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Usefulness of Ct value in acute respiratory infections caused by respiratory syncytial virus A and B and influenza virus A (H1N1)pdm09, A (H3N2) and B☆

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ABSTRACT

Introduction: Acute respiratory infections of viral cause are very frequent entities. The difficulty in evaluating the detection of a virus in these entities could be solved by determining the viral load.
Methods: A prospective study on the mean Ct value (cycle threshold value) detected against RSV-A, RSV-B and influenza A (H1N1)pdm09, A (H3N2) and B viruses in patients of different origin and age was performed. Detection was performed using a commercial molecular amplification (RT-PCR) technique.
Results: Different mean Ct values were detected for each virus. In RSV infections, no differences were observed between those caused by RSV-A or RSV-B in children. Depending on the patient's age, the only statistical significance was observed in those included in the 0–4 month groups for RSV-A and this group and the 5–12 months group for RSV-B (higher values). A lower viral load was detected in adult patients than in paediatric patients. In influenza infections, no statistical significance was observed in the mean values detected in patients from the Red Centinela ("sentinel network", a Spanish network of doctors aimed at research and surveillance of diseases), those diagnosed in the adult emergency room or in hospital admissions. In the adult patients admitted to the ICU, only a slightly lower mean value was observed in those infected with influenza A (H1N1)pdm09, but without statistical significance. There were no patients admitted to the ICU with influenza B infection.
Conclusion: The detection of viral load could be a good tool for the evaluation, monitoring and prognosis of acute viral respiratory infections. With the exception of those caused by RSV, no significant differences were observed in influenza infections except in younger paediatric patients.

Utilidad del valor Ct en las infecciones respiratorias agudas causadas por el virus respiratorio sincitial A y B y los virus gripales A (H1N1)pdm09, A (H3N2) y B

Palabras clave:
Infecciones respiratorias agudas
Virus respiratorio sincitial
Virus gripales
Valor Ct
Carga viral

RESUMEN

Introducción: Las infecciones respiratorias agudas de causa viral son unas entidades muy frecuentes. La dificultad para valorar la detección de un determinado virus en estas entidades podría solucionarse con la determinación de la carga viral.
Métodos: Se ha realizado un estudio prospectivo sobre el valor medio de los Ct (cycle threshold value) detectados frente al VRS-A, VRS-B y los virus gripales A (H1N1)pdm09, A(H3N2) y B en pacientes de diferente procedencia y edad. La detección se ha realizado mediante una técnica de amplificación molecular (RT-PCR) comercial.

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Resultados: Se han detectado valores medios de Ct distintos para cada virus. En las infecciones por VRS, no se han observado diferencias entre las causadas por el VRS-A o VRS-B en pediatría. De acuerdo con la edad de los pacientes solo se ha observado significación estadística en los incluidos en los grupos de 0-4 meses para el VRS-A y este grupo y el de 5-12 meses para el VRS-B (valores más elevados). En los pacientes adultos se ha detectado una carga viral menor que en los pediátricos.
En las infecciones gripales no se ha observado significación estadística en los valores medios detectados en los pacientes procedentes de la Red Centinela, en los diagnosticados en las urgencias de adultos ni en los ingresados hospitalarios. En los pacientes adultos ingresados en la UCI solo se ha observado un valor medio algo más bajo en los infectados por el virus gripal A (H1N1)pdm09 pero sin significación estadística. No hubo ningún paciente ingresado en la UCI con infección por gripe B.
Conclusión: La detección de la carga viral podría ser una buena herramienta para la evaluación, seguimiento y pronóstico de las infecciones respiratorias agudas víricas. A excepción de las causadas por el VRS, no se han observado diferencias significativas en las infecciones gripales, salvo en los pacientes pediátricos de menor edad.

