[haematologica]
2004;89:1248-1252

Cytomegalovirus reactivation during alemtuzumab therapy for chronic lymphocytic leukemia: incidence and treatment with oral ganciclovir

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A B S T R A C T

Background and Objectives. Although alemtuzumab (campath-1H) has been successfully used in patients with untreated or previously treated chronic lymphocytic leukemia (CLL), a variable incidence of cytomegalovirus (CMV) reactivation has been described. No prospective reports currently provide results of the use of oral ganciclovir as pre-emptive therapy in patients with CMV reactivation during alemtuzumab treatment.

Design and Methods. We designed a prospective study in 12 patients with pretreated CLL with the aim of evaluating the incidence of CMV reactivation during alemtuzumab treatment and the role of oral ganciclovir as pre-emptive therapy and in preventing CMV organ disease.

Results. In the 12 CLL patients being treated with alemtuzumab, 8 patients (66%) had CMV reactivation, as detected by antigenemia and/or CMV DNA. No patient showed clinical evidence of CMV disease. The alemtuzumab was discontinued and the patients were immediately treated with oral ganciclovir 1000 mg tid. After a median of 14 days of antiviral therapy all patients achieved negative CMV polymerase chain reaction (PCR) assays and/or antigenemia. No patients showed further CMV reactivation up to the end of the study.

Interpretation and Conclusions. CMV reactivaction, studied with periodic analysis of antigenemia and PCR, is frequent in previously treated CLL patients receiving alemtuzumab therapy although only sporadic cases of CMV disease have been reported. Using oral ganciclovir, the response to therapy was prompt, there was no progression to CMV disease, and no relevant clinical toxicity, thus sparing unnecessary hospitalization. Oral ganciclovir may be used as pre-emptive therapy in all patients who develop CMV reactivation during alemtuzumab treatment.

Key words: chronic lymphocytic leukemia, alemtuzumab, CMV, pre-emptive therapy, oral ganciclovir.

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Infections are a prominent feature in patients with chronic lymphocytic leukemia (CLL) because of the typical immunosuppression induced by both the disease itself and its treatment, this latter being particularly relevant when patients have received several chemotherapeutic regimens. Alemtuzumab is a humanized IgG1 class of immunoglobulin which takes effect activating both complement and antibody-dependent cell-mediated cytotoxicity (ADCC) against CD52 antigen, which is a glycosylphosphatidylinositol-anchored glycoprotein expressed on all mature lymphocytes (and at especially high levels on many malignant lymphocytes), monocytes and spermatozoa, while it is not expressed by hematopoietic stem cell progenitors.[1] Several published studies describe the efficacy of alemtuzumab in previously treated CLL. The dose is 10 or 30 mg three times a week administered intravenously or subcutaneously for a variable time ranging from 6 weeks to 16 weeks.[2-5] After alemtuzumab administration the lymphocyte count falls rapidly in the peripheral blood, reaching few or no circulating lymphocytes at a median of 4 weeks after starting treatment. Cytomegalovirus (CMV) reactivation during alemtuzumab therapy has been described in various studies. In most of these studies the CMV reactivation was detected by CMV DNA in peripheral blood mononuclear cells (PBMC) by polymerase chain reaction (PCR) or by pp65 antigenemia.[6] These studies revealed a CMV reactivation rate of 16%-33%, during the period of lymphocyte depletion, with the period of T-cell depletion being particularly important; this is supported by the fact that the expression of CD52 antigen is higher in normal T cells than in normal B cells.[2-4,6-8] Recently, the burden of

CMV viremia during alemtuzumab therapy was evaluated in patients with relapsed/refractory disease receiving famcyclovir prophylaxis; 15% of cases had detectable CMV viremia at a median of 28 days after the first dose of alemtuzumab, without clinical evidence of CMV disease.[9] In patients with lymphoid malignancy, without specific prophylactic therapy, the probability of CMV pneumonia following alemtuzumab treatment was 0.6%.[10]

Furthermore, patients experiencing CMV reactivation were successfully treated with oral ganciclovir.[3] Oral ganciclovir has been shown to be an effective prophylactic agent against CMV in patients receiving renal or liver transplant;[11-12] on the other hand, there is only one report in the literature regarding the use of oral ganciclovir as pre-emptive therapy.[13] On this background, we designed a prospective study with the aim of evaluating the incidence of CMV reactivation during alemtuzumab treatment in patients with CLL as well as the role of oral ganciclovir as pre-emptive treatment and preventing CMV organ disease.

