



**HAL**  
open science

## **Low Frequency of Cytomegalovirus Infection during Exacerbations of Inflammatory Bowel Diseases.**

Nicolas Lévêque, Hédia Brix-Benmansour, Thierry Reig, Fanny Renois, Déborah Talmud, Véronique Brodard, Jean-François Coste, Christophe de Champs, Laurent Andréoletti, Marie-Danièle Diebold

► **To cite this version:**

Nicolas Lévêque, Hédia Brix-Benmansour, Thierry Reig, Fanny Renois, Déborah Talmud, et al.. Low Frequency of Cytomegalovirus Infection during Exacerbations of Inflammatory Bowel Diseases.. Journal of Medical Virology, 2010, 82 (10), pp.1694. 10.1002/jmv.21877 . hal-00570698

**HAL Id: hal-00570698**

**<https://hal.science/hal-00570698>**

Submitted on 1 Mar 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## Low Frequency of Cytomegalovirus Infection during Exacerbations of Inflammatory Bowel Diseases.

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-1790.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	20-May-2010
Complete List of Authors:	Lévêque, Nicolas; Faculté de médecine, université de Reims Champagne Ardenne, IFR53/EA-4303; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire Brix-Benmansour, Hédia; Centre Hospitalier Universitaire, Service de Gastro-entérologie Reig, Thierry; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire Renois, Fanny; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire; Faculté de médecine, université de Reims Champagne Ardenne, IFR53/EA-4303 Talmud, Deborah; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire; Faculté de médecine, université de Reims Champagne Ardenne, IFR53/EA-4303 Brodard, Véronique; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire Coste, Jean-François; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire De Champs, Christophe; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire Andréoletti, Laurent; Centre Hospitalier Universitaire, Unité de Virologie Médicale et Moléculaire; Faculté de médecine, université de Reims Champagne Ardenne, IFR53/EA-4303 Diebold, Marie-Danièle; Centre Hospitalier Universitaire, Laboratoire d'Anatomopathologie
Keywords:	Cytomegalovirus, inflammatory bowel diseases, exacerbation, real-time quantitative PCR



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

# Low Frequency of Cytomegalovirus Infection during Exacerbations of Inflammatory Bowel Diseases.

## *Running title*

### Cytomegalovirus infection in Inflammatory Bowel Diseases

Nicolas Lévêque<sup>1,2\*</sup>, Hedia Brix-Benmansour<sup>3</sup>, Thierry Reig<sup>1</sup>, Fanny Renois<sup>1,2</sup>,  
Déborah Talmud<sup>1,2</sup>, Véronique Brodard<sup>1</sup>, Jean-François Coste<sup>1</sup>,  
Christophe De Champs<sup>1</sup>, Laurent Andréoletti<sup>1,2</sup>, Marie-Danièle Diebold<sup>4</sup>

<sup>1</sup> Unité de Virologie Médicale et Moléculaire, Centre Hospitalier Universitaire, Reims;

<sup>2</sup> IFR 53/EA-4303 (DAT /PPCIDH), Faculté de Médecine, Reims;

<sup>3</sup> Service de Gastro-entérologie, Centre Hospitalier Universitaire, Reims, France;

<sup>4</sup> Laboratoire d'Anatomopathologie, Centre Hospitalier Universitaire, Reims, France;

\*Corresponding author: Nicolas Lévêque, Laboratoire de Virologie, Service de Microbiologie, Hôpital Robert Debré, Avenue du Général Koenig, 51092 REIMS Cedex, France. Tel: (33) 3 26 78 39 93; Fax: (33) 3 26 78 41 34; Electronic address: [nleveque@chu-reims.fr](mailto:nleveque@chu-reims.fr)

**Abstract (248 words):**

Although numerous reports have described inflammatory bowel diseases (IBD) complicated with cytomegalovirus (CMV) infection, the virus participation as an exacerbating factor remains unclear. The aim of this study was thus to clarify the clinical significance of CMV infection complicating exacerbation and to correlate CMV detection with various characteristics in IBD patients. Sixty-seven colonic biopsies obtained from 53 patients admitted for IBD exacerbation were retrospectively analyzed by real-time PCR assay. The CMV genome was detected in 7 (10.4%) colonic biopsies related to 7 patients (3 ulcerative colitis and 4 Crohn's diseases). Among the patients with IBD studied, patients with evidence of CMV infection were older ( $p= 0.047$ ), were more likely male gender (relative risk [RR] 4.48; 95% confidence interval [CI] 0.94-21.36), received corticosteroids (RR 3.2; CI 0.79-13.02) or azathioprine (RR 3.17; CI 0.80-12.57) treatments, presented more extended lesions (RR for rectum-sigmoid-left colon 3.75 (0.0-69.37) and for pancolitis 2.45 (0.36-16.23)), and had a more severe disease (RR 3.3; CI 0.87-12.48) than those without CMV infection. Viral loads measured in the colonic mucosa of infected patient ranged from 5 to 236961 genome copies by microgram of total extracted DNA. No relationship was observed between the severity of the disease and the viral load level. Furthermore, CMV disappeared in 5 infected IBD patients in remission without antiviral agents. In conclusion, these results showed infrequent CMV detection in colonic biopsies of IBD patients during exacerbation leaving open the question of the relationship between CMV reactivation and the onset or the severity of IBD exacerbation.

