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Clinical severity of enteric viruses detected using a quantitative molecular assay compared to conventional assays in the Global Enteric Multicenter Study

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Abstract

Background: Quantitative molecular assays are increasingly used for detection of enteric viruses.

Methods: We compared the clinical severity using modified Vesikari score (mVS) of enteric viruses detected by conventional assays (enzyme immunoassays [EIA] for rotavirus and adenovirus 40/41 and conventional polymerase chain reaction for astrovirus, sapovirus, and norovirus) and a quantitative molecular assay (TaqMan Array Card [TAC]) among children aged 0–59 months in the Global Enteric Multicenter Study. For rotavirus and adenovirus 40/41, we compared severity between EIA-positive and TAC-positive cases assigned etiologies using different cycle threshold (CT) cutoffs.

Results: Using conventional assays, the median (interquartile range) mVS was 10 (8, 11) for rotavirus, 9 (7, 11) for adenovirus 40/41, 8 (6, 10) for astrovirus, sapovirus, and norovirus GII, and 7 (6, 9) for norovirus GI. Compared to rotavirus EIA-positive cases, the median mVS was 2 and 3 points lower for EIA-negative/TAC-positive cases with CT<32.6 and 32.6≤CT<35, respectively (p-value<.0001). Adenovirus 40/41 EIA-positive and EIA-negative/TAC-positive cases were similar, regardless of CT cutoff.

Conclusions: Quantitative molecular assays compared to conventional assays, such as EIA, may influence severity of identified cases, especially for rotavirus. Cutoffs to assign etiology for quantitative assays should be considered in the design and interpretation of enteric virus studies.

Summary:

Children under 5 with rotavirus had the highest clinical severity compared to other gastrointestinal viruses. Rotavirus and adenovirus 40/41 cases detected only with quantitative molecular assays were slightly less severe compared to cases detected with conventional enzyme immunoassays.

Keywords

viral gastroenteritis; quantitative molecular assay; clinical severity; pediatric diarrhea; diagnostics

Introduction

Acute gastroenteritis (AGE) is a leading cause of morbidity and mortality among children under 5 years of age [1]. While bacteria and parasites are common causes of AGE in low-income settings, viruses are the main cause of pediatric AGE globally, accounting for up to two-thirds of AGE in the first year of life [2, 3]. Rotavirus is the most common viral cause of AGE, but norovirus, adenovirus types 40/41, sapovirus, and astrovirus are increasingly recognized as important contributors to the burden of viral gastroenteritis, particularly since the introduction of rotavirus vaccines into childhood immunization programs [2–4].

Several studies have attempted to quantify the burden of viral gastrointestinal pathogens, but there are few studies comparing the clinical severity of different viral gastrointestinal pathogens [5–9]. Understanding the clinical severity of different viral gastrointestinal pathogens can help inform prioritization of vaccine and antiviral development. Viruses other than rotavirus can cause severe AGE, but their severity typically is lower than rotavirus in the limited studies that have investigated this [5–7, 10], even in settings with introduction of rotavirus vaccine [8].

Studies have found that the choice of diagnostic method (e.g., enzyme immunoassays (EIA) or molecular assays such as polymerase chain reaction (PCR)) can influence pathogen detection [2, 11]. However, there is limited evidence describing how the clinical severity of cases detected using different diagnostic methods may vary. This is particularly relevant for rotavirus and adenovirus 40/41, which historically have been detected using EIA and are now more frequently tested for by molecular methods [12]. For example, adenovirus 40/41 was detected at a five-fold higher incidence in re-evaluations of the Global Enteric Multicenter Study (GEMS) and the Malnutrition and Enteric Disease (MAL-ED) study when using quantitative molecular assays compared to EIA, indicative of previously underestimated burden of this pathogen. Given the increase in molecular methods in recent years, it is valuable to understand how cases detected by molecular methods compare clinically to those detected by the more conventional EIAs.

In the current study, we aimed to describe and compare the clinical severity of the principal viral gastrointestinal pathogens detected using conventional methods, including EIAs for rotavirus and adenovirus 40/41 or multiplex reverse transcriptase PCR with gel electrophoresis for other viral etiologies, versus quantitative molecular assays. These findings will characterize the severity of illnesses diagnosed using quantitative molecular methods, aid interpretation of longitudinal data with multiple diagnostic methods, and help inform case definitions for surveillance activities, vaccine trials, and vaccine effectiveness studies.

Methods

Study population

The study population comprised children enrolled as cases with moderate-to-severe diarrhea (MSD) in GEMS. GEMS was a case-control study conducted in 7 countries (Kenya, Mali, Mozambique, The Gambia, Bangladesh, India, and Pakistan) from December 1, 2007

through March 3, 2011 [13]. A MSD case in GEMS was defined as a child aged 0–59 months who sought care at a sentinel health center for acute (onset within the previous 7 days) diarrhea (three or more loose stools within the previous 24 hours) and had at least one of the following criteria: sunken eyes, loss of skin turgor, intravenous hydration, dysentery, or hospital admission. Study procedures are described in detail elsewhere [13]. None of the countries in GEMS had introduced rotavirus vaccine at the time of this study. Children without diarrhea were enrolled as controls but were not included in this secondary analysis of symptomatic clinical profiles.

