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ORIGINAL INVESTIGATION

Increased frequency of JC-polyomavirus detection in rheumatoid arthritis patients treated with multiple biologics

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Abstract Progressive multifocal leukoencephalopathy (PML) represents a rare but potentially fatal reactivation of JC-polyomavirus (JCPyV) recently also reported in patients with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis (RA) treated with rituximab. The aim of the present study was to analyse the pattern of JCPyV infections in patients with RA undergoing treatment with biologic agents. Urine and blood samples were analysed from 80 patients for antibody levels and/or the presence of JCPyV DNA. Genotyping of the control region and VP1 was performed for all JCPyV DNA-positive specimens. Viremia of JCPyV was only temporarily detected in two patients, and these viruses did not carry any mutations associated with the occurrence of PML. JCPyV DNA was prevalent in initial urine samples of 33 % of all patients. RA patients who have consecutively been treated with two or more biologic agents revealed significantly higher prevalence of JCPyV DNA in the urine compared to RA patients treated with their first biologic agent. The presence of JCPyV DNA in the urine closely correlated to JCPyV antibody positivity, and therefore, antibody titres were higher in RA patients who had consecutively received two or more biologic agents over time. Therefore, the overall number of biologic agents had an impact on the pattern of JCPyV detection in this study. Hence, JCPyV antibody screening might be useful as part of the PML risk stratification for RA patients in the future.

Keywords Rheumatoid arthritis · TNF-blocker · Rituximab · JC-polyomavirus

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder, predominantly characterized by synovitis that ultimately leads to structural damage of the joints associated with progressive disability. Through the use of biologic agents like cytokine inhibitors in patients refractory to conventional DMARD therapy and cell-depleting therapies such as rituximab (RTX) in patients who fail TNF inhibitors, remission or at least low disease activity (LDA) has become an achievable goal. However, despite decreasing the inflammatory burden, the use of biologic agents has been associated with an increased rate of serious infections. In particular, patients with latent viral infections are at a higher risk for reactivation as demonstrated recently by an increased incidence of herpes zoster in patients receiving TNF inhibitors [1].

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) and is caused by the lytic replication of JCPyV in oligodendrocytes. PML was most often observed in patients with haematological malignancies and HIV-1 infections. Recently, the use of different therapeutic monoclonal antibodies (natalizumab, efalizumab and RTX) has been associated with an increased risk of PML [2, 3]. In the case of natalizumab, which is used in patients with refractory multiple sclerosis (MS), the risk of PML varies from <0.11/1000 to 7.8/1000 [2] and increases over time. The underlying mechanism of action is thought to involve the inhibition of migration of lymphocytes to the CNS and/or the proliferation of bone marrow cells, known to be a site of JCPyV persistence. Therefore, screening for JCPyV antibodies was implemented in clinical practice to identify subgroups of MS patients with high or low risk of developing PML during natalizumab treatment. In the case of RTX, approved as a second-line biologic treatment in RA patients, the risk of PML is rare (4–5/100,000) but clearly higher than in the general population (0.3/100,000) or in RA patients in general (0.4/100,000) [3–5]. Since the depletion of CD20(+)-B cells by RTX treatment leads to the proliferation of bone marrow cells, JCPyV reactivation in RA patients might also be facilitated by RTX. Until now, patterns of JCPyV infections in RA patients are largely unknown, particularly in patients undergoing treatment with biologic agents.

Methods

Urine ($n = 222$) and blood ($n = 172$) samples unstructuredly taken from routine diagnostics were retrospectively analysed from 80 RA patients treated with biologics. All patients were informed about the risk of developing PML under immune suppressive therapy and gave their consent for this study. In 57 cases, multiple urine samples could be analysed, including baseline samples before the start of RTX treatment in 16 patients. Blood samples were tested for the presence of JCPyV DNA in parallel with urine samples. In addition, levels of serum antibodies were determined, in most patients at a single time point. DNA extraction was performed using the MagnaPUre LC 2.0 (input volume of 500 µl/100 µl elution volume), and JCPyV DNA was determined using assays from TIB MOLBIOL or Astra diagnostics. The antibody detection method was based on GST-capture ELISA in combination with fluorescent bead technology using affinity-purified JCV VP1 bacterially expressed as glutathione S-transferase (GST) fusion protein and affinity-purified directly on glutathione-coated beads as antigen [6]. A Luminex analyser identified the internal colour of the individual beads and quantified bound human Ig [expressed as median fluorescence intensity (MFI) of at least 100 beads per colour per serum] through the reporter fluorescence of bound secondary reagents). JCPyV genotyping was performed by amplifying and sequencing the control region and VP1 using previously published nested PCR protocols [7]. Categorical variables were analysed using the Fisher's exact test.

