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JCV-Specific Immune Responses in HIV-1 Patients with Progressive Multifocal Leukoencephalopathy

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Abbreviations: cART, combination antiretroviral therapy; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; IFN-g, interferon-gamma; IgG, immunoglobulin G; IgM, immunoglobulin M; JCV, JC virus; PBMC, peripheral blood mononuclear cells; PML, progressive multifocal leukoencephalopathy; SFU, spot-forming units; VLP, virus-like particles

Abstract

Background: Progressive multifocal leukoencephalopathy (PML) is a frequently fatal disease caused by uncontrolled polyomavirus JC (JCV) in severely immunodeficient patients. We investigated the JCV-specific cellular and humoral immunity in the Swiss HIV Cohort Study.

Methods: We identified PML cases (n=29) as well as 3 matched controls per case (n=87) with prospectively cryopreserved PBMC and plasma at diagnosis. Nested controls were matched according to age, gender, CD4+ T-cell count and decline. Survivors (n=18) were defined as being alive for >1 year after diagnosis.

Results: Using interferon-γ ELISpot, we found that JCV-specific T-cell responses were lower in non-survivors compared to their matched controls (p=0.08) which was highly significant for laboratory- and histologically confirmed PML cases (p=0.004). No difference was found between PML survivors and controls or for CMV-specific T-cell responses. PML survivors showed significant increases in JCV-specific T-cells (p=0.04) and IgG response (p=0.005). IgG responses in survivors were positively correlated with CD4+ T-cell count (p=0.049), and negatively with HIV RNA load (p=0.03).

Conclusions: PML non-survivors have selectively impaired JCV-specific T-cell responses compared to CD4+ T-cell matched controls, and fail to mount JCV-specific antibody responses. JCV-specific T-cell and IgG responses may serve as prognostic markers for patients at risk.

Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS) caused by lytic replication of the human polyomavirus JC (JCV) in oligodendrocytes [1]. PML is observed in the setting of profound cellular immunodeficiency as encountered in hematological malignancy, after hematopoietic stem cell transplantation and more recently after exposure to potent anti-lymphocyte drugs such as natalizumab [2-5]. In the last 25 years, however, PML has been most frequently diagnosed in connection with the HIV-1/AIDS epidemic [6]. Although different steps in the pathogenesis of PML have been elucidated [7-9], its somewhat erratic manifestation even in the setting of HIV/AIDS suggests that additional factors might be at play. Several lines of evidence suggest that JCV-specific cellular immunodeficiency is presumably a key factor [10-12]. As no antivirals of clinically documented efficacy are available, treatment aims at improving JCV-specific immunity. This task is difficult to achieve in the setting of cancer, chemotherapy, and transplantation [13]. Moreover, most patients are diagnosed, when neurological signs are present, typically reflecting extensive CNS disease with little time for such interventions. In HIV-AIDS, however, the availability of combination antiretroviral therapy (cART) has significantly improved the prognosis, and largely reduced the incidence rates of PML [14, 15]. Nevertheless, the mortality still approximates 50% within the first year [16, 17]. Recent data indicated that JCV-specific cytotoxic T-lymphocytes in the peripheral blood at diagnosis may be associated with better outcome, but independent studies in prospectively collected patients are missing [10, 18].

Methods

Study Participants and Setting

The Swiss HIV Cohort Study (SHCS) is a prospective, observational study of HIV-1 infected adults initiated in 1988 approved by the local IRBs [19]. The diagnosis “PML” has been encoded in the SHCS datasheet as either “definitive” if proven by histology, or as “presumptive” according to clinical, radiological or laboratory evidence i.e. JCV detected by PCR in cerebrospinal fluid (CSF). Between January 1995 and August 2006, 67 patients with the diagnosis “PML” were identified in the SHCS database and were confirmed by independent chart review. Accordingly, the diagnosis PML was classified as: i) “possible” by typical clinical and neuroradiologic findings; ii) “laboratory-confirmed” by detection of JCV DNA in CSF; iii) “definitive” by histology of brain biopsy or autopsy [20]. The neuroradiological findings on MRI were evaluated according to combined clinical and radiological criteria as elaborated in a recent consensus statement for the critical radiological distinction between multiple sclerosis and PML [9]. Patients with PML were enrolled in the study, if viable cryopreserved PBMC samples were available at the time of PML diagnosis which led to the exclusion of 38 PML cases. Of the included 29 PML patients (definitive 4, laboratory-confirmed 10, possible 15), cryopreserved PBMC samples were available within +/-6 months of diagnosis for 23 (79%) patients. Since PBMC samples were cryopreserved yearly, we aimed at obtaining one PBMC sample at diagnosis, one up to 2.5 years before and one up to 1.5 years after PML diagnosis. Plasma samples were cryopreserved every six months and all available samples within an interval between 2.5 years before up to 1.5 years after diagnosis were included. Cases and controls were selected based on the SHCS database of December 2006, and the follow-up data were based on the SHCS database of July 2008.

