NEED FOR EARLY AND ACCURATE DIAGNOSIS OF LOCAL CMV REACTIVATION FOR THE PROPER TREATMENT OF INFLAMMATORY BOWEL DISEASE

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BACKGROUND

Inflammatory bowel disease (IBD) is an autoimmune chronic inflammation of the gastrointestinal tract. The primary forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Bloody diarrhea, abdominal pain, loss of appetite and weight are the most common symptoms of the disease. Diagnosis is generally based on clinical simptoms, findings of colonoscopy and bowel biopsy. Microbiological infections such as Cytomegalovirus (CMV) can be similar. Differential diagnosis is important to choose correct therapy. Medical treatment of IBD is individualised to each patient and depends on the type and severity of the disease, as well as other prognostic factors. Medical management of inflammatory bowel disease may require immunosuppression, such as steroids, azathioprin and TNF-alfa inhibitors. TNF-alfa inhibitors are used especially in patients with severe or therapy resistant IBD.

Cytomegalovirus is a double-stranded DNA virus and a member of the *Herpesviridae* family. Primary CMV infection remains generally asymptomatic, but then the virus enters a latency phase. This latent infection can reactivate when the conditions are appropriate for, especially in immunocompromised patients. The reactivation of CMV is usually locally in different organs and tissues.

Cytomegalovirus local reactivation is more frequently in sever or steroid-refractory UC patients. Some recent studies demonstrated that immunosuppressant therapy are ineffective if CMV is detected in the colon mucosa. Early CMV identification is recommended by the guidelines of the European Crohn's and Colitis Organization in cases of IBD relapse. In case of sever colitis with CMV detected in the mucosa antiviral therapy should be initiated.

Cytomegalovirus reactivation of the inflamed colonic mucosa cannot be recognized only by endoscopy, histology or serology. Correct and rapid diagnosis is essential to the success of the therapy. Early CMV diagnosis is important especially for those patients who are resistant to first line treatment. Different laboratory diagnostic methods exist and are used for the detection of CMV. Only a few technics are suggested for the current diagnosis of CMV reactivation in the colonic mucosa. The serology is necessary to identify those patients who already contacted with CMV. Hematoxylin-eosin is not sensitive enough and often leads to false negative results. Immunohistochemistry is much more sensitive and also suitable for quantitative assessments. The most widespread technique in the routine diagnostics is serology and immunohistochemistry, but all the routine diagnostic tools are limited in the case of immunocompromised individuals.

The molecular biological techniques are based on the detection of CMV DNA in biopsy tissues. Real-time quantitative PCR methods are very sensitive and can detect low level viral reactivation, too.

AIM

The aim of this study was to determine the Cytomegalovirus DNA load in blood samples and especially in biopsy tissues in patients suffering from inflammatory bowel disease. Monitoring the viral load we also wanted to find out IBD severity is related to the CMV reactivation. For this purpose, real-time PCR assay was used.

METHODS

Virus reactivation was studied by CMV specific real-time polymerase chain reaction (PCR) in tissue samples obtained from the inflamed colonic mucosa. The quantitative PCR assay (Geenproof) helps monitoring the progress of the CMV infection, the efficacy of therapy and also let us make difference between systemic and local CMV reactivations. Our laboratory can measure the viral DNA in different clinical sample types including blood, urine and bowel biopsy tissues. We apply different commercial kits for nucleic acid extraction (Qiagene, Roche). In order to differentiate between systemic and local viral reactivation, parallel assays are made with the blood and biopsy tissues.

Results were expressed as the number of viral DNA copies/ml (blood) and copies/mg in (tissue samples), respectively. The study period when clinical samples were submitted to our laboratory for the CMV DNA measuring lasted from January of 2012 to August of 2016.

Investigated patients were predominantly suffering from ulcerative colitis (UC) and Crohn's disease (CD), but unclassified IBD and other disease cases also occurred in low percentage. According to the widely accepted criteria, clinical diagnosis was based on endoscopy and histology, as well as clinical symptoms. Individuals were treated with conventional therapies. Antiviral treatment (gancyclovir, 5 mg/kg intravenously) was added to the therapy when clinical symptoms of the infection were presented by the patient, and CMV DNA was detected in the colon mucosa.

