Journal of Clinical Virology 66 (2015) 103–106

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

# Toxic megacolon and human *Cytomegalovirus* in a series of severe ulcerative colitis patients

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ARTICLE INFO

*Article history:*
Received 3 March 2015
Received in revised form 10 March 2015
Accepted 12 March 2015

*Keywords:*
Cytomegalovirus
Ulcerative colitis
Megacolon

ABSTRACT

*Background:* Human *Cytomegalovirus* (HCMV) infection has been reported to be a cause of refractory ulcerative colitis (UC). Toxic megacolon (TM) is a rare but severe complication of an acute attack of UC.
*Objectives:* Aim of this study is to evaluate in a case-control study the association between HCMV and TM.
*Study design:* All patients who were admitted at Medicine Department of V. Cervello Hospital in Palermo (tertiary referral center) for a severe UC flare-up complicated by the onset of TM (diameter of the transverse colon > 6 cm) between January 1990 and November 2011 were identified through the electronic database. A total of 24 consecutive patients (16 male/8 female) with TM were identified. Each case of TM were individually matched by sex, age, extent of the underlying disease to 24 severe UC controls who did not develop TM. A further non matched control population of 48 severe UC was included.

Haematoxilin and eosin stain, immunohistochemical procedure and nested polymerase chain reaction were performed to detect HCMV genes and proteins on rectal biopsies or surgical specimens. Pp65 antigenemia was performed in order to diagnose any possible systemic infection. HCMV frequency was compared between patients with and without TM during follow-up, using Fisher's Exact test.
*Results and conclusions:* HCMV was detected in histological specimens of 11 patients (46%) with TM compared to 2 (9%) severe UC matched controls ($P = 0.0078$) and 7 (14%) unmatched controls ($p = 0{,}003$). In severe colitis the presence of HCMV is more frequently associated with TM.


## 1. Background

TM is a well recognized, potentially fatal complication of UC and denotes a clinical syndrome of systemic toxicity accompanied by radiographic evidence of colonic dilatation. First described in 1950, TM is a potentially lethal complication of inflammatory bowel disease (IBD) or infectious colitis, characterized by total or segmental non-obstructive colonic dilatation of at least 6 cm associated with systemic toxicity. Criteria for the diagnosis of TM in adults were proposed by Jalan et al. [1].

Among infective cause *Clostridium* difficile [3], *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter* and, above all in AIDS patients, *Cryptosporidium*, *Entameba* and HCMV were recognized.

A prospective study evaluated the prevalence rate of HCMV infection among a population of consecutive IBD patients with severe acute colitis, as well as the prevalence of this infection among the subgroups of steroid refractory patients. The prevalence of HCMV infection was 21% (9 out of 42 patients) [2]. The prevalence rate of HCMV infection in the steroid-refractory group was 33% (4 out of 12 patients) which approximate the previous estimate in a previous series [3].

In one series, evidence of HCMV infection was found in the resected colon of 6 of 46 patients with UC. Five of these six patients but only 2 of the 40 patients without HCMV infection had TM [4]. Further cases reports described the association between HCMV and TM [5–7] but it is not clear if HCMV is more frequent in TM than in severe colitis.

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http://dx.doi.org/10.1016/j.jcv.2015.03.009
1386-6532/

**Table 1**
Patient and disease characteristics of TM patients and control group.

| | TM patient | Control matched | Controls Not matched |
|---|---|---|---|
| No. of patients (M/F) | 24 (16/8) | 24 (16/18) | 48 (31/17) |
| Age at admission | 45, 5 (15–70) | 37, 4 (18–76) | 39 (18–71) |
| Disease extent | | | |
| # Pancolitis N. (%) | 15 (62.5%) | 14 (58%) | 20 (41%) |
| # Left-sided N. (%) | 9 (37.5%) | 10 (42%) | 28 (59%) |
| Steroid–dependance | 11 (46%) | 16 (66%) | 15 (31%) |
| Previous therapy | | | |
| Mesalamine | 24 (100%) | 24 (100%) | 36 (100%) |
| Azathioprine | 5 (21%) | 13 (54%) | 10 (20%) |

**Table 2**
Clinical characteristics and outcomes of patients with TM and HCMV+

| | Age/sex | Relapse/onset | Duration of disease (year) | Extent of disease | Outcome |
|---|---|---|---|---|---|
| 1 | F/53 | R | 31 | P | C |
| 2 | M/48 | R | 16 | T | C |
| 3 | M/63 | O | 0 | P | C/E |
| 4 | M/45 | O | 0 | P | C/E |
| 5 | F/75 | R | 20 | P | E |
| 6 | F/70 | R | 11 | T | A |
| 7 | M/46 | R | 16 | L | C |
| 8 | M/17 | R | 2 | P | C |
| 9 | M/22 | R | 8 | P | C |
| 10 | M/55 | R | 11 | P | C |
| 11 | M/32 | R | 2 | P | C |

#P = pancolitis, T = transverse, L = left colitis, §C = colectomy, A = antiviral therapy, E = exitus

## 2. Objectives

Aim of this study was to retrospectively assess the frequency of HCMV infection in a consecutive series of severe UC complicated by TM compared to a control group.

