



# No association between human herpesvirus or herpesvirus saimiri and idiopathic pulmonary fibrosis

To the Editor:

Idiopathic pulmonary fibrosis (IPF) is a rare, progressive and irreversible lung disease leading to death within 3 to 5 years of diagnosis [1]. The current hypothesis concerning pathophysiology is that chronic alveolar epithelial cell injury leads to an aberrant reparative response resulting in chronic architecture remodelling of the lung. By inducing alveolar cell injury, viral infection or reactivation has been proposed to play a role in the pathogenesis of IPF and could act as a trigger for acute exacerbation [2]. Herpesviruses are particular suspects since a higher prevalence of Epstein–Barr virus (EBV) and human herpesvirus 8 (HHV-8) has been identified in lung samples from IPF patients [2–7]. However, many of these studies were performed in a period where steroids and immunosuppressive drugs were the standard of care for IPF patients. Mouse models have also suggested the involvement of human herpesviruses as an enhancer of the fibrotic response in lung, by inducing pro-fibrotic factors [8]. Herpesvirus saimiri (HVS), a nonpathogenic gamma herpesvirus infecting squirrel monkeys, has been associated with IPF in one study [9]. This finding has not been confirmed yet. The role of torque teno virus (TTV) has been previously suspected in acute exacerbation of IPF [10].

The aim of this study was to assess the presence of TTV, HVS and human herpesviruses DNA, using real-time PCR assays, in lung samples from IPF and control patients to evaluate their potential role in IPF pathogenesis.

Lung samples were obtained from 19 IPF patients (median (interquartile range (IQR)) age 58.0 (51–62) years), at time of diagnosis (n=1) or lung transplant (n=18) (table 1). IPF was diagnosed according to 2011 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association criteria. 13 patients were receiving antifibrotic drugs (pirfenidone n=10, nintedanib n=3), in combination with prednisone (<10 mg·day<sup>-1</sup>) in one patient; one patient received prednisone alone (<10 mg·day<sup>-1</sup>); and five patients received no specific treatment. Lung samples used as controls were obtained after cancer surgery, away from the tumour, for seven patients, and at time of lung transplant for two patients presenting evolved COPD (median (IQR) age 69 (60.0–71.0) years). All controls were free of systemic corticosteroids or immunosuppressive drugs at time of surgery. Populations were comparable for smoking status and environmental exposure, but there were significantly more females in the control group (4/9 (44.4%) versus 2/19 (10.5%)), and this group was slightly older (mean age 69 years versus 58 years). Presence of human herpesviruses DNA was assessed using commercial PCR assays: herpes simplex virus (HSV) type 1 (HSV-1), type 2 (HSV-2) and varicella-zoster virus (VZV) with RealStar Alpha Herpesvirus PCR kit (Altona Diagnostics GmbH, Germany); EBV and human cytomegalovirus (hCMV) with Artus EBV and CMV kits (Qiagen, Germany); and HHV-6, HHV-7 and HHV-8 with the HHV-6, -7 and -8 R-gene kit (Argene, France). TTV DNA was detected with TTV R-gene kit (Argene). We developed a real-time PCR assay amplifying part of the DNA polymerase gene of HVS. Briefly, 10 µL of extracted DNA was added to 2 µL of FastStart DNA MasterMix (Roche, Germany), 2.4 µL of 25 mM magnesium chloride, 0.2 µL of 20 µM forward and reverse primers (5'-TTAAAATAAATGTGCCTACAATT-3' and 5'-TGCTTATTTGTAGCTTTCATT-3', respectively),



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**There is a high prevalence of human herpesviruses in lung samples of IPF patients but this does not differ from controls, neither regarding prevalence, viral load levels nor co-infection rates. Herpesvirus saimiri DNA is not detected in any lung samples.** <https://bit.ly/2ZrKiDj>

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TABLE 1 Patient characteristics and virology results