Diagnostics

A fresh stool specimen collected from each case at enrollment was tested by multiple standardized diagnostic methods. Rotavirus and adenovirus were detected using enzyme immunoassays (EIA) and norovirus, sapovirus, and astrovirus were detected using multiplex reverse transcriptase (RT) PCR with gel electrophoresis [14]. Adenovirus positive samples were tested for serotypes 40 and 41 by EIA, and norovirus genogroups I and II were differentiated by RT-PCR. An age- and site-stratified random sample of 5,304 cases had stool specimens retested by the TaqMan Array Card (TAC; Thermo Fisher, Carlsblad, CA, USA) [11]. The TAC is a probe-based quantitative real-time PCR (qPCR) assay for detection of 32 enteropathogens, including rotavirus, norovirus, sapovirus, astrovirus, and adenovirus types 40/41 [15]. The cycle threshold (CT) values were used as an inverse measure of pathogen quantity, where a one-unit increase corresponds to a doubling in quantity of nucleic acid [11]. Validation of this assay, quantitative PCR setup, and quality control procedures have been published in detail elsewhere [11, 15].

Modified Vesikari Score

To assess illness severity, a 17-point modified Vesikari score (mVS) was calculated as previously adapted for GEMS (Supplemental Table 1) [16, 17]. This mVS is a weighted score based on the following: duration of diarrhea, maximum number of diarrheal episodes within 24 hours, vomiting ≥3 times within 24 hours, fever, dehydration, and treatment. A child was considered moderately to severely dehydrated if two or more of the following were present: lethargic or unconscious on arrival; sunken eyes; drank poorly or unable to drink; skin pinch—goes back very slowly (>2 seconds). A child was considered mildly dehydrated if two or more of the following were present: restless/irritable on arrival; sunken eyes; thirsty, drank eagerly; skin pinch—goes back slowly (1–2 seconds). Treatment was categorized as outpatient without intravenous (IV) fluid, outpatient with IV fluid, or hospitalization. Standardized interviews with parents or primary caretakers obtained data on duration and frequency of diarrhea and vomiting at enrollment. The entire duration of diarrhea which was collected using a memory aid card during two weeks of follow-up after enrollment [13].

Statistical analysis

The proportion of positive test results (i.e., prevalence) and median (interquartile [IQR]) mVS for each viral pathogen were calculated using conventional and TAC results, and for multiple definitions of a TAC-positive: any positive detection (CT<35), diarrhea-associated CT values [11], highly diarrhea-associated CT values [11], isolated as only highly diarrhea-

associated pathogen, and receiver operating characteristic (ROC) cutoff CT values [11] (Table 1). The CT cutoff values were based on the original reanalysis of GEM using TAC and factor in CT values detected in control samples [11]. Given the variability in disease burden by age, we conducted a sensitivity analysis comparing mVS for each pathogen stratified by age 0–11 months and 12–59 months. Given heterogeneity in disease burden and potential variability in ascertainment of mVS characteristics by site, we also conducted a sensitivity analysis excluding one country at a time [4]. For each viral pathogen, TAC CT values were plotted against the mVS and overlaid with a linear regression line and Pearson correlation coefficients estimating the statistical significance of linear correlation between CT and mVS. A sensitivity analysis was conducted excluding children with more than one highly diarrhea-associated pathogen detected to exclude co-infections.

Given that rotavirus and adenovirus 40/41 were the only two viruses originally tested for by EIAs, we compared the mVS, individual characteristics of severity, and demographic factors between cases detected by EIA and TAC for rotavirus and adenovirus 40/41. We compared three mutually exclusive groups: 1) EIA-positive (irrespective of TAC results), 2) EIA-negative but TAC-positive with highly diarrhea-associated CT values (henceforth referred to as EIA-negative/TAC-attributable), 3) EIA-negative but TAC-positive without highly diarrhea-associated CT values (henceforth referred to as EIA-negative/TAC-unattributable). To define TAC attribution, we used the highly diarrhea-associated cutoff for rotavirus (CT=32.6) and adenovirus (CT=22.7) derived from the original qPCR GEMS analysis [11]. Stratifying TAC detections by this more stringent cutoff allows for evaluation of how use of cutoffs with quantitative molecular methods influences clinical severity. Categorical variables were compared using Chi-square tests and continuous variables were compared using Wilcoxon rank sum test with two-tailed p-values.

Ethical review

The GEMS protocol was approved by ethics committees at the University of Maryland, Baltimore, MD, USA, and at every field site. Written informed consent was obtained from the parent or primary caretaker of each participant before initiation of study activities.

