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TURKISH INFLAMMATORY BOWEL DISEASE SOCIETY RECOMMENDATIONS ON SELECTED TOPICS OF CROHN'S DISEASE

TÜRK İNFLAMATUVAR BARSAK HASTALIKLARI DERNEĞİNİN CROHN HASTALIĞI İLE İLGİLİ SEÇİLMİŞ KONULARDA ÖNERİLERİ

What is the most accurate method for the diagnosis of cytomegalovirus (CMV) enteritis or colitis?

Sitomegalovirus enteritinin ve kolitinin tanısında en doğru yöntem nedir?

Key words: Crohn's disease, cytomegalovirus, diagnosis

Anahtar kelimeler: Crohn hastalığı, sitomegalovirus, tanı

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INTRODUCTION

Cytomegalovirus (CMV) infection is a common viral infection with a reported incidence rate of 40% and 100% (1). Primary CMV infection is seen at an early period of life. Its seroprevalence is higher than 70% in adults. After the primary infection, the virus goes into the latent state particularly in the white blood cells and in the endothelial cells of the body. In order to consider CMV disease, the clinical symptoms of CMV infections (fatigue, high fever, leucopenia, atypical lymphocytosis, thrombocytopenia, mild to moderate serum aminotranspherase elevation etc.) should be present. CMV disease is one of the most common complications, developing after an immunosuppressive therapy. CMV disease can be seen anywhere in the gastrointestinal tract, from the mouth to the rectum. Diagnostic identification is difficult via endoscopic methods. Ulcers are common with co-existing hemorrhagia (2-4).

CMV colitis should be excluded prior to increasing the immune-modulator therapy in cases with inflammatory bowel diseases that are refractory to the immune-modulator treatment (5).

METHODS

We used the systematic literature search of 3e Initiative, in order to find the answer to the question which is not quite clear in ECCO 2006 guideline: "What is the most accurate test for the diagnosis of CMV enteritis or colitis?"

Systematic literature search was performed on the Pubmed database. Also, other studies were revealed via literature reference search. "Cytomegalovirus enterocolitis" was picked as a key word to search the publications in English, and trials with subjects older than 18 years of age were included in the study. The sensitivity and specificity of the methods used in the diagnosis of CMV disease were determined in the study via statistical analysis.

RESULTS

433 studies were found in the systematic literature search regarding the diagnostic methods of CMV enterocolitis. Recurrent studies; studies based on the series including less than 5 cases; studies including pediatric and adult patients collectively; editorials; collective publications referring to different keywords and targeting different sce-

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narios other than ours; studies in languages other than English; and studies with insufficient statistical data were excluded. Totally 18 studies were found to be appropriate for the assessment in terms of the determined criteria. While evaluating the studies, it was aimed to determine both the sensitivity and the specificity and also the priority of the diagnostic methods used for CMV enterocolitis. Types of the 18 analyzed studies were demonstrated in table 1 (Table 1).

Methods that will be used for the diagnosis of CMV enterocolitis

Only a very few number of CMV infections result in clinical diseases. When CMV disease is suspected in an inflammatory bowel disease, different techniques are available for the diagnosis.

a) Serology

Due to the high seroprevalence, diagnostic value of the serology is limited for the determination of an active infection in the adult population. However, the use of CMV specific antibodies may be beneficial in the diagnosis of the new onset infections (CMV IgM positivity (+), elevation in titration of IgG). Cases in the risk group for CMV reactivation can be identified by this method (1, 6). The sensitivity and the specificity values of the serology that we determined were 63.7% and 99.5%, respectively (Table 2).

b) Conventional Culture

It depends on the principle of virus isolation in the fibroblast tissue culture. Blood, tissue, urine, spittle and sputum samples can be used for conventional culture. Despite its high specificity (89% to 100%), the sensitivity of this test is quite low (45% to 78%). The other disadvantages are the long incubation period, the insufficient virus quantity and the high rate of false negativity (7). As a result of our 3e systematic literature search, we found the specificity of this test as 99.8% and the sensitivity as 42.6% (Table 2).

Histopathology and Immunohistochemistry (IHC)

In cases with serious colitis, the rate of CMV is reported to be 21% to 34% in the colon tissue. This rate is between 33-36% in steroid refractory cases. For detection of CMV infection in the tissue or in the biopsy samples, the specificity and sensitivity of the combination of histopathology and IHC (monoclonal antibodies against CMV immediate early antigen) is quite high (8). In the tissues, histopathological detection of CMV can be provided by routine H&E staining of typical intranuclear and intracytoplasmic inclusion. Although this method is accepted as a "gold standard" for the diagnosis of CMV active disease, viral inclusions can not be easily seen, for they are very rare. Also; superficial mucosal biopsies may not always be diagnostic, since CMV inclusion bodies are observed mainly around mucosal vessel walls or ulcer bed (9, 10, 11). Sensitivity of IHC is higher than H&E staining. Susceptibility rates of up to 93% have been reported (7). In addition, in the ECCO recommendations on prevention-diagnosis and on approaching to opportunistic infections in inflammatory bowel diseases, it is also noted that, specificity of histopathological and immunohistochemical investigations in CMV disease is up to 100% (8). The specificity of histopathology was 100% while the sensitivity was 23.2% in the 3e systematic literature search (Table 2).

