Monitoring respiratory syncytial virus in patients infected in different epidemiological seasons

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Introduction

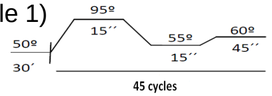
Human respiratory syncytial virus (HRSV) is a mayor etiologic agent of lower tract infections and a leading cause of hospitalizations among infants. Relating viral load and viral dynamics to disease severity will expand our understanding of RSV pathogenesis.

Objective

The monitoring of HRSV-infected patients in different epidemiological seasons.

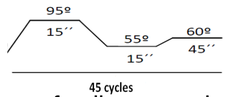
Material and methods

- ✓ **Study period:** December 2014 – April 2016
- ✓ **Patients:** 14140 (mean age: 35.50±32.45 years old; range: 1 month-104 years)
- ✓ **Samples:** 18874 respiratory samples
- ✓ **Nucleic Acid Extraction:** Magnapure automatic extractor (Roche)
- ✓ **HRSV quantification: Multiplex real-time RT-PCR (IA, IB, HRSV)**
  - Reagent: TaqMan Fast Virus 1-Step Master Mix (ABI, USA)
  - Primers/Probes: (Table 1)
  - Thermal profile:



Ct HRSV → Dilutions of HRSV amplicon harbouring plasmid → Copies of HRSV genome

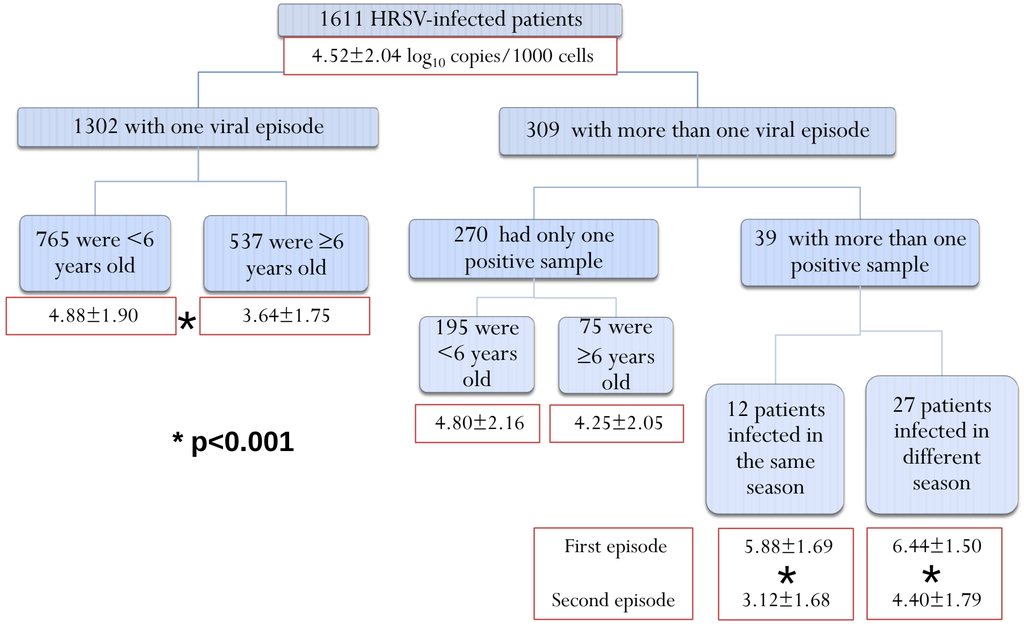
- ✓ **Cell quantification: Single real-time PCR (β-globin gene)**
  - Reagent: Brilliant III Ultra-Fast QPCR Master Mix (Agilent, USA)
  - Primers/Probes: (Table 1)
  - Thermal profile:



Ct β-globin gene → Dilutions of cell suspensions to detect β-globin gene → Number of cells

- **HRSV viral load: Ratio of copies of viral genome/number of cells (log_{10} copies/1000 cells)**

Results



*** p<0.001**

Table 1. Primers used for quantification of HRSV (F gene) and β-globin gene

Target	Primer		Probe	
	Name	Sequence (5'-3')	Name	Sequence (5'-3')
VSR-A	VSRA-TR-S	GCCAGTGGCATTGCTGTAT	VSR-VIC	AGAAGTGAACAAGATCAA
	VSRA-TR-A	CTGACTACGGCCTTGTTTGT		
VSR-B	VSRB-TR-S	GCAAGTGGTATAGCTGTAT		
	VSRB-TR-A	CTGACTACAGCTTTGTTTGT		
β-globin	Beta-TR-S	ACACAACTGTGTTCACTAGC	Beta-FAM	TGCATCTGACTCCTGAGGA
	Beta-TR-A	CCAACTTCATCCACGTTCACC		

Conclusions

1) The detection of HRSV was more frequently associated to children less than 6 years and with a higher viral load.

2) Viral load in those patients infected in different seasons was lower in the second infection than in the first one.

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