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Cytomegalovirus colitis in inflammatory bowel disease and after haematopoietic stem cell transplantation: diagnostic accuracy, predictors, risk factors and disease outcome

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ABSTRACT

Background Concurrent cytomegalovirus (CMV) colitis in inflammatory bowel disease (IBD) and after haematopoietic stem cell transplantation (HSCT) is an important clinical entity associated with high rates of morbidity and mortality.

Methods A retrospective study of 47 patients with IBD and 61 HSCT patients was performed regarding the evaluation of diagnostic accuracy of applied methods, predictors, risk factors for CMV disease manifestation, the proportion of patients with antiviral treatment and disease outcome.

Results The sensitivity of quantitative PCR (qPCR) with a cut-off value of >250 copies/mg for CMV colitis in patients with IBD and HSCT patients was 79% and 92%, respectively. Predictors for CMV colitis in the IBD cohort were anaemia and the presence of endoscopic ulcers. Glucocorticoids, calcineurin inhibitors and >2 concurrent lines of treatment with immunosuppressive drugs could be identified as risk factors for CMV colitis in the IBD cohort with an OR of 7.1 (95% CI 1.7 to 29.9), 21.3 (95% CI 2.4 to 188.7) and 13.4 (95% CI 3.2 to 56.1), respectively. Predictors and risk factors for CMV gastroenteritis in the HSCT cohort was the presence of endoscopic ulcers (OR 18.6, 95% CI 3.3 to 103.7) and >2 concurrent lines of treatment with immunosuppressive drugs. Antiviral therapy was administered in 70% of patients with IBD and 77% of HSCT patients with CMV disease. 71% of antiviral-treated patients with IBD showed an improvement of their disease activity and 14% underwent colectomy. The mortality rate of HSCT patients was 21% irrespective of their CMV status.

Conclusions In addition to the implementation of histological methods, qPCR may be performed in patients with suspected high-risk IBD and HSCT patients for CMV colitis. Independent validations of these results in further prospective studies are needed.

Summary box

What is already known about this subject?

- Gastrointestinal cytomegalovirus (CMV) disease is especially prevalent in immunosuppressed patients with inflammatory bowel disease or after haematopoietic stem cell transplantation. CMV can be detected by histological staining methods or by PCR with different diagnostic accuracies.

What are the new findings?

- Our findings consolidate the diagnostic certainty of the quantitative PCR in intestinal tissue, which showed an acceptable sensitivity for diagnosing CMV colitis. This study is the first that evaluated the diagnostic certainty of the cut-off value of >250 copies/mg in patients after allogeneic stem cell transplantation. The low sensitivity of the histological and immunohistochemical examination is in line with data from the literature. Anaemia and the presence of endoscopic ulcers seem to be predictive factors for CMV colitis. The use of glucocorticoids and immunosuppressive agents as well as concurrent administration of more than two lines of immunosuppressive drugs increased the risk for CMV colitis.

How might it impact on clinical practice in the foreseeable future?

- The additional use of quantitative PCR for detection of gastrointestinal CMV disease manifestation in patients with inflammatory bowel disease and after haematopoietic stem cell transplantation may help facilitate timely diagnosis of CMV disease and improve outcome. We believe that the identified risk factors and predictors help increase the awareness among physicians in the diagnosis of CMV disease.

BACKGROUND

Patients with inflammatory bowel disease (IBD) under immunosuppressive therapy and haematopoietic stem cell transplantation (HSCT) are at an increased risk for cytomegalovirus (CMV) infection and disease given the virus' tropism for inflamed tissue.[1 2]

Interestingly, patients with medically refractory ulcerative colitis (UC) are at the highest risk for CMV disease compared with severe Crohn's disease (CD), and patients with pouchitis.[3–7]

Following HSCT, CMV infection occurs in up to 25%, and gastrointestinal (GI) CMV disease manifests in 10% of these cases. The mortality rate of these patients is highly increased and can approach up to 80%.[8] Early and accurate differentiation between GI graft versus host disease (GVHD) and CMV diseases is critical for the clinical management, because of the fundamentally different treatment strategies. For patients with IBD, early detection and rapid initiation of antiviral treatment for CMV disease seems to reduce the mortality and colectomy rate.[9]

The major challenge in the management of patients with IBD and HSCT is the differentiation between acute IBD exacerbation or acute GVHD and CMV colitis. In order to differentiate these conditions, endoscopic examinations have to be performed with sampling of tissue biopsies.