Patient ID	Sex	Age years	Tobacco exposure <sup>#</sup>	Environmental exposure <sup>¶</sup>	Condition	HRCT pattern	Histology	IPF treatment	HSV-1	HSV-2	VZV	hCMV viral load (log <sub>10</sub> copies per 10 <sup>6</sup> cells)	EBV viral load (log <sub>10</sub> copies per 10 <sup>6</sup> cells)	HHV-6 viral load (log <sub>10</sub> copies per 10 <sup>6</sup> cells)	HHV-7	HHV-8	HVS	TTV viral load (log <sub>10</sub> copies per 10 <sup>6</sup> cells)	Viral DNA detected		
#01	Male	55	1	0	IPF	UIP	UIP	None					3.1						ND	EBV	
#02	Male	44	0	0	IPF	UIP	UIP	Pirfenidone					3.3							EBV	
#03	Male	49	1	0	IPF	Possible UIP	Probable UIP	None					2.4	2.4					3.1	EBV, HHV-6, TTV	
#04	Male	62	1	0	IPF	UIP	UIP	Pirfenidone and prednisone (exacerbation)						2.6						HHV-6	
#05	Male	66	1	1	IPF	UIP	UIP	Prednisone						2.1					2.1	HHV-6, TTV	
#06	Male	68	1	0	IPF	Probable UIP	UIP + Exacerbation	Pirfenidone											2.5	TTV	
#07	Male	58	0	0	IPF	UIP	UIP	None					2.4						2.9	EBV, TTV	
#08	Male	74	1	1	IPF	Possible UIP	UIP	Nintedanib					3.3							EBV	
#09	Male	50	1	0	IPF	Possible UIP	UIP	None					2.0	2.2					3.4	EBV, HHV-6, TTV	
#10	Male	61	1	0	IPF	Possible UIP	UIP	Nintedanib					3.1	2.4						EBV, HHV-6	
#11	Male	57	1	1	IPF	Possible UIP	UIP	Pirfenidone					3.9		+					EBV, HHV-7	
#12	Male	51	1	0	IPF	UIP	UIP + Exacerbation	None				1.6		2.0						hCMV, HHV-6, TTV	
#13	Male	60	1	1	IPF	UIP	UIP	Pirfenidone					2.7	1.9					1.8	EBV, HHV-6, TTV	
#14	Male	61	0	0	IPF	Possible UIP	UIP	Pirfenidone						1.7						2.7	HHV-6, TTV
#15	Male	64	1	1	IPF	UIP	UIP + Exacerbation	Pirfenidone					3.8							1.7	EBV, TTV
#16	Female	46	0	1	IPF	Possible UIP	UIP	Pirfenidone						2.2							HHV-6
#17	Male	57	1	1	IPF	UIP	UIP + Exacerbation	Nintedanib				5.8		1.6						7.5	hCMV, HHV-6, TTV
#18	Male	58	1	0	IPF	UIP	UIP	Pirfenidone					2.9	2.3	+					2.2	EBV, HHV-6, HHV-7, TTV
#19	Female	50	1	0	IPF	UIP	UIP + Exacerbation	Pirfenidone					0.3	1.8	+					3.8	EBV, HHV-6, HHV-7, TTV
#21	Male	69	0	0	Adenocarcinoma	NA	NA	NA						3.2						2.3	HHV-6, TTV
#22	Female	71	1	0	Adenocarcinoma	NA	NA	NA					2.9							3.8	EBV, TTV
#23	Female	77	1	0	Adenocarcinoma	NA	NA	NA												3.0	TTV
#24	Female	73	0	0	Epidermoid carcinoma	NA	NA	NA					4.0		+					1.9	EBV, HHV-7, TTV
#25	Male	52	1	0	Adenocarcinoma	NA	NA	NA				1.8								3.0	hCMV, TTV
#26	Male	71	2	0	Adenocarcinoma	NA	NA	NA						2.7						2.2	HHV-6, TTV
#27	Female	68	2	0	Epidermoid carcinoma	NA	NA	NA					3.0							2.2	EBV, TTV
#28	Male	60	1	0	COPD/emphysema	NA	NA	NA					2.6	2.8						2.4	EBV, HHV-6, TTV
#29	Male	52	1	1	COPD/emphysema	NA	NA	NA						2.9						2.5	HHV-6, TTV

Abbreviations: HRCT: high-resolution computed tomography; IPF: idiopathic pulmonary fibrosis; HSV: herpes simplex virus; VZV: varicella-zoster virus; hCMV: human cytomegalovirus; EBV: Epstein-Barr virus; HHV: human herpesvirus; HVS: herpesvirus saimiri; TTV: torque teno virus; UIP: usual interstitial pneumonia; NA: not applicable; ND: not done. #: current=2, former=1, never=0; ¶: yes=1, no=0.

