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JCPyV microRNA in plasma inversely correlates with JCPyV seropositivity among long-term natalizumab-treated relapsing-remitting multiple sclerosis patients

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Abstract Sensitive biomarkers are needed to better manage multiple sclerosis (MS) patients for natalizumab (NTZ)-associated risk of progressive multifocal leukoencephalopathy (PML). A currently used risk stratification algorithm, mainly based on JC polyomavirus (JCPyV) serology, has not led to a reduction of PML incidence. Therefore, this study was designed to evaluate the presence and prevalence of JCPyV miRNAs in plasma of NTZ-treated MS patients, and to explore their biomarker potential for NTZ-associated PML risk assessment. Altogether, 102 plasma samples from 49 NTZ-treated and 28 interferon-beta (IFN-β)-treated relapsing-remitting MS patients, and 25 healthy controls (HCs) were analyzed for jcv-miR-J1-5p (5p miRNA) and jcv-miR-J1-3p (3p miRNA) expression. The overall detection rate of 5p miRNA was 84% (41/49) among NTZ-treated patients, 75% (21/28) among IFN-β-treated patients, and 92% (23/25) in HCs. Relative 5p miRNA expression levels were lower in NTZ-treated patients as compared to patients treated with IFN-β ($p = 0.027$) but not to HCs. Moreover, 5p miRNA expression inversely correlated with anti-JCPyV antibody index among JCPyV seropositive long-term NTZ-treated

patients ($r = -0.756$; $p = 0.002$). The overall detection rate of 3p miRNA was low. Our results suggest that JCPyV miRNA in plasma may be linked to the reactivation of persistent JCPyV, to enhanced virus replication, and eventually to the risk of developing PML among NTZ-treated MS patients. However, further study is warranted in a larger data set including samples from PML patients to confirm the clinical relevance of JCPyV miRNA as a sign of/in viral reactivation, and to identify its potential to predict developing PML risk.

Keywords Anti-JCPyV antibody index · Biomarker · IFN-β · JCPyV miRNA · Multiple sclerosis · Natalizumab · PML

Introduction

Natalizumab (NTZ, Tysabri®) is one of the most effective therapies for active relapsing-remitting multiple sclerosis (RRMS) (Polman et al. 2006). Regardless of its efficacy, long-term use (mostly more than 18 months) is associated with increased risk of progressive multifocal leukoencephalopathy (PML), caused by reactivation of JC polyomavirus (JCPyV) (Clifford et al. 2010). Primary JCPyV infection is encountered in childhood in 60–80% of the human population, and asymptomatic lifelong persistent infection is established (Ferenczy et al. 2012). PML is a rare consequence of reactivation of latent virus, followed by lytic infection of oligodendrocytes and astrocytes under immunosuppressive conditions (Khalili et al. 2007). Although the exact mechanisms of JCPyV reactivation leading to PML lack experimental evidence, two main conceptions prevail (Wollebo et al. 2015). Rearrangements may emerge in the genome of persistent archetype virus either in B cells where immunoglobulin gene rearrangement machinery can be exploited, or by homologous recombination events taking place during virus replication. According to the second conception, the proportions of existing

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viral quasispecies in the JCPyV population of the individual may change as a response to alterations in immunological and other circumstances. In order to cause PML, the virus has to enter the brain and replicate under immunosuppressive conditions (Wollebo et al. 2015). Currently, the risk of PML among NTZ-treated MS patients is stratified using three parameters: anti-JCPyV-antibody (Ab) status, prior use of immunosuppressants, and duration of NTZ treatment (Bloomgren et al. 2012).

Presently, the measurement of serum or plasma anti-JCPyV Ab is widely used to establish past exposure to JCPyV infection, and Ab level expressed as JCPyV Ab index helps to stratify patients at PML risk (Plavina et al. 2014). The rationale behind JCPyV Ab determination is to identify individuals who have Ab as a sign of past exposure and who thus have persistent JCPyV in their organism, whereas JCPyV Ab-negative individuals are thought not to have encountered the virus. However, there has been no reduction in the NTZ-associated PML incidence concomitant with the utilization of JCPyV serology (Cutter and Stuve 2014), and thus it seems insufficient to evaluate complete risk. Moreover, as 60–70% of the MS patients are JCPyV Ab positive, further tools are needed to narrow down high PML risk among patients receiving NTZ (Kolasa et al. 2016; Olsson et al. 2013). Previously evaluated tools based on leukocyte cell membrane markers such as CD11a, CD49d, and CD62L (Basnyat et al. 2015; Jilek et al. 2013; Schwab et al. 2013), and quantification of JCPyV load in the blood or urine still lack applicability in a clinical setting (Chen et al. 2009; Jilek et al. 2010; Rudick et al. 2010). Specifically, quantification of JCPyV DNA from urine and plasma is not sufficiently sensitive to rule out PML risk (Rudick et al. 2010).