**Key-words:** Cytomegalovirus, inflammatory bowel diseases, exacerbation, real-time quantitative PCR.

## Introduction

The Inflammatory Bowel Diseases (IBDs) contain mainly two types of chronic intestinal disorders: Crohn's disease and ulcerative colitis [Abraham and Cho, 2009]. They correspond to chronic inflammatory bowel disorders involving most often the ileum and the colon in Crohn's disease, and the colon and the rectum in ulcerative colitis. IBDs peak onset is in persons aged 15 to 30 [Loftus and Sandborn, 2002]. Their prevalence doubled during the past 50 years and is estimated at this day at 11 cases for 10,000 inhabitants [Shanahan and Bernstein, 2009]. Like all other immune-mediated inflammatory diseases, natural history of IBDs is characterised by periods of exacerbation and remission [Levenstein et al., 2000; Doherty and Cheifetz, 2009]. Several environmental triggers including nonsteroidal anti-inflammatory drugs, antibiotics, bacterial and viral infections were identified to be responsible for the development of IBDs exacerbation [Lakatos et al., 2009; Lidar et al., 2009]. Among viral aetiologies, cytomegalovirus (CMV), a member of the herpes virus family, was reported to cause opportunistic infection in patients suffering from IBDs, usually immunosuppressed, and therefore presumably at increased risk of active infection and disease [Rowshani et al., 2005; Kandiel et al., 2006; Criscuoli et al., 2006].

CMV was associated for the first time with IBDs in 1961 when it was detected in a case of ulcerative colitis [Powell et al., 1961]. Since then, some reports have assumed a triggering role of CMV infection in the onset or the worsening of IBDs [Cooper et al., 1977; Eyre Brook and Dundas, 1986; Criscuoli et al., 2006; Kandiel et al., 2006; Orloff et al., 1989]. CMV was thus found to cause immunosuppression and to increase the severity of inflammatory diseases [Pofelski et al., 2007; Maher and Nassar, 2009]. Furthermore, CMV detection was associated with poor prognosis and steroid refractoriness [Cottone et al., 2001; Kambham et al., 2004; Maconi et al., 2005]. In most cases, CMV infections were caused by a reactivation of a latent virus due to immunosuppressive therapy; acute CMV infections were rare [Hommes et al., 2003]. Among these studies, the observed incidence of active CMV infection in IBDs patients varied widely with regard to patients' selection (ulcerative colitis or non selected patients, mild to moderate disease, severe colitis, steroid-resistant colitis, urgent colectomy for colitis) and diagnostic methods (type of sample and diagnostic method used for analysis) [Kaufman et al., 1999; Vega et al., 1999; Wada et al., 2003; Kishore et al., 2004; Takahashi and Tange, 2004; Domènech et al., 2008; Kim et al., 2010]. Incidence was thus reported to range from 0.5 to 100% and was always higher in IBDs patients than in the control groups studied [Wakefield et al., 1992; Papadakis et al., 2001]. However, despite the above-

1  
2  
3 mentioned investigations, the strength of association between CMV infection and IBDs  
4 remains controversial [Pfau et al., 2001; Kim et al., 2010]. In fact, the relationship of  
5 superimposed CMV infection with severity and clinical outcome of IBDs is not well  
6 established, since the virus is known to replicate coincidentally due to the inflammatory  
7 disease itself and to the associated immunosuppressive therapy. Furthermore, as far as the  
8 treatment is concerned, a review of the literature does not affirm that antiviral treatment is  
9 mandatory to go into remission when CMV is detected in biopsy specimens or in peripheral  
10 blood [De Saussure et al., 2004; Matsuoka et al., 2007].  
11

12  
13 In this context, the aim of this 4-year retrospective study was to evaluate the incidence  
14 of CMV infections in a cohort of non selected IBDs patients (ulcerative colitis and Crohn's  
15 disease) using a real-time quantitative PCR assay in colonic biopsies sampled during  
16 exacerbation. It was also attempted to correlate CMV infection with various demographic,  
17 therapeutic and clinical variables in order to define a clinical index of suspicion for CMV  
18 infection in IBDs patients. The last objective was to assess the role of the CMV quantitation  
19 in biopsy specimens from the gastrointestinal mucosa for the diagnosis and the management  
20 of IBDs exacerbation by relating the viral load to clinical, histological data and to the medical  
21 treatment outcome.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Patients and Methods

### *Patients and samples*

All patients admitted in the gastroenterology department of the Reims University hospital between May 2005 and May 2009 for IBDs exacerbation were investigated including both outpatients and inpatients. The diagnosis of IBD was based on clinical, endoscopic, radiologic, and histologic parameters. The Lichtiger index (LI) was selected as the representative clinical index to determine the severity of the disease [Lichtiger et al., 1994]. Biopsies were obtained during colonoscopy for histological examination of inflammatory activity and routinely addressed to the virological laboratory for CMV detection. Samples were stored at  $-80^{\circ}\text{C}$  until further processing for CMV DNA. Five millilitres venous blood were also obtained from colonic biopsies CMV positive patients to quantify CMV viremia by real-time PCR. Demographic, therapeutic and clinical profiles of all investigated patients (age, sex, IBD type, Lichtiger index, extent of the disease, severity of exacerbation and treatment) were retrospectively collected. Informed consent was obtained from each included patient. The present study was conducted by the University hospital of Reims (Champagne Ardenne, France) and was approved by the hospital's Ethics Committee.

