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# Herpesvirus infections in immunocompromised patients : treatment, treatment failure and antiviral resistance

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# Failure of preemptive treatment of cytomegalovirus infections and antiviral resistance in stem cell transplant recipients

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## ABSTRACT

*Background:* Treatment of cytomegalovirus (CMV) infections after stem cell transplantation (SCT) does not always lead to a rapid viral response. The causes of treatment failure may be either viral resistance or immunological failure to control viral replication. This study investigated the response to preemptive treatment in CMV infections in order to define risk factors for treatment failure, including the role of antiviral resistance.
*Methods:* Adult recipients of allogeneic T-cell depleted SCT were studied retrospectively (n = 92). CMV infections were treated with (val)ganciclovir according to a CMV DNA load based preemptive strategy. Treatment failure was defined as a CMV DNA load of 1000 copies/ml or more after at least 2 weeks of treatment. Resistance was analyzed by nucleotide sequence analysis of the UL97 and UL54 genes in the first CMV DNA positive sample and in samples during treatment failure.
*Results:* Treatment failure occurred in 26 of the 47 preemptively treated patients (55%) and in 39 of 86 (45%) treatment episodes. The risk of treatment failure was increased during first treatment episodes ($p = 0.01$) and during the use of immunosuppressive medication ($p = 0.02$). Antiviral resistance was found in only 1 patient (4%) with treatment failure.
*Conclusions:* A slow response to preemptive antiviral treatment occurred frequently in CMV infections in SCT recipients. Antiviral resistance was observed but played a minor role in treatment failure.

## INTRODUCTION

Preemptive therapy of cytomegalovirus (CMV) infections based on virological monitoring has been proven effective and has become common practice after hematopoietic stem cell transplantation (SCT) in many centres. Quantitative viral load measurements can additionally be used for monitoring treatment responses. In this way, it has been shown that preemptive treatment of CMV infections after SCT does not always lead to a rapid, complete and sustained viral response. By sensitive assays such as real-time PCR, viral DNA can be detected for days to weeks after treatment,[1;2] frequently considerably longer as compared to detection of pp65 antigenemia. The clinical significance of prolonged CMV DNAemia is not always clear. It may predict recurrence of CMV DNAemia or development of CMV disease, but persistent infections may also be cleared spontaneously or respond to a repeated treatment course without complications.[1;3;4] The causes of viral DNA persistence despite antiviral treatment may be viral resistance on the one hand or immunological failure to control viral replication on the other. Risk factors associated with treatment failure and in particular the role of resistant CMV in a preemptive treatment setting after SCT are largely unknown.

In guiding treatment of persistent CMV infections, it is important to know the contribution of the various causes of treatment failure. This study investigated the occurrence and risk factors of failure of preemptive treatment of CMV infections in SCT patients, focusing on the possible role of antiviral resistance.

## METHODS

### *Patient data*

Consecutive adult recipients of an allogeneic T-cell depleted stem cell transplant between 2005 and 2008 at risk for CMV (donor and/or recipient CMV seropositive) were included (n = 92). Follow-up was one year after transplantation. T cell-depleted transplantation was performed either according to a reduced intensity conditioning protocol or a myeloablative conditioning regimen as described previously [5-7]. In the absence of graft versus host disease (GvHD) or graft failure, patients received donor lymphocyte infusion for treatment of mixed chimerism or relapsed disease, at least 3 months (median of 187 days) after transplantation.

***CMV treatment***

CMV DNA loads in EDTA plasma were determined prospectively by quantitative real-time PCR as previously described.[8] A preemptive treatment protocol[8] with valganciclovir (vGCV, 900 mg twice daily for 14 days) was applied based on CMV DNA load. Treatment was initiated if the load exceeded $10^4$ copies/ml or was above $10^3$ copies/ml combined with an at least tenfold increase in comparison to the preceding week. Subsequent DNAemia episodes were treated if the load was above $10^5$ copies/ml or above $10^4$ copies/ml and increasing at least tenfold in one week. Treatment was continued as long as the CMV DNA load was above these thresholds. Symptomatic CMV infection was treated with intravenous ganciclovir (GCV, 5 mg/ kg twice daily for 14 days). Oral ganciclovir was not applied.