JCPyV belongs to human polyomaviruses, which are nonenveloped, double-stranded DNA viruses with genomes of approximately 5130 nucleotides. JCPyV encodes a pre-microRNA which is processed into two mature microRNAs (miRNAs), jcv-miR-J1-5p (5p miRNA) and jcv-miR-J1-3p (3p miRNA), that appear late in infection to autoregulate early gene expression (Seo et al. 2008). jcv-miR-J1-5p is unique for JCPyV, but jcv-miR-J1-3p shares identical sequence with BK polyomavirus (BKPyV)-encoded bkv-miR-B1-3p, and thus its origin cannot be differentiated (Seo et al. 2008). Polyomavirus encoded miRNAs are known to play a key role in controlling viral replication through downregulation of large T (tumor) antigen (LTAg) expression (Seo et al. 2008). They also modify host immune responses by controlling killer receptor NKG2D-mediated killing of virus-infected cells by natural killer (NK) cells through downregulation of the stress-induced ligand ULBP3 (Bauman et al. 2011).

The biomarker potential of circulating human miRNAs in blood has been studied for NTZ-associated PML (Munoz-Culla et al. 2014), but studies on JCPyV-specific miRNAs for their potential as biomarkers of PML risk in NTZ-treated patients are still lacking. Recent studies signify the potential of jcv-miR-J1-5p as a biomarker of past JCPyV infection as it can be frequently detected in plasma, urine, and CSF of both JCPyV seropositive and seronegative healthy individuals and immunosuppressed patients (Lagatie et al. 2014; Pietila et al. 2015). Therefore, jcv-miR-J1-5p could serve as a more sensitive biomarker of JCPyV infection than serology. JCPyV miRNA has been recently proposed as a potential biomarker for JCPyV infection in the gastrointestinal tract, and expression of these microRNAs has been further shown in brain tissues from PML patients (Link et al. 2014; Seo et al. 2008). In the present study, we characterized JCPyV miRNA expression in the plasma of NTZ-treated RRMS patients as well as in patients treated with interferon-beta (IFN-β), and in healthy controls (HCs) to evaluate the utility of these miRNAs in PML risk assessment.

Patients and methods

Patients

This cross-sectional study included a total of 102 subjects of whom 49 RRMS patients had been treated with NTZ, 28 patients with IFN-β (21 patients with IFN-β -1a sc 22 μg and 7 patients with IFN-β -1a sc 44 μg), and 25 subjects were HCs. MS patients were enrolled consecutively from four Finnish MS outpatient departments. Approximately 75% (58/77) of our MS patients were female, representative of the global gender distribution among MS patients (Kira 2014). The HCs were selected to have similar distribution. The clinical characteristics of the patients are shown in Table 1. All patients underwent clinical and neurological examinations before blood sampling. The diagnosis of MS was based on the revised McDonald Criteria (Polman et al. 2005), and the diagnosis was definite. Neurological disability was evaluated by the expanded disability status scale (EDSS) score (Kurtzke 1983). No cases of PML developed among the patients enrolled in this study. The study was approved by the Ethics Committee of Tampere University Hospital, and all subjects gave informed consent.

Determination of the anti-JCPyV antibody index

A confirmatory second generation ELISA (STRATIFY JCPyV™ DxSelect) was used to test sera of the MS patients for anti-JCPyV Ab at Unilabs, Copenhagen, Denmark. A screen index value of less than 0.2 was considered anti-JCPyV Ab negative, and of greater than 0.4 as anti-JCPyV Ab positive. The samples with a screen index between 0.2 and 0.4 were evaluated with a supplementary confirmatory inhibition test, and samples showing greater than 45%

Table 1 Clinical characteristics of MS patients and healthy controls

Characteristics	NTZ-treated $n = 49$	IFN-β-treated $n = 28$	HCs $n = 25$	p value
Sex (F/M)[a]	38/11	20/8	18/7	
Age (years)[b]	38.3 ± 7.6 (23–52)	35.6 ± 10 (20–53)	33.3 ± 11.3 (22–60)	NS
Disease duration (years)[b]	9.2 ± 5.3 (1.8–22.4)	4.3 ± 5.1 (0.2–18.1)	–	<0.001
EDSS[b]	2.6 ± 1.8 (0–6.5)	1.3 ± 1.3 (0–5.5)	–	0.001
Number of relapses[b,c]	2.0 ± 1.0 (0–4)	–	–	–
Duration of treatment (years)[b]	2.8 ± 1.4 (0.4–5.8)	2.5 ± 3.0 (0.1–13.1)	–	NS
Anti-JCPyV Ab seropositivity[a]	24 (49%)	12 (43%)	–	NS
Anti-JCPyV Ab index[d,e]	1.0 (0.3–3.1)	1.9 (0.7–2.9)	–	NS

IFN-β interferon-β, *HC* healthy controls, *EDSS* Expanded Disability Status Scale, *JCPyV* JC polyomavirus, *NS* not significant, − not applicable

[a] Number of patients

[b] Mean ± SD (range)

[c] Two years before starting natalizumab

[d] Median (range)

[e] Anti-JCPyV antibody index of seropositive patients

inhibition in blocking with specific antigen were classified as anti-JCPyV Ab positive (Lee et al. 2013). The anti-JCPyV-Ab index was only determined for the MS patients, not for HCs.

