Viral gastroenteritis in hospitalized patients: Evaluation of immunochromatographic methods for rapid detection in stool samples

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Viral gastroenteritis in hospitalized patients. Evaluation of immunochromatographic methods for rapid detection in stool samples

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1. BACKGROUND

Acute gastroenteritis (AGE) remains the second cause of death in children under five years, mainly in developing regions [1]. In developed countries, mortality is low, but the number of hospitalizations is a public health problem [2,3]. The etiology of AGE includes bacteria, parasites and viruses, but the latter are considered the main cause of diarrhea in children [4]. Norovirus and rotavirus are the most frequent associated viruses, followed by enteric adenovirus, astrovirus and sapovirus [5-9]. Other viruses such as enterovirus, are also detected in AGE, although their true role in the etiology is not well known [10-12].

Currently, detection of viral antigens in stool samples performed by enzyme immunoassay or immunocromatografic (ICG) techniques are recommended because of their simplicity and low cost. Genome amplification methods are more sensitive than immune analytical tests but its application for the routine clinical diagnosis of AGE is still limited [13].

In this study, two commercial ICG tests, one for simultaneous detection of rotavirus species A (RV-A) and adenovirus (HAdV) types 40/41, and the other for norovirus (NoV) genogroups GI/II were evaluated by comparison of the results to those obtained with specific PCR assays for the same viruses. In addition, clinical and epidemiological characteristics of the viral AGE infections were analyzed.

2. PATIENTS AND METHODS

2.1 Patients.

In this study, 100 stool samples collected from patients with AGE symptoms for etiological diagnosis at the hospital's microbiology laboratory were included. The inclusion criteria for the patients were any child or adult with diarrhea (several and fluid stools) who attend the Hospital "Puerta de Hierro" in Madrid (Spain) as outpatients or through the emergency room between February and July 2018. Most of the AGE patients were children (84/100, 84%) (mean age 3 years; range, 3 months-15 years) but 16 were adults (mean age 58 years; range 23-83 years). Female/male ratio was 1.2. Only 14 (14%) patients required admission to the hospital (nine children under one year and two adults over 65 years). Prevalent symptoms included diarrhea (100%), fever (28%), vomiting (28%) and abdominal pain (13%). Additionally, half of the patients (all children) referred to have received at least two doses of RV-A vaccine. In Spain, vaccination against RV-A is not included in the routine immunization schedule currently, but it is recommended by the Spanish Association of Paediatrics [14]. Both vaccines (Rotarix and RotaTeq) are available. The schedule is two doses for monovalent vaccine (at two and four months of age) and three doses for the pentavalent one (at two, four and six months of age).

2.2 Bacterial and parasitic detection.

All faecal samples were analyzed for enteric bacteria by conventional culture methods [15], and for *Giardia spp.* and *Cryptosporidium spp.* detection, an ICG test was used (Crypto-Giardia IC, CerTestBiotec, Spain).

2.3 Viral detection.

Two commercial ICG techniques were used, one for simultaneous detection of RV-A and HAdV antigens, *Rota-AdenoCard* (Materlab S.L., Madrid, Spain) and other for detection of NoV antigens, *NorovirusCard* (Materlab). In all cases, the stools were initially diluted in 150µL of the supplied extraction buffer and subsequently analyzed according to the manufacturer's instructions.

To determine the sensitivity and specificity of the ICG methods, results were classified as positive or negative based on the PCR results, which were considered the gold standard. For that purpose, an aliquot of each sample was sent to the Laboratory of Enterovirus and Viral Gastrointestinal

79 infections in the National Centre for Microbiology. Viral RNA/DNA was purified from the stool
80 samples and RV-A, HAdV and NoV were detected using specific PCR assays described
81 previously [16-18].
82 Presence of other enteric viruses, i.e. astrovirus (HAstV) and enterovirus (EV), was also tested
83 using PCR methods previously published [19, 20].
84 **2.4 Genotyping**.
85 Characterization of RV-A, HAdV, NoV, and HAstV genotype was performed by sequencing of the
86 resulted PCR amplicons and BLAST analysis http://blast.ncbi.nlm.nih.gov. In the case of EV, the
87 sequencing of the amplified fragment (in 5'-non-coding region) allowed to identify the species but
88 not the specific type.

