Serum biomarkers of polyomavirus infection and risk of lung cancer in never smokers

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Background: Lung cancer in never smokers is a significant contributor of cancer mortality worldwide. In this analysis, we explored the role of nine human polyomaviruses, including JC virus (JCV), BK virus (BKV) and Merkel cell virus (MCV), in lung cancer development in never smokers as there are data to support that polyomaviruses are potentially carcinogenic in the human lung.

Methods: We used multiplex serology to detect serum antibodies to polyomaviruses in a nested case–control design combining lung cancer cases and controls from four cohort studies – NYU Women’s Health Study (NYU-WHS), Janus Serum Bank, Shanghai Women’s Health Study and Singapore Chinese Health Study (SCHS).

Results: The final analyses included 511 cases and 508 controls. Seroprevalence for each polyomavirus showed significant heterogeneity by study, but overall there were no statistically significant differences between cases and controls. In total, 69.1% of the cases and 68.7% of the controls were seropositive for JCV VP1 antibody. Seropositivity for BKV was higher at 89.0% in cases and 89.8% in controls and lower for MCV at 59.3% in cases and 61.6% in controls. Similar results were obtained after adding an additional retrospective case–control study (Xuanwei study) to the analysis.

Conclusions: Our results do not support the hypothesis that seropositivity for polyomaviruses is associated with increased lung cancer risk in never smokers. Future research to evaluate relationship between polyomavirus infection and lung carcinogenesis should focus more on evaluating the presence of virus or viral nucleic acids (DNA or RNA) in lung tumour samples.
a need to investigate other risk factors associated with lung cancer in never smokers and one potential area of further research is in carcinogenesis associated with infectious agents. This is especially relevant as there is evidence to support that chronic inflammation from factors such as infection may be involved in lung carcinogenesis (Engels, 2008; Shiels et al, 2015). An infectious aetiology can be relevant to lung cancer in both ever- and never smokers, but it might be easier to identify in the latter group because of the lack of potential confounding by tobacco smoking.

Polyomaviruses are non-enveloped DNA viruses that may be potentially carcinogenic in the human lung. The first two identified human polyomaviruses – JCV and BKV – were first isolated in 1971 (Gardner et al, 1971; Padgett et al, 1971). From 2007 onwards, eight more human polyomaviruses have been identified (Allander et al, 2007; Gaynor et al, 2007; Feng et al, 2008; Schowalter et al, 2010; van der Meijsden et al, 2010; Buck et al, 2012). Serological studies till now suggest that polyomaviruses subclinically infect the general population with rates ranging from 35 to 90% Among the polyomaviruses known to date, JC virus (JCV) and Merkel cell virus (MCV) have attracted the strongest attention for a possible role in human cancer because of their presence in various tumours, their oncogenic potential in several animal models and the interaction with the Rb family of tumour suppressor genes (Bouvard et al, 2012). It has been suggested that the presence of JCV correlates with lung cancer and various other human neoplasms, including colorectal, gastric, prostate, oesophageal cancers, brain tumours and B-cell lymphoma (Weber and Major, 1997; Galateau-Salle et al, 1998; Reiss and Khalili, 2003; Del Valle et al, 2004, 2005; Hori et al, 2005; Theodoropoulos et al, 2005; Murai et al, 2007). In animal studies also, there are reported pulmonary tumours derived from JCV T-antigen (TAg) in a transgenic mouse model (Noguchi et al, 2013). Further investigation of polyomavirus infection in lung cancer is needed to explore a potential association.

The gold standard to investigate the role of transforming viral agents in human carcinogenesis is by detection of persistent, biologically active virus infection in the target organ. However, this is difficult to explore in lung cancer especially as tumour samples are available only from a selected group of cases undergoing surgery, and are difficult to obtain from unbiased series of non-cancer controls. In these circumstances, use of validated serological markers represents a promising alternative, especially in studies aimed at establishing the presence of an association rather than obtaining a precise estimate of its magnitude. Polyomaviruses studied so far lead to strong and over time stable antibody responses to structural viral proteins, that is, the major capsid protein VP1 (Kjaerheim et al, 2007; Antonsson et al, 2010a) and are highly sensitive cumulative markers of past infection. Recently, multiplex serology assays have been developed that allows simultaneous measurement of antibody responses to multiple proteins (Waterboer et al, 2005). In this analysis, we explored the role of polyomaviruses in lung cancer development in never smokers using a multiplex assay to detect serum antibodies to capsid proteins of these polyomaviruses. We have used a nested case–control design to pool data from four prospective cohort studies and also one retrospective study.