RNA extractions

Total RNA was extracted from 620 μl of plasma using the mirVana™ PARIS™ RNA and native Protein Purification Kit (Ambion, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. Total RNA was eluted into 95 μl of elution solution and spiked with 5 μl of cel-39-3p miRNA (5 fmol/μl, Integrated DNA Technologies, Coralville, IA, USA). This synthetic *Caenorhabditis elegans* miRNA was used to control the success of reverse transcription and miRNA amplification, as well as for result normalization (see below). All RNA preparations were stored at −70 °C until analyzed.

TaqMan miRNA assays

For reverse transcription (RT) and miRNA detection, TaqMan miRNA assays (Thermo Fisher Scientific) were used. The specific targets were jcv-miR-J1-5p, bkv-miR-B1-3p/jcv-miR-J1-3p (identical sequences), and cel-miR-39-3p. Each 15 μl RT reaction mixture contained 1× RT buffer, 0.25 mM of each dNTP, 1× RT primer, 3.33 U/μl MultiScribe RT enzyme, 0.25 U/μl RNase inhibitor, and 10 ng of total RNA. RT reactions were incubated 30 min at 16 °C, 30 min at 42 °C, and 5 min at 85 °C. If real-time PCR was performed directly after RT, the tubes were cooled to 4 °C, but for longer storage, the reactions were placed in −20 °C as instructed by the manufacturer.

For PCR amplification, the Applied Biosystems® 7500 Real-Time PCR System (Thermo Fisher Scientific) was used. Each 10 μl RT reaction contained 1.3 μl of diluted (1:2) RT reaction, 1× TaqMan® assay mixture, and 1× TaqMan® Universal Master Mix II, no UNG (Thermo Fisher Scientific). All miRNA assays were performed in three replicate reactions in the following conditions: enzyme activation in 95 °C for 10 min, after which 40 cycles of 15 s denaturation in 95 °C and 1 min annealing and extension in 60 °C was performed. In each 96-microwell plate, three replicate no template controls (NTC) were run for each master mix. The functionality of the miRNA assays was confirmed using synthetic oligonucleotides (Integrated DNA Technologies) representing the target sequence of each specific miRNA assay as templates.

Baseline and threshold values were set manually for each PCR run according to instrument instructions. The same baseline values were applied in all runs, and threshold was set to the exponential phase of the amplification curves. The sample was interpreted as positive if a Ct cycle was obtained for two or three of the replicates, and then a mean Ct was calculated (Supplementary Table 1). Mean Ct was calculated independent of the amplitude of variation between replicates. If only one of the replicates gave a signal, the sample was not interpreted as true positive. Even though there is no consensus available for normalization of polyomavirus miRNA expression levels in plasma, we used Ct values of spiked synthetic *Caenorhabditis elegans* (cel-miR-39-3p) miRNA to normalize the JCPyV miRNA expression in each patient and HC (ΔCt). We calculated the relative miRNA expression of sick versus healthy individuals by using standard delta delta Ct ($2^{-\Delta\Delta Ct}$) method (Auvinen 2017). cel-miR-39-3p does not share homology with any known human miRNAs and it is widely used as spike-in control (Kroh et al. 2010).

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in the detection rate of JCPyV miRNA between different groups were assessed using Fisher's exact test. A non-parametric, two-tailed Mann–Whitney U test was used to compare the differences between the clinical parameters and JCPyV miRNA expression levels in the different patient groups. Spearman's correlation coefficient was used to explore the association between the relative miRNA expression and clinical parameters. A p value less than 0.05 was considered statistically significant.

Results

Clinical data

NTZ-treated patients had a longer disease duration and higher EDSS scores than IFN-β-treated patients, but the age of the patients did not differ between the groups (Table 1). In the NTZ-treated group, 46 out of 49 patients had been previously treated with other immunomodulatory or immunosuppressive (IS) drugs: 15 patients with IFN-β only, 29 patients with IFN-β, and other immunomodulatory drugs and two with only IS drug. The remaining three patients did not have any previous treatment. In the IFN-β-treated group, no patients had any previous medication with any other immunomodulatory or IS drugs. Two years before initiating NTZ therapy, almost all (96%) patients had had relapses (mean $2.0 \pm$ SD 1.0 (range 0–4, 17 patients had one relapse, 18 patients had two relapses, six patients had three relapses, and six patients had four relapses)). During therapy, relapses were observed in seven (14%) patients (four patients had one relapse each and three patients had two relapses each) among whom the NTZ treatment duration ranged from 1.7 to 5.8 years. The seroprevalence of anti-JCPyV Ab was 49% among NTZ-treated and 43% among IFN-β-treated patients.

