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Predictive value of JC virus PCR in cerebrospinal fluid in the diagnosis of PML

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A B S T R A C T

Objective: To assess the predictive value of JC virus (JCV) PCR in cerebrospinal fluid (CSF) in the diagnosis of pro-gressive multifocal leukoencephalopathy (PML).

Methods: We conducted a retrospective database query to identify patients with positive CSF JCV PCR. Clinical features, final diagnosis and quantitative PCR results were obtained.

Results: A positive CSF JCV PCR had a PPV of 10.4% for the diagnosis of PML. A weakly positive PCR had a PPV of 1.6%, whereas a moderately to highly positive PCR had a PPV of 92.3%. A PPV of 0.0% was observed in immuno-competent patients and in patients without compatible clinical or radiological features.

Conclusions: A false-positive CSF JCV PCR is highly prevalent in our clinical practice. This test should be reserved for patients with a clinical suspicion of PML and the quantitative result of the PCR should be taken into account when making the diagnosis of PML.

1. Introduction

Progressive multifocal leukoencephalopathy (PML) is a neuro-infectious disease caused by the JC polyomavirus (JCV). It is highly uncommon in immunocompetent persons and almost always occurs in the setting of an acquired immunosuppressive condition, with AIDS and natalizumab treatment being the most frequent (Bauer et al., 2015). Because of atypical clinical and radiological features, diagnosis is often not straightforward. According to the current diagnostic criteria, definite PML can be diagnosed in two ways (Berger et al., 2013). First, in case a brain biopsy has been performed, a definite diagnosis can be established based on histopathology. A second manner to definitely diagnose PML is through compatible clinical and radiological features together with a positive cerebrospinal fluid (CSF) JCV polymerase chain

reaction (PCR). The presence of either compatible clinical or radiological features is considered as 'probable PML'. In practice, brain biopsy is only rarely performed and clinical and radiological features lack specificity. Hence, diagnosis heavily relies on CSF JCV PCR. According to available literature, qualitative CSF JCV PCR is regarded as highly sensitive and specific with a high positive predictive value (PPV) for the diagnosis of PML (Berger et al., 2013; Fong et al., 1995; Iacobaeus et al., 2009; McGuire et al., 1995).

We wanted to analyze the PPV of quantitative CSF JCV PCR testing in clinical practice. Our second aim was to assess whether a particular JCV PCR profile in accompanying blood and/or urine samples would be of additional diagnostic value. Finally, we performed a literature review, identifying series where CSF JCV PCR was performed to calculate its PPV and compare it to this cohort's PPV.

2. Materials and methods

2.1. Database query

A query searching for all JCV PCRs performed on clinical samples was performed on the database of the clinical laboratory of the University

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Hospitals Leuven between January 2004 and December 2014. For all patients with a positive CSF JCV PCR, the final diagnosis was obtained and verified (based on clinical course, radiological features and/or brain biopsy) by examining the corresponding electronic medical record. Additionally, clinical and radiological features at the time of lumbar puncture were obtained. The query was conducted with permission from the local ethics committee (local study code S57675).

2.2. PCR

CSF, plasma and urine samples were extracted by means of NucliSens easyMAG (bioMérieux, Marcy l'Etoile, France). All samples were extracted within a time lag of 48 hours. Deoxyribonucleic acid (DNA) extracts subsequently were analyzed with in-house real-time PCR Taqman (ABI7900) according to the method previously described (Beuselink et al., 2005; Herman et al., 2004). JC virus and BK virus are both a polyomavirus strain causing disease in humans. Both viruses cause distinct infections in immunocompromised patients, i.e. neurological disease and renal disease, respectively. Therefore, a common quantitative real-time PCR detecting both JC and BK virus was developed in the year 1997. The primers of the in-house JC/BK PCR target a region of the large T antigen present both in JC and BK virus (Beuselink et al., 2005; Herman et al., 2004). These primers were used to amplify DNA and detection was done with a probe complementary to an internal target sequence present in both BK and JC virus PCR amplicons (Herman et al., 2004). For each virus, a standard curve was used to quantify the amount of DNA; based on duplicate internal standard samples, the quantifiable range was constituted by values between 2.7 log copies/mL (i.e. 500 copies/mL) and 6.7 log copies/mL (i.e. 5,000,000 log copies/mL). Hence, the following cut-offs were therefore used in routine samples: weakly positive (<2.7 log copies/mL, not quantifiable), moderately positive (≤6.7 log copies/mL, quantifiable), strongly positive (>6.7 log copies/mL, not quantifiable).