Identification of Controls

From the SHCS database, each PML case was matched with three control patients without PML. The date of PML diagnosis was defined as “time 0”. Cases and controls were matched according to predefined criteria at time 0: i) Calendar year (+/- 1 year), ii) time since first positive HIV test (equal or at most 2 years longer for controls), iii) age (+/- 10 years), iv) gender, v) $CD4^+$ T-cell count (+/- 25 cells/µl) at time 0, and 1 or 2 years before. In addition, controls had to have PBMC samples drawn at the same time points (+/- 6 months) as the cases relative to time 0. For 11 (38%) PML cases, $CD4^+$ T-cell counts prior to diagnosis were unavailable; hence, matching for these cases was only performed according to the time 0 $CD4^+$ T-cell count. To find enough controls, we relaxed the criteria for 34 (39%) of the controls: calendar year (+/- 3 years) for 14 controls, $CD4^+$ T-cell count at time 0 (+/- 50 cells/µl) for 11 controls and for prior $CD4^+$ T-cell counts fro 20 controls despite available prior $CD4^+$ T-cell data for the case.

Enzyme immunoassay (EIA) and ELISpot Assay (ESA)

For the EIA, virus-like particles (VLP) were produced by expressing the JCV capsid protein 1 in insect cells from a recombinant baculovirus and EIA were performed as previously described [21, 22]. JCV-specific cellular immune responses were measured in 58 samples from 29 PML cases and 104 samples from 70 controls due to non-viable PBMC. For the ESA, interferon-γ (IFN-g) production by JCV-specific T-cells was quantified after PBMC stimulation with overlapping 15mer peptide pools from JCV large T-antigen (LT) and VP1 capsid protein (VP1) of the JCV Mad-1 strain [23]. The CMV seroprevalence was 83% in the PML cases and 85% in the controls which corresponded well to the overall CMV seroprevalence of 83.4% recorded in the SHCS. For JCV, the IgG seroprevalence was 93% in the cases and 90% in the controls. CMV-specific cellular immune responses were measured in 51 samples from 24 CMV IgG seropositive cases and in 98 samples from 59 IgG seropositive controls. For CMV-specific PBMC stimulation, corresponding 15mer peptide pools spanning CMV pp65 were used [24]. IFN-g spots were counted by using an ESA reader (Cellular Technologies Ltd., Shaker Heights, USA). The number of spot forming unit (SFU) per well was calculated from duplicates after subtraction of the negative control.

Statistical Methods

We first visualized the course of the 4 immunological markers (JCV- and CMV-specific cellular immune responses and JCV-specific IgG and IgM activity) in PML cases over time. In PML non-survivors, only marker values before PML diagnosis were available. In PML survivors, we tested whether marker values systematically increase or decrease at PML diagnosis by using a linear mixed model with fixed effects for the mean marker levels before and after diagnosis, random effects for the patient-specific marker value levels before and after diagnosis and measurement error. We then concentrated on the marker values at time 0 (i.e. at the date of PML diagnosis or at the respective matching date for controls) defined as the closest available measurements to that time point. We compared these marker values between cases and controls using a logistic regression with Firth bias reduction and adjustment for the matching. This method has been shown to be an alternative to exact conditional regression for matched pairs, especially for continuous markers where the latter often leads to highly discrete or even degenerate conditional distributions [25]. The analysis was separately repeated for survivors and non-survivors, and their respective controls. As sensitivity analyses, we adjusted for (log-transformed) HIV RNA, excluded patients with possible PML diagnosis or counted only patients who died due to PML as non-survivors. In a second step, we compared PML survivors and non-survivors with respect to their time 0 immunological marker values (with or without adjustment for $CD4^+$ T-cell count) using again logistic regression with Firth bias reduction. Finally, we explored correlations between immunological markers, $CD4^+$ T-cell counts and HIV RNA. Both correlations between patients of the measurement at time 0 and correlations within patients over time were studied. For the latter, we calculated correlations for each patient contributing at least 2 measurements and then tested whether the proportion of patients with positive correlations was statistically different from 0.5 (the expected proportion if the true correlation is 0). Throughout, we used Spearman's rank correlation for quantifying the strength of the association.

The immunological markers were log-transformed prior to all analyses with “1” added to JCV- and CMV-specific cellular immune response values prior to log-transformation to deal with values of zero. All reported confidence intervals are two-sided 95% intervals and tests were performed at the two-sided 5% level. If not otherwise reported, p-values for the comparisons of continuous or categorical covariates between groups were based on the Wilcoxon test or Fisher's exact test, respectively. We used R v2.51 (R Foundation for Statistical Computing, Vienna, Austria) [26] for all analyses and the contributed R package logistf for Firth's penalized-likelihood logistic regression.

Results

Patients' characteristics

We identified 29 patients with cryopreserved PBMCs and plasma at the time of diagnosis. Table 1 summarizes the baseline characteristics of the enrolled PML cases. The median age was 39 years, 17% were female and 41% had prior AIDS-defining diseases. Median $CD4^+$ T-cell counts at diagnosis were 102 cells/µl with plasma HIV RNA of 4.18 log_{10} copies/mL. Two-thirds were treated with cART before PML diagnosis. The 11 non-survivors (38%) deceased after a median of 72 days with PML as reason for death in 9 (82%). The 18 survivors were characterized by higher $CD4^+$ T-cell counts at diagnosis (p=0.02).

