POLYMERASE CHAIN REACTION AS DIAGNOSTIC TOOL FOR HUMAN CYTOMEGALOVIRUS INFECTION IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Cytomegalovirus and Inflammatory Bowel Disease

Human cytomegalovirus (CMV), member of the *Herpesviridae* family. Cytomegalovirus infection can be range from asymptomatic or mild disease in immunocompetent hosts to severe and sometimes fatal illness in immunocompromised patients. Its prevalence has been estimated to range from 30% to 100% worldwide. Primary infection with CMV is often followed by the lifelong persistence in the host organism. Latent form of the virus can be found in various cell types like myeloid progenitors, monocytes, and endothelial cells. 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.

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). Although the exact cause of IBD is still unknown, genetic and environmental factors, as well as immune-regulatory disorders plays a role in the development of inflammation. Inflammatory bowel disease treatment is based on immunosuppressive agents.

Cytomegalovirus reactivation is often affecting the gastrointestinal tract, considered as a complicating factor in IBD patients. Either primary infection or virus reactivation can induce inflammation in the intestinal mucosa, often resulting severe lesions or ulcers, similar to IBD relapse. Contribution of local CMV reactivation can induce exacerbation of IBD, or result resistance to therapy. Early CMV identification is recommended by the guidelines of the European Crohn's and Colitis Organization in cases of IBD relapse, if virus infection or reactivation is expected. Untreated CMV reactivations increase the risk of steroid-resistance, therapy refractory complications in IBD, especially in UC. In case of sever colitis with CMV detected in the mucosa antiviral therapy should be initiated.

Correct and rapid diagnosis is essential to the success of the therapy. Different laboratory diagnostic methods exist and are used for the detection/identification of CMV. 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.

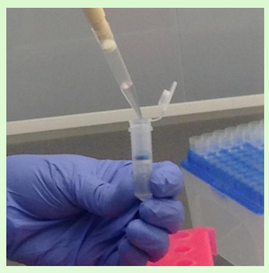
Aim

We aimed to prepare a nucleic acid based polymerase chain reaction (PCR) diagnostic protocol to separate systemic and local CMV reactivation, and also monitor the viral load in different types of samples, especially in bowel biopsy.

Methods

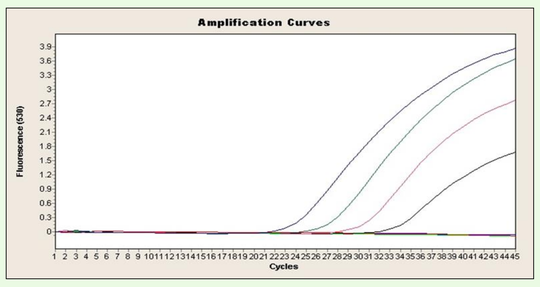
Molecular biological methods like PCR can be a useful tools for early diagnosis and monitoring viral infection and reactivation.

We used quantitative real-time CMV PCR assay (Geneproof, specificity 100% CMV virus, sensitivity 95%) to detect CMV. We applied a method that allows us to measure CMV blood (plasma) and other clinical sample types (urine and tissue biopsies), as well. We use manual nucleic acid isolation from the samples, an average of 25 mg tissue and 200 µl of concentrated urine, but from blood samples the viral DNA was extracted with nucleic acid isolation automata.

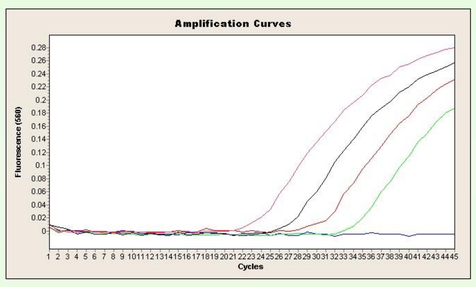


Systemic or general CMV reactivation	Local CMV reactivation
CMV detectable: **- *blood sample*** **- *blood and tissue samples***	**CMV detectable:** **- *tissue sample***
Urine: In cases of primary CMV infection or general virus reactivation, the appearance of the virus is expected between the days of 45-60. Though it is generally excreted into the urine, the process itself is subjected to some individual variations.	

In order to check the efficacy of the extraction and PCR amplification, an internal standard was applied during the isolation process in each case. According to the type of sample, three different kind of commercial kits (Qiagen for tissues, Geneproof for urine, and Roche for blood samples) were applied for the viral DNA extraction.



CMV PCR amplification curves (530 nm)



Internal Control PCR amplification curves (560 nm)

The viral DNA detection is based on the amplification of a specific conservative sequence of a single-copy gene encoding the 4 IE antigen. The PCR product was detected with fluorescence labelled probes. The presence of CMV is indicated by the timely increase in fluorescence intensity of FAM (530 nm) fluorophore, while the internal standard is detected by JOE (560 nm) fluorophore.

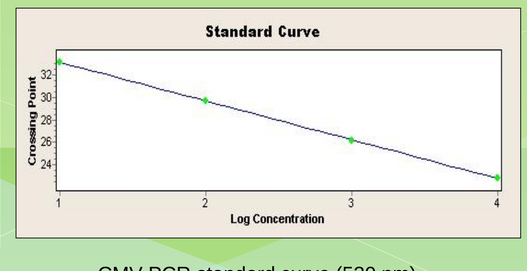
Virus concentration was calculated according to the formula indicated on the picture below, and expressed as the number of viral DNA copies per ml (blood or urine) and copies per mg (tissue samples), respectively.

Performing parallel measurements in all sample types let us not only detect but even distinguish between systemic and local CMV infections/reactivations.