For validation of quantification, we used plasmid derived JCV clones with the complete JCV genome cloned in the EcoRI site of the multiple cloning site of the pBR322 vector (courtesy of TH Weber, Marienkrankenhaus, Hamburg, Germany). The inserted sequence was quantitated using ultraviolet spectrophotometry to calculate the copy number for a master standard positive control.

From 2007 onwards, we yearly participate at the QCMD External quality assessment scheme for JCV/BK virus with good results.

The detection limit of the PCR was lower than 500 copies/mL, but 100% of the samples were detected at the cutoff of 500 copies/mL. Since February 29th 2012, PCR reaction is repeated on samples with weakly positive Ct (cycle threshold) values higher than 37.3 (i.e. <2.7 log copies/mL) and only the repeat value is reported. Weakly positive samples were negative after repetition in 67% of the cases. In every PCR run a positive and negative control is included, the run is repeated in case of positivity in the negative control.

2.3. Literature review

For the literature review, the PubMed database was searched with the following terms: 'JCV', 'polyoma', 'PCR' and 'PML'. Papers concerning series of patients with CSF JCV PCR were included and the number of true- and false-positive PCRs were deducted.

2.4. Statistics

Odds ratio (OR) and PPV were performed in GraphPad Prism 7.01 (GraphPad Software, San Diego, USA) using Fisher's exact test and the Wilson-Brown method. Significance level was set at 0.05. ROC analysis was performed with R statistical software 3.2.2 (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org>). The optimal threshold was defined by the Youden index.

3. Results

The query (Fig. 1) yielded a total of 1130 patients in whom CSF JCV PCR was performed. In 233 (20.6%) of these patients PCR was positive. After excluding patients from other hospitals (of whom we did not have access to the electronic medical record), 135 (57.9%) patients with a positive CSF JCV PCR remained in the final analysis (later referred to as the *index population*).

Fourteen patients were finally diagnosed with PML whereas 121 received another diagnosis, resulting in a PPV of 10.4% (95% CI: 6.3–16.7%) (Fig. 1). Alternative diagnoses comprise toxic-metabolic encephalopathy (n = 34), central nervous system (CNS) infection (n = 27; i.e. bacterial, viral, fungal and parasitic meningitis/encephalitis), CNS tumor (n = 17; e.g. lymphoma, neoplastic meningitis and astrocytoma), CNS inflammatory disease (n = 16; e.g. multiple sclerosis, paraneoplastic encephalitis and vasculitis), neurodegenerative disease (n = 10; e.g. Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis and vascular dementia), posterior reversible encephalopathy syndrome (n = 6), primary headache (n = 3), psychiatric disorder (n = 3), stroke (n = 3) and cranial nerve palsy (n = 2). Of these non-PML cases, 77 (63.6%) were immunocompromised due to immunosuppressive medication (n = 43; one patient treated with natalizumab), chemotherapy (n = 15), AIDS (n = 12), hematological malignancy (n = 5) or primary immune deficiency (n = 2).

Subsequently, the *index population* was stratified according to the degree of PCR positivity. Only two of 122 patients with a weakly positive (i.e. <2.7 log copies/mL, not quantifiable) PCR were diagnosed with PML (PPV = 1.6%, 95% CI: 0.3–5.8%), whereas 12 of the 13 patients with a moderately or strongly positive (≥2.7 log copies/mL) PCR received a final diagnosis of PML (PPV = 92.3%, 95% CI: 66.7–99.6%) (Fig. 1). Twelve patients had a moderately positive (≤6.7 log copies/mL) PCR, of whom 11 were diagnosed with PML. The one patient without PML but with a moderately positive PCR had a viral load of 2.85 log copies/mL, which is only marginally above the quantification threshold (Fig. 2a). Most PML patients with a moderately positive PCR had viral loads above 3.0 log copies/mL (Fig. 2a). Only one patient had a strongly positive (>6.7 log copies, not quantifiable) PCR, he was diagnosed with PML (Fig. 2a). In total 14.3% (2/14) of PML patients had a weakly positive CSF JCV PCR.

Next, we performed an ROC analysis to identify the viral load with optimal cut-off value to detect PML patients in case of a positive CSF JCV PCR. ROC analysis identified an area under the curve (AUC) of 0.93 (95% CI: 0.83–1.00) (Fig. 2b) with an optimal threshold of 2.82 log copies to detect PML patients. This corresponds to a sensitivity of 86% (95% CI: 57–98%) and a specificity of 99% (95% CI: 95–100%).