PATIENTS AND METHODS

Study design and population. Our current study is a nested case–control study of cases identified from four established prospective cohorts. Never-smoking cases of lung cancer were selected among members of two prospective cohort studies of Europeans – NYU Women’s Health Study (WHS) (Toniole et al, 1995) and Janus Serum Bank (Toniole et al, 1995) and two prospective studies of Asians – Shanghai Women’s Health Study (SWHS) (Zheng et al, 2005) and Singapore Chinese Health Study (SCHS) (Koh et al, 2010). The four cohorts have been established in the 1970s–1990s, and several rounds of follow-up have been successfully conducted, enabling the identification of incident cancer cases. Controls were matched to cases on the basis of the following established criteria: gender, never-smoking status, age at entry, calendar period of entry and other relevant criteria that are specific to each cohort (e.g. recruitment area). Consent has been obtained from all participants for future use of serum samples collected at the time of enrolment into the cohort in each of the four participating studies. We also included a retrospective case–control study in Asian Women from Xuanwei, China (Lan et al, 2002; Shen et al, 2009) in the secondary analysis. In this study, serum samples from cases were obtained after diagnosis. Study approval was also obtained from the Institutional Review Board at Icahn School of Medicine at Mount Sinai and from ethical review boards for each participating cohort.

Laboratory analysis. A 100 μl prediagnostic serum sample was obtained from each case and control from all four participating cohort studies and shipped to the Division of Molecular Diagnostics of Oncogenic Infections at the German Cancer Research Center (Deutsches Krebsforschungszentrum, DKFZ) in Heidelberg for the analysis of serological markers of infection with polyomaviruses. Serological analyses were performed by fluorescent bead-based multiplex serology as previously described (Waterboer et al, 2005; Kjaerheim et al, 2007; Michel et al, 2009; Antonsson et al, 2010a), allowing simultaneous quantification of antibodies up to 100 antigens in a high-throughput assay. Briefly, full-length viral proteins were expressed in bacteria in fusion with an N-terminal glutathione S-transferase (GST) domain. Glutathione crosslinked to casein was covalently bound to fluorescence labelled polystyrene beads (SeroMap; Luminex Corp., Austin, TX, USA), and GST-fusion proteins were affinity-purified directly on the beads. Plasma dilutions were incubated with the same volume of mixed bead sets, at a final dilution of 1:000. Bound antibodies were detected with biotinylated goat–anti human IgG (H + L) secondary antibody and streptavidin-R-phycoerythrin. Beads were examined in a Luminex 200 analyzer (xMAP, Luminex Corp.) that identifies the different bead types by their internal colour and quantifies the antibody bound to the viral antigen on the different bead types via the median R-phycocerythrin fluorescence intensity (MFI) of at least 100 beads of each bead type (Waterboer et al, 2005; Kjaerheim et al, 2007). The serological analyses included viral capsid protein-1 (VP1) and T-antigens (Tag) of nine human polyomaviruses: JC virus (JCVp1, JCTag), BK virus (BKVp1, BKTag), KI virus (KI VP1), WU virus (WU VP1), trichodysplasia spinulosa-associated polyoma virus (TSV VP1, TSV Tag), Merkel cell polyoma virus (MCV344 VP1, MCV small T Ag, MCV large T Ag), human polyoma virus 6 (HPyV6 VP1, HPyV6 Tag), human polyoma virus 7 (HPyV7 VP1, HPyV7 Tag) and human polyoma virus 10 (HPyV10 VP1, HPyV10 Tag). With respect to serological markers of polyomavirus infection, the Pearson’s correlation coefficients for the net MFI values for 188 sera analysed on two consecutive days for antibodies to BKV and JCV VP1 range from 0.83 to 1.00 (median, 0.96) (Antonsson et al, 2010a).

