after a loading dose was sufficient for maintenance of normal hematologic parameters and cobalamin levels during a follow-up period of 12 months. Our study challenges the conclusion that biweekly 1 mg vitamin B_{12} treatment is sufficient and questions hematologic parameters and plasma cobalamins as measures of inadequate vitamin B_{12} status.

Elevated plasma concentrations of MMA and tHcy can be used as biochemical markers to aid in the diagnosis of vitamin B_{12} deficiency and to monitor the response to cobalamin supplementation. In our study, more than 75% of the patients with congenital vitamin B_{12} deficiency treated biweekly with 1 mg oral vitamin B_{12} for more than five years had normal levels of MMA and tHcy, but only 33% had a normal holo-TC. This observation agrees with the view that holo-TC is an early marker of a suboptimal vitamin B12 homeostasis.[12]

In conclusion, biweekly treatment with 1 mg vitamin B_{12} seems to normalize indices of vitamin B_{12} status in some, but not all of the patients with congenital vitamin B_{12} deficiency. We, therefore, believe that a more frequent intake of oral vitamin B_{12} is required, but we question whether a daily dose of 1-2 mg vitamin B_{12} is needed.

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Key words: oral vitamin B12 treatment, holo-TC, cobalamin, inherited vitamin B12 deficiency

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Monitoring for cytomegalovirus and Epstein-Barr virus infection in chronic lymphocytic leukemia patients receiving i.v. fludarabine-cyclophosphamide combination and alemtuzumab as consolidation therapy

The combination of fludarabine and cyclophosphamide (FC) has become the standard of care in chronic lymphocytic leukemia (CLL) patients. Due to the well-recognized F-related immunosuppression,[1] a higher risk of opportunistic infections could be expected by adding another immunosuppressive agent. Randomized trials[2-4] do not report a significantly higher rate of infections in patients receiving FC combination versus F alone as first-line therapy, although they do not focus on reactivation of latent viral infections. However, the combination of FC and dexamethasone has been associated with Epstein-Barr virus (EBV) infection in heavily pre-treated patients with lymphoproliferative diseases[5] and with EBV-related histological transformation of CLL.[6] In addition, sporadic cases of cytomegalovirus (CMV) infection on F-based therapy have been reported.[7] The susceptibility to opportunistic infections related to defective cell-mediated immunity is even higher with alemtuzumab (AL),[8] with particular concern for CMV infection. We report results from our surveillance program for CMV and EBV infections in patients treated with FC and AL as consolidation of response.

Sixty-seven CLL patients ≤ 65 years old (median age 54 yrs) received FC combination for progressive disease defined according to NCIWG criteria (as first-line therapy in 53 and second-line in 14). All patients had signed an informed consent before inclusion in treatment and monitoring programs. Median time to treatment was 36 months (range: 1-79 mos) for first-line patients, while median interval from previous therapy to FC administration was 15 months (range 5-32 mos). FC consisted of F

25 mg/m^2/d i.v. and C 250 mg/m^2/d i.v. for three days every four weeks for 4-6 courses. Corticosteroids were not routinely administered. All patients received anti-*Pneumocyctis Carinii* (PC) prophylaxis and oral acyclovir or valacyclovir.

According to institutional treatment protocol, 17 responding patients (CR and PR according to NCIWG) with residual disease at 4-color flow cytometry underwent AL consolidation 3-4 months after completion of FC, at the dose of 30-60mg/wk for 3-10 weeks (median: 6 wks). AL was administered i.v. in 5 patients and s.c. in 12. The median cumulative dose of AL was 360 mg (range: 90-540). In these patients anti-infectious prophylaxis was maintained.