### *Quantitative Real-time PCR*

Five milligrams of colonic tissues were subjected to proteinase K (600 mAnson-U/ml) (Merck Novagen®) digestion in extraction buffer containing 20mM Tris-HCl pH 8.3 (Sigma®), and 0.5% SDS (Sigma®) 30 minutes in a water bath at  $56^{\circ}\text{C}$ . DNA for real-time PCR assay was then extracted from the supernatant using EasyMAG® (BioMérieux, France) according to the manufacturer's instructions. The assay integrating PCR inhibitors detection was performed using an iCycler IQ<sup>TM</sup> (BioRad) as described previously [Najioullah et al., 2001]. Briefly, the oligonucleotide primers used for CMV DNA amplification were constructed to detect the late HXLF-4 transmembrane protein gene. The upstream primer was 5-ACCAACATAAGGACT TTTCACACTTTT-3 and the downstream primer was 5-GAATACAGACTTAGAGCTCGGGGT-3. The 6-carboxyfluorescein-labeled probe was 5-CTGGCCAGCACGTATCCCAACAGCA-3. The PCR conditions were incubation at  $95^{\circ}\text{C}$  for 10 minutes, 45 cycles of  $95^{\circ}\text{C}$  for 15 seconds, followed by incubation at  $60^{\circ}\text{C}$  for 15 seconds and  $72^{\circ}\text{C}$  for 40 seconds.



1  
2  
3 Total DNA was quantified at 260/280 nm using a spectrophotometer. CMV viral load levels  
4 were then expressed as the number of CMV DNA copies by microgram ( $\mu\text{g}$ ) of extracted  
5 DNA.  
6  
7  
8  
9

### 10 *Statistical Analysis*

11 Statistical analysis to determine the characteristics associated with CMV infection in IBDs  
12 patients was conducted by utilizing the Chi-squared test or two-tailed Fisher exact test to  
13 compare qualitative variables, and Student's t-test for quantitative variables. These analyses  
14 were carried out with SPSS 11.0 program (SPSS, Paris, France). A  $p$  value  $<0.05$  was  
15 considered statistically significant. The 95% confidence intervals (95% CI) were calculated  
16 for the relative risk using Taylor series confidence intervals with Epi Info version 6 software  
17 (Center for Disease Control USA).  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## RESULTS

### *Demographic and clinical parameters of investigated patients with exacerbate IBD*

From May 2005 to May 2009, 53 patients were admitted in the Gastroenterology department of the Reims university hospital for IBD exacerbation. Forty-two patients (79.2%) presented a single event of exacerbation while 9 (17%) had two, one had three and one (1.9%) had 4. Sixty-seven colonic biopsies, corresponding each to one exacerbation phase, were thus collected from the 53 patients during the 4-year study. Among the 53 included patients, 20 (37.7%) had ulcerative colitis (sex-ratio M/F: 0.7) while 33 (62.3%) had Crohn's disease (sex-ratio M/F: 0.45). At the time of their assessment in the study, the median age of ulcerative colitis patients was  $40 \pm 14.6$ , while it was  $37 \pm 16.1$  in Crohn's disease patients. In addition, mean duration of IBD in these patients was 9.8 years. As defined by Lichtiger index, 42 (62.7%) selected patients had clinically moderate to severe IBD at time of exacerbation. IBD described pancolitis in 25.9 %. Eight patients (14.8%) were resistant to steroids while 12 (22%) were steroid-dependent. Thirteen (24%) presented with a history of colectomy. All the demographic, therapeutic and clinical characteristics of the IBD patients are shown in table 1.

### *Prevalence of active CMV infection during IBD exacerbation*

The CMV genome was found in the colonic mucosa by PCR in 7 (10.4%) of the 67 colonic biopsies corresponding to 7 patients (13.2%): 3 (15%) of those with ulcerative colitis and 4 (12.1%) of those with Crohn's disease. CMV infection incidence was not statistically significant between the two types of IBD.

### *Factors associated with CMV infection*

Table 2 summarizes the association of various clinical characteristics with or without CMV infection in IBDs patients. Due to the small number of CMV infection, statistical analysis was conducted on overall IBDs patients with no distinction between ulcerative colitis and Crohn's disease. On statistical analysis patients with evidence of CMV infection were older ( $p=0.047$ ), were more often male gender (relative risk [RR] 4.48; 95% confidence interval [CI] 0.94-21.36), had been more often on corticosteroids (RR 3.2; CI 0.79-13.02) or azathioprine (RR 3.17; CI 0.80-12.57) treatments than those without CMV infection. Furthermore, infected patients presented more extended lesions (RR for rectum-sigmoid-left colon 3.75 (0.0-69.37) and for pancolitis 2.45 (0.36-16.23)), and had a more severe disease (RR 3.3; CI 0.87-12.48). Interestingly, female gender, IBD type, smoking, administration of biologics, dependence or

1  
2  
3 resistance to corticosteroids and requirement for surgical treatment, were not statistically  
4 associated with CMV detection in the colonic tissue (table 2).  
5  
6  
7

8  
9 *Relationship between viral load in colonic biopsies and clinical management and outcome of*  
10 *exacerbation*

11 CMV DNA was detected and quantified in 7 biopsy specimens corresponding to 7 IBD  
12 exacerbations (table 3). Furthermore, one patient (P48) underwent a second endoscopy 2  
13 weeks after initial CMV detection for virological and histological monitoring, which was thus  
14 not considered as a second IBD flare. Viral load was then correlated to the treatment, in  
15 particular the administration of antiviral therapy, and to the clinical outcome of the IBD  
16 exacerbation. In the positive studied biopsies, CMV DNA load ranged from 5 to 236961  
17 genome copies/ $\mu$ g of total extracted DNA with a median viral load of 37433 copies (table 3).  
18 Among the 7 CMV positive patients, 6 did not receive antiviral therapy. In 5 patients, the  
19 course of the disease was significantly improved by intensifying immunosuppressive therapy  
20 resulting in the decrease of abdominal pain, colitis lesions and diarrhea. For the last patient  
21 without antiviral therapy, the patient P1 who presented Crohn's disease, the IBD evolution  
22 required surgical treatment (table 3).  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

