



# SARS-CoV-2 Spike Protein and Viral RNA Persist in the Lung of Patients with Post-COVID Lung Disease.

Mark E. Fraser<sup>1</sup>, Patricia Smith<sup>1</sup>, Rabab ElMergawy<sup>1</sup>, Guang-Sheng Lei<sup>2</sup>, Ankeeta Koirala<sup>1</sup>, Radha Patel<sup>1</sup>, Haylee Wynn<sup>1</sup>, Matthias Clauss<sup>1</sup>, Ryan F. Relich<sup>2</sup>, Homer L. Twigg III<sup>1</sup>.

<sup>1</sup> Department of Pulmonary, Critical Care, Sleep, and Occupational Medicine, Indiana University School of Medicine  
<sup>2</sup> Department of Pathology and Laboratory Medicine, Indiana University School of Medicine

## Introduction

SARS-CoV-2 has resulted in over 100 million cases<sup>1</sup> with ~2% of cases severe enough to merit hospitalization.<sup>2</sup> In severe cases, up to 70% have persistent respiratory symptoms or imaging findings consistent with ongoing inflammation.<sup>3</sup> We and others have described the presence of usual interstitial pneumonia and cryptogenic organizing pneumonia in patients with post-viral lung disease, including COVID-19 infection. We hypothesize that persistent SARS-CoV-2 antigens well after acute infection may be driving this response.

## Objectives

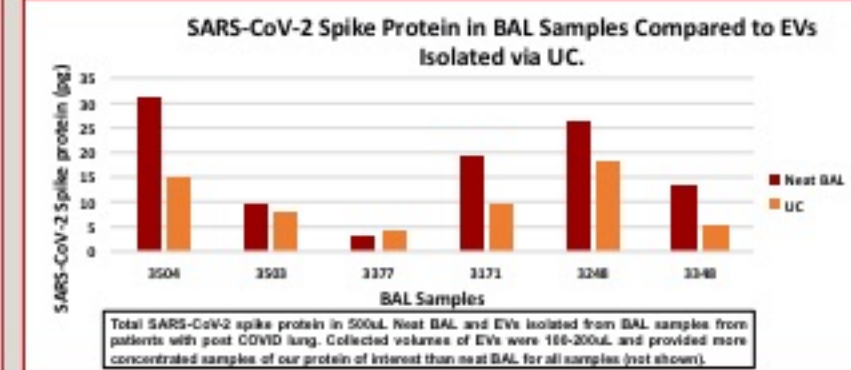
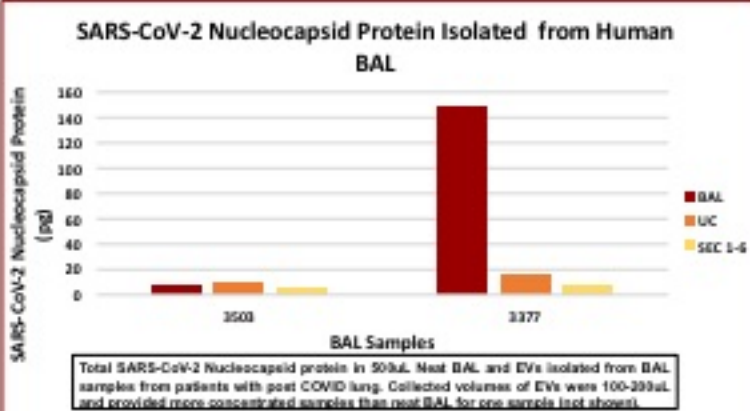
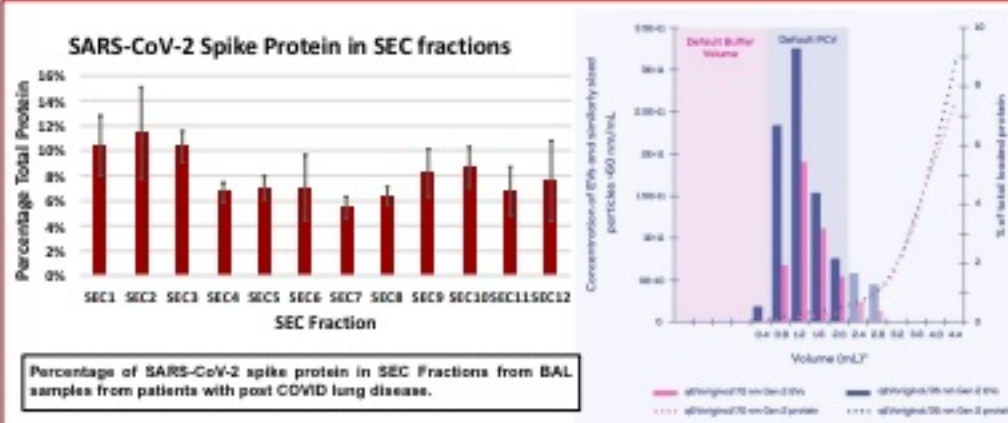
- Demonstrate presence of SARS-CoV-2 proteins and RNA in acellular BAL from patients with history of severe COVID-19 via ELISA.
- Isolate Extracellular Vesicles from BALs to determine if these are sources of residual spike protein and nucleocapsid protein well after acute infection has resolved.

