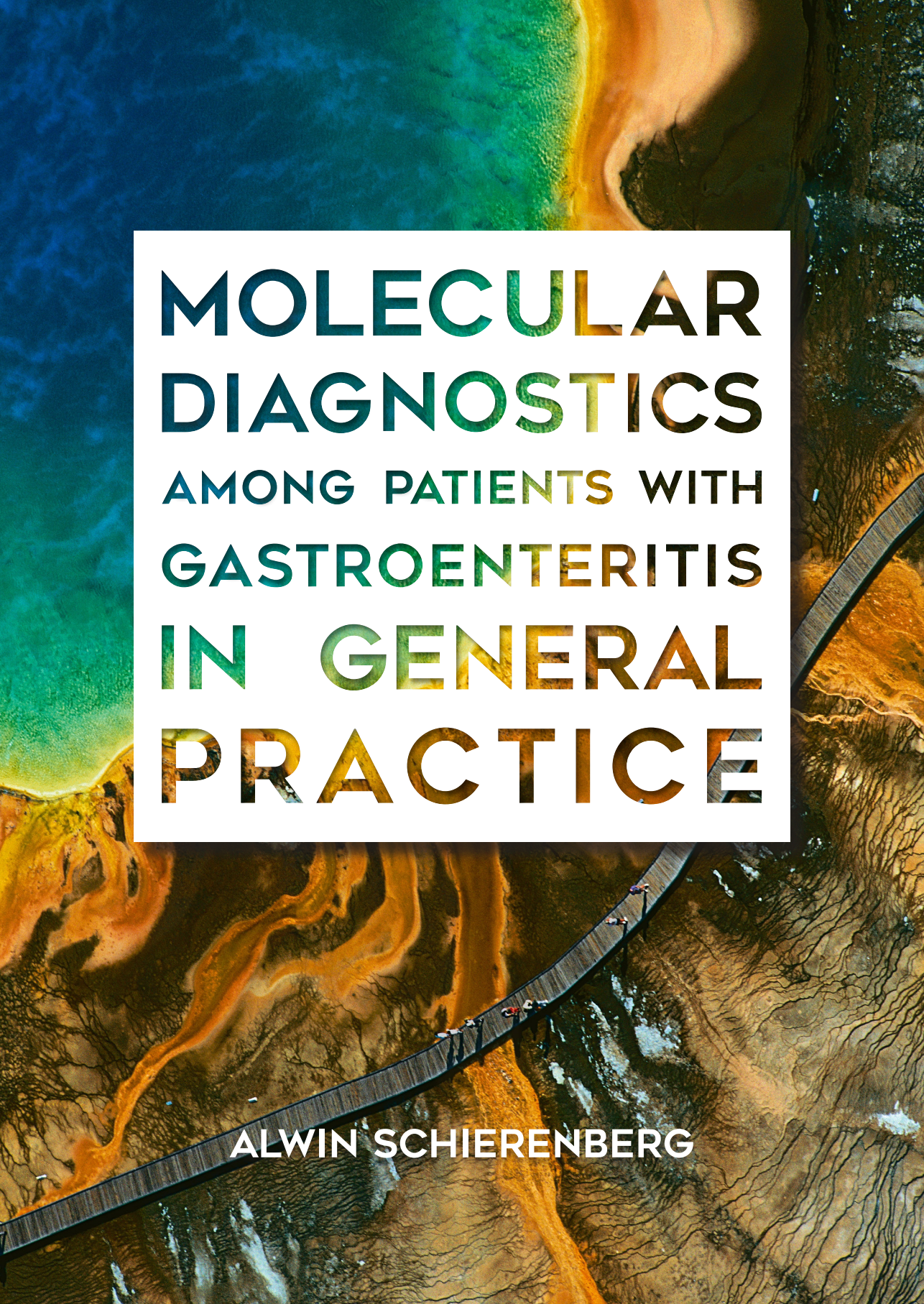
MOLECULAR DIAGNOSTICS AMONG PATIENTS WITH GASTROENTERITIS IN GENERAL PRACTICE
ALWIN SCHIERENBERG

Molecular Diagnostics among Patients with Gastroenteritis in General Practice

Alwin Schierenberg

About the cover

The Grand Prismatic Spring in Yellowstone National Park is the largest hot spring in the United States and was named after its striking resemblance to the rainbow dispersion of white light through an optical prism. Although this hot spring is mainly known amongst tourists for its stunning coloration, the Yellowstone's geyser pools also harbor bacteria that enabled a major scientific breakthrough. In 1969, Thomas Brock, a biologist at Indiana University, found the bacterium *Thermus aquaticus* thriving in the near-boiling waters of these springs. The DNA polymerase of *T. aquaticus*, better known as Taq polymerase, ultimately became the backbone of polymerase chain reaction (PCR). The ability of Taq polymerase to survive the high temperatures required to denature DNA during PCR, meant that PCR could be performed without having to add fresh polymerase each cycle. Brock's discovery enabled PCR to become an indispensable (diagnostic) tool in microbiology.

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Molecular Diagnostics among Patients with Gastroenteritis in General Practice

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(met een samenvatting in het Nederlands)

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CONTENTS

CHAPTER

General introduction

Gastroenteritis is one of the most frequently occurring infections, with a relatively high consultation rate in general practice. Although the disease course is favorable and gastroenteritis is self-limiting for the large majority of patients, the infection may be complicated in high-risk groups, such as immune-compromised patients, frail elderly or young children. In those patients, fecal diagnostics may be indicated to guide management. Traditionally, general practitioners used microbiological culture and microscopy to identify bacteria and parasites causing gastroenteritis. In recent years, molecular-based techniques have become available as an alternative to traditional diagnostic modalities. Molecular diagnostic testing increases the accuracy of stool testing and decreases the time to diagnosis. We set out to evaluate the impact of the introduction of molecular testing on the clinical management and the associated healthcare costs of patients with gastroenteritis that present in general practice.

Gastroenteritis

Gastroenteritis (GE) is an inflammation of the lining of the gastrointestinal tract, including the stomach, small and large intestines. GE usually presents with mild to severe diarrhea that may be accompanied by loss of appetite, nausea, vomiting, cramps, and discomfort in the abdomen. It is usually caused by infection with a microorganism, then referred to as infectious gastroenteritis (IGE), but can also be caused by ingestion of chemical toxins or drugs. The term (infectious) gastroenteritis is commonly referred to as *acute diarrhea* or *infectious intestinal disease* (IID). Every year, around 1 in 3 persons experience an episode of gastroenteritis (GE).[1,2] Most cases of GE in the community have a viral etiology (33.5%)[3], primarily Norovirus (16%).[3,4] *Campylobacter* is the most frequent bacterial cause of GE (1.3-4.6%), whereas *Giardia* is the most frequently detected parasite (0.8-5%). Other bacteria and parasites, such as *Salmonella, Yersinia, Shigella, Clostridium difficile,* and *Entamoeba histolytica*, are less frequent in community cases of GE (0-0.4%).[3,4]

Clinical management of GE in general practice

In the Netherlands, 5 to 12% of patients with GE consult their general practitioner (GP). With 240 to 600 thousand consultations annually, GE poses a substantial burden on primary healthcare.[1,2] Also in patients presenting with GE to their GP, viruses are the most frequent cause (16-35%), but bacteria (15-18%) and parasites (3-9%) are more often detected when compared to community cases with GE.[4,5] The protozoa *D. fragilis* and *Blastocystis spp.* are identified in up to 40% of the GE cases in general practice.[5,6]

Although GE is usually self-limiting – in over 90% of the patients symptoms resolve within 10 days – the disease course may be complicated in immune-compromised and fragile patients, such as infants and elderly.[7] To guide clinical management, GPs may request diagnostic feces testing (DFT) for high-risk patients with the purpose of identifying a causal microorganism. However, clinical practice guidelines (CPG) for general practice in the United Kingdom, the Netherlands and Scandinavian countries advocate restrictive use of DFT in patients with GE symptoms. This is primarily because of the favorable prognosis and the limited benefit of antibiotic treatment, even in GE with bacterial etiology.[7–9] The CPG on acute diarrhea of the Dutch College of General Practitioners recommends to consider DFT only if patients have a) severe illness with fever, frequent watery stools, or bloody or mucosal stools, b) compromised immunity, c) increased risk of disease transmission, as is the case for healthcare workers or food handlers.[7,8] The Dutch and UK CPGs recommend against routine testing for *D. fragilis* and *Blastocystis spp*. For patients with chronic abdominal discomfort and diarrhea, DFT and subsequent antibiotic treatment can be considered after ruling out other causes of diarrhea.[7,10] This restrictive policy is based on the absence of evidence for the effectiveness of antibiotic therapy for *D. fragilis* or *Blastocystis* spp. Infections.[11–17] Up to now, the clinical significance of these protozoa remains unestablished. However, it remains unknown if and how often GPs prescribe antibiotic treatment after identifying these protozoa.

In case of positive DFT results, CPGs generally recommend antibiotic treatment only for patients who are likely to clinically benefit from treatment (e.g. severely ill patients) or because of public health reasons, which overlaps with the indications for DFT.[7,18] This means that even after the identification of a presumed causal microorganism, subsequent antibiotic treatment is recommended only for those patients who had an indication for DFT. Empirical antibiotic treatment, i.e. treatment based on clinical suspicion of bacterial or parasitic GE without confirmation by DFT, is discouraged.[7,8] Although it was documented for earlier versions of the Dutch CPG that compliance with diagnostic indications was only moderate, it is not known how the current guideline is used by GPs and to what extent they adhere to guideline recommendations for DFT and antibiotic treatment.

Conventional diagnostic feces testing

Traditionally, microbiological culture and microscopy were used to identify bacteria and parasites in stools. These conventional DFT techniques are still widely used in many parts of the world, but are laborious and time-consuming and generally have a low diagnostic yield.[6,19–22] Successful bacterial culture is largely dependent on the quality of the fecal sample. Fresh stool samples are ideally processed within 2 hours of collection to ensure *Shigella* and

Campylobacter remain viable for culture.[23] As such short processing time is hardly feasible for samples submitted in primary care, cultured samples may generate false-negative results in patients with a *Shigella* or *Campylobacter* infection. Non-culture dependent testing, using molecular techniques generally are more sensitive with less dependency on sampling quality.[24] Also, some pathogenic bacteria are difficult to culture (e.g. *Clostridium difficile*) or cannot be distinguished from commensal non-pathogenic bacteria through culture, which is the case for *E. coli* and toxin-producing diarrheagenic *E. coli* species.[25] Microscopy is labor intensive and requires extensive technical expertise.[26] Also, as sensitivity can be low due to the intermittent shedding of parasites, multiple samples are required (i.e. triple feces testing) in order to reduce the day-to-day variability in parasite shedding, which poses an additional burden on patients.[27] In general, the collection of an adequate fresh stool sample – especially in the case of watery diarrhea – can be a burden to patients and samples may prove unsuitable for testing when the sampled quantity is too small.

Developments in diagnostic testing in general practice

In the last decade, the diagnostic possibilities in general practice have advanced considerably. At the same time, the use of diagnostic tests, in general, has increased substantially. For example, in the UK an average annual increase of 8.7% was observed for laboratory tests between 2000 and 2015.[28] It is plausible that a similar rise in testing occurred for DFT, but this was not studied. Moreover, in the UK GPs increasingly perform tests for "strategic, non-medical reasons", such as on patient's request. In addition, part of the increase is related to the transition of healthcare services from hospital to general practice.[29,30] Both factors put a strain on primary healthcare providers in order to meet the patients' expectations and health system requirements. Similar developments occur in the Netherlands,[31] where general practice today provides in-practice diagnostic services, such as point-of-care tests to measure hemoglobin levels for the diagnosis of anemia and CRP to diagnose pneumonia or COPD exacerbations,[32,33] as well as extended primary care laboratory services, providing the full spectrum of diagnostic tests for support of clinical practice. Furthermore, developments have progressed to more technologically advanced tests, such as molecular polymerase chain reaction (PCR). Initially used for the diagnosis of sexually transmitted diseases,[34] PCR has also become available as a diagnostic tool in general practice for the diagnosis of IGE. After molecular-based testing for chlamydia became available, the greatest proportionate rise in testing was observed in general practice. This was accompanied by a substantial increase in costs per diagnosis.[35] Although substantial health gains were anticipated, these were not confirmed. In general, traditional diagnostic tests are often replaced by novel diagnostic methods before proper evaluation in an experimental setting or in clinical practice.

Molecular diagnostic feces testing

Polymerase chain reaction (PCR) testing detects the presence of DNA or RNA from micro-organisms in feces samples. Through copying (amplification) of nucleic acids strands of DNA or RNA, it enables for detection of bacteria (e.g. *Salmonella*, *Shigella*, entero-invasive *E. Coli*, *Yersinia*, *Campylobacter.* and *Plesiomonas*), parasites (e.g. *Giardia, Entamoeba histolytica, Dientamoeba, Cryptosporidium and Blastocystis*) and viruses (e.g. Norovirus, Adenovirus, Rotavirus, Sapovirus, Astrovirus). PCR has become available for high throughput diagnostic testing at lower costs, partly due to the expiration of key patents in 2006 and extensive automation.[36] In order to improve the diagnostic accuracy and patient-friendliness of DFT in general practice, multiplex PCR-based DFT has increasingly replaced conventional techniques.[7,22,37,38] PCR allows for highly sensitive identification of multiple enteropathogens in a single stool sample with shorter turnaround times, improved convenience and diagnostic yield as compared to conventional techniques.[22,37,39] While conventional DFT requires up to 4 days before results become available, PCR-based DFT generally takes less than 24 hours. Because of the short turn-around times, GPs may be more inclined to refrain from initiating empirical antibiotic treatment pending PCR test results. Although these advantages could potentially benefit patient management, healthcare use, and associated costs, an evaluation of the introduction of PCR-based DFT in general practice has not yet been performed. Furthermore, PCR-based DFT has some (theoretical) drawbacks that need consideration. For example, the clinical significance of an identified microorganism may not be clear as PCR cannot discriminate between viable and non-viable microorganisms, and between pathogenic and non-pathogenic levels of the microorganism that present in the intestines. This can be a substantial problem, given the high incidence of asymptomatic carriage of infectious agents like for example for *Salmonella*.[40]

In summary, PCR-based DFT may benefit patients with GE in general practice, as it provides an accurate diagnosis and a timely administration of antibiotic treatment, but conversely may also harm patients by increasing the risk of overdiagnosis and overtreatment. Although two hospital-based studies demonstrated that PCR may potentially decrease GE-related healthcare costs and stimulate targeted antibiotic therapy, these results cannot be generalized to general practice.[21,38,41] We, therefore, set out to investigate the impact of PCR-based DFT in patients with GE in primary care.

