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ORIGINAL ARTICLE

The detection of the cytomegalovirus DNA in the colonic mucosa of patients with ulcerative colitis is associated with increased long-term risk of proctocolectomy: results from an outpatient IBD clinic

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Abstract

Purpose Cytomegalovirus (CMV) infection has been found to be associated with a reactivation of ulcerative colitis (UC) and with an impaired response to medical therapy. In the past, only limited data were available on the long-term outcome for UC patients with positive tissue CMV-PCR in the colonic mucosa.

Methods Between January 2010 and April 2015, we performed a qualitative PCR screening for CMV DNA in one biopsy from most actively inflamed rectal mucosa (tCMV-PCR). All tCMV-PCR-positive patients received 900 mg of valganciclovir b.i.d. for at least 15 days. We analyzed the association of the tCMV-PCR status with the time to steroid-free remission (SFR) and with the risk of proctocolectomy during the further course.

Results One hundred eight consecutive patients (50 women, 58 men, median age 41 years, median UC duration 6 years) with active UC not responding to anti-inflammatory medication were analyzed. Eight of the 24 tCMV-PCR-positive patients (33.3%) compared to ten of the 84 tCMV-PCR-negative patients (11.9%) underwent proctocolectomy during a median follow-up of 52 months ($p < 0.005$). The median time from CMV diagnosis to colectomy was 501 days (median, interquartile range (IQR): 170, 902 days) in tCMV-PCR-positive and 958 days (IQR: 287, 1328 days) in tCMV-PCR-negative patients ($p < 0.01$). The median time to SFR was 126 days in tCMV-PCR-positive patients vs. 63 days in tCMV-PCR-negative patients ($p < 0.01$).

Conclusions The detection of the CMV DNA in the colonic mucosa of patients with active UC is associated with a longer time to steroid-free UC remission and with an increased rate and earlier need of proctocolectomy.

Keywords Ulcerative colitis · Cytomegalovirus · Proctocolectomy · Polymerase chain reaction

Introduction

The human cytomegalovirus (CMV) is a member of the Herpesviridae family containing double-stranded DNA. After primary infection, the virus persists in the body and is located mainly in myeloid and endothelial cells. CMV infection is of particular interest with regard to ulcerative colitis (UC) that combines an inflammation in the colon and the need for long-term maintenance of immunosuppressive therapy. Both can facilitate the reactivation of a latent CMV infection [1].

The reactivation of a latent CMV infection in patients with UC is associated with an increased risk of colectomy [2, 3], mortality [4, 5], and health care utilization [4] suggesting an underlying pathogenic role. In addition, antiviral therapy

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seems to improve the clinical outcomes for patients otherwise resistant to immunosuppressive therapy [6, 7]. A recent meta-analysis concluded that antiviral therapy is associated with an 80% reduction in proctocolectomy risk for CMV-positive patients with corticosteroid-refractory UC [8]. Therefore, a prompt diagnosis and early administration of antiviral therapy may improve the clinical outcomes for certain high-risk individuals.

Current guidelines suggest that in patients with refractory IBD flares, a CMV infection should be excluded before escalating immunosuppressive therapy. Whereas in the past, only CMV DNAemia, positive CMV immunohistochemistry, and positive histology (inclusion bodies) were considered strong evidence for a relevant CMV infection, the last 10 years have seen a shift towards tissue-based diagnostics, e.g., tissue CMV polymerase chain reaction (tCMV-PCR) was advised [9–11].

In contrast, several authors have observed positive CMV markers in patients without any obvious impact of CMV on IBD evolution. This has led to the idea that CMV infection or reactivation could be considered as an "innocent bystander" or "byproduct" of an acute UC flare [12, 13, review in 14].

Given these uncertainties, we have developed and validated a standard of care for CMV diagnosis and treatment in outpatients with UC not responding to anti-inflammatory medication. The aim of this study was to compare the long-term clinical courses of tCMV-PCR-negative and tCMV-PCR-positive UC patients in a retrospective chart analysis after at least 3 years of follow-up.