JCV- and CMV-Specific Cellular Immune Response

Longitudinal measurements of all parameters including JCV- and CMV-specific T-cell response before and after PML diagnosis are shown in Figure 1. In PML non-survivors, JCV-specific T-cell responses were low, with little variation over time. By contrast, PML survivors developed a statistically significant increase in JCV-specific T-cell responses at PML diagnosis (p=0.04, crude median of all measurements until diagnosis 4 [IQR 0 – 12] and after diagnosis 10 [IQR 5 – 30]). No significant change was observed for CMV-specific immune response in survivors (p=0.22).

In the case-control analysis, PML non-survivors tended to have lower JCV-specific T-cell responses than their matched controls (p=0.08). The odds of developing PML and surviving decreased by a factor of 0.29 if the JCV-specific T-cell response increased by a factor from 0 to 10. This trend was statistically significant when the analysis was performed with laboratory-confirmed and definitive PML cases (p=0.004). Similarly, statistical significance was reached when adjusted for HIV RNA (p=0.03) or restricted to the non-survivors who died because of PML (p=0.049). By contrast, no difference for JCV-specific T-cell response was found comparing PML survivors and their controls, or for CMV-specific T-cell responses in any group (Table 2, Figure 2). JCV-specific T-cell responses correlated with 1-year survival in PML patients, but reached significance only for laboratory-confirmed or definitive PML cases ($p=0.02$) (Table 3). No correlation of CMV-specific immune responses with 1-year survival was observed.

JCV specific T-cell responses at PML diagnosis (or at the respective time 0 for controls) were detectable in 36% of non-surviving cases compared to 67% of patients surviving PML and 61% of controls. The median JCV T-cell response measured in SFU per 10^6 PBMC at diagnosis was 10 (IQR 0 – 16) in PML survivors, 0 (IQR 0 – 5) in PML non-survivors, and 5 (IQR 0 – 15) in controls. Median CMV-specific immune response at time 0 was 98 (IQR 37 – 1494) in PML survivors, 425 (IQR 43 – 1898) in PML non-survivors, and 400 (IQR 105 – 1854) in controls (Figure 2). CMV-specific T-cell responses were significantly higher than JCV-specific T-cell responses in all 3 groups (all $p<0.01$).

JCV-Specific Humoral Immune Response

JCV-specific antibodies were analyzed in 99 and 98 samples from 29 cases, respectively, and in 283 samples from 87 controls. IgG and IgM showed little variation over time in non-survivors (Figure 1). In survivors, however, we observed a statistically significant increase in IgG ($p=0.005$, crude median of all measurements until diagnosis 0.43; IQR 0.32 – 1.40; and after diagnosis 2.86; IQR 1.76 – 3.10), but no significant change for IgM responses ($p=0.22$).

At diagnosis, 93% of cases were JCV IgG seropositive. Seventeen of 18 (94%) of PML survivors had IgG activities of ≥ 0.22 OD compared to only 5/11 (45%) of PML non-survivors. In the controls, 78/87 (90%) were seropositive at time 0, and 62/87 (71%) had IgG activities of ≥ 0.22 OD. Median IgG activity at time 0 was 1.08 in PML survivors, 0.16 in PML non-survivors, and 0.33 in controls. The respective values for IgM were 0.054, 0.063, and 0.055 (Figure 2). PML non-survivors had similar JCV-specific IgG antibodies compared to matched controls. By contrast, PML survivors had significantly higher JCV-specific IgG antibodies than matched controls (p=0.003). The same was true if this analysis was adjusted for HIV RNA load (p=0.003). IgM antibodies tended to be higher in PML patients compared to matched controls without reaching statistical significance (Table 2).

Higher IgG values at diagnosis were associated with a significantly better 1-year survival of PML cases in the univariate analyses which was also true after adjustment for the $CD4^+$ T-cell count. However, the result was not significant when cases with possible PML were excluded. No association between IgM values and survival of PML cases was observed (Table 3).

Role of $CD4^+$ T-cell counts and HIV RNA for JCV-specific immune responses

Between-patient correlations of CD4+ T-cell counts, HIV RNA and the 4 markers of JCV-specific and CMV-specific immune responses at time 0 were generally not strong: In PML cases, the only rank correlations that exceeded 0.5 in absolute value were those between IgG and IgM (rank correlation 0.52, p=0.004) and between $CD4^+$ T-cells and HIV RNA (rank correlation -0.65, p<0.001) whereas in controls, the absolute value of the rank correlations never exceeded 0.5. The examined parameters showed little systematic variation over time for PML non-survivors (Figure 1). Therefore within-patient associations were only calculated for PML survivors. Significant longitudinal associations in survivors were found between IgG and $CD4^+$ T-cells (positive association in 76% of the 17 analysable patients, p=0.049), between IgG and HIV RNA load (negative association in 80% of patients, p=0.03) and between $CD4^+$ T-cells and HIV RNA load (negative association in 87% of patients, p=0.007).

