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Evaluation of a rapid antigen detection test for the diagnosis of emergency influenza A H1N1 pandemic in a pediatric population

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Abstract

Introduction: The immunochromatographic capillary method (ICC) provides rapid results that can help the individual treatment of patients. The aim was to evaluate the diagnostic accuracy of an ICC rapid test for detection of influenza virus in pediatric emergencies.

Material and methods: 225 samples were collected from pediatric patients and tested by ICC and RT-PCR (as gold standard).

Results: Overall, the ICC sensitivity and specificity values were 51% and 100%, respectively. Sensitivity in throat swabs and nasal aspirates was 46.6% and 52.6% respectively. In regards to gernder, flu was diagnosed by PCR in 21 out of 110 males (19.1%) and 32 out of 115 females (27.8%).

Conclusion: Although the ICC displayed limited sensitivity, the excellent positive predictive value it could be useful in the presumptive diagnosis of emergency.

Keywords

Immunochromatographic capillary, RT-PCR, influenza A H1N1

Resumen

Título: Evaluación de un test de detección antigénica rápido para el diagnóstico de urgencias de gripe A H1N1 pandémica en la población pediátrica

Introducción: La inmunocromatografía capilar (ICC) ofrece resultados rápidos que pueden ayudar al tratamiento individual de los pacientes. El objetivo ha sido evaluar la eficacia diagnóstica de un test rápido de ICC para la detección virus gripales y su aplicación en urgencias pediátricas.

Material y métodos: Se analizaron 225 muestras mediante ICC y RT-PCR (método de referencia) procedentes de pacientes pediátricos.

Resultados: La sensibilidad y especificidad media hallada para la ICC en el conjunto de muestras fue del 51 y 100%, respectivamente. La sensibilidad en frotis faríngeos fue del 46,6% y en aspirados nasales fue del 52,6%. En relación con el sexo, mediante PCR se diagnostica la gripe en 21/110 hombres (19,1%) y 32/115 mujeres (27,8%).

Conclusión: La ICC presenta una sensibilidad limitada, aunque por su excelente especificidad sería útil su uso en el diagnóstico presuntivo de urgencias.

Palabras clave

Inmunocromatrografía capilar, RT-PCR, gripe A H1N1

Introduction

in June 2009, the World Health Organization declared a state of pandemic by the emergence of a new influenza A H1N1 virus which affected mainly to young-adults and paediatric population.[1,2] This new virus can be detected by different techniques such as cell culture, genomic amplification and rapid antigen detection. Cell culture has the drawback of being a cumbersome and long response time technique with 24-48 hours on average to get a result.[3] On the other hand, rapid antigen detection techniques have as inconvenient a lack of sensivity.[4] Therefore, real time RT-PCR provides the best combination of sensitivity, specificity and response time. In addition, it is considered as the gold-standard method for influenza A H1N1 virus detection.[5]

In clinical practice, different rapid tests based on membrane-enzyme immunoassay or immunochromatographic methods are available for detection of influenza antigens in a few minutes.

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These tests provide rapid results that can help the clinician in the individual treatment of patients.[6] The aim of this study was to evaluate the diagnostic accuracy of a test based on immunochromatrographic method (ICC) for rapid detection of influenza viruses.

Material and methods

During the epidemic H1N1 influenza season, nasal aspirates and throat swabs samples were collected from all the paediatric patients (aged 16 or younger) prospectively diagnosed with influenza according to strict clinical criteria in our hospital setting. Inclusion criteria included the presence of six symptoms or clinical criteria outside the epidemic period and at least four in the epidemic period of the following: sudden onset, fever, chills, fatigue and prostration, myalgia, cough, flu patient contact and upper respiratory symptoms.

All samples were tested using an ICC test (Directigen EZ fluA + B, Becton & Dickinson, USA) for antigen detection of influenza viruses type A and/or B simultaneously, obtaining a preliminary result in 15 minutes. Subsequently, the samples were assessed by a real time (RT-PCR) (Applied Biosystems, USA) detecting influenza genome by using the M protein and the hemagglutinin genes as amplification targets. The real time RT-PCR was carried out in the thermocycler 7500 Fast (Applied Byosistems, USA), using the primers and the probes recommended by the CDC for H1N1 influenza virus diagnosis.[5] The results of the rapid test and PCR were interpreted separately without the knowledge of one or other. This technique was used as gold-standard method to assess the perfomance of the ICC test. We used the Epidat 3.1 for calculating the validity of diagnostic tests. Data were compared by the chi-square, using the SPSS 15.0.