Overall detection rate of JCPyV miRNA

The presence of JCPyV-encoded 5p and 3p miRNAs was studied in altogether 102 plasma samples obtained from 77 RRMS patients and 25 HCs. The results showed that the overall detection rate for 5p miRNA was 80.5% among MS patients and 92% in HCs. In groups based on current medication, the overall detection rate for 5p miRNA was 84% among NTZ-treated patients, and 75% among IFN-β-treated patients. However, the differences in detection rates were not found statistically significant ($p > 0.5$). Furthermore, the detection rate of 5p miRNA among JCPyV seropositive and seronegative patients receiving either NTZ or IFN-β was similar (Table 2). Surprisingly, the rate of 5p miRNA detection among JCPyV seronegative patients treated with NTZ was higher than among IFN-β-treated patients (Table 2), although the difference was not statistically significant ($p > 0.5$). The anti-JCPyV antibody assay was not available for HCs, and therefore we were not able to correlate miRNA detection rates to JCPyV antibody status among HCs.

We could not detect true positive 3p miRNA signals indisputably in any of the samples. Occasional amplification, with Ct cycle for just one replicate, was obtained in samples of four MS patients and four HCs. Only one sample from a HC gave positive signal for 3p miRNA with high Ct cycles for two replicates (Supplementary Table 1). Therefore, we were unable to assess 3p miRNA expression levels in different patient groups and in HCs.

Assessment of relative 5p miRNA expression levels in different patient cohorts

The 5p miRNA expression levels were found to be similar in the whole MS cohort compared to HCs ($p = 0.06$). In groups based on current medication, the expression levels were found to be lower in NTZ-treated patients as compared to patients treated with IFN-β ($p = 0.027$) but not as compared to HCs ($p = 0.454$). IFN-β-treated patients had considerably higher levels of 5p miRNA than NTZ or HCs as shown by fold change in Fig. 1 ($p = 0.001$). 5p miRNA expression levels between JCPyV seropositive and seronegative patients in the different MS cohorts, treated either with NTZ or IFN-β, were found to be similar ($p > 0.5$).

NTZ-treated patients were further stratified according to their anti-JCPyV Ab index into groups with high risk of developing PML (anti-JCPyV Ab index >1.5, $n = 9$) and low risk group of developing PML (anti-JCPyV Ab index ≤1.5, $n = 40$) (Plavina et al. 2014). The levels of 5p miRNA expression did

Table 2 jcv-miR-J1-5p detection rates in the different patient groups. The overall detection rate among all groups was 85/102 (83%)

	All MS patients	NTZ-treated	IFN-β-treated	HCs	p value
All	62/77 (80.5%)	41/49 (84%)	21/28 (75%)	23/25 (92%)	>0.5
JCPyV Ab⁺	30/36 (83%)	20/24 (83%)	10/12 (83%)	NA	>0.5
JCPyV Ab⁻	32/41 (78%)	21/25 (84%)	11/16 (69%)	NA	>0.5

JCPyV Ab⁺ anti-JCPyV antibody positive, *JCPyV Ab⁻* anti-JCPyV antibody negative, *NA* not available
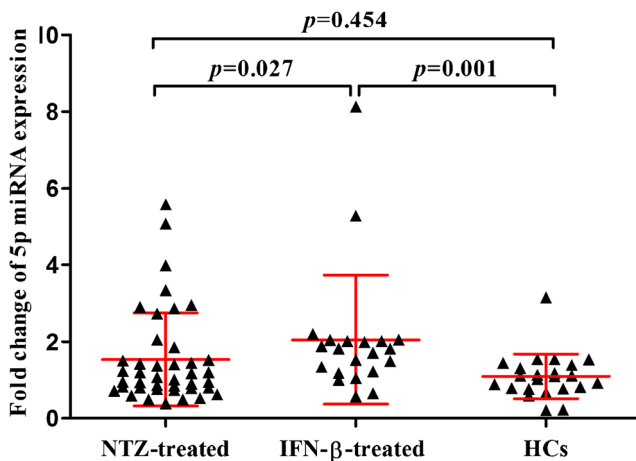
Fig. 1 Scatterplot of relative jcv-miR-J1-5p expression levels. *Bars* indicate mean ± standard deviation. The values on the *y*-axis reflect fold change of 5p miRNA expression among individual NTZ-treated patients and IFN-beta-treated patients as compared to HCs. The scatterplot of the HCs (on the *right*) presents the variation among individual HCs. Plasma levels of 5p miRNA were lower among NTZ-treated patients (*n* = 49) compared to IFN-beta-treated patients (*n* = 28), whereas the expression levels remained similar to those among HCs (*n* = 25). Significantly enhanced levels were observed among IFN-beta-treated patients as compared to HCs. In the calculation of relative expression levels or fold changes, the mean Ct value of HCs was subtracted from the values of each NTZ- or IFN-beta-treated patient, and thus the mean value among HCs is equal to 1

Fig. 2 Inverse correlation between jcv-miR-J1-5p expression and the anti-JCPyV Ab index among JCPyV seropositive patients treated long-term with NTZ

not differ between the high-risk group and low-risk group of developing PML (*p* = 0.38).