***Data collection***

Patient and treatment data were collected retrospectively from patient charts. The recorded baseline data are shown in Table 1. The follow-up data included the use of antiviral medication, the use of immunosuppressive medication (therapeutic, i.e. non-prophylactic, use of systemic corticosteroids, cyclosporine, rituximab) and the total lymphocyte count, signs and symptoms of CMV end-organ disease[9] and patient survival. Renal function was recorded to check for adequate dosing of antiviral medication.

***Analysis of resistance***

Analysis of resistance was performed by nucleotide sequence analysis of CMV DNA from plasma samples as previously described.[10] The amplified region ranged from codon 370 to 708 of the *UL97* gene, covering all previously described mutations.[11] Sequence analysis of codon 262 to 1169 of the *UL54* gene was performed on DNA isolates with *UL97* mutations or from patients using foscarnet or cidofovir. Sequences were compared to the sequence of the GCV susceptible AD169 strain and to pre-treatment samples from the patient. Baseline resistance was determined in the first sample from each patient containing at least 1000 copies/ ml of CMV DNA. Resistance during treatment was determined by analysis of subsequent plasma samples containing at least 1000 copies/ ml of CMV DNA after at least two weeks of treatment of CMV infection. Genotypic resistance was defined as the presence of resistance-associated mutations that have been published previously (as proven by marker transfer).

***Statistical analysis***

CMV DNAemia was defined as any detectable CMV DNA load in plasma. A treatment episode was defined as a period of antiviral treatment of 14 days. Treatment failure was

*Table 1. Baseline characteristics of the 92 patients.*

| | Patients | |
|---|---|---|
| | n | (%) |
| **Sex** | | |
| Male | 54 | (59) |
| **Age** | | |
| Years (mean, range) | 48 | (21-70) |
| **Underlying disease** | | |
| ALL | 9 | (10) |
| AML | 29 | (32) |
| CLL | 3 | (3) |
| CML | 9 | (10) |
| MDS | 5 | (5) |
| MM | 14 | (15) |
| Other | 23 | (25) |
| **Transplant type** | | |
| Peripheral blood stem cells | 91 | (99) |
| Bone marrow | 1 | (1) |
| **Conditioning regimen** | | |
| Myeloablative | 48 | (52) |
| Non-myeloablative | 44 | (48) |
| **Donor type** | | |
| Matched sibling donor | 51 | (55) |
| Matched unrelated donor | 39 | (42) |
| Other[1] | 2 | (2) |
| **Serostatus donor** | | |
| CMV-seropositive | 56 | (61) |
| **Serostatus recipient** | | |
| CMV-seropositive | 75 | (82) |
| **Serostatus combination** | | |
| Donor -/ recipient + | 36 | (39) |
| Donor +/ recipient - | 17 | (19) |
| Donor +/ recipient + | 39 | (42) |
| **Total** | **92** | (100) |

Abbreviations: ALL = acute lymphoblastic leukaemia, AML = acute myelogenous leukaemia, CLL = chronic lymphocytic leukaemia, CML = chronic myelogenous leukaemia, MDS = myelodysplastic syndrome, MM = multiple myeloma

[1] haplo-identical related donor (n = 1), mismatched related donor (n = 1)

defined as the presence of at least 1000 copies/ ml of CMV DNA in plasma at the end of a treatment episode. In view of the repeated measurement of treatment failure as a binary variable (failure yes/no) within patients, the outcome was modelled as a repeated mea-

sures logistic regression. Parameters were estimated using the Generalized Estimating Equations procedure in SPSS 16 (SPSS Inc., Chicago, IL, USA) with first-order autoregressive correlation structure and a robust estimation procedure. Univariable analyses of potential predictors was performed with p-values <0.20 as a criterion for possible inclusion in a multivariable model.

## RESULTS

***CMV infections***

Plasma samples and treatment data were available from all 92 transplanted patients at risk for CMV (donor and/or recipient CMV seropositive). Baseline characteristics of the patients are shown in Table 1. CMV DNAemia was detected in 67 of all 92 patients (73%). Pre-emptive treatment for CMV infections was administered in 47 of 67 patients with CMV DNAemia; in total 96 treatment episodes occurred of which 86 were evaluable with respect to treatment response. CMV treatment was initiated after a median of 33 days after transplantation. CMV disease occurred in five patients; three patients with pneumonitis, one patient with encephalitis and one patient with retinitis. During the first year after transplantation 30 patients (33%) died, including the five patients with CMV disease.