Statistical analysis. To calculate the association between markers of JCV and other polyomavirus infection and lung cancer risk, odds ratios and 95% confidence intervals were calculated using logistic regression in which lung cancer status was the dependent variable (outcome) and positivity to each serological markers was the main independent variables (determinant). Seropositivity for each polyomavirus was defined as virus-specific VP1 antibody ≥ 250 MFI and ≥ 400 MFI for Tag (except for seropositivity for MCV small TAg where MFI ≥ 200 considered to be seropositive).
Cut points were determined, and were chosen for each PyV by visual inspection of frequency distribution curves (percentile plots) for the inflection points of all sera tested as done in prior studies (Karagas et al., 2006; Michael et al., 2008; Paulson et al., 2010; Antonsson et al., 2010e). Analyses were also repeated after stratifying by gender, race/ethnicity, histology and quantiles of the serological marker MFI. All analyses were performed using STATA 11 (College Station, TX, USA).

## RESULTS

The study included 511 cases and 508 controls from four cohort studies; baseline characteristics for each study are listed in Table 1. Shanghai Women’s Health Study included only Asian women, Janus Serum Bank included both European men (39.4%) and women (60.8%), NYU-WHS had only women mostly of European descent (79.7%), while SCHS only had Asian participants. There were variations in mean age of sample collection (range: 45.1–65.8 years) and mean age at diagnosis (range: 64.6–70.9 years) between the four studies. Another major difference in the four studies was in the time interval between sample collection and cancer diagnosis. Majority of the participants in Janus and NYU-WHS had samples collected more than 10 years before the time of cancer diagnosis. Conversely, in SCHS and SWHS studies, most of the participants had samples collected within 10 years of cancer diagnosis. Age at sample collection, gender and race/ethnicity were similar between cases and controls in all of the five studies. For instance, MCV showed seroprevalence against VP1 in cases ranging from 36.0 to 76.7%. Overall, seroprevalence for each polyomavirus was higher in the Asian Cohorts compared with cohorts with Caucasian patients. For example, seropositivity for JCV VP1 in Janus study (predominantly Caucasian) was 59.5% in cases compared with 77.2% in SWHS and 75.4% in SCHS (Asian cohorts).

In addition, we did not find any differences in seropositivity between cases and controls in our stratified analysis based on time from sample collection to cancer diagnosis (Table 4) or gender. We also divided the participants based on their MFI into quartiles for each polyomavirus and then compared the mean MFI between the quartiles for cases and controls to explore for any potential association between antibody levels and seropositivity. However, cases and controls did not differ significantly in any of these analyses.

### DISCUSSION

Our study is the largest epidemiological study in never smokers to investigate if infection with polyomaviruses as measured by

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### Table 1. Baseline characteristics of participating studies