Results

None of the patients developed neurological symptoms suggestive of PML at any time point. JCPyV DNA was only detected once in the blood of two patients during RTX treatment (6 and 12 months after the last RTX cycle, respectively), whereas all other blood samples ($n = 170$) tested negative. The sequence analysis of these two JCPyV isolates in the control region and VP1 did not reveal any mutations related to the occurrence of PML. Both viruses were archetype-like in the control region and could be assigned to JCPyV genotypes 2a and 4, respectively, according to the VP1 sequences.

The initial detection rate of JCPyV DNA in the urine of patients was 32.5 % (26/80). Neither the age of patients nor the average time since treatment with the first biologic started nor the frequency of methotrexate (MTX) co-administration was different between JCPyV-positive and JCPyV-negative patients. However, the average number of biologics that had consecutively been used for RA treatment was significantly higher in the group of patients tested positive for JCPyV DNA (Table 1). Particularly, the exposure to adalimumab and infliximab was significantly higher in this group of patients. Overall, JCPyV DNA prevalence in the urine was significantly higher in RA patients who had previously been treated with more than one biologic agent over time [1 biologic agent: 6/35 (18.2 %) vs. ≥2 biologic agents: 20/45 (42.6 %), ($p < 0.05$) (Fig. 1)]. In 55 patients, follow-up urine samples collected during ongoing TNF inhibitor treatment [$n = 4$: 347 (±69) days], after start of RTX treatment [$n = 16$: 688 (±294) days and additional RTX-cycles 1.2 (±1.0)] and during ongoing RTX treatment [$n = 35$: 416 (±238) days and additional RTX-cycles: 1.5 (±0.9)] were tested and showed identical results as the initial testing in the majority of patients. Two patients tested negative only once and several times positive ($n = 5$ and $n = 6$, respectively), and for two other patients, an initially positive result was followed by one or two negative results after start of RTX treatment. In one case, two negative results preceded two positive results during ongoing RTX treatment.

In a subset of patients ($n = 69$), JCPyV antibodies in serum could be analysed and correlated with the detection

Table 1 JCPyV DNA in the urine of RA patients

	RA patients tested for JCPyV DNA in urine		
	Negative ($n = 54$)	Positive ($n = 26$)	
Age (mean age, years)	60.7 (±12.7)	58.7 (±9.6)	n.s.
Female/male	44/10	19/7	
Coadministration of MTX	$n = 27$	$n = 8$	n.s.
Biologic agents consecutively used for RA treatment			
Etanercept	$n = 24$	$n = 13$	n.s.
Infliximab	$n = 10$	$n = 12$	$p < 0.05$
Adalimumab	$n = 15$	$n = 14$	$p < 0.05$
Golimumab	$n = 0$	$n = 1$	n.s.
Certolizumab	$n = 5$	$n = 2$	n.s.
Anakinra	$n = 4$	$n = 5$	n.s.
Tocilizumab	$n = 6$	$n = 1$	n.s.
Abatacept	$n = 1$	$n = 0$	n.s.
Rituximab	$n = 30$	$n = 17$	n.s.
Mean number of biologic agents consecutively used for RA treatment	1.8 (±1.0)	2.5 (±1.2)	$p < 0.05$
Mean time after first biologic agent was started (days)	1559 (±1343)	2038 (±992)	n.s.