Results

Among 9,439 MSD cases enrolled into the GEMS study from Dec 1, 2007-March, 3, 2011, the proportion of any viral detection using conventional methods was 33.9% (n=3,202); 18.5% (n=1,747) for rotavirus, 5.2% (n=495) for norovirus genogroup GII, 2.8% (n=265) for norovirus genogroup GI, 3.4% (n=325) for sapovirus, 2.5% (n=238) for astrovirus, and 2.5% (n=235) for adenovirus 40/41 (Table 2).

As reported previously for GEMS [11], the re-analysis of 5,304 specimens using TAC influenced the detection for viruses differentially, with minimal variability between EIA and TAC for rotavirus detection, but higher detection of other viral pathogens using TAC, especially for adenovirus 40/41 (Table 2). The prevalence varied based on the definition of a TAC-positive (Table 2). For example, the prevalence of adenovirus 40/41 was 28.3% using the any detection definition (CT<35) and was 4.2% when using the more stringent cutoff of highly diarrhea-associated CT (CT<22.7), compared to 2.3% when using EIA.

Modified Vesikari Score among MSD cases in GEMS with viral pathogens detected

Using conventional diagnostics, rotavirus had the highest mVS (median 10 [8, 11]), while adenovirus 40/41 had the second highest mVS (median 9 [7, 11]). All other viral pathogens had a median mVS of 8 (Table 2). The mVS for rotavirus was statistically higher than all other viral pathogens ($p<0.05$ for all comparisons). These trends were similar in the sensitivity analysis excluding one country at a time and when stratifying by age (Supplemental Table 2). The most notable differences were for vomiting and treatment, with rotavirus and adenovirus 40/41 having the highest proportions of cases experiencing ≥3 episodes of vomiting or requiring hospitalization or IV fluids (Supplemental Table 3).

Using TAC (any detection, CT<35), the median mVS was lower for rotavirus and adenovirus 40/41 than when using EIAs, but rotavirus still had the highest mVS compared to all viral pathogens (median 9 vs 8 for all other pathogens, Table 2). Rotavirus remained the highest median mVS regardless of the TAC-positive definition (Table 2). The more conservative definition of 'highly diarrhea-associated quantity' that attributed etiology of these pathogens to fewer cases than any-detection TAC positivity resulted in higher median mVS scores for rotavirus, norovirus GII, and adenovirus 40/41. The median mVS for sapovirus and astrovirus were consistently 8, regardless of the TAC-positive definition used to attribute etiology to these pathogens. There was a significant correlation between decreasing CT values and increasing mVS for rotavirus, but a much less pronounced pattern between lower CT values and higher mVS for adenovirus 40/41 and norovirus GII, and no correlation between CT and mVS for the other viruses (Figure 1). Findings were consistent when excluding children with more than one pathogen detected below a highly diarrhea-associated CT value (Supplemental Figure 1).

Individual mVS characteristics, EIA-positive compared to EIA-negative/TAC-positive

Rotavirus—There were 959 rotavirus cases positive by EIA and 331 rotavirus cases negative by EIA but with TAC detection, of which 153 were considered attributable based on a highly diarrhea-associated cutoff (CT<32.6) and 178 were not-attributable (32.6≤CT<35). There were 121 children with EIA-positive and TAC-negative, who had a median (IQR) mVS of 9 (7, 10). The median mVS of rotavirus EIA-positive cases was 10 [8, 11], 2 points higher than the mVS of EIA-negative/TAC-attributable (8 [6, 10], p<.0001) and 3 points higher than the mVS of EIA-negative/TAC-unattributable cases (7 [5, 10], p-value<.0001). These differences remained consistent when excluding children with EIA-positive and TAC-negative specimens (potentially false-positives). The difference in overall mVS appears to be driven by differences in four severity characteristics (Figure 2; Table 3). EIA-negative/TAC-attributable and EIA-negative/TAC-unattributable cases had longer durations of diarrhea compared to EIA-positive, however they were less likely to have vomiting ≥3 times within 24 hours, less likely to have mild or moderate/severe dehydration, and less likely to require hospitalization or IV fluids. EIA-negative/TAC-attributable cases were also more likely to have had longer duration of diarrhea at study enrollment compared to EIA-positive cases.

Adenovirus 40/41—There were 123 adenovirus 40/41 cases positive by EIA and 1,381 adenovirus 40/41 cases negative by EIA but with TAC detection, of which 108 were considered attributable based on a highly diarrhea-associated cutoff (CT<22.7) and

1,273 were not-attributable (22.7≤CT<35). There were 4 cases with EIA-positive and TAC-negative (potentially false-positive). The mVS distribution was similar for adenovirus 40/41 EIA-positive (median 9 [IQR 7, 11]) compared to EIA-negative/TAC-attributable (9 [7, 11], p=0.84), but the median was 1 point higher among EIA-positive compared to EIA-negative/TAC-unattributable (8 [6, 10]) (p-value=0.02, Table 3). For EIA-positive compared to EIA-negative/TAC-attributable, the only difference in clinical severity characteristics was that EIA-negative/TAC-attributable were less likely to have ≥7 episodes of diarrhea within 24 hours (Figure 2, Table 3). Duration of diarrhea, vomiting ≥3 times within 24 hours, fever, dehydration, treatment, age, and gender were similar between EIA-positive and EIA-negative/TAC-attributable. For EIA-positive compared to EIA-negative/TAC-unattributable, EIA-negative/TAC-unattributable were less likely to have ≥7 episodes of diarrhea within 24 hours and less likely to have vomiting ≥3 times within 24 hours. Duration of diarrhea, fever, dehydration, treatment, age, and gender were similar between EIA-positive and EIA-negative/TAC-unattributable cases.