## Materials & Methods

Bronchoalveolar lavage (BAL) samples sent to the Clinical BAL Laboratory at Indiana University for evaluation of possible post-COVID lung disease were studied. BAL was filtered through nylon gauze and then centrifuged at 400 g for 10 minutes to pellet cells and obtain acellular supernatants. Acellular BAL fluid was analyzed for spike protein using a commercially available ELISA kit (Kerafast) and for spike protein RNA using a laboratory-developed SARS-CoV-2 qualitative real-time PCR to determine the presence of viral RNA. In preliminary work, extracellular vesicles (EVs) were isolated from 500uL BAL samples by Size Exclusion Chromatography (SEC) where samples were filtered through a 0.65 micron filter, and then collected using Izon qEV Original columns in fractions 1-6 followed by concentration utilizing ultracentrifugation at 150,000xg for 90 minutes, and through a separate Ultracentrifugation only method (UC) where samples were filtered through a 0.65 micron filter and then ultracentrifuged twice at 150,000xg for 90 minutes each time, and analyzed for spike protein and nucleocapsid protein using a commercially available ELISA kit (Kerafast).

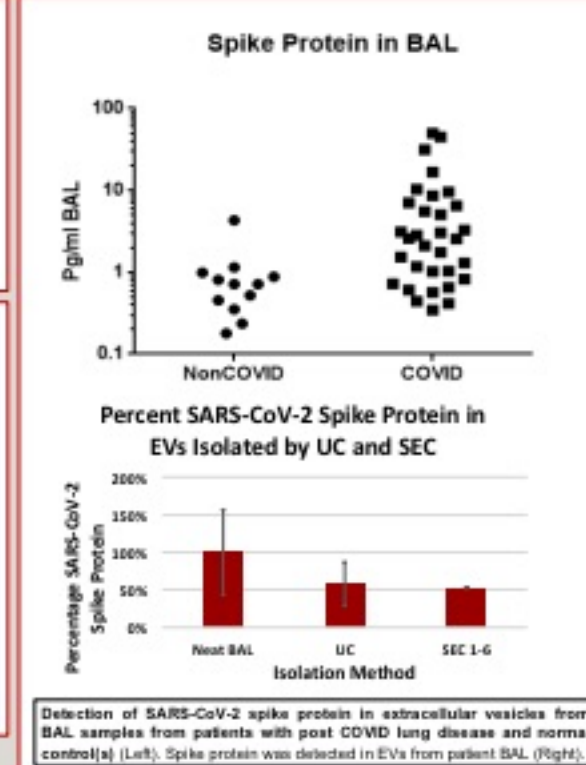
## Results

Clinical post COVID BA sample characteristics				
	Acute infection (n=8)	Normal BAL diff (n=10)	Neutrophilic Diff (n=14)	Lymphocytic Diff (n=19)
Age	67.5 ± 6.5	56.0 ± 12.9	55.2 ± 13.8	56.4 ± 11.6
Age ≥ 60	7/8	4/10	7/13	8/19
Days post COVID + Median (range)	9 (0-21)	175.5 (88-810)	181.5 (27-632)	99 (28-730)
Immunosuppressed	4/5	4/8	5/14	12/17



