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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.

Lung cancer in never smokers is estimated to be the seventh leading cause of cancer mortality worldwide (Sun *et al*, 2007), with an incidence of 10–15 cases per 100 000 person-years in the United States and Europe (Wakelee *et al*, 2007; Thun *et al*, 2008). Recognised causes of lung cancer in never smokers include second-hand smoke (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004; Boffetta *et al*, 2009), indoor radon (National Research Council (US), 1999; Samet *et al*, 2009), indoor coal burning (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2010), occupational carcinogens (Zeka *et al*, 2006) and outdoor air pollution (Loomis *et al*, 2013). In addition, association of lung cancer risk with genetic variants on chromosome 5p15.33 (Hsiung *et al*, 2010; Truong *et al*, 2010), 6q (Amos *et al*, 2010) and 13q31.3 (Li *et al*, 2009) has been identified in never smokers. However, these known causes explain only a small proportion of never-smoking lung cancer cases (Sisti and Boffetta, 2012). There is

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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 Meijden *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) (Toniolo *et al*, 1995) and Janus Serum Bank (Toniolo *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-phycoerythrin 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 $\geqslant$250 MFI and $\geqslant$400 MFI for TAg (except for seropositivity for MCV small TAg where MFI$\geqslant$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 (P-value >0.05). Addition of Xuanwei study to the analysis increased the study size to 720 cases and 717 controls. As Xuanwei was a retrospective cohort study in Asian women, the samples were collected around the time of cancer diagnosis.

The results with seropositivity for each polyomavirus are listed in Table 2. The percentage of participants seropositive for each polyomavirus was similar between cases and controls after pooling results from all four studies. Seventy per cent of the cases and 67.9% of the controls were seropositive for JCV VP1 antibody. On combining results for seropositivity to either JCV VP1 or JCV TAg, 72.0% of the cases and 71.5% of the controls were seropositive. Similarly, we found no significant differences in cases and controls for each of the polyomaviruses for VP1 antibody seropositivity as well as combined seropositivity for VP1 and TAg (positive for at least one). These seroprevalences for polyomaviruses were also similar for cases and controls after including Xuanwei study (retrospective case-control study) in the analysis. The largest histological group was adenocarcinoma (58% of all cancers) and analysis restricted only to adenocarcinoma cases and their matched controls in presented in Table 2. There were no significant differences in seropositivity for polyomaviruses on stratified analysis although the subgroups for other histologies other than adenocarcinoma were small in size.

Seropositivity for each polyomavirus VP1 stratified by study is listed in Table 3. In the stratified analysis by study, seroprevalence for each polyomavirus showed significant heterogeneity by study but overall there were no statistical significant differences between cases and controls in any of the five studies. For instance, MCV showed seroprevalence against VP1 in cases ranging from 36.0 to 76.7%. Overall, seropositivity 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 interval 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

Table 1. Baseline characteristics of participating studies

	Cases[a] ($n=511$)	Controls[a] ($n=508$)	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%)	[b]
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.
[a]Studies included in primary analysis – Janus, NYU-WHS, SCHS, SWHS.
[b]Xuanwei study is a retrospective cohort study so samples were collected after the time of cancer diagnosis.