33 CMV detection in colonic biopsy was associated with the administration of intravenous  
34 ganciclovir followed by oral valganciclovir for only one patient, the patient P48 (table 3).  
35 Antiviral treatment was administrated because of the severity of the clinical symptoms. Rapid  
36 and significant decrease of viral load was observed both in the colonic biopsy and in the blood  
37 samples, since CMV DNA became undetectable in the patient's plasma 3 weeks after the  
38 beginning of the antiviral treatment (figure 1). At the same time, immunosuppressive therapy  
39 was also reinforced by the association of intravenous corticosteroids and anti-TNF alpha  
40 therapy to the previous azathioprine and mesalamine treatment. The course of the disease  
41 improved rapidly, since the Lichtiger score decreased from 12 at time of admission to 1 one  
42 month later.  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## DISCUSSION

In this study, 67 colonic biopsies sampled from 53 patients suffering from exacerbation of IBD were analyzed retrospectively. CMV was detected in 7 biopsies corresponding to 7 exacerbations that occurred in 7 different patients establishing a prevalence of CMV infection at 10.4%. The prevalence rates of CMV infection among IBDs populations reported previously ranged from 0.5% to 72% [Wakefield et al., 1992; Papadakis et al., 2001]. Studies including patients presenting acute severe colitis or steroid-refractory colitis described the highest CMV prevalence [Cottone et al., 2001; Criscuoli et al., 2004; Minami et al., 2007; Yoshino et al., 2007; Maher et al., 2008]. Only 3 studies could be legitimately compared with the present work. These studies have determined CMV prevalence by PCR assays in colonic biopsies obtained from non selected IBD patients. In 1992, Wakefield et al. highlighted the prevalence of the CMV infection of 72%, whereas Kishore *et al.* in 2004 and Dimitroulia *et al.* in 2006 described the prevalence of 16% and 33%, respectively [Wakefield et al., 1992; Kishore et al., 2004. Dimitroulia et al., 2006]. This prevalence, variable from one study to another, was significantly higher than the one reported here. The most obvious difference holds in the technique of PCR employed, nested for Wakefield, standard for Kishore and Dimitroulia or real-time PCR assay in the present study. Considering the high sensitivity of the previously published real-time PCR assay used in this work, lower CMV prevalence may be due to the over-representation of Crohn's disease patients (62.3% of the IBD population studied) [Najioullah et al., 2001]. Prior reports showed a higher incidence of CMV in ulcerative colitis patients compared to Crohn's disease patients [Loftus et al., 1994; Vega et al., 1999; Cottone et al., 2001; Kim et al., 2010]. Furthermore, the therapeutic protocols, some of them at higher risk of causing CMV reactivation, and the percentage of seropositive patients for CMV among IBD studied patients could vary from a hospital to another and thus generate differences in viral infection prevalence.

Demographic and clinical characteristics of IBDs patients presenting or not CMV infection were then compared to try to determine specific profile and predisposing factors to viral infection (table 2). Unfortunately, in spite of a 4-year recruitment, only a small number of CMV positive biopsies were obtained limiting thus the significance of the statistical analysis of collected data. Because of the small number of infected patients, the remaining analysis was conducted on all the IBDs population with no distinction between ulcerative colitis and Crohn's disease patients. Several risk factors of developing CMV infection were this way identified (table 2). The first characteristic was that patients with CMV infection

1  
2  
3 were significantly older. The second risk factor was a treatment by corticosteroids or  
4 azathioprine. This result was in agreement with the data reported previously demonstrating a  
5 statistically significant association between azathioprine or a high-dose systemic  
6 corticosteroids therapy and the detection of CMV in the colonic biopsies of IBDs patients  
7 [Cottone et al., 2001; Wada et al., 2003; Yoshino et al., 2007; Domènech et al., 2008].  
8 Domènech detected the CMV only among corticosteroid-refractory ulcerative colitis patients  
9 treated for 7 to 10 days with a high-dose systemic corticosteroids therapy. The prevalence  
10 observed was 32% [Domènech et al., 2008]. More generally, high CMV detection rates were  
11 logically associated with the administration of intensive immunosuppressive therapies.  
12 Yoshino detected the genome of CMV in 57% of the colonic biopsies (17 biopsies out of 30)  
13 of patients suffering from ulcerative colitis refractory to immunosuppressive therapies,  
14 including corticosteroids and immunomodulators [Yoshino et al., 2007]. It is interesting to  
15 note that the present study confirmed that anti-Tumor Necrosis Factor (TNF) alpha therapy,  
16 known to be highly immunosuppressive, did not seem to be significantly associated with  
17 CMV infection [D'Ovidio et al., 2008]. The continuation of this study should allow to  
18 improve the knowledge of clinical characteristics associated to CMV infection and of factors  
19 predisposing to CMV infection in IBDs patients.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