Relationship between JCPyV miRNA expression and anti-JCPyV antibody index

Association between plasma 5p miRNA levels and anti-JCPyV Ab index was further studied by correlation analyses along with other clinical characteristics of MS patients (age, disease duration, drug duration, and EDSS). There were no significant correlations found between 5p miRNA expression and clinical characteristics of patients. Among all NTZ-treated patients (*n* = 49), and patients who had been treated more than 18 months (*n* = 40), 5p miRNA expression did not correlate with anti-JCPyV Ab index. Interestingly, a significant inverse correlation between 5p miRNA expression and anti-JCPyV Ab index was established among those patients (*n* = 19) who had been treated long-term with NTZ and were JCPyV seropositive (*r* = −0.756, *p* = 0.002, Fig. 2). The mean duration of NTZ treatment among JCPyV seropositive MS patients was 3.0 years (SD ±1.3 years, range 1.6–5.3 years). Moreover, further analyses by linear regression model showed that the observed correlation between relative 5p miRNA expression levels and anti-JCPyV Ab indices was not affected when adjusted for age, sex, or for both (data not shown).
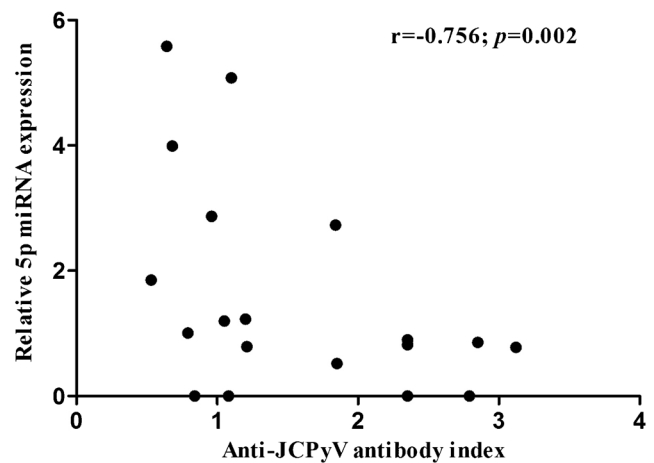
Discussion

When considering appropriate medication for MS patients, risk of PML has remained a major challenge for clinicians, because in addition to NTZ also other effective MS drugs such as fingolimod and dimethyl fumarate have been reported to increase PML risk in MS patients (Faulkner 2015). This has led to the urgent need of reliable and sensitive prognostic markers to better manage the patients for PML risk on an individual basis. Since polyomavirus microRNAs circulating in biological fluids currently hold potential as diagnostic markers of several diseases (Martelli and Giannecchini 2017), we analyzed the presence of JCPyV encoded miRNAs in plasma and explored their biomarker potential in NTZ-therapy-associated PML risk assessment in MS patients.

Altogether, 83% of plasma samples 5p miRNA could be detected, whereas credible signals for 3p miRNA were obtained only for one sample from a HC. Although previous studies have suggested that the 3p miRNA is expressed at lower rates as compared to the 5p miRNAs (Lagatie et al. 2013; Seo et al. 2008), such low detection rate was surprising. In our previous study, we showed high detection rates of the 3p miRNA in plasma, urine, and CSF samples of transplant and other immunosuppressed patients using similar assays (Pietila et al. 2015). We are not able to provide an exhaustive explanation to this discrepancy, except for the differences in the studied patient populations and different real-time PCR platforms used in the two studies. The functionality of all the assays was verified using synthetic oligonucleotide templates. The spike-in controls in all samples were valid. A LightCycler 480 platform (Roche Applied Science, Penzberg, Germany) was used in the previous study, which may partially explain the differences in detection rates and Ct cycles. It has been shown earlier that the results obtained using different real-time PCR platforms are not directly comparable (Lu et al. 2010;

Silvy et al. 2005). Furthermore, our preliminary data of BKPyV miRNA detection from renal transplant patients (Virtanen et al., unpublished), show high detection rates for bkv-miR-B1-3p/jcv-miR-J1-3p, suggesting clear differences in 3p detection rates between different patient populations. However, the low detection rate of 3p miRNA in this study does not interfere with our interpretation of 5p miRNA analyses.