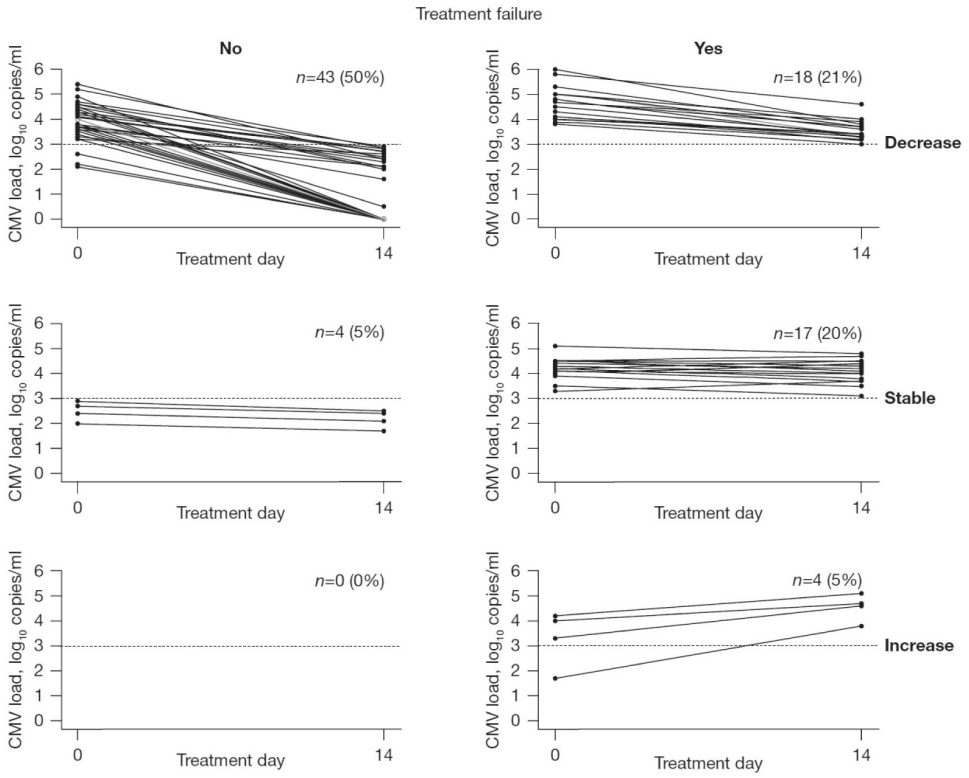
***Treatment failure***

Treatment failure occurred in 55% of the treated patients (26 of 47), corresponding to failure in 39 of 86 (45%) treatment episodes. In 4 of the failing episodes (4.7%) the viral load increased during treatment, in 17 episodes (19.7%) the viral load remained stable above 1000 copies/ml despite treatment and in 18 episodes (20.9%) the viral load decreased but remained above 1000 copies/ml (Figure 1). Nineteen patients (73%) experienced a single episode of treatment failure, three patients (12%) had persistent treatment failure lasting multiple episodes of antiviral treatment and four patients (15%) had recurrent episodes of treatment failure.

In 25 of the 26 patients (96%) treatment failure occurred during the first treatment episode. The cumulative duration of all treatment episodes in the first year after transplantation was 15 and 29 days (median) in the patients without and with treatment failure respectively. CMV disease was present in 4 of 26 treated patients with (recurrent or persistent) treatment failure (15%) and in 1 of 21 patients (4.8%) without treatment failure (odds ratio 3.63, $p = 0.27$).