Next, to assess whether the indication for CSF JCV PCR testing is related to the PPV, clinical and radiological features at the time of lumbar puncture were analyzed (Table 1). Analysis irrespective of the immune status revealed a PPV of 0.0% (95% CI: 0.0–4.8%) when compatible clinical and radiological features were absent. PPV gradually increased with increasing compatible features, reaching a PPV of 48.0% (95% CI: 30.0–66.5%) in case of both compatible clinical and radiological features. Analysis taking into account the immune status revealed a PPV of 0.0% (95% CI: 0.0–8.0%) in immunocompetent patients. In immunocompromised patients, PPV was 15.4% (95% CI: 9.4–24.2%), with the PPV again gradually increasing with increasing compatible features, reaching 70.6% (95% CI: 46.9–86.7%) in case of both compatible clinical and radiological features (i.e. patients with 'definite PML'). For this latter group of patients clinical features, radiological features, underlying disorder, immune suppression state, diagnosis and JCV PCR titer are displayed in Table 2. When the quantitative result of the CSF JCV PCR was also taken into account, PPV further increased to 100.0% (95% CI: 74.1–100.0%).

Next, we analyzed the performance of CSF JCV PCR before versus after implementing repeated testing of weakly positive samples. Before implementation, 10 out of 128 patients had PML (PPV = 7.8%, 95% CI:

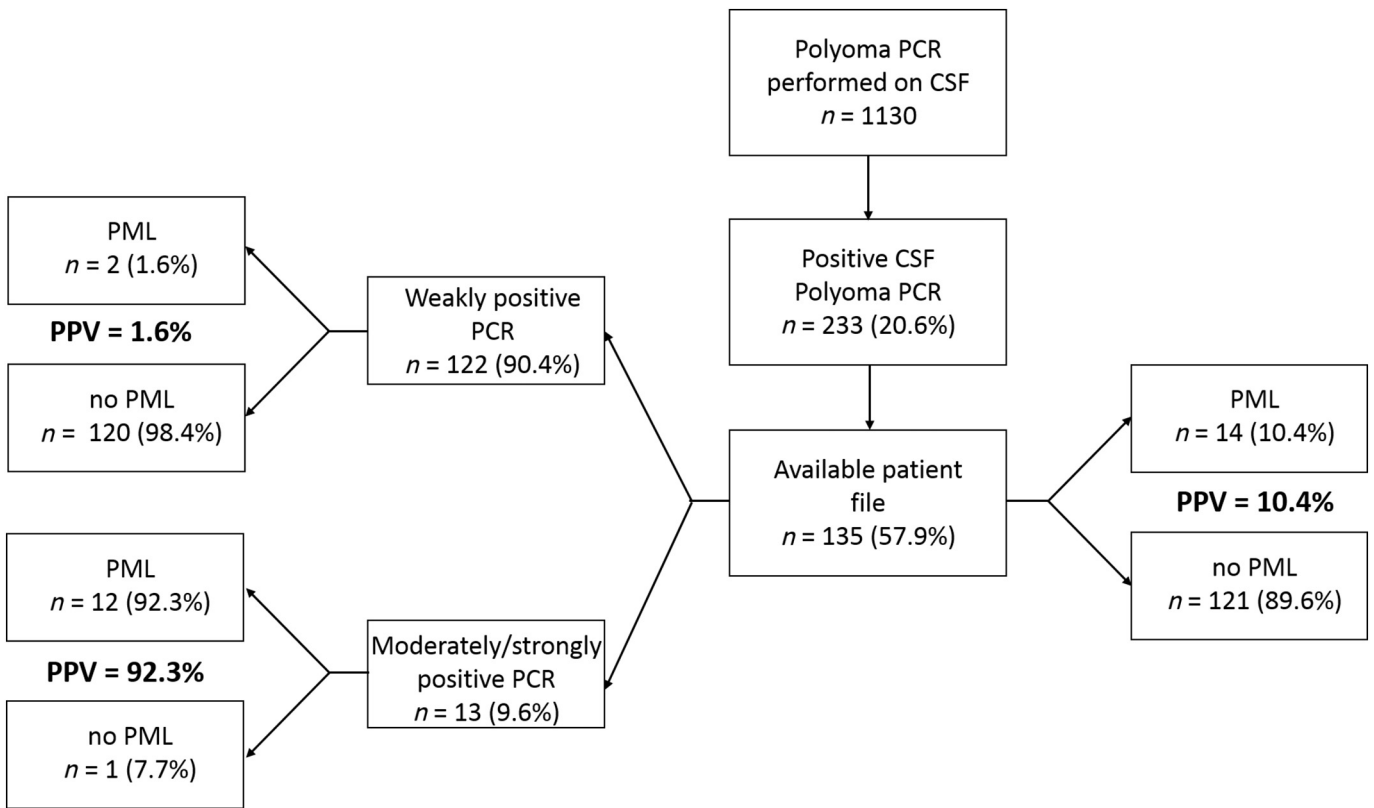


Fig. 1. Flowchart of data analysis. The query yielded 1130 patients with JCV PCR performed on CSF, of whom 233 were positive. For 135 of these patients we were able to obtain a patient file. A weakly positive PCR is defined as a viral load <2.7 log copies/mL, whereas a moderately/strongly positive PCR is defined as a viral load ≥ 2.7 log copies/mL.

4.3–13.8%), whereas afterwards 4 out of 7 patients had PML (PPV = 57.1%, 95% CI: 25.0%–84.2%). Hence, retesting of weakly positive PCRs significantly decreased the amount of false positives (OR = 0.06, 95% CI: 0.02–0.27, $P = 0.002$).

Next, we aimed to assess whether JCV PCR results in blood and/or urine might have an additional diagnostic value. Blood JCV PCR was

performed in 27 patients of the *index population* (Fig. 3). Twenty (74.1%) PCRs were positive. Three (15%) of the patients with a positive blood JCV PCR had PML, whereas three (42.9%) of the patients with a negative blood JCV PCR had PML. Hence, additionally having a positive blood PCR does not increase the chances of having PML when compared to a negative blood PCR (OR = 0.24 (95% CI: 0.04–1.39, $P = 0.29$)).

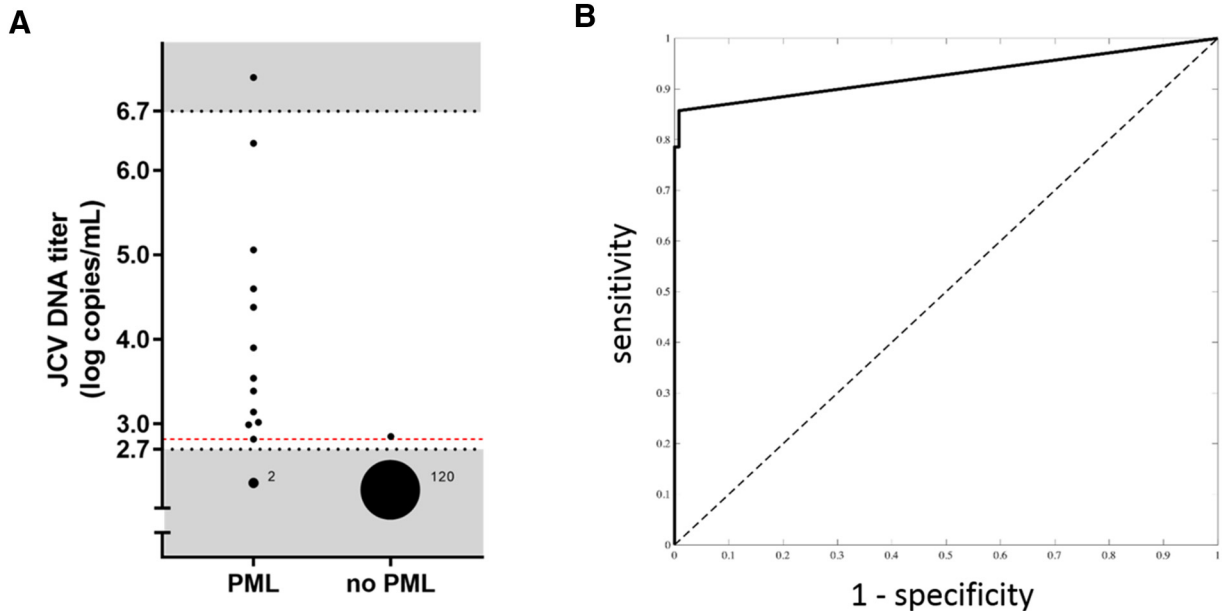


Fig. 2. Qualitative result of CSF JCV PCR. (A) Distribution of the 135 index patients according to qualitative result of CSF JCV PCR. The lower part (gray) represents patients with a weakly positive (<2.7 log copies/mL, not quantifiable) PCR. The middle part (white) represents patients with a moderately positive (<6.7 log copies/mL, quantifiable) PCR. The upper part (gray) represents one patient with a strongly positive (>6.7 log copies/mL, not quantifiable) PCR who was diagnosed with PML. Red dashed line indicates optimal cut-off value as obtained with Youden index. (B) ROC curve of JCV DNA viral load to predict diagnosis of PML (AUC = 0.93).