Consistent with other studies, we found equal rates of 5p miRNA in JCPyV seronegative and seropositive patients indicating that a negative JCPyV Ab result does not necessarily mean the absence of JCPyV infection (Lagatie et al. 2014). Indeed some recent NTZ-related PML cases have been reported in MS patients in whom no anti-JCPyV Ab had been detected (Gagne Brosseau et al. 2016). Recent studies have reported higher levels of 5p miRNA in various clinical samples such as in tissue samples of patients with colonic neoplasia compared to healthy subjects (Link et al. 2014), as well as in PBMC and in exosomes obtained from plasma and urine of NTZ-treated MS patients as compared to untreated and healthy subjects (Giovannelli et al. 2015). Our present work is the first to show differential expression of JCPyV 5p miRNA in MS patients treated with NTZ or IFN-β, and to display the association of 5p miRNA with JCPyV seropositivity in NTZ-treated MS patients. Indeed, in a previous attempt, an association with developing PML risk in NTZ-treated patients positive for JCPyV DNA in blood or urine could not be established (Rudick et al. 2010).

It has been suggested that subclinical reactivation of JCPyV may occur in NTZ-treated patients with MS (Chen et al. 2009). Upon reactivation, increased viral replication may be accompanied by rearrangements of archetype JCPyV, involving putative alterations within the non-coding control region (NCCR). JCPyV-carrying archetype NCCR is mainly present in asymptomatic individuals whereas viral strains with rearranged NCCR forms are frequently found in patients with PML (Martelli and Giannecchini 2017). Increased transcription, DNA replication, and expression of viral gene products would allow better immune detection of infection by both innate and adaptive immune systems of the host. NTZ treatment has further been shown to increase cellular immune responses specific to virus and myelin proteins in peripheral blood (Jilek et al. 2010). On the other hand, considerable downregulation of archetype BKPyV DNA replication has been shown due to viral microRNA expression, which helps to maintain persistent virus in a healthy host despite a functional immune system (Broekema and Imperiale 2013). In analogy, downregulation of miRNA expression would release viral early gene transcription and DNA replication from negative regulation and, in the case of JCPyV, enable efficient replication and, consequently, putative emergence of rearranged neurotropic strains. Along these lines, our present study on plasma samples revealed lower levels

of 5p miRNA in NTZ-treated patients as compared to IFN-β-treated patients, but the levels were similar to HCs. Although no PML cases were included in our patient population, the observed reduced levels of 5p miRNA among patients treated with NTZ suggest a possible involvement in support of viral reactivation. However, regulation of JCPyV miRNA expression is not well studied, although some very rare LTAg mutants have been reported which do not encode microRNAs, with putative effect on viral replication and host immune response (Chen et al. 2014).

Furthermore, growing evidence suggests that JCPyV miRNAs, similar to other viral miRNAs, can reside in exosomes, and that the exosomal miRNAs may have a role in viral persistence and reactivation (Martelli and Giannecchini 2017). Remarkably, these miRNAs have been detected in exosomes obtained from plasma and urine of both JCPyV-DNA positive and JCPyV-DNA negative MS patients (Giovannelli et al. 2015), suggesting that these miRNAs may provide new element in identifying patients at risk of PML.

Interestingly, we found higher levels of 5p miRNA in IFN-β-treated patients as compared to NTZ-treated and HCs. The different miRNA levels that we observed in these two groups of patients may either be a direct consequence of a particular treatment, or an off-target phenomenon, and it should be evaluated further. To date, there have been no reported cases of PML due to reactivation of JCPyV in either IFN-β or glatiramer acetate-treated MS patients. In addition to antiviral activity of IFN-β, it has been shown that the treatment leads to the reduction of JCPyV viral load in peripheral blood of MS patients (Delbue et al. 2007), which might be associated with the higher 5p miRNA levels found in the present study. However, further investigations are needed to explain the mechanism and impact of high 5p miRNA expression observed in our MS patients treated with IFN-β.

It is noteworthy that 5p miRNA levels correlated inversely with anti-JCPyV Ab index in JCPyV seropositive patients treated long-term with NTZ. At present, there are no further reports available on the association between anti-JCPyV Ab and JCPyV miRNA expression patterns in MS. However, two recent studies have reported inverse correlations of 5p miRNA expression: with JCPyV T-Ag expression in colorectal cancer tissues (Link et al. 2014) and with JCPyV DNA load in blood and CSF of HIV patients who are at risk of developing PML (Rocca et al. 2015), confirming that miRNA expression may indeed restrict virus replication in order to suppress host immune responses. Nevertheless, considering the observed correlation, further studies are needed to confirm the clinical relevance of the relationship between 5p miRNA and anti-JCPyV Ab index in identifying individual patients at risk of developing PML.

Our study has some limitations. The number of patients enrolled was relatively small. Further, the assessment of viral loads, and JCPyV antibodies among HCs, as performed in a previous study (Lagatie et al. 2014), would have enabled

additional analyses on the impact of our microRNA findings, but the antibody assay was only available for MS patients in the present study.