3. RESULTS

3.1 Evaluation of ICG assays.

Of the total 100 samples analyzed, 15 (15%) were positive for RV-A according to ICG test, and all but one, were confirmed by PCR (one false positive sample). In addition, RV-A was detected by PCR in two more patients (two false negative samples). With respect to HAdV infections, this virus was identified in six samples (6%), but only three of them were confirmed by PCR (three false positives). Other sample was positive only by PCR (one false negative). Finally, NoV was detected in a single sample by ICG assay, whereas four samples (4%) resulted positive by PCR, including the ICG-positive sample (three false negatives). Hence, the sensitivity and specificity of the evaluated ICG techniques were between 57.1 and 88.8%, and 96.9 and 100%, respectively (Table 1).

3.2 Clinical and epidemiological characterization of viral AGE infections.

To complete the etiologic diagnosis of AGE cases, all samples were analyzed for HAstV and EV. HAstV was detected in one sample (1%), while 12 (12%) were positive for EV. The most frequently detected virus was RV-A (16%), followed by EV (12%), HAdV and NoV (4% each) and HAstV (1%). Co-infections with EV were observed in three patients, two with RV-A and one with AdV, and with rhinovirus in one NoV-positive patient. In addition, in one patient, RV-A and NoV were co-detected. Therefore, at least one viral pathogen was identified in 32 (32%) of the samples (Table 2).

RV-A infections mainly affected children under five years but also an older child and a 60-years-old patient. Patients infected with HAdV, HAstV or EV were children under six years of age. NoV infections were also detected in young children (1-4 years). RV-A and HAdV infections occurred along the study-period (February-July) whereas NoV and EV were detected between April and July. Regarding clinical presentation, there was no association between symptoms and a specific viral infection. Only in 6 (43%) of the 14 patients who required hospitalization, an infection by RV-A (N=5) or EV (N=1) was detected.

RV-A genotypes identified were G2 (N=1), G3 (N=2), G9 (N=5) and G12 (N=8). All HAdV were type 41 and the unique HAstV was type 1. NoV were GI.3 (N=1), GI.4 (N=2) and GII.21 (N=1).

With respect to EV-positive samples, two EV belonged to species A, eight to species B and two were confirmed as rhinovirus.

Finally, RV-A was detected in five (10%) of the 50 children who had received RV-A vaccine, all of them were young children between 4 months and 2.5 years of age. The genotypes identified were G12 (N=3) and G9 (N=2).

3.3 Bacterial and parasitic detection.

Campylobacter sp. was detected in 11 samples (11%), *Salmonella sp.* in two (2%) and *Shigella sp.* in one specimen (1%). In addition, one sample (1%) resulted positive for *Giardia spp.* and *Cryptosporidium spp.* test. Two *Campylobacter*-positive patients were coinfected with viruses, NoV and EV, respectively.

4. DISCUSSION

Viral gastroenteritis is a common cause of morbidity and mortality worldwide [1]. Rapid diagnosis allows us to determine the underlying etiology and prognosis, and reduce unnecessary use of antibiotic treatment [13, 21, 22]. In this study, two commercial ICG tests (Materlab), for RV/HAdV and NoV detection respectively, have been evaluated comparing the results obtained by PCR methods. This is the first study reporting on these ICG tests. Overall, RV-A/HAdV ICG test showed good sensitivity and specificity (>80%). For RV-A, other commercial ICG methods evaluated previously [23-25] had also high sensitivity and specificity. For HAdV, however, not all reported assays showed good sensitivity [23, 24]. Our results suggest that Materlab RV-A/HAdV ICG test could be a used as an alternative choice for rapid screening of these viruses in stool specimens, especially in the context of an epidemic outbreak. On the contrary, ICG test for NoV antigen showed lower sensitivity (<60%), similarly to the values reported in other evaluated NoV ICG methods (between 23 and 59%) [23, 26], which is consistent with the fact that development of the ICG technique for these viruses usually presents more difficulties. Therefore, currently the most appropriate method for NoV detection should be the genomic amplification by PCR.

