The role of CMV in steroid-resistant ulcerative colitis: A systematic review

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Received 4 December 2008; received in revised form 2 March 2009; accepted 3 March 2009

Abstract

Background and aims: Steroid-resistance presents a management challenge in ulcerative colitis. How steroid-resistance occurs is unknown, but cytomegalovirus infection, often unrecognised, may be the cause in some patients. Current evidence and therapeutic recommendations are examined. Methods: A systematic review of PubMed and EMBASE databases was performed. Search and exclusion criteria are defined in the text. Results: Heterogeneity of experimental design and definitions of key terms were notable. Criteria for cytomegalovirus disease, infection or detection varied, as did definitions of steroid-resistance. CMV infection defined by antigenaemia or serology was common in patients on steroids and associated with a higher rate of steroid-resistance (41.66–61% versus 0–68% in steroid-responsive patients). Colonic mucosal cytomegalovirus disease detected by histopathology was associated with intravenous steroid-resistance in 5–36%, compared to 0–10% of steroid-responsive patients. CMV colitis has rarely been reported in association with ulcerative colitis without steroids or other immunomodulators. CMV colitis in healthy individuals is so exceptional as to be the topic of case reports. Conclusion: Ulcerative colitis and its treatment put patients at risk of CMV infection or reactivation. A distinction is necessary between CMV disease (colitis) and CMV infection. Only colonic mucosal CMV infection detected by histopathology appears clinically relevant and appropriate for antiviral therapy. CMV antigenaemia may be associated with steroid-resistance, but may also be a self-limiting marker of viral reactivation. The impact of CMV on steroid-resistance is complicated by inconsistencies in the literature. Coherent definitions of clinically relevant CMV infection and steroid-resistance are needed.

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1. Introduction

Ulcerative colitis (UC) is a relapsing-remitting condition. During an acute relapse, the mainstay of treatment is corticosteroid therapy. Although highly effective for many patients, a proportion of patients do not respond. The reasons are unclear, but reports suggest that cytomegalovirus (CMV) may often be overlooked. The prevalence of CMV infection appears to be particularly high in steroid-resistant colitis, raising the question of whether CMV is cause or consequence of a relapse or therapeutic refractoriness. 36% of an Italian cohort of steroid-resistant Crohn’s disease and UC patients with acute severe colitis were found to be CMV positive by immunohistopathology and buffy coat PCR. The authors concluded that CMV is a frequent cause of refractory colitis. A Japanese study detected CMV using immunohistopathology in 21% of colectomy specimens, all of which (almost by definition) were steroid-resistant. An American group found that 25% of a small number (n = 40) of patients with refractory UC were CMV positive using immunohistochemistry, compared to 2.5% of 40 patients with treatment-responsive colitis (p = 0.007). These data suggest that CMV may be associated with or cause about a third of cases of refractory colitis, but this is much higher than is recognised in clinical practice, which in turn raises a number of questions.

How robust is this evidence? How should CMV “infection” be defined in the clinical context? What, then, is the role of antiviral therapy? Should all UC patients have CMV assessment before therapy? How is steroid-refractoriness defined? This article reviews the literature, discusses experimental design and proposes some answers that need to be confirmed by appropriate trials.

2. Methods

A systematic review of PubMed and EMBASE databases was performed, using the terms inflammatory bowel disease; colitis, ulcerative, Crohn’s disease, drug resistance, cytomegalovirus infection, cytomegalovirus, CMV, requiring colectomy, steroid resistant, steroid refractory, treatment refractory and treatment resistant. Free text and Medical Subject Heading (Mesh) strategies were deployed. Exclusion criteria were: studies not published in the English language; those inaccessible to Oxford University e-resources; those unrelated to humans or irrelevant to the topic; and case reports/case series.