Aim and outline

The aim of this thesis was to gain insight into the effect of the introduction PCR-based DFT testing on primary care management and costs of GE, as well as in the current management of GE by GPs.

The general design of this study is described in **chapter 2**. First, the effect of the introduction of PCR-based DFT on healthcare use and associated healthcare costs among patients consulting for GE is studied in **chapter 3**. In **chapter 4,** we focus on the effect of replacing conventional parasite DFT (i.e. the triple feces test) with more sensitive protozoal PCR testing on the detection and antibiotic treatment of *D. fragilis* and *Blastocystis* spp. **Chapter 5** describes the frequency of antibiotic treatment for GE, the compliance with CPG recommendations for antibiotic treatment and the results of routine antimicrobial susceptibility testing (AST) in patients who underwent molecular diagnostic feces testing. In **chapter 6,** we investigate the adherence of Dutch GPs to the Dutch general practice CPG on acute diarrhea and a potential change after PCR introduction. Finally, the potential clinical benefit of PCR-based broad-panel DFT compared to current practice targeted DFT, will be evaluated in **chapter 7**.

To conclude this thesis, a summary and discussion of the main findings are presented in **chapter 8**. The discussion will review our principal findings in the context of previous research and provide suggestions for future research and clinical management.

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CHAPTER

Design of the PROUD study: PCR feces testing in outpatients with diarrhea

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ABSTRACT

Background

Infectious intestinal disease (IID) is an important cause of morbidity in developed countries and a frequent reason for general practitioner (GP) consultation. In recent years polymerase chain reaction (PCR) based techniques have gradually replaced conventional enteropathogen detection techniques like microscopy and culture in primary care patients suspected of IID. PCR features testing of multiple enteropathogens in a single fecal sample with shorter turnaround times and greater sensitivity compared to conventional techniques. However, the associated costs and benefits have not been quantified. Furthermore, primary care incidence and prevalence estimates of enteropathogens associated with IID are sparsely available and predominantly based on conventional techniques. The PROUD-study (PCR diagnostics in Outpatients with Diarrhea) determines: 1) health (care) effects and 2) cost-effectiveness of PCR introduction in primary care patients suspected of IID; 3) occurrence of major enteropathogens in primary care patients suspected of IID.

Methods

A before-after cohort study will be performed of patients with suspected IID consulting a GP in the Utrecht General Practitioner Network (UGPN), covering the before period (2010-2011) with conventional testing and the after period (2013-2014) with PCR testing. Prospective study data on patient characteristics and primary outcome measures (i.e. healthcare use and disease outcome) will be collected from electronic patient and laboratory records in 2015 and 2016. The effect of PCR introduction is investigated by comparing the primary outcome measures and their associated healthcare costs between the conventional period and the PCR period and is followed by a cost-effectiveness analysis. To determine the occurrence of enteropathogens associated with IID in primary care, routine care feces samples from the year 2014 will be screened using PCR.

Discussion

The PROUD-study will quantify the costs and effects of the introduction of PCR techniques for enteropathogens in primary care patients suspected of IID and generate up-to-date and sensitive estimates of enteropathogen occurrence among primary care patients.

BACKGROUND

Despite high hygienic standards and socioeconomic level, infectious intestinal disease (IID) remains a major cause of morbidity in developed countries, with a reported incidence of 19-83 cases/100 person years.[1–5] The direct healthcare costs for all-cause gastroenteritis in the Netherlands have almost doubled in the last decade and are estimated at €147 million per year.[6]

In primary care, IID is among the most frequent reasons for consultation,[7] but generally requires supportive treatment only as m0ost IID episodes are self-limiting. According to Dutch guidelines, microbiological feces testing to detect the causative pathogen is only recommended for high-risk patients that may require antimicrobial treatment or pose a substantial transmission risk to others, such as healthcare or food-production workers.

In recent years, molecular-based feces testing using Polymerase Chain Reaction (PCR) techniques have become available for primary care use, replacing conventional microbiological diagnostic techniques like culture and microscopy. PCR feces testing allows detection of multiple enteropathogens in a single sample with shorter turnaround times and greater sensitivity compared to conventional methods.[8,9] Implementation of PCR initially requires a substantial investment, but can potentially lead to an overall cost reduction by extensive automation. Due to its potential added clinical value, primary care diagnostic laboratories in the Netherlands have increasingly replaced conventional techniques by PCR testing. To what extent the introduction of PCR in primary care has affected detection rates of causative enteropathogens, disease outcome and the use of healthcare resources, such as antibiotic prescribing and feces testing has not yet been determined. Before further implementation of PCR feces testing in primary care can be recommended, it is crucial to identify its effects and to evaluate if PCR feces testing in patients with suspected IID is cost-effective in comparison to conventional testing.

Here the study design and rationale of the PROUD-study (PcR feces testing in OUtpatients with Diarrhea), a primary care-based study on IID and PCR introduction in the Netherlands, is described.

METHODS

Objectives

The primary objectives of the PROUD study are:

1. To determine the effects of PCR introduction for enteropathogen detection in primary care on important aspects of *healthcare use* among patients consulting for suspected IID, including the rate of microbiological feces testing, (antibiotic) drug prescription, reconsultation and referral to a medical specialist, and on their *disease outcome*, including IID duration and confirmed enteropathogens.
2. To determine the cost-effectiveness of PCR feces testing in primary care in comparison to conventional testing taking both a program perspective, including only testing costs, and a healthcare payer perspective, including both testing costs and other direct healthcare costs.
3. To determine the occurrence of major enteropathogens as detected by PCR testing among primary care patients with suspected IID.

Secondary objectives investigated in the PROUD study are outlined in Supplement 1.

Study design

To evaluate the introduction of PCR feces testing and improve clinical management of IID in primary care, a before-after cohort study will be performed, including prospective data of a 2-year 'before' period with conventional testing and a 2-year 'after' period with PCR testing, and excluding a one-year wash-in period in which PCR testing is introduced. A before-after study design is adopted while the novel diagnostic technique (PCR) is already implemented in the region of our institute. Besides this reason, a prospective randomized design would require substantial human and financial resources in order to recruit sufficient patients to study the primary objectives. The main advantage of a before-after study design is that it prevents interference with usual clinical care (i.e. GPs are not aware of the on-going study) and laboratory logistics, and the use of prospective study data, therefore representing routine clinical practice.

To determine primary care occurrence of major enteropathogens as detected by PCR feces testing, a nested 1-year microbiological study with full panel enteropathogens PCR testing for IID causing bacteria, parasites, and viruses, will be performed.

Study population

The study population includes patients registered with a general practice affiliated with both the Utrecht General Practitioner Network (UGPN) and Saltro Diagnostic Center. The UGPN database contains pseudonymous routine healthcare data extracted from the Electronic Medical Records (EMR) of 225 GPs in metropolitan Utrecht with approximately 330,000 patients enlisted (in 2013). The general practices contributing to the database contain a representative sample of the Dutch population. The GPs working in participating practices are trained in the correct use of International Classification of Primary Care (ICPC) coding and have on average 10 years' experience in systematic coding of disease episodes.[10] Saltro Diagnostic Center is a large primary care laboratory operating in the UGPN service area and replaced conventional enteropathogen testing by PCR in April 2012.

In the before-after study, subjects eligible for inclusion are patients consulting a UGPN practitioner with suspected IID in the before period (2010-2011) or after period (2013-2014) (Figure 1, population 1a and 1b). Suspected IID is defined as a consultation coded with ICPC D11 (diarrhea), D70 (gastrointestinal infection) or D73 (suspected infectious gastroenteritis). A one-year wash-in period (2012) is excluded from the analysis to account for adaptation to the new PCR strategy.

In the microbiological study, all patients referred by an UGPN physician to Saltro Diagnostic Center for microbiological feces testing in 2014 were included (Figure 1, population 3a and 3b). Therefore, the microbiological study population includes all PCR tested UGPN patients, regardless of the assigned ICPC codes.

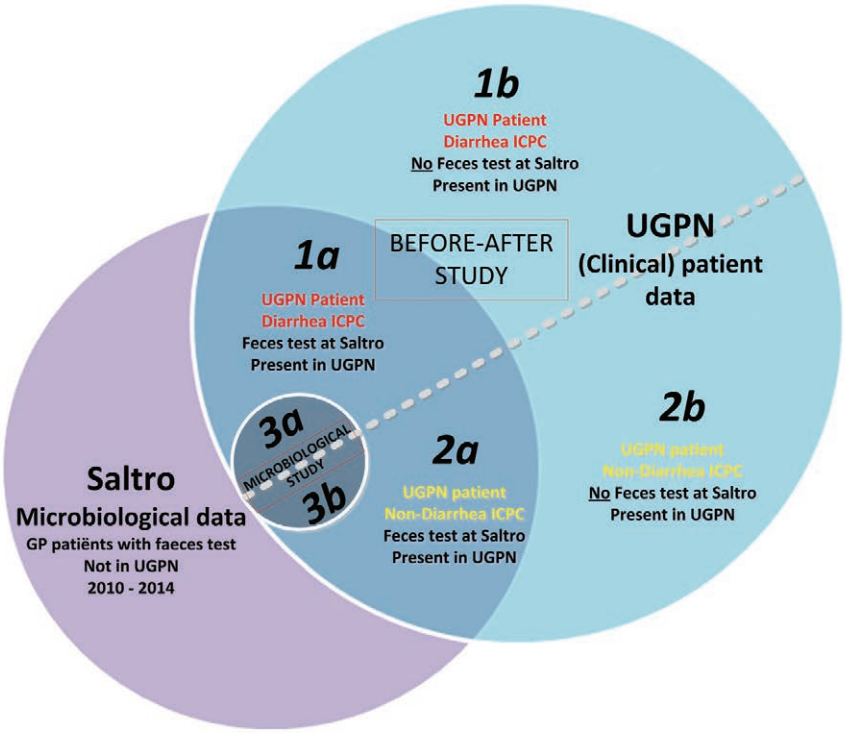
Measurements before-after study

Clinical patient data

For each patient with an episode of suspected IID, routine care data on *patient demographics*, *healthcare use,* and *disease outcome* are extracted from the EMR. *Patient demographics* include age, gender, ICPC coded co-morbidities, immunocompromised status (e.g. chronic immunosuppressive therapy, chronic renal and/or liver disease, current malignancy, and chemotherapy) and other assumed IID risk factors present at the first time of consultation for suspected IID (Supplement 2). *Healthcare use* per disease episode (defined as a period between the first and last consultation for the same indication with a minimum of 60 days) includes drug prescriptions, assigned ICPC codes (Supplement 3), and the number and type of consultations per episode. *Disease outcome* includes duration per episode and enteropathogens identified.

Mortality is not measured, as it is not part of the UGPN data and not retrievable via Municipal Administration (GBA) since direct identification of included subjects is not possible.

Figure 1. Sources and composition of the study population of 'before' and 'after' cohorts. Blue circle: Clinical patient data from Utrecht General Practitioner Network (UGPN) of 2010-2014. Purple circle: feces testing result from Saltro Diagnostic Center of 2010-2014. 1/2a: UGPN patient with a feces test. 1/2b: UGPN patient without feces test. 1a/b: UGPN patients with a coded episode of suspected Infectious Intestinal Disease (IID). 2a/b: UGPN patients without a coded episode of suspected IID. 3a/b: Patients included in the microbiological study with/without a coded episode of suspected IID.



Linkage of clinical patient data with feces test data

To link (clinical) patient data with corresponding microbiological test results, all patients identified in the UGPN database are linked with the laboratory records from Saltro Diagnostic Center by a 'trusted third party' using a pseudonymization procedure in accordance with the Dutch Health Insurance Portability and Accountability Act of 1996.

Feces testing

Results for 14 enteropathogens (Table 1) of patients with suspected IID who underwent conventional feces testing (microscopy, culture and/or enzyme immunoassay [EIA]) in the before period and with primarily PCR testing in the after period, are gathered. For both methods, the relative sensitivity, specificity, and efficiency will be determined, also proving a basis for the cost-effectiveness analysis (objective 2).

Table 1. IID causing enteropathogens (n=14) included in "before-after" study.

Enteropathogen	Before test		After test	
	Test method	**Identification method**	**Test method**	**Identification method**
Campylobacter spp.	Culture	Campylobacter selective agar, hippurate hydrolysis identification	PCR	LightMix Modular Gastro Bacteria
Clostridium difficile	EIA	ImmunoCard Toxine A/B	EIA	ImmunoCard Toxine A/B
Salmonella spp.	Culture	XLD agar, Vitek identification	PCR	TIB MOLBIOL LightMix Modular Gastro Bacteria
Shigella spp.	Culture	XLD agar, Vitek identification	PCR	TIB MOLBIOL LightMix Modular Gastro Bacteria
Plesiomonas spp	Culture	XLD agar, Vitek identification	PCR	TIB MOLBIOL LightMix Modular Gastro Bacteria
Yersinia spp.	Culture	CIN agar, Vitek identification	PCR	TIB MOLBIOL LightMix Modular Gastro Bacteria
Blastocystis hominis	Microscopy	Direct with accumulation (Ridley)	PCR	TIB MOLBIOL LightMix Modular Gastro Parasites
Cryptosporidium spp.	Microscopy	Direct with accumulation (Ridley)	PCR	TIB MOLBIOL LightMix Modular Gastro Parasites
Dientamoeba fragilis	Microscopy	TFT	PCR	TIB MOLBIOL LightMix Modular Gastro Parasites
Entamoeba histolytica	Microscopy	Direct with accumulation (Ridley)	PCR	TIB MOLBIOL LightMix Modular Gastro Parasites
Giardia spp.	Microscopy	Direct with accumulation (Ridley)	PCR	TIB MOLBIOL LightMix Modular Gastro Parasites
Adenovirus 40/41	ICS	R-Biopharm RIDA Quick Adeno/Rotavirus	ICS	R-Biopharm RIDA Quick Adeno/Rotavirus
Norovirus	ICS	R-Biopharm RIDA Quick Nonvirus	ICS	R-Biopharm RIDA Quick Nonvirus
Rotavirus	ICS	R-Biopharm RIDA Quick Adeno/Rotavirus	ICS	R-Biopharm RIDA Quick Adeno/Rotavirus

CIN= Cefsulodin-Irgasan-Novobiocin, EIA= Enzyme Immunoassay, ICS= Immunochromatographic strip, TFT= Triple Feces Test, XLD= Xylose-lysine-Deoxycholate

Measurements for microbiological study

For the microbiological study (objective 3), fecal samples sent for microbiological testing from all participating UGPN practices to Saltro Diagnostic Center in 2014 are tested with PCR (Supplement 4) for 19 of the enteropathogens as described in Table 2.

