the pathogen,

2) Release of proinflammatory mediators following recognition of the pathogen pattern receptor by the immune system and subsequent activation of auto-reactive lymphocytes,

3) Binding of microbial super-antigens with major histocompatibility complex class 2 molecules and activation of non-specific auto-reactive T cells via T cell receptors,

4) Activation of auto-reactive T cells by molecular similarity of pathogenic antigens,

5) Epitope proliferation, and,

6) Prolongation of lives of auto-reactive lymphocytes by pathogenic agents (10).

Among the viral agents, EBV is the most discussed. EBV, which is observed in 90% of adults, behaves according to the hygiene and prevalence hypotheses. It may remain silent in B cells, its nuclear antigen is similar to the MBP epitope, and it may result in the formation of antigens on the B cell surface that can activate T cells (15,16). When MS and non-MS tissue samples were examined, it was detected in 90% of MS cases and 25% of non-MS cases (17). Unlike EBV IgG and IgM, Epstein-Barr nuclear antigen-1 positivity has been found to increase the risk of MS/clinically isolated syndrome (18). Although studies attempt to reveal the viral agent-disease relationship, they do not contribute

to diagnosis. No difference was found between the disease groups during this study, EBV antibody results did not change the diagnosis of the disease, and active infection could not be shown in any patients.

VZV is a common virus that was previously immunized by vaccination. It shows a distribution that fits the prevalence hypothesis, and its incidence increases with years, as MS. VZV may remain silent in the posterior root ganglia after varicella or may cause nerve inflammation called shingles. The virus DNA was observed in the first days of the MS attack, but not after the attack (12). In a study comparing anti VZV and anti-CMV IgG titers in 800 patients with MS and 1000 controls, higher titer values were found in patients with MS (19). Another interesting observation was the negative correlation between CMV and the development of MS. It was stated that there was a need to determine whether a previous disease history had a real protective effect in pediatric and adult patients with MS (20).

Ischemic stroke is a common health problem that occurs with alterations in the arterial wall, which lead to deterioration of blood flow, and is often observed in advanced age. It includes non-modifiable risk factors such as age, sex, family history, as well as modifiable risk factors such as hypertension, obesity, hypercholesterolemia, and smoking. Atherosclerosis is seen as the most common cause in older age, whereas vasculitic processes, cardiac causes, and coagulation disorders are more prominent in younger patients. A recent study investigated the contribution of vasculitis examinations to diagnosis in more than 3500 young people (aged 18-45 years), and it was found to be very low and only recommended if there was clinical suspicion (21).

It has been investigated in the past that viral agents might also have an effect on changes in the arterial wall besides a cause-process relationship. It is known that VZV virus can invade arteries directly and develop stroke (22). It was shown serologically in the atherosclerotic vascular wall of patients and their DNAs were identified. However, it is known that two-thirds of VZV-mediated strokes occur during/after VZV infection/reactivation and can be clinically and/or serologically demonstrated. Antiviral therapy may also be beneficial during activation (23). The risk of stroke and myocardial infarction (MI) were shown to increase during infection/post-infection periods, but vaccination was found to have no effect on risk (24,25,26).

CMV infects smooth muscle structures in immunocompromised individuals; however, its status in individuals with normal immune systems is not clear (22). Transient anticardiolipin antibody syndrome and a relationship with stroke was reported during infection in one case (27). Although CMV antibody titers were observed to be high in patients who underwent surgery for atherosclerotic plaques (28), no relationship was found in a prospective study investigating whether CMV and HSV antibodies could be markers of atherosclerosis (29). In a study examining the relationship between CMV seropositivity and MI risk in advanced age in terms of T cell subtypes and chronic immune activation, it was stated that the risk was increased and the possible cause was the chronic inflammation process (30).

Studies investigating the relationship between HSV type 1 and 2 and stroke have reported that stroke almost always occurs during encephalitis or meningitis (31).

In the present study, there was no difference between the MS, stroke, and other diseases groups in terms of the results of the above-mentioned viruses, similar to the EBV-related results, and the diagnoses were unchanged. It was concluded that the use of these tests for routine screening during the diagnosis of MS and stroke was not beneficial because "they do not have discriminative value".

Study Limitations

Our major limitations were that this study had a retrospective design and the relatively smaller sample size may have limited the generalization of the findings.

Conclusion

Serum ANA, anti-dsDNA, CMV, EBV, VZV, HSV1-2 IgG or IgM tests do not contribute to the diagnosis and differential diagnosis of MS and stroke. In tissue or cerebrospinal fluid studies, investigation of antibody titer differences and specific antigens may be important for the pathophysiology of MS and stroke, they are considered worthless procedures for routine screening without clinically significant findings.

Ethics

Ethics Committee Approval: Ethical consent was obtained from Adnan Menderes University Faculty of Medicine Non-Interventional Ethics Committee (protocol no: 2017/1134).

Informed Consent: Retrospective study.

Peer review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: N.K., A.Ş., Design: N.K., A.Ş., U.O.A., Data Collection or Processing: N.K., A.Ş., Analysis or Interpretation: N.K., A.Ş., B.K., Literature Search: N.K., A.Ş., Writing: N.K., A.Ş.

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