CMV infection was monitored by measuring pp65 positive cells/2×10^5 peripheral blood leukocytes (PBL) until November 2005, and after then by detection of CMV DNA in whole blood by a quantitative realtime PCR assay.[9] EBV monitoring was performed by measuring EBV DNA copies/10^5 PBMC by an in-house developed realtime PCR. Briefly, a 59 nt fragment of the BamH1 C/W fragment of EBV genome was amplified using the following primers: EBVSMF 5'-AGCGGGTC-TATGGTTGGCT-3' and EBVSMR 5'-GCTTATTC-CTCTTTTCCCCTCTAAA-3' in the presence of a FAM-labeled MGB TaqMan probe (EBVSM 5'-CGCTGCTGC-TATC-3') using a 7300 Real Time PCR System (Applied Biosystem, Foster City, CA, USA) and standard reagents (TaqMan® Unversal Master Mix, Applied Biosystem, Warrington, Cheshire, UK). The thermal profile was as follows: one cycle at 95°C for 10 mins., followed by 40 cycles at 95°C for 15 secs., and 60°C for 60 secs. A plasmid containing the amplified EBV fragment was used as external quantification standard. Patients were monitored every two weeks during FC therapy, monthly for three months after FC completion and weekly during AL consolidation. Absolute CD4 T-cell counts and CD8 T-cell counts were serially measured in peripheral blood by flow cytometry. Before starting FC therapy, CMV and EBV serologies were consistent with prior infection; no patients had detectable viral load for CMV or EBV DNAemia. The median number of FC courses was 4 (range: 2-6). During FC therapy, CMV infection was never detected, while a transient increase in EBV DNAemia was observed in 17 asymptomatic patients (median peak level: 108 copies/2×10^5 PBMC, range: 15-650). Two patients had dermatomal Varicella-Zoster infection and 2 had Herpes Simplex infection. Absolute CD4 T-cell counts significantly decreased from a median baseline value of 490/µL (range: 142-1965) to 167/µL (median post-FC value; range: 71-680) (p<0.05; Wilcoxon matched pairs test). The CD8 T-cell count decreased from a baseline median value of 520/µL to a median value of 399µ/L after FC therapy, although this was not statistically significant. During AL consolidation, CMV was detected in the blood of 9 patients (52%) with antigenemia peak levels ranging from 3 to 70 pp65 positive cells/2×10^5 PBL (3 pts) or DNAemia peak levels ranging from 3500 to 7900 CMV DNA copies/mL whole blood (6 pts). Median time to infection was three weeks from starting AL treatment. Ganciclovir was administered as pre-emptive therapy (as per institutional protocol) in 5 asymtomatic patients and in 4 patients presenting symptoms (fatigue, fever, cough, arthralgia) that could not be unequivocally linked with CMV infection (peak CMV value in these 4 pts: 70 pp65 positive cells/2×10^5 PBL; 3500, 4600, 7900 CMV DNA copies /mL whole blood respectively). Eight patients (47%) showed an increase in EBV DNA load with peak levels ranging from 95 to 6700 copies/10^5 PBMC (median: 624); EBV DNA load of 6700 copies/10^5 PBMC was detected in one patient with documented EBV-related nodal disease progression of CLL. Three patients were simultaneously positive for CMV and EBV DNA. Detection of CMV and EBV DNA in blood was not related to the underlying disease stage or the cumulative dose of AL. All CMV and EBV-positive patients recovered from infectious episodes. From the results of large randomized trials,[3,4,10] FC combination therapy does not appear to be associated with a marked increase in infections. However, in these studies viral screening was not performed, despite recorded episodes of fever of unknown origin for which a viral pathogenesis could not be ruled out. In this respect, our results indicate that reactivation of latent viral infections is uncommon in patients receiving FC combination despite CD4 T-cell depletion. Specific PCR-based assays for CMV and EBV DNAemia should be performed in symptomatic patients in whom other infectious causes are considered unlikely and in heavily pretreated patients, as the occurrence of opportunistic infections is related to disease duration and activity, and extensive prior treatment.[11] CMV infection is a well-documented event in patients receiving AL and guidelines on the management of this complication have been published.[12] We confirm the risk of CMV and EBV infection following AL administration also in the consolidation setting, and point out the need for a surveillance program including sequential determination of EBV DNA load in PBMC as well as CMV load in whole blood.

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