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Rotavirus, vaccine failure or diagnostic error?

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ABSTRACT

Immunochromatography (ICG) is highly used in clinical settings for rotavirus (RV) diagnosis. The specificity of the tests differs by brand type and is not 100%, therefore its use when the prevalence of the disease is low (i.e. in vaccinated children) may result in a proportion of false positive diagnoses.

In some areas, vaccine effectiveness studies or surveillance is done using ICG. Our objective was to estimate the validity of ICG test in vaccinated children, and estimate the number of false positive results in the Valencian Region of Spain, where all RV infections are diagnosed using ICG and are not confirmed by PCR.

Population based registries were used to identify all results from the RV antigen tests performed between January 2008 and June 2012 in children under 37 months. Hospitalization and vaccination status of the patients were obtained by linking different databases through a unique identification number. The Positive Predictive Value of the ICG test depending on the vaccination status of the child, hospitalization and the rotavirus season was estimated by a Bayesian model of latent classes.

Of the 48,833 tests with valid results, 9429 were done in vaccinated children, and of those 3963 (42%) during the rotavirus season. The prevalence of positive results in vaccinated varied from 2.9 to 21.4% of the tests depending on the hospitalization and seasonality. The estimated PPV also varied from 27.1 to 84.6% when stratified by these two parameters. Globally it is calculated that approximately 267 out of the 520 (51.3%) positives in vaccinated children were false positive tests.

The large percentage of false positives, due to an excessive number of tests in vaccinated and out of the RV season, if interpreted as vaccine failures, can cause a loss of confidence in the vaccine and lower the estimates of vaccine effectiveness.

1. Introduction

Rotavirus (RV) is the most common agent that causes diarrhea in children under 5 years of age [1]. Since 2006 there are two authorized vaccines against RV in Europe. Both have an effectiveness over 90% to prevent RV hospitalizations and above 70% for mild to moderate infections attended in the primary care [2,3].

For the diagnosis of RV infection there are simple and reproducible techniques that detect the presence of the VP6 antigen (main protein of the intermediate capsid) in feces. Immunochromatography (ICG) is widely used as it is easy to perform and is not very expensive. The sensitivity and specificity of different ICG tests vary with the commercial brands [4–6], and there are important differences among the package inserts and studies on the field when comparing ICG with the gold standard PCR (protein chain reaction) [5,7–11]. While inserts show a high sensitivity, specificity and positive predicted value, they show very wide confidence intervals that reach up to 50%. Two studies on the accuracy of ICG for RV antigen detection have been recently published with discordant results, that can be partly explained by different in house PCR or specimen processing, or even an improvement in the manufacturing process [5,6]. Therefore, regular assessments of the diagnostic performance of ICG assays are recommended in order to accurately diagnose RV infections.

The PPV indicates the probability of a positive result being a true positive and depends on the test characteristics (sensitivity and specificity) and also on the prevalence of the disease [12]. In areas with high rotavirus vaccine coverage, the low prevalence of the disease decreases the PPV of the tests. In Australia, after RV universal vaccination started, only 28–37% of the positive results by ICG could be confirmed with PCR, which means that 60–70% of these results could be considered false positives [11]. Therefore, their epidemiological surveillance of RV disease requires either a more specific technique, like ELISA, or PCR confirmation of the

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ICG positive results [13]. Studies on the field have described positive predictive values (PPV) between 85% and less than 45% in unvaccinated during the rotavirus season [4,11].

False positive ICG results may result in both a loss of confidence in the vaccine from parents or health providers and also biased analysis of vaccine effectiveness.

In Spain, parents pay for the vaccine, and a vaccination coverage of around 50% has been reached in the Autonomous Region of Valencia [14,15]. RV infections in this region are tested by using ICG but not confirmed by PCR, independently of the vaccination status. Therefore, our objective was to estimate the validity of ICG test for the detection of rotavirus antigen in feces in subjects vaccinated, and estimate the number of false positive results yielded in the Valencian Region of Spain.

2. Design and population studied

Study performed using the health databases of the Valencian Community. All of them can be linked through a unique personal identification number (SIP) [14].

RedMIVA (Red de Vigilancia Microbiologica de Valencia) gathers all the results from all the public microbiology laboratories of the Valencian Community [3,16]. All results from the RV antigen tests done between January 2008 and June 2012 in children under 37 months were obtained. Of the total of tests, those unprocessed or with indeterminate results were dismissed. The decision of doing or not a fecal test was not systematic and depended on the health department and the pediatrician. All the microbiology labs used ICG tests of different brands.