Table 2. Seropositivity of polyomaviruses and association with lung cancer

	Cases		Controls			
	n	%	*n*	%	Odds ratio (95% CI)	*P*-value
JCV (VP1)	353	69.1	349	68.7	1.02 (0.78–1.33)	0.90
JCV (VP1 ± TAg)	368	72.0	363	71.5	1.03 (0.78–1.35)	0.84
BKV (VP1)	455	89.0	456	89.8	0.93 (0.62–1.38)	0.71
BKV (VP1 ± TAg)	455	89.0	456	89.8	0.93 (0.62–1.38)	0.71
KI (VP1)	434	84.9	445	87.6	0.80 (0.56–1.14)	0.22
WU (VP1)	490	95.9	492	96.9	0.76 (0.39–1.47)	0.41
TSV (VP1)	391	76.5	387	76.2	1.02 (0.76–1.36)	0.90
TSV (VP1 ± TAg)	392	76.7	388	76.4	1.02 (0.76–1.36)	0.13
MCV (VP1)	303	59.3	313	61.6	0.91 (0.71–1.17)	0.45
MCV (VP1 ± small TAg ± large TAg)	305	59.7	314	61.8	0.91 (0.71–1.18)	0.49
HPyV6 (VP1)	393	76.9	399	78.5	0.91 (0.68–1.22)	0.53
HPyV6 (VP1 ± TAg)	393	76.9	400	78.7	0.90 (0.67–1.21)	0.48
HPyV7 (VP1)	330	64.6	332	65.4	0.97 (0.75–1.25)	0.80
HPyV7 (VP1 ± TAg)	333	65.2	334	65.8	0.97 (0.75–1.26)	0.85
HPyV10 (VP1)	492	96.3	496	97.6	0.63 (0.30–1.30)	0.21
HPyV10 (VP1 ± TAg)	492	96.3	497	97.8	0.57 (0.27–1.22)	0.15
Analysis including adenocarcinoma only						
JCV (VP1)	207	69.5	196	66.7	1.14 (0.80–1.61)	0.47
JCP (VP1 ± TAg)	216	72.5	204	69.4	1.16 (0.81–1.66)	0.41
BKV (VP1)	265	88.9	268	91.2	0.78 (0.45–1.34)	0.37
BKV (VP1 ± TAg)	265	88.9	268	91.2	0.78 (0.45–1.34)	0.37
KI (VP1)	251	84.3	258	87.8	0.75 (0.47–1.19)	0.22
WU (VP1)	285	95.6	287	97.6	0.53 (0.21–1.36)	0.19
TSV (VP1)	230	77.2	219	74.5	1.16 (0.79–1.69)	0.44
TSV (VP1 ± TAg)	230	77.2	220	74.8	1.14 (0.78–1.66)	0.5
MCV (VP1)	162	54.4	184	62.6	0.71 (0.51–0.99)	0.04
MCV (VP1 ± small TAg ± large TAg)	163	54.7	184	62.6	0.72 (0.52–1.00)	0.05
HPyV6 (VP1)	226	75.8	239	81.3	0.72 (0.49–1.07)	0.11
HPyV6 (VP1 ± TAg)	226	75.8	240	81.6	0.71 (0.47–1.05)	0.09
HPyV7 (VP1)	188	63.1	204	69.4	0.75 (0.54–1.06)	0.11
HPyV7 (VP1 ± TAg)	190	63.8	205	69.7	0.76 (0.54–1.08)	0.12
HPyV10 (VP1)	282	94.6	285	96.9	0.56 (0.24–1.28)	0.17
HPyV10 (VP1 ± TAg)	282	94.6	286	97.3	0.49 (0.21–1.17)	0.11

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, SCHS, SWHS. Seropositivity for each polyomavirus VP1 defined as MFI ⩾ 250 and MFI ⩾ 400 for each TAg (except for MCV small Ag where seropositivity ⩾ 200 MFI).

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 that 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 analysis of JCV TAg in 103 lung carcinomas and 18 normal lung tissues, 68% of lung carcinomas were positive, compared with only 11% of normal lung tissue ($P<0.05$), indicating that JCV may be involved in lung carcinogenesis (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

Table 3. Seropositivity of polyomaviruses and association with lung cancer stratified by participating study

	Cases		Controls			
	n	%	*n*	%	Odds ratio (95% CI)	*P*-value
Janus						
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	79.0	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	[a]	
NYU-WHS						
JCV (VP1)	34	53.1	35	54.7	0.94 (0.47–1.88)	0.86
BKV (VP1)	55	85.9	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)	42	65.6	44	68.8	0.87 (0.42–1.82)	0.71
MCV (VP1)	51	79.7	49	76.6	1.20 (0.52–2.78)	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	[a]	
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)	156	75.4	169	82.0	0.67 (0.42–1.08)	0.1
HPyV7 (VP1)	148	71.5	146	70.9	1.03 (0.67–1.58)	0.89
HPyV10 (VP1)	199	96.1	201	97.6	0.62 (0.20–1.92)	0.41
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	0.63 (0.39–1.00)	0.05
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; *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).
[a]Odds ratio cannot be calculated as 100% seropositivity for polyomavirus in cases.