Methods

Standard of care

At an institutional conference in December 2009, the authors TK, AB, PD, CK, and NT from an approved outpatient IBD clinic suggested the following standard of care: patients with UC not responding to anti-inflammatory medication should undergo endoscopic biopsy for tCMV-PCR on the same day. Patients in whom CMV is detected should get a prescription of the oral antiviral agent valganciclovir 900 mg b.i.d. for 15 days. Baseline immunosuppressive therapy and concomitant medication was continued throughout the 15 days of valganciclovir 900 mg b.i.d. with the exception of systemic steroids. Steroids could be tapered in case of clinical improvement of the UC flare.

In case of a complete clinical remission, valganciclovir should be stopped and baseline medical therapy only should be continued. In the case of clinical improvement only, immunosuppressive therapy should be intensified and valganciclovir should be continued for 60 days at a dosage of 450 mg q.d. If the UC deteriorates, a re-endoscopy and control tCMV-PCR should be done. If positive, the patient should be admitted to the hospital for intravenous ganciclovir therapy. If negative, anti-TNF therapy should be started immediately.

The rationale for this sequence was based on own experience in clinical practice, one prescription package containing 60 tablets of valganciclovir tablets, and its agreement with the "International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation" as published in early 2010 [15]. In brief, this guideline strongly suggested initial therapy with valganciclovir 900 b.i.d. no shorter than 2 weeks. The use of secondary prophylaxis was reported as very variable across transplant centers but was often recommended (range 1–3 months) [15]. For practical and economical reasons, we, therefore, recommended 60 days of 450 mg q.d. in case of intensified immunosuppressive therapy.

The ongoing disease courses were documented in electronic patient charts as part of the usual clinical routine.

Chart review

A retrospective chart review was done for all UC patients who had colorectal tissue samples sent for tCMV-PCR testing from January 2010 to April 2015. The following data were sampled: gender, body mass index, year of birth, year of UC manifestation, endoscopic extend of UC, date of start of the current UC flare, date of tCMV-PCR, dosage of systemic steroids, and use of thiopurines and biologics before and after the current flare, CRP, hemoglobin, leucocytes, platelets, evidence of infectious diarrhea, number of prescribed valganciclovir packages, CMV-associated systemic complications, date of steroid-free remission, proctocolectomy date, and repeated UC flares with CMV testing during follow-up, last follow-up.

Tissue diagnostic

Using a sterile endoscopic biopsy forceps, one or two mucosal samples were taken from the mucosal region showing the endoscopically highest inflammatory activity as judged by the endoscopist. These biopsies for molecular analysis were always taken first of all the biopsies to avoid formalin contact of the forceps or the endoscope-working channel. Samples were stored and shipped at room temperature in 1 ml of sterile saline 0.9%. After DNA extraction utilizing the High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH), the RealStar® CMV PCR Kit 1.0 (altona Diagnostics GmbH, Hamburg, Germany) was applied. This test consists of two processes in a single tube assay: PCR amplification of target DNA and internal control and simultaneous detection of PCR amplicons by fluorescent dye-labeled probes. The following real-time PCR instruments and agents were used: LightCycler® CMV Quant Kit (Roche Diagnostics GmbH) with LightCycler® 2.0 (until September 2012) and LightCycler® 480 II (since October 2012). Based on prior work that found no IHC positivity in the setting of negative

tissue PCT [16, 17], only t-CMV-PCR-positive intestinal tissues with concurrent tissue samples sent for histology were investigated by immunohistochemistry (IHC). IHC was performed on paraffin-embedded samples using monoclonal mouse anti-CMV antibodies (clones CCH2 + DDG9, Dako Denmark) according to the manufacturer's recommendations.