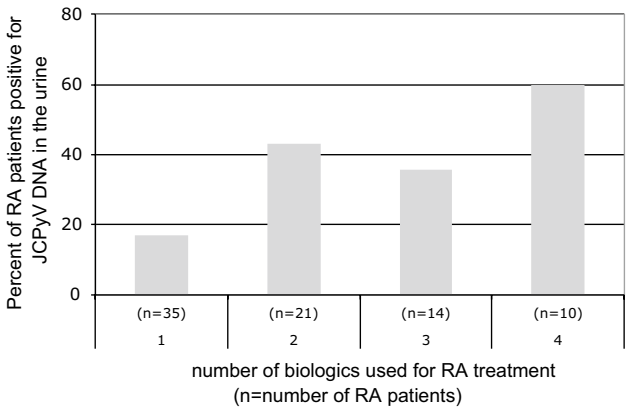

Fig. 1 JCPyV DNA in the urine of (RA) patients and the number of biologic agents consecutively used for their treatment (including rituximab)

of JCPyV DNA in the urine. JCPyV antibody titres were significantly higher in patients who tested positive at least once for JCPyV DNA in the urine [JCPyV DNA positive: 4871 (±2741) MFI vs. JCPyV DNA negative: 1461 (±1802) MFI, $p < 0.0001$]. Accordingly, patients who had been treated with more than one biologic agent over time had higher titres of JCPyV antibodies. However, four patients were found to be seronegative for JCPyV antibodies (<400MFI) despite being DNA positive in the urine. Another four patients had high levels of antibodies (>4500MFI) but were consistently negative in the urine (Table 2).

Table 2 Correlation between JCPyV antibodies in serum and the detection of JCPyV DNA in the urine

JCPyV antibodies titres (MFI)	Detection of JCPyV DNA in the urine	
	Negative ($n = 45$)	Positive ($n = 24$)
Mean (MFI)	1460 (±1802)	4871 (±2741)
MFI < 400 (negative)	16 (35.5 %)	4 (16.7 %)
MFI > 4500 (highly positive)	4 (8.8 %)	16 (66.6 %)

Discussion

Neither RTX treatment nor treatment with other biologic agents significantly increased the frequency of JCPyV viremia in this cohort of RA patients. A comparable study analysing JCPyV viremia in MS patients treated with natalizumab yielded similar results which appear to reflect the transient nature of JCPyV viremia [8]. Even though it may be speculated that JCPyV reactivation generates mutated JCPyV variants that reach the CNS through the blood, JCPyV DNA was not consistently detected in the blood of patients developing PML [9]. Interestingly, in our study, none of the two viruses detected in the blood of RA patients carried a mutation previously associated with PML [10, 11], and they could be assigned to different JCPyV genotypes. In summary, even though viremia of pathogenic JCPyV appears to be essential for the development of PML, its transient nature precludes its usefulness in screening or diagnostic algorithms.

The overall JCPyV DNA detection in the urine could be correlated with the number of biologics consecutively used for RA treatment in this study. As a limitation, the small number of patients precludes any specific assumption of the influence of single biologics, especially, since the correlation between JCPyV DNA and specific biologics might be confounded by the subsequent availability of biologics over time or by co-administrated drugs or by group-specific differences between patients treated with one or more biologic agents over time. The frequency of JCPyV DNA detection in patients receiving their first biologic treatment regime was lower than in healthy individuals from Japan indicating probably also regional differences [12]. Concordantly, Egli et al. [7] reported a point prevalence of 19 % for the detection of JCPyV DNA in the urine of healthy Swiss blood donors with increasing frequencies in male donors and the older age groups. The effect of immunosuppression on the prevalence of JCPyV DNA is controversially discussed [13]. Whereas HIV-1-infected patients did not show increased frequencies of JCPyV DNA in the urine [14, 15], different results had been published from patients with autoimmune diseases. The positive correlation between the numbers of biologic agents consecutively used for RA treatment and the JCPyV DNA detection in the urine is consistent with a previous study reporting an increased likelihood of JCPyV DNA prevalence in the urine of patients with autoimmune diseases receiving high-dose corticosteroid and cytotoxic agents [16]. Similar, young patients with Crohn's disease ($n = 18$) were also reported to be positive for JCPyV DNA in the urine in 33 %, which temporarily increased to 55 % during infliximab treatment [17]. In addition, it is important to note that previous immunosuppressive treatments also increase the risk of JCPyV associated PML in MS patients starting treatment with natalizumab [2]. Moreover, most recently, a national population-based study in Sweden found that patients with RA themselves might have an increased risk of PML compared to the general population [18], as it was also shown for patients with systemic lupus erythematosus (SLE) [4]. Therefore, patterns of JCPyV infection may be related to treatment regimens as well as to the underlying disease process.