Introduction

Acute respiratory infections (ARIs) of viral cause are entities which affect the entire population, although their morbidity peaks at the start or end of life. Most of these infections occur each year during the winter, resulting in the so-called epidemic seasons, basically assigned to those caused by the respiratory syncytial virus (RSV) in the infant population[1] and to that caused by influenza in the general population.[2]

Molecular biology techniques applied to establishing the viral aetiology of ARIs has made it possible in recent years to determine the range of viruses involved in these conditions.[3] Despite this, RSV and influenza are the viruses with the greatest impact on public health, which should be actively monitored.[1,2]

Detection using molecular techniques, generally reverse transcription polymerase chain reaction (RT-PCR), of a specific virus in a respiratory sample of a patient with an ARI is usually considered to be a definitive aetiological diagnosis, as it is detected in less than 2% of asymptomatic patients.[4] However, the clinical translation of this virus means that its presence can be detected both in mild catarrhal or respiratory processes as well as severe respiratory symptoms with a risk of associated mortality.[1,2]

One option for trying to predict or determine the significance of a virus in these patients would be the quantification of the viral load in the respiratory sample. The hypothesis would be that with a higher viral load there is higher morbidity or mortality associated with the infectious process.[5–7]

For the past three years, we have been using a commercial RT-PCR technique for this viral diagnosis. It is initially qualitative, i.e. it detects whether the viruses are present or absent. However, the Ct (cycle threshold) value is presented in the final result. This expresses the cycle in which the detection system considers that the sample is positive for a certain virus (its value ranges between 0 and 40).[3] In this way, the samples with a high viral load have a low Ct value and those with a low viral load have a higher Ct value (they need more amplification cycles to cross the positivity threshold).

With this concept and the Ct value obtained in each respiratory sample, we studied the ARIs caused by RSV and influenza viruses, and analysed its mean value in different age groups and hospital location.

Material and methods

A prospective study was carried out on the mean Ct value of patients with an ARI caused by RSV and influenza viruses. All respiratory samples, nasopharyngeal aspirate for children <3 years-old and pharyngeal smears for children <3 years-old, were analysed using a commercial molecular technique (RT-PCR), which simultaneously and differentially detects 19 different respiratory viruses (Allplex, Seegen; North Korea). In addition to the sample being positive, the Ct value, in which the automated detection system considered the sample of a specific virus to be positive, was recorded. RSV antigen detection was performed in 77 samples using commercial immunochromatography (Binax RSV, Alere, Barcelona).

To avoid bias, the first 150 respiratory samples positive for RSV-A, RSV-B, influenza A (H1N1)pdm09, influenza A (H3N2) and influenza B (total 750 samples) were analysed. For this, the influenza A (H1N1)pdm09 and influenza B values corresponded to the 2015–2016 influenza season (due to its predominance) and the influenza A (H3N2) values corresponded to the cases from the 2016–2017 season (for the same reason). To avoid interference, samples which had co-infections with any of the viruses studied were removed. The Ct values of patients with RSV-A and RSV-B corresponded to both seasons.

The patients were classified according to age, children (<14 years) or adults (>14 years). In the ARIs caused by RSV, a classification was made between those that presented with a single viral infection and those that presented with a mixed infection (co-infection). Similarly, the mean value of the Cts was evaluated in relation to the samples which were previously positive in the antigen detection test (only in the paediatric population).

Patients with an ARI caused by the influenza viruses were classified in terms of their location at the time positivity was detected, into the following groups: those from the influenza sentinel surveillance network (SN), those from adult hospital emergency departments (AHED), those admitted to the Intensive Care Unit (ICU) and paediatrics.

Statistical analyses were performed using the SPSS 21 program (http://www.spss.com), considering $p < 0.05$ as a significant value.

Results

The mean Ct value detected in the samples positive for RSV-A was 23.75 (range 17.02–33.76) and 24.49 for RSV-B (range 15.29–36.68). In patients with co-infection with another respiratory virus, its mean value was 22.94 for RSV-A and 23.44 for RSV-B.

A significant difference was noted between the mean Ct value observed and that observed in adult patients infected with RSV-A (32.46; $p < 0.05$) and with RSV-B (27.85; $p < 0.05$). This significance was also observed in those samples which were positive in the antigen detection test, both for RSV-A (19.18; $p < 0.05$) and for RSV-B (19.16; $p < 0.05$) (Table 1).

The mean Ct value observed in the patients with infection caused by the influenza A virus (H1N1)pdm09 was 31.02