Statistics and ethical considerations

In order to compare two groups, we used Fisher's exact test for nominal variables and Student's *t* test or Wilcoxon-Mann-Whitney tests for continuous variables as appropriate. Time-to-event analysis was performed using Kaplan–Meier estimates and Cox proportional hazard regression models. This retrospective analysis of pseudonymized electronic patient charts was approved by the University Hospital Ethics Committee of Jena University, Germany; no. 2018-1149.

Results

Primary and per-protocol patients cohort

One hundred and twelve consecutive patients (51 women, 62 men, median age 40 years, median UC duration 6 years, median BMI 23.5) with active UC not responding to anti-inflammatory medication were tested for tCMV-PCR between November 2009 and April 2015. From this cohort, four patients underwent proctocolectomy for cancer or high-grade dysplasia and had to be excluded, resulting in 108 patients for a per-protocol analysis (Fig. 1). The baseline characteristics of tCMV-PCR-positive and tCMV-PCR-negative patients are shown in Table 1.

The baseline characteristics did not differ between tCMV-PCR-positive and tCMV-PCR-negative patients. Time periods and steroid dosages were reported as median and interquartile range (1st quartile; 3rd quartile). Frequencies were reported as absolute numbers and percentages.

CMV and colectomy: univariate analysis

Eight out of 24 tCMV-PCR-positive patients (33.3%) compared to only ten out of 84 tCMV-PCR-negative patients (11.9%) who underwent proctocolectomy during follow-up ($p = 0.026$). Furthermore, tCMV-PCR-positive patients showed a shorter time to proctocolectomy than tCMV-PCR-negative patients ($p = 0.008$ in log rank; Fig. 2).

The cumulative probabilities of colectomy (estimate ± standard error) were 4.9 ± 2.4% in tCMV-PCR-negative patients and 13.0 ± 7.0% in tCMV-PCR-positive patients at 1 year. At 3 and 5 years, the probabilities were 7.4 ± 2.9% and 14.6 ± 4.4% in tCMV-PCR-negative patients, and 31.5 ± 9.9% and 42.9 ± 13.3% in tCMV-PCR-positive patients, respectively.

We found no association between tCMV-PCR positivity and neither CRP or hemoglobin concentrations, nor leucocyte or platelet counts (data not shown). Repeated tCMV-PCR-positive UC flares did not further increase the risk of proctocolectomy (data not shown). The cumulative valganciclovir dosage to remit the index CMV-associated UC flare was not significantly associated with proctocolectomy risk.

Proctocolectomy risk: multivariate analysis

In a univariate Cox regression analysis, tCMV-PCR positivity was associated with an increased hazard of colectomy (hazard ratio [HR] 3.284; 95% CI 1.296–8.371; $p = 0.012$). In addition, patients with pancolitis showed a statistically non-significant trend towards being at risk of colectomy (HR 2.271; 95% CI 0.895–5.761; $p = 0.08$). After an adjustment for the extent of colitis, being a tCMV-PCR-positive remained an independent risk factor for colectomy (adjusted HR 3.287; 95% CI 1.293–8.354; $p = 0.012$).

Steroid dosage at the time of CMV biopsy and risk of proctocolectomy

Systemic steroid dosages at the time of biopsy for tCMV-PCR were available for 105 patients. The proctocolectomy risk in patients without systemic steroids at that time was 16.7% compared to 17.9% in patients with steroids. Analysis of the four quartiles of steroid dosages neither revealed an increasing risk with higher steroid dosages nor any additional effect on tCMV detection ($p = 0.66$ in Fisher's exact test).

Quantitative tCMV-PCR and risk of proctocolectomy

The focus of our study was on how the presence of mucosal CMV influenced the proctocolectomy risk in everyday practice. Quantification of the CMV copy number in tCMV-PCR-positive biopsies revealed median tissue concentrations of 12,100 IU/ml (interquartiles 525–166,250 IU/ml). In tCMV-PCR-positive patients, there was no association of quantitative tCMV-PCR with the outcome or with immunosuppression (data not shown).