Discussion

This analysis of a large multi-center study in seven low-and-middle income countries evaluated the impact of etiologic assay utilized on the characterization of clinical severity of AGE caused by a variety of viral pathogens. Assessments of the severity of AGE by etiology might be expected to find discordant results depending on whether conventional assays, such as EIAs for rotavirus and adenovirus 40/41 or conventional PCR for other viral etiologies, are used versus a quantitative molecular diagnostic (TAC) because of differences in sensitivity for detecting pathogens. While the choice of assay and TAC-positive definition had a large impact on the detection of viral pathogens, particularly for adenovirus 40/41, there was less variability in the overall clinical severity of cases when using these different case definitions. However, for rotavirus and adenovirus 40/41, EIA-positive cases did have higher proportions of certain severe clinical markers compared to EIA-negative/TAC-positives cases, particularly for rotavirus. The use of more sensitive quantitative molecular assays such as TAC, even when using stringent quantitative cutoffs used to assign etiology, may detect some less severe cases of rotavirus and adenovirus 40/41.

Rotavirus was consistently the most severe pathogen for both cases detected by EIA and cases detected by TAC, regardless of the TAC-positive definition. There are now four rotavirus vaccines pre-qualified by WHO and implemented in over 120 countries globally [18], which have dramatically reduced the burden of severe rotavirus gastroenteritis in these settings [19]. Our findings support a large body of literature indicating the high clinical severity of rotavirus illnesses [10], and should further motivate continued implementation of rotavirus vaccination in more countries as well as increasing vaccine coverage in countries that have already implemented vaccination [20]. While this study was conducted prior to rotavirus vaccine introduction in these countries, a subsequent study called Vaccine Impact on Diarrhea in Africa (VIDA) was conducted with similar methodology to GEMS in three of the original GEMS countries following rotavirus vaccine introduction, and found that rotavirus remained the highest clinical severity viral pathogen despite reduced burden in settings of vaccine introduction [8]. These findings support the need for ongoing efforts to develop next-generation rotavirus vaccines to improve the effectiveness of rotavirus

vaccines, particularly in low-income settings that report disproportionately lower vaccine effectiveness, higher disease burden, and continued (albeit dramatically reduced) severe rotavirus gastroenteritis [21].

Our findings also help describe the clinical characteristics of moderate to severe cases of rotavirus detected by TAC only; these cases were in general less severe than those detected by EIA, with lower proportion of cases with high frequency vomiting, moderate or severe dehydration, and hospitalization or IV fluids, regardless of whether the cases were TAC-attributable or TAC-unattributable based on quantitative cutoffs. Our findings suggest that TAC may also detect rotavirus cases that present later for medical care when viral excretion might be expected to be lower. However, given that only approximately a quarter of cases were detected by TAC only, the inclusion of these less severe cases did not dramatically reduce the population level distribution estimates of mVS for rotavirus. Rotavirus also had the most notable correlation between decreasing CT values (which is inversely related to viral load) and increasing mVS, similar to a smaller study on rotavirus in India [22], indicating that severe rotavirus disease may be associated with higher levels of viral shedding. We found no association or less pronounced associations between CT values and mVS for the other non-rotavirus viral pathogens, consistent with studies evaluating viral load and severity for norovirus [23, 24], indicating that this relationship between viral shedding and severe disease may not apply to other (non-rotavirus) viral causes of AGE. There is currently no standardized approach for attributing CT values to etiologies. In addition to the definitions explored in this analysis to assign TAC results to etiologies, attributable fraction estimates have also been used to adjust for the CT detection among controls [13].

Adenovirus 40/41 detected using EIA had the second highest mVS distribution following rotavirus, consistent with the VIDA study conducted in post-vaccine introduction settings [8]. The definition a TAC-positive case dramatically impacted prevalence of adenovirus 40/41 (ranging from 4.2% to 28.3%), although there was marginal difference in the mVS distributions. The mVS distribution was similar among EIA-positive and EIA-negative/TAC-attributable adenovirus 40/41 cases, and EIA-negative/TAC-unattributable cases only had slightly lower distribution of mVS, with minimal differences between the three groups for individual clinical severity markers. These differences in severity were much less dramatic than the differences seen when comparing rotavirus cases detected by EIA and TAC. This could be a reflection of a lower sensitivity of the EIA for adenovirus 40/41, contrary to the EIA for rotavirus which is quite sensitive [4]. Unlike rotavirus, which had only a relatively low proportion of cases detected by TAC only, over 90% of adenovirus cases were detected by TAC-only, although primarily categorized as TAC-unattributable. These findings suggest that much of the difference in the prevalence may be due to EIA test sensitivity rather than illness characteristics (e.g., less severe disease). These findings support other studies that indicate EIA for adenovirus 40/41 may miss severe adenovirus 40/41 cases [2, 11, 25]. Future studies should continue to use molecular methods for adenovirus 40/41 detection and continue to evaluate and validate appropriate CT or viral load thresholds for quantitative assays.