Conclusion

Taken together, our results suggest that the presence of JCPyV miRNA in plasma may indicate asymptomatic JCPyV infection in MS patients and therefore may hold potential as a biomarker for PML risk assessment. However, due to the lack of PML samples in our study, these preliminary findings should be evaluated further in a larger set of longitudinal samples including samples from NTZ-associated PML patients before and after the onset of the disease to better define the predictive value of JCPyV miRNA. Furthermore, this study confirms our previous observation that JCPyV miRNA can be determined in plasma of MS patients and healthy subjects (Pietila et al. 2015), and further suggests that JCPyV serostatus actually underestimates the true infection rates.

Compliance with ethical standards

Conflict of interests The authors have no conflict of interests to declare.

References

Auvinen E (2017) Diagnostic and prognostic value of MicroRNA in viral diseases. Mol Diagn Ther 21:45–57

Basnyat P, Hagman S, Kolasa M, Koivisto K, Verkkoniemi-Ahola A, Airas L, Elovaara I (2015) Association between soluble L-selectin and anti-JCV antibodies in natalizumab-treated relapsing-remitting MS patients. Mult Scler Relat Disord 4:334–338

Bauman Y, Nachmani D, Vitenshtein A, Tsukerman P, Drayman N, Stern-Ginossar N, Lankry D, Gruda R, Mandelboim O (2011) An identical miRNA of the human JC and BK polyoma viruses targets the stress-induced ligand ULBP3 to escape immune elimination. Cell Host Microbe 9:93–102

Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, Lee S, Plavina T, Scanlon JV, Sandrock A, Bozic C (2012) Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med 366:1870–1880

Broekema NM, Imperiale MJ (2013) miRNA regulation of BK polyomavirus replication during early infection. Proc Natl Acad Sci U S A 110:8200–8205

Chen CJ, Burke JM, Kincaid RP, Azarm KD, Mireles N, Butel JS, Sullivan CS (2014) Naturally arising strains of polyomaviruses with severely attenuated microRNA expression. J Virol 88:12683–12693

Chen Y, Bord E, Tompkins T, Miller J, Tan CS, Kinkel RP, Stein MC, Viscidi RP, Ngo LH, Koralnik IJ (2009) Asymptomatic reactivation of JC virus in patients treated with natalizumab. N Engl J Med 361:1067–1074

Clifford DB, De Luca A, Simpson DM, Arendt G, Giovannoni G, Nath A (2010) Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. Lancet Neurol 9:438–446

Cutter GR, Stuve O (2014) Does risk stratification decrease the risk of natalizumab-associated PML? Where is the evidence? Mult Scler 20:1304–1305

Delbue S, Guerini FR, Mancuso R, Caputo D, Mazziotti R, Saresella M, Ferrante P (2007) JC virus viremia in interferon-beta -treated and untreated Italian multiple sclerosis patients and healthy controls. J Neuro-Oncol 13:73–77

Faulkner M (2015) Risk of progressive multifocal leukoencephalopathy in patients with multiple sclerosis. Expert Opin Drug Saf 14:1737–1748

Ferenczy MW, Marshall LJ, Nelson CD, Atwood WJ, Nath A, Khalili K, Major EO (2012) Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 25:471–506

Gagne Brosseau MS, Stobbe G, Wundes A (2016) Natalizumab-related PML 2 weeks after negative anti-JCV antibody assay. Neurology 86:484–486

Giovannelli I, Martelli F, Repice A, Massacesi L, Azzi A, Giannecchini S (2015) Detection of JCPyV microRNA in blood and urine samples of multiple sclerosis patients under natalizumab therapy. J Neuro-Oncol 21:666–670

Jilek S, Mathias A, Canales M, Lysandropoulos A, Pantaleo G, Schluep M, Du Pasquier RA (2013) Natalizumab treatment alters the expression of T-cell trafficking marker LFA-1 alpha-chain (CD11a) in MS patients. Mult Scler 20:837–842

Jilek S, Jaquiery E, Hirsch HH, Lysandropoulos A, Canales M, Guignard L, Schluep M, Pantaleo G, Du Pasquier RA (2010) Immune responses to JC virus in patients with multiple sclerosis treated with natalizumab: a cross-sectional and longitudinal study. Lancet Neurol 9:264–272

Khalili K, White MK, Lublin F, Ferrante P, Berger JR (2007) Reactivation of JC virus and development of PML in patients with multiple sclerosis. Neurology 68:985–990

Kira J (2014) Disease concept, etiology and mechanisms of multiple sclerosis. Nihon Rinsho 72:1884–1894

Kolasa M, Hagman S, Verkkoniemi-Ahola A, Airas L, Koivisto K, Elovaara I (2016) Anti-JC virus seroprevalence in a Finnish MS cohort. Acta Neurol Scand 133:391–397

Kroh EM, Parkin RK, Mitchell PS, Tewari M (2010) Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods 50:298–301

Kurtzke JF (1983) Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33:1444–1452