Table 1
Relation between PPV and clinical and radiological features Patients were categorized according to the presence/absence of compatible clinical ('clinical +/-') and radiological ('radiological +/-') features as specified in the diagnostic criteria (Berger et al., 2013). Patients were also categorized according to immune status (i.e. immunocompetent versus immunocompromised).

			(n)	(%)	PML diagnosis (n)	PPV	CI 95%
Irrespective of immune status			135	100.0	14	10.4	6.3–16.7
	Clinical -	Radiological -	77	57.0	0	0.0	0.0–4.8
	Clinical +	Radiological -	28	20.7	1	3.6	0.2–17.7
	Clinical -	Radiological +	5	3.7	1	20.0	1.0–62.4
	Either clinical or radiological +		33	24.4	2	6.1	1.1–19.6
	Clinical +	Radiological +	25	18.5	12	48.0	30.0 – 66.5
Immunocompromised			91	67.4	14	15.4	9.4–24.2
	Clinical -	Radiological -	50	54.9	0	0.0	0.0–7.1
	Clinical +	Radiological -	22	24.2	1	4.5	0.2–21.8
	Clinical -	Radiological +	2	2.2	1	50.0	2.6–97.4
	Either clinical or radiological +		24	26.4	2	8.3	1.5–25.8
	& viral load ≥ 2.7 log copies/mL		1	0.7	1	100.0	5.1–100.0
<i>Probable PML</i>							
	Clinical +	Radiological +	17	18.7	12	70.6	46.9 – 86.7
<i>Definite PML</i>							
	& viral load ≥ 2.7 log copies/mL		11	8.1	11	100.0	74.1–100.0
Immunocompetent			44	32.6	0	0.0	0.0–8.0

Urine JCV PCR was performed in 32 patients of the *index population* (Fig. 3). Thirty-one (96.9%) of these PCRs were positive and one was negative. Five (16.1%) of the patients with positive urine JCV PCR had PML. The one patient with a negative urine JCV PCR was not diagnosed with PML. Although odds ratio can't be calculated, additionally having a positive urine PCR does not increase the chance of having PML when compared to a negative urine PCR.

Finally, JCV PCR was performed on both urine and blood in 19 patients of the *index population* (Fig. 3). Sixteen of these index patients had positive PCRs both in blood and urine. Three (18.7%) of those were diagnosed with PML. In three patients the PCR was negative in blood and positive in urine, one of these patients was diagnosed with PML. There were no patients with negative PCR in both blood and

urine, or positive PCR in blood and negative in urine. Hence, additionally having a positive PCR in both blood and urine does not increase the chance of having PML when compared to a negative PCR in blood and/or urine (OR = 0.46 (95% CI: 0.04–8.74, P = 0.53)).

Review of the literature identified fifteen studies where JCV PCR had been performed on CSF in a large (n > 30) series of patients (Table 3) (Alvarez-Lafuente et al., 2007; Behzad-Behbahani et al., 2003; Ferrante et al., 1997, 1998; Fink et al., 2006; Fong et al., 1995; Gibson et al., 1993; Hammarin et al., 1996; Iacobaeus et al., 2009, 2013; Korálnik et al., 1999; McGuire et al., 1995; Perrons et al., 1996; Vago et al., 1996; Weber et al., 1994). In most of these studies, the studied population consisted of HIV patients and healthy controls. However, three studies (Alvarez-Lafuente et al., 2007; Ferrante et al., 1998; Iacobaeus

Table 2
Features of patients with 'definite PML'. Clinical features, radiological features, underlying disorder, immune suppression state, diagnosis and JCV PCR titer of immunocompromised patients with compatible clinical and radiological features. Abbreviations: CNS, central nervous system; PRES, posterior reversible encephalopathy syndrome; DWI, diffusion weighted imaging; T1+, enhancement on T1 with Gadolinium; SLE, systemic lupus erythematosus; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome.