Results

A total of 225 samples (including 49 throat swabs and 176 nasal aspirates) were prospectively collected from an equal number of patients (110 males and 115 females; ranging from 0 to 16 years old). The mean and the median age was 4.52 (SD= ±5.8) and 2 years old, respectively. One hundred and sixty out of the 225 patients (71%) were 5 years old or younger.

The PCR detected 53 positive cases (15 from throat swabs and 38 from nasal aspirates samples) corresponding to 23.6% of the patients, whereas ICC detected 27 positive cases (7 from throat swabs and 20 from nasal aspirates samples) corresponding to 12% of the patients. No false positive results were obtained from the ICC technique since all the postive cases were also positive by PCR.

Overall, the sensitivity and specificity of the ICC technique was 51% and 100%, respectively. Different parameters from the ICC test compared to the PCR gold-standard method are displayed in table 1.

TABLE 1

Validity of diagnostic tests for ICC compared with PCR

	Value	*CI (95%)*	
Sensitivity ICC (%)	50.94	36.54	65.35
Especificity ICC (%)	100.00	99.71	100.00
PPV (%)	100.00	98.15	100.00
PNV (%)	86.87	81.91	91.83
Prevalence (%)	23.56	17.79	29.32
Likelihood ratio +	0.51	0.39	0.67
Likelihood ratio –	0.49	0.37	0.65

CI: confidence interval; PPV: predictive positive value; PNV: predictive negative value.

Sensitivity was also calculated according to the specimen, obtaining values of 46.6% for pharyngeal swabs and 52.6% for nasal aspirates.

The ICC/PCR ratios for the overall positive samples, positive throat swabs and positive nasal aspirates samples were 27/53 (p= 0.002), 7/15 (p= 0.005) and 20/38 (p= 0.001), respectively.

In regards to gender, 21 out of 110 male patients (19.1%) and 32 out of 115 female patients (27.8%) were positive according to PCR gold-standard method, respectively (p= 0.001). The ICC technique detected 10 out of 110 male patients (9.1%) and 17 out of 115 female patients (14.8%) as positive cases (p <0.0001).

Discussion

Despite fears of a possible collapse of national health systems around the world during the declared influenza pandemic in June 2009, the impact was less than expected, with incidences similar to those observed in other flu seasons.

The influenza incidence for the pediatric patients included in this study was nearly 25% of the suspected cases according to the PCR gold-standard method. This result is similar to those obtained from other studies,[7] although higher incidences (ranging around 40%), have also been reported in other series.[8,4] Thus, an influenza-like illness could be caused by other viral respiratory infections in pediatric patients.[9] Therefore, screening for other respiratory viruses could be of great interest to improve the diagnosis and management of viral respiratory infections.

In our study, the sensitivity of the Directigen ICC rapid diagnostic test (Becton-Dickinson) displayed a limited value, detecting only 27 out of the 53 cases confirmed by PCR (50.9%). This result is similar to those found in other studies performed in Spain and USA, reporting sensitivities of 46 and 49%, respectively.[4,8] However, the Directigen ICC rapid diagnostic test displayed higher sensitivities such as 70% and 67% in other studies conducted in Spain and Italy, respectively.[10,11] Some other rapid tests from other companies provided lower sensi-

tivities, ranging from 18 to 50%.[12-14] According to the literature, discrepancies in test sensitivity may be due to several factors, including patient characteristics and viral load. Indeed, our data showed that the ICC sensitivity obtained from nasal aspirate samples was somewhat higher than that from throat swabs, possibly related to different viral load kinetics.[15] In addition, performance factors such us reading and interpretation of results could also explain some other discrepancies.[4] Thus, further studies with a larger sample size are needed to assess the reliability of this technique as well the factors possibly related to the discrepancies cited above.

Regarding specificity, the Directigen ICC rapid test displayed values of 100%, in accordance with previously published series using the same test.[4,10,11] A specificity of 96.5% has also been reported using Directigen in an American study.[8]

In regards to gender results, the sensitivity (using both the ICC and PCR methods) found in females was significantly higher than that found in males. Since the ICC sensitivity mainly depends on viral load,[4] this might be a factor that influences this result. However, no gender differences regarding sensitivity performance have been reported according to the literature.[16-18]

In conclusion, the Directigen ICC technique displayed limited sensitivity in our study which was conducted in pandemic phase. Thus, non-epidemic conditions might limit the use of this test. On the other hand, an excellent positive predictive value was found, providing a rapid and reliable diagnosis at the Emergency Room in epidemic seasons. However, the low negative predictive value of the Directigen ICC represents the major disadvantage for implementing this technique since a high portion of samples should be assessed by PCR to rule out influenza infection.

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