0.2 µL of 10 µM FAM-probe (5'-FAM-AGACGGGCGCCGCCACAAAAGC-BHQ1-3') and 5 µL of DNase- and RNase-free water. PCR was performed with the following protocol: 8 min at 95°C, then 45 amplification cycles (95°C for 10 s, 48°C for 10 s and 65°C for 30 s). Positive controls consisted of dilutions of HVS strain C488 (ATCC VR-1414) viral stock, ranging from 2000 to 0.002 50% tissue culture infective dose. Human albumin gene was amplified to normalise number of viral DNA copies to cell input [11]. Prevalence of viral infections in IPF and control samples were compared using the Mann-Whitney and Fishers exact tests, with a significance level defined as p-value below 0.05.

At least one human herpesvirus DNA was detected in 26 out of 28 (93%) lung samples, with no difference between IPF patients (n=18/19) and controls (n=8/9; p=1.00) (table 1). HSV-1, HSV-2, VZV and HHV-8 DNA were not detected in any sample. hCMV DNA was detected in two out of 19 (10.5%) IPF samples and in one out of nine (11.1%) control samples (p=1.0). HHV-6 was detected in 12 out of 19 (63.2%) IPF samples and in four out of nine (44.4%) control samples (p=0.43), and, among HHV6-positive biopsies, median viral load was lower for IPF than for controls (2.2 and 2.9 log<sub>10</sub> copies per 10<sup>6</sup> cells, respectively; p=0.004). EBV DNA was detected in 12 out of 19 (63.2%) IPF samples and in four out of nine (44.4%) control samples (p=0.43), and median EBV viral load did not differ between IPF and controls (3.00 and 2.95 log<sub>10</sub> copies per 10<sup>6</sup> cells, respectively; p=0.72). The mean number of human herpesvirus DNAs was 1.53 in IPF patients and 1.11 in controls (p=0.10). The rate of human herpesvirus co-infections did not significantly differ between IPF and controls (n=9/19 and n=2/9, respectively, p=0.25). TTV DNA was detected in 12 out of 18 (66.7%) IPF samples and nine out of nine control samples (p=0.07), and median TTV viral load did not differ between both groups (2.70 versus 2.40 log<sub>10</sub> copies per 10<sup>6</sup> cells; p=0.70). Interestingly, TTV DNA was detected among all IPF patients presenting an episode of acute exacerbation (5/5 patients). HVS DNA was not detected in any lung samples.

In this study, we found a high prevalence of human herpesviruses, with an expression of at least one human herpesvirus in 93% of samples, and no difference in prevalence of viral infections between IPF patients and controls. This is concordant with the high prevalence of human herpesviruses in the general population. EBV and HHV-6 were the most frequently identified herpesviruses.

Previous studies reported a higher expression of EBV and/or HHV-8 in IPF patients [3, 5-]. However, those studies were performed prior to the results of the PANTHER-IPF study [12]. Samples were obtained from patients frequently treated with corticosteroids or immunosuppressive therapy that may have allowed reactivation of latent herpesviruses. In our study, most of our patients were not receiving corticosteroids or immunosuppressive therapy, with only two patients receiving low-dose prednisone at the time of lung sampling. Levels of immunosuppression in IPF patients were similar to controls, as emphasised by the similar TTV DNA levels. Moreover, as previously reported, TTV DNA was always detected in patients presenting an acute exacerbation of IPF at the time of lung transplantation [10]. Compared with previous studies, we reported a higher prevalence of human herpesviruses infections in control patients [3, 5, 13]. This may be partly due to differences with previous control populations, including a higher median age of control patients in our study, but it is probably mainly explained by the higher sensitivity of specific PCR assays compared with hybridisation assays, or to immunohistochemistry that can only detect active infections [3, 5].

We did not detect HVS DNA in any lung sample, suggesting that HVS is not involved in IPF pathogenesis, a result consistent with our current knowledge on this virus. However, these results differ from those of FOLCIK *et al.* [9], who detected HVS DNA in all IPF samples, using a hybridisation assay.

This study has several limitations, notably the limited sample size, a slight imbalance in sex ratio and age, and our inability to formally differentiate latent and productive infections with PCR assays. However, the similar prevalence of human herpesvirus infections and the absence of HVS DNA in controls and IPF patients do not support a specific role for those viruses in IPF.

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