Table 1. Analyzed study types

Type of study	Number of studies
Prospective	15
Case control	1
Retrospective	2

c) "Shell Vial Assay" (SVA)

Although being a quicker method compared with the conventional culture method, this method also has a low sensitivity rate. In the comparative studies of PCR and SVA, sensitivity of SVA method is reported to be between 35% and 44% (12, 13). As a result of a 3e systematic literature search, sensitivity and specificity of this test were found to be 42.8% and 98.4%, respectively (Table 2).

d) CMV pp65 Antigen Test

Detection of late structural protein pp65 in leucocytes is provided by immune-fluorescent staining of pp65 specific monoclonal antibodies. This test can be applied to blood and cerebrospinal fluid. Results are obtained within 8-24 hours. If it is not available to measure the viral load in immunosuppressive patients with PCR, in some centers, this test is quick enough to screen the infection and the anti-viral treatment. The rates of sensitivity and specifity have been reported between 60% to 100% and 83% to 100%, respectively. Eric E et al found the sensitivity of pp65 Ag method as 87.5% and specificity as 92.9% in their comparative study in which they have performed viral cultu-

Table 2. Sensitivity and specificity of laboratory methods which are used for CMV enterocolitis diagnosis

Diagnostic Method	Sensitivity %	Specifity %	PPV* %	NPV** %
Histopathology	23.2	100	100	23.2
CMV IgM	63.7	99.5	99.5	65.3
Viral Culture	42.6	99.8	98.7	85.5
Shell Vial Assay	42.8	98.4	87.6	86.9
CMV pp65 Ag	83.7	96.3	95.8	85.9
CMV pp67 mRNA	50.3	90.3	90.1	51.2
PCR	91.0	92.1	94.5	87.4
Real-Time PCR	85.4	77.7	57.2	93.9

*:Positive Predictive Value

**:Negative Predictive Value

re technique. However since this is a semi-quantitative method and also the evaluation of the results is subjective; major disadvantages exist for this method (1.14). Regarding the CMV pp65 Ag, we have found the sensitivity and specificity as 96.3% and 83.7% respectively (Table 2).

e) CMV pp67 mRNA NASBA

Pp67-mRNA encodes the structural tegument protein which is expressed in the late phase of CMV replication. The late mRNA expression of CMV in leucocytes shows active replication and dissemination of CMV. NASBA (nucleic acid sequence based amplification) assay method can specifically amplify RNA sequences. Conflicting results have been obtained from the studies, comparing the CMV pp67 mRNA assay and other methods for the detection of CMV infection. For example Koenig et al. have showed that pp67 mRNA has less sensitivity than either quantitative PCR or CMV pp65 Ag test. Blank et al have demonstrated that it has a higher specificity and a positive predictive value compared with CMV pp65 Ag test and viral culture. However, in this study, sensitivity of CMV pp 67 mRNA was detected lower than both the quantitative PCR and CMV pp65 Ag (15, 16). As a result of 3e systematic literature search, the sensitivity and specificity of CMV pp67 mRNA were found as 50.3% and 90.3%, respectively (Table 2).

f) CMV DNA Tests

CMV DNA can be detected with the PCR technology, which is used in detecting the viral nucleic acids (DNA or RNA). Either qualitative or quantitative PCR techniques can be used. The quantitative PCR is more sensitive than the qualitative PCR method. The PCR method can be performed with whole blood, plasma, leukocytes, in buffy-coat samples, bronchoalveolar lavage fluid, the target organ tissue or the stool. PCR results in whole blood are more sensitive than in plasma. Moreover, as a non-invasive method, CMV DNA investigation of colonic disease in stool has a high sensitivity than investigating in blood and tissue PCR (1, 6, 7, 17).

Being a rapid process (16-48 hours), the high sensitivity and requirement of samples in fewer amounts are the advantages of PCR. In two different studies, Piiaparinen et al and Sia et al found high rates for the sensitivity (91% and 84%, respectively) and specificity (94% and 95%, respectively) of the PCR method for CMV diagnosis (18, 19). The disadvantages of this technique are the usage of different techniques and assays, the usage of different quantitation methods, collection of different tissue samples and undetermined cut-off values for CMV disease (7). Specific detection of replicative cycle-dependent viral transcription is aimed via Real-Time PCR which is another detection method of CMV DNA. Despite its high sensitivity, its specifity is low due to the detection of some residual viral DNA contaminations (12, 20). According to our 3e systematic search, regarding the diagnosis of the CMV disease we determined the sensitivities of PCR and real-time PCR as 91.0% and 85.4%, and the specificities as 92.1% and 77.7%, respectively (Table 2).

CONCLUSION

Although only a small number of CMV infections progress to develop clinical diseases, in the presence of CMV enterocolitis mimicking Crohn's disease activation, they have severe clinical courses and have been reported to cause high colectomy rates. Therefore, it is important to determine CMV enterocolitis by the most rapid and accurate methods in patients with severe enterocolitis. National recommendations for "Diagnosis of CMV enterocolitis in Crohn's disease", which were created according to the expert opinions in the literature reviews, have been presented in the box.

Recommendation:

When patients with Crohn's disease have a concomitant severe colitis, the preferred tests for differential diagnosis of CMV enterocolitis in the circumstances of our country:

1. *Primarily, histopathologic examination and immunohistochemical assessments (CMV antibody staining in tissue) should be performed* ***(EL 4, RG C).***
2. *In order to increase the effectiveness of the histopathologic examination, it is recommended to collect samples from the ulcer bed* ***(EL 5, RG D)***
3. *In the event of negative histopathologic examination, but with continuous clinical suspicion*
   - ***a.*** *Conventional tissue or stool or whole blood PCR (center facilities) and/or*
   - ***b.*** *pp65 antigen examination must be conducted* ***(EL 2c, RG B)***

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