Previous studies which examined the diagnostic accuracy of haematoxylin and eosin (H&E) staining have shown a sensitivity of only ~10%.[10–12] Therefore, an adjunct method to further improve the diagnostic value of histological techniques immunohistochemistry (IHC) is recommended. Using this technique, the sensitivity can be increased up to ~78%.[11 13] However, in order to achieve adequate sensitivity a high number of biopsy samples must be examined and a trained pathologist must be available at all times.[14] Due to these limitations, quantitative PCR (qPCR) analysis in intestinal tissue specimens was described as a useful addition to clinical and endoscopic findings for diagnosing CMV GI disease.[15]

The aim of this study was to examine the diagnostic accuracy of the above-mentioned methods hypothesising that the additional use of quantitative CMV-DNA-PCR (qPCR) in intestinal tissue increases the detection rate of CMV colitis. We further evaluated the risk factors for GI CMV disease in patients with IBD and HSCT patients and analysed the disease outcome in these cohorts in a tertiary referral centre.

METHODS AND MATERIALS

Study population

We initially reviewed the medical records of 902 patients with IBD treated in the Clinic for Gastroenterology and Gastrointestinal Oncology of the University Medical Center Goettingen between January 2005 and December 2016, and 217 HSCT patients between January 2003 and December 2016. We included 47 patients with IBD and 67 HSCT patients who were treated between 2009 and 2017. All patients with IBD were identified from a computerised database of University Medical Center Goettingen using the International Classification of Diseases for disease coding CD K50 and UC K51, respectively. Initially, medical records of outpatients and inpatients were analysed for this study.

The diagnosis of IBD was based on clinical, laboratory, endoscopic, radiologic, and histological parameters. We included only adult patients with IBD with known CMV status, complete medical records, symptomatic patients undergoing endoscopic evaluation to assess the disease severity, biopsies for CMV detection (for histology and PCR) and regular follow-ups. Patients with mild symptoms and clinically a low probability of CMV disease did not undergo CMV testing and were therefore excluded.

First, stool cultures were analysed in all patients with aggravated symptoms to exclude bacterial (clostridium difficile, salmonella, yersinia, campylobacter and shigella) or viral (adenovirus, norovirus and rotavirus) infection. After exclusion of an infection, intravenous steroids were used as the first-line treatment for acute exacerbation management. If clinical symptoms did not improve under corticosteroid treatment, endoscopy was performed to exclude other causes of symptom aggravation and to collect an intestinal tissue biopsy.

The HSCT patients were identified from the computerised database of the Clinic for Gastroenterology and Gastrointestinal Oncology of the University Medical Center Goettingen using Clinic WinData software by E&L medical systems, Erlangen, Germany. We searched for patients who underwent endoscopic examination due to GI symptoms or suspicion of GI-GVHD. In contrast to patients with IBD, only a small part of the HSCT patients underwent endoscopic procedures. These adult patients had malignant and non-malignant haematological diseases and underwent a myeloablative or non-myeloablative HSCT at the Clinic for Haematology and Oncology of the University Medical Center Goettingen. The inclusion criteria were: (1) known CMV status, (2) complete medical records and (3) regular follow-ups. Patients with GVHD were excluded.

Clinical GI-GVHD was defined as the presence of diarrhoea, persistent nausea and vomiting, and abdominal pain with or without ileus with positive GI-GVHD histological findings.[16]

The conditioning regimens and GVHD prophylaxis were selected according to ongoing protocols at the University Medical Center. The patients were monitored for active CMV infection via blood analysis (CMV antigen and/or qPCR). Patients with positive CMV infection results were treated with antiviral therapy.

The following data were collected from all included patients: demographics, laboratory results, endoscopic findings, histology, current use of steroids, thiopurines, calcineurin inhibitors, mTOR inhibitors or biologics as well as antiviral treatment.

Endoscopic procedures

For endoscopic evaluation conventional endoscopic instruments (Olympus, Tokyo, Japan) were used, and biopsy specimens were obtained endoscopically from severely affected and from normal appearing areas.