Discussion

PML is a rare disease and affects almost exclusively patients with impaired immune functions as a consequence of chemotherapy, transplantation or therapy for autoimmune diseases. The highest rates of up to 8% have been reported in HIV-AIDS in the pre-cART era which have significantly declined after introducing cART [6, 16, 27]. Our nested case control study of patients prospectively enrolled in the Swiss HIV cohort study provides evidence that low T-cell immunity specifically directed at JCV is a key feature of the disease. Moreover, the study of immune responses before and after PML diagnosis supports the notion that mounting of a JCV-specific humoral and cellular immune response correlates with a better prognosis. Although limited to 29 cases, our study represents one of the largest and most comprehensive prospective, longitudinal investigations of both humoral and cellular immunity before and after PML diagnosis. Importantly, the nested controls were not only matched for age, gender, and date of manifestation, but also for $CD4^+$ T-cell count and slope of decline. Although a declining $CD4^+$ T-cell count is known to progressively increase the risk of opportunistic complications including PML [17, 28], our results suggest that the frequency of JCV-specific T-cell response is indeed a critical determinant. The lack of JCV-specific immunity would explain the sporadic onset of disease in HIV-1 patients with $CD4^+$ T-cell counts below 100/µL, as well as the exceptional cases with $CD4^+$ T-cell counts well above 200/µL. This is further supported by the fact that T-cell responses to CMV were high and not significantly different between PML-survivors, non-survivors and controls.

Up to now, there is no prospective evaluation of JCV immunity before PML diagnosis. However, the correlation of JCV-specific T-cell immunity, particularly of specific $CD8^+$ T-cells with survival has been suggested previously [10, 11, 29]. Despite methodological differences concerning in vitro the separation and expansion of T-cell subpopulations in those studies, their results seem consistent with our direct ex vivo data of PBMC [10, 11]. $CD8^+$ T-lymphocyte are critical to JCV-specific cytotoxicity, but it is now clear for cytomegalovirus and adenovirus that stable antiviral control also requires specific $CD4^+$ T-cells [30].

The JCV IgG seroprevalence of 91% at PML diagnosis was comparable to the reported prevalence in other studies [31], but significantly higher than the seroprevalence of 58% found in healthy blood donors using the same assay [22]. In the latter study, we observed an increasing IgG seroprevalence with age in line with significant JCV (re-)exposure which might play a role in some cases of PML [22]. We found that higher JCV-specific IgG activity at diagnosis was associated with better survival and that the rise in JCV IgG levels correlated with increasing $CD4^+$ T-cell counts and decreasing HIV RNA loads. This dynamic pattern emphasises the role of a functional immune response which is improved by cART through interfering with HIV replication mediated $CD4^+$ T-cell loss. Among the non-survivors, five of 11 patients were (re-) treated with cART within 3 months of PML diagnosis which likely was too late to modify the outcome.

PML survivors were able to mount higher humoral immune response than controls despite still impaired cellular immune responses. The correlation of humoral immunity with PML prognosis was previously investigated in 62 PML patients from the pre-cART era. Intrathecal IgG production was seen in 47 of 62 patients without clinical and biological correlations [32]. This argues against a major role of the humoral immunity in the pre-cART era, but should be readdressed now.

Some limitations of our study should be noted. First, given the poor prognosis of PML, the identification of surrogate markers would be desirable predicting PML before clinical manifestation. Although prospectively collected samples of the SHCS provide a unique aspect to our case-control study, we were unable to unambiguously distinguish control patients from cases at risk for PML by immunological markers alone. Possibly, other markers or their combination may be more suitable such as the detection of JCV in plasma or in

PBMC by PCR. Second, the diagnosis of PML is generally a late-stage presentation. In the setting of HIV-AIDS, this implies missed opportunities including HIV diagnosis, care and cART [33]. These factors also affect the availability of study samples prior to PML diagnosis. Third, a significant number of our patients particularly of the "survivors" fell into the category of "possible" PML, but with typical clinical and radiological MRI signs. Also, we cannot exclude that patients with "possible" diagnosis were diagnosed earlier and had therefore a better outcome due to earlier introduction of cART. Fourth, immune reconstitution syndrome (IRS) has recently emerged as a fatal complication of PML of HIV/AIDS treated with cART [34] and is reported to occur in up to 23% of all PML patients [35]. Therefore, some of the "non-survivors" with good immune responses might fall into this group. It is tempting to speculate that the higher JCV-specific humoral immune response in "survivors" could modulate a protective effect against IRS by neutralizing antigen. Interestingly, in a recent study, no difference in mortality of PML patients with or without IRS was observed [36]. Finally, the overall JCV-specific T-cell responses were low. In healthy donors and HIV-1 infected patients with CD4 > 300/µL, the median ELISpot SFU was 45 per 10^6 PBMCs showing a significant difference between JCV and CMV-specific T-cell response. Nevertheless, our results are comparable to earlier studies using other methods such as in-vitro stimulation assays to amplify responses which are time-consuming and may introduce a bias in the activated T-cell repertoire [37].

In conclusion, PML remains a significant threat in severely immunocompromised patients including HIV-AIDS and other immunodeficiencies. Our data reveal JCV-specific defects in the T-cell repertoire indicating that the JCV-specific cellular immunity is a critical determinant in PML patients irrespective of the $CD4^+$ T-cell counts. Both, cellular and humoral immunity might be used as prognostic markers future clinical studies.