| Cases* (n = 511) | Controls* (n = 508) | Janus | NYU-WHS | SCHS | SWHS | Xuanwei study b |
|-----------------|-------------------|-------|---------|------|------|-----------------|
| n, Cases/controls | – | 126/124 | 64/64 | 114/114 | 207/206 | 209/209 |
| Age at sample collection | Mean ± s.d. | 56.0 ± 11.0 | 56.1 ± 11.1 | 45.1 ± 8.7 | 55.0 ± 8.1 | 65.8 ± 7.8 | 57.7 ± 8.50 | 50.7 ± 9.6 |
| Gender | Female | 433 (84.7%) | 430 (84.7%) | 152 (60.8%) | 128 (100%) | 170 (74.6%) | 413 (100%) | 418 (100%) |
| Race/ethnicity | White | 177 (34.6%) | 175 (34.5%) | 250 (100%) | 102 (79.7%) | – | – | – |
| | Asian | 322 (63.0%) | 321 (63.2%) | – | 2 (1.6%) | 228 (100%) | 413 (100%) | 418 (100%) |
| | Other | 12 (2.4%) | 12 (2.3%) | – | 24 (18.8%) | – | – | – |
| Age at diagnosis | Mean ± s.d. | 66.9 ± 9.9 | – | 65.3 ± 11.0 | 70.9 ± 9.4 | 70.5 ± 8.1 | 64.6 ± 9.2 | 50.6 ± 9.4 |
| Histology | Adenocarcinoma | 592 (58.1%) | – | 65 (51.6%) | 32 (50%) | 74 (64.9%) | 127 (61.4%) | 185 (88.5%) |
| | Squamous cell | 54 (5.2%) | – | 7 (5.6%) | 5 (7.8%) | 5 (4.4%) | 10 (4.8%) | 24 (11.5%) |
| | Small cell | 214 (21.0%) | – | 13 (10.3%) | 11 (17.2%) | 20 (17.5%) | 63 (30.4%) | – |
| Interval between sample collection and cancer diagnosis (years) | ≤ 5 | 135 (26.4%) | – | 9 (7.1%) | 4 (6.3%) | 61 (53.5%) | 61 (29.5%) | – |
| | 5–10 | 172 (33.7%) | – | 7 (5.6%) | 10 (15.6%) | 49 (42.3%) | 106 (51.2%) | – |
| | > 10 | 204 (39.9%) | – | 110 (87.3%) | 50 (78.1%) | 4 (3.5%) | 40 (19.3%) | – |

Abbreviations: Janus – Janus Serum Bank; n – number; NSCLC – non-small cell lung cancer; NYU-WHS – NYU Women’s Health Study; SCHS – Singapore Chinese Health Study; SWHS – Shanghai Women’s health study; s.d. – standard deviation.

Studies included in primary analysis – Janus, NYU-WHS, SCHS, SWHS.

Xuanwei study is a retrospective cohort study so samples were collected after the time of cancer diagnosis.
seroprevalence and quantity of antibodies against viral structural proteins are associated with lung cancer risk. After pooling serological results from four cohort studies using a nested case–control design, we did not find any significant differences in serological measurements of antibodies against each of the polyomaviruses between the cases and controls. Similar results were obtained from an additional retrospective case–control study. Our results do not support the hypothesis that seropositivity for polyomaviruses is associated with increased lung cancer risk in never smokers.

Serological studies have indicated asymptomatic JCV infection in up to 80–90% of the adult population (Weber and Major, 1997; DeCaprio and Garcea, 2013), which is similar to what reported in our analysis and, which may be activated in immunodeficient patients, resulting in progressive multifocal leukoencephalopathy (Frisque et al, 1984; White and Khalili, 2004, 2005). JC virus can transform cells, as shown by effects such as growth in agar, rapid division, prolongation of life span, unstable multicentric chromosomes, centric and acentric rings, and the ability to form dense foci in culture (Frisque et al, 1984). Intravenous or intracranial inoculation of JCV into experimental animals has been found to cause astrocytomas, glioblastomas, neuroblastomas and medulloblastomas (Reiss and Khalili, 2003). In addition, transgenic mice expressing the JCV T-antigen developed pituitary adenomas or malignant peripheral nerve sheath tumours (Gordon et al, 2000; Reiss and Khalili, 2003). The molecular mechanisms underlying oncogenesis by JCV could centre on its encoded regulatory products, T-antigens and agnoprotein. The JCV T-antigen can inactivate p53 and members of the pRb family, and deregulate the Wnt signalling pathway through stabilisation of β-catenin to promote uncontrolled proliferation and immortal survival (Ricciardiello et al, 2001; Khalili et al, 2003; Niv et al, 2005; White and Khalili, 2005). A number of studies investigating JCV in lung cancer tissue samples have been performed. In a Japanese study by the same group, 25 of 62 lung cancers had TAg compared with only 4 of 23 normal lung tissues (Zheng et al, 2007). In another study by the same group, 25 of 62 lung cancers had TAg compared with only 4 of 23 normal lung tissues (P = 0.048) (Abdel-Aziz et al, 2007). In contrast, an Italian study reported that only one tumour sample out of 78 was positive (Giuliani et al, 2007). KI and WU polyomaviruses have also been identified in respiratory tract