For CMV detection, biopsies were taken from the edge of ulcers during endoscopic procedures. Biopsy specimens were fixed immediately in a 10% buffered formalin solution for H&E and IHC staining or in 0.9% saline solution for qPCR, respectively. To identify endoscopic findings characteristic for CMV disease in our cohorts, we analysed ulcerative features such as longitudinal or geographical ulcers, deep or punched-out ulcers, and mucosal defects. The endoscopic findings were defined according to previously published reports.[17–19]

CMV infection/disease

We collected results on CMV diagnostic tests and analysed which patients were treated with antiviral therapy (ganciclovir, foscarnet intravenously or valganciclovir orally). Patients with suspicion of CMV infection with GI involvement underwent serological and endoscopic tests for CMV detection.

CMV infection was defined as a positive result for CMV IgM antibody and/or qPCR in blood serum. CMV disease was defined by the presence of CMV infection associated with clinical signs, for example, fever, pain, diarrhoea, vomiting, and so on.

Due to the absence of a gold standard we defined the diagnosis of CMV colitis by the presence of inclusion bodies detected microscopically by two experienced pathologists using H&E and/or CMV IHC staining and/or qualitative PCR in GI biopsies of macroscopic lesions found by endoscopy at the time of the analysis as well as by qPCR from intestinal tissue specimens. H&E and IHC staining for CMV using anti-CMV monoclonal antibodies DDG9 and CCH2 (Dako, Code GA752, Denmark) as well as qualitative PCR were performed routinely in the Institute of Pathology of the University Medical Center Goettingen according to the manufacturer's instructions. CMV antibody and qPCR measurement were also performed routinely in the Institute of Microbiology of the University Medical Center Goettingen according to the manufacturer's instructions.

For real-time PCR, DNA was extracted from intestinal biopsies with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics, Mannheim, Germany) using the MagNA Pure LC 2.0 instrument according to the manufacturer's instructions. Purified DNA was eluted in a volume of 200 µL, and 10 µL was applied for qPCR analysis with the RealStar CMV PCR Kit 1.2 (Altona Diagnostics, Hamburg, Germany) licensed for diagnostic use. External positive controls ranging from 10 to 10^4 CMV units and an internal heterologous amplification system are supplied to determine the detection limit, the pathogen load and to identify possible PCR inhibition. The specificity of the assay is guaranteed by an oligonucleotide probe, which binds exclusively to the amplified product. The limit for CMV detection was <20 copies/mg tissue.

Statistical analysis

Quantitative variables are described as medians and percentages. Categorical data were analysed using Fisher's exact test. For continuous data, a Mann-Whitney test was performed. Odds ratio (ORs) were calculated when appropriate. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were used by using a conventional two-by-two table.

P value <0.05 after Bonferroni correction for multiple testing was considered statistically significant. The analysis was performed with GraphPad Prism V.5.

RESULTS

Study population and diagnostic accuracy for CMV testing

The clinical database included 902 patients with IBD and 217 HSCT patients. Ninety-five per cent (855/902) of the adult patients with IBD had no clinical signs of CMV disease, had incomplete medical records or missing CMV diagnostic and were excluded. Five per cent (47/902) of patients with IBD with known CMV status including serum and intestinal tissue CMV-PCR as well as H&E and IHC staining and with active clinical disease signs were further divided into two groups: a CMV negative (−) group (57%, 27/47) and a biopsy-proven CMV colitis group (43%, 20/47). The median Mayo Score for patients with UC in CMV (−) group was 7 compared with 10 in CMV colitis group. Patients with CD in the CMV (−) group had a median Harvey-Bradshaw Index of 14 compared with 17 in CMV colitis group. In the CMV (−) group, 136 biopsies (median 5) were taken compared with 160 biopsies (median 8) in CMV colitis group. A median of 3 biopsies was evaluated by qPCR and by histological staining methods (H&E and IHC).

In the HSCT cohort, 72% (156/217) of the adult patients were excluded due to lack of data. Twenty-eight per cent (61/217) of the HSCT patients with proven CMV status including serum and intestinal tissue CMV-PCR as well as H&E and IHC staining were divided into three groups: a CMV (−) group (36%, 22/61), a CMV infection group (31%, 19/61) and a biopsy-proven CMV colitis group (33%, 20/61). In CMV (−) group, 140 biopsies (median 6) were taken, and in CMV infection group 129 biopsies (median 7) and in CMV colitis group 173 biopsies (median 9) were taken. A median of 3 biopsies was evaluated by qPCR and by histological staining methods (H&E and IHC). Four patients of the CMV infection group were diagnosed with CMV pneumonitis and one with CMV encephalitis, respectively. They were diagnosed by detection of CMV-DNA in the bronchoalveolar lavage and in the cerebrospinal fluid using the PCR method.