Hospitalizations due to RV were obtained from the hospital discharge database- Minimum Basic Data Set (MBDS), where the diagnosis and procedures are gathered along with the evaluation of the medical activity. The codification system used is the International Classification of Diseases (9th version), clinical modification [17]. The use of MBDS is mandatory for all public hospitals. All hospitalizations due to gastroenteritis (001–009) and rotavirus gastroenteritis (008.61) during the study period (2008–2012) were obtained. It was considered that a diagnostic test was associated with a hospitalization, when the date of the result was from 5 days prior to admission to 15 days after discharge.

The subjects' vaccination status was obtained from the Vaccination Registry (Sistema de Información Vacunal – SIV) [14,18]. This database registers the vaccines administered in all public and most of the private vaccination centers. Children were considered vaccinated 15 days after the administration of the first dose of the vaccine [14]. Protection was considered with at least one dose, because previous studies in our Community have shown high protection with partial vaccination [14]. It is estimated that among all rotavirus vaccine doses distributed in Valencia during 2009–2012, most (86%) were registered in SIV as administered in children aged <1 year [14].

This was a sub analysis of an effectiveness RV vaccine study in the Valencian Community, approved by the Ethical Committee of Investigation of the General Directorate of Public Health/Public Health Research Center.

2.1. Statistical analysis

A descriptive analysis of the RV ICG test results was done based on the vaccination status of the child, the rotavirus season and hospitalization, using frequencies and percentages. Cases were categorized in two age groups: 0–24 months and 25–36 months.

A Bayesian model of latent classes was developed for the estimation of the PPV for ICG tests, also with this method we estimated, in absence of a gold standard, the unknown diagnostic test parameters: prevalence, sensibility and specificity. The number of true positive and false negative were the latent data (missing information without gold standard) which followed a binomial distribution with PPV and PFN (false negative probability) probabilities of success respectively. At the same time these probabilities depended on the prevalence, the sensibility and the specificity [19]. Due to the large number of commercial ICG brands used in the different hospitals, and the lack of consensus of the diagnosis accuracy of these tests, we considered beta distributions as informative priors for the sensitivity and the specificity with an average of 99% and 96% respectively [5]. In the case of the prevalence an uninformative prior (beta(1,1)) was considered. The posterior of these parameters were beta distributions resulted from the conjugation between the beta prior with the binomial likelihood [19].

The Markov chain Monte Carlo (MCMC) approach was employed to estimate the posterior distributions. The model was developed taking into account the vaccination status of the child, the rotavirus season and the severity of the disease (hospitalized or not) [4,9]. The rotavirus season was considered between December and April [14].

For more details of the model see Appendix 1.

All analyses were carried out with R (Foundation for Statistical Computing, Vienna, Austria) version 3.0.3. and WinBUGS (Alastair Stevens TOP) version 1.4.

3. Results

Of the 44,618 RV antigen tests requested; 1053 samples were not processed and 732 of the result were inconclusive, so for the study 42,833 diagnostic tests were considered (Table 1). Of these, 35,589 (83.1%) came from children under 25 months of age and essentially in non-hospitalized children (31,577 tests).

In vaccinated children, 9429 tests were performed; 3946 (42%) during the RV season, and 468 in hospitalized subjects, of these only 201 (42.9%) during the RV season.

Overall, the greater percentage of positive results was observed in non-vaccinated, hospitalized children and during the rotavirus season (Table 1). In the younger age group, the percentage of positive test varied from 2.9 to 61.7%, depending on the vaccine status, hospitalization, and rotavirus circulation season.

The estimated PPV according to the vaccine status, rotavirus season and hospitalization is depicted in Table 2. In vaccinated children it is close to 30% when it is outside of the RV season, which means approximately that 7 out of every 10 positive results are false positives, and increases in hospitalized (Table 2).

The PPV is above 80% in unvaccinated hospitalized children, but decreases to 34.8% if the sample came from an outpatient out of the RV season.

With these data, it was estimated that of the 520 positive results in vaccinated children 267 (51.3%) could be false positives. In non-vaccinated the number of false positives was estimated to be 5676 (18.2%)

The cost of every diagnostic test by ICG in the Valencian Community is 22.45€ [20], which means that 987,608€ were invested in 5 years for the RV diagnosis in children up to 3 years. Of these, 486,536€ were spent outside the rotavirus season, when the efficiency of the test is low and therefore the positive result is difficult to interpret.

4. Discussion

In Valencia, with an approximate annual birth cohort of 45,000 children, more than 9000 antigen RV detection tests by ICG are done every year in children under 3 years, highlighting that half of them are done to vaccinated children or outside of the rotavirus

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