Diagnosis	Clinical features	Radiological features	Underlying disorder	Immune suppression	JCV titer (log copies)
1 PML	Subacute focal neurological deficit	Multifocal T2 & DWI hyperintense lesions	SLE	Mycophenolate	6.32
2 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Hematological malignancy	Rituximab	< 2.70
3 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Solid organ transplant	Mycophenolate Cyclosporine	3.54
4 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	HIV	AIDS	5.06
5 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	HIV	AIDS	4.60
6 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Hematological malignancy	Rituximab	> 6.70
7 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Solid organ transplant	Mycophenolate Tacrolimus	3.39
8 PML	Subacute focal neurological deficit	Multifocal T2 & DWI hyperintense lesions	Hematological malignancy	Leukopenia	4.38
9 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Bone marrow transplant	Cyclosporine	3.02
10 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	HIV	AIDS	2.99
11 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Hematological malignancy	Rituximab	2.82
12 PML	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	HIV	AIDS	3.90
13 CNS lymphoma	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Solid organ transplant	Rituximab	< 2.70
14 CNS cystinosis	Subacute cognitive deterioration	T2 hyperintense lesion	Solid organ transplant	Cyclosporine	< 2.70
15 CNS lymphoma	Subacute focal neurological deficit	T2 and T1+ hyperintense lesion	HIV	AIDS	< 2.70
16 Septic embolisms	Subacute focal neurological deficit	Multifocal T2 hyperintense lesions	Gynecological malignancy	Chemotherapy	< 2.70
17 PRES	Seizures	Multifocal T2 hyperintense lesions	Solid organ transplant	Tacrolimus	< 2.70

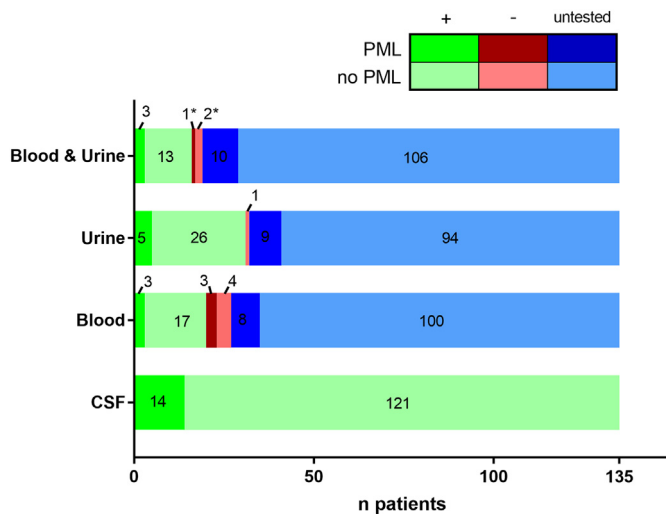


Fig. 3. JCV PCR in blood and urine. Representation of the distribution of the 135 index patients with positive CSF JCV PCR according to qualitative result of JCV PCR on blood and/or urine. PML cases are indicated in dark shades. The patients indicated with an asterisk (*) had a negative PCR in blood and a positive PCR in urine.

et al., 2009) investigated multiple sclerosis (MS) patients, one study (Behzad-Behbahani et al., 2003) investigated patients with meningitis or encephalitis and one study (Iacobaeus et al., 2013) investigated patients with neuropsychiatric systemic lupus erythematosus. Only four of the studies were published less than 15 years ago (Alvarez-Lafuente et al., 2007; Fink et al., 2006; Iacobaeus et al., 2009, 2013). Only four of the studies used a quantitative method (Alvarez-Lafuente et al., 2007; Iacobaeus et al., 2009, 2013; Korálnik et al., 1999). Pooling of these literature data showed that CSF JCV PCR was positive in 152 of 174 PML cases while it was falsely positive in 31 of 1847 controls, resulting in a sensitivity of 87.4% (95% CI: 81.6–91.5%), a specificity of 98.3% (95% CI: 97.6–98.8%) and a PPV of 83.1% (95% CI: 77.0–87.8%).

4. Discussion

We evaluated the performance of JCV PCR on CSF, blood and urine for the diagnosis of PML in clinical practice. Although sensitivity and specificity was not deducible from our data, we calculated a PPV of 10.4% for a positive CSF JCV PCR. This is significantly lower than the 83.1% derived from literature. There are several possible explanations for this.

First, since the PCRs in this study have been performed in a real-world clinical context, no indication criteria were applied and the decision to perform CSF JCV PCR was at the discretion of the treating physician. Since PML has a very low prevalence, the low PPV might reflect a relatively high index of suspicion for PML among physicians. Indeed, the majority (57.0%) of patients didn't exhibit compatible clinical or radiological features at the time of the lumbar puncture and a considerable proportion (32.6%) of patients was not immunocompromised. However, even in immunocompromised patients with both compatible clinical and radiological features (i.e. 'definite PML') the PPV was only 70.6%, indicating that even in patients with a solid indication for CSF JCV PCR testing a false-positive result is frequent.