Table 1 shows the diagnostic accuracy of the used methods for diagnosing CMV colitis in the IBD cohort. According to a previous published study,[15] we also examined the diagnostic accuracy of the qPCR cut-off level of >250 copies/mg and could show that it has the highest sensitivity for diagnosing a CMV colitis in colonic tissue, followed by the qPCR of colonic tissue and the serum qPCR analysis. The sensitivity, specificity, PPV and NPV for H&E staining method were 0%, 100%, 0% and 70%

Table 1 Diagnostic accuracy of the methods for diagnosing a CMV colitis in the IBD cohort

Diagnostic method for CMV detection	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CMV-DNA-PCR from serum (n=47)	65	100	100	79
CMV-DNA-PCR of TS (n=47)	70	100	100	82
CMV-DNA-PCR >250 copies/mg of TS (n=11)	79	83	92	63
Owl eyes in H&E of TS (n=47)	0	100	0	70
IHC of TS (n=47)	14	100	100	73

CMV, cytomegalovirus; IBD, inflammatory bowel disease; IHC, immunohistochemistry; NPV, negative predictive value; PPV, positive predictive value; TS, tissue sample.

as well as for IHC staining method were 14%, 100%, 100% and 73%, respectively.

Table 2 shows the diagnostic accuracy of the applied methods for diagnosing a CMV colitis in the HSCT cohort. The highest sensitivity, specificity, PPV and NPV for diagnosing a CMV colitis showed the qPCR >250 copies/mg from GI tissue analysis, followed by qPCR of colonic tissue and the serum qPCR analysis. The IHC staining method showed a slightly higher diagnostic accuracy than H&E for detecting CMV colitis.

The median qPCR of the colonic tissue of the CMV colitis group in the IBD cohort was 1100 copies/mg (range: 36–950 000). In the CMV colitis group of the HSCT cohort the median qPCR of GI tissue was 6500 copies/mg (range: 180–27 000 000).

Table 2 Diagnostic accuracy of the methods for diagnosing a CMV colitis in the HSCT cohort

Diagnostic method for CMV detection	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CMV-DNA-PCR from serum (n=61)	80	57	46	85
CMV-DNA-PCR of TS (n=61)	80	100	100	91
CMV-DNA-PCR >250 copies/mg of TS (n=11)	92	88	92	88
Owl eyes in H&E of TS (n=61)	5	96	50	37
IHC of TS (n=61)	5	100	100	38

CMV, cytomegalovirus; HSCT, haematopoietic stem cell transplantation; IHC, immunohistochemistry; NPV, negative predictive value; PPV, positive predictive value; TS, tissue sample.

Predictors and risk factors associated with GI CMV disease

The clinical and laboratory characteristics as well as risk factors of the IBD cohort are presented in table 3.

After Bonferroni correction for multiple testing, anaemia and presence of endoscopic ulcers turned out to be predictive factors for CMV colitis in the IBD cohort. The use of glucocorticoids and calcineurin inhibitors (cyclosporine A and tacrolimus) as well as concurrent use of more than two lines of immunosuppressive drugs were risk factors for patients with IBD to develop CMV colitis. Thrombocytes and leucocytes as well as C-reactive protein (CRP) were not significantly associated with CMV colitis (table 3). Gender, age, administration of biologics (infliximab, adalimumab, vedolizumab), antimetabolites (methotrexate, 6-mercaptopurin, azathioprine) or 5-aminosalicylates were not associated with CMV colitis.

The median duration of steroid treatment was 3 weeks before initiation of the CMV diagnosis.

The clinical and laboratory characteristics as well as risk factors of the HSCT cohort are presented in table 4.

Within the CMV (−) and CMV colitis group only visible ulcerative lesions during endoscopy were significantly associated as a predictive factor for CMV colitis (p=0.0003) with an OR of 18.6 (95% CI 3.3 to 103.7). The concurrent use of more than two immune-modulating drugs in the HSCT cohort was found to be a risk factor for CMV GI disease in the CMV colitis group compared with CMV (−) group (p=0.002) with an OR of 8.6 (95% CI 2.1 to 35.3). A higher percentage of patients in the CMV colitis group compared with the CMV (−) group were treated with immunosuppressive agents and mycophenolate mofetil but results were not significant. Gender, age and diagnosis of a GVHD as well as treatment with glucocorticoids or other medications (rituximab, alemtuzumab and thalidomide) were not significantly different between the HSCT cohorts.