## 3. Study design

This was a retrospective study conducted in a single tertiary referral center.

In our unit since 1975, all the patients admitted for severe ulcerative colitis were evaluated clinically with the Truelove and Witts criteria [8] and with a plain X-ray of the abdomen to evaluate the diameter of the transverse colon. Patient was diagnosed to have megacolon if the diameter of the transverse colon was >6 cm. Patients during admission repeated plain X-ray of abdomen in relation to the clinical course.

Health records from 1990 to 2011 were electronically searched through a database for patients with the diagnosis of " megacolon"; exclusion criteria were infectious or ischemic colitis, Hirschsprung disease (HD) and the diagnosis of TM without a clear diagnosis of IBD. Each case of TM were individually matched by sex, age, extent of the underlying disease, year of onset of illness, to one severe UC controls who during the clinical course did not develop TM. A further control non matched population of 48 severe UC was identified from a cohort followed up from 2000 to 2004 in which patients matched with TM were excluded.

A total of 24 consecutive patients with TM (16 male/8 female; median age at diagnosis 45, 5 years) were identified. The study methodology was as follows: from 1990 to 1998 patients hospitalized for severe colitis underwent proctoscopy with rectal biopsy without air insufflations in those who developed steroid resistance after 5 days of conventional therapy. In case of megacolon proctoscopy was performed at diagnosis of this complication. From 1998 to 2011, patients underwent proctoscopy with rectal biopsy at the ward admission without waiting for the response to steroid therapy. Up to 1998 were identified a first series of 11 patients, and from 1999 to 2011 were identified a second series of 13 patients. Basic characteristics, medication and disease history are listed in Table 1.

Abdominal radiographs at the time of diagnosis with TM and subsequent radiographs during that admission, were compared with radiograph of the age-matched UC-controls and with unmatched controls.

### 3.1. Laboratory techniques

Surgical specimens or rectal biopsies were evaluated by a dedicate pathologist in order to identify the presence of HCMV by performing:

- Light microscopy with Haematoxilin and Eosin (H&E) stain in order to document the microscopic disease activity and allow the detection of cytomegalic cells, markers of infected viral cells.
- Immunohistochemical (ICH) procedure for HCMV performed on a paraffin-embedded section with monoclonal mouse antibodies anti-Human CMV (clone BM204) and conjugated to a peroxidase-labeled amino acid polymer by peroxidase-antiperoxidase (PAP) method in order to detect viral proteins. Nuclear or cytoplasmic antigen was identified by the typical brown reaction product of the PAP method.
- Nested polymerase chain reaction ($_n$PCR) by using two pairs of primers annealed to the gB region of HCMV. Primers used for the first-round product and second-round PCRs are as follows (5′–3′): first-round primer 1, GAGGACAACGAAATCCTGTTGGGCA; first-round primer 2, GTCGACGGTGGAGATACTGCTGAGG; second-round primer 3, ACCACCGCACTGsAGGAATGTCAG; and second-round primer 4, TCAATCATGCGTTTGAAGAGGTA, to obtain a HCMV fragment of 100 bp.

The PCR amplification products were run on 2% agarose gel and stained with ethidium bromide and visualized under ultraviolet light.

On peripheral blood was also performed:

- Pp65 antigenemia consist of the detection of the viral pp65 tegument protein by immunofluorescence in polymorphonuclear neutrophils (PNML), previously recovered from a blood buffy-coat. The result was expressed as the number of positive cells per 200.000 PNML.

### 3.2. Statistical analysis

Presence of HCMV were compared between patients with TM and 2 controls population, using chi-square statistics, Fisher's Exact test. Data were analyzed using the software package SPSS 15150 (SPSS Inc., Chicago, IL, USA).

## 4. Results

Among the 24 patients identified in the series, HCMV was detected in histological specimens of 11 patients (46%) (Table 2) with severe UC complicated with TM, compared to 2 (9%) matched controls ($P = 0.0078$) and 7 (14%) ($p = 0{,}003$) unmatched controls.

Among the TM HCMV + patients, the virus was identified in rectal biopsies in seven and in surgical specimen in four. The rate of steroid-resistance in patients with TM was 45% (5/11) among the first series and 53% (7/13) among the second series. Steroid resistance was 21% among matched (5/24) and 20% (10/48) among un matched controls.