In addition to RV-A, NoV and HAdV, the presence of HAstV and EV was also investigated. Overall, viral infection was detected in 32% of the total patients. This percentage is greater than the one described in the USA (26%) by Osborne et al. [27], but it is below that observed in other European countries (Italy, UK, Switzerland) or Japan (36-59%), although in most of these publications, the authors analyzed a greater number of different viruses than we did [28-31].

RV-A were the most frequently detected viruses in our series. This result coincides with those published in some European countries and Japan [28, 29, 30, 32] where vaccination against RV has not been implemented in a systemic manner yet as in Spain. However, in the USA, where vaccination has been included nationwide for a few years, the incidence of RV infections has decreased considerably [27, 33]. The detection percentage observed for HAstV infection was similar to those described in other reports [6, 30, 31] but for both HAdV and NoV, it was significantly lower (4%) compared to other series (11-23%) [28, 30, 31, 34]. In the case of HAdV infections, differences in the sensitivity of PCR assays or in the extraction methods used could be related with the different detection frequency. For NoV, the cause could be related to the fact

158 that NoV infections are usually milder than RV infection, not requiring hospital admission [9]. In
159 addition, NoV infections are more frequent in the autumn-winter months [35] and the study period
160 only spanned from February to July. Finally, with regard to EV infections, these viruses are not
161 considered the main cause of AGE [10] and are not usually included in the differential diagnosis.
162 In this series, EV has been detected as the only etiologic agent in 7% of the samples. The studies
163 published by Gosert et al. [29] and Chansaenroj et al. [36] obtained similar incidence results (5-
164 6%) and in a report conduced in Italy [31], this percentage was even higher (17%) suggesting that
165 in those cases where no other pathogen is detected, AGE symptoms could be due to the infection
166 by these picornaviruses.
167 RV-A genotyping showed that G9 and G12 were predominant serotypes. Both are considered
168 emerging genotypes in Europe [32]. Recent studies [37, 38] have suggested these vaccines may
169 exert different immunological pressures that influence the diversity of circulating strains.
170 Therefore, the emergence of these genotypes could be related with the introduction of vaccination
171 programs. In this study, almost 60% of children reported having been received a dose of vaccine
172 against RV at least. However, in five vaccinated children an RV-A was detected. Of them, three
173 were correctly vaccinated and one received two of the three doses, but all at least one year before
174 RV infection. Only one child, patient ID17, had received a single dose of vaccine when she
175 suffered the AGE. However, genotypes detected in these patients were G9 or G12, which are not
176 part of the used vaccines that only cover the genotypes G1-4 [39]. These data suggest that
177 immunization against G9 and G12 RV-A of current vaccines could be partial in some cases, but
178 further surveillance studies are necessary to confirm this hypothesis.
179 Although enteric bacteria and parasites were detected in 15 cases, the etiologic diagnosis was
180 reached in less than 50% of the patients studied. Different causes such as receiving some type
181 of medication could be associated with a lack of diagnosis but still, it is necessary to develop and
182 incorporate to routine diagnosis new techniques, either immunological or molecular, to detect the
183 maximum possible pathogens involved in AGE to improve the prognosis and management of
184 affected patients, especially those with a higher risk of complications such as young children or
185 the elderly [4].
186 In conclusion, the evaluated ICG methods showed good sensitivity and specificity and they are
187 useful for a rapid diagnosis of AGE viral infections. However, molecular detection and

characterization of the genotypes are essential for epidemiological surveillance of these infections, especially RV infections which vaccination is being implemented worldwide currently.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration. Collection of clinical and microbiologic data was approved by the institutional ethics board of the hospital and informed consent was obtained.