Routinely available laboratory parameters were analysed as risk factors for CMV (+) and CMV colitis in the HSCT cohort. None of the evaluated parameters, such as haemoglobin, thrombocytes and leucocytes as well as CRP levels were associated with the CMV status in the HSCT cohort (table 4).

Antiviral therapy and disease outcome of the IBD and HSCT cohorts

An antiviral therapy was given in 70% (14/20) of the patients with IBD in the CMV colitis group. Seventy-one per cent (10/14) of the patients with IBD who were treated with antiviral agents showed an improvement of their clinical disease activity compared with 96% (26/27) of patients in the CMV (−) group. Fourteen per cent (2/14) of antiviral-treated patients with CMV colitis and 4% (1/27) of CMV (−) group patients (all patients with UC) underwent colectomy. The mortality rate of the CMV colitis group was 10% (2/20) compared with 0% in the CMV (−) group. The cause of mortality of these two patients with CMV colitis was sepsis. Both patients were treated with an antiviral regimen. The median duration

Table 3 Clinical and laboratory characteristics as well as risk factors of the IBD cohort

IBD n=47	CMV (–) n=27	CMV colitis n=20	OR (95% CI)	P value
Ulcerative colitis, n (%) Crohn's disease, n (%)	18 (67) 9 (33)	15 (75) 5 (25)		0.75
Gender (M/F), n (%)	17/10 (63/37)	11/9 (55/45)		0.76
Age (mean, SD)	39.5 (17–77)	45.6 (18–73)		0.47
Haemoglobin, g/L (mean, SD)	120 (±21)	100 (±42)		0.009*
Thrombocyte, cells $\times 10^3$/µL (mean, SD)	387 (±183)	314 (±204)		0.32
Leucocyte, cells $\times 10^3$/µL (mean, SD)	10 (±4)	9 (±6)		0.16
CRP, mg/dL (mean, SD)	20 (±32)	32 (±29)		0.08
Endoscopic ulcers, n (%)	4 (15%)	15 (75%)	17.3 (3.9 to 74.8)	0.0001*
Medication, n (%)				
► Glucocorticoids	12 (44)	17 (85)	7.1 (1.7 to 29.9)	0.006*
► Immunosuppressants	1 (4)	9 (30)	21.3 (2.4 to 188.7)	0.0009*
► Biologics	9 (33)	4 (20)		0.35
► Antimetabolite	7 (26)	3 (15)		0.48
► 5-Aminosalicylates	17 (63)	5 (25)		0.02
Number of immunosuppressive medications >2	4 (15%)	14 (70%)	13.4 (3.2 to 56.1)	0.0002

Immunosuppressants: tacrolimus, ciclosporine A.
Biologics: infliximab, adalimumab, vedolizumab.
Antimetabolite: methotrexate, 6-mercaptopurin, azathioprine.
*Corrected for Bonferroni.
CMV, cytomegalovirus; CRP, C-reactive protein;IBD, inflammatory bowel disease.

Table 4 Clinical and laboratory characteristics as well as risk factors of the HSCT cohort

HSCT n=61	Group A CMV (–) n=22	Group B CMV (+) n=19	Group C CMV colitis n=20	P value A versus B	P value A versus C
Gender (M/F), n (%)	14/8 (64/36)	11/8 (58/42)	10/10 (50/50)	0.76	0.53
Age (mean, SD)	51.5 (13.3)	52.9 (12.1)	51.2 (11.2)	0.65	0.67
Haemoglobin, g/dL (mean, SD)	10 (±2)	10 (±4)	9 (±1)	0.94	0.76
Thrombocyte, cells $\times 10^3$/µL (mean, SD)	123 (±106)	135 (±87)	128 (±74)	0.54	0.61
Leucocyte, cells $\times 10^3$/µL (mean, SD)	6 (±6)	6 (±4)	6 (±3)	0.35	0.39
CRP, mg/dL (mean, SD)	56 (±45)	78 (±78)	74 (±72)	0.59	0.56
Endoscopic ulcers, n (%)	2 (9)	4 (21)	13 (65)	0.39	0.0003*†
GVHD, n (%)	3 (14)	4 (21)	3 (15)	0.68	0.98
Medication, n (%)					
► Glucocorticoids	17 (77)	13 (68)	14 (70)	0.73	1.74
► Immunosuppressants	19 (86)	17 (90)	20 (100)	0.98	0.23
► MMF	8 (36)	7 (37)	2 (10)	0.99	0.07
► Others	2 (9)	2 (11)	3 (15)	0.98	0.66
Number of immunosuppressive medications >2	7 (32%)	4 (21%)	16 (80%)	0.50	0.002*‡