Design and Methods

Starting from November 2002, we used alemtuzumab at a dose of 10 mg i.v. three times a week for 10 weeks in 12 pre-treated CLL patients (8 males and 4 females). All patients had been previously treated with at least two lines of chemotherapy (median 2.5; range 2-5), including chlorambucil, CHOP, COP, fludarabine, autologous peripheral blood stem cell transplantation (APBSCT), with or without immunotherapy with rituximab. The median age of the patients was 61.5 years (range 50-78 years). At the time of treatment five patients were in stage B/II, six in stage C/IV and one in stage A/progressive. The median lymphocyte count in peripheral blood (PB) was 45,030/μL (range 1,070-14,8640/μL) and the median lymphocytic infiltration of bone marrow (BM) aspiration was 80% (range 36-93%). Before treatment with alemtuzumab the patients had normal concentrations of T lymphocyte subsets and immunoglobulin levels. The median total T-lymphocyte count ($CD3^+$ cells) was 1515/μL (range 908-5990/μL) (normal values 1185-1540/μL), the median T-helper lymphocyte count ($CD4^+$ cells) was 796/μL (range 374-1582/μL) (normal values 670-950/μL), and the median T-suppressor lymphocyte count ($CD8^+$ cells) was 549/μL (range 192-1650/μL) (normal values 504-695/μL). The concentration of IgG was 1080 mg/dL (range 503-1992 mg/dL) (normal values 800-1800 mg/dL), that of IgA 57 mg/dL (range 26-200 mg/dL) (normal values 70-400 mg/dL) and that of IgM 52 mg/dL (range 19-108 mg/dL) (normal values 50-250 mg/dL). At baseline, all patients had undetectable CMV-DNA and negative CMV antigenemia but were positive by serology. The planned prophylaxis was oral acyclovir 800 mg tid and trimethoprim-sulfamethoxazole 960 mg bid for two consecutive days a week, to be used from the start of alemtuzumab to two months after the end of treatment. During follow-up we assessed peripheral blood cell counts, liver and kidney function before each administration of alemtuzumab.

CMV reactivation surveillance

Antigenemia and CMV DNA were studied weekly until two months after alemtuzumab treatment had been discontinued. CMV infection was monitored by the CMV pp65 antigenemia assay (Light Diagnostics CMV pp65 Antigenemia IFA KIT, Chemicon International Inc., Temecula, USA), according to the manufacturer's instructions. Positive results consisted of one or more CMV antigen-positive cells per set of triplicate wells (expressed as number of positive cells per 2×10^5 leukocytes). Samples were referred as *not evaluable* when there were insufficient leukocytes to perform the test. The detection of CMV DNA in whole blood was performed by amplification of two different genome regions, the *MIE* gene and the *gpB* gene, as confirmation of positivity.[14,15] Consecutive positive results were evaluated quantitatively using a real-time automatic PCR performed by Q-CMV AmplyMIX *MIEA* (Amplimedical-BIOLINE, Turin, Italy) according to the manufacturer's instructions. Serial dilutions of CMV standard (Q-CMV AmpliSTANDARD, Amplimedical-BIOLINE), within the range of 10 to 10^6 copies/reactions, were amplified in duplicate to generate a standard curve for CMV quantification in clinical samples. The threshold value was estimated at 200 copies/mL of whole blood.

Criteria for defining CMV reactivation and disease

According to Leruez-Ville, CMV reactivation is defined as the presence of at least one pp65 antigen-positive cell per 100,000 leukocytes; however, in this study, the number of detectable CMV DNA copies, measured by real-time PCR, were used to make decisions about preemptive therapy.[16] CMV disease was diagnosed from the association of clinical symptoms with virologic confirmation of CMV infection of an organ (e.g. from bronchoalveolar lavage fluid, cerebrospinal fluid, or biopsy of the stomach, duodenum, brain, liver, heart or kidney).

Results

During alemtuzumab therapy, the PB lymphocyte count rapidly decreased reaching values below 500/μL at a median of 3 weeks (range 1-7 weeks) and remaining so for 4 weeks (range 1-9) after the discontinuation

Table 1. Patients' characteristics.