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### Table 2. Seropositivity of polyomaviruses and association with lung cancer

| Polyomavirus | Cases | Controls |
|--------------|-------|----------|
| JCV (VP1)    | 207   | 196      |
| JCV (VP1 + TAg) | 266  | 240      |
| BKV (VP1)    | 285   | 287      |
| BKV (VP1 + TAg) | 320  | 219      |
| KI (VP1)     | 320   | 240      |
| WU (VP1)     | 320   | 240      |
| TSV (VP1)    | 320   | 240      |
| TSV (VP1 + TAg) | 320  | 240      |
| MCV (VP1)    | 320   | 240      |
| MCV (VP1 + TAg) | 320  | 240      |
| MCV (VP1 + small TAg) | 320 | 240     |
| HPyV6 (VP1)  | 226   | 239      |
| HPyV6 (VP1 + TAg) | 285  | 287      |
| HPyV7 (VP1)  | 188   | 204      |
| HPyV7 (VP1 + TAg) | 190  | 205      |
| HPyV10 (VP1) | 282   | 285      |
| HPyV10 (VP1 + TAg) | 282 | 285     |

Abbreviations: BKV = BK virus; CI = confidence interval; HPyV6 = human polyoma virus 6; HPyV7 = human polyoma virus 7; HPyV10 = human polyoma virus 10; JCV = JC virus; MCV = Merkel cell polyoma virus; n = number; TSV = trichodysplasia spinulosa-associated polyoma virus. Studies included in primary analysis = Janus, NYU-WHS, SHS, SHWS. Seropositivity for each polyomavirus VP1 defined as MFI > 250 and MFI > 400 for each TAg (except for MCV small Ag where seropositivity > 200 MFI).

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specimens, especially in children with respiratory tract infections (Ren et al., 2008; Okada et al., 2013). This raises the question whether KI or WU subclinical infection in the respiratory tract may lead to chronic inflammatory changes and ultimately lung carcinogenesis. Merkel cell virus is associated with Merkel cell carcinomas in immunocompromised hosts (Feng et al., 2008).

There have been studies conducted to investigate the prevalence of MCV in lung tumours and these have shown variable results with some studies showing MCPyV DNA present in more than 15% of the tumours (Joh et al., 2010). One of these studies also demonstrated the expression of large T RNA transcripts and antigen in lung cancer, thus indicating possible viral integration (Hashida et al., 2013). A Greek study also reported MCV DNA in 9.1% of lung tumours. Moreover, increased BRAF expression and decreased Bcl-2 expression were noted in MCV DNA-positive samples (Lasithiotaki et al., 2013). These results suggest that viral DNA integration may potentially activate cancer pathways and is an area for future investigation.

Recent developments have resulted in increased specificity and sensitivity of serological markers of past infection with known and suspected oncogenic viruses. Multiplex serology allows quantitative analysis of antibodies to multiple recombinant structural and