Second, there is an important difference in the constitution of the control population (i.e. non-PML patients). The studies reported so far made use of selected groups of control patients (mostly HIV and MS patients) who were often even asymptomatic. On the contrary, our control population was composed of clinical cases among whom PML was often in the differential diagnosis. We believe that these, often critically ill, patients have a higher chance of hematogenic or urinary contamination of the liquor sample, either in vivo or during lumbar puncture. The finding in our series that the vast majority of CSF positive patients also has a positive blood and/or urine JCV PCR (if tested) supports this hypothesis.

Third, because of the real-world clinical context, with CSF samples often analyzed at the same moment as highly positive urine or blood samples, a non-specific reaction in some CSF samples during PCR might explain some false-positive samples. Accordingly, since the implementation of repeated testing of weakly positive samples a lower false-positive rate has indeed been observed.

Fourth, since this is a single center study, it is likely that differences in technique (e.g. PCR primers) may be partially responsible for the difference in PPV observed when compared to published series. Of notice, since the PCR method used here is also able to detect BK polyomavirus DNA, some false-positive PCRs might be explained by presence of BK polyomavirus DNA in the CSF sample. Also, the PCR assay used is an in-house test with a limit of quantification set at 500 copies/mL based on plasmid as mentioned in Herman et al. (Herman et al., 2004). The limit of detection was later on determined at 300 copies/mL. It is important to realize that results of this type of studies may differ, given the differences in performance characteristics of the assays used and the fact that there is no standardization of JCV PCR assays. This also explains the large range in the PPV (0 to 100%) of JCV PCR to diagnose PML found in literature. On the other hand, since most reported studies were performed more than 15 years ago, it is likely that the current PCR method has a higher sensitivity than before, implicating that weakly positive CSF samples might have been missed previously. The finding that, when stratified/dichotomized, a weakly positive CSF JCV

Table 3

Literature review Literature review of CSF JCV PCR in PML patients and controls. Fifteen studies were identified, with an overall positive predictive value of 83.1%.

Study	Quantitative PCR?	PML (n)	Controls (n)	True positive (n)	False positive (n)	PPV (%)
Gibson et al. (1993)	No	13	41	10	0	100.0
Weber et al. (1994)	No	3	30	3	0	100.0
Fong et al., (1995)	No	23	48	17	2	89.5
McGuire et al. (1995)	No	26	130	24	11	68.6
Hammarin et al. (1996)	No	20	192	20	0	100
Vago et al. (1996)	No	13	16	8	0	100
Perrons et al. (1996)	No	23	67	19	0	100
Ferrante et al. (1997)	No	12	52	11	0	100
Ferrante et al. (1998)	No	0	175	0	11	0.0
Korálnik et al. (1999)	Yes	14	92	13	1	92.9
Behzad-Behbahani et al. (2003)	No	0	151	0	2	0.0
Fink et al. (2006)	No	27	143	27	1	96.4
Alvarez-Lafuente et al. (2007)	Yes	0	73	0	2	0.0
Iacobaeus et al. (2009)	Yes	0	515	0	1	0.0
Iacobaeus et al. (2013)	Yes	0	122	0	0	N/A
TOTAL		174	1847	152	31	83.1
Current study	Yes	14	N/A	14	121	10.4

PCR has a PPV of only 1.6% whereas a moderately to strongly positive PCR has a PPV of 92.3%, supports this hypothesis. This latter rate approaches the range of the 83.1% calculated from literature data.

In total, we identified 121 patients with a false-positive CSF JCV PCR. Since the vast majority ($n = 120, 99.2\%$) had a weakly positive (< 2.7 log copies/mL) viral load, it could be argued to implement this threshold for diagnosis of PML. This is supported by the study by Iacobaeus et al. which identified one false-positive patient with a viral load of 2.01 log copies/mL, falling within our range of 'weak positivity' (< 2.7 log copies/mL) (Iacobaeus et al., 2009). Unfortunately, in the other studies of our literature search either a qualitative PCR method was used or the viral load was not mentioned. On the contrary, implementing/establishing a higher threshold will result in a significantly lower sensitivity for several reasons. First, in our series 2 out of 14 PML patients had a viral load of less than 2.7 log copies/mL. Second, two small series (Clifford et al. (Clifford et al., 2010) and Dahlhaus et al. (Dahlhaus et al., 2013)) of patients with natalizumab associated PML reported a high rate of weakly positive (< 2.7 log copies/mL) CSF JCV PCRs (57% and 53%, respectively). Third, several cases of PML with negative CSF JCV PCR (i.e. 'false negatives') have been reported (Babi et al., 2015; Landry et al., 2008). So implementing a threshold around 2.7 log copies (e.g. 2.82 based on the present ROC analysis) would indeed give rise to a considerable reduction of false-positive PCRs, but in turn some PML patients would be missed.