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References

1. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ and Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet 1971;1:1257-1260

2. Garcia-Suarez J, de Miguel D, Krsnik I, Banas H, Arribas I and Burgaleta C. Changes in the natural history of progressive multifocal leukoencephalopathy in HIV-negative lymphoproliferative disorders: impact of novel therapies. Am J Hematol 2005;80:271-81

3. Langer-Gould A, Atlas SW, Green AJ, Bollen AW and Pelletier D. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. N Engl J Med 2005;353:375-81

4. Kleinschmidt-DeMasters BK, Tyler KL. Progressive multifocal leukoencephalopathy complicating treatment with natalizumab and interferon beta-1a for multiple sclerosis. N Engl J Med 2005;353:369-74

5. Van Assche G, Van Ranst M, Sciot R, et al. Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease. N Engl J Med 2005;353:362-8

6. Berger JR, Kaszovitz B, Post MJ and Dickinson G. Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. A review of the literature with a report of sixteen cases. Ann Intern Med 1987;107:78-87

7. Major EO, Amemiya K, Tornatore CS, Houff SA and Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev 1992;5:49-73

8. Weber F, Goldmann C, Kramer M, et al. Cellular and humoral immune response in progressive multifocal leukoencephalopathy. Ann Neurol 2001;49:636-42

9. Kappos L, Bates D, Hartung HP, et al. Natalizumab treatment for multiple sclerosis: recommendations for patient selection and monitoring. Lancet Neurol 2007;6:431-41

10. Du Pasquier RA, Kuroda MJ, Zheng Y, Jean-Jacques J, Letvin NL and Koralnik IJ. A prospective study demonstrates an association between JC virus-specific cytotoxic T lymphocytes and the early control of progressive multifocal leukoencephalopathy. Brain 2004;127:1970-8

11. Gasnault J, Kahraman M, de Goer de Herve MG, Durali D, Delfraissy JF and Taoufik Y. Critical role of JC virus-specific CD4 T-cell responses in preventing progressive multifocal leukoencephalopathy. Aids 2003;17:1443-9

12. Berger JR. JCV-specific CD4 T cell response: another piece of the puzzle in explaining some aspects of AIDS associated PML. Aids 2003;17:1557-9

13. Crowder CD, Gyure KA, Drachenberg CB, et al. Successful outcome of progressive multifocal leukoencephalopathy in a renal transplant patient. Am J Transplant 2005;5:1151-8

14. Berger JR, Houff S. Progressive multifocal leukoencephalopathy: lessons from AIDS and natalizumab. Neurol Res 2006;28:299-305

15. De Luca A, Ammassari A, Pezzotti P, et al. Cidofovir in addition to antiretroviral treatment is not effective for AIDS-associated progressive multifocal leukoencephalopathy: a multicohort analysis. AIDS 2008;22:1759-67

16. Tassie JM, Gasnault J, Bentata M, et al. Survival improvement of AIDS-related progressive multifocal leukoencephalopathy in the era of protease inhibitors. Clinical Epidemiology Group. French Hospital Database on HIV. Aids 1999;13:1881-7

17. Berenguer M, Prieto M, Palau A, et al. Severe recurrent hepatitis C after liver retransplantation for hepatitis C virus-related graft cirrhosis. Liver Transpl 2003;9:228-35

18. Koralnik IJ, Du Pasquier RA, Kuroda MJ, et al. Association of prolonged survival in HLA-A2+ progressive multifocal leukoencephalopathy patients with a CTL response specific for a commonly recognized JC virus epitope. J Immunol 2002;168:499-504

19. Ledergerber B, von Overbeck J, Egger M and Luthy R. The Swiss HIV Cohort Study: rationale, organization and selected baseline characteristics. Soz Praventivmed 1994;39:387-94

20. Cinque P, Koralnik IJ and Clifford DB. The evolving face of human immunodeficiency virus-related progressive multifocal leukoencephalopathy: defining a consensus terminology. J Neurovirol 2003;9 Suppl 1:88-92

21. Viscidi RP, Rollison DE, Viscidi E, et al. Serological cross-reactivities between antibodies to simian virus 40, BK virus, and JC virus assessed by virus-like-particle-based enzyme immunoassays. Clin Diagn Lab Immunol 2003;10:278-85

22. Egli A, Infanti, L., Dumoulin, A., Buser, A., Samarides, J., Stebler, C., Gosert, R., Hirsch, H.H. Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. J Infect Dis 2008:in press

23. Binggeli S, Egli A, Schaub S, et al. Polyomavirus BK-Specific Cellular Immune Response to VP1 and Large T-Antigen in Kidney Transplant Recipients. Am J Transplant 2007;7:1131-9

24. Egli A, Binet I, Binggeli S, et al. Cytomegalovirus-specific T-cell responses and viral replication in kidney transplant recipients. J Transl Med 2008;6:29

25. Heinze G, Schemper M. A solution to the problem of separation in logistic regression. Stat Med 2002;21:2409-19

26. R Development Core Team: R: A language and environment for Statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. 2008

27. Albrecht H, Hoffmann C, Degen O, et al. Highly active antiretroviral therapy significantly improves the prognosis of patients with HIV-associated progressive multifocal leukoencephalopathy. Aids 1998;12:1149-54

28. Berger JR, Levy RM, Flomenhoft D and Dobbs M. Predictive factors for prolonged survival in acquired immunodeficiency syndrome-associated progressive multifocal leukoencephalopathy. Ann Neurol 1998;44:341-9