| Table 3. Seropositivity of polyomaviruses and association with lung cancer stratified by participating study |
|--------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Cases | Controls | Odds ratio (95% CI) | P-value |
| n | % | n | % | |
| JCV (VP1) | 75 | 59.5 | 77 | 62.1 | 0.90 (0.54–1.49) | 0.68 |
| BKV (VP1) | 105 | 83.3 | 106 | 85.5 | 0.85 (0.43–1.68) | 0.64 |
| KI (VP1) | 113 | 89.7 | 116 | 93.6 | 0.60 (0.24–1.50) | 0.28 |
| WU (VP1) | 119 | 94.4 | 118 | 95.2 | 0.86 (0.28–2.65) | 0.8 |
| TSV (VP1) | 100 | 79.4 | 99 | 79.8 | 0.97 (0.52–1.80) | 0.93 |
| MCV (VP1) | 84 | 66.7 | 79 | 63.7 | 1.14 (0.68–1.92) | 0.62 |
| HPyV6 (VP1) | 97 | 77.0 | 98 | 79.9 | 0.89 (0.49–1.62) | 0.7 |
| HPyV7 (VP1) | 69 | 54.8 | 78 | 62.9 | 0.71 (0.43–1.18) | 0.19 |
| HPyV10 (VP1) | 126 | 100.0 | 124 | 100.0 | * | |
| NYU-WHS | | | | | |
| JCV (VP1) | 34 | 53.1 | 35 | 54.7 | 0.94 (0.47–1.88) | 0.86 |
| BKV (VP1) | 55 | 83.3 | 57 | 89.1 | 0.75 (0.26–2.16) | 0.59 |
| KI (VP1) | 57 | 89.1 | 57 | 89.1 | 1 (0.33–3.03) | 1.00 |
| WU (VP1) | 56 | 87.5 | 63 | 98.4 | 0.11 (0.01–0.92) | 0.04 |
| TSV (VP1) | 52 | 65.6 | 44 | 68.8 | 0.87 (0.42–1.82) | 0.71 |
| MCV (VP1) | 51 | 79.7 | 49 | 76.6 | 1.20 (0.52–2.70) | 0.67 |
| HPyV6 (VP1) | 49 | 76.6 | 47 | 73.4 | 1.18 (0.53–2.63) | 0.68 |
| HPyV7 (VP1) | 37 | 57.8 | 36 | 56.3 | 1.07 (0.53–2.15) | 0.86 |
| HPyV10 (VP1) | 64 | 100.0 | 63 | 98.4 | * | |
| SWHS | | | | | |
| JCV (VP1) | 156 | 75.4 | 151 | 73.3 | 1.11 (0.72–1.73) | 0.63 |
| BKV (VP1) | 193 | 93.2 | 188 | 91.3 | 1.32 (0.64–2.73) | 0.45 |
| KI (VP1) | 183 | 88.4 | 182 | 88.4 | 1.01 (0.55–1.84) | 0.99 |
| WU (VP1) | 206 | 99.5 | 204 | 99.0 | 2.02 (0.18–22.45) | 0.57 |
| TSV (VP1) | 164 | 79.2 | 163 | 79.1 | 1.01 (0.63–1.62) | 0.98 |
| MCV (VP1) | 127 | 61.4 | 138 | 67.0 | 0.78 (0.52–1.17) | 0.23 |
| HPyV6 (VP1) | 75 | 156 | 169 | 82.0 | 0.67 (0.42–1.08) | 0.1 |
| HPyV7 (VP1) | 48 | 71.5 | 46 | 70.9 | 1.03 (0.67–1.58) | 0.89 |
| HPyV10 (VP1) | 64 | 96.1 | 63 | 98.4 | * | |
| SCHS | | | | | |
| JCV (VP1) | 88 | 77.2 | 86 | 75.4 | 1.10 (0.60–2.03) | 0.76 |
| BKV (VP1) | 102 | 89.5 | 105 | 92.1 | 0.73 (0.29–1.80) | 0.49 |
| KI (VP1) | 81 | 71.1 | 90 | 79.0 | 0.65 (0.36–1.20) | 0.17 |
| WU (VP1) | 109 | 95.6 | 107 | 93.9 | 1.43 (0.44–4.63) | 0.56 |
| TSV (VP1) | 85 | 74.6 | 81 | 71.1 | 1.19 (0.67–2.14) | 0.55 |
| MCV (VP1) | 41 | 36.0 | 47 | 41.2 | 0.80 (0.47–1.37) | 0.42 |
| HPyV6 (VP1) | 91 | 79.8 | 85 | 74.6 | 1.35 (0.72–2.51) | 0.34 |
| HPyV7 (VP1) | 76 | 66.7 | 72 | 63.2 | 1.17 (0.68–2.01) | 0.58 |
| HPyV10 (VP1) | 103 | 90.4 | 108 | 94.7 | 0.52 (0.19–1.46) | 0.21 |
| Xuanwei study | | | | | |
| JCV (VP1) | 153 | 73.2 | 170 | 81.3 | 1.03 (0.39–1.00) | 0.95 |
| BKV (VP1) | 160 | 76.6 | 161 | 77.0 | 0.97 (0.62–1.53) | 0.91 |
| KI (VP1) | 190 | 90.9 | 186 | 89.0 | 1.24 (0.65–2.35) | 0.52 |
| WU (VP1) | 208 | 99.5 | 207 | 99.0 | 2.01 (0.18–22.33) | 0.57 |
| TSV (VP1) | 181 | 86.6 | 181 | 86.6 | 1 (0.57–1.76) | 1 |
| MCV (VP1) | 113 | 54.1 | 101 | 48.3 | 1.26 (0.86–1.85) | 0.24 |
| HPyV6 (VP1) | 58 | 27.8 | 68 | 32.5 | 0.80 (0.52–1.21) | 0.29 |
| HPyV7 (VP1) | 23 | 11.0 | 19 | 9.1 | 1.24 (0.65–2.35) | 0.52 |
| HPyV10 (VP1) | 193 | 92.3 | 197 | 94.3 | 0.73 (0.34–1.59) | 0.44 |