The other viral pathogens, norovirus GI and GII, sapovirus and astrovirus, were generally less severe than rotavirus and adenovirus 40/41. The high infectiousness and susceptibility for norovirus illness across the age spectrum results in large overall burden [26, 27], and some norovirus GII illness with more conservative TAC-positive definitions had higher clinical severity, similar to analysis of norovirus severity in the VIDA study [28]. There are several norovirus vaccine candidates in development, which, if effective, may help alleviate norovirus burden [29]. Sapovirus, while part of the same family as noroviruses, typically has lower clinical severity than norovirus infection, both of which are less severe than rotavirus [30]. Our findings also help support that while astrovirus and sapovirus can cause severe illness, they represent a lower proportion of viral illness and sometimes with less severity.

This study was subject to a number of limitations. First, the GEMS study was conducted from 2007 through 2011 in countries with no rotavirus vaccine immunization programs during that time period. However, the relative performance of these assays would not be expected to differ over this time period. The VIDA study, designed similarly to GEMS from 2015–2018 in 3 of the GEMS African sites following rotavirus vaccine introduction, found consistent results with rotavirus and adenovirus 40/41 continuing to have the highest mVS. Second, this study was designed to only enroll children with moderate-to-severe diarrhea based on set criteria and thus may not be generalizable to the entire spectrum of AGE, including milder illness [13]. Third, it is unclear if the 121 rotavirus cases that were EIA-positive and TAC-negative were false-positives, or whether there were other unknown factors that may have influenced specimens testing positive by EIA but negative by TAC. Fourth, the mVS was adapted to the data collected in GEMS [17], and thus is not directly comparable with the original VS that may be used in other studies. However, this study is strengthened by the simultaneous comparison of multiple viral pathogens, enabling valid internal comparisons of the severity of these viruses. Also, this analysis did not account for differences in severity related to prior exposure and immunity or genetics [31, 32]. As this analysis was designed as exploratory, multiple comparisons were not adjusted for in statistical analyses.

In summary, these findings inform the clinical severity of the five main viral pathogens that cause severe acute gastroenteritis and provide evidence regarding the severity of cases identified using EIA compared with molecular methods for rotavirus and adenovirus 40/41. The comparison of EIA and molecular techniques for both rotavirus and adenovirus 40/41 may also help inform interpretation of surveillance platforms with changing diagnostics and is relevant to the clinical interpretation of viral detections identified with the increased use of multipathogen molecular panels. Some adenovirus 40/41 illnesses were also notably severe, and molecular detection highlights the previously underappreciated burden of this pathogen, which may inform vaccine development efforts for vaccines against this virus. Our findings reinforce the need to increase access to rotavirus vaccines and continued development of next-generation vaccines, given the high severity of illness this virus can cause. Case definitions for surveillance activities, vaccine trials, and vaccine effectiveness studies may need to incorporate the use of quantitative cutoffs when evaluating severe disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability:

The data are available in the GEMS Repository: https://clinepidb.org/ce/app/.