CMV and time to steroid-free remission

The exact time of steroid-free remission (SFR) was available for 101 of 108 patients. The median time to SFR was 126 days (IQR 53, 313 days) in tCMV-PCR-positive patients, which was twice as long as in tCMV-PCR-negative patients (median 63 days, IQR 36, 120 days, $p = 0.0083$, Student's *t* test).

Thiopurines or anti-TNFs were more often necessary in tCMV-PCR-positive patients in order to achieve SFR, whereas no significant differences were found for anti-TNFs and thiopurines alone (Fig. 3).

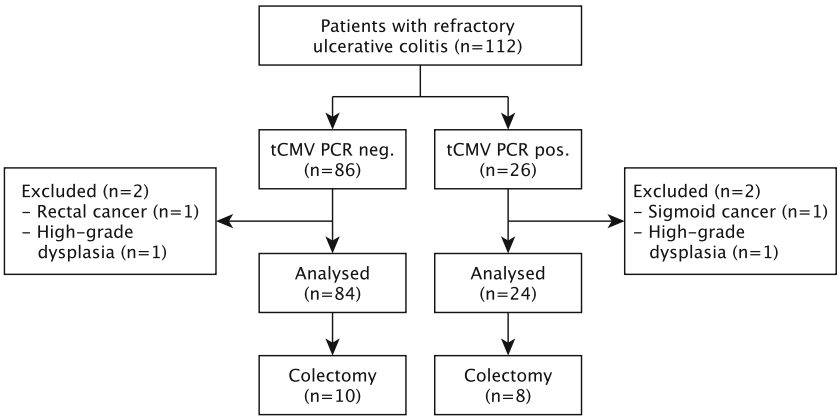

Fig. 1 Flowchart for patient inclusion and follow-up

Response to CMV treatment

All but two tCMV-PCR-positive patients and all but five tCMV-PCR-negative patients achieved clinical remission. Remarkably, three patients out of the tCMV-PCR-positive patients had to be hospitalized as remission could not be achieved in the outpatient setting. Two of them had no response to oral valganciclovir—assumingly due to high stool frequency with impaired intestinal absorption. Both came into remission through a course of intravenous ganciclovir and were treated further with oral valganciclovir for 1 month. The third patient was hospitalized due to the clinical suspicion of meningitis (see below).

CMV immunohistochemistry vs. tCMV-PCR

In 16 of 24 tCMV-PCR-positive biopsies, CMV immunohistochemistry (CMV-IHC) could be performed and was positive in eight (50%) patients. Only one of them had to undergo colectomy. CMV inclusion bodies were not detected in any hematoxylin and eosin (H&E) stained samples.

Serum CMV-PCR vs. tissue CMV-PCR

In 41 patients, whole-blood CMV-PCR was performed within 14 days before or after tCMV-PCR was investigated. Out of the tCMV-PCR-positive patients, 7/16 (43.8%) patients were blood-CMV-PCR-positive (bCMV-PCR). Out of the tCMV-

Table 1 Baseline characteristics

		tCMV-PCR-positive ($n = 24$)	tCMV-PCR-negative ($n = 84$)	P value
Follow-up	Months	54.4 (37; 71.7)	51.7 (40; 70)	0.87
Women	*n* (%)	9 (37.5%)	41 (48.8%)	0.3
Age	Years	43 (31;54)	39 (31; 50)	0.54
BMI	kg/m^2	22.7 (21.5; 27.5)	23.5 (21.7; 27.5)	0.37
UC duration	Years	5.7 (2.3; 11.6)	6.6 (2.7; 15.1)	0.52
UC extension*				
Proctitis	*n* (%)	1 (4.2%)	10 (11.9%)	0.24
Left side	*n* (%)	16 (66.7%)	47 (56.0%)	
Extensive	*n* (%)	7 (30.0%)	22 (26.2%)	
Start bleeding before baseline	Days	72 (41; 158)	74 (40; 126)	0.87
Systemic steroids	*n* (%)	16 (66.7%)	41 (48.8%)	0.12
Systemic steroids	mg	37.7 (20; 60)	30 (15; 40)	0.28
Systemic steroids	Weeks	7 (1.5; 37)	3 (1.5; 10.5)	0.23
Anti-TNFs at baseline	*n* (%)	4 (20.0%)	17 (20.3%)	0.70
Systemic steroids + anti-TNFs	*n* (%)	1 (4.2%)	3 (3.6%)	0.89
Systemic steroids + thiopurine	*n* (%)	6 (25.0%)	9 (10.7%)	0.07
Systemic steroids + thiopurine + antiTNFs	*n* (%)	0	1	0.59