33 CMV viral loads in colonic biopsies were then determined using a real-time PCR  
34 assay and normalized as the number of CMV DNA copies by  $\mu\text{g}$  of extracted DNA. Although  
35 real-time PCR assay was used previously for detection of CMV infection in patients with  
36 IBDs, this study is the first to define the level of CMV replication in the intestine tissue  
37 [Matsuoka et al., 2007; Minami et al., 2007; Yoshino et al., 2007]. The value of viral  
38 quantitation, in contrast to the qualitative PCR assays, is to define the level of replication of  
39 the virus and then to try to link it to the severity of the disease or to give it a prognostic value.  
40 Furthermore, in case of antiviral treatment the quantitative PCR can also allow to follow the  
41 therapeutic effectiveness through the decrease of the viral load [Baldanti et al., 2008]. In this  
42 report, the viral loads measured among IBDs CMV positive patients ranged from 5 to 236961  
43 genome copies/ $\mu\text{g}$  of total extracted DNA. Interestingly, the CMV replication level was not  
44 related to the severity of the exacerbation, since viral loads at 5 and 236961 copies  
45 respectively detected in the P17 and P27 patients were associated with moderate exacerbation,  
46 whereas viral load at 1834 in patient P1 corresponded to severe disease and poor outcome  
47 (table 3). A limitation of this aspect in the present study was again the small number of  
48 positive biopsies to conclude on the basis of viral quantitation for the prognosis of IBD  
49 exacerbation evolution. Sampling error should also be considered, since CMV replication  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 within the colonic mucosa could be focal. Therefore, the possibility of underestimating CMV  
4 viral load in case of severe disease cannot be entirely excluded.  
5  
6

7 Despite the association between CMV and the extent and the severity of the disease  
8 suggested by the statistical analyses, the results obtained in this study did not provide a clear  
9 evidence of the pathogenicity of CMV in IBDs exacerbation. Five of the 7 CMV positive  
10 patients went into remission by reinforcement of immunosuppressive therapies without  
11 administration of antiviral treatment (table 3). Clinical improvement in spite of the lack of  
12 antiviral medication was reported previously in many studies [De Saussure et al., 2004;  
13 Matsuoka et al., 2007; Kim et al., 2010]. In this series, only one patient among the 7 IBD  
14 patients presenting colonic biopsy positive for the CMV received ganciclovir. Clinical  
15 improvement, consisting in the decrease of the Lichtiger score from 12 to 1 within 3 weeks,  
16 was then observed along with the decrease of the viral load in the plasma as well as in the  
17 colonic mucosa. However, clinical improvement could not be only related to the antiviral  
18 treatment responsible for CMV viral load decrease, since the immunosuppressive therapy was  
19 introduced concomitantly. The good correlation noticed between plasma and colonic mucosa  
20 viral loads was an interesting diagnostic data to consider a possible virological monitoring of  
21 the patient by examination of blood samples instead of colonic biopsies.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

33 In summary, the aim of this 4-year retrospective study was to clarify the clinical  
34 significance of CMV infection complicating IBDs exacerbation. The results showed  
35 infrequent CMV detection in colonic biopsies of IBDs patients without any correlation  
36 between the severity of the disease and the viral load levels in the colonic mucosa.  
37 Furthermore, CMV disappeared in most infected IBDs patients without antiviral treatment. In  
38 conclusion, the relationship between CMV reactivation and the onset or the severity of IBDs  
39 exacerbation remains debatable.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1.** Demographic, therapeutic and clinical characteristics of IBD investigated patients at time of exacerbation. Numbers in parentheses indicate percentage. Ranges are indicated in brackets.

Characteristic	Overall IBD patients
Median age (years)	38.5 [16; 81]
Duration of disease (years)	9.8 [0.5; 21.8]
Male	23 (43.4)
Female	30 (56.6)
Crohn's disease	33 (62.3)
Ulcerative colitis	20 (37.7)
Smoker	10 (23.8)
Extent of disease	
Rectum	16 (29.6)
Sigmoid	6 (11.1)
Left colon	4 (7.4)
Rectum Sigmoid	6 (11.1)
Rectum Sigmoid Left colon	4 (7.4)
Colitis	4 (7.4)
Pancolitis	14 (25.9)
Clinical severity of the disease	
Mild	12 (22.2)
Moderate	32 (59.3)
Severe	10 (18.5)
Medications at time of the assessment in the study	
None	8 (14.8)
Mesalamine	24 (44.4)
Corticosteroids	7 (13.0)
Azathioprine	16 (29.6)
Methotrexate	6 (11.1)
Anti-TNF $\alpha$	11 (20.4)
Corticosteroid-dependant	12 (22.2)
Corticosteroid-resistant	8 (14.8)
History of colectomy	11 (20.4)



**Table 2.** Demographic, therapeutic and clinical characteristics of IBD patients with or without detection of CMV genome in their intestinal tissue samples. Numbers in parentheses indicate percentage. ND, not done. CI, confidence interval.