Age/Sex	*Prior chemotherapy*	*Disease status*	*Lymphocyte count (per μL)*	*Week of CMV Ag⁺*	*Week of PCR⁺ (quantitative level)**	*Lymphocytes at CMV reactivation (per μL)*
51/m	CHOP, CTX, APBSCT	B/II	36000	6th week Ag n.e.	6th week PCR + ($7,5\times10^2$)	120
78/m	CHL, COP, Fludarabine	C/IV	67280	8th week Ag n.e.	8th week PCR + (2×10^2)	180
61/f	Fludarabine, Rituximab	B/II	56830	6th week Ag n.e.	6th week PCR + (2×10^2)	140
56/m	CHL+PDN, Fludarabine, Splenectomy	C/IV	55110	4th week Ag+ (2 cells)	4th week PCR + ($2,3\times10^3$)	180
56/m	CHL, Fludarabine, CHOP	B/II	50490	7th week Ag + (2cells)	7th week PCR +	700 ($1,2\times10^3$)
50/f	Fludarabine, CTX	B/II	32390	5th week Ag + (3 cells)	5th week PCR + ($4,3\times10^4$)	90
70/m	CHL+PDN, CHOP, Fludarabine, Rituximab, RT	C/IV	148640	5th week Ag + (6 cells)	5th week PCR + ($3,2\times10^4$)	140
74/f	CHOP, CHL	C/IV	58470	7th week Ag + (5 cells)	7th week PCR + (n.d.)	40
72/f	CHL, CTX	A/Progressive	39570	No	No	n.a.
57/m	Fludarabine, CTX, APBSCT, Rituximab, CHOP	C/IV	1070	No	No	n.a.
62/m	CHL, Fludarabine	B/II	16400	No	No	n.a.
68/m	CHOP, COP	C/IV	10860	No	No	n.a.

*n.e.: not evaluable; n.a.: not applicable; n.d.: not done; *viral copies/mL.*

of the treatment. Except for mild, common, infusion-related side effects due to cytokine release (fever, chills, rigor, rash, nausea and emesis, fatigue, headache, myalgia, diarrhea) observed during the first week, the patients tolerated alemtuzumab treatment well. Two patients developed severe neutropenia (PMN< 500 μL) during the alemtuzumab treatment and required granulocyte-colony stimulating factor. No patients developed bacterial or fungal diseases. During the treatment eight patients (66.6%) showed CMV reactivation: 1 patient was positive for antigenemia (CMV DNA not done), 4 patients were positive for CMV DNA and antigenemia, and 3 patients were positive only for CMV DNA, antigenemia being undetectable because of the low white blood cell count. No patients showed clinical evidence of CMV disease: in particular, we did not observe fever, pneumonia or increased concentrations of liver enzymes. CMV reactivation appeared after a median of 6 weeks (range: 4-8 weeks) of treatment when the median PB lymphocyte count was 140/μL (range 40-180/μL). The alemtuzumab and acyclovir prophylaxis were discontinued and the patients were treated immediately with oral ganciclovir 1000 mg tid. During treatment with oral ganciclovir, CMV antigenemia and CMV DNA were studied weekly until the indicator tests became negative. After a median of 14 days (range 9-19 days) of antiviral therapy all patients had achieved negative CMV PCR assays and undetectable antigenemia; oral ganciclovir was, therefore, discontinued and the acyclovir prophylaxis and alemtuzumab treatment were resumed. No significant myelotoxicity was observed during the treatment with oral ganciclovir: the median absolute neutrophil count was 1860 mm^3 (range 1020-2890) and 1790 mm^3 (range 900-2250) before and at the end of oral ganciclovir treatment, respectively. In the first two months after alemtuzumab discontinuation no patient showed further CMV reactivation in the weekly tests of CMV antigenemia and CMV DNA. After a median follow-up of 8 months (range 2-14 months) since the end of alemtuzumab therapy, we

have not observed any cases of CMV reactivation, assessed monthly on the basis of CMV antigenemia and PCR analysis of CMV DNA, or CMV disease.