Immunosuppressants: tacrolimus, ciclosporine A, everolimus, sirolimus.
Others: rituximab, alemtuzumab, thalidomide.
*Corrected for Bonferroni.
†OR of endoscopic ulcer group A versus C: 18.6 (95% CI 3.3 to 103.7).
‡OR of number of immunosuppressive medications >2 group A versus C: 8.6 (95% CI 2.1 to 35.3).
CMV, cytomegalovirus; CRP, C-reactive protein; GVHD, graft versus host disease;HSCT, haematopoietic stem cell transplantation; MMF, mycophenolate mofetil.

between diagnosis of CMV colitis and colectomy was 10 weeks and between deaths was 8 weeks.

Overall, 77% (30/39) of the HSCT patients with CMV infection/disease were treated with an antiviral therapy. Particularly, 79% (15/19) of the CMV (+) and 75% (15/20) of the CMV colitis group received an antiviral therapy, respectively. The mortality rate of the HSCT patients with regard to their CMV status was not different. Overall, the mortality rate was 21% (13/61) in the HSCT cohort. Particularly, 18% (4/22) of the patients from the CMV (−) group, 16% (3/19) of the patients from the CMV (+) group and 30% (6/20) of patients from the CMV colitis group died, respectively. The median duration between diagnosis of CMV gastroenteritis and death was 6 weeks.

DISCUSSION

In line with previous published data the present study demonstrates that the sensitivity of H&E and IHC in the IBD and HSCT cohorts was extremely low.[11 12] Due to the low diagnostic performance of these two techniques, alternative diagnostic methods should be established in the future, for example, qPCR analysis. To date, only few published studies have evaluated the diagnostic accuracy of qPCR amplification assay in the intestinal mucosa. Briefly, these studies support the greatest accuracy for CMV detection as reviewed by Pillet *et al*.[20] Only few studies indicated a correlation between H&E/IHC staining and qPCR results.[21 22] Accordingly, this suggests that detection of low copies of CMV-DNA may determine a latent infection and therefore a cut-off value of the viraemia may distinguish CMV infection from CMV disease.[1] Roblin *et al*[15] demonstrated that a cut-off value of >250 copies/mg of tissue is predictive of resistance to immunosuppressive therapy for a CMV disease with a sensitivity of 100% and a specificity of 66%. In the present study, the highest sensitivity and specificity was shown for qPCR results with a cut-off value of >250 copies/mg tissues for the IBD and HSCT cohort. One could argue that the new method is too sensitive having the risk of being false positive. The limitation of this diagnostic method is the lack of standardisation. Therefore, the comparison of the results between different studies is difficult and accepted cut-off values of CMV-DNA load for assessing CMV disease have to be defined.

To the best of our knowledge, this is the first study which evaluates the diagnostic accuracy for the cut-off value of >250 copies/mg GI tissue for an HSCT cohort. In contrast, the sensitivity of H&E and IHC staining was only 5% in the HSCT cohort, respectively. Therefore, some HSCT patients would be diagnosed false negatively and probably receive the insufficient treatment due to differential diagnosis of GVHD.[23–25]

In this study, two predictors (anaemia and presence of endoscopic ulcers) and three risk factors (the use of corticosteroids or calcineurin inhibitors and concurrent use of more than two lines of immunosuppressive drugs) for CMV colitis were found in the IBD cohort. In the HSCT cohort, only the presence of ulcers predicted a GI CMV disease and the concurrent use of more than two immunosuppressive drugs was a risk factor. The administration of corticosteroids in the IBD cohort is a well-known risk factor for CMV disease.[22 26–28] With regard to immunosuppressive therapy cyclosporine A and tacrolimus were risk factors for developing CMV colitis in the IBD cohort. This is consistent with previously published studies in which the use of calcineurin inhibitors emerged as risk factor for CMV involvement of the intestine.[21 29] Regarding anaemia, we presume that these findings are closely related to the chronic inflammation and maybe the immunosuppressive medications resulting in myelosuppression.

