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Clinical significance of real time PCR panel in children with acute gastrointestinal symptoms

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Clinical significance of real time PCR panel in children with acute gastrointestinal symptoms – RealCAGI

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Abstract
Acute gastroenteritis is one of the most common reasons for pediatric emergency visits in both general and pediatric emergency departments. For most of the children rehydration is the only therapy needed. However, a range of bacterial pathogens may need accurate diagnosis and targeted antimicrobial therapy. Use of molecular multiplex testing has increased detection of pathogens in children with acute gastrointestinal symptoms. The PCR tests currently available enable rapid identification of gastrointestinal pathogens, with the test results often being available during the day sample was taken. However, it is unclear which of the patients are most likely to benefit from testing. Also, there is considerable uncertainty about the cost-effectiveness of the multiplex panels used to test for suspected infectious gastroenteritis in hospital and community settings. We have demonstrated that acute gastrointestinal symptoms are one of the most common diagnoses and a major cost in high-income population.

We hypothesize that real time multiplex PCR testing for gastrointestinal pathogens at pediatric emergency department setting could allow earlier initiation of appropriate antibiotics, reduce use of unnecessary antibiotics and improve identification of conditions in need for follow-up.

To assess the diagnostic performance of alternative sample types, we investigate the use of rectal swab and vomit in detecting gastrointestinal pathogens.

To estimate the usefulness of real time multiplex PCR testing, we will conduct investigator-driven academic clinical trial with randomized controlled study setting conducted at the Pediatric Emergency Department of Oulu University Hospital. For eligibility, children aged under 16 years arriving to pediatric emergency due to acute gastrointestinal symptoms will be assessed. After obtaining the written consent, fecal specimens will be collected by the nurses from the first stool after arriving to hospital. The pediatric nurses also collect rectal swab samples from each child. Vomit is also collected, if vomiting occurs.

The composite primary outcome consists of three outcomes which are evaluated using medical records: 1) correctly targeted antimicrobial treatment, 2) untargeted antimicrobial treatment and 3)

identification of conditions that require specific follow-up. Secondary outcomes, evaluated by medical records and electronic survey sent to families two weeks after the study visit, include: proportion of correctly targeted antimicrobial treatment, proportion of untargeted antimicrobial treatment, proportion of conditions in need for hospitalization or specific follow-up, time needed for clinician to receive the results of the samples, length of hospital stay, time to correct diagnosis, resolution of symptoms, laboratory and radiology costs, total costs, need for surgical consultation and proportion of patients needing surgical procedure, proportion of unscheduled revisits and proportion of correctly used hospital infection control measures.

In addition to the clinical trial conducted with fecal samples, we aim to assess the usability of rectal swab samples and vomit samples in identification of gastrointestinal pathogens.

1. BACKGROUND

Diarrheal diseases are the second leading infectious cause of mortality globally, after lower respiratory infections, among children younger than 5 years. Although childhood mortality due to diarrhea has declined substantially during previous decades, diarrhea is responsible for 500 000 deaths annually (GBD 2017). Acute gastroenteritis is one of the most common reasons for pediatric emergency visits in both general and pediatric emergency departments (Pöyry et al. 2021, Yoong et al. 2021). For most of the children rehydration is the only therapy needed. However, a range of bacterial pathogens may need accurate diagnosis and targeted antimicrobial therapy (Guarino et al. 2014).

In previous observational studies, use of molecular multiplex testing for all children who presented to the ED with acute gastroenteritis has increased detection of pathogens and decreased return visits (Pavia et al. 2023). However, it is unclear which of the patients most likely to benefit from testing. Also, there is considerable uncertainty about the cost-effectiveness of the multiplex panels used to test for suspected infectious gastroenteritis in hospital and community settings (Freeman et al. 2017).

Collection of fecal samples can be challenging and time consuming as children do not necessarily defecate during emergency visit. Finding the pathogens with recto-anal swab, that could be analyzed with real time multiplex PCR could significantly decrease the time to correct diagnosis. Rectal swabs have been reported to be reasonable option as sample collection method for real time PCR in children with or without diarrhea (Kabayiza et al. 2013) and in adults with norovirus infection (Sidler et al. 2014). Similarly, vomit samples have been reported eligible in identification of noroviral infection. (McHugh et al. 2018). The literature concerning usability of rectal swabs or vomit samples with multiplex PCR is however lacking.