*not known for 5 patients

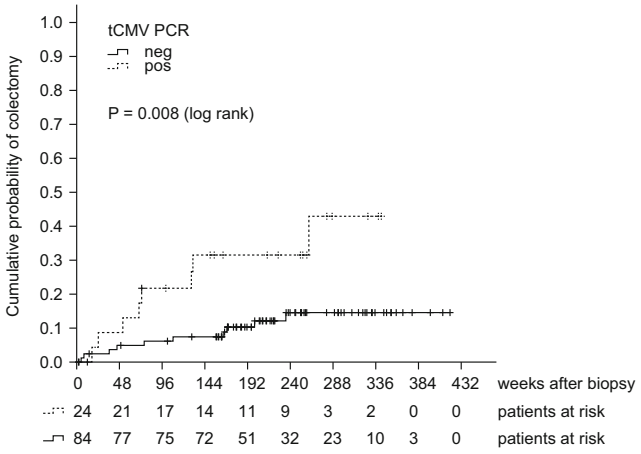

Fig. 2 The cumulative probability of colectomy was higher in tCMV-PCR-positive patients and proctocolectomy—if necessary—was done earlier than in tCMV-PCR-negative patients

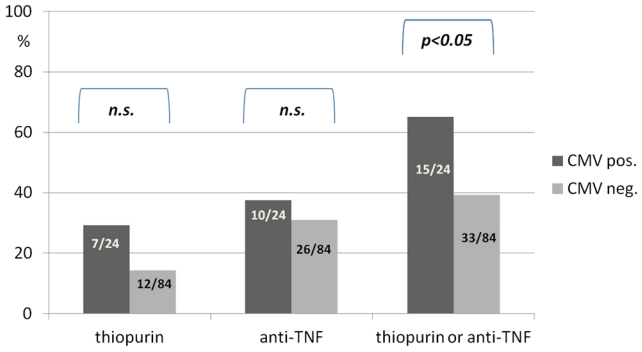

Fig. 3 Thiopurines or anti-TNFs were significantly more often necessary in order to achieve steroid-free remission in tCMV-PCR-positive patients

PCR-negative patients, 1/25 (4%) was bCMV-PCR-positive. Thus, whole-blood PCR had 44% sensitivity and 96% specificity to indicate the presence of CMV DNA in the biopsies. The associated positive and negative predictive values were 88 and 73%, respectively. In bCMV-PCR positive patients, time to SFR was median 125 days (interquartile range: 53, 314 days), in bCMV-PCR negative patients 125 days (interquartile range: 30, 313 days); $p = 0.99$. We, therefore, do not see evidence that tCMV-PCR-positive plus bCMV-PCR-positive patients better response to valganciclovir therapy than tCMV-PCR-positive/bCMV-PCR-negative patients.

Interestingly, extraintestinal manifestations of CMV disease were only found in one patient with both bCMV-PCR as well as tCMV-PCR positivity. In this case, CMV DNA was also found in cerebrospinal fluid, and meningitis was treated with intravenous ganciclovir. Despite successful treatment of meningitis, proctocolectomy had to be performed 170 days after positive tCMV-PCR due to a refractory course of the UC without CMV relapse.