Abbreviations: BKV = BK virus; CI = confidence interval; HPyV6 = human polyoma virus 6; HPyV7 = human polyoma virus 7; HPyV10 = human polyoma virus 10; JCV = JC virus; MCV = Merkel cell polyoma virus; MFI ≥250 and MFI ≥400 for each TAg (except for MCV small TAg where seropositive if MFI ≥200). *Odds ratio cannot be calculated as 100% seropositivity for polyomavirus in cases.
and infectious agents (Littman, 2006). These agents can drive lung cancer through different mechanisms, such as the expression of oncoproteins, which can alter cell cycle regulation. A marker for malignancies driven by these viral oncogenes, such as E6 and E7, has also been observed in patients with HPV-associated cancer (Paulson et al., 2007). Development of strong antibody responses to oncogenic proteins, specifically those encoded by E6 and E7, can be diagnostic early on. Transformation-associated proteins could be diagnostic markers for malignancies driven by these viral oncogenes.

In a study by Poulin and DeCaprio (2006), the seroprevalence of SV40 in the general population was reported to be anywhere between 9 and 12.7%. Kojima and colleagues (2012) found a significantly lower seroprevalence of SV40 in the general population. However, other studies with conflicting results have shown SV40 seropositivity to be higher. For example, one study found SV40 seropositivity to be higher in patients with malignant mesothelioma in some studies (Poulin and DeCaprio, 2006; Mazzoni et al., 2012; Comar et al., 2014). Moreover, other studies with conflicting results have shown that this low level seroprevalence to SV40 is actually an artefact due to cross-reactivity with other related human polyomaviruses such as BKV and JCV.

One strength of our study is the large sample size, which allowed us to explore associations with lung cancer stratified by interval between sample collection and cancer diagnosis. This approach is important as it can help identify the timing of infection and its relation to cancer development. By using a high-throughput assay, we were able to measure the seroprevalence of polyomaviruses in parallel, allowing us to determine markers that can be diagnostic for lung cancer. However, the cutoff value used to define seropositivity in our study is somewhat arbitrary, although carefully examined (Gossai et al., 2016). We did perform sensitivity analyses and found the seroprevalence data to be very robust to alterations in the cutoff.

### Table 4. Seropositivity of polyomaviruses and association with lung cancer stratified by interval between sample collection and cancer diagnosis