Outcome measures

In the before-after study the outcome measures are: *healthcare use*; operationalized as the proportions of feces testing, (antibiotic) drug prescribing, number of GP consultations per disease episode and specialist referrals during each period among patients consulting their GP for suspected IID, and *disease outcome*; operationalized as confirmed IID indicated by a positive test result and disease duration defined as the number of days between the first and the last consultation of the episode (objective 1).

In the economic evaluation, several outcome measures for *costs* and *effects* are included. For *costs* healthcare costs, testing costs and total costs (healthcare and testing costs) per episode and in total, are included. Included *effects* per disease episode are the proportion of feces testing, detected relevant enteropathogens, antibiotic prescription, reconsultation and referral in the before and after periods. To compare conventional testing to PCR testing (objective 2), the difference in *costs* will be compared to a difference in the mentioned *effects* and expressed as a cost-effectiveness ratio (CER), for example, the additional costs per detected relevant enteropathogen.

In the microbiological study, the outcome measure is the absence or presence per enteropathogen in the collected feces samples detected by PCR (objective 3).

Statistical analysis

Objective 1

To estimate the effect of PCR introduction on *healthcare use* and *disease outcome* the above-mentioned outcome measures are compared between the patient cohorts in the before and after period, taking into account potential differences in patient characteristics and comorbidities between the two cohorts (Supplement 2). Differences in continuous and categorical outcome measures are quantified by Mann-Whitney U tests and Fisher's exact tests, respectively. To assess the independent effect of the introduction of PCR on the proposed outcome measures, an interrupted time series analysis is performed incorporating potential confounding variables including age, gender, policy deductibles of health care insurance and patient co-morbidities (DM, COPD, asthma, cardiovascular diseases, inflammatory bowel disease

Table 2. IID causing enteropathogens (n=19) included in PCR testing for microbiological study

Enteropathogen	PCR assay	PCR system	Positive test
Campylobacter spp., *Salmonella spp., Shigella spp., Plesiomonas spp., Yersinia spp.*	TIB MOLBIOL LightMix® Modular Gastro Bacteria	LightCycler 480 II	Ct <45
Clostridium difficile	R-Biopharm RIDA®GENE Clostridium difficile Toxin A/B	LightCycler 480 II	Ct <45
EHEC/STEC, EPEC	R-Biopharm RIDA®GENE E.Coli Stool Panel 1	LightCycler 480 II	Ct <45
Blastocystis hominis, Cryptosporidium spp., Dientamoeba fragilis, Entamoeba histolytica, Giardia spp.	TIB MOLBIOL LightMix® Modular Gastro Parasites	LightCycler 480 II	Ct <45
Adenovirus 40/41, Astrovirus, Norovirus, Rotavirus, Sapovirus	Laboratory Developed Test	ABI 7500	Ct <45

EHEC= Enterohaemorrhagic Escherichia coli, STEC= Shiga toxin-producing Escherichia coli, EPEC= Enteropathogenic Escherichia coli

[IBD], irritable bowel syndrome [IBS], and immunocompromising disease and medication). This type of analysis uses segmented regression to measure changes in level and slope in the before period compared to the after period to control for secular trends in the data,[11] making an adjustment for individual-level characteristics unnecessary.[12]

Objective 2

Healthcare costs are calculated by multiplying the extracted healthcare resources used with their unit cost prices according to the Dutch guidelines.[13, 14] Test costs are calculated based on the unit cost prices according to the Dutch Healthcare Authority (NZA) tariffs, material and overhead costs for conventional and PCR testing. All costs are expressed for the year 2015 and considered both undiscounted and discounted (i.e. 4%). To determine the cost-effectiveness of PCR testing compared to conventional feces testing, a cost-effectiveness analysis (CEA) is performed from both a program perspective (i.e. testing costs only) and from a healthcare payer perspective (i.e. healthcare costs and testing costs) respectively, and expressed as CERs. Subgroup analysis is performed for various age groups and for all pathogen-groups (i.e. bacteria, parasites, and virus testing). Finally, cost-effectiveness for scenarios with different levels for PCR costs and cut-off values for PCR sensitivity (Ct-values) are evaluated. Adjustment for potential confounding variables is performed as described above. It is assumed that within the study periods no alterations in the healthcare setting will occur that significantly influence the results, like changes in personnel (e.g. GP) and background changes in GP population.

Objective 3

To determine the incidence of enteropathogens in primary care patients suspected of IID as detected by PCR testing, the proportion and 95% CI of individual and combined infections are calculated. First using the number of patients with microbiological testing performed as the denominator, and secondly by extrapolation to the general population of patients that visit the GP with suspected IID through standardization by age, gender, patient comorbidities (as described under objective 1) and episode ICPC code according to the distribution in the complete cohort of 2014.

Power calculations

We assessed the statistical power of the before-after study, covering a 2-year "before" period with conventional testing and a 2-year "after" period with PCR testing and excluding a one-year wash-in period in between. These calculations were based on the minimal detectable difference between the two study periods for two clinically important outcomes: the proportions of IID patients in whom diagnostic testing is performed and the proportion of prescribed antibiotics. Two-tailed Fisher's exact tests were performed using a significance level of 5% and a power of 90%. Based on previous evidence it is anticipated that between 4,615 and 11,539 of the 330,000 UGPN patients annually consulted their GP with suspected IID (Figure 1, population 1a/b),[1] of which around 12% (554-1,385) are tested for enteropathogens in the before period (Figure 1, population 3a).[15] The minimal detectable difference in the proportion of diagnostic tests performed between the two periods ranges between 1 to 1.6% depending on the actual number of suspected IID cases. Anticipating a 27% antibiotic prescription rate among patients with suspected IID in the conventional period, around 9,230-23,078 patients per year were expected to receive an antibiotic prescription.[15] Depending on the actual number of suspected IID cases, the minimal detectable difference in antibiotic prescribing rates ranged between 1.3 and 2.1%.

For the one-year microbiological study with PCR testing, the achievable precision in estimates for enteropathogen proportions was evaluated through an exploration of the 95% confidence interval (CI) widths over a range of plausible enteropathogen proportions. As described above we expected at least 554-1,385 UGPN patients per year with an episode of suspected IID and a feces test. The width of the CI ranged between ±0.2% for the least expected pathogen (*Shigella* spp.) and based on the largest estimated sample size, to ±3.4% for the most prevalent pathogen (*Blastocystis hominis*) and smallest sample size (Supplement 5).

It was concluded that a before-after study including two periods of 2 years and microbiological study including a 1-year period of PCR testing were sufficient to study our primary objectives.

Ethical approval

The act on medical research involving human subjects does not apply to this study and therefore official approval of this study by the Institutional Review Board (IRB) of the University Medical Center Utrecht was not required (IRB-number: 13-480).

DISCUSSION

The PROUD study will quantify the effects of the introduction of PCR feces testing and its cost-effectiveness in primary care patients with suspected IID and may guide further large-scale implementation of PCR testing in primary care. It will also describe the epidemiology of IID and etiology of enteropathogens detected with PCR, both relevant for clinical practice and healthcare policymaking.

Conventional and molecular techniques for the detection of enteropathogens exhibit different test characteristics, where PCR-based testing has a lower turnaround time and its increased sensitivity may yield 1.4 to 3-fold higher detection rate.[8, 9, 16, 17] The latter leads to a higher diagnostic yield, but may also detect non-relevant microorganisms. It is therefore important to include the effects of the test results on patient management in the evaluation of introducing PCR-based testing, rather than exclusively focusing on the technical performance of these diagnostic techniques.

Strengths

The use of routine study data and a before-after study design prevents interference with routine clinical care in primary care patients with suspected IID, excluding bias introduced by an observer effect.[20] However, the before-after study design may be susceptible to bias but has previously been used successfully to evaluate the cost-effectiveness of an infection control program to reduce nosocomial respiratory syncytial virus transmission and an intervention to reduce the spread of influenza during the H1N1 pandemic.[18, 19]

The possibility to use the UGPN database, including 330,000 patients, allows us to study sufficient numbers of patients in a relatively short time period. Naturally, our study domain is restricted to patients consulting their GP for IID, which we consider the relevant population of

this research question. Lastly, the use of a third trusted party enables for the merging of patient data on an individual level and ensures the anonymity of included patients.

Limitations

Ideally, a randomized controlled trial comparing effects between two diagnostic strategies without extraneous (other than the diagnostic strategy) factors would have been performed. However, a prospective randomized design would require substantial human and financial resources in order to recruit sufficient patients to study the primary objectives and exceeds the available budget. Moreover, as PCR feces testing was already implemented in routine practice when initiating the PROUD study, a comparison to conventional techniques was logistically unfeasible.

In the economic evaluation, a restricted perspective has to be taken, as we only have information on testing costs and potential savings in healthcare costs in primary care. Potential positive monetary and health effects due to, for example, reduced hospitalization, earlier detection of an outbreak leading to reduced monitoring costs and the prevention of disease complications (e.g. sepsis, sequelae) through timely diagnosis and appropriated treatment were not included as this information is lacking in the consulted databases. Therefore, this economic evaluation will lead to conservative CER estimates but will resemble the costs and effects that are relevant for the primary care domain.

Furthermore, a one-year microbiological study will potentially be more prone to fluctuations when compared to studies including multiple years. Yet, yearly variation is mainly observed for enteric viruses, whereas for more clinically relevant bacterial and parasitic enteropathogens fluctuations are less common.

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CHAPTER

Economic evaluation of molecular diagnostic feces testing in primary care patients with gastroenteritis

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ABSTRACT

Background

In recent years, diagnostic feces testing (DFT) by polymerase chain reaction (PCR) has gradually replaced conventional culture and microscopy for enteropathogen detection in primary care. However, the effect of this altered diagnostic approach on primary healthcare consumption and costs is unclear.

Methods

We performed a cost analysis using electronic patient records from a large Dutch general practice network. Within this network, conventional DFT was replaced by PCR testing in 2012. All gastroenteritis (GE) episodes from 2010-2011 ("pre-PCR period"; conventional DFT) and 2013-2014 ("post-PCR period"; PCR testing) were extracted. For each GE episode, we extracted data on DFT, antibiotic prescriptions, consultations, and specialist referrals. Healthcare resource consumption and direct healthcare costs (DHC) were calculated for each GE episode. We estimated the effect of the DFT method on healthcare resource consumption and calculated the incremental DHC – attributable to the use of PCR-based DFT – while adjusting for secular time trends, seasonality and differences in case-mix in both study periods.

Results

A total of 10,947 GE episodes were identified in the pre-PCR period and 13,276 in the post-PCR period. In the post-PCR period there were increased rates in testing for bacteria (RR=1.40; [95%CI=1.30-1.52]) and parasites (1.66; [1.55-1.79]), number of GE related referrals for DFT (1.29; [1.22-1.38]), telephone consultations (1.23; [1.18-1.28]), prescription of antiprotozoal drugs (2.49; [2.14-2.90]), referrals to an internal medical specialist (1.97; [1.67-2.32]), and a decrease in GE related home visits (0.71; [0.64-0.79]). Average DHC per GE episode were €76.2 before and €93.4 (95%CI=€92.8-93.9) after PCR introduction, while adjusting for cohort differences. The estimated adjusted incremental DHC – attributable to the change in DFT method – was €17.1 (95%CI=€16.6-17.6) per GE episode, which translated to a 22.4% (21.8-23.1%) increase in DHC, of which 79% resulted from increased healthcare resources consumption. Extrapolated to the Dutch population, the incremental DHC of PCR-based DFT introduction is €7.4 million (95%CI=€7.2-€7.6 million) per year.

Conclusion

The introduction of PCR-based DFT in primary care is associated with a 22% cost increase per GE episode, of which 79% was attributable to healthcare resources consumption and 21% to PCR-based bacterial testing costs.

INTRODUCTION

In Western countries, 20-80% of the people experience an episode of gastroenteritis (GE) each year.[1–3] Estimated average direct healthcare-costs (DHC) per GE episode in the community varies between €14-32 in the Netherlands and €75-155 in the United States. Costs for the subset of patients seeking healthcare attendance are significantly higher (€472 per case in the US).[4] reflecting the additional cost of diagnostic workup and clinical management. In the Netherlands, 5-12% of patients' with acute diarrhea consult their general practitioner (GP).[2,5,6] GE can be caused by a range of bacteria, parasites, and viruses, and differentiation between these infectious agents – based on clinical signs and symptoms – can be difficult.[5,7,8] As most episodes of GE are self-limiting, primary care practice guidelines recommend to refrain from diagnostic feces testing (DFT). For immune compromised patients, those with a severe disease course, or in case of an increased epidemiologic risk of spreading (e.g. food-handlers), guidelines recommend DFT and pathogen guided follow-up treatment or infection control measures.[9,10]

Conventional DFT techniques like culture, microscopy, and antigen detection are still widely used in many parts of the world, but are laborious and time-consuming and generally have a low diagnostic yield.[8,11–14] Also, the sensitivity of conventional parasite testing can be low due to the intermittent shedding of parasites. This requires the examination of multiple samples (i.e. triple feces testing), which poses an additional burden on patients.[15] For all these reasons, molecular multiplex Polymerase Chain Reaction (PCR)-based DFT has increasingly replaced conventional techniques.[9,14,16,17] Multiplex PCR allows for highly sensitive identification of multiple enteropathogens in a single stool sample with shorter turnaround times, improving convenience and diagnostic yield as compared to conventional techniques.[14,16,18] More sensitive and rapid pathogen detection by PCR-based DFT may improve GE management and patient satisfaction in primary care. On the other hand, it may also result in overdiagnosis and overtreatment of clinically irrelevant pathogens, and increased healthcare costs. Therefore we evaluated the effects of replacing conventional DFT with PCR testing in primary care patients with GE on healthcare resource consumption and associated direct healthcare costs (DHC).