The PubMed search revealed 45 papers, of which 35 were then excluded for irrelevance or unavailability. The 10 remaining ranged in level of evidence from prevalence studies to cohort or case-control studies. A similar search of EMBASE produced 63 papers. After exclusions, all but one paper overlapped with those found on PubMed. There were no randomised controlled trials or systematic reviews in the Cochrane database.

3. Analysis

3.1. What does CMV infection mean?

Although the population seroprevalence of CMV is high, including in developing countries, primary infection in healthy individuals is usually asymptomatic. The virus exists in a latent state thereafter. However, reactivation is common. Immunocompromised patients are known to be more susceptible to reactivation of latent CMV, which can then present as a variety of clinical syndromes or diseases.

Prior to the development of highly active anti-retroviral therapy, 21–44% of acquired immunodeficiency syndrome (AIDS) patients suffered CMV-related disease including retinitis, encephalitis, polyradiculopathy and mononeuritis. Bone marrow transplant recipients are also at increased risk, with CMV infection having wide-ranging detrimental effects, including graft rejection.

A distinction must therefore be made between CMV infection, where a person is CMV-positive on polymerase chain reaction (PCR) or serological testing, and CMV disease, where clinical symptoms are manifest. In the current context, the term disease refers to CMV colitis, causing bloody diarrhoea, abdominal cramps, or fever that might otherwise be attributed to UC. It is this that is considered a possible cause of steroid-resistance. The distinction is usually achieved by light microscopy of rectal or colonic
biopsy specimens, since serological or nucleic acid amplification tests generally identify patients with coexistent rather than causative infection. This distinction has an impact on the detection and implied causation of CMV in the severity of relapse in ulcerative or steroid-resistance.

3.2. How is CMV best detected?

3.2.1. Serology
IgG antibodies reflect exposure and given that up to 70% of the population is seropositive, a positive result is uninformative. Conversely, about 30% of inflammatory bowel disease patients will therefore not be at risk of CMV reactivation since they will not have had previous infection. IgG serology is a fast way of identifying this sub-group and excluding CMV reactivation from the differential diagnosis. IgM antibodies, on the other hand, indicate recent infection, but are not specific to colonic disease. CMV antigenaemia assays, which involve staining for CMV in peripheral blood leucocytes, are open to subjective interpretation and assays can be positive without gastrointestinal involvement. Antigenaemia assays have largely been replaced by CMV detection and quantification through molecular techniques such as PCR.

3.2.2. Histopathology
Histopathological examination of colorectal mucosal biopsies, stained with haematoxylin and eosin (H&E), appears to be a reliable method of detecting colonic mucosal CMV disease (colitis) that is clinically relevant (Fig. 1)(Table 1). Clinical relevance in this context means colitis that deteriorates or persists despite oral corticosteroids. CMV inclusion bodies are found in greatest number around mucosal blood vessels or at the base of ulcers, so superficial mucosal biopsies may not always be accurate. Nevertheless, in most clinically relevant CMV disease in UC, the CMV inclusions are numerous and easily seen on H&E stained mucosal biopsies. Some studies have used colectomy specimens, but whilst specific, is clearly only applicable in retrospect.

Standard H&E staining has been reported to be 92–100% specific, but often insensitive (10–87%). The pivotal question about sensitivity is the reference technique for CMV infection and CMV disease (colitis) used by the investigators, since many infections have little clinical relevance with a virus that is so highly prevalent. Immunohistochemistry (IHC) has been found to be more sensitive than H&E staining (78–93%), but is generally used only to confirm findings by conventional light microscopy unless the specific question of CMV colitis has been raised by the clinician.

3.2.3. PCR
Molecular diagnostic techniques such as real-time PCR are used to detect and quantify CMV viraemia. They are sensitive, but not specific for clinically relevant CMV colitis. In one study, 24 patients with UC due to undergo colectomy for refractoriness to medical treatment (corticosteroids and in some cases ciclosporin or infliximab) were compared with patients undergoing colonic resection for colorectal cancer. Although CMV was detected by PCR in 13% and by histopathology in 4% of UC cases, compared with none of the controls, the difference between cases and controls was not significant. The low CMV detection rate is notable in a patient population that was highly immunocompromised, but the numbers are too small to draw definitive conclusions. Furthermore, the clinical relevance remains unclear, because it is not shown that treatment for CMV would have altered the outcome of colectomy.