Detection of SARS-CoV-2 in BAL fluid by PCR and culture.							
Sample No.	PCR Ct Value	Culture Result	Days Post Positive Test	Sample No.	PCR Ct Value	Culture Result	Days Post Positive Test
BAL # 3171	32.36	Not Detected	153	BAL # 3151	37.02	Not Detected	172
BAL # 3183	33.53	Not Detected	37	BAL # 3275	31.54	Not Detected	280
BAL # 3184	33.25	Not Detected	65	BAL # 3292	ND	Not Detected	90
BAL # 3188	33.91	Not Detected	51	BAL # 3295	30.54	Not Detected	425
BAL # 3189	11.81	Detected	0	BAL # 3326	32.86	Not Detected	37
BAL # 3192	42.58	Not Detected	177	BAL # 3298	39.69	Not Detected	281
BAL # 3213	35.15	Not Detected	264	BAL # 3315	33.34	Not Detected	134
BAL # 3232	40.94	Not Detected	120	BAL # 3316	32.89	Not Detected	284
BAL # 3251	30.82	Not Detected	139	BAL # 3323	32.78	Not Detected	59
BAL # 3263	37.1	Not Detected	17	BAL # 3333	35.66	Not Detected	218
BAL # 3267	37.8	Not Detected	21	BAL # 3347	31.33	Not Detected	27
BAL # 3269	ND	Not Detected	146	BAL # 3348	ND	Not Detected	626
BAL # 3257	37.55	Not Detected	186	BAL # 3352	30.89	Detected	7
BAL # 3248	32.06	Not Detected	248	BAL # 3361	30.1	Detected	427
BAL # 3187	28.79	Not Detected	25	BAL # 3369	33.2	Not Detected	34
BAL # 3219	35.24	Not Detected	88	BAL # 3372	35.2	Not Detected	401
BAL # 3207	40.8	Not Detected	286	BAL # 3377	30.33	Detected	38
BAL # 3200	38.7	Not Detected	179	BAL # 3399	ND	Not Detected	730
BAL # 3156	38.99	Detected	0				

7 pre-pandemic BALs were studied. 1/7 specimens had a PCR Ct of 37.55. Thus we conservatively are calling any sample with a PCR Ct <35 as positive.



## Summary

Spike protein, nucleocapsid protein, and viral RNA persist in BAL from patients with post-COVID lung disease up to two years after acute infection. Preliminary data from EV isolations suggests that EVs are the primary source of residual SARS-CoV-2 proteins.

## Future Work

- Continue analyzing additional BAL samples for spike and Nucleocapsid via ELISA
- Western Blot for Spike protein and nucleocapsid proteins within isolated EVs
- ELISA and Western Blot for Tetraspanin proteins CD9, CD63, and CD81 for EV confirmation
- Proteomics to be performed on isolated EVs to determine cellular origin/possible spike protein, nucleocapsid, and viral RNA reservoir

## References

1. Center for Disease Control. COVID Data Tracker Weekly Review. <https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/index.html>
  2. Menachemi N, Dixon BE, Wools-Kaloustian KK, Yiannoutsos CT, Halverson PK. How Many SARS-CoV-2-Infected People Require Hospitalization? Using Random Sample Testing to Better Inform Preparedness Efforts. *J Public Health Manag Pract.* May-Jun 01 2021;27(3):246-250. doi:10.1097/PHH.0000000000001331
  3. Han X, Fan Y, Alwalid O, et al. Six-month Follow-up Chest CT Findings after Severe COVID-19 Pneumonia. *Radiology.* Apr 2021;299(1):E177-E186. doi:10.1148/radiol.2021203153
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