METHODS

Study design

A cost analysis was performed using routine primary care data from the Julius General Practitioner Network (JGPN) from the period 2010-2014. Within the practices in this study cohort (described below), PCR replaced conventional DFT from April 2012 onwards. We excluded data from the year 2012 to account for an adaptation period for the introduction of PCR-based DFT. Conventional DFT included culture for bacteria and triple feces testing for parasites. PCR-based DFT included a panel of selected bacteria and parasites (Chapter 2, Table 1). To adjust for any potential underlying time trend in the requests of diagnostic testing, we examined the requests for viral DFT in the same time periods. Since the diagnostic method for enteric viruses – using immunochromatographic strip tests – did not differ between the periods, it can serve as a negative control group. In patients with an episode of GE, we compared the mean healthcare resource consumption between the first period with conventional DFT and the subsequent period with PCR-based DFT. Costs of DFT, GP consultations, prescriptions for antibiotic therapy, and referrals to a specialist were included in our analysis if they were incurred within 60 days of a recorded GE episode. Costs associated with the negative control group (viral DFT) were not included in the economic evaluation.

Study cohort

The cohort contains routine care data from the Julius General Practitioner Network (JGPN) and microbiological test data from Saltro Diagnostic Center. The JGPN database consists of pseudonymized healthcare data from 45 primary care practices with 160 GPs in the greater Utrecht area, the Netherlands, with approximately 290,000 patients listed (reference date: 31-7-2012).[19] The database contains information on all routine care GP contacts. Every contact is coded according to the International Classification of Primary Care (ICPC)[20] and prescriptions are listed according to the WHO guidelines for Anatomical Therapeutic Chemical (ATC) classification.[21] GPs working in participating practices are trained in the correct use of ICPC coding and have on average 10 years' experience in systematic coding of disease episodes.[22] Saltro Diagnostic Center is a primary care laboratory that provides diagnostic services to primary care professionals in the central region of the Netherlands. Approximately 96% of the GPs in the JGPN network use their laboratory facilities for microbiological testing. JGPN and Saltro databases were linked using a standardized, GDPR proof pseudonymization procedure.[19] More elaborate information on the study design and population can be found in the study design statement.[23]

Data collection

Patient characteristics

For each GE episode, we collected patient information with clinical relevance for the management of GE, such as patients' age, gender, comorbidity such as intestinal disorders, malignant disease, diabetes mellitus (DM), COPD, asthma, immunocompromising disorders, and chronic pharmacotherapy with acid-suppressive medication, immunosuppressants, and chemotherapy (Supplement 6). An episode of GE was defined as consultations labeled with International Classification of Primary Care (ICPC)-codes D11 (Diarrhea), D70 (Gastrointestinal infection), or D73 (Gastroenteritis presumed infection), and with a maximum episode duration of 60 days.

Healthcare consumption data

We quantified the total GE-related healthcare consumption per GE episode. For each DFT order, we determined whether testing was performed for bacteria and parasites, and the number and type of pathogens testing positive. GP consultations included in-office consultations, home visits, and telephone consultations. Antibiotic prescriptions included all antibiotics recommended for the treatment of GE by the Dutch primary and secondary care clinical practice guidelines[9,10]. These included *intestinal anti-infectives* (paromomycin and vancomycin), *antibacterials for systemic use* (co-trimoxazole, erythromycin, azithromycin, ciprofloxacin, teicoplanin, fidaxomicin), and *antiprotozoal agents* (metronidazole and clioquinol). We also included referrals to pediatricians and internal medicine specialists (such as gastroenterologists). Indirect GE-related costs, such as productivity loss, were not considered.

Costing of health care interventions

Costing data for DFT were based on the order request costs plus the laboratory costs, which were obtained from 15 regional primary care laboratories for the year 2016. Costs were averaged to determine the mean cost per test. Unit resource costs for consultations and specialist referrals were based on the latest guideline prices of the Dutch National Health Care Institute (2015).[24] Medication unit costs were based on the Pharmacy Purchase Price index of the Dutch National Health Care Institute. Per drug, the total costs were calculated by multiplying the prescribed quantity with the dosage specific unit costs and including a dispensing fee of €6.- per prescription.[25] All costs were adjusted to 2016 prices using the Dutch consumer price index, when applicable.[26]

Analysis

Baseline patient characteristics, crude healthcare resource consumption per 1000 GE episodes, and associated costs per GE episode were compared between the two periods using the Pearson's chi-squared test for dichotomous variables and the Kruskal-Wallis test for continuous and categorical variables.

The adjusted incremental DHC per GE episode – attributable to the introduction of PCR-based DFT – were estimated in three steps. First, the change in healthcare resource consumption after the introduction of PCR-based DFT was estimated for each healthcare resource, by calculating the rate ratios (RR) using negative binomial regression to correct for expected overdispersion. Second, resources with a significant change in RR after PCR introduction ($p<0.05$) were selected to calculate the incremental attributable DHC. Third, we multiplied the predicted resource use per episode (using the previously described negative binomial regression models) by the resource list price (appropriate for that period, see Table 2) and summed all costs. The mean difference between the pre- and post PCR period was calculated and the 95% confidence interval (CI) was estimated using bootstrapping (n=30,000). To discriminate between costs attributable to the change in resource costs (i.e. for DFT) and those attributable to a change in healthcare consumption, we performed the above-described regression analyses using the resource costs from the pre-PCR period. This provided the adjusted incremental DHC per GE episode attributable to healthcare consumption only. All regression analyses were adjusted for (1) potential differences in case-mix before and after PCR introduction using a predefined set of confounding factors,[23] (2) seasonal influences on resource consumption using two Fourier terms, and (3) for holidays and the first week after each holiday[27]. We additionally established if a correction for a secular time trend of underlying requesting behavior was necessary. Therefore, we estimated the linear time trend (per study month) for viral DFT between 2010 and 2014, using negative binomial regression analysis and above-mentioned adjustment methods. If a significant monthly baseline trend in viral testing was found ($p<0.05$), the estimate was included in the models for all resources as a fixed regression coefficient (i.e. $\gamma=\beta_{\text{trend}}\times\text{study month}$.). Separately for patients with and without DFT, we additionally calculated the crude and adjusted incremental DHC post-PCR introduction per GE episode, using the above-mentioned procedures.

Finally, we calculated the yearly attributable incremental DHC for the Dutch population by multiplying the adjusted incremental DHC per GE episode by the total yearly number of GE episodes in Dutch primary care, which we estimated using the average observed GE incidence

in our primary care database for the post-PCR (see Results section) and a population count of 17,081,507 (reference date: 1-11-2017).[28]

All statistical analyses were conducted in R 3.3.2, using the *tableone*, *dplyr, boot and mice* packages.[29–33]

Missing data

For some electronic medical record systems used in the JGPN practices, data on consultations and specialist referrals could not be extracted. Data for these variables were imputed in R using the *mice* package.[29] The imputation model included variables on age, gender, consultations, comorbidity, chronic medication use, DFT requests, antidiarrheal drugs prescription, antibiotic prescription, and referrals. The imputation process was performed using predictive mean matching, which was considered appropriate for the imputation of missing numeric data.

RESULTS

Patients characteristics

We identified 10,947 GE episodes (incidence rate=24.7/1000 person-years) before PCR introduction and 13,271 episodes (25.7/1000 person-years) after PCR introduction (p<0.001) (Table 1). Compared with the pre-PCR period, patients in the post-PCR period were on average younger (32.3 years versus 33 years, p=0.03), less often female (54.2% versus 57.2%, p<0.001), more often had intestinal and immunocompromising comorbidity, and used corticosteroids and antacids more often (Table 1).

Missing data

Data on consultations and specialist referrals could not be extracted for 231 (1%) and 4,421 (18%) of the GE episodes, respectively. Data for one or both variables were missing in 2018 (18.4%) and 2,481 (18.7%) episodes in the pre- and post-PCR cohort, respectively (Supplement 7). Overall, 221 (2%) episodes before PCR and 10 (0.1%) episodes after PCR introduction had missing data on consultation, while 1940 (17.7%) and 2481 (18.7%) episodes had missing data on specialist referrals, respectively (Table 1, footnote 3). Details on the imputed data are provided in Supplement 7.

Table 1. Baseline characteristics of primary care patients presenting with GE during conventional DFT (2010-2011) and PCR-based DFT (2013-2014). Table includes all the available data. Data regarding imputation can be found in Supplement 7.

Patient characteristics	GE episodes		p-value
	Before PCR introduction N*=10,947	After PCR introduction N*=13,271	
Age, mean (SD)	33.04 (27.49)	32.28 (27.06)	0.030
Gender, Female	6,266 (57.2)	7,199 (54.2)	<0.001
Comorbidity[1]			
Intestinal	1,255 (11.5)	1,639 (12.4)	0.036
Malignancy	267 (2.4)	353 (2.7)	0.297
Immunocompromising disease	338 (3.1)	505 (3.8)	0.003
DM	846 (7.7)	996 (7.5)	0.530
COPD	403 (3.7)	467 (3.5)	0.521
Asthma	923 (8.4)	1,397 (10.5)	<0.001
Chronic medication use[1]			
Systemic corticosteroids	480 (4.4)	713 (5.4)	<0.001
Chemotherapy	25 (0.2)	51 (0.4)	0.041
Immunosuppressants	51 (0.5)	76 (0.6)	0.291
Acid suppressive drug	2,090 (19.1)	3,016 (22.7)	<0.001
DFT orders			<0.001
None	9,483 (86.6)	10,842 (81.7)	
1 order	1,263 (11.5)	2,219 (16.7)	
2 orders	173 (1.6)	186 (1.4)	
3 orders	28 (0.3)	22 (0.2)	
4 orders	0 (0.0)	2 (0.0)	
Type of DFT[2]			
Bacteria	1,053 (9.6)	1,871 (14.1)	<0.001
Bacteria (positive) with AST	140 (13.3)	284 (15.2)	0.182
Parasites	1,042 (9.5)	2,073 (15.6)	<0.001
Viruses	142 (1.3)	306 (2.3)	<0.001
Consultations[3]			
Total, mean (SD)	1.75 (1.72)	1.93 (1.98)	<0.001
In-office, mean (SD)	1.09 (1.13)	1.14 (1.25)	0.006
Home visits, mean (SD)	0.12 (0.52)	0.09 (0.49)	<0.001
Telephone,mean (SD)	0.57 (1.08)	0.71 (1.26)	<0.001

Patient characteristics	GE episodes		p-value
	Before PCR introduction N*=10,947	After PCR introduction N*=13,271	
Antibiotic prescriptions			
Any	791 (7.2)	1,316 (9.9)	<0.001
Intestinal anti-infectives	40 (0.4)	41 (0.3)	0.519
Antibacterials for systemic use	530 (4.8)	624 (4.7)	0.633
Antiprotozoal agents	240 (2.2)	687 (5.2)	<0.001
Specialist referrals[4]			
Any	286 (3.2)	660 (6.1)	<0.001
Internal medicine	173 (1.9)	406 (3.8)	<0.001
Pediatrician	108 (1.2)	226 (2.1)	<0.001

AST=Antibiotic Susceptibility Testing; DFT=Diagnostic Feces Testing; PCR=Polymerase Chain Reaction;
*Numbers represent counts (%), unless indicated else, and are based on non-imputed data;
[1]See Supplement 6 for the specification of comorbidity and medication groups;
[2]Multiple types of stool tests can be included in one order;
[3]Data on consultations were missing in 221 (2%) cases before PCR and 10 (0.1%) after PCR;
[4]Data on specialist referrals were missing in 1940 (17.7%) cases before PCR and 2481 (18.7%) after PCR.

Healthcare consumption and costs

Direct healthcare-related costs for consultations, DFT, medication, and referral are specified in Table 2, Table 3 and Supplement 8. After PCR introduction, the unadjusted total DHC per GE episode increased from €76.2 before to €94.4 (difference €18.2, p<0.001) (Table 4). This was due to increased use of DFT, to more in-office and telephone consultations, and to more referrals to an internal medicine specialist. This increase in expenses was primarily attributable to the increased use and the higher costs of DFT (€13 per GE case) and to the rates of bacterial testing (131/1000 before versus 183/1000 after PCR introduction) and parasite testing (102 versus 170/1000) increased, with accompanying incremental costs of €6.6 and €4.6 per GE episode, respectively (Table 4).

Trend in resource consumption

After adjustment, the rate of virus DFT per month did not alter between 2010 and 2014 (RR= 0.998, 95%CI=0.992-1.002). Therefore, we concluded that correction for an underlying secular time trend of requesting behavior was not indicated.

Table 2. Unit resource costs for diagnostic feces testing and antibiotic susceptibility testing (AST). Costs are averaged tariffs of primary care laboratories (n=16).

Stool test type	Period	N[1]	Mean costs per test[2]	Mean costs for AST per isolate[2]
DFT order bench fee	Before/After	15	€ 12.5	-
Culture	Before	6	€ 66.3	€ 44.6
Triple Feces Test	Before	4	€ 97.9	-
PCR bacteria	After	11	€ 91.4	€ 79.8
PCR parasites	After	11	€ 87.5	-

AST=Antibiotic Susceptibility Testing;
[1]Not every laboratory offered the complete range of stool tests included in the study;
[2]All costs were updated to 2016 according to the consumer price index.