In transplant medicine, studies have shown that although positive antigenaemia and PCR results may not by themselves constitute CMV disease, a quantitative cut-off point in the levels of each can help identify patients at risk of going on to develop CMV disease. There are at present no data with respect to inflammatory bowel disease.

In contrast, a Japanese study reported that PCR detected CMV in the inflamed mucosa of 17 (57%) of thirty patients with ulcerative colitis refractory to immunomodulators and in none of four treatment-responsive patients. The disparity in numbers suggests that patient-selection in this study was highly biased, since the response to immunomodulators is generally estimated to be around 70%. Furthermore, histopathological analysis with H&E and IHC in this

1 Clinically relevant in this context means colitis that deteriorates or persists despite corticosteroid therapy.
study detected CMV in only one of the 17 patients found to be positive with PCR. The authors gave antiviral therapy to those identified as CMV-positive by PCR and intensified immunomodulator therapy in CMV-negative patients, reporting “a high remission rate”, but numbers are too small and the lack of controls make it inappropriate to draw any conclusions. In the Consensus on Opportunistic Infections, ECCO guidelines recommend tissue (not serology) PCR or IHC as the methods of choice for excluding CMV colitis. When speed is essential, such as for patients with acute severe colitis facing emergency colectomy, urgent H&E staining can provide an answer faster than PCR or IHC, within 12 h.

### 3.3. CMV and steroid-resistance

It is conceivable that the association between CMV and steroid-resistance is simply a marker of higher viral reactivation rates in immunocompromised patients and reflects impaired cellular immunity caused by corticosteroids. Data suggesting an association between CMV and steroid-resistance therefore need critical appraisal, since the association may be artefactual.

To deal with retrospective studies first, CMV was identified by H&E and IHC-staining in 8/39 colectomy specimens, all performed for steroid-resistant UC. Since 6/8 with CMV had their colectomy for an acute severe attack, the authors reasonably suggested that CMV could cause an acute deterioration and should be considered in steroid-resistant cases. Another group used IHC to classify the surgical specimens of severe UC into CMV-positive (5/5) or CMV-negative specimens (2/8) (p = 0.005). The median daily dose of steroid was significantly higher in the CMV-dense group than the CMV-negative group (p = 0.008).

In a well-designed study, Maconi examined surgical specimens of 77 UC patients and their corresponding colonoscopy biopsy specimens, taken up to 12 months before colectomy. H&E staining and IHC were used to detect CMV. 55/77 patients had steroid-refractory UC and these were compared with 6/77 patients who underwent colectomy because of toxic megacolon, 7/77 for dysplasia or cancer and 9/77 due to inability to control symptoms in the absence of steroids. Although the proportion of CMV-positive specimens in steroid-refractory patients was higher than the non-refractory group, the difference was not statistically significant (p = 0.123). When clinical characteristics of the patients were examined in relation to CMV-positivity, only current, systemic use of corticosteroids was significantly associated (p = 0.03). The authors conclude that these data support an association between CMV and steroid-resistance, but interpret it as a reflection on viral reactivation due to corticosteroid therapy, rather than CMV being a causal factor. The time between biopsy and colectomy, however, did not affect the CMV detection rate. This means that if their theory about CMV reactivation through immunosuppression is correct, the CMV rates should be higher at the time of the surgery, since colectomy is the final therapeutic stage for patients completely refractory to immunomodulators. This is consistent with another cohort study, which found no association between steroid therapy and CMV positivity.

