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Original article

Monitoring of Epstein-Barr virus (EBV)/cytomegalovirus (CMV)/varicella-zoster virus (VZV) load in patients receiving tocilizumab for rheumatoid arthritis

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ABSTRACT

Introduction: IL-6 is involved in viral immunosurveillance. We studied the effect of tocilizumab (TCZ) on the evolution in viral load (VL) for the Epstein-Barr virus (EBV), cytomegalovirus (CMV) and varicella-zoster virus (VZV) in patients with rheumatoid arthritis (RA).
Methods: EBV, CMV and VZV loads were prospectively determined in whole blood of 22 RA patients at TCZ initiation and during treatment follow-up. A difference of 0.5 log10 or of threefold copies/mL between two VL was considered significant.
Results: There were 20 (91%) women, (mean age of 57.8 ± 11.2 years, mean disease duration 11.3 ± 9.7 years) with 16 (73%) seropositive and 16 (73%) erosive patients. TCZ was administered alone for 8 patients (36.7%) or in combination with methotrexate for 11 patients (50%). At baseline, the EBV VL was positive in 8 patients with a mean VL value of 1777.2 ± 3518.3 (3.5 ± 0.4 log10) copies/mL. Only one patient had a positive CMV VL with 2337 copies/mL (3.4 log10). The VZV VL was negative in all patients. After 9.2 ± 4.8 months, EBV VL became negative in 6 of 8 patients ($P = 0.01$) and did not significantly vary in the remaining 2 patients. CMV VL became also negative. No VL (EBV, CMV, VZV) became positive. A positive EBV VL did not correlate with disease activity or with inflammatory biomarkers (ESR and CRP).
Conclusion: TCZ does not seem to increase the VL of EBV, CMV or VZV. Studies involving larger patient populations are necessary.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease in which several cytokines are involved. Some of these cytokines are targets for RA therapy, such as tumor necrosis factor (TNF) alpha and interleukin 6 (IL-6) [1]. In the past decade, RA treatment has significantly improved with the introduction of biological disease-modifying antirheumatic drugs (bDMARDs) like tumor necrosis factor inhibitors (TNFi) [2], abatacept (a costimulatory blocker), rituximab (a B cell antibody) [3] and tocilizumab (TCZ) (an IL-6 inhibitor) [4,5]. More recently, tofacitinib (a Janus kinase inhibitor) [6] has been considered in cases of bDMARD failure. TCZ is a monoclonal antibody that competitively inhibits the binding of IL-6 to its receptor. IL-6 is a cytokine that plays a crucial role in the maintenance of immune system function, such as by perpetuating inflammation and stimulating the differentiation of hematopoietic cells, notably into cytotoxic T-lymphocytes. Although the safety of TCZ is reassuring [7], it is plausible that IL-6 receptor blockade through interaction with TCZ could alter immunosurveillance for viruses or cancer.

It is already known that RA patients have an impaired immune response to the Epstein-Barr virus (EBV) [8,9]. EBV can cause infectious mononucleosis and a number of malignancies in immunocompromised individuals. It is uncertain if there is an increased risk of lymphoma in RA patients, regardless of whether these patients are taking bDMARDS [10,11]. Previous studies have presented IL-6 as an autocrine growth factor for EBV-immortalized B cells [12], which increases the risk of lymphoma. In order

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to prevent associated EBV lymphoma, it is interesting to know the EBV infection status of RA patients and monitor their viral load under biological treatment [13]. Previous studies revealed that TNFi do not significantly influence EBV load in RA patients [14,15]. Prospective monitoring of EBV has been studied only in patients with juvenile idiopathic arthritis (JIA) treated with TCZ and MTX [16]. Moreover, severe CMV and VZV infection have been reported in patients with RA treated by TCZ [17,18]. Given the multiple functions of IL-6 in virus immunosurveillance [12], it is of interest to monitor the viral load in RA patients receiving TCZ.

2. Methods

We conducted a prospective monocenter study of 22 patients treated with TCZ (IL-6 inhibitor, Roactemra®) for RA in accordance with the current guidelines [19] in the Rheumatology department of the University hospital of Clermont-Ferrand, France. All patients fulfilled the 2010 ACR/EULAR diagnostic criteria and gave their informed consent. This is an ancillary study of the TEFRA study, which obtained approval for the local ethical committee (CPP SUD EST VI, IRB: 00008526).

In each patient, EBV, CMV and VZV loads were measured at baseline and once during treatment follow-up: for EBV load determination, the EBV R-gene Quantification kit; for VZV load, the HSV1-HSV2-VZV R-gene kit (Biomérieux, France); for CMV load, Abbott Real time CMV kit (Abbott Diagnostics). All these kits quantify EBV, CMV and VZV DNA in whole blood. For each virus, the detection threshold and the quantification threshold were respectively 624 copies/mL and 1000 copies/mL for EBV, 62.4 IU/mL and 780 IU/mL for CMV and 250 copies/mL and 500 copies/mL for VZV. Differences in EBV, CMV and VZV loads between two blood samples of threefold (copies/mL) or more 0.5 log10 were considered significant. The determination of the viral load is used to assess viral reactivation, and the virus replication level. Serological tests were not used because they are not relevant for the monitoring of patients and evaluating the potential impact of immunosuppressive therapy. The second VL measure was the last viral load control for each patients in order to have the longest follow-up.