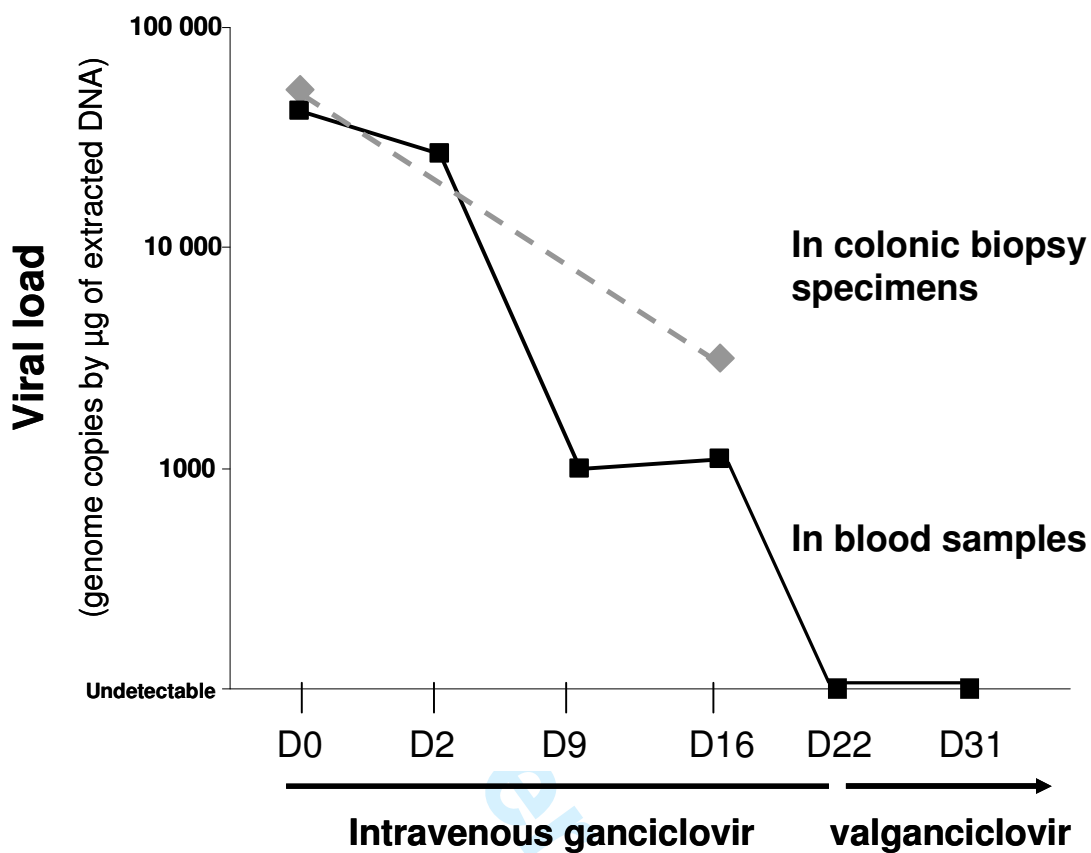
Characteristics	CMV positive	CMV negative	Relative risk [95% CI]
Age > 38 years old	5 (71.4)	29 (48.3)	2.43 [0.51-11.64]
Male	5 (71.4)	19 (39.1)	4.48 [0.94-21.36]
Crohn's disease	4 (57.1)	38 (63)	0.79 [0.19-3.26]
Ulcerative colitis	3 (42.9)	22 (37)	1.26 [0.31-5.18]
Smoker	1 (14.3)	11 (22.0)	0.58 [0.08-4.39]
Extent of disease			
Rectum	1 (14.3)	15 (31.9)	0.4 [0.05-3.03]
Sigmoid	0 (0)	6 (12.8)	ND
Left colon	1 (14.3)	3 (6.4)	2.08 [0.33-13.33]
Rectum Sigmoid	1 (14.3)	5 (10.6)	1.33 [0.19-9.27]
Rectum Sigmoid Left colon	1 (14.3)	2 (4.3)	2.83 [0.48-16.60]
Colitis	0 (0)	4 (8.5)	ND
Pancolitis	3 (42.9)	11 (23.4)	2.14 [0.55-8.41]
Clinical severity of the disease			
Mild	1 (14.3)	11 (23.4)	0.58 [0.08-4.39]
Moderate	3 (42.9)	29 (61.7)	0.52 [0.13-2.08]
Severe	3 (42.9)	7 (14.9)	3.3 [0.87-12.48]
Medications at time of the assessment in the study			
None	1 (14.3)	8 (17.0)	0.83 [0.11-6.11]
Mesalamine	3 (42.9)	19 (40.4)	1.09 [0.27-4.4]
Corticosteroids	2 (28.6)	4 (8.5)	3.2 [0.79-13.02]
Azathioprine	4 (57.1)	12 (25.5)	3.17 [0.80-12.57]
Methotrexate	0 (0)	6 (12.8)	ND
Anti-TNF $\alpha$	0 (0)	11 (23.4)	ND
Corticosteroid-dependant	1 (14.3)	11 (23.4)	0.58 [0.08-4.39]
Corticosteroid-resistant	0 (0)	8 (17.4)	ND
History of colectomy	2 (28.6)	11 (23.4)	1.26 [0.28-5.75]



**Table 3.** Description of the relationship between CMV viral load in colonic biopsies with the severity of the disease, the use of immunosuppressive and antiviral therapies and the outcome of the exacerbation.

Patients	CMV viral load (copies by $\mu\text{g}$ of total DNA)	Severity of the disease	Immunosuppressive therapy at onset of exacerbation	Immunosuppressive therapy at remission of exacerbation	Antiviral treatment	Exacerbation outcome
P 1	1834	Severe	AZA	AZA, CS, MES	None	Poor with colectomy
P 15	4677	Severe	AZA, CS	AZA, CS	None	Remission
P 17	5	Moderate	MES	CS, MES	None	Remission
P 27	236961	Moderate	AZA	AZA, CS	None	Remission
P 34	145	Moderate	MES	MES	None	Remission
P45	845	Mild	None	CS	None	Remission
P 48 B1	51812	Severe	AZA, CS, MES	Anti-TNF $\alpha$ , AZA, CS,	<i>IV</i>	Remission
P 48 B2	3189	Moderate	<i>IV</i> CS, AZA, MES	MES	ganciclovir	

AZA : Azathioprine ; CS : corticosteroids ; MES : Mesalamine; *IV* : intravenous



**Figure 1.** Comparative monitoring of CMV DNA load levels in colonic biopsy specimens and in blood samples in patient P48 after applying two successive antiviral therapies.