Discussion

CMV in inflamed mucosa indicates a severe clinical course

In this first cohort of non-selected UC patients not responding to anti-inflammatory medication in an outpatient setting, CMV DNA was detected in the inflamed mucosa in 22% of the cases. A positive tCMV-PCR was associated with a higher proctocolectomy risk, earlier surgery, a longer time to steroid-free remission, and a higher need for thiopurines and/or anti-TNFs in the later course of the UC.

Five earlier published studies focused on the impact of CMV detection on the need of proctocolectomy in the further course of UC. In one retrospective Canadian study comprising 31 IBD patients with evidence of CMV in IHC and 581 IBD patients with negative CMV-IHC, 29% of the CMV-IHC-positive patients but only 11% of the CMV-IHC-negative patients required colectomy (0.007) [2].

Only one retrospective study from Japan correlated tCMV-PCR in UC patients with endoscopic activity. In 12 tCMV-PCR-positive patients, one colectomy was necessary, but in the 34 tCMV-PCR-negative patients, not one patient underwent surgery. However, no follow-up times were reported [18]. In another retrospective Japanese study of 118 UC patients with predominantly moderate to severe UC flares, the pp65 CMV antigenemia assay was used and a median follow-up of 3 years was reported [3]. The authors presented a colectomy rate of 30% without differences in pp65-positive and pp65-negative patients. This markedly exceeds the 17% in our overall per-protocol cohort but seems to be well comparable to the 33% colectomy rate in our tCMV-PCR-positive patients. However, the average period between the measurement of the CMV antigenemia assay and the start of antiviral therapy was 10.8 ± 13.4 days in the study by Inokuchi [3]. One could hypothesize that this could be too long for any positive effect on CMV-triggered UC activity in already hospitalized high-risk UC patients.

A third Japanese study reporting 149 in-patients with acute severe UC, CMV colitis was diagnosed histopathologically and/or using immunohistochemical analysis in one third of the patients. During admission, 14% of the CMV-positive patients versus 9% of the CMV-negative patients underwent colectomy. Eventually, this difference was not significant due to a short follow-up, but this study furthermore reported the need for rescue therapy. This need was 2.3-fold higher in the CMV-positive patients [19]. Even in our cohort, CMV was associated with a longer time to steroid-free remission and increased use of systemic immunosuppressants.

In a very recent published study, 25 tCMV-PCR-positive and 154 tCMV-PCR-negative patients with Crohns disease, indeterminate colitis, or UC were retrospectively analyzed with regard to the surgical risk. Twenty-seven percent of the tCMV-PCR-negative patients underwent surgery (type not specified).

Interestingly, only 80% of the tCMV-PCR-positive patients received antiviral therapy. Of those treated, 40% underwent surgery vs. 80% of the patients who were not treated for CMV [20]. These surgery rates were much higher than in our cohort of immediately CMV-treated UC patients who—in case of surgery—all underwent proctocolectomy. Furthermore, we were able to show that time to proctocolectomy was shorter in tCMV-PCR-positive than in tCMV-PCR-negative patients.

Summarizing these six—including ours—studies in order to conclude the long-term effects of CMV positivity on the colectomy risk is difficult as all studies were retrospective and used different inclusion criteria, different detection methods for defining CMV reactivation, superinfection or disease, and very different follow-up periods.

Tissue CMV-PCR is more sensitive than other detection methods

At the time when we decided our institutional sequence for CMV diagnostic and therapy in 2009, clinical guidelines favored blood tests and CMV-IHC. However, some retrospective studies demonstrated that tCMV-PCR could be a better diagnostic method than IHC or blood tests [5, 18, 21]. We, therefore, focused on tCMV-PCR but blood tests were allowed as found necessary. For the retrospective data collection in this report, bCMV-PCR was available for 38% of the patients of the per-protocol cohort between 14 days before and 14 days after t-CMV-PCR. Confirming a recent systematic review and meta-analysis, we found an insufficient sensitivity but high specificity of the bCMV-PCR results in comparison to tCMV-PCR [22 and references within]. So far, a gold standard for CMV detection in refractory UC patients has not been determined. This is mainly due to the very broad variations in study protocols including self-made CMV-PCR and CMV-IHC protocols making results non-comparable across studies. One advantage of our study could be the continuous use of one commercially available controlled CMV-PCR assay.