We have demonstrated that acute gastrointestinal symptoms are one of the most common diagnoses and a major cost in high-income population (Pöyry et al. 2021).

2. RESEARCH HYPOTHESIS AND AIMS OF THE STUDY

Clinical significance of real time PCR panel for gastrointestinal pathogens

We hypothesize that real time multiplex PCR testing for gastrointestinal pathogens at pediatric emergency department setting could enhance initiation of appropriate antibiotics, decrease use of inappropriate antibiotics and increase identification of conditions in need for a particular follow-up or hospitalization.

The aim of the study is to evaluate the clinical significance of real time multiplex gastrointestinal PCR panel at pediatric emergency department.

Diagnostic study about alternative sample types for detecting gastrointestinal pathogens

We also hypothesize that rectal swab samples are a rapid and competent sample collection technique in identification of gastrointestinal pathogens with multiplex PCR in children with acute gastrointestinal symptoms.

Based on earlier literature, we hypothesize that rectal swab samples are rapid and competent sample collection technique in identification of gastrointestinal pathogens with multiplex PCR in children with acute gastrointestinal symptoms (Kabayiza et al. 2013). We also hypothesize that as in identification of norovirus, vomit samples could be utilized with multiplex PCR testing as well (McHugh et al. 2018). We aim to evaluate the sensitivity and specificity of rectal swab and vomit samples in relation to gold standard fecal samples.

3. METHODS

3.1 Clinical significance of real time PCR panel for gastrointestinal pathogens (RCT)

Study design and oversight

This is an investigator-driven academic clinical trial with randomized controlled study setting conducted at the Pediatric Emergency Department of Oulu University Hospital. The samples are processed in the NordLab clinical microbiology laboratory at Oulu University Hospital, where the diagnostic device is already in clinical use. The device has been as acquired by NordLab under national procurement legislation.

Study population and inclusion criteria

For eligibility, children aged under 16 years arriving in pediatric emergency due to acute gastrointestinal symptoms (vomiting and/ or diarrhea and/or abdominal pain and/or watery stool) will be assessed. Pediatric nurses in the ED screen children for eligibility upon arrival. Exclusion criteria are the need for cardiopulmonary resuscitation, immediate transfer to the intensive care unit, haemato-oncological disease or other severe immunosuppression, bloody diarrhea or a clinical suspicion of typhoid/paratyphoid fever. Otherwise, patients with comorbidities or prior antibiotic treatment are included. The multiplex PCR test is used only in children admitted to the study or those that meet the exclusion criteria.

Randomization

Before initiation of the study, a biostatistician who is not involved in the data collection creates a randomization list using a computer-generated random sequence of numbers, randomization being performed in permuted blocks of sizes varying randomly between 4 and 6. After obtaining written informed consent from each child's legal guardian and from children older than 6 years, the ED nurse opens the next opaque randomization envelope to assign the participant to either the intervention or control group (Figure 1).