| Time Interval | Cases (n (%)) | Controls (n (%)) | Odds ratio (95% CI) | P-value |
|--------------|--------------|-----------------|---------------------|---------|
| < 5 years    |              |                 |                     |         |
| JCV (VP1)    | 101 (74.8)   | 98 (73.1)       | 1.09 (0.63–1.88)    | 0.75    |
| BKV (VP1)    | 125 (92.6)   | 119 (88.8)      | 1.58 (0.68–3.64)    | 0.29    |
| KI (VP1)     | 109 (80.7)   | 109 (81.3)      | 0.96 (0.52–1.77)    | 0.9     |
| WU (VP1)     | 131 (97.0)   | 128 (95.5)      | 1.54 (0.42–5.57)    | 0.51    |
| TSV (VP1)    | 100 (74.1)   | 101 (75.4)      | 0.93 (0.34–1.62)    | 0.81    |
| MCV (VP1)    | 75 (55.6)    | 74 (55.2)       | 1.01 (0.63–1.64)    | 0.96    |
| HPyV6 (VP1)  | 105 (77.8)   | 102 (76.1)      | 1.10 (0.62–1.94)    | 0.75    |
| HPyV7 (VP1)  | 92 (68.2)    | 83 (61.9)       | 1.31 (0.80–2.17)    | 0.29    |
| HPyV10 (VP1) | 129 (95.6)   | 126 (94.0)      | 1.37 (0.46–4.05)    | 0.58    |
| 5–10 years   |              |                 |                     |         |
| JCV (VP1)    | 131 (76.2)   | 125 (72.7)      | 1.20 (0.74–1.95)    | 0.46    |
| BKV (VP1)    | 155 (90.1)   | 159 (92.4)      | 0.75 (0.35–1.58)    | 0.45    |
| KI (VP1)     | 142 (82.6)   | 150 (90.1)      | 0.52 (0.27–0.98)    | 0.04    |
| WU (VP1)     | 169 (98.3)   | 167 (97.1)      | 1.69 (0.40–7.17)    | 0.48    |
| TSV (VP1)    | 138 (80.2)   | 127 (73.8)      | 1.44 (0.87–2.39)    | 0.16    |
| MCV (VP1)    | 89 (51.7)    | 102 (59.3)      | 0.74 (0.48–1.13)    | 0.16    |
| HPyV6 (VP1)  | 133 (77.3)   | 145 (84.3)      | 0.64 (0.37–1.09)    | 0.1     |
| HPyV7 (VP1)  | 115 (66.9)   | 119 (69.2)      | 0.90 (0.57–1.41)    | 0.64    |
| HPyV10 (VP1) | 162 (94.2)   | 164 (98.3)      | 0.29 (0.08–1.06)    | 0.06    |
| > 10 years   |              |                 |                     |         |
| JCV (VP1)    | 121 (59.3)   | 126 (62.4)      | 0.88 (0.59–1.31)    | 0.53    |
| BKV (VP1)    | 175 (85.8)   | 178 (88.1)      | 0.81 (0.46–1.45)    | 0.49    |
| KI (VP1)     | 183 (89.7)   | 189 (89.6)      | 1.01 (0.53–1.92)    | 0.97    |
| WU (VP1)     | 190 (93.1)   | 197 (95.7)      | 0.34 (0.12–0.97)    | 0.05    |
| TSV (VP1)    | 153 (75.0)   | 159 (78.7)      | 0.61 (0.31–1.29)    | 0.38    |
| MCV (VP1)    | 139 (68.1)   | 137 (67.8)      | 1.01 (0.67–1.54)    | 0.95    |
| HPyV6 (VP1)  | 155 (76.0)   | 152 (75.3)      | 1.04 (0.66–1.64)    | 0.86    |
| HPyV7 (VP1)  | 123 (60.3)   | 130 (64.4)      | 0.84 (0.56–1.26)    | 0.4     |
| HPyV10 (VP1) | 201 (98.5)   | 201 (99.5)      | 0.33 (0.03–3.23)    | 0.34    |

Abbreviations: BKV = BK virus; CI = confidence interval; HPyV6 = human polyoma virus 6; HPyV7 = human polyoma virus 7; HPyV10 = human polyoma virus 10; JCV = JC virus; MCV = Merkel cell polyoma virus; n = number; TSV = trichodysplasia spinulosa-associated polyoma virus. Seropositivity for each polyomavirus VP1 defined as MFI > 250 and MFI > 400 for each TAg (except for MCV small TAg where seropositive if MFI > 200).
as BKV and JCV (Poulin and DeCaprio, 2006; Kjaerheim et al., 2007; Shah, 2007).

In conclusion, we did not find an increased lung cancer risk is association with seropositivity for polyomaviruses including JCV. Therefore, future research should focus on exploring viral replication in tumour in combination with serological markers of infection especially as polyomavirus exposure can vary considerably across different populations and geographical areas as demonstrated by our study.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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