Formula to Quantitative detection

$$\text{Copy/ml} = \frac{\text{SC x EV}}{\text{IV}}$$

SC = sample concentration (copy / µl)
EV = elution volume (µl)
IV = isolation volume (ml)



CMV PCR standard curve (530 nm)

Results

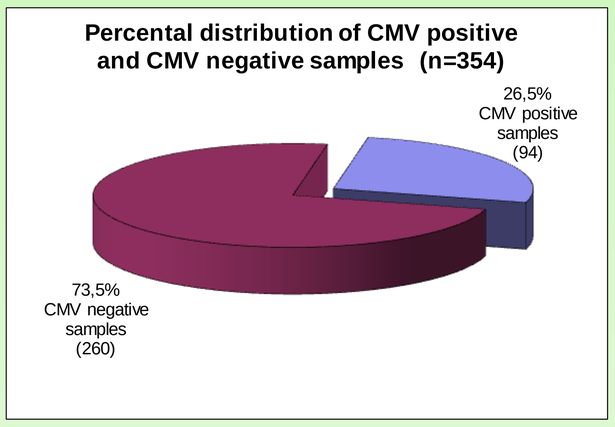
We examined 354 clinical samples (159 gastroenterological (GI) patients) from 2012 to 2016. From these patients 141 (88,05%) had IBD (CD: n=48, 34%; UC: n=93, 65,9%). The average age of GI patients were 41±14 years, sex ratio M/F:0,93).

One hundred eighty-three (51,7%) samples were collected from blood, 140 (39,5%) from tissue (colon-, rectum- or sigma-bowel biopsy) and 31 (8,8%) urine. Ninety-four (26,5%) samples were found positive for CMV DNA, and 260 (73,5%) samples were detected negative for viral DNA.

From the 94 CMV positive samples was found to be CMV DNA positive, with a distribution of 61 (tissue biopsy 64,8%); 30 (blood 31,9%) and 3 (urine 3,2%) among the different samples.

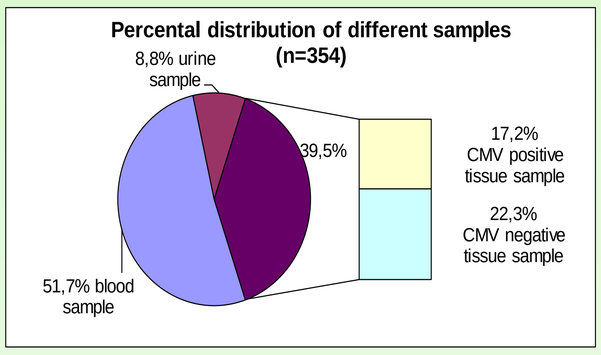
CMV reactivation was demonstrated in 61 (n=94, 64,8%) tissues biopsy. We could measure the local gastrointestinal CMV reactivation in 50 (n=61, 81,9%) tissues samples, however, we could determine systemic CMV reactivation only in 11 (n=61, 18%) cases among the CMV positive tissues samples.

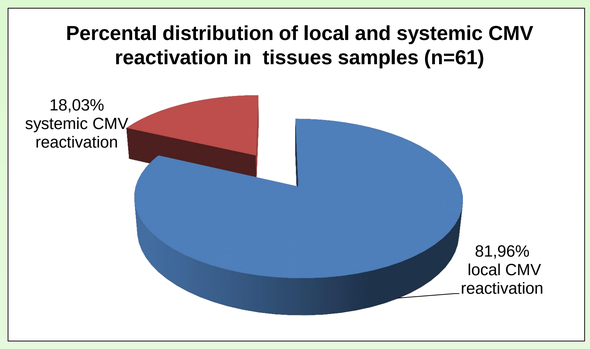
CMV reactivation was demonstrated in 49 (n=159, 30,8%) patients, of them reactivation localized only to the gastrointestinal tract in 32 (n=49, 65,3%) cases. Although we could prove the systemic CMV reactivation or infection only in 17 (n=49, 34,7%) patient, resulting improvement of the clinical symptoms in most of them (n=23, 82,10%).

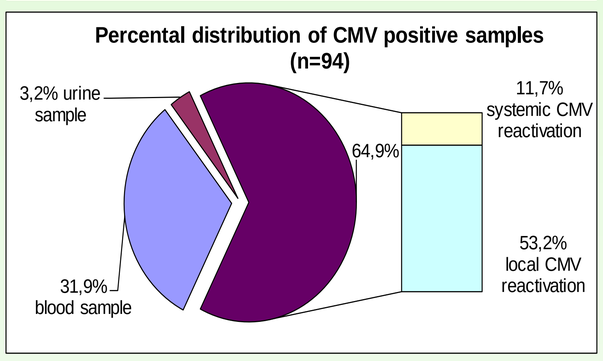


CMV results (n=354)

	Sample number	CMV DNA positive	CMV DNA negative
Blood sample	183	16,4%	83,6%
Urine sample	31	9,7%	90,3%
Tissue sample	140	43,6%	56,4%
Total samples	354	26,5%	73,5%







Results of CMV positive and negative patients (n=159)

	CMV negative patients	CMV positive patiens
	110 (69,2%)	49 (30,8%)
local CMV reactivation	-	32 (20,1%)
systemic CMV reactivation	-	17 (10,7%)

Conclusions

-Quantitative PCR assay helps us in the diagnosis and monitoring of the CMV infection from different biological samples, and also to follow therapy.

-The applied protocol is qualified to make a difference between systemic and local CMV infections/reactivations.

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