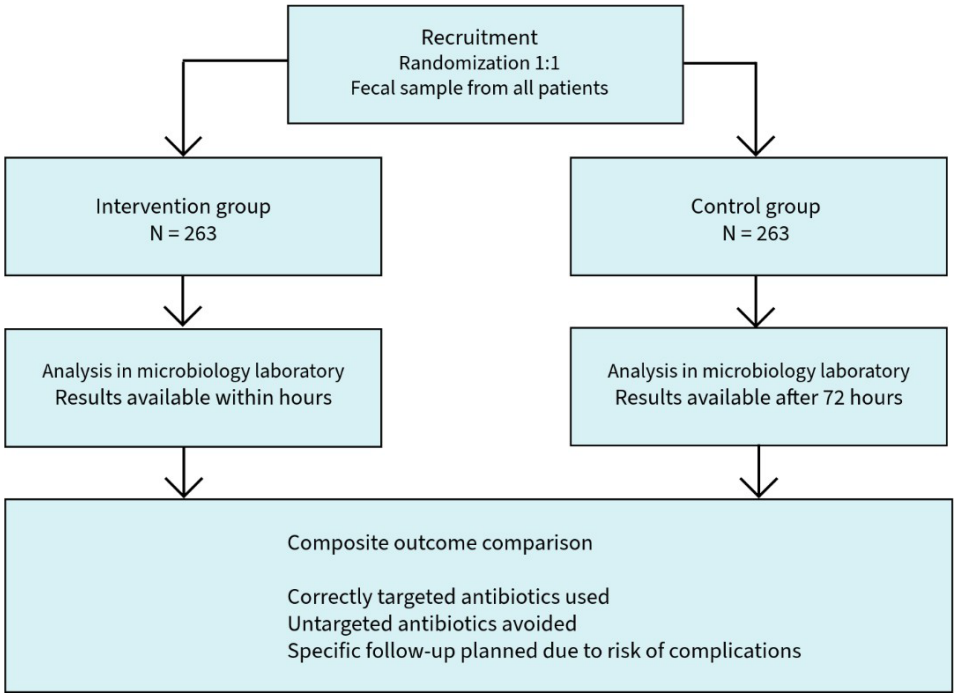

Figure 1. Randomized, controlled trial study flow chart regarding the clinical benefit of active testing.

Sample Collection and Pathogen Detection

After obtaining the written consent, fecal specimens will be collected by the nurses from the first stool after arriving in hospital. We collect fecal samples from all the children in both groups (n =

526) following the IVD (in vitro diagnostic) protocols. Also, all fecal samples will be processed according to IVD protocols to ensure reliability of the results. The pediatric nurses also collect rectal swab samples from all children. If vomiting occurs, vomit samples are also collected.

Fecal specimens will be collected by the pediatric nurses from the first stool after arriving in the hospital. Sterile swab such as Copan Regular Flocked Swab 502CS01 will be used to insert the specimen into Cary-Blair medium (Copan FecalSwab) according to manufacturer's instructions. Rectal swab samples and vomit samples are collected using the same swab-kits. Swab is inserted approximately 2 cm into the rectum and rotated 3-4 times before removal. In case of vomiting, the swab is rotated in the gastric secretion. The specimens are then inserted into Cary-Blair medium solution, similarly to fecal samples.

Fecal samples will be tested with QIAstat-Dx gastrointestinal panel 2, which can detect 13 gastrointestinal bacterial species, 5 viral pathogens and 4 different parasitic species with results ready within 70 minutes (Table 1). Positive test result for *Clostridium Difficile* is complemented with an addendum "Clostridium Difficile is part of normal gut microbiota in children under 2 years of age". Following the IVD protocols, only fecal sample results are available to care taking physicians. Rectal swab and vomit results are only used for the methodological analysis (Figure 2). Threshold cycle (Ct) values for all samples will be recorded to estimate pathogen loads in different samples.

Study results are available to the clinicians mostly within a few hours from receiving the fecal sample in the intervention group. In the control group the results are delayed until 72 hours. By analyzing fecal samples in both groups, we ensure safety, transparency and enable a pathogen-specific impact analysis.

If the child is admitted to hospital and the test is considered necessary the following day, a new sample may be taken in addition to study samples.

Table 1. Pathogens detected by the QIAstat-Dx Gastrointestinal Panel 2

- Adenovirus F40/F41
- Astrovirus
- Norovirus (GI/GII)
- Rotavirus A
- Sapovirus (GI, GII, GIV, GV)
- *Campylobacter* (*C. jejuni, C. coli* and *C. upsaliensis*)
- *Clostridium difficile* (toxin A/B)
- Enteroaggregative Escherichia coli (EAEC)
- Shigella/Enteroinvasive *Escherichia coli* (EIEC)
- Enteropathogenic *Escherichia coli* (EPEC)
- Enterotoxigenic *Escherichia coli* (ETEC) lt/st
- *Plesiomonas shigelloides*
- *Salmonella spp.*
- Shiga-like toxin-producing Escherichia coli (STEC) stx1/stx2* (including specific identification of E. coli O157 serogroup within STEC)
- *Vibrio vulnificus*
- *Vibrio parahaemolyticus*
- *Vibrio cholerae*
- *Yersinia enterocolitica*
- *Cryptosporidium*
- *Cyclospora cayetanensis*
- *Entamoeba histolytica*
- *Giardia lamblia*

*Shiga-like toxin-producing E. coli (STEC) genes (stx1 and stx2) are differentiated by QIAstat-Dx Gastrointestinal Panel 2

Follow-up

Primary outcome and most secondary outcomes will be assessed using medical record review. For selected secondary outcomes, we will follow the patients with electronic survey sent via e-mail two weeks after the study visit. The survey is established, and the answers are retained on the *Red-CAP* -platform. With the survey we estimate recovery of the child (duration of symptoms, return to day care or school). If the symptoms have not resolved by two weeks, an automized follow-up questionnaire will follow four weeks after the study visit.