We observed a PPV of CSF JCV PCR of 0.0% in immunocompetent patients as well as in patients without compatible clinical or radiological features, indicating that CSF JCV PCR testing should be reserved for patients with a certain degree of clinical suspicion of PML, as suggested by the current diagnostic criteria (Berger et al., 2013). On the other hand, this also implies that a positive CSF JCV PCR in a patient without any clinicoradiological suspicion of PML (i.e. 'coincidentally positive') is almost always false positive and hence no further measures, except repeated PCR testing, should be taken. On the other end of the diagnostic spectrum, PPV of CSF JCV PCR in immunocompromised patients with compatible clinical and radiological features (i.e. the highest clinical suspicion possible) was 70.6%, supporting the view of restrictive indication for CSF JCV PCR testing. However, this also implies that almost one out of three patients with 'definite PML' according to the diagnostic criteria eventually turns out not to have PML. This suggests clinicians should be on the outlook not to miss alternative (often treatable) diagnoses. Importantly, taking into account the quantitative aspect (i.e. viral load) of the PCR increased the PPV to 100.0% and may thus be of critical aid in solidifying the diagnosis. Similarly, in patients with 'probable PML' the PPV was only 8.3%, while taking into account the viral load increased the PPV to 100.0%, again indicating the importance of the viral load and exclusion of alternative diagnoses.

We observed that repeated testing of weakly positive samples significantly decreases the rate of false-positive PCRs. This indicates that, in spite of rigorous laboratory protocols and precautions, the PCR can still be falsely positive. This is of high clinical importance, especially in real-world clinical practice where the index of suspicion for PML is very high and JCV PCR testing is performed even in patients without compatible clinical and radiological features. Therefore, pretest probability is generally low in clinical practice. Repeated testing of weakly positive samples in a clinical context of diagnostic uncertainty may partially correct for this and is hence recommended. Generally, it is recommended to reserve CSF JCV PCR for patients with compatible clinical and/or radiological features. This is in line with the current diagnostic criteria for PML, where patients with a positive CSF JCV PCR in the absence of typical clinical and radiological features are considered 'possible PML' (Berger et al., 2013).

Predictive value of JCV PCR on urine and blood was low (Fig. 3). We could not identify a specific JCV PCR profile on CSF, blood and urine being highly suggestive of PML. In fact, it was clear that the majority of patients with a positive CSF JCV PCR also had a positive PCR on blood and urine (if tested). So JCV PCR on blood and urine has no

additional value in the diagnosis of PML in patients with a positive CSF JCV PCR. We observed a relatively high proportion of positive JCV PCR in blood (i.e. 74.1% – 20 out of 27 tested individuals), which is markedly higher than the supposed proportion of JCV viremia in healthy individuals (ranging from 0% to 30% (Boukoun et al., 2016; Delbue et al., 2007; Haghghi et al., 2019; Rocca et al., 2015)). However, a significantly higher proportion has been noted in several disease states (e.g. 46.1% in MS patients (Delbue et al., 2007) and 67% in HIV patients (Rocca et al., 2015)). Therefore, as the majority of patients in our cohort was immunocompromised and/or seriously ill, this proportion is actually in line with previous data.

To conclude, in clinical practice a moderately to strongly positive CSF JCV PCR (i.e. ≥ 2.7 log copies/mL) is strongly suggestive of PML. In case of a weakly positive CSF JCV PCR (i.e. < 2.7 log copies/mL) diagnosis of PML is unlikely but not excluded, and repeated testing is recommended. Altogether it seems prudent not to rely blindly on the qualitative result of a CSF JCV PCR in the diagnosis of PML, but to consider the quantitative result (i.e. viral load) together with clinical and radiological data.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

BS and MS acquired, analyzed and interpreted the data and performed a literature research. VS was involved with microbiological testing and conducted the database query. KB was involved with microbiological testing. VS, GM, GC and AW analyzed and interpreted the data. All authors were involved in drafting and revising the manuscript.

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