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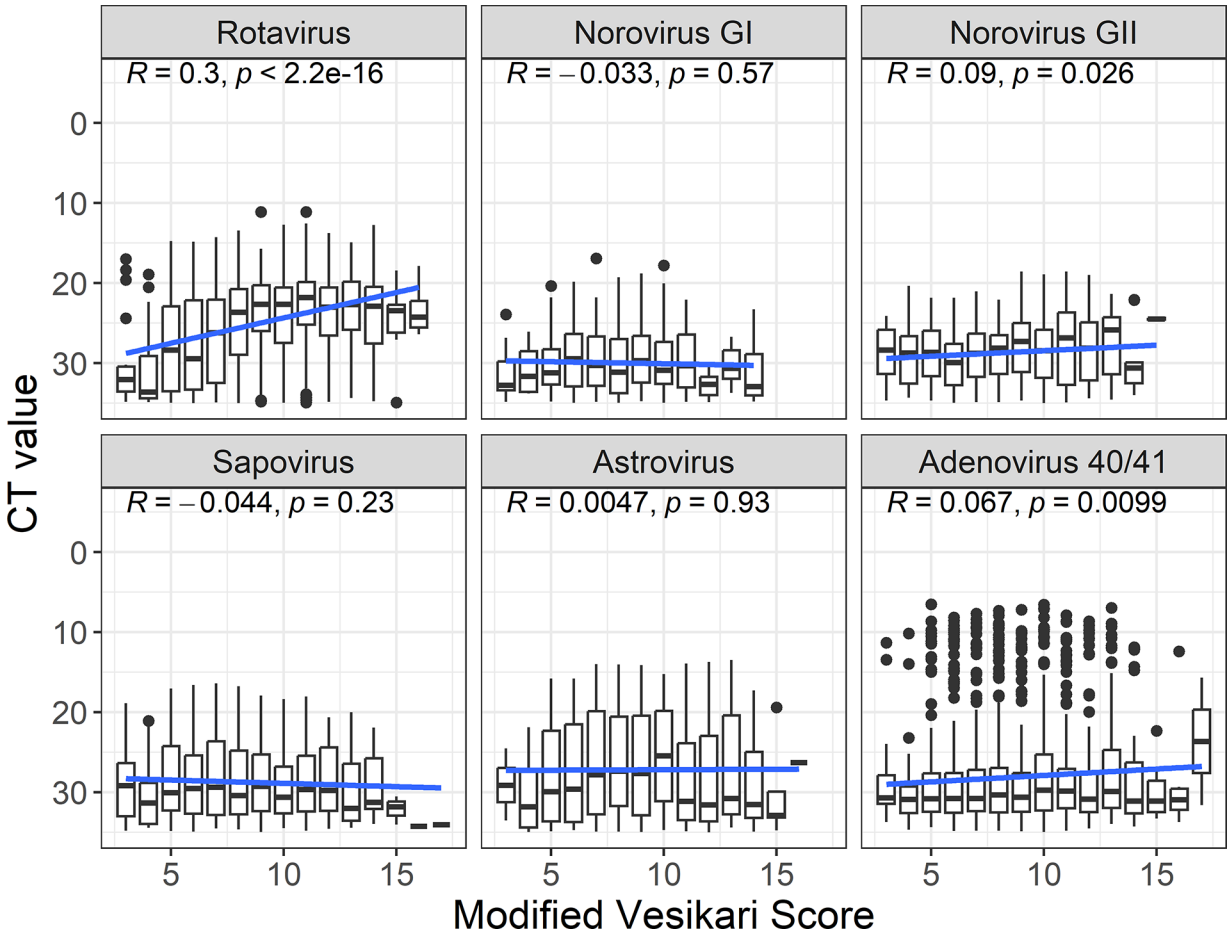

Figure 1. Boxplots of TAC diagnostic cycle threshold (CT) by modified Vesikari score for each of the six viral pathogens overlaid with linear regression (solid line) and corresponding Pearson correlation coefficient estimates (R).

The centerlines of the boxplots represent the median, ends of the boxes represent the interquartile range, and the solid dots are outliers if an observation is 1.5 times the interquartile range.

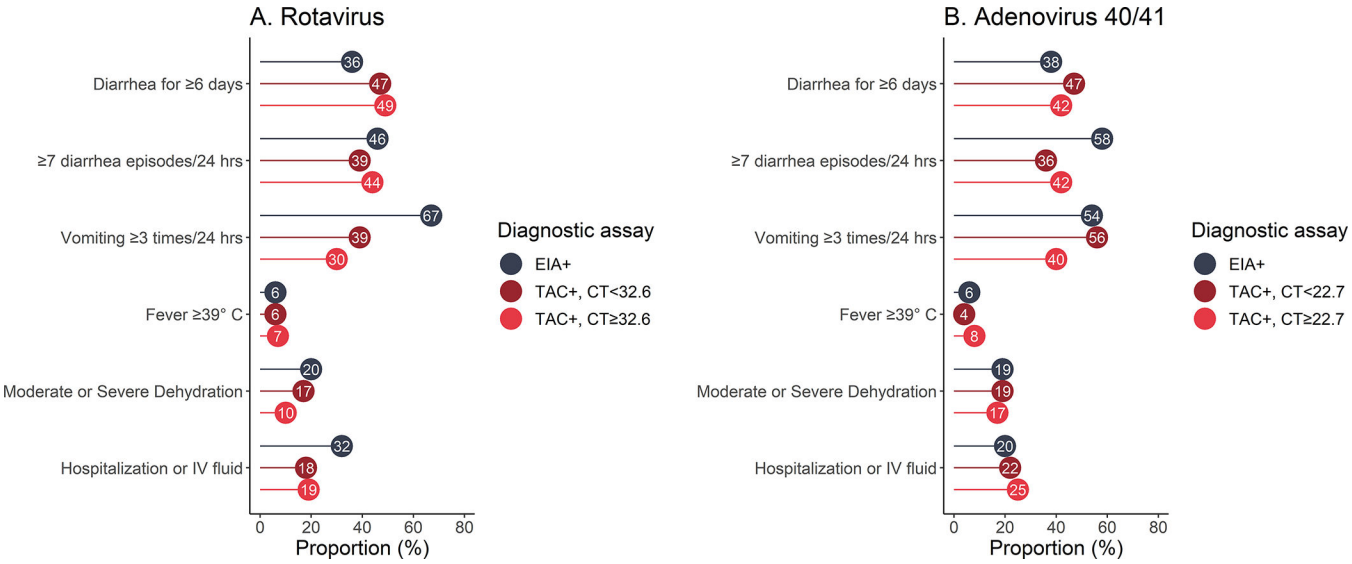

Figure 2. Proportions of cases with individual markers of clinical severity of AGE among EIA-positive cases irrespective of TAC results, EIA-negative but TAC-positive cases with highly diarrhea-associated cycle threshold values, and EIA-negative but TAC-positive cases without highly diarrhea-associated CT values, for both rotavirus (A) and adenovirus 40/41 (B).