Lagatie O, Van Loy T, Tritsmans L, Stuyver LJ (2014) Viral miRNAs in plasma and urine divulge JC polyomavirus infection. Virol J 11:158–166

Lagatie O, Tritsmans L, Stuyver LJ (2013) The miRNA world of polyomaviruses. Virol J 10:268–288

Lee P, Plavina T, Castro A, Berman M, Jaiswal D, Rivas S, Schlain B, Subramanyam M (2013) A second-generation ELISA (STRATIFY JCV DxSelect) for detection of JC virus antibodies in human serum

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and plasma to support progressive multifocal leukoencephalopathy risk stratification. J Clin Virol 57:141–146

Link A, Balaguer F, Nagasaka T, Boland CR, Goel A (2014) MicroRNA miR-J1-5p as a potential biomarker for JC virus infection in the gastrointestinal tract. PLoS One 9:e100036

Lu S, Smith AP, Moore D, Lee NM (2010) Different real-time PCR systems yield different gene expression values. Mol Cell Probes 24:315–320

Martelli F, Giannecchini S (2017) Polyomavirus microRNAs circulating in biological fluids during viral persistence. Rev Med Virol 27: e1927

Munoz-Culla M, Irizar H, Castillo-Trivino T, Saenz-Cuesta M, Sepulveda L, Lopetegi I, Lopez de Munain A, Olascoaga J, Baranzini SE, Otaegui D (2014) Blood miRNA expression pattern is a possible risk marker for natalizumab-associated progressive multifocal leukoencephalopathy in multiple sclerosis patients. Mult Scler 20: 1851–1859

Olsson T, Achiron A, Alfredsson L, Berger T, Brassat D, Chan A, Comi G, Eraksoy M, Hegen H, Hillert J, Jensen PE, Moiola L, Myhr KM, Oturai A, Schippling S, Siva A, Sorensen PS, Trampe AK, Weber T, Potts J, Plavina T, Paes D, Subramanyam M, Wiendl H, Dib H, Uren D, Hemmer B, Buck D (2013) Anti-JC virus antibody prevalence in a multinational multiple sclerosis cohort. Mult Scler 19:1533–1538

Pietila T, Nummi M, Auvinen P, Mannonen L, Auvinen E (2015) Expression of BKV and JCV encoded microRNA in human cerebrospinal fluid, plasma and urine. J Clin Virol 65:1–5

Plavina T, Subramanyam M, Bloomgren G, Richman S, Pace A, Lee S, Schlain B, Campagnolo D, Belachew S, Ticho B (2014) Anti-JCV antibody levels in serum or plasma further define risk of natalizumab-associated PML. Ann Neurol 76:802–812

Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW, Investigators AFFIRM (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 354:899–910

Polman CH, Reingold SC, Edan G, Filippi M, Hartung HP, Kappos L, Lublin FD, Metz LM, McFarland HF, O'Connor PW, Sandberg-Wollheim M, Thompson AJ, Weinshenker BG, Wolinsky JS (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald criteria". Ann Neurol 58:840–846

Rocca A, Martelli F, Delbue S, Ferrante P, Bartolozzi D, Azzi A, Giannecchini S (2015) The JCPYV DNA load inversely correlates with the viral microrna expression in blood and cerebrospinal fluid of patients at risk of PML. J Clin Virol 70:1–6

Rudick RA, O'Connor PW, Polman CH, Goodman AD, Ray SS, Griffith NM, Jurgensen SA, Gorelik L, Forrestal F, Sandrock AW, Goelz SE (2010) Assessment of JC virus DNA in blood and urine from natalizumab-treated patients. Ann Neurol 68:304–310

Schwab N, Schneider-Hohendorf T, Posevitz V, Breuer J, Gobel K, Windhagen S, Brochet B, Vermersch P, Lebrun-Frenay C, Posevitz-Fejfar A, Capra R, Imberti L, Straeten V, Haas J, Wildemann B, Havla J, Kumpfel T, Meinl I, Niessen K, Goelz S, Kleinschnitz C, Warnke C, Buck D, Gold R, Kieseier BC, Meuth SG, Foley J, Chan A, Brassat D, Wiendl H (2013) L-selectin is a possible biomarker for individual PML risk in natalizumab-treated MS patients. Neurology 81:865–871

Seo GJ, Fink LH, O'Hara B, Atwood WJ, Sullivan CS (2008) Evolutionarily conserved function of a viral microRNA. J Virol 82:9823–9828

Silvy M, Mancini J, Thirion X, Sigaux F, Gabert J (2005) Evaluation of real-time quantitative PCR machines for the monitoring of fusion gene transcripts using the Europe against cancer protocol. Leukemia 19:305–307

Wollebo HS, White MK, Gordon J, Berger JR, Khalili K (2015) Persistence and pathogenesis of the neurotropic polyomavirus JC. Ann Neurol 77:560–570