RESULTS

During this study various biological samples were submitted for CMV real-time PCR. We studied 354 different clinical samples (blood, bowel biopsy and urine) obtained from 159 patients (mean age 41±14 years, sex ratio M/F: 0,93), 93 with colitis ulcerosa (58,9%), 47 with Crohn's disease (29,5%), 17 with unclassified IBD (10,7%) and 2 with other gastrointestinal diseases (1,25%). Patients were treated in accordance with the current guidelines. CMV-DNA was detected in 61 (64,8%) intestinal biopsy representing of the 94 CMV positive samples found entirely in all analysed samples (26,5%). The biopsies taken from inflamed bowel, the number of CMV-DNA copies distributed as follows.

41 (n=94, 43,6%) CMV-DNA positive cases (34 UC, 7 CD) were treated with gancyclovir resulting in an improvement of the clinical symptoms in 27 (n=41, 65,8%) cases (24 UC, 3 CD). In some of the inflammatory bowel disease patients also presenting intestinal CMV-DNA positivity, anti-viral treatment even resulted in a dramatic recovery from the disease. Ten (n=41, 24,3%) IBD patients with repeatedly identified CMV-DNA positivity were among the cases who were needed colectomy.

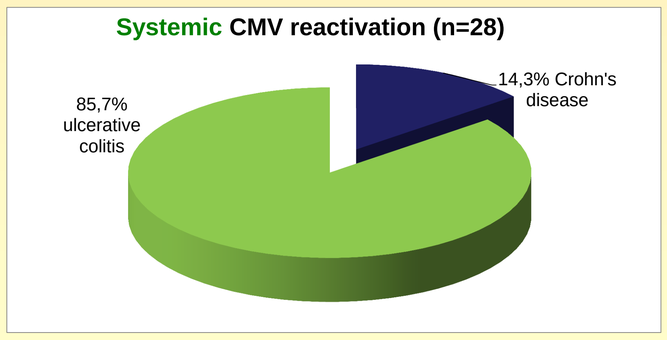
Correlation between CMV copies number and the efficacy of antiviral treatment

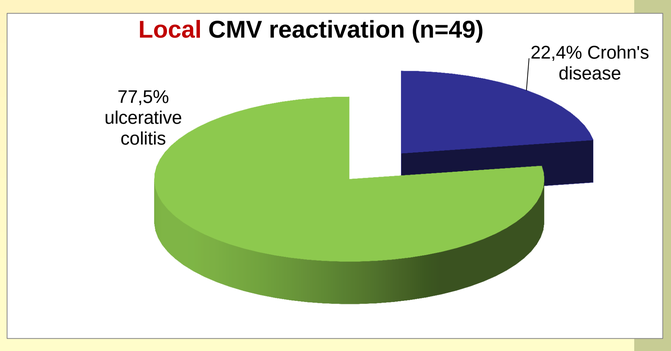
Systemic CMV reactivation	Blood	Tissue	Antiviral therapy	Improve of symptom	Colectomy
Blood samples (CMV DNS copy/ml)					
< 100	11	-	3	3	-
100-1000	6	-	2	2	-
> 1000	2	-	2	1	-
Blood samples (CMV DNS copy/ml) + tissue samples (CMV DNS copy/mg)					
< 100	1	**2**	**1**	**1**	-
100-1000	5	**6**	**4**	**4**	**2**
> 1000	5	**3**	**3**	**2**	**1**
Total number of samples	30	**11**	**15**	**13**	**3**

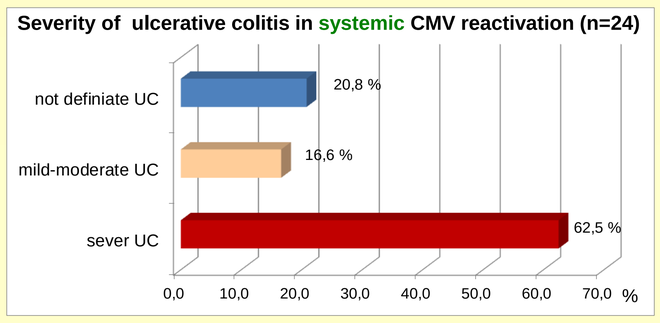
Local CMV reactivation	Tissue	Antiviral therapy	Improve of symptom	Colectomy
Tissues samples (CMV DNS copy/mg)				
<100	**28**	**13**	**5**	**5**
100-1000	**15**	**8**	**6**	-
>1000	**7**	**5**	**3**	**2**
Total samples number	**50**	**26**	**14**	**7**