Table 1.

Cycle threshold cutoffs for assigning etiology based on previously published criteria[a].

	Rotavirus	Norovirus GI	Norovirus GII	Sapovirus	Astrovirus	Adenovirus 40/41
Quantitative cutoffs for each pathogen						
Diarrhea-associated quantity	35.0	n/a	27.6	31.6	25.5	35.0
Highly diarrhea-associated quantity	32.6	n/a	23.4	n/a	22.2	22.7
ROC Cutoff	26.9	n/a	28.8	34.1	28.1	30.2

ROC= receiver operating characteristic; n/a=not applicable

[a]The quantitative cutpoints for each pathogen were based off of the previously published qPCR analysis by Liu et al [11], with diarrhea-associated quantities defined as all quantities above the point at which the 95% confidence interval of the odds ratio comparing quantities in cases and control exceeded 1, highly diarrhea-associated quantities defined as all quantities above the point at which the point estimate of the odds ratio exceeded 2, and ROC cutoff as the quantity that maximally discriminates case-control status.

Table 2.

Prevalence and modified Vesikari scores of viral pathogens detected among GEMS cases of moderate-to-severe diarrhea (N=9,439) using conventional test results and TAC molecular test results among a sub-set with additional testing (n=5,304).

	Rotavirus	Norovirus GI	Norovirus GII	Sapovirus	Astrovirus	Adenovirus 40/41
Prevalence, number (%)						
AGE cases (N=9,439)						
Conventional test results	1747 (19)	265 (3)	495 (5)	325 (3)	238 (3)	235 (2)
Subset tested by TAC (N=5,304)						
Conventional test results	959 (18)	152 (3)	281 (5)	182 (3)	141 (3)	123 (2)
TAC-positive[a]						
Any detection (CT<35)	1169 (22)	293 (6)	608 (11)	734 (14)	388 (7)	1500 (28)
CT< Diarrhea-associated quantity	1169 (22)	N/A[c]	251 (5)	457 (9)	155 (3)	1500 (28)
CT< Highly diarrhea-associated quantity	977 (18)	N/A[c]	60 (1)	N/A[d]	111 (2)	223 (4)
Isolated as only highly diarrhea-associated pathogen[b]	775 (16)	N/A[c]	40 (1)	N/A[d]	60 (1)	121 (3)
CT<ROC Cutoff	787 (15)	N/A[c]	313 (6)	683 (13)	193 (4)	709 (13)
Modified Vesikari Score, median (IQR)						
Conventional test results (among subset with TAC testing)	10 (8, 11)	7 (6, 9)	8 (6, 10)	8 (6, 10)	8 (6, 10)	9 (7, 11)
TAC-positive						
Any detection (CT<35)	9 (7, 11)	8 (6, 10)	8 (6, 10)	8 (6, 10)	8 (6, 10)	8 (6, 10)
CT< Diarrhea-associated quantity	9 (7, 11)	N/A[c]	8 (7, 11)	8 (6, 10)	8 (6, 10)	8 (6, 10)
CT< Highly diarrhea-associated quantity	10 (8, 11)	N/A[c]	10 (7, 11)	8 (6, 10)	8 (6, 10)	9 (7, 11)
Isolated as only highly diarrhea-associated pathogen[b]	10 (8, 11)	N/A[c]	9.5 (7, 11)	N/A[d]	8 (7, 10)	9 (7, 11)
CT<ROC Cutoff	10 (8, 11)	N/A[c]	8 (7, 10)	8 (6, 10)	8 (7, 10)	8 (6, 11)

CT=cycle threshold; IQR=interquartile range; N/A=not available; ROC=receiver operating characteristic; TAC=TaqMan Array Card

[a]The quantitative CT cutoffs for each pathogen were based off of the original qPCR analysis, with diarrhea-associated quantities defined as all quantities above the point at which the 95% confidence interval of the odds ratio comparing quantities in cases and control exceeded 1, highly diarrhea-associated quantities defined as all quantities above the point at which the point estimate of the odds ratio exceeded 2, and ROT cutoff as the quantity that maximally discriminates case-control status [11].

[b]The denominator excluding cases with more than 1 highly attributable pathogen was 4745

[c] Norovirus GI was not associated with diarrhea using conventional or TAC diagnostics, thus no CT threshold were provided in Liu et al 2016.

[d] No highly diarrhea-associated quantity threshold was provided for sapovirus.