Sample size calculation

With a composite primary outcome consisting of 1) use of correctly targeted antibiotic treatment, 2) avoiding incorrect antibiotic treatment and 3) identification of conditions in need for a precise follow-up, we regard a clinically significant difference if the proportion of children with a positive composite outcome is 20 % in the intervention group and 10 % in the control group. With an α error of 5% and a statistical power of 80 % and using 1:1 randomization, with an assumption of 20 % loss in retaining requested fecal samples, we need 263 children in intervention group and 263 controls, for a total of 526 children. We use all children with alternative sample types (rectal swab or vomit) for the diagnostic analysis.

Primary outcome

To assess the test's impact on clinical decision making, primary outcome composite includes three questions:

1) Was correctly targeted antimicrobial treatment started
2) Was untargeted antimicrobial treatment avoided
3) Was specific follow-up planned due to risk of complications (e.g. EHEC and hemolytic uremic syndrome)

within 72 hours from initial contact in Pediatric Emergency Room?

When any of these questions is answered YES, the composite variable is considered positive. Antimicrobial treatment is considered appropriate if a bacterial pathogen has been found and the patient meets criteria for severe gastroenteritis (aged under 1 year or immunocompromised or hospitalized due to gastroenteritis).

Secondary outcomes are proportion of correctly targeted antimicrobial treatment, proportion of conditions in need for hospitalization or specific follow-up, time needed for clinician to receive the results of the samples, length of hospital stay, time to correct diagnosis, resolution of symptoms, laboratory and radiology costs, total costs, need for surgical consultation and proportion of patients needing surgical procedure, proportion of unscheduled revisits, proportion of correctly isolated patients or correct use of personal protective equipment and the number of consultations for pediatric infectious diseases specialist.

3.2 Diagnostic analysis

For the diagnostic analysis, we will assess the usability of rectal swab and vomit samples for detecting gastrointestinal pathogens within both arms of the RCT. (Figure 2) We will conduct a diagnostic study, in which the PCR testing of the fecal samples is considered the gold standard. We will calculate the sensitivity and specificity of rectal swab and vomit samples analyzed with the PCR device in relation to the fecal sample results. Also, we will collect a VAS score on how unpleasant the rectal swab sample collection was in relation to the collection of fecal samples. The VAS scores are questioned from children 7-15 years of age, and from parents of children younger than 7 years of age. We will also calculate the time to the result as compared to the use of fecal samples in same patients.