All these three risk factors show that CMV affects predominantly patients with IBD with severe flare-ups. The endoscopic presence of colonic ulcers was an independent predictor for the manifestation of CMV colitis in the HSCT cohort. Only one previously published study in HSCT patients reported the association between CMV gastritis and endoscopic findings but no colonoscopic evaluation was conducted.[30] In patients with IBD, inconsistent results exist regarding the presence of ulcers at endoscopy and CMV disease. While some studies indicate an association between endoscopic ulcers and CMV disease[5 17 19 31] others do not.[15 32]

In our study, 70% of patients with CMV colitis of the IBD cohort and 77% of patients with CMV colitis of the HSCT cohort were treated with an antiviral therapy, according to their physician's decision. One could speculate why not all CMV diseased patients were treated with antiviral agents. Retrospectively, four arguments may have contributed to the decision not to treat 30% of the patients with IBD and 23% of the HSCT patients despite a positive CMV detection: (1) some patients of both cohorts had a low viraemia by qPCR analysis from the intestinal tissue specimens, (2) the disease activity of few patients recovered spontaneously, (3) in few cases the underlying disease was lethal, especially in the HSCT cohort and (4) in some patients a decrease of the immunosuppressive medication was sufficient, especially in the IBD cohort. With regard to IBD, it is difficult to draw conclusions about the role of antiviral CMV therapy because of the lack of evidence-based medicine data. Various gastroenterology societies recommend an antiviral treatment when severe flare-ups of IBD exhibit CMV markers in inflamed tissue, but recommendations on antiviral drug or treatment duration do not exist. Based on the results of mostly retrospective studies, an antiviral therapy is started with ganciclovir followed by oral valganciclovir.[20 33] Data on the mortality rate of patients with IBD with CMV disease are lacking. In our study, the mortality rate of patients with IBD was much lower compared with the HSCT cohort. Interestingly, the mortality rate in the HSCT cohort was not significantly different from the CMV (−), CMV (+) and CMV colitis group. We therefore conclude that the rate of morbidity

and mortality in HSCT patients is much higher due to the underlying disease and increased immunosuppressive treatment.[34] Moreover, only the manifestation of GVHD increases the rate of mortality to approximately 50%–70%,[35] and an additional CMV disease manifestation increases the mortality rate to ~80%.[8]

The limitations of this study include its retrospective design at a single centre. The number of the included patients in our study was small. We also included patients with a higher probability of CMV disease according to clinical symptoms which could reflect a positive selection. However, the European Crohn´s and Colitis Organisation (ECCO) consensus for opportunistic infections does not recommend screening for subclinical CMV infection in patients with IBD.[36] The treatment strategy was based on both physician's decisions and the patients' preferences. The present study included two heterogeneous cohorts, which might have been different from previous IBD and HSCT studies evaluating the clinical course of CMV disease.

Nevertheless, this retrospective study provides evidence that qPCR is more sensitive to diagnose CMV colitis. Moreover, qPCR is investigator independent while histological staining has high interobserver variations because of the individual experience of the pathologists. Therefore, future prospective trials should employ qPCR and cut-off limits as well as histological methods in a multicentre setting to confirm these initial findings.

CONCLUSIONS

In addition to the application of histological methods, qPCR may be performed in patients with IBD and after HSCT who are suspected for CMV colitis. This was a retrospective single-centre study with limitations such as an increased probability of causing bias due to the fact that there were few patients included. Therefore, (1) it is necessary to design further prospective and multicentre studies, (2) to evaluate which diagnostic method for diagnosing clinical relevant CMV colitis in patients with IBD and HSCT patients is most appropriate, (3) to validate the identified risk factors for CMV colitis, (4) to determine the ideal therapeutic regimen and (5) to define the required treatment duration as well as (6) to document the outcome of antiviral therapy.

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Contributors AA conceived the study, analysed and interpreted the data and edited the manuscript. EM assisted in data analysis, and wrote the manuscript. KT acquired the data and contributed to the statistical analysis. NCM, SCBB, SK, VE and AN edited significant sections and revised the manuscript. All authors read and approved the final version of the manuscript.

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