**Acknowledgments**

The authors thank Lysiane Bourdairé, Harmony Lapie, Petra Hutig and Dorine Lebrun for their technical help during this study. They also thank Ms Jocelyne Wuibout to have kindly reviewed the English of this paper.

**Conflict of interest statement**

None of the authors have conflict of interest or funding source to declare in connection with this study.

For Peer Review

## References

- Abraham C, Cho JH. 2009. Inflammatory bowel disease. *N Engl J Med* 361:2066-2078.
- Baldanti F, Lilleri D, Gerna G. 2008. Human cytomegalovirus load measurement and its applications for pre-emptive therapy in patients undergoing hematopoietic stem cell transplantation. *Hematol Oncol* 26:123-130.
- Cooper HS, Raffensperger EC, Jonas L, Fitts WT Jr. 1977. Cytomegalovirus inclusions in patients with ulcerative colitis and toxic dilation requiring colonic resection. *Gastroenterology* 72:1253-1256.
- Cottone M, Pietrosi G, Martorana G, Casà A, Pecoraro G, Oliva L, Orlando A, Rosselli M, Rizzo A, Pagliaro L. 2001. Prevalence of cytomegalovirus infection in severe refractory ulcerative and Crohn's colitis. *Am J Gastroenterol* 96:773-775.
- Criscuoli V, Casà A, Orlando A, Pecoraro G, Oliva L, Traina M, Rizzo A, Cottone M. Severe acute colitis associated with CMV: a prevalence study. *Dig Liver Dis* 2004 36:818-820.
- Criscuoli V, Rizzuto MR, Cottone M. 2006. Cytomegalovirus and inflammatory bowel disease: is there a link? *World J Gastroenterol* 12:4813-4818.
- De Saussure P, Lavergne-Slove A, Mazon MC, Alain S, Matulcerative colitishansky C, Bouhnik Y. 2004. A prospective assessment of cytomegalovirus infection in active inflammatory bowel disease. *Aliment Pharmacol Ther* 20:1323-1327.
- Dimitroulia E, Spanakis N, Konstantinidou AE, Legakis NJ, Tsakris A. 2006. Frequent detection of cytomegalovirus in the intestine of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 12:879-884.
- Doherty GA, Cheifetz AS. 2009. Management of acute severe ulcerative colitis. *Expert Rev Gastroenterol Hepatol* 3:395-405.
- Domènech E, Vega R, Ojanguren I, Hernández A, Garcia-Planella E, Bernal I, Rosinach M, Boix J, Cabré E, Gassull MA. 2008. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. *Inflamm Bowel Dis* 14:1373-1379.
- D'Ovidio V, Vernia P, Gentile G, Capobianchi A, Marcheggiano A, Viscido A, Martino P, Caprilli R. 2008. Cytomegalovirus infection in inflammatory bowel disease patients undergoing anti-TNFalpha therapy. *J Clin Virol* 43:180-183.
- Eyre Brook IA, Dundas S. 1986. Incidence and clinical significance of colonic cytomegalovirus infection in idiopathic inflammatory bowel disease requiring colectomy. *Gut* 27:1419-1425.

- 1  
2  
3 Hommes DW, Sterringa G, van Deventer SJ, Tytgat GN, Weel J. 2004. The pathogenicity of  
4 cytomegalovirus in inflammatory bowel disease: a systematic review and evidence-  
5 based recommendations for future research. *Inflamm Bowel Dis* 10:245-250.  
6  
7  
8 Kambham N, Vij R, Cartwright CA, Longacre T. 2004. Cytomegalovirus infection in steroid-  
9 refractory ulcerative colitis: a case-control study. *Am J Surg Pathol* 28:365-373.  
10  
11 Kandiel A, Lashner B. 2006. Cytomegalovirus colitis complicating inflammatory bowel  
12 disease. *Am J Gastroenterol* 101:2857-2865.  
13  
14  
15 Kaufman HS, Kahn AC, Iacobuzio-Donahue C, Talamini MA, Lillemoe KD, Hamilton SR.  
16 1999. Cytomegaloviral enterocolitis: clinical associations and outcome. *Dis Colon*  
17 *Rectum* 42:24-30.  
18  
19  
20 Kim JJ, Simpson N, Klipfel N, Debose R, Barr N, Laine L. 2010. Cytomegalovirus Infection  
21 in Patients with Active Inflammatory Bowel Disease. *Dig Dis Sci* [Epub ahead of print].  
22  
23 Kishore J, Ghoshal U, Ghoshal ULCERATIVE COLITIS, Krishnani N, Kumar S, Singh M,  
24 Ayyagari A. 2004. Infection with cytomegalovirus in patients with inflammatory bowel  
25 disease: prevalence, clinical significance and outcome. *J Med Microbiol* 53:1155-1160.  
26  
27  
28 Lakatos PL. 2009. Environmental factors affecting inflammatory bowel disease: have we  
29 made progress? *Dig Dis* 27:215-225.  
30  
31  
32 Levenstein S, Prantera C, Varvo V, Scribano ML, Andreoli A, Luzi C, Arcà M, Berto E,  
33 Milite G, Marcheggiano A. 2000. Stress and exacerbation in ulcerative colitis: a  
34 prospective study of patients enrolled in remission. *Am J Gastroenterol* 95:1213-1220.  
35  
36  
37 Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S.  
38 1994. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J*  
39 *Med* 330:1841-1845.  
40  
41  
42 Lidar M, Langevitz P, Shoenfeld Y. 2009. The role of infection in inflammatory bowel  
43 disease: initiation, exacerbation and protection. *Isr Med Assoc J* 11:558-63.  
44  
45  
46 Loftus EV Jr, Alexander GL, Carpenter HA. 1994. Cytomegalovirus as an exacerbating factor  
47 in ulcerative colitis. *J Clin Gastroenterol* 19:306-309.  
48  
49  
50 Maconi G, Colombo E, Zerbi P, Sampietro GM, Fociani P, Bosani M, Cassinotti A, Casini V,  
51 Russo A, Ardizzone S, Porta M, Bianchi Porro G. 2005. Prevalence, detection rate and  
52 outcome of cytomegalovirus infection in ulcerative colitis patients requiring colonic  
53 resection. *Dig Liver Dis* 37:418-423.  
54  
55  
56 Maher MM, Nassar MI. 2009. Acute cytomegalovirus infection is a risk factor in refractory  
57 and complicated inflammatory bowel disease. *Dig Dis Sci* 54:2456-2462.  
58  
59  
60