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Competing interests

None declared

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393 **Table 1.** Evaluation of the Materlab (Mlab) ICG techniques for the detection of rotavirus A (RV-
394 A), human adenovirus (HAdV) 40/41 and norovirus (NoV) GI/GII, by comparison with PCR results.
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Virus	Method	N° positive samples (%)	Sensitivity*	Specificity*
RV-A	**ICG Mlab**	15 (15%)	88.9% (63.9-98.1)	98.8% (92.7-99.9)
	PCR	16 (16%)		
HAdV 40/41	**ICG Mlab**	6 (6%)	80% (29.9-98.9)	96.9% (90.7-99.2)
	PCR	4 (4%)		
NoV GI/II	**ICGMlab**	1 (1%)	57.1% (20.2-88.2)	100% (95.2-100)
	PCR	4 (4%)		

*sensitivity calculated as: true positives/(true positives+false negatives); specificity calculated as: true negatives/(true negatives+false positives). Lower and upper limits for a 95% confidence interval are shown in brackets.

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420 **Table 2.** Patients with stool sample positive for rotavirus A (RV-A), human adenovirus 40/41
421 (HAdV), norovirus (NoV), astrovirus (HAstV) and enterovirus (EV) by PCR. Results of bacterial
422 and parasitic detection were also included.

ID patient	sex	age	symptoms date	RV-A vaccine	RV-A-PCR positive	HAdV-PCR positive	NoV-PCR positive	HAstV-PCR positive	EV-PCR positive	bact/paras-positive
5	M	1 yr	Feb-18	no	RV-A G9.P4					
6	M	2 mo	Feb-18	no	RV-A G12.P8					
7	M	2 yr	Feb-18	yes		HAdV 41				
11	M	23 yr	Feb-18	no						Shig
14	F	1 yr	Mar-18	yes	RV-A G9.P4					
15	M	2 yr	Mar-18	no	RV-A G12.P8					
16	M	8 yr	Feb-18	no	RV-A G12.P8					
17	F	4 mo	Feb-18	yes	RV-A G12.P8					
22	M	1.5 mo	Feb-18	no	RV-A G9.P4					
26	F	9 yr	Mar-18	yes						Campy
30	M	1 yr	Mar-18	na				HAstV- 1		
38	F	5 yr	Mar-18	no						Salm
39	M	5 yr	Mar-18	yes						Campy
43	M	2 yr	Mar-18	yes	RV-A G12.P8					
46	M	4 yr	Mar-18	yes						Campy
47	M	4.5 mo	Mar-18	na		HAdV 41				
53	F	13 yr	Mar-18	no						Campy
54	F	1 yr	Apr-18	yes						Campy
56	F	2 yr	Apr-18	yes					Rino	Campy
58	M	1 yr	Apr-18	yes			NoV GI.4			
59	F	1 yr	Apr-18	no						Campy
60	M	60 yr	Apr-18	no	RV-A G3.P8					
62	F	4 yr	May-18	no						Salm
63	M	5 yr	Apr-18	no	RV-A G9.P4					
64	M	85 yr	Apr-18	no						Campy
67	M	3 yr	May-18	yes	RV-A G9.P8				EV-B	
68	M	4 yr	May-18	no	RV-A G2.P4				EV-B	
70	M	1 yr	May-18	yes					EV-B	
73	F	3 yr	May-18	no	RV-A G12.P8					
74	M	1.5 mo	May-18	no	RV-A G3.P8					
75	F	3 yr	May-18	no		HAdV 41				
76	M	1 yr	Jun-18	na	RV-A G12.P8					
77	M	6 yr	Jun-18	yes		HAdV 41			EV-B	
81	F	2 yr	Jun-18	yes					EV-B	
84	F	1.5 mo	Jun-18	no					EV-A	
85	M	2 yr	Jun-18	yes						Cripto/Gard
86	M	2 yr	Jun-18	yes					EV-A	Campy
87	F	4 yr	Jun-18	no					EV-B	
88	F	1 yr	Jun-18	yes						Campy
89	M	4 yr	Jun-18	no			NoV GII.P21		Rino	
91	M	1 yr	Jun-18	yes					EV-B	
90	M	1 yr	Jul-18	yes			NoV GI.P4			Campy
92	F	1 yr	Jul-18	yes					EV-B	
94	M	1 yr	Jul-18	yes	RV-A G12.P8		NoV GI.3			

Campy: Campylobacter sp.; Salm: Salmonella sp.; Shig: Shigella sp; Cripto/Gard: Cryptosporidium spp/Giardia spp; Rino: rinovirus; EV-A: enterovirus species A; EV-B: enterovirus species B.

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