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

	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 (0.54–1.62)	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)	155 (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)	169 (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)	181 (89.6)	1.01 (0.53–1.92)	0.97
WU (VP1)	190 (93.1)	197 (97.5)	0.34 (0.12–0.97)	0.05
TSV (VP1)	153 (75.0)	159 (78.7)	0.81 (0.51–1.29)	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).

regulatory proteins in parallel in a high-throughput fashion (Dillner, 2005; Waterboer *et al*, 2005). This increases the sensitivity and in comparison with conventional screening ELISA increases the specificity of antibody detection as it has been shown for HPV (Dillner, 2005; Waterboer *et al*, 2005), polyomaviruses (Kjaerheim *et al*, 2007) and *Helicobacter pylori* (Michel *et al*, 2009). The protein antigens used in the assay for our analysis have been shown to allow very sensitive and virus-type-specific antibody detection (Waterboer *et al*, 2005; Kjaerheim *et al*, 2007; Paulson *et al*, 2010; Liang *et al*, 2012; Teras *et al*, 2015). Moreover, there is ample evidence supporting the use of serological markers in detection of polyomaviruses. Systemic infections, as observed with all 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 thus highly sensitive cumulative markers of past infection, while antibodies to polyomaviral T-Antigens are rare and usually of low titer. However, strong antibody responses to the large TAg of MCV have been found to be very significantly associated with Merkel cell carcinoma (Paulson *et al*, 2010). Development of strong antibody responses to oncogenic proteins E6 and E7 has also been observed in patients with HPV-associated cervical (Meschede *et al*, 1998) or oropharyngeal (Zumbach *et al*, 2000; Liang *et al*, 2012) cancers, thus suggesting that antibodies to early, transformation-associated proteins could be diagnostic markers for malignancies driven by these viral oncogenes.

One strength of our study is the large sample size after pooling data and samples from five large lung cancer studies in comparison with previous studies exploring association between lung cancer and infectious agents (Littman *et al*, 2005; Chaturvedi *et al*, 2010; Koshiol *et al*, 2011; Anantharaman *et al*, 2014). Moreover, in four of the five studies, we had access to samples collected before tumour development, thus avoiding potential bias from reverse causality and inadequate selection of controls. Our study also had a representation from different geographical areas. Moreover, in addition to JCV and BKV, we report the seroprevalence results for seven other human polyomaviruses that have been identified recently (after 2007) and, therefore, data for these polyomaviruses in literature are limited. One limitation of our study is that the MFI cutoff value used to determine seropositivity in this 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. Also, we observed variation on seroprevalence of polyomaviruses based on ethnicity and geographical area which may make the interpretation of results difficult. In addition, we did not adjust for other potential confounders such as second-hand smoking and radon exposure as data for these confounders were not available across all five studies. We also did not adjust for multiple comparisons, but this may not be clinically relevant as we did not find significant association between polyomaviruses seroprevelence and lung cancer risk. We have not included measurement of polyomavirus Simian virus 40 (SV 40) seroprevalence in our study. Simian virus 40 seroprevalence has been reported to be anywhere between 9 and 12.7% in the general population and higher at 26% in patients with malignant mesothelioma in some studies (Poulin and DeCaprio, 2006; Mazzoni *et al*, 2012; Comar *et al*, 2014). However, other studies with conflicting results have shown that this low level seroprevalnce to SV40 is actually an artefact due to cross-reactivity with other related human polyomaviruses such

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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