The question is better answered by a more recent prospective study of steroid-responsive and -refractory patients, which included groups with inactive UC on maintenance azathioprine or mesalazine therapy, as well as healthy controls with a normal colonoscopy and no family history of UC. CMV was detectable by H&E, IHC and PCR only in the steroid-refractory group, in 32% of 19 patients. There were, however, no differences in clinical characteristics between the CMV-positive and CMV-negative steroid-refractory patients, although a higher proportion of the CMV-positive patients underwent colectomy. Another Italian group detected CMV in the biopsies of 4 of 12 (33%) steroid-resistant

<table>
<thead>
<tr>
<th>Study</th>
<th>Method of CMV detection</th>
<th>Overall (%)</th>
<th>Steroid-refractory (%)</th>
<th>Steroid-responsive (%)</th>
<th>Other (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottone 2001</td>
<td>H&amp;E and IHC</td>
<td>–</td>
<td>36 (7/19)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kambham 2004</td>
<td>IHC</td>
<td>–</td>
<td>25 (10/40)</td>
<td>2.5 (1/40)</td>
<td>–</td>
</tr>
<tr>
<td>Criscuoli 2004</td>
<td>H&amp;E</td>
<td>–</td>
<td>5 (2/40)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Takahashi 2004</td>
<td>H&amp;E</td>
<td>17 (7/42)</td>
<td>33 (4/12)</td>
<td>10 (3/30)</td>
<td>–</td>
</tr>
<tr>
<td>Maconi 2005</td>
<td>H&amp;E</td>
<td>–</td>
<td>27 (15/55)</td>
<td>9.1 (2/22)</td>
<td>–</td>
</tr>
<tr>
<td>Kojima 2006</td>
<td>H&amp;E</td>
<td>–</td>
<td>1.3 (1/72)</td>
<td>N/A</td>
<td>16 (5/32, severe colitis)</td>
</tr>
<tr>
<td>Domènech 2008</td>
<td>H&amp;E and/or IHC</td>
<td>–</td>
<td>8.3 (6/72)</td>
<td>N/A</td>
<td>25 (8/32, severe colitis)</td>
</tr>
</tbody>
</table>

*a* H&E: Haematoxylin and eosin staining of colonic mucosal biopsies. 

*b* IHC: Immunohistochemistry of colonic mucosal biopsies.
patients admitted with acute severe colitis and only 3 out of 30 steroid responsive patients (10%), although again there were no substantial clinical differences between patients with and without infection.33

Consequently, the question of whether CMV causes steroid-resistance needs to be seen in both a clinical context and in relation to the definition of CMV infection. Immuno-suppressed patients are known to be susceptible to CMV reactivation, but studies must distinguish between serological reactivation of CMV (infection) and tissue damage (“disease”, or colitis) caused by CMV. In addition, the association of CMV with steroid use is more specific than for “immunosuppressant” drugs in general. Infliximab may not exacerbrate CMV. When 11 patients with inflammatory bowel disease before and after treatment with infliximab were assessed, 9 were seropositive and CMV was detected in colonic biopsies of 3 patients prior to treatment. After treatment, there was no worsening of colonic disease and in one patient colonic CMV became undetectable.34

Reactivation of CMV during immunosuppressive therapy is usually a self-limiting phenomenon and screening for latent CMV infection before starting an immunomodulator is unlikely to be useful.14 In contrast, when there is an episode of steroid-refractory acute severe colitis, the possibility of superimposed CMV disease should be considered and treated if possible. Cases of CMV colitis occurring prior to steroid therapy in inflammatory bowel disease patients have been reported, but are so rare as to be confined to case reports.35,36

3.4. What is the role of antiviral therapy in CMV colitis?

The risks and benefits of CMV anti-viral therapy need to be considered before decisions are taken either to treat CMV disease or infection, or as adjunctive treatment for steroid-resistant colitis, or even as prophylaxis for patients starting steroids who have positive CMV serology or CMV viraemia. CMV anti-viral drugs have the potential for serious side-effects, including bone marrow suppression, pulmonary and neurological dysfunction, so robust evidence of benefit is needed.