Another unanswered question is the quantitative influence of CMV on the course of UC. One retrospective study reported that five or more CMV-IHC-positive cells per biopsy section were indicative of a greater colectomy risk [23]. Another retrospective comparative study of CMV-IHC and bCMV-PCR from the same authors reported an association between the highest viremia quartile and a positive CMV-IHC-result which may justify the administration of an antiviral treatment in selected patients [24]. In our cohort, however, we were not able to show that quantitative bCMV-PCR results permitted a better prediction of the subsequent course of UC than the qualitative ones alone. Moreover, neither a quantitative association between tCMV-PCR or CMV-IHC copy numbers nor the recent finding of an increased risk of surgery in patients with positive CMV-IHC or inclusion bodies in H&E stains in addition to a positive tCMV-PCR [20] could be replicated in our study.

Only one retrospective study investigated—and excluded—a beneficial influence of subsequent intensification of immunosuppressive therapy in addition to antiviral therapy. In this multinational study spanning 12 years, altogether 110 hospitalized UC patients from 23 centers were tested positively for CMV [25]. The colectomy rate at 12 months was almost 35%. This is much more than in our ambulatory care study, where only 5 and 13% of tCMV-PCR-negative and tCMV-PCR-positive patients, respectively, had to undergo colectomy. This marked difference could be explained by the IBD care setting. In our outpatient cohort, UC patients with no response to anti-inflammatory therapy and with no clinical signs of circulatory or vigilance impairment were immediately investigated for CMV. Every patient with a positive tCMV-PCR got immediate antiviral therapy, even despite low CMV concentrations in some patients and a positive CMV-IHC result in only 50%. This closely meshed proactive procedure could prevent aggravation of CMV-associated UC but could also lead to unnecessary antiviral therapy in distinct patients with a transient or a non-UC-worsening presence of CMV DNA in the colonic mucosa.

Limitations

In addition to its retrospective nature, our study has further limitations. The assumption of "active UC not responding to anti-inflammatory medication" was mainly based on anamnestic ratings at a consultation and clinical assessment but not on measurements of more objective activity scores such as the Mayo score. Only one or two mucosal biopsies were taken for tCMV-PCR. Therefore, CMV detection could have been false-negative in some patients [26]. Furthermore, we did not repeat endoscopy and tCMV-PCR in clinically improved patients after the valganciclovir course. The efficacy of antiviral therapy may therefore not be concluded from our data. Finally, our clinically driven approach is not able to distinguish between CMV colonization and CMV infection. This may induce an overtreatment of patients with non-pathogenic colonic CMV colonization. An actual comparative study of different CMV detection methods showed that low CMV DNA levels in non-immunosuppressed patients were not a risk factor for the development of a more severe IBD. However, an exact cutoff between harmless and harmful CMV viral load could not be established in this study and elsewhere—especially not in immunosuppressed UC patients [27].

Conclusion

Our presented study is the first to describe the significant influence of CMV on the course of UC in outpatients. So far,

no randomized controlled trial has been performed to evaluate the efficacy of antiviral therapy in UC patients with a CMV infection, and it is an ongoing debate on which patients and treatment schedules are appropriate [25, 28]. Further prospective controlled research should focus on a "clinically relevant" CMV infection in patients with active UC.

Authors' contributions TK, AB, PD, CK, and NT developed the standard of care, cared for all patients, and did the endoscopic biopsies. RK did the PCRs. ES did the immunohistochemistry. WS did the retrospective chart analysis and provided raw data for statistical analysis. This study is part of the doctorate thesis of WS. TB, AS, and NT conceived the study. TB and NT did the statistical analyses. AS conducted the doctorate thesis procedure of WS. All authors have read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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