29. Du Pasquier RA, Clark KW, Smith PS, et al. JCV-specific cellular immune response correlates with a favorable clinical outcome in HIV-infected individuals with progressive multifocal leukoencephalopathy. J Neurovirol 2001;7:318-22

30. Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J Exp Med 1998;188:2199-204

31. Knowles WA, Pipkin P, Andrews N, et al. Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. J Med Virol 2003;71:115-123

32. Weber T, Trebst C, Frye S, et al. Analysis of the systemic and intrathecal humoral immune response in progressive multifocal leukoencephalopathy. J Infect Dis 1997;176:250-4

33. Wolbers M, Bucher HC, Furrer H, et al. Delayed diagnosis of HIV infection and late initiation of antiretroviral therapy in the Swiss HIV Cohort Study. HIV Med 2008;9:397-405

34. Hirsch HH, Kaufmann G, Sendi P and Battegay M. Immune reconstitution in HIV-infected patients. Clin Infect Dis 2004;38:1159-66

35. Tan K, Roda R, Ostrow L, McArthur J and Nath A. PML-IRIS in patients with HIV infection. Clinical manifestations and treatment with steroids. Neurology 2009;7:7

36. Falco V, Olmo M, del Saz SV, et al. Influence of HAART on the clinical course of HIV-1-infected patients with progressive multifocal leukoencephalopathy: results of an observational multicenter study. J Acquir Immune Defic Syndr 2008;49:26-31

37. Lima MA, Marzocchetti A, Autissier P, et al. Frequency and phenotype of JC virus-specific CD8+ T lymphocytes in the peripheral blood of patients with progressive multifocal leukoencephalopathy. J Virol 2007;81:3361-8

Legend to the figures

Figure 1.

Laboratory markers over time in PML survivors and non-survivors: HIV viral load (copies/ml), $CD4^+$ T-cell count (cells/µl), JCV- and CMV-specific cellular immune response (Interferon-γ Spot forming units/Million PBMC), and JCV-specific IgG and IgM antibody activity (optical densities 492 nm).

Figure 2.

Cellular and humoral immunity in PML cases at the time of PML diagnosis and in matched controls. P-values correspond to comparisons of cases versus controls in PML survivors and non-survivors separately with adjustment for matching as described in the methods section. Box plots of data are drawn in grey, individual measurements as black dots.

Figure 1. Laboratory markers over time in PML survivors and non-survivors: HIV viral load (copies/ml), CD4 cell count (cells/µl), JCV and CMV specific cellular immune response (Interferon-y SFU/Million PBMC), and JCV VLP IgG and IgM response (Antibody activity (OD 492 nm)), Local weighted regression curve indicated a bold line.