In a prospective study of CMV anti-viral therapy for steroid-resistant, moderate–severe UC, patients with CMV antigenaemia were treated with ganciclovir if oral prednisolone was clinically ineffective after 1–2 weeks.37 A CMV antigenaemia assay, by direct immunostaining of peripheral blood buffy coat, was used to divide 47 patients into CMV-positive (n=16) and CMV-negative (n=31) groups, but H&E and IHC staining of colonic mucosal biopsies was also performed. Steroid-resistance was more common in the CMV-positive group (n=13/16 vs 9/31, p=0.001). CMV-positive patients also had more severe colitis, whether judged endoscopically or by histopathology (p=0.016 and 0.013 respectively). Of these CMV-positive patients, 12/16 were given ganciclovir, while 3/16 needed colectomy and one improved on steroids. Of those given ganciclovir, 8/12 improved and became CMV-negative on the antigenaemia assay. However, the remaining 4 patients also became CMV-negative and did not improve, suggesting that resolution of CMV antigenaemia is insufficient to bring about clinical improvement. These are very small numbers, so no conclusion other than proof-of-concept for the effect of ganciclovir is possible. Placebo-controlled studies are needed.38

A separate study examined 19 steroid-resistant patients with UC (n=16) or Crohn’s colitis (n=3), also using a CMV antigenaemia assay, as well as H&E with IHC staining for CMV in rectal biopsies to separate CMV-positive or -negative patients.3 Of these 19 steroid-resistant patients 7/19 (36%) were CMV-positive both by antigenaemia assay and histopathology. Steroid-resistant, CMV-negative patients (n=11 UC, 1 Crohn’s) were started on ciclosporin, while CMV-positive patients (n=5 UC, 2 Crohn’s) were given antiviral therapy (ganciclovir n=4, or foscarnet n=2). One patient was not treated with ganciclovir because CMV was only later detected in the surgical specimen. 5/6 of patients treated with antiviral therapy went into remission and became CMV-negative. The remaining patient underwent colectomy due to lack of response to antiviral therapy. Of the remaining 12 (CMV-negative) patients, it is not stated how many came to colectomy. The authors concluded that CMV is a frequent cause of steroid-resistant colitis and recommended rectal biopsy to look for CMV in these circumstances.

In a further prospective study, colonic CMV was detected in 32% of 19 steroid-refractory patients with active UC.32 Two of these patients underwent colectomy due to clinical deterioration and the remaining four were treated with ganciclovir and ciclosporin. All four patients cleared colonic CMV after treatment, although clinical remission was achieved in only three, with the remaining patient undergoing colectomy. Although consistent with a beneficial effect of CMV anti-viral therapy in steroid-resistant colitis, small numbers and lack of controls make it impossible to make therapeutic recommendations. ECCO guidelines currently recommend that CMV-antiviral therapy with cessation of immunomodulators is appropriate for CMV colitis complicating UC, but that chemoprophylaxis before immunomodulator therapy is not indicated.14

3.5. How is steroid-resistant colitis defined?

Steroid-resistance and -refractoriness are very variably defined, which contributes to confusion24 (Table 2). European guidelines on the management of UC define steroid-refractory colitis as those patients who have active disease despite prednisolone up to 0.75 mg/Kg/day over a period of 4 weeks,19 but needs qualification. This is because the definition is likely to evolve as the threshold for using anti-TNF therapy changes and other therapeutic options develop. Steroid-resistance, however, is not defined in the ECCO guidelines, although steroid-dependence is defined as patients who are either unable to reduce steroids below the equivalent of prednisolone 10 mg/d within 3 months of starting steroids, without recurrent active disease, or who have a relapse within 3 months of stopping steroids. In addition, the term “chronic active disease” can refer to patients dependent on, refractory to or intolerant of steroids, meaning its use as a clinical definition is no longer recommended.19 More specifically, the definition of steroid-resistance is dependent on the length of time in which a clinical response is judged to have occurred. This will in turn directly influence the timing of a CMV assay and alternative/antiviral therapy initiated. Inconsistency in the definition of
steroid-resistance will in turn affect the reported prevalence of CMV, because the virus may be more prevalent in cases where steroid therapy is continued for longer.