Estimated effect of PCR introduction on resource consumption

After adjustment for pre- and post-PCR cohort differences (Table 5, footnote 1), PCR introduction was associated with an increase in the rate of DFT for bacteria (RR=1.40; 95%CI=1.30-1.52) and parasites (1.66; 1.55-1.79), with more separate (initial and follow-up) requests for DFT per episode (1.29; 1.22-1.38), more telephone consultations (1.23; 1.18-1.28), more prescriptions of antiprotozoal drugs (2.49; 2.14-2.90), and more referrals to an internal medicine specialist (1.97; 1.67-2.32). Furthermore, the number of home visits decreased (0.71; 0.64-0.79, Table 5). The number of antibiotic susceptibility tests, in-office consultations, and referrals to a pediatrician also appeared increased after PCR introduction, but after adjustment these trends were non-significant.

Table 3. Direct healthcare-related resources and associated costs.

Category	Resource	Costs[1]	Source
Medication	Symptomatic	*	Dutch NHCI
	Antibiotics	*	Dutch NHCI
Consultations	In-office	€ 33	Dutch NHCI
	Home visit	€ 50	Dutch NHCI
	Telephone	€ 17	Dutch NHCI
Specialist referrals	Internal medicine	€ 91	Dutch NHCI
	Pediatrician	€ 101	Dutch NHCI

NHCI=National Health Care Institute;
[1]All costs were updated to 2016 according to the consumer price index;
*Depending on medication (see Supplement 8 for elaborate costing specification).

Table 4. Changes in healthcare resource consumption and costs per GE episode before and after PCR introduction.

Healthcare Resource	Crude IR/1000 GE episodes				Crude mean costs/GE episode			
	Before	After	Δ	p-value	Before	After	Δ	p-value
DFT								
Bacteria[1]	131	183	52	<0.001	€ 6.6	€ 13.2	€ 6.6	<0.001
Bacteria positive (AST)[1]	13	23	10	<0.001	€ 0.6	€ 1.8	€ 1.2	<0.001
Parasites[1]	102	170	67	<0.001	€ 10.0	€ 14.6	€ 4.6	<0.001
DFT orders[2]	155	201	46	<0.001	€ 1.9	€ 2.5	€ 0.6	<0.001
Consultations								
In-office	1,098	1,137	38	0.014	€ 36.6	€ 37.8	€ 1.3	0.014
Home visits	121	87	-34	<0.001	€ 6.1	€ 4.4	-€ 1.7	<0.001
Telephone	571	706	135	<0.001	€ 9.8	€ 12.1	€ 2.3	<0.001
Antibiotics[3]								
Intestinal anti-infectives	4	4	0	0.656	€ 0.1	€ 0.4	€ 0.3	0.165
Antibacterials for systemic use	56	55	0	0.982	€ 0.6	€ 0.6	€ 0.0	0.656
Antiprotozoal agents	25	61	36	<0.001	€ 0.3	€ 1.1	€ 0.8	<0.001
Specialist referrals								
Internal medicine	19	39	19	<0.001	€ 1.8	€ 3.5	€ 1.8	<0.001
Paediatrician	18	22	4	0.021	€ 1.8	€ 2.3	€ 0.4	0.021
Total	-	-	-	-	€ 76.2	€ 94.4	€ 18.2	<0.001

Δ=Difference; IR=Incidence Rate; AST=Antibiotic Susceptibility Testing; DFT=Diagnostic Feces Testing; GE=gastroenteritis;

[1]Crude mean costs for DFT cannot be directly inferred from crude incidence rate as test prices differed for conventional and PCR-based DFT;

[2.] Includes initial and follow-up DFT orders per episode, irrespective of the type of test performed (i.e. bacteria, parasites or both);

[3]Crude mean costs for antibiotics cannot be directly inferred from the crude incidence rate as categories represent composite groups with various antibiotics.

Direct health costs associated with PCR introduction

The adjusted DHC after PCR introduction were €93.4 (95%CI=€92.8-93.9) per GE episode (Table 6), resulting in an estimated incremental DHC attributable to PCR introduction of €17.1 (95%CI=€16.6-17.6) per GE episode. This translates to an increase of 22.4% (21.8-23.1%) in DHC (Table 6), of which 79% (€13.5 per GE episode) was attributable to the increase in healthcare resources consumption and 21% (€3.6 per GE episode) to the higher costs of PCR-based bacterial testing. In GE patients receiving DFT, the adjusted incremental DHC were €44.5 (42.8-46.2) per GE episode, compared to €0.5 (0.0-0.9) for patients that did not receive DFT. For those tested, costs therefore increased by 18.5% (17.8-19.2%) and for those not tested by 1.0%

(0.0-1.8%). Extrapolated to the total Dutch population (see Analysis section for computation), the incremental DHC of PCR-based DFT in primary care patients with GE is estimated to be € 7.4 million (95%CI=€7.2-€7.6 million) annually.

DISCUSSION

Main findings

The replacement of conventional culture and triple feces testing techniques with molecular-based stool testing increased overall healthcare consumption, mainly because of more testing for bacterial and parasites, resulting in more consultations and antibiotic prescriptions. Together these changes were associated with a 22% cost increase per GE episode, of which 79% was attributable to healthcare resources consumption and 21% to PCR-based bacterial testing costs.

Strengths and limitations

We used data from a large routine primary care cohort, consisting of a representative sample of the general Dutch population,[22] including a large number of consecutive GE cases and four study years. The inclusion of a one year adaptation period provided the opportunity for GPs to adjust to the replacement of conventional DFT with PCR. Furthermore, we checked for an underlying trend of resource consumption using a control group, we corrected for cohort differences. Compared to aggregated data, the use of individual patient data prevents ecological fallacy (false inference for an individual level based on aggregate data-analysis) and enables appropriate correction for confounding factors. This level of detail was essential for the validity of our study, because of patient differences in the dynamic cohort during the study. Lastly, by including all major DHC categories, this study provides a good overview of the total and incremental costs of PCR introduction within the primary care setting.

The main limitations of our study are its non-randomized design, the lack of a fully independent control group and the absence of health outcomes and quality-of-life measurements. An independent control group would most adequately detect potential underlying trends in resource use. Individual (or cluster) randomization of the intervention would fully account for confounding.[34] However, this was logistically not feasible due to the implementation of PCR-based DFT prior to the initiation of this study. Therefore, an approach was adopted where an underlying trend of viral DFT (if present) and differences in patient characteristics would be corrected for in our analysis using regression analysis. Such an approach can serve as an

adequate design for measuring the impact of interventions already implemented.[27,35] The fact that we did not consider health outcomes, such as quality of care, patient-related quality of life and non-medical costs (e.g. productivity loss) prohibits us to draw any conclusions on the clinical utility of PCR-based DFT in primary care.[34]

Interpretation of results

The effect of introducing PCR-based stool testing on associated healthcare costs and clinical management has been evaluated in two other studies. These hospital-based studies demonstrated a decrease in costs associated with patient isolation for severe GE cases and an increase in targeted rather than empirical therapy.[8,17,36] As such, these results have limited generalizability to primary care. In the current study, the introduction of PCR-based DFT was associated with a significant increase in direct healthcare costs, incurred mainly due to the increased rate of DFT in patients with GE and the increased costs of bacterial testing. For the Dutch population, the incremental DHC of PCR-based DFT in primary care patients with GE would sum up to €7.4 million per year. These incremental costs only represent a fraction (approximately 2%) of the total estimated costs for all food-related illness of €468 million yearly.[37]

We only considered the effects of PCR introduction on direct healthcare costs and although some gain may be expected from an earlier and more accurate causal diagnosis in patients with GE, like reduced disease duration, most patients have a self-limiting and relatively short disease course, without the need for antibiotics, specialist referral or hospitalization.[9] It is plausible that a substantial proportion of the change after PCR introduction we found represents over-testing. This is supported by the fact that four out of five patients do not have an identifiable evidence based indication for stool testing.[38]

The rise in DFT use may well be explained by the shorter turnaround time and improved user-friendliness and diagnostic yield compared to conventional techniques, lowering the threshold for GPs to request DFT.[14,16,18] However, other potential explanations must be stipulated. In the UK, the use of diagnostic tests (mainly imaging, laboratory tests) in primary care markedly increased between 2000 and 2015.[39] A plausible cause for these developments in the UK is that GPs increasingly perform tests for "strategic, non-medical reasons" (such as on patient's request) and struggle with the transition of services from secondary to primary care,[40,41] analogous to the current practice in the Netherlands.[42] Both of these factors put a strain on primary healthcare providers in order to meet the patients' expectations. However, in the UK an average yearly increase of 8.7% was observed for laboratory tests (data on DFT not available),[39]

while in our study an increase of 66% was seen between 2010-11 and 2013-14 for parasite DFT (Table 5). Therefore, previously described factors, linked to PCR-based testing, clearly play an important role.

Table 5. Crude and adjusted rate ratio's for healthcare resource consumption associated with PCR introduction.

Resource category	Healthcare resource	Crude RR for post-PCR period	Adjusted RR for post-PCR period (95%CI)[1]
DFT			
	Bacteria	1.40 (1.31-1.49)*	1.40 (1.30-1.52)*
	AST for bacteria positive	1.77 (1.43-2.15)*	1.19 (0.97-1.45)[2]
	Parasites	1.66 (1.54-1.78)*	1.66 (1.55-1.79)*
	DFT orders[3]	1.30 (1.22-1.38)*	1.29 (1.22-1.38)*
Consultations			
	In-office	1.03 (1.01-1.06)*	1.02 (0.99-1.04)
	Home visits	0.72 (0.66-0.78)*	0.71 (0.64-0.79)*
	Telephone	1.24 (1.20 1.28)*	1.23 (1.18-1.28)*
Antibiotics			
	Intestinal anti-infectives	0.89 (0.59-1.35)	0.83 (0.51-1.34)
	Antibacterials for systemic use	1.00 (0.90-1.11)	0.95 (0.84-1.08)
	Antiprotozoal agents	2.43 (2.12-2.80)*	2.49 (2.14-2.90)*
Specialist referrals			
	Internal medicine	2.00 (1.70-2.36)*	1.97 (1.67-2.32)*
	Pediatrician	1.25 (1.04-1.50)*	1.17 (0.98-1.4)

AST=Antibiotic Susceptibility Testing; DFT=Diagnostic Feces Testing; PCR=Polymerase Chain Reaction; RR=Rate Ratio;

[1]Corrected for seasonal fluctuations, age, gender, intestinal comorbidities, diabetes mellitus, COPD, asthma, malignancies, immunocompromising disorders, immunosuppressive therapy, acid-suppressive medication, and holidays;

[2]AST was included in further cost calculations as the unit costs differed before and after PCR introduction (see Table 1);

[3] Includes initial and follow-up DFT orders per episode, irrespective of the type of test performed (i.e. bacteria, parasites or both);

*Statistically significant change in resource consumption ($p<0.05$).

Table 6. Total DHC and incremental DHC post-PCR per GE episode.

Patient group	Total DHC (95%CI)			Incremental DHC post-PCR (95%CI)		
	Crude pre-PCR[1]	Crude post-PCR[1]	Adjusted post-PCR[2]	Crude DHC[1]	Adjusted attributable[2]	Percentage[2]
All GE patients	€ 76.2	€ 94.4	€93.4 (92.8-93.9)	€ 18.2	€ 17.1 (16.6-17.6)	22.4 (21.8-23.1)
GE with DFT	€ 240.5	€ 282.9	€285.0 (283.3-286.7)	€ 42.3	€ 44.5 (42.8-46.2)	18.5 (17.8-19.2)
GE without DFT	€ 50.9	€ 52.2	€51.4 (50.9-51.8)	€ 1.4	€ 0.5 (0.0-0.9)	1.0 (0.0-1.8)

DFT=Diagnostic Feces Testing, DHC=direct healthcare costs, PCR=Polymerase Chain Reaction.
[1]Includes all resources.
[2]Only resources with a significant change after PCR introduction were included. DHC were corrected for seasonal fluctuations, age, gender, intestinal comorbidities, diabetes mellitus, COPD, asthma, malignancies, immunocompromising disorders, immunosuppressive therapy, acid-suppressive medication and holidays

Implications for research and practice

We earlier reported that in the same primary care cohort 80% of DFT testing was not in line with current guideline recommendations.[38] Although more timely and accurate diagnosis through PCR-based DFT in primary care may potentially provide health gain and more cost-effective GE management, we think that increased guideline adherence by GPs is conditional to effectuate this in practice. Therefore, future research should identify the motives for current test practices in order to provide a tailored approach to improve guideline adherence. Furthermore, as it is known that GPs often ignore guideline recommendations that lack scientific evidence,[43,44] the current test indications should be critically appraised.

CONCLUSIONS

The introduction of PCR-based DFT in primary care is associated with a significant increase in the number of tests requests and with a 22% cost increase per GE episode, of which 79% was attributable to healthcare resources consumption and 21% to PCR-based bacterial testing costs. In order to maintain adequate clinical care and to control costs, adherence to clinical practice guideline indications for stool testing should be evaluated and stimulated, where necessary.

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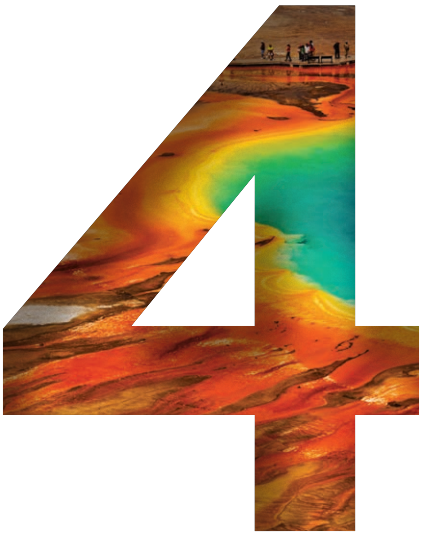
CHAPTER



Substantially increased detection and treatment of Blastocystis and Dientamoeba in patients with gastroenteritis after the introduction of molecular stool testing

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Submitted

ABSTRACT

Background

The clinical relevance of detecting *Dientamoeba fragilis* and *Blastocystis* spp. in stool samples from gastroenteritis (GE) patients is unknown.