In each patient, demographic characteristics (age, gender) RA characteristics (disease duration, seropositive status, disease activity, concomitant treatment), but also clinical characteristics such as tender joint count (TJC), swollen joint count (SJC), visual analog scale pain score (VAS, 0–100 mm) and at last laboratory results such as erythrocyte sedimentation rate (ESR, mm after 1 hour) and serum *C*-reactive protein level (CRP, mg/L), anti-cyclic citrullinated peptide antibody (ACPA) and rheumatoid factor (RF), were collected at baseline and during the follow-up visits. This enabled disease activity to be assessed based on the Disease Activity Score-28 joints (DAS-28ESR and DAS-28CRP) at the time of blood sampling.

2.1. Statistical analysis

Statistical analysis was performed using Stata 13 software (Stata Corp LP, College Station, TX, US). The tests were two-sided, with a type I error set at $\alpha = 0.05$. Quantitative data were presented as mean ± standard deviation or median [interquartile range] according to statistical distribution (assumption of normality assessed using the Shapiro–Wilk test) and categorical parameters, such as the number of patients and associated percentages. In a paired context, the usual tests were performed: paired Student's *t*-test or Wilcoxon test for quantitative parameters and McNemar's test for categorical data.

3. Results

3.1. Patient characteristics

Twenty-two patients were analyzed. There were 20 (91%) women with a mean age of 57.8 ± 11.2 years and a mean disease duration of 11.3 ± 9.7 years. Sixteen (73%) of patients were seropositive and 16 (73%) erosive. TCZ was administered alone (36.4%) or in combination with MTX (50%). Of these 22 patients, 14 (63.6%) were taking synthetic DMARDs (MTX alone: $n = 8$, MTX + hydroxychloroquine [HCQ]: $n = 1$, MTX + salazopyrine: $n = 1$, MTX + HCQ + salazopyrine: $n = 1$, HCQ alone: $n = 2$, HCQ + leflunomide: $n = 1$). In patients receiving MTX, the mean dosage was 13.9 ± 4 mg per week. Twelve (54.5%) patients were receiving glucocorticoids, with a mean daily dosage of 6.5 ± 1.5 mg. TCZ was administered every four weeks at a mean dosage of 511.8 ± 125 mg, or 8 ± 0.8 mg/kg.

3.2. Monitoring viral loads

Prior to the administration of TCZ, EBV was detected in eight patients (mean load 1777.2 ± 3518.3 [3.5 ± 0.4 log10] copies/mL). Only one patient had a positive CMV load with 2337 copies/mL (3.4 log10). VZV was not detected in any patient at any time during the observation period. After 9.2 ± 4.8 months of follow-up, EBV load became negative in 6 of 8 patients ($P = 0.01$) and did not significantly vary in the other patients. CMV load became also negative. None of the viral loads (EBV, CMV, VZV) became positive during the follow-up and no lymphoma cases were reported.

The treatments of positive EBV patient were collected on Table 1. Among 6 patients with EBV, VL became negative after TCZ perfusions, 3 (50%) did not take any DMARDS.

3.3. Disease activity parameters

The RA activity as measured by the DAS-28 significantly decreased after six months (DAS-28ESR 2.8 ± 1.1 vs 5.1 ± 0.9 at baseline, DAS-28CRP 3.3 ± 0.9 vs 4.9 ± 0.7 at baseline [$P < 0.001$]). A positive EBV load was not correlated with disease activity (DAS-28ESR, DAS-28CRP) or inflammatory biomarkers (ESR and CRP).

4. Discussion

According to our study, we did not find any significant increase in EBV, CMV or VZV load in patients treated with TCZ for RA. Furthermore, EBV load became negative in six of eight patients and we did not find any cases of viral reactivation during follow-up. CMV load became also negative. To the best of our knowledge, this is the first report monitoring longitudinal EBV, CMV and VZV load in RA patients treated with TCZ. Kawada et al. monitored viral load for EBV and other herpes viruses in four JIA patients treated with MTX and TCZ and found that neither drug influenced EBV or CMV viral load [16]. According to the literature, TCZ was associated with exacerbated latent viral infection in three RA case reports, but the context was generally unusual. Ogawa et al. reported on the case of a woman who developed hemophagocytosis and capillary leak syndrome due to an exacerbation of a chronic active EBV infection after a single TCZ infusion. They suggested a breakdown in EBV immunosurveillance due to IL-6 interference. The patient had a positive viral load before receiving anti-IL-6 agents, but she also had a history of Hodgkin's disease that had previously regressed after MTX withdrawal [20]. Van Duin et al. reported a case of pneumonitis due to CMV reactivation in a 41-year-old RA patient [17]. Kopf et al. have demonstrated through studies with IL-6-deficient mice the role of IL-6 in clearance of viral infections [21]. Varicella-zoster virus (VZV) is maintained after primary infection throughout