In univariable analysis (Table 2), the risk of treatment failure was increased during first treatment episodes, during the therapeutic use of immunosuppressive medication and in patients with a higher CMV load at the start of treatment. Conditioning regimen, donor

*Figure 1. Cytomegalovirus (CMV) treatment episodes categorized according to viral kinetics and treatment failure.*




Treatment course and failure could be evaluated in 86 of 96 treatment episodes (14 days of antiviral treatment). Treatment failure was defined as the presence of at least 1000 copies/ ml of cytomegalovirus DNA in plasma after at least two weeks of treatment. A change in viral load of ≥ 0,5 $^{10}$log copies/ml was considered an increase or decrease.

type, CMV serostatus, lymphocyte counts and the timing after SCT were not significant predictors of treatment failure. Underdosage of antiviral medication (defined as a dosage lower than valganciclovir 900 mg twice daily or ganciclovir 5 mg/kg twice daily excluding cases of dose adjustments for impaired renal function) was associated with a decreased occurrence of treatment failure (Table 2). In multivariable analysis (Table 2), the risk of treatment failure was increased during first treatment episodes and during the use of immunosuppressive medication.

***Antiviral resistance***

Resistance development could be monitored by analyzing baseline samples from 58 of 67 patients with CMV DNAemia and follow-up samples from 23 of 26 patients with

*Table 2. Treatment failure.*

| | Treatment failure | | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|---|---|
| | No | Yes | | | | | | |
| | (% of episodes) | (% of episodes) | Odds-ratio | 95% confidence interval | *p*-value | Odds-ratio | 95% confidence interval | *p*-value |
| **Sex** | | | | | | | | |
| Male | 51,3 | 48,7 | 1,26 | (0,53-2,96) | 0,60 | | | |
| Female | 57,4 | 42,6 | | reference category | | | | |
| **Age,** years, median | 53,0 | 51,0 | 0,97 | (0,94-1,01) | 0,15 | 0,98 | (0,94-1,03) | 0,49 |
| **Underlying disease** | | | | | | | | |
| ALL | 46,2 | 53,8 | [b] | | | [b] | | |
| AML | 63,3 | 36,7 | [b] | | | [b] | | |
| CLL | 50,0 | 50,0 | [b] | | | [b] | | |
| CML | 71,4 | 28,6 | [b] | | | [b] | | |
| MDS | 66,7 | 33,3 | [b] | | | [b] | | |
| Other | 45,2 | 54,8 | [b] | | | [b] | | |
| **Conditioning regimen** | | | | | | | | |
| Myeloablative | 50,0 | 50,0 | 1,61 | (0,67-3,86) | 0,29 | | | |
| Non-myeloablative | 61,1 | 38,9 | | reference category | | | | |
| **Donor type** | | | | | | | | |
| Matched sibling donor | 54,5 | 45,5 | 1,19 | (0,61-2,34) | 0,61 | | | |
| Matched unrelated donor | 53,5 | 46,5 | 1,25 | (0,65-2,40) | 0,51 | | | |
| Other | 60,0 | 40,0 | | reference category | | | | |
| **CMV serostatus donor (D)/ recipient (R)** | | | | | | | | |
| D -/ R + | 53,1 | 46,9 | 1,20 | (0,50-2,87) | 0,69 | | | |
| D +/ R - | 50,0 | 50,0 | 1,40 | (0,43-4,50) | 0,58 | | | |
| D +/ R + | 58,1 | 41,9 | | reference category | | | | |
| **Treatment onset** | 58,0 | 44,0 | 1,00 | (0,99-1,00) | 0,47 | | | |
| day after SCT, median | | | | | | | | |

*Table 2. Continued.*

| | Treatment failure | | Univariable analysis | | | Multivariable analysis | | |
|---|---|---|---|---|---|---|---|---|
| | No | Yes | | | | | | |
| | (% of episodes) | (% of episodes) | Odds-ratio | 95% confidence interval | *p*-value | Odds-ratio | 95% confidence interval | *p*-value |
| **Ranking of treatment** | | | | | | | | |
| First episode | 44,4 | 55,6 | 4,05 | (1,60-10,28) | 0,003* | 4,44 | (1,39-14,16) | 0,01* |
| Subsequent episode | 65,9 | 34,1 | | reference category | | | reference category | |
| **Underdosage of antiviral medication** | | | | | | | | |
| No | 51,3 | 48,7 | | reference category | | | reference category | |
| Yes | 87,5 | 12,5 | 0,15 | (0,06-0,35) | 0,000* | 1,01 | (0,33-3,04) | 0,99 |
| **CMV load at start of treatment** | 3,8 | 4,2 | 2,31 | (1,25-4,30) | 0,01* | 2,16 | (0,68-6,87) | 0,19 |
| $^{10}$log copies/ml, median | | | | | | | | |
| **Immunosuppressive medication** [a] | | | | | | | | |
| No | 62,7 | 37,3 | | reference category | | | reference category | |
| Yes | 37,0 | 63,0 | 2,82 | (1,14-6,98) | 0,03* | 4,10 | (1,23-13,66) | 0,02* |
| **Lymphocyte count** | 0,27 | 0,15 | 0,70 | (0,43-1,15) | 0,16 | 0,58 | (0,25-1,33) | 0,20 |
| $\cdot 10^9$ cells/litre, median | | | | | | | | |
| | 55 | 45 | | | | | | |
| **Total** | | | | | | | | |
| | (n = 47) | (n = 39) | | | | | | |