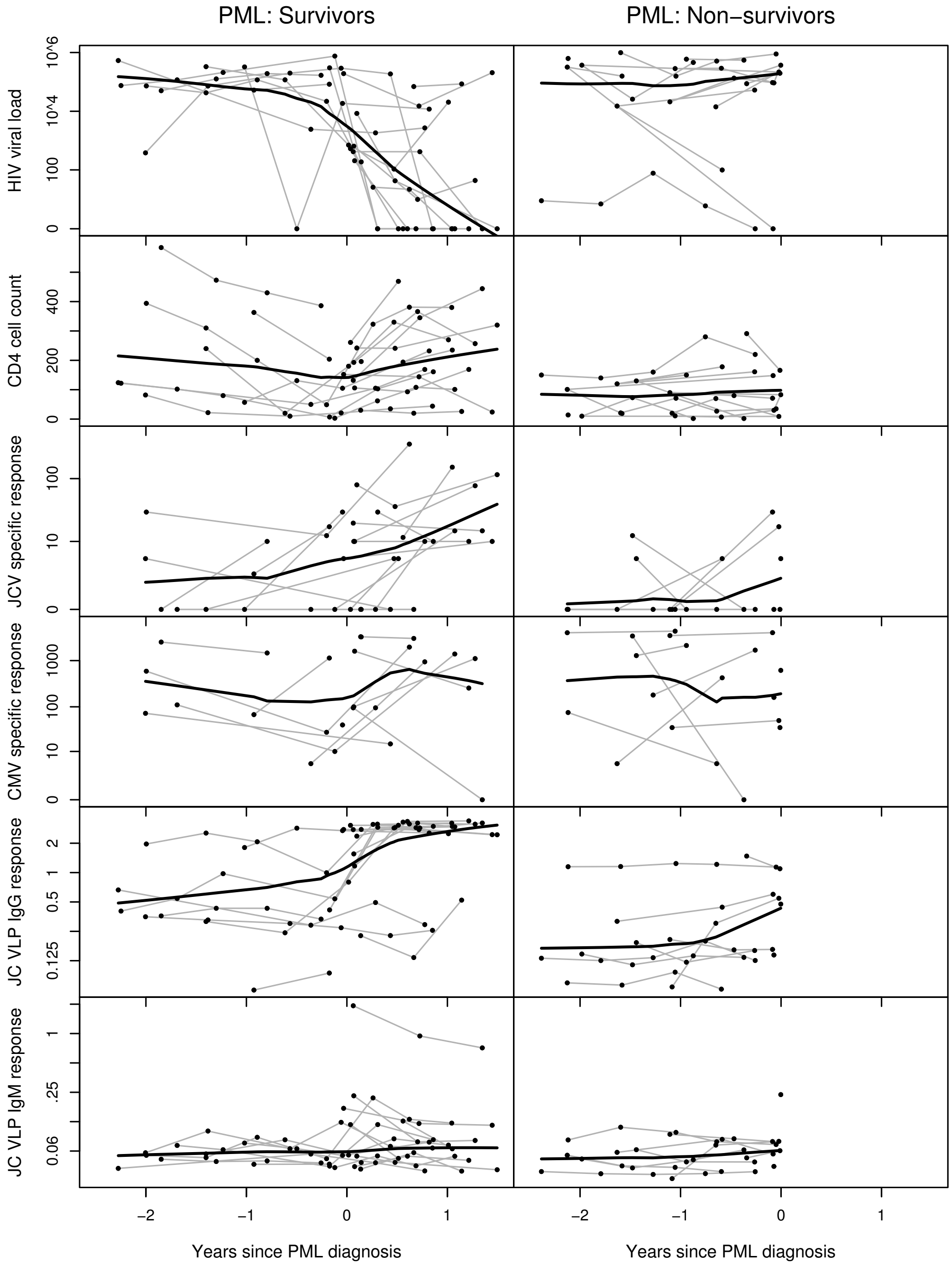

Figure 2. Cellular and humoral immunity in PML cases at the time of PML diagnosis and in matched controls. p-values correspond to comparisons of cases versus controls in PML survivors and non-survivors separately with adjustment for matching as described in the methods section. Boxplots of data drawn in gray, individual measurements as black dots.

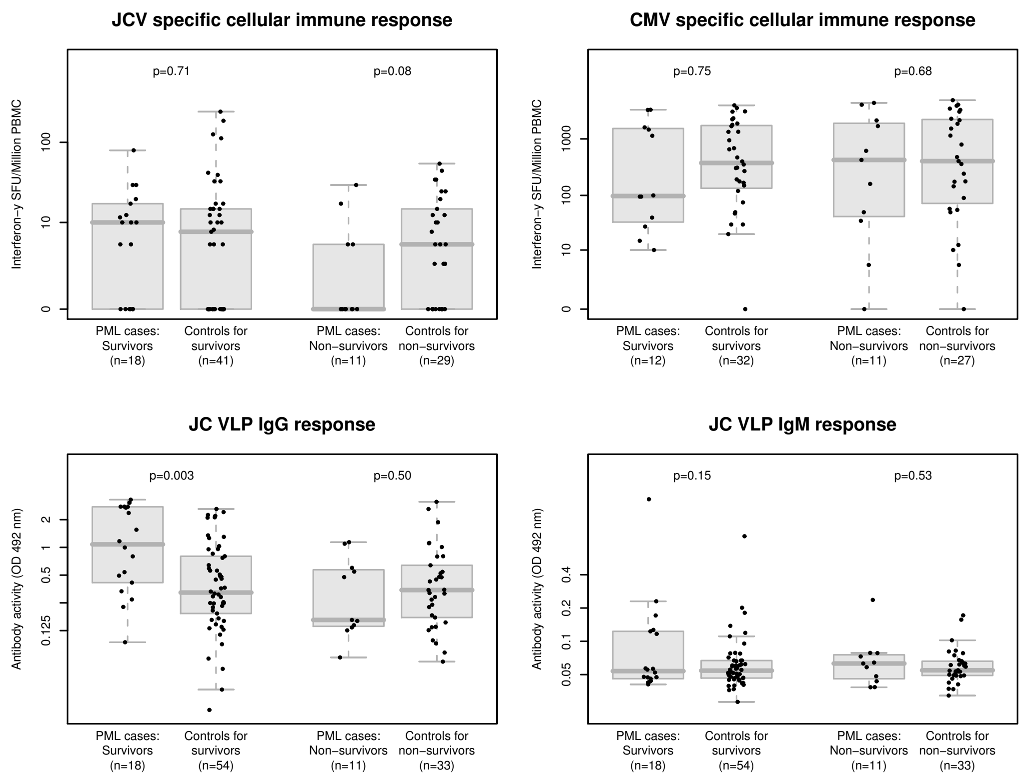

Table 1. Baseline characteristics for the 29 PML cases.

