31st ECCMID Online 9 – 12 July 2021

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Characterisation of the lung virome in patients with Legionnaires' disease

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With the support of the « *Association des Internes et des Anciens Internes en Pharmacie des Hôpitaux de Lyon* »

Introduction

Legionnaires' disease (LD) due to the bacterium *Legionella* is a pneumonia for which mortality remains high (10%, up to 30%). Only few studies have explored the lung bacteriome of LD patients[1,2] and data on the lung virome are still lacking.

Aim

We aimed to characterise the diversity, richness and composition of the lung virome at the initial time and during the course of LD, and to determine whether viral signatures were associated with characteristics of LD patients.

Material & methods

We performed a shotgun viral metagenomic process adapted from Bal et *al.*[3] on 63 lung samples collected at the initial time (n=33) and during follow-up time points (n=30, from day 3 (D3) to D21) from 33 patients with LD enrolled in the french cohort ProgLegio.

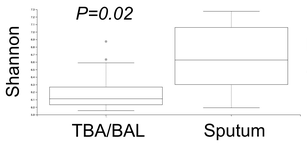
Viral metagenomic process

Sample types (n=63)
- 23 sputa
- 38 tracheobronchial aspirates (TBA)
- 2 bronchoalveolar lavages (BAL)

1. **Fluidification**
   - Dithiothreitol (v/v)
2. **Viral enrichment**
   - Centrifugation 6000G 10 min
   - RNA internal quality control (IQC) + DNA IQC
   - Filtration 0,8µm
   - Dilution (1:4 BAL & sputa; 1:10 TBA)
   - TURBO™ DNAse 37°C 90'
3. **Total nucleic acids extraction**
   - MagNA Pure 24 System (Roche)
   - Ethanol precipitation
4. **Random amplification**
   - WTA2 (Whole Transcriptome Amplification)
   - Spin column-based purification
5. **Library / Sequencing**
   - Nextera XT / Nextseq 2*150 bp Illumina
6. **Bioinformatic analyses**
   - Pipeline (aligning softwares: bwa mem, DIAMOND)

Results

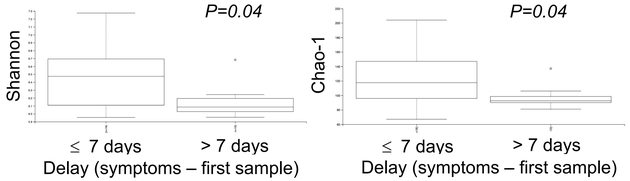
Less diversity of virome in TBA/BAL than in sputa



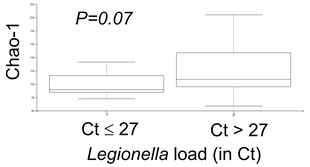
N = 33 initial samples
Mann-Whitney test

Low viral diversity and richness in samples collected lately after the onset of LD symptoms

N = 33 initial samples
Mann-Whitney tests



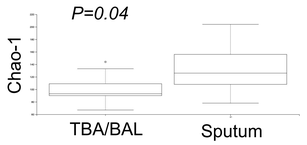
Trend of low viral richness in respiratory samples with high *Legionella* load



N = 33 initial samples
Mann-Whitney test

Ct = Cycle threshold of *Legionella* PCR

Less richness of virome in TBA/BAL than in sputa



N = 33 initial samples
Mann-Whitney test

Eight viral families were significantly more prevalent in sputum than in TBA/BAL

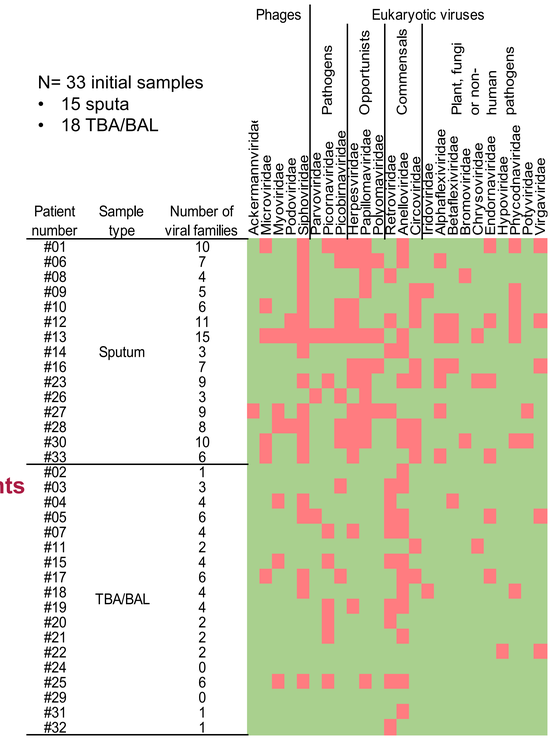
Papillomaviridae (73% *vs* 6%, *p*=0.0001)
Herpesviridae (73% *vs* 11%, *p*=0,0004)
Siphoviridae (87% *vs* 28%, *p*=0.0013)
Alphaflexiviridae (47% *vs* 0%, *p*=0.0015)
Circoviridae (53% *vs* 11%, *p*=0,02)
Picobirnaviridae (53% *vs* 11%, *p*=0.02)
Phycodnaviridae (40% *vs* 6%, *p*=0.03)
Polyomaviridae (27% *vs* 0%, *p*=0.03)

Herpesviridae reactivations highlighted in 21 LD patients

- Overall, *Herpesviridae* reads were detected in 48% (30/63) samples corresponding to 64% (21/33) of LD patients
- We had follow-up samples from D3 to D21 for 16 patients
  - At D0
    - **80% (4/5) were *Herpesviridae*-positive in sputa**
    - None (0/11) were *Herpesviridae*-positive in TBA/BAL
  - Between D3-D21
    - **100% (5/5) were *Herpesviridae*-positive in sputa**
    - **64% (7/11) were *Herpesviridae*-positive in TBA/BAL,** orotracheal intubation was reported in 78% (6/7) of them

A total of 27 viral families found in LD respiratory samples

N= 33 initial samples
- 15 sputa
- 18 TBA/BAL



Patient number	Sample type	Number of viral families
#01	Sputum	10
#06		7
#08		4
#09		5
#10		6
#12		11
#13		15
#14		3
#16		7
#23		9
#26		3
#27		9
#28		8
#30		10
#33		6
#02	TBA/BAL	1
#03		3
#04		4
#05		6
#07		4
#11		2
#15		4
#17		6
#18		4
#19		4
#20		2
#21		2
#22		2
#24		0
#25		6
#29		0
#31		1
#32		1

Green: absence of the viral family; red: presence of the viral family.

Conclusions

- Lung virome data during the course of LD showed a large variety of viral families and are depending on sample type.
- A *Herpesviridae* reactivation was observed for 21 patients (64%), among them 8 became positive during the follow-up. Reactivations of *Herpesviridae* according to the site of the reactivation (sputum or TBA/BAL) could be related to immunosuppression and/or orotracheal intubation.
- Low viral diversity and richness was found in late initial samples, which could be impacted by the lung load of *Legionella* and by the immune response to the bacterial infection.

References

[1] Mizrahi H, et *al*. Sci Rep. 2017;7(1):40114.
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[3] Bal A, et *al.* BMC Infect Dis. 2018;18(1):1-10.

Contact information

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