The prevalence of JCPyV antibodies found in this study (75 %) was in line with previous studies reporting seroprevalences from 63 to 82 % in patients older than 50 years [6, 7, 19]. In average, the antibody titres were higher in patients with detectable JCPyV DNA in the urine than in patients tested negative for JCPyV DNA, even though MFI titres cannot be considered per se as a quantitative result. A correlation between JCPyV antibody reactivities and the detection of JCPyV DNA in the urine was also noticed in MS patients. [20]. But, not all patients with detectable JCPyV DNA in the urine displayed JCPyV antibodies, while some patients had high titres of antibodies without any JCPyV DNA detection in the urine. These discordant results might be related to JCPyV persistence in different compartments (kidney/bone marrow), a false-negative result of the antibody assay or a false-negative result of the PCR. In this study, two urine samples of two patients were tested negative although other samples from the same patient were tested previously and thereafter always positive (5 and 6 positive samples, respectively). This might be explained by the presence of PCR inhibitors, which inhibit the amplification of targeted nucleic acids and can especially be found in the urine or stool of patients [21]. For MS patients treated with natalizumab, only the presence of JCPyV antibodies using a specifically validated assay could be correlated with a risk of developing PML during treatment with natalizumab [2, 9]. A direct comparison [22] of an ELISA adapted version of the assay used in this study showed a good correlation with the two-step-enzyme-linked-immunosorbent assay [23] used for PML risk stratification in MS patients and was even proposed to be included in the case definition of natalizumab-associated PML for detecting the cerebrospinal fluid JCPyV antibody index [24]. However, there is at the moment no reference material available to validate different assays applying different strategies for detecting JCPyV antibodies, by this all so far published results might have been confounded by the choice of assay [13, 25]. Interestingly, also in the case of Merkel cell polyomavirus (MCPyV), a positive correlation between virion-specific antibody titres and viral loads at all tested anatomical sites has been reported [26].

In summary, patterns of JCPyV infections seemed to be cumulatively influenced by biologics used for treatment of RA patients. It remains unclear whether this results indeed from the sequential use of various immunosuppressive agents including biologics with a different mode of action or whether this represents a more treatment refractory patient population. Due to the limited statistical power of this study with a small number of patients and with various treatment regimens, no specific effect can be assigned to specific biologics. The increasing number of patients treated with multiple biologic agents over time and a potentially cumulative effect of previous immunosuppressive treatment regimens on patterns of JCPyV infections warrant and need further validation. The proposed prophylactic measurements of JCPyV DNA or the detection of JCPyV antibodies for PML risk assessment in RA patients have considerable limitations, namely low positive or negative predictive values or lacking standardization. At least in the case of natalizumab, the detection of JCPyV antibodies is routinely used for risk assessment in MS patients; however, additional studies are needed to define specific criteria for risk assessment of PML in patients with rheumatoid diseases. It might additionally be that the detection

of cellular immunity could be helpful in the future for PML risk assessment, since CD8+ *T* cells were discussed to be protective against PML [27], and recently, the importance of CD4+ cells for polyomavirus-specific *T* cell immunity was highlighted [28, 29]. It has also to be considered in future that biologics (e.g. rituximab) might directly influence JC antibody titres, even though consecutively testing of sera from the same patient obtained at different time points yielded similar results in all patients (data not shown). Overall, further studies are needed to define the diagnostic workup (including the standardization of assays and definition of cut-offs) for the PML risk assessment of patients with rheumatoid diseases. The rare risk of developing PML in patients with rheumatic diseases and a great variety of treatment regimens using biologic and/or immunosuppressive agents in these patients underscore the need of developing specific criteria of probably multiple factors for risk assessment in patients with rheumatic diseases.

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Conflict of interest None.

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