3.6. Can steroid-resistance be explained by CMV increasing the severity of the colitis?

CMV appears to have a particular tropism for inflamed mucosa. If this indeed true, then higher tropism of CMV would be expected in more severe cases, which in turn could increase the severity and therefore increase steroid-resistance. This would bias any investigation into the role of CMV in steroid-resistance. Steroid-resistance, however, should not be seen as synonymous with severe colitis, although the terms have regrettably often been used interchangeably. It is important to draw a distinction between biological severity and colitis that is “severe” (or more serious) as a consequence of resistance to treatment.

When the CMV detection rates in 40 steroid-refractory UC and Crohn’s disease patients were compared with 40 steroid-responsive patients and 40 non-colitis controls, the rate was significantly higher in the refractory group (25% vs 2.5%, p = 0.007, as detected by IHC). The refractory group was more likely to have clinically severe disease (p = 0.006), as detected by IHC). The refractory group was more likely to have clinical disease (p = 0.001), as defined by the Truelove & Witts’ criteria. When the respective mucosal biopsies or colectomy specimens of those groups were compared, those from the refractory group were more likely to have mucosal erosion/ulceration (60% vs 35%, p = 0.006), crypt destruction (p = 0.013), or architectural distortion (p = 0.0027), although the number of sections with grade 2/3 inflammation (1 = mild, 2 = moderate, 3 = severe) did not differ significantly. There was, however, no association between CMV density and the severity of inflammation.

A different approach retrospectively compared the CMV detection rate in colectomy specimens classified according to surgical indication: acute severe UC (n = 32), steroid-refractory UC (n = 72) and dysplasia/neoplasia (n = 22). CMV detected by H&E and IHC staining was more common in those with acute severe colitis (n = 8 with IHC) than in either the refractory UC group (6 with IHC) or the neoplasia groups (n = 0), p < 0.03. This is consistent with more severe inflammation being a confounding factor, but does not address whether this is cause or effect for CMV. It is difficult to address in experimental design, since it would require a steroid-resistant group without severe inflammation. Such a group would have relatively quiescent disease and not require systemic steroids.

4. Conclusions

There is significant heterogeneity in experimental design of studies on CMV, ulcerative colitis and steroid-resistance. Distinctions need to be made between colonic mucosal CMV disease (CMV colitis), identified by standard histopathology and confirmed by immunohistochemistry; and CMV infection with antigenemia quantified by PCR, or positive CMV serology. There is sufficient evidence to conclude that CMV disease increases the biological severity of colitis and is the cause of acute severe colitis in a small minority (around 3–5%), not all of whom are on immunosuppressive therapy. The diagnostic possibility should be considered at an early stage, in patients with a high fever, rapid deterioration of previously stable disease, or no response to intensive therapy within 3 days. Such patients may be appropriate candidates for anti-viral therapy, but randomised controlled trials are needed to identify this cohort and elucidate the response to anti-viral therapy. There is only circumstantial evidence that CMV infection detected by viraemia contributes to steroid-resistance in patients with UC who do not have biologically severe disease. The possibility that steroids simply cause self-limiting viral reactivation has to be considered. There is insufficient evidence that the benefits of anti-viral therapy in these patients outweigh the risks and no evidence for chemoprophylaxis against CMV reactivation.

Acknowledgements

Thanks to Tatjana Petrinic and Neal Thurley, outreach librarians at the Cairns Library, Oxford University for their help in formulating literature search terms and using the databases.

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