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Table 3.

Modified Vesikari score, individual markers of clinical severity of AGE, and patient demographics comparing cases that tested positive by EIA, negative by EIA but positive by TAC with highly-diarrhea CT values, and negative by EIA but positive by TAC without highly diarrhea CT values, for both rotavirus and adenovirus 40/41.

	Rotavirus					Adenovirus 40/41				
	EIA positive	EIA negative and TAC positive				EIA positive	EIA negative and TAC positive			
	Reference	CT<32.6		32.6≤CT<35		Reference	CT<22.7		22.7≤CT<35	
	N (%)	N (%)	p-value	N (%)	p-value		N (%)	p-value	N (%)	p-value
Cases	N=959	N=153		N=178		N=123	N=108		N=1273	
Median Vesikari (IQR)	10 (8, 11)	8 (6, 10)	<.0001	7 (5, 10)	<.0001	9 (7, 11)	9 (7, 11)	0.84	8 (6, 10)	0.02
Modified Vesikari Characteristics										
Duration of diarrhea during entire illness (days)										
1—4	497 (52)	70 (46)	0.02	74 (42)	0.003	63 (51)	49 (45)	0.34	601 (47)	0.65
5	118 (12)	11 (7)		16 (9)		13 (11)	8 (7)		131 (10)	
≥6	344 (36)	72 (47)		88 (49)		47 (38)	51 (47)		541 (43)	
Median (IQR)										
Duration of diarrhea at study enrollment										
1—4	888 (93)	130 (85)	0.004	158 (89)	0.22	115 (94)	96 (89)	0.18	1121 (88)	0.06
5	41 (4)	11 (7)		11 (6)		7 (6)	7 (6)		79 (6)	
≥6	30 (3)	12 (8)		9 (5)		1 (1)	5 (5)		73 (6)	
Median (IQR)										
Max no. diarrhea/24 hrs.										
3—6	515 (54)	93 (61)	0.10	99 (56)	0.64	52 (42)	69 (64)	0.001	739 (58)	0.0007
≥7	444 (46)	60 (39)		79 (44)		71 (58)	39 (36)		534 (42)	
Vomiting ≥3/24 hrs										
No	316 (33)	94 (61)	<.0001	125 (70)	<.0001	57 (46.3)	48 (44) (44.4)	0.77	767 (60)	0.003
Yes	643 (67)	59 (39)		53 (30)		66 (54)	60 (56)		506 (40)	
Fever										
<37.0	479 (50)	76 (50)		89 (50)		67 (54)	50 (46)		662 (52)	
37.1—38.4	374 (39)	62 (41)	0.83	66 (37)	0.87	44 (36)	49 (45)	0.36	445 (35)	0.76

	Rotavirus					Adenovirus 40/41				
	EIA positive	EIA negative and TAC positive				EIA positive	EIA negative and TAC positive			
	Reference	CT<32.6		32.6≤CT<35		Reference	CT<22.7		22.7≤CT<35	
	N (%)	N (%)	p-value	N (%)	p-value		N (%)	p-value	N (%)	p-value
38.5—38.9	47 (5)	5 (3)		11 (6)		4 (3)	5 (5)		63 (5)	
≥39	59 (6)	10 (6)		12 (7)		8 (7)	4 (4)		103 (8)	
Dehydration [a]										
None	183 (19)	58 (38)	<.0001	103 (58)	<.0001	27 (22)	28 (26)	0.73	409 (32)	0.07
Mild	585 (61)	69 (45)		58 (33)		73 (59)	59 (55)		650 (51)	
Moderate/Severe	191 (20)	26 (17)		17 (10)		23 (19)	21 (19)		214 (17)	
Treatment										
Hospitalization	302 (31)	28 (18)	<.0001	34 (19)	<.0001	25 (20)	24 (22)	0.58	318 (25)	0.10
Outpatient with IV fluid	120 (13)	8 (5)		6 (3)		14 (11)	8 (7)		84 (7)	
Outpatient without IV fluid	537 (56)	117 (76)		138 (78)		84 (68)	76 (70)		871 (68)	
Demographic variables										
Age (months)										
0–11	502 (52)	71 (46)	0.18	70 (39)	0.005	58 (47)	54 (50)	0.41	560 (44)	0.10
12–23	336 (35)	55 (36)		76 (43)		49 (40)	35 (32)		445 (35)	
24–59	121 (13)	27 (18)		32 (18)		16 (13)	19 (18)		268 (21)	
Female	425 (44)	68 (44)	0.98	74 (42)	0.50	62 (50)	47 (44)	0.30	560 (44)	0.17

[a]A child was considered mildly dehydrated if 2 or more of the following were present: Restless/irritable on arrival/admission; sunken eyes; thirsty, drank eagerly; skin pinch—goes back slowly (1–2 seconds). A child was considered moderately to severely dehydrated if 2 or more of the following were present: lethargic or unconscious on arrival/admission; sunken eyes; drank poorly or unable to drink; skin pinch—goes back very slowly (>2 seconds)