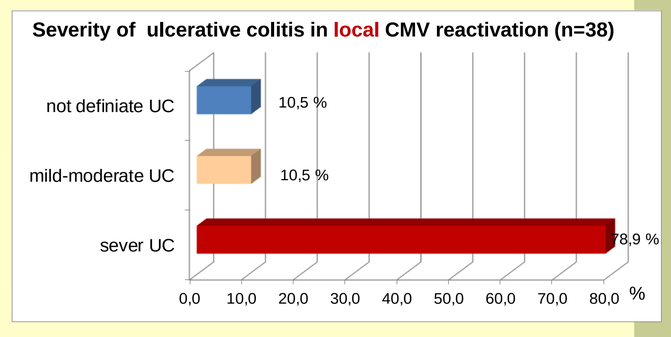
Comments: one patient was treated with gancyclovir on divers occasions; more than one samples belong to one patient; this table contains the total CMV positive cases including colitis ulcerosa, Crohn's disease, unclassified IBD and other diseases too.

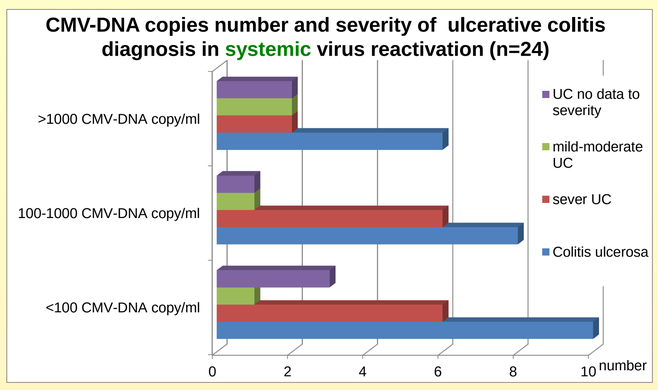
The rate of local and systemic virus reactivation were larger in patients with ulcerative colitis than in other IBD cases. If CMV-DNA was found in patients with ulcerative colitis, the basic disease also proved to be more severe. In the majority of the severe and mild-moderate ulcerative colitis cases, <100 and 100-1000 copies of viral DNA was found. Introduction of the gancyclovir treatment to UC patients with justified local or systemic virus reactivation, also led to the substantial improvement of the clinical symptoms.