Characteristics *	Summary statistic**			p-value***
	All patients (N=29)	Survivors (N=18)	Non-survivors (N=11)	
Age (years)	39 (36, 45)	37 (34, 44)	42 (39, 45)	0.13
Female gender	5 (17%)	2 (11%)	3 (27%)	0.34
PML diagnosis				
- Definitive	4 (14%)	2 (11%)	2 (18%)	0.09
- Laboratory-confirmed	10 (34%)	4 (22%)	6 (55%)	
- Possible	15 (52%)	12 (67%)	3 (27%)	
Years since first positive HIV test	8.20 (2.72, 11.34)	7.10 (0.51, 9.14)	10.81 (8.30,13.19)	0.03
CD4 cell count (cells/µl)	102 (35, 152)	119 (82, 192)	35 (18, 107)	0.02
CD8 cell count (cells/µl)	700 (455, 1137)	698 (546, 1030)	700 (425, 1168)	0.74
HIV viral load ($\log_{10}$ copies/ml)	4.18 (2.62, 5.28)	3.88 (2.62, 4.88)	4.98 (2.79, 5.41)	0.22
Prior AIDS-defining conditions	12 (41%)	9 (50%)	3 (27%)	0.27
cART status at PML diag.				
- cART naive	10 (34%)	7 (39%)	3 (27%)	0.45
- cART treated	10 (34%)	7 (39%)	3 (27%)	
- cART interrupted	9 (31%)	4 (22%)	5 (45%)	
cART (re-)initiated within 3 months of PML diagnosis?				
- In cART naïve patients	7 / 10 (70%)	6 / 7 (86%)	1 / 3 (33%)	0.10
- In cART pre-treated patients	4 / 9 (44%)	3 / 4 (75%)	1 / 5 (20%)	

* Time-dependent covariates evaluated at the time of PML diagnosis.
** Median (Interquartile range, IQR) for continuous variables, n (%) for categorical variables.
*** Survivors vs. non-survivors; based on Wilcoxon test for continuous variables and Fisher's exact test for categorical variables.

Table 2: Comparison of PML cases and their matched controls: Odds of developing PML based on cellular and humoral immunity at time 0 in all patients and in PML-survivors and non-survivors (and their matched controls) separately. Results based on logistic regression using Firth's bias reduction adjusted for matching.

Parameter	All PML patients and their controls		Patients with laboratory-confirmed or definitive diagnosis and their controls	
	Odds-ratio (95% CI)	p-value	Odds-ratio (95% CI)	p-value
JCV-specific cellular immune response *(estimates by 10-fold higher of parameter+1)*				
All patients	0.77 (0.36, 1.62)	0.48	0.20 (0.03, 0.68)	0.008
Survivors	1.19 (0.48, 3.05)	0.71	0.50 (0.09, 2.08)	0.34
Non-survivors	0.29 (0.05, 1.13)	0.08	0.03 (0.00, 0.46)	0.004
CMV-specific cellular immune response *(estimates by 10-fold higher of parameter+1)*				
All patients	0.85 (0.46, 1.54)	0.60	0.56 (0.19, 1.47)	0.24
Survivors	0.87 (0.35, 2.07)	0.75	1.41 (0.18, 11.50)	0.73
Non-survivors	0.85 (0.38, 1.88)	0.68	0.44 (0.11, 1.30)	0.14
JCV VLP IgG response *(estimates by 2-fold higher parameter value)*				
All patients	1.31 (1.01,1.75)	0.04	1.14 (0.83, 1.63)	0.42
Survivors *	1.73 (1.19, 2.70)	0.003	1.46 (0.91, 2.94)	0.13
Non-survivors	0.86 (0.53, 1.34)	0.50	0.88 (0.52, 1.43)	0.62
JCV VLP IgM response *(estimates by 2-fold higher parameter value)*				
All patients	1.45 (0.91, 2.40)	0.12	2.39 (0.99, 6.24)	0.053
Survivors	1.44 (0.87,2.48)	0.15	3.08 (1.02,11.11)	0.047
Non-survivors	1.48 (0.41, 5.32)	0.53	1.51 (0.35, 6.65)	0.56

* If results are based on non log-transformed JCV VLP IgG response instead, which is equally sensible based on the data, results for survivors are significant both in all survivors (p=0.001) and in survivors with laboratory-confirmed or definitive diagnosis and their controls (p=0.03).

Table 3. Logistic survival for estimating the effect of cellular and humoral immunity at the time of PML diagnosis on one-year survival. Univariate analyses and analyses adjusted for CD4 cell count based on logistic regression using Firth's bias reduction.

Parameter	All PML patients		Patients with laboratory-confirmed or definitive diagnosis	
	Odds-ratio (95% CI)	p-value	Odds-ratio (95% CI)	p-value
JCV-specific cellular immune response				
(estimates by 10-fold higher of parameter+1)				
- unadjusted	0.36 (0.09, 1.20)	0.10	0.06 (0.00, 0.62)	0.02
- adjusted for CD4 cell count	0.45 (0.11, 1.57)	0.21	0.11 (0.00, 1.56)	0.11
CMV-specific cellular immune response				
(estimates by 10-fold higher of parameter+1)				
- unadjusted	1.00 (0.46, 2.19)	1.00	0.67 (0.12, 2.00)	0.49
- adjusted for CD4 cell count	1.23 (0.53, 3.04)	0.63	1.15 (0.13, 19.97)	0.88
JCV VLP IgG response				
(estimates by 2-fold higher parameter value)				
- unadjusted	0.51 (0.27, 0.84)	0.007	0.62 (0.30, 1.08)	0.09
- adjusted for CD4 cell count	0.54 (0.28, 0.90)	0.02	0.67 (0.28, 1.31)	0.24
JCV VLP IgM response				
(estimates by 2-fold higher parameter value)				
- unadjusted	0.84 (0.34, 1.49)	0.58	0.46 (0.12, 1.39)	0.17
- adjusted for CD4 cell count	0.88 (0.36, 1.57)	0.68	0.29 (0.00, 1.30)	0.12