Predictors of treatment failure were analyzed by repeated measures logistic regression using the Generalized Estimating Equations procedure in SPSS 16 with first-order autoregressive correlation structure and a robust estimation procedure. For multivariable analysis potential predictors were included with p-values <0.20 in univariable analysis.
Abbreviations: ALL = acute lymphoblastic leukaemia, AML = acute myelogenous leukaemia, CLL = chronic lymphocytic leukaemia, CML = chronic myelogenous leukaemia, MDS = myelodysplastic syndrome, MM = multiple myeloma
[a] Therapeutic use of (combinations of) corticosteroids (22 episodes), cyclosporine (21 episodes) or rituximab (11 episodes), mainly for graft-versus-host disease or post-transplant lymphoproliferative disorder; [b] not determined because of insufficient power for analysis of various small strata; *significant at a 0.05 level.

treatment failure. Each patient with treatment failure was investigated for antiviral resistance in samples every two weeks until the CMV DNA load fell below 1000 copies/ml. The median number of samples for each patient was 2 (range 1-7). Most follow-up samples (75%) were retrieved after two weeks of treatment (range 2-13 weeks of treatment). No resistance associated mutations were found in the *UL97* gene in the 58 pre-treatment samples. Resistance at treatment failure was found in 1 of 23 patients (4%). In this previously described patient[12] with recurrent treatment failure, CMV DNAemia first occurred at day 27 after transplantation, which was treated with vGCV (900 mg twice daily). Subsequently, the patient was treated with vGCV prophylaxis (450 mg twice daily) because of persistent low levels of CMV DNA. However, from day 136 onward, CMV DNA loads steadily increased and eventually progression to CMV encephalitis occurred. Viral DNA with the resistance associated A594V mutation in the *UL97* gene was found in plasma and cerebrospinal fluid samples from day 136 onwards, after 109 days of antiviral treatment. In the other 22 patients with treatment failure, no resistance associated mutations were found in the *UL97* gene (30 samples) or the *UL54* gene (7 samples).

## DISCUSSION

In this study, the response to preemptive antiviral treatment of CMV infections after SCT and the role of antiviral resistance in treatment failure were studied. Based on regular monitoring of CMV DNA in plasma, CMV DNA levels during treatment were analyzed. Approximately half of the patients still had CMV DNAemia of at least 1000 copies/ml after a standard course of antiviral treatment of two weeks. Antiviral resistance played only a minor role in such persistent CMV DNAemia, with resistance-associated mutations found in only one patient with treatment failure and in none of the baseline samples.

The definition of treatment failure as a CMV DNA load of at least 1000 copies/ ml after at least two weeks of treatment was chosen to include those patients in whom prolongation of treatment is commonly considered. It appeared that patients with treatment failure according to our definition indeed had a longer duration of treatment for CMV than patients without treatment failure. Treatment failure can also be defined using viral dynamics and, for example, treatment failure defined as less then 2log decrease in viral load after 14 days of treatment would have resulted in a comparable prevalence of failure in 49% of the episodes (MTvdB *et al.*, data not shown). Although the persistence of viral DNA in plasma is no direct proof of ongoing viral replication, it is likely that the

half-life of viral DNA is short and, therefore, that plasma DNAemia is a correlate for recent viral replication.[13] This view is supported by the fact that viral loads decreased rapidly in many patients in our study.