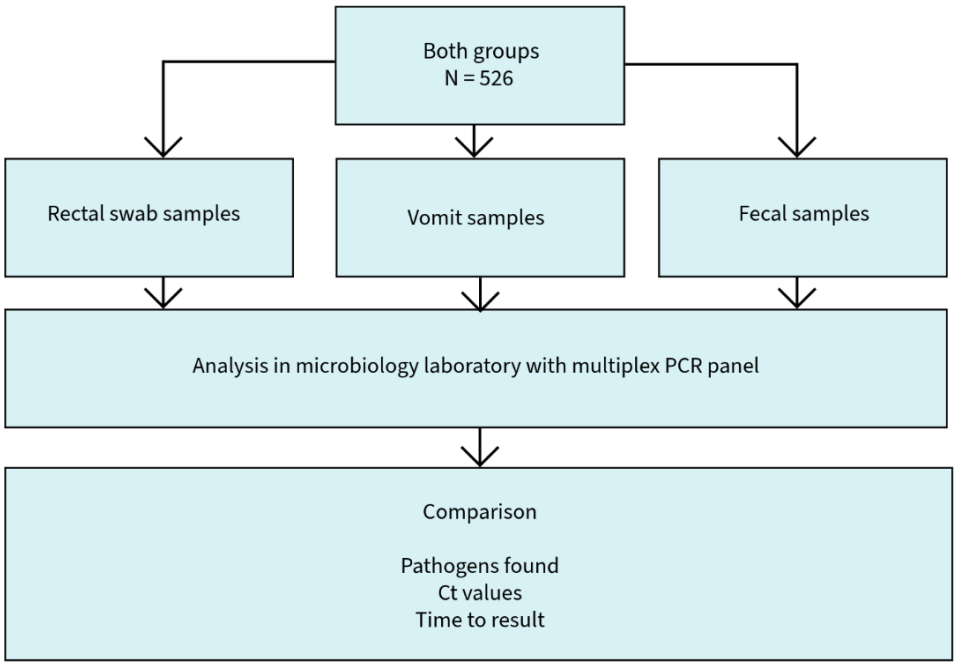

Figure 2. Diagnostic analysis regarding the detection of pathogens in alternative sample types.

4. IMPLEMENTATION

Schedule

The study will be conducted at the Pediatric Emergency Room at the Oulu University Hospital. Recruiting of patients will start in 2025. We estimate that we can recruit 5 patients weekly. Therefore, the estimated duration of the recruiting process will be approximately 2 years until 2027. However, recruiting is continued at least for one epidemiological year to maximize coverage of different epidemics and causative gastrointestinal pathogens. Results of the study will be analyzed and published during 2028.

5. ETHICAL CONSIDERATIONS

The study will not include any additional blood samples. Apart from the possible transportation of fecal samples received at home, there will be no additional visits to hospital due to the study. The equipment used in the study is already in clinical use. We collect written informed consent from all participants and participating is voluntary. Before initiation of the study, we will request approval from Ethical Committee of Oulu University Hospital, Finland.

6. RISK ASSESMENT AND POTENTIAL IMPACT

This is a low-risk project. Most challenging part of this study is recruiting the children. However, gastrointestinal symptoms are one of the most common reasons for pediatric emergency visits. The study does not contain any painful procedures. We have an experienced study nurse with whom we have successfully recruited thousands of study patients in numerous projects in case the pediatric nurses need any support in the recruiting process. We also have a highly experienced and well-funded study group with background of several successfully conducted clinical studies in emergency department settings.

This study is based on a relevant hypothesis and focuses on one of the major patient groups in pediatric emergencies. The randomized controlled setting is scientifically relevant. If successful, it could improve identification of conditions in need for antibiotic treatment or follow-up and on the other hand, decrease testing and revisits in conditions they aren't necessary in. Also, it could reduce unnecessary use of antibiotics in children and therefore reduce long term adverse effects on gut microbiome and children's health.

7. FINANCIAL CONSIDERATIONS

This is an independent academic investigator driven project. Financially, the study contains only low risk. As the *QIAstat-Dx Gastrointestinal Panel 2* is already actively in clinical use at Oulu University Hospital, the fecal sample testing in the active group of the RCT (N = 263) is carried out as a part of accustomed hospital patient care. To purchase the rest of the cartridges needed for the study, procurement contract is made with Qiagen Finland. These analysis costs will be covered partly with grants received by the pediatric infectious diseases study group and partly with support of The Association of Friends at the University Children's Hospitals (Lastenklinikoiden kummit). Doctoral researcher's work is funded by personal grants. The study nurse is already hired by the study group partly with state research funding (VTR) received by the study group, so there will be no additional personnel related costs.

8. STUDY GROUP

- Joni Kalermo, MD, PhD student
- Suvi Mattila, MD, PhD, Co-supervisor of PhD thesis
- Kimmo Halt MD, PhD
- Hilla Pöyry MD, PhD
- Minna Honkila MD, PhD
- Niko Paalanne MD, PhD
- Terhi Ruuska-Loewald, MD, PhD, Professor, Principal supervisor of PhD thesis

All members of the study group are affiliated to Research Unit of Clinical Medicine, University of Oulu and Department of Pediatrics and Adolescent Medicine, Oulu University Hospital.

9. AMENDMENTS

Added "pediatric infectious diseases specialist consultations" to secondary outcomes (16.10.2025).

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