Discussion

CMV reactivaction, studied with periodic analyses of antigenemia and PCR, is frequent in previously treated CLL patients on alemtuzumab therapy although only sporadic cases of CMV disease have been reported. The introduction of both prophylaxis and pre-emptive therapy, guided by close monitoring of CMV reactivation, are probably responsible for the low incidence of CMV disease with alemtuzumab. Pre-emptive therapy with intravenous ganciclovir or foscarnet, based on early detection of CMV reactivation, has become the most commonly used strategy for preventing CMV disease after allogeneic stem cell transplantation. This model has rapidly been extended to patients on alemtuzumab therapy but with some modifications tailored to the different clinical setting. Oral ganciclovir was the drug of choice because of its ease of administration and mild side effects, although its bioavailability is poor.[9,10] Moreover, Razonable *et al.* showed the efficacy of oral ganciclovir as pre-emptive therapy of CMV in liver transplant recipients.[13] All patients tolerated the orally administered ganciclovir well, without nausea, vomiting or gastrointestinal pain during its use. Oral administration of a drug is usually associated with lower overall costs, greater safety and convenience than is intravenous administration, and this may improve patients' adherence to treatment and quality of life. In this study we found a 66.6% rate of CMV reactivation during alemtuzumab therapy and then prospectively evaluated the efficacy of oral ganciclovir from the day of CMV reactivation, as detected by PCR and/or antigenemia studies, until the disappearance of CMV. Oral ganciclovir was chosen on the basis of its ready availability; furthermore, although valganciclovir has been recently reported to be safe and efficacious when given as a single daily dose, its role in patients with CMV reactivation after alemtuzumab therapy has not yet been addressed.[17] There is evidence in literature of a correlation between CMV antigenemia and the results of real time PCR assays for CMV DNA; Leruez-Ville' recently found that the results obtained with the two techniques were significantly correlated in 558 blood samples.[16] Usually a low level of antigenemia was observed and in 3 patients antigenemia was not evaluable. CMV PCR had, as expected, a higher sensitivity; furthermore, when quantitative CMV PCR was used, a high number of copies was detected in most of the patients, suggesting that this method could be preferred to the classical CMV antigenemia test, at least in these patients. (Table 1). In our experience (*data not shown*) and in accordance with published data, the CMV DNA levels in samples with positive antigenemia (from 1 to 10 cells) were within the range of 200 to 130,000 copies/mL.[16,18] A low level of CMV DNA associated with negative antigenemia or a *not evaluable* test because of insufficient leukocytes may be related to the high sensitivity of the molecular assay. On the other hand, these values may be interpreted as an early marker of CMV reactivation and may be a predictor of subsequent CMV disease. In fact, several studies showed that real-time PCR in blood allowed CMV replication to be detected earlier than CMV antigenemia.[16,18-20] Whether patients showing only CMV antigenemia and/or PCR positivity require specific antiviral therapy still remains questionable considering the very low incidence of CMV symptoms or CMV disease. In a recent study of patients affected by lymphoid malignancies who were receiving a brief course of alemtuzumab plus rituximab (maximum 4 weeks of treatment), antiviral therapy was used only if positive CMV antigenemia was concomitant with symptoms although a precise definition is lacking. In that series 27% of patients developed CMV antigenemia and among them 54% became symptomatic, but 69% of patients still required antiviral treatment.[21]

We successfully used oral ganciclovir in all patients with CMV reactivation. The response was prompt and there was no progression to CMV disease, no relevant clinical toxicity and unnecessary hospitalization was, therefore, avoided. Oral ganciclovir may be used as pre-emptive therapy in all patients who develop CMV reactivation (measured by antigenemia and/or PCR assay) during alemtuzumab treatment in order to avoid progression to CMV disease. However, given the lack of CMV disease and the lack of CMV reactivation once negative assays have been achieved, we then suggest that the ganciclovir therapy is stopped and alemtuzumab therapy recommenced.

LL and PP conceived and design the study; PCh and MT collected data; SS wrote the manuscript; PCa and GF were responsible for microbiological tests, AC was responsible for pre-emptive therapy, GL critically revised the manuscript. The authors reported no potential conflicts of interest.

This work was supported in part by the Associazione Italiana per la Ricerca contro il Cancro (AIRC) Milan, Italy.

Manuscript received July 22, 2004. Accepted August 7, 2004.

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