- 1  
2  
3 Matsuoka K, Iwao Y, Mori T, Sakuraba A, Yajima T, Hisamatsu T, Okamoto S, Morohoshi  
4 Y, Izumiya M, Ichikawa H, Sato T, Inoue N, Ogata H, Hibi T. 2007. Cytomegalovirus is  
5 frequently reactivated and disappears without antiviral agents in ulcerative colitis  
6 patients. *Am J Gastroenterol* 102:331-337.  
7  
8  
9  
10 Minami M, Ohta M, Ohkura T, Ando T, Ohmiya N, Niwa Y, Goto H. 2007. Cytomegalovirus  
11 infection in severe ulcerative colitis patients undergoing continuous intravenous  
12 cyclosporine treatment in Japan. *World J Gastroenterol* 13:754-760.  
13  
14  
15  
16 Najioullah F, Thouvenot D, Lina B. 2001. Development of a real-time PCR procedure  
17 including an internal control for the measurement of HCMV viral load. *J Virol Methods*  
18 92:55-64.  
19  
20  
21 Orloff JJ, Saito R, Lasky S, Dave H. 1989. Toxic megacolon in cytomegalovirus colitis. *Am J*  
22 *Gastroenterol* 84:794-797.  
23  
24  
25 Papadakis KA, Tung JK, Binder SW, Kam LY, Abreu MT, Targan SR, Vasiliauskas EA.  
26 2001. Outcome of cytomegalovirus infections in patients with inflammatory bowel  
27 disease. *Am J Gastroenterol* 96:2137-2142.  
28  
29  
30 Pfau P, Kochman ML, Furth EE, Lichtenstein GR. 2001. Cytomegalovirus colitis  
31 complicating ulcerative colitis in the steroid-naive patient. *Am J Gastroenterol* 96:895-  
32 899.  
33  
34  
35 Podolsky DK. 2002. Inflammatory bowel disease. *N Engl J Med* 347:417-429.  
36  
37 Pofelski J, Heluwaert F, Roblin X, Morand P, Gratacap B, Germain E, Brion JP, Salon C,  
38 Bonaz B. 2007. Cytomegalovirus and cryptogenic inflammatory bowel disease.  
39 *Gastroenterol Clin Biol* 31:292-296.  
40  
41  
42 Powell RD, Warner NE, Levine RS, Kirsner JB. 1961. Cytomegalic inclusion disease and  
43 ulcerative colitis; report of a case in a young adult. *Am J Med* 30:334-340.  
44  
45  
46 Rowshani AT, Bemelman FJ, van Leeuwen EM, van Lier RA, ten Berge IJ. 2005. Clinical  
47 and immunologic aspects of cytomegalovirus infection in solid organ transplant  
48 recipients. *Transplantation* 79:381-386.  
49  
50  
51 Shanahan F, Bernstein CN. 2009. The evolving epidemiology of inflammatory bowel disease.  
52 *Curr Opin Gastroenterol* 25:301-305.  
53  
54  
55 Takahashi Y, Tange T. 2004. Prevalence of cytomegalovirus infection in inflammatory bowel  
56 disease patients. *Dis Colon Rectum* 47: 722-726.  
57  
58  
59 Vega R, Bertrán X, Menacho M, Domènech E, Moreno de Vega V, Hombrados M, Cabré E,  
60 Ojanguren I, Gassull MA. 1999. Cytomegalovirus infection in patients with  
inflammatory bowel disease. *Am J Gastroenterol* 94:1053-1056.

1  
2  
3 Wada Y, Matsui T, Matake H, Sakurai T, Yamamoto J, Kikulcerative colitishi Y, Yorioka M,  
4 Tsuda S, Yao T, Yao S, Haraoka S, Iwashita A. 2003. Intractable ulcerative colitis  
5 caused by cytomegalovirus infection: a prospective study on prevalence, diagnosis, and  
6 treatment. *Dis Colon Rectum* 46:S59-65.  
7  
8

9  
10 Wakefield AJ, Fox JD, Sawyerr AM, Taylor JE, Sweenie CH, Smith M, Emery VC, Hudson  
11 M, Tedder RS, Pounder RE. 1992. Detection of herpesvirus DNA in the large intestine  
12 of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain  
13 reaction. *J Med Virol* 38:183-190.  
14  
15

16  
17 Yoshino T, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, Matsuura M, Ohmori K, Sakurai  
18 T, Nagayama S, Hasegawa S, Sakai Y, Chiba T. 2007. Usefulness of quantitative real-  
19 time PCR assay for early detection of cytomegalovirus infection in patients with  
20 ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis*  
21 13:1516-1521.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60