Objective

To evaluate the effects of replacing triple feces test (TFT) with more sensitive protozoal polymerase chain reaction (PCR) on the detection and antibiotic treatment of *D. fragilis* and *Blastocystis* spp. among GE patients in primary care.

Methods

After identifying GE episodes in a large primary care registry, we collected protozoal stool testing results and prescriptions for metronidazole and clioquinol. The absolute difference (AD) in the detection and targeted antibiotic treatment rate were compared between 2010-11 (TFT) and 2013-14 (PCR testing).

Results

We identified 10,947 GE episodes during 2010-11 and 13,271 episodes during 2013-14. In GE patients, protozoal stool testing increased from 9.5% (n=1042) with TFT to 15.6% (n=2073) with PCR testing (AD=6.1%, 95%CI=5.3-6.9). *D. fragilis* or *Blastocystis* spp. was detected in 2.4% and 35.6% of the TFT and PCR stool tests, respectively (AD=33.2%, 31-35). Among GE patients with stool testing for protozoa, targeted antibiotic treatment for *D. fragilis* or *Blastocystis* spp. increased from 1.1% (n=11) to 14.5% (n=300) after PCR introduction (AD=13.4%, 12-15) and from 0% to 1.3% (n=27) for clioquinol (95%CI=1-2%).

Conclusion

Replacing conventional TFT stool testing by a protozoal PCR panel in primary care patients with GE is associated with a sizable increase in protozoal stool testing, and the detection and antibiotic treatment of *Blastocystis* spp. and *D. fragilis*. This stresses the need for clarification of the clinical significance of these protozoa.

INTRODUCTION

Dientamoeba fragilis and *Blastocystis* spp. have been linked to gastrointestinal complaints for over 100 years, but the introduction of highly sensitive molecular polymerase chain reaction (PCR) stool testing has revealed that these protozoa are also highly prevalent in asymptomatic children and adults.[1,2] Also, there is no conclusive evidence from randomized placebo-controlled trials that antibiotic therapy of *D. fragilis* or *Blastocystis* spp. infections provide clinical benefit.[3–9] Hence, Dutch and UK primary care guidelines on gastroenteritis (GE) recommend antibiotic treatment of *D. fragilis* or *Blastocystis* spp. infections only in patients with chronic abdominal discomfort and diarrhea, after exclusion of other potential causes.[10,11] Laboratory facilities for primary care providers in de municipality of Utrecht, The Netherlands, have switched from traditional triple feces testing (TFT) to PCR stool testing in 2012. Reasons for this transition are the higher sensitivity, (subtype) specificity, and more convenient sampling (single instead of three samples). These factors may have an effect on the stool testing request and detection rate,[12] and – in light of their uncertain pathogenicity – overdiagnosis and overtreatment of *D. fragilis* and *Blastocystis* spp. We, therefore, assessed the impact of the introduction of PCR-based stool testing for protozoa on the request rate of stool testing in primary care patients with GE, and on the detection and antibiotic treatment rate of *D. fragilis* and *Blastocystis* spp.

MATERIALS AND METHODS

We performed a before and after cohort study using routine primary care registration data from the Julius General Practitioner Network (JGPN), which contains the electronic patient records of around 290,000 patients from 160 GPs. All records with an episode of GE (ICPC D11, D70 or D73 coded consultation, using a 60-day timeframe) between 2010-2011 (conventional TFT) and between 2013-2014 (after the introduction of PCR stool testing) were selected. In 2012, PCR stool testing was introduced by the JGPN serving primary care laboratory (Saltro Diagnostic Center). Stool test results included data on *D. fragilis*, *Blastocystis* spp. and frequently tested pathogenic protozoa (i.e. *Giardia lamblia*, *Entamoeba histolytica* or *Cryptosporidium*), and were obtained by pseudonymous linkage of GE episodes in JGPN to laboratory records (for details on the study design see).[13] For each episode, we collected the patient's age, gender, comorbidity (Supplement 9) and protozoal stool test results, where applicable. Furthermore, we collected prescription data on nationally registered antimicrobials for protozoal disease in primary care, i.e. clioquinol (P01AA02) and metronidazole (P01AB01). Targeted antibiotic treatment was defined as the treatment of stool test confirmed monoinfection with *D. fragilis*,

Blastocystis spp. or pathogenic protozoa. Empirical treatment was defined as the antibiotic treatment of patients with a GE episode, but without prior stool testing or despite a negative test result.

We calculated all outcome measures per period and subsequently compared them. The GE consultations rate was calculated per 1000 person-years and compared using the rate ratio test. Patient characteristics and stool test rates were compared using Pearson's chi-squared test for dichotomous variables and the Kruskal-Wallis test for continuous and categorical variables. The proportions of protozoa detected in patients with stool testing for protozoa and the proportion receiving targeted treatment were compared the absolute difference (AD) in incidence using the risk difference test with 95% confidence interval (CI). The proportion of GE episodes with empirical treatment was compared using Pearson's chi-squared test. We compared the gender-specific treatment proportions to assess whether co-occurring non-intestinal infections that require metronidazole, such as bacterial vaginitis and trichomoniasis, inflated the number of targeted and empirical antibiotic prescriptions. Finally, we calculated the attributable number of antibiotic prescriptions related to the change in stool test technique, assuming that PCR stool testing for protozoa was implemented nationwide. Statistical analysis was conducted in R 3.3.2 (*tableone and fmsb* packages).

RESULTS

We identified 10,947 GE episodes in the TFT period (JGPN population=444,029 person-years, incidence rate=24.7 GE episodes per 1000 person-years) and 13.271 episodes in the PCR testing period (JGPN population=515,811 person-years, 25.7/1000 person-years, p<0.001) (Supplement 10). The median consultation rate per GE episode increased from 2 (IQR: 1-4) during the TFT period, to 3 (IQR: 2-4) during the PCR testing period (p=0.001). No significant differences were observed in the distribution of potential confounders in the two periods (Supplement 11).

Diagnosing D. fragilis and Blastocystis spp.

The proportion of GE episodes with stool testing for protozoa increased from 9.5% (n=1.042) in the TFT period to 15.6% (n=2,073) after PCR introduction (AD=6.1%, 95%CI=5.3-6.9) (Table). The rate of simultaneous stool testing for other types of pathogens among patients tested for protozoa increased from 64% to 77% for bacteria (p<0.001) and from 7% to 10% for viruses (p=0.01) after the introduction of PCR (Supplement 11).

Table. Stool test results and antibiotic treatment for protozoal infections in primary care GE patients.

Pathogen	Stool test results and *Antibiotic treatment*	TFT (%)	PCR (%)	Absolute difference (95%CI)
N (% of GE episodes)		1,042 (9.5)	2,073 (15.6)	6.1% (5.3-6.9)*
D. fragilis and/or *Blastocystis* spp.	Positive	25 (2.4)	738 (35.6)	33.2 (31-35)*
	Without pathogenic protozoal co-infection*	25 (2.4)	711 (34.3)	31.9 (30-34)*
	Metronidazole[1]	11 (1.1)	300 (14.5)	13.4 (12-15)*
	Clioquinol[2]	0 (0.0)	27 (1.3)	1.3 (1-2)*
D. fragilis	Positive	20 (1.9)	477 (23.0)	21.1 (19-23)*
	Monoinfection	20 (1.9)	260 (12.5)	10.6 (9-12)*
	Metronidazole[2]	10 (1.0)	130 (6.3)	5.3 (4-7)*
	Clioquinol[2]	0 (0.0)	17 (0.8)	0.8 (0-1)**
Blastocystis spp.	Positive	5 (0.5)	451 (21.8)	21.3 (19-23)*
	Monoinfection	5 (0.5)	216 (10.4)	9.9 (9-11)*
	Metronidazole[2]	1 (0.1)	85 (4.1)	4 (3-5)*
	Clioquinol[2]	0 (0.0)	2 (0.1)	0.1 (0-0)
Pathogenic protozoa[1]	Positive	10 (1.0)	193 (9.3)	8.4 (7-10)*
	Without D. fragilis and/or Blastocystis spp. co-infection	10 (1.0)	93 (4.5)	3.5 (2-5)*
	Metronidazole[2]	8 (0.8)	70 (3.4)	2.6 (1.7-3.5)*

*p<0.001; **p<0.01.
[1] Infection with Giardia spp, Entamoeba histolytica and/or Cryptosporidium spp.
[2] Treatment for a microbiologically confirmed monoinfection.

D. fragilis, Blastocystis spp., or both were identified in 2.4% (n=25) of the episodes tested with TFT and in 35.6% (n=738) of the episodes with PCR stool testing (AD=33.2%, 31-35) (Table). In patients with a stool test confirmed *D. fragilis* or *Blastocystis* spp. infection (n=738), none of the TFT tested patients and 27 (4%) of the PCR tested patients were positive for co-occurring frequently tested pathogenic protozoa (i.e. *Giardia lamblia*, *Entamoeba histolytica* or *Cryptosporidium*). The total detection rate of pathogenic protozoa in patients with stool testing increased from 1% (n=10) with TFT to 9.3% (n=193) with PCR stool testing (AD=8.4%, 7-10), of which 100 (52%) were also positive for *D. fragilis* or *Blastocystis* spp.

Antibiotic prescription

Targeted metronidazole treatment for a confirmed *D. fragilis* or *Blastocystis* spp. monoinfection was prescribed in 1% (n=11) of the GE episode with TFT and 14.5% (n=300) of the episodes with PCR stool testing (AD=13.4%, 12-15). Targeted clioquinol treatment for these protozoa was prescribed in none of the GE episodes with TFT and 1.3% (n=27) of the episodes with PCR stool testing (95%CI=1-2%). Targeted metronidazole treatment for confirmed pathogenic protozoal infections was prescribed in 0.8% (n=8) of the GE episodes with TFT and 3.4% (n=70) of the episodes with PCR stool testing (AD=2.6% ,1.7-3.5). Among all patient who presented at the GP with symptoms of gastroenteritis, the empirical prescription rate of metronidazole decreased from 1.9% to 1.5% (AD=−0.4%, −0.8-−0.1) after PCR introduction, while empirical clioquinol prescription remained equally low before (0.1%) and after (0.2%) PCR introduction (AD=0.1%, 0-0.2). No differences were observed in empirical and targeted antibiotics prescription rates for males and females (Supplement 12).

Based on protozoal stool testing rate of 15.6% in patients with GE (25.7/1000 person-years) and a 14.7% increase in the targeted antibiotic treatment of *D. fragilis* or *Blastocystis* spp. after PCR introduction, 10,087 additional antibiotic courses are annually prescribed in the Netherlands (n=17,114,616, April 2017).

DISCUSSION

Main findings

We demonstrated that the introduction of PCR protozoal stool testing was accompanied with a 6% absolute increase in protozoal testing, a 33% absolute increase in the detection, and a 15% absolute increase in the antibiotic treatment of *Blastocystis* and *Dientamoeba* infections.

Strengths and limitations

Strictly speaking, this observational study only provides evidence on associations between the observed increase in both stool testing and detection rates, and antibiotic prescription frequency, not on causality. Furthermore, adjustment for potential confounders was not possible due to the low number of *D. fragilis* and *Blastocystis* spp. cases in the TFT period and the lack of clinical determinants in the patient data. However, we did not observe any significant differences in the occurrence of the available potential confounding factors (e.g. age and incidence of comorbidity) between the two study periods. Still, other factors may have contributed to the observed differences. For example, a generally increasing trend in the use of

diagnostic tests in primary care, due to for example an increased patient demand for diagnostic testing, may partly account for the observed increase in stool testing rate. Finally, we were not able to fully adjust for other indications of metronidazole therapy, like bacterial vaginitis and trichomoniasis, whereas these diagnostic data were not available, and treatment for vaginitis is often prescribed empirically. However, we did not identify differences in the prescriptions rates between gender. Taken together, we consider it unlikely that potential differences between the before and after study populations can be fully explained by extraneous factors.

Implications for clinical practice

Although previous studies indicated high incidences of *D. fragilis* and *Blastocystis spp.* in asymptomatic children and adults,[1,2] our finding indicate that GPs more readily initiate antibiotic treatment now that PCR stool testing is implemented. This does not necessarily indicate overdiagnosis and overtreatment, but it does further stress the need for clarification of the clinical significance of these protozoa. Until then it is important that GPs follow current clinical practice guidelines and reserve antibiotics for patients with chronic abdominal discomfort and diarrhea, and after ruling out other causes.[10,11]

CONCLUSION

Replacing conventional TFT stool testing by a protozoal PCR panel in primary care patients with GE is associated with a sizable increase in protozoal stool testing, and the detection and accompanying antibiotic treatment of *Blastocystis* spp. and *D. fragilis.* Further studies need to evaluate if these findings indicate overdiagnosis and overtreatment or that protozoal PCR testing has a clinical benefit to patients.

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CHAPTER

Antibiotic treatment of gastroenteritis in primary care

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ABSTRACT

Background

Gastroenteritis (GE) is a frequent occurring reason for consulting a general practitioner. Yet little is known about antibiotic prescribing in primary care patients with GE. In this study, we quantified empirical and targeted antibiotic treatment of GE, compliance with recommendations from primary care clinical practice guidelines (CPGs) and the degree of antimicrobial resistance in patients receiving diagnostic feces testing (DFT).

Methods

We performed a cohort study using routine care data of 160 general practitioners, including electronic patient records from 2013 to 2014. GE episodes were extracted and linked to microbiological laboratory records to retrieve the results of DFT. For each episode, data on patient characteristics, DFT results including antimicrobial resistance testing, and antibiotic prescriptions were collected.

Results

We identified 13,217 GE episodes. Antibiotic treatment was prescribed in 1,163 (8.8%) episodes, most frequently with metronidazole (*n* = 646, 4.9%), azithromycin (*n* = 254, 1.9%) or ciprofloxacin (*n* = 184, 1.4%). Treatment was empirical for 641 (5%) GE episodes, of which 30% (*n* = 191) followed the CPG-recommended antibiotic choice. Targeted treatment following DFT results was prescribed for 537 GE episodes (4%), of which 99% (*n* = 529) followed CPG recommendations. Non-susceptibility to first- or second-choice antibiotics was demonstrated in three *Salmonella* isolates (9%–13% of all isolates) and one *Campylobacter* isolate (1%).