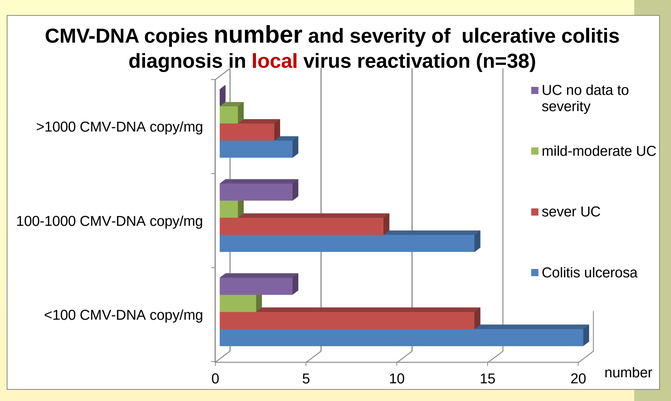


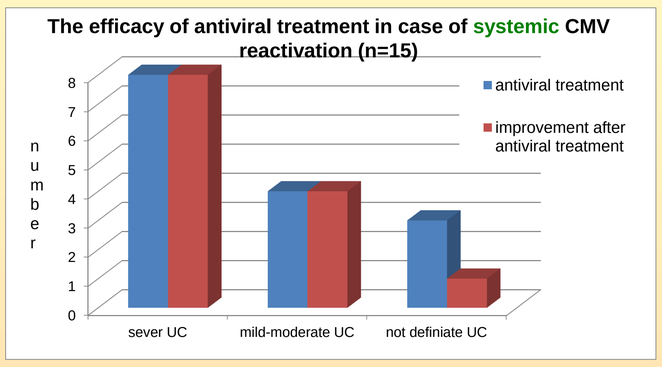


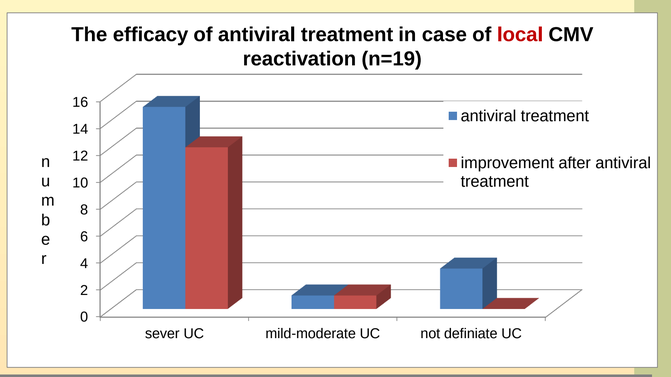


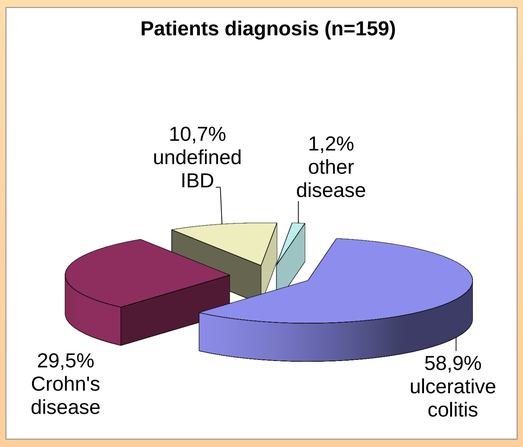


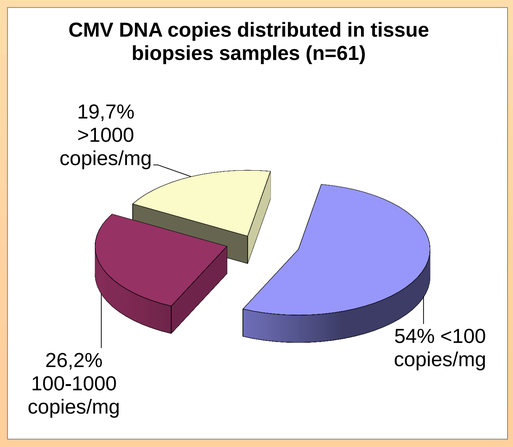












CONCLUSIONS

Rapid and exact detection of CMV from inflamed colonic mucosa seems essential for choosing the right treatment in severe or steroid-refractory IBD.

This laboratory assistance could present a strong support for the clinician in applying the most appropriate treatment to inflammatory bowel disease patients, that might well pay off in economical terms, as well.

Together, detection of CMV in blood and bowel tissue seems to be useful. The clinical outcome of patients with CMV reactivation in colon mucosa was improved after antiviral treatment. Gancyclovir was particularly efficient in cases when we could measure high virus DNA copy numbers and it seems to be more effective in patients with high numbers of CMV DNA.

The laboratory can help clinicians when performing assessment for CMV in patients with IBD during the routin clinical practice. Furthermore, the reduced morbidity and mortality caused by undiagnosed CMV disease can also gain benefit from the collaboration between the clinician and the laboratory.

In summary, our results are similar to the data found in the most recent literature and justify the initiative that detection of CMV infection or reactivation from the inflamed mucosa seems essential for the apropriate treatment in sever or steroid-refractory IBD, especially in ulcerative colitis. If CMV DNA is detectable, antiviral treatment seems not just beneficial, but also a necessity for these patients. Nevertheless, in spite of this novice approach to the therapy, colectomy still remains anavoidable.

We plan an overall prospective randomised study in co-operation with the clinicians in order to optimize the management of IBD patients with CMV infection or reactivation.

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