The rate of treatment failure of 45% of all episodes was somewhat higher than observed in an earlier study in our hospital (Leiden University Medical Center, Leiden, The Netherlands), which found failure in 20-25% of episodes, despite comparable patient and episode characteristics.[3] This may be due to a longer duration of follow up in the current study. Our results are comparable to two other studies where rising antigenemia under treatment occurred in 45% of patients[4] and PCR positivity was found in 39% of patients after treatment.[14] A recent prospective cohort study showed persistent CMV infection after 21 days of antiviral treatment in only 4 of 59 (7%) SCT patients.[15] The different definition of treatment failure (21 versus 14 days and inclusion of DNAemia as well as antigenemia, viral culture and histopathology) probably contributed to this variation. Differences in antiviral treatment regimen, which was not further specified, may also play a role. A lack of association between treatment failure and CMV disease in our study may be explained by the small number of patients with CMV disease due to the effective preemptive treatment strategy.

In our study, all patients treated for CMV DNAemia were tested for resistance, regardless of symptoms or clinical suspicion of resistance. Most previous publications on resistance comprised case reports or small case series of patients with CMV disease and did not assess the overall prevalence of resistant CMV. Only a few earlier studies are available in which resistance has been systematically studied in persistent CMV infections in SCT patients undergoing preemptive treatment.[4;15;16] A prospective study found resistance in 1 of 4 SCT patients with persistent viral replication.[15] Low-level resistance (as determined by phenotypic methods) was found in 1 of 15 patients with rising antigenemia levels under treatment[4] and in none of 10 patients with positive PCR results after two weeks of antiviral treatment.[16] In the setting of recurrent CMV after preemptive treatment, one study observed resistance in 1 of 13 patients.[17] These results are in accordance with the low prevalence of resistance found in our study, demonstrating that this does not provide a likely explanation for the majority of cases with treatment failure. There is a small chance that *UL54* mutations have been missed due to our strategy of only analyzing samples from patients harbouring *UL97* mutants or from patients who had been treated with the antivirals (foscarnet and cidofovir) that directly target the viral DNA polymerase *UL54.*[18;19] However, previous studies have shown that the majority of resistance-associated mutations are found the *UL97* gene.[15;20]

Treatment failure was found most often during first treatment episodes. Probably, the development of CMV-specific immunity during CMV infection facilitates treatment responses in subsequent episodes. Furthermore, the use of immunosuppressive medication, mainly for GvHD or post-transplant lymphoproliferative disorder treatment, during antiviral treatment increased the risk of treatment failure. Likewise, in previous studies, the use of high-dose corticosteroids was a significant risk factor for persistent or increasing pp65 antigenemia.[4;21] Obviously, the time needed to clear DNAemia or antigenemia is related to the amount of virus at start of treatment[22] and, indeed, in univariable analysis, a high viral load at start of treatment was associated with a higher risk of treatment failure. In multivariable analysis this effect was insignificant, however, suggesting a more complex relationship between viral load, treatment failure, failure of immunological recovery and, likely, viral resistance. A sustained lack of (CMV-specific) T-cells after transplantation, for example due to the use of immunosuppressive medication, probably allows for high viral loads and, hence, persistent or recurrent CMV infection.[23] This necessitates repeated and prolonged antiviral treatment and may also lead to the development of antiviral resistance.

Indeed, the prolonged treatment with a low dose of vGCV in the only patient with a *UL97* mutant, may have led to the development of resistance due to the erroneous use of secondary prophylaxis during an ongoing viral reactivation.[12] Surprisingly, suboptimal dosages of antiviral medication were not associated with treatment failure, but were administered more often during successful treatment episodes. This appears to be the confusing result of the off-protocol use of secondary prophylaxis with vGCV 900 mg once daily in certain patients. Those patients had a low viral load after a standard treatment course and then received secondary prophylaxis with low dose valganciclovir. Either spontaneously or due to the antiviral treatment, CMV DNAemia was rapidly cleared. This unexpected association was insignificant in multivariable analysis.

In conclusion, in SCT patients, CMV infections with a slow response to antiviral treatment occurred frequently. Antiviral resistance was observed but played a minor role in treatment failure.

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MB designed and performed the study research, analysed the data and wrote the paper, EM designed the study, AV analysed the data, CB performed the research, RW analysed the data, CH contributed clinical data, EC analysed the data, AK designed the study.

## DISCLOSURE STATEMENT

The authors declare no competing interest.

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