Conclusions

Antibiotic treatment of GE in primary care is relatively infrequent, with 1 in 11 episodes treated. Empirical treatment was more frequent compared with targeted treatment and mostly with non-CPG-recommended antibiotics. However, treatment based upon DFT results followed CPG recommendations.

INTRODUCTION

Every year, around one in three persons experiences an episode of gastroenteritis (GE).[1,2] In the Netherlands, 5%–12% of patients with GE consult their general practitioner (GP). With 240,000–600,000 consultations annually, GE is imposing a substantial burden on primary healthcare.[1,2] Because GE is predominantly of viral etiology and usually self-limiting,[2,3] routine prescription of antibiotics is not recommended in clinical practice guidelines (CPGs) for primary care.[4,5] Yet little is known about the frequency and pattern of antibiotic prescribing for GE in primary care. Additionally, antimicrobial resistance (AMR) of *Campylobacter* spp. and *Salmonella* spp. to ciprofloxacin was found to be considerable in the Netherlands,[6] but primary-care-specific AMR data are lacking. These data are, however, important to inform guideline development on optimal antibiotic treatment. Internationally, AMR of *Salmonella* spp., *Shigella* spp. and *Campylobacter*spp. to first- and second-choice antibiotics is widespread and considered to be a substantial public health problem.[7–11]

Here we report the frequency and determinants of antibiotic treatment for GE in primary care, the compliance with CPG recommendations on antibiotic treatment and the results of routine antimicrobial susceptibility testing (AST) in patients who underwent molecular diagnostic feces testing (DFT).

METHODS

Study design

We performed a retrospective cohort study using the routine primary care data from the Julius General Practitioner Network (JGPN), containing pseudonymized routine primary care data from 45 general practices (with 160 GPs and around 290,000 patients) in the academic primary care network of Utrecht, the Netherlands. Participating centers included both small- to medium-size practices and large primary care centers, all located in a typical Western European (sub)urban environment. The database contains detailed information on all patient contacts (telephone and practice consultations and home visits) of the participating GPs during office hours. Diagnoses during these contacts are coded according to the International Classification of Primary Care (ICPC)[12] and antibiotic prescriptions are registered according to the WHO guidelines for Anatomical Therapeutic Chemical (ATC) classification.[13] GPs of participating practices are trained in the correct use of ICPC coding and have on average 10 years experience in systematic coding of disease episodes.[14]

We selected patients with at least one GE episode that presented to GPs participating in the JGPN between 2013 and 2014. An episode of GE was defined as a GP contact with assigned ICPC code D11 (diarrhea), D70 (infectious diarrhea) or D73 (suspected infectious diarrhea). Multiple GP contacts for GE within a 60 day period were counted as one episode. More elaborate information on the study design and population can be found in the study design statement.[15]

Data collection

For each GE episode, we collected information on age, gender, number of GP contacts per disease episode, comorbidities, immunocompromising disorders, immunosuppressive therapy (Supplement 6), antimicrobial drug prescriptions and, if performed, results of DFT. According to the Dutch primary care guideline on GE, DFT and antibiotic treatment are only recommended in patients with severe illness, those with compromised immunity or those with increased risk of disease or pathogen transmission from a public health perspective (i.e. food handlers or healthcare professionals).[4,16] DFT results were retrieved from Saltro Diagnostic Center, the main regional primary care laboratory, and linked to the JGPN cohort by a pseudonymization procedure performed by a 'trusted third party' in accordance with privacy regulations. Approximately 96% of the GPs in JGPN use the laboratory facilities of Saltro Diagnostic Center for performing microbiological diagnostics.

We extracted data on all ATC-coded antimicrobial drug prescriptions that are presently recommended for the treatment of GE by the Dutch primary and secondary care CPGs.[4,16] These included 'intestinal anti-infectives' (paromomycin and vancomycin), 'antibacterials for systemic use' (co-trimoxazole, erythromycin, azithromycin, ciprofloxacin, teicoplanin, fidaxomicin) and 'antiprotozoal agents' (metronidazole and clioquinol). Antibiotic treatment was classified as either empirical or targeted, the latter defined as antibiotics prescribed in the presence of positive DFT results and empirical defined as prescriptions that were issued for GE episodes without DFT being performed, initiated pending DFT results or with negative DFT results. CPGs in the Netherlands advocate restrictive use of empirically prescribed antibiotics for GE, but recommend considering empirical treatment with azithromycin in severely ill or immunocompromised patients with suspected bacterial infection. In these CPGs, empirical treatment of patients with suspected parasitic GE is not recommended.[4,16] Targeted treatment was defined as an antibiotic prescription for a GE episode where a presumed causal pathogen was identified through DFT. For both empirical and targeted treatment, we assessed whether the prescribed antibiotic was in accordance with the recommendations described in the Dutch CPGs[4,16] (Supplement 13).

Table 1. Characteristics of patients with GE episodes with and without antibiotic treatment. Numbers are counts of patients with percentages (%) unless otherwise indicated.

Patient characteristic	Antibiotic treatment (*n* = 1,163)	No antibiotic treatment (*n* = 12,108)	*P*
Age (years), median (IQR)	35 (12.5–53)	28 (4–53)	<0.001
Female	651 (56.0)	6,548 (54.1)	0.227
Number of consultations, median (IQR)[a]	3 (1–4)	1 (1–2)	<0.001
Comorbidities[b]			
Any	369 (31.7)	3,616 (29.9)	0.197
Intestinal	169 (14.5)	1,470 (12.1)	0.02
Malignancy	36 (3.1)	317 (2.6)	0.384
Diabetes mellitus	73 (6.3)	923 (7.6)	0.108
COPD	54 (4.6)	413 (3.4)	0.036
Asthma	136 (11.7)	1,261 (10.4)	0.191
Immunocompromising condition	52 (4.5)	453 (3.7)	0.245
Chronic medication[b]			
Systemic corticosteroids	97 (8.3)	616 (5.1)	<0.001
Chemotherapy	4 (0.3)	47 (0.4)	1
Immunosuppressants	11 (0.9)	65 (0.5)	0.118
Acid-suppressive drug	290 (24.9)	2,726 (22.5)	0.065
DFT			
Any DFT	684 (58.8)	1,745 (14.4)	<0.001
Bacteria	493 (42.4)	1,378 (11.4)	<0.001
Parasites	632 (54.3)	1,457 (12.0)	<0.001
Viruses	78 (6.7)	228 (1.9)	<0.001
Any positive	560 (81.9% of tests)	517 (29.6% of tests)	<0.001
Bacterium positive	107 (21.7% of tests)	177 (12.8% of tests)	<0.001
Parasite positive	481 (76.1% of tests)	350 (24.0% of tests)	<0.001
Virus positive	3 (3.8% of tests)	17 (7.5% of tests)	0.396

[a]In-office consultations, home visits and telephone consultations per GE episode.
[b]See Supplement 6.

Table 2. Empirical and targeted antibiotic treatment for GE episodes (n = 13,271). Numbers are counts of patients with percentages.

Antibiotic	GE episodes with treatment[a]		
	empirical[b] (%)	targeted[c] (%)	empirical/targeted (% of GE)
Any prescription	641 (100)	537 (100)	1,163 (8.8)
Metronidazole	205 (32)	442 (82)	646 (4.9)
Azithromycin	191 (30)	65 (12)	254 (1.9)
Ciprofloxacin	165 (26)	19 (4)	184 (1.4)
Co-trimoxazole	56 (9)	3 (1)	59 (0.4)
Clioquinol	31 (5)	27 (5)	57 (0.4)
Erythromycin	12 (2)	1 (0)	13 (0.1)
Paromomycin	4 (1)	3 (1)	7 (0.1)
Vancomycin	3 (0)	2 (0)	5 (<0.1)
CPG recommended	191 (30)	529 (99)	–

[a]Results in each column are not mutually exclusive as multiple antibiotics could have been prescribed for each GE episode.
[b]Prescriptions issued for a GE episode without DFT, preceding a DFT result or after a negative DFT result.
[c]Prescriptions issued after reporting of a positive DFT result.

Microbiological diagnostic testing

The presence of viruses was tested by immunochromatographic strip tests for each individual pathogen (i.e. adenovirus 40/41, norovirus, rotavirus). Bacteria and parasites were tested for each pathogen group by real-time multiplex PCR methods, which are described elsewhere.[15] Bacterial pathogen testing included *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter* spp., and *Plesiomonas* spp. and parasite testing included *Blastocystis hominis*, *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica/dispar,* and *Giardia* spp. We performed AST on bacterial isolates using disc diffusion for *Campylobacter* and automated susceptibility testing systems (Vitek 2, bioMérieux) for other bacteria. *Campylobacter* isolates were tested for resistance to ciprofloxacin, erythromycin, and tetracycline; other bacteria were tested for resistance to ciprofloxacin, co-trimoxazole, amoxicillin, and amoxicillin/clavulanic acid.

Analysis

We calculated the incidence rate of GP consultation for GE in the population by dividing the total number of GE episodes by the person-years of observation. We assessed the proportion of episodes where treatment performed with any antibiotic and for each antimicrobial agent. Patient characteristics were compared between episodes with and without antibiotic treatment, using Pearson's χ^2 test statistic for dichotomous variables and the Kruskal–Wallis

test for continuous and categorical variables. Variation in antibiotic treatment proportions among months and age groups was assessed using Pearson's χ^2 test statistic; a subsequent pair-wise comparison was performed and included Holm's correction for multiple testing.[17] We classified antibiotic prescriptions as either empirical or targeted and calculated proportions of GE episodes treated according to CPG-recommended antibiotic choices. Furthermore, for each gastrointestinal pathogen species detected through DFT, we assessed the proportion of infections treated with antibiotics.

Finally, antibiotic resistance levels were assessed for bacterial species detected with DFT by calculating the proportions and 95% CIs of resistant isolates for each antibiotic, but only for those bug-drug combinations with AST results available for at least 20 isolates per species. Statistical analysis was conducted in R 3.3.2, using the *tableone*, *dplyr*, *xts* and *rms* packages.

Results

Between January 2013 and December 2014, the JGPN database comprised a total of 515,811 person-years from 277,578 enlisted patients. In total, 13,217 episodes of GE were recorded in the database, resulting in an overall incidence rate of primary care consultations for GE of 26 per 1000 person-years (Figure 1).

Antibiotic treatment of GE

Antibiotic treatment was prescribed in 1,163 (8.8%) episodes (Table 1), with 1,393 antibiotic prescriptions in total (105 per 1000 GE episodes). In most episodes (n = 982, 84.5%) a single antibiotic was prescribed. In 145 (12.5%) and 36 (3%) episodes two and three or more antibiotics were prescribed, respectively. Metronidazole was most frequently prescribed (n = 646, 4.9% of all GE episodes), accounting for more than half of the antibiotic treatment courses for GE, followed by azithromycin (n = 254, 1.9% of all GE episodes), ciprofloxacin (n = 184, 1.4%), co-trimoxazole (n = 59, 0.4%) and clioquinol (n = 57, 0.4%) (Table 2).

Determinants of antibiotic treatment

Compared with patients without antibiotic treatment, those treated were older (median 35 versus 28 years, $P < 0.001$), more often had intestinal comorbidity (14.5% versus 12.1%, $P = 0.02$) and chronic obstructive pulmonary disease (4.6% versus 3.4%, $P = 0.036$) and were more often prescribed systemic corticosteroids (8.3% versus 5.1%, $P < 0.001$). Patients treated with antibiotics had on average more GP consultations per episode (median 3 versus 1, $P < 0.001$) and higher DFT testing rates (59% versus 14%, $P < 0.001$). Patients who were 0 and 1–4 years old (1.7% and 5%, respectively) were less frequently treated with antibiotics than older (>4 years)

patients (between 8.1% and 11.4%, $P < 0.05$) (Figure 2a). Prescription rates differed significantly between the months of the year ($P = 0.003$) (Figure 2b).

Figure 1. Flow chart of primary-care GE episodes and antibiotic treatment.

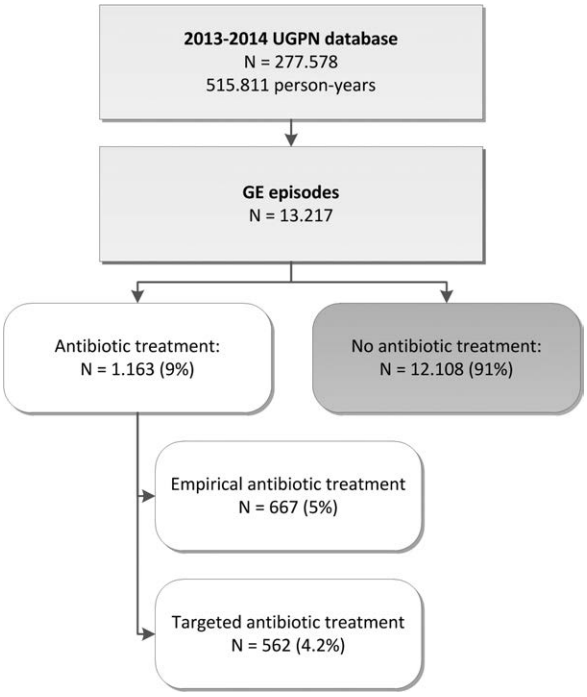
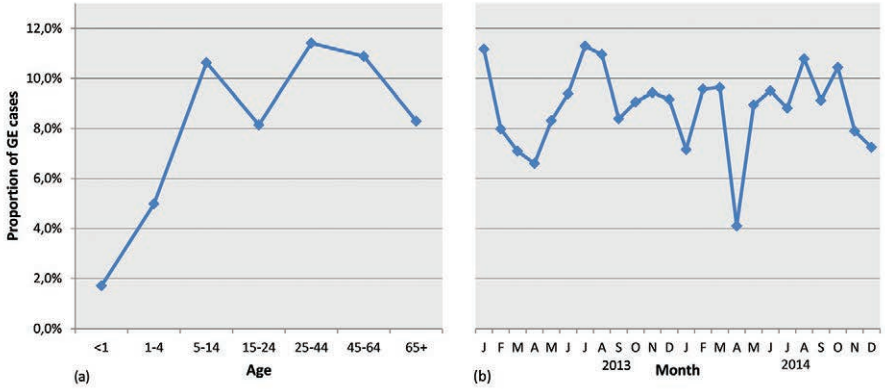


Figure 2. (a) Antibiotic treatment of patients with GE stratified by age category. (b) Monthly (***n*** = 24) antibiotic prescription for patients with GE cases (***n*** = 13,217).



Empirical and targeted antibiotic treatment

Empirical treatment was prescribed in 641 (4.9%) of the patients presenting with GE episodes; 479 (74.7%) of these 641 patients did not have a DFT request, 41 (6.4%) received the prescription before DFT results became available and 121 (18.9%) received the prescription after a negative DFT result. Targeted treatment was prescribed in 537 (4.1%) of the patients presenting with a GE episode. Empirical prescription preceded subsequent targeted antibiotic prescription in 1.3% (n = 15) of the GE episodes with treatment (Table 2).

Azithromycin, which is the recommended choice for empirical treatment according to the CPGs, was prescribed in 30% (n = 191) of the empirically treated patients. Metronidazole (n = 205, 32%) and ciprofloxacin (n = 165, 26%) were also prescribed frequently, even though they are not recommended for empirical treatment (Table 2).

Metronidazole was most frequently prescribed as targeted antibiotic treatment (n = 442, 82%) (Table 2). The antibiotic choice for targeted treatment was in accordance with CPG recommendations in 99% (n = 529) of GE episodes with targeted antibiotics.

Pathogen-specific antibiotic treatment

Routine DFT testing identified a total of 1,437 potential GE-causative enteropathogens in 2,429 GE episodes. Overall, 751 (52%) of the identified enteropathogens were treated with a CPG-recommended antibiotic and 32 (2%) with a non-recommended antibiotic. The remaining 654 patients (46%) with identified enteropathogens did not get antibiotic treatment (Table 3). In the patients with a confirmed bacterial or protozoal enteropathogen, an antibiotic was prescribed in 32% (n = 94) and in 58% (n = 657), respectively. *Blastocystis* and *Dientamoeba* were relatively often treated with antibiotics, in 52% (n = 234) and 60% (n = 292) of the positive patients, respectively. Patients receiving targeted treatment for bacteria or parasites had more GP contacts per GE episode (median 4 versus 2 contacts, $P < 0.001$) and higher specialist referral rates (9% versus 5%, $P = 0.05$) compared with patients that did not receive treatment. Other patient characteristics (age, comorbidities and chronic medication use) did not significantly differ between those treated and those not treated.

Antimicrobial susceptibility

Microbiological culture and AST were performed in 144 (71%) of 204 episodes in which *Campylobacter* isolates were detected and in 23 of 30 (77%) episodes in which *Salmonella* was detected. One *Campylobacter* isolate (1%, 95% CI 0.1%–4%) was resistant to erythromycin (Table 4). Three *Salmonella* isolates (13%, 95% CI 3%–35%) were resistant to

ciprofloxacin and two to co-trimoxazole (9%, 95% CI 2%–30%). None of the patients with resistant bacteria was treated with an antibiotic for which resistance was demonstrated.

Table 3. Pathogen-specific antibiotic treatment for GE and accordance with first- and second-choice CPG-recommended antibiotics

Pathogen	Infection count	Antibiotic treatment, no. of patients (%)		
		CPG recommended[a]	not CPG recommended	no treatment
Bacteria	293	94 (32)	11 (4)	188 (64)
Clostridium difficile	12	10 (83)	–	2 (17)
Campylobacter	204	67 (33)	–	127 (62)
Shigella	36	10 (28)	1 (3)	25 (69)
Salmonella	30	6 (20)	–	24 (80)
Yersinia	7	1 (14)	–	6 (86)
Plesiomonas	4	–	–	4 (100)
Parasites	1,124	657 (58)	20 (2)	447 (40)
Giardia lamblia	160	140 (88)	3 (2)	17 (11)
D. fragilis	477	283 (59)	9 (2)	185 (39)
B. hominis	451	226 (50)	8 (2)	217 (48)
E. histolytica/dispar	18	8 (44)	–	10 (56)
Cryptosporidium	18	–	–	18 (100)
Viruses	20	–	1 (5)	19 (95)
Adenovirus	3	–	–	3 (100)
Norovirus	10	–	–	10 (100)
Rotavirus	7	–	1 (14)	6 (86)
Total	1,437	751 (52)	32 (2)	654 (46)

[a]See Supplement 13 for specification of CPG-recommended antibiotics.

Table 4. Number of isolates (for ***n*** ≥ 20) tested for antimicrobial resistance and proportion of resistant isolates for each bacterial species

Antibiotic[a]	*Campylobacter* spp.			*Salmonella* spp.		
	isolates, *n*	resistant isolates		isolates, *n*	resistant isolates	
		n	percentage (95% CI)		*n*	percentage (95% CI)
Ciprofloxacin	147	90	61 (53–69)	**23**	**3**	**13 (3–35)**
Erythromycin[b]	**144**	**1**	**1 (<0.1–4)**	–		–
Tetracycline	147	60	41 (34–50)	–		–
Co-trimoxazole	–		–	**23**	**2**	**9 (2–30)**
Amoxicillin	–		–	23	5	22 (8–44)
Amoxicillin/clavulanic acid	–		–	23	0	0 (0–18)

[a]Indicated in bold are the first- and second-choice antibiotics recommended by the Dutch CPGs for treatment of infectious GE (see Supplement 13).[4,16]
[b]Resistance to erythromycin tends to correspond with cross-resistance to other macrolides, such as azithromycin.[20]

DISCUSSION

Main findings

We found that GPs prescribe antibiotic treatment in 1 in 11 patients who presented with a GE episode in primary care. Metronidazole is most frequently prescribed, followed by azithromycin and ciprofloxacin. About half of antibiotic treatment for GE is prescribed on an empirical basis, without microbiological test results available. Although azithromycin is the first-choice empirical treatment according to the CPGs, metronidazole was more frequently prescribed, closely followed by azithromycin and ciprofloxacin. Targeted treatment is, in nearly all cases, in agreement with CPG recommendations. After DFT confirmation, GPs refrain from antibiotic treatment in most patients with bacterial infections, but they do treat the majority of patients with parasitic infections. AST results suggest that almost all *Campylobacter* and *Salmonella* infections in patients with DFT are susceptible to the antibiotics currently recommended by CPGs.

Interpretation of results

Antibiotic treatment of GE is less frequent compared with other infectious diseases in primary care.[18,19] For example, antibiotic treatment is prescribed in 55% of the patients with acute otitis

media antibiotic treatment is prescribed, and 14% of the episodes with acute upper respiratory tract infections.[19]

When receiving antibiotic treatment for GE, more than half (55%) of the prescriptions are made empirically, mainly in patients without testing for enteropathogens. This proportion seems relatively high when considering that CPGs discourage the prescription of empirical antibiotics.[4,16] Specifically, metronidazole and ciprofloxacin are frequently prescribed as empirical treatment, despite the recommendation of CPGs for azithromycin as empirical treatment for suspected bacterial GE.[4,16] Possibly, GPs prescribe metronidazole when suspecting a parasitic infection based on current clinical presentation or due to previous parasitic infection of the patient or of family members. Ciprofloxacin is often prescribed as pre-emptive therapy for travelers' diarrhea,[4,16] which potentially explains the relatively high empirical prescription rate of this antibiotic. Although with GE the prescription of empirical antibiotics may be justified in some patients, it remains to be investigated whether this can be considered good clinical practice and whether CPGs should expand the recommendations for empirical treatment of GE in primary care, including alternative antibiotics such as metronidazole and ciprofloxacin.[20] Our study was, however, not designed to answer this question.

Microbiological testing may allow empirically started antibiotic treatment to be switched to targeted treatment. Our findings, though, demonstrate that GPs await microbiological test results to guide subsequent antibiotic treatment. In fact, only 1.3% of all empirical treatment episodes were followed by targeted antibiotic treatment.

We found a positive association between age and antibiotic prescribing, with antibiotics prescribed the least in children under 4 years old. This contrasts with other (mainly respiratory) infections, where antibiotic prescription rates are highest in younger children.[19] Apparently, GPs consider GE in children as mild and self-limiting, and presumably of viral origin.[2,21]

Only 32% of the patients with GE episodes in which a potential bacterial cause of infection was identified with DFT were actually treated with antibiotics. These patients had fewer GP contacts and lower specialist referral rates compared with patients with targeted treatment, suggesting a benign and self-limiting course of GE. As GPs did not regard *post hoc* antibiotic treatment necessary in these patients, it remains unclear what the actual indication for the diagnostic testing was. It can be argued that, if no clear indication for treatment exists, DFT has no direct clinical value and can be withheld in these patients. Furthermore, around 60% of the GE episodes in which DFT revealed *Blastocystis* and *Dientamoeba* were treated

with antibiotics, although the clinical relevance of these parasites in GE remains unclear. As treatment of these parasites may not always be clinically beneficial[22–25] and in many cases even ineffective,[24,26,27] further research needs to evaluate the role of antibiotics in the treatment of these parasites and identify when antibiotic treatment of primary care patients with GE is appropriate.

Strengths and limitations

We used a large routine-care dataset, consisting of a representative sample of the general Dutch population,[14] and included detailed laboratory results of molecular PCR feces testing to determine infection status.

The main limitation of our study is that we could only perform a descriptive analysis of diagnostic practices and antibiotic use in a primary care population. We were not able to relate determinants of diagnostic behavior of GPs and antibiotic prescribing to the clinical disease course, as the data did not provide such in-depth information. The same holds for the assessment of CPG adherence to the prescription of no, empirical or targeted antibiotic treatment. Finally, we only obtained AMR data for a small number of isolates. The usefulness of these data in guiding the antibiotic policy of CPGs is therefore limited.

CONCLUSIONS

Antibiotic treatment of GE in primary care is relatively infrequent, with 1 in 11 episodes treated. Empirical treatment was more frequent compared with targeted treatment and was mostly with non-CPG-recommended antibiotics. However, treatment based upon DFT results followed CPG recommendations. As bacterial infections are largely untreated, the clinical value of DFT in patients with suspected bacterial GE is unclear. Non-susceptibility to first- or second-choice antibiotics was infrequent.

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CHAPTER

Guideline adherence for diagnostic feces testing in primary care patients with gastroenteritis

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ABSTRACT

Background

Gastroenteritis (GE) is a common reason for primary care consultation. Dutch clinical practice guidelines (CPG) recommend diagnostic feces testing (DFT) only in primary care patients with severe illness, comprised immunity or increased transmission risk. For its superior accuracy, shorter turnaround time and ease of use, polymerase chain reaction (PCR)-based DFT has largely replaced conventional techniques. It is unknown whether this changed CPG adherence.

Objective

To quantify the effect of PCR introduction on adherence to CPG indications for DFT in primary care patients with GE.

Methods

We performed a cohort study using routine care data of 225 GPs. Episodes of GE where DFT was performed were extracted from electronic patient records. Presenting symptoms were identified and adherence to CPG indications for DFT assessed in two randomly drawn samples of each 500 patients, one from the period before PCR introduction (2010–11) and one after (2013). The association between PCR introduction and adherence was estimated using multivariable regression analysis.

Results

In 88% of all episodes relevant presenting symptoms were reported, most often 'frequent watery stool' (58%) and 'illness duration >10 days' (40%). DFT was performed in 15% of episodes before PCR introduction and in 18% after. Overall, in 17% the DFT request was considered adherent to the CPG, 16% before PCR introduction and 18% after (adjusted OR 1.2, 95% CI 0.9–1.7).

Conclusion

Overall adherence to CPG indications when requesting DFT in primary care patient with GE was 17%. Implementation of PCR-based DFT was not associated with a change in CPG adherence.

INTRODUCTION

Gastroenteritis (GE) is a major cause of morbidity in developed countries, with 2–8 in every 10 persons experiencing an episode of GE per year.[1,2] Although most patients have an uncomplicated and self-limiting disease course,[3] in the Netherlands still 14 per 1000 patients annually consult their GP with complaints of GE.[4] To determine the causal pathogen and to guide potential antimicrobial treatment, the GP can request diagnostic feces testing (DFT). Most clinical practice guidelines (CPGs) on the management of GE define criteria for DFT primarily on pragmatism and consensus, as solid scientific evidence is lacking. In countries with a strong primary care system like the UK and the Netherlands,[5] CPGs advocate restrictive use of DFT in patients presenting with GE symptoms, largely because of the favorable prognosis and the limited benefit of antibiotic treatment, even in GE with bacterial etiology.[3,6,7] The Dutch primary care CPG on acute diarrhea recommends to consider DFT only in patients with severe illness with fever, frequent watery or bloody/mucosal stools; in patients with comprised immunity; or in those with an increased risk of disease transmissions, such as in health care workers or food handlers.[3,6] Consequently, DFT is not perceived as routine care for primary care patients with GE, but should only be 'considered' for high-risk patients. CPG adherence is, therefore, most appropriately assessed when focusing on patients receiving DFT, and not on those in which the GP refrained from testing.

Two European studies in primarily outpatient children with GE demonstrated that full CPG adherence is low (3–34%),[8,9] and that adherence is most commonly violated by performing DFT in the absence of appropriate indications.[8] In two other studies, the average compliance with CPG recommendations among GPs in the Netherlands was estimated at around 60%.[10,11] However, one of these did not include the CPG on acute diarrhea,[10] while the other quantified adherence as refraining from requesting DFT by the GP for all patients with GE.[11] We, therefore, believe that these studies do not provide appropriate information to conclude to what extent Dutch GPs adhere to the current CPG for GE when requesting DFT in both children and adults.

Furthermore, in the last decade, molecular polymerase chain reaction (PCR)-based techniques have increasingly replaced conventional techniques like culture and microscopy.[3,12–14] PCR allows for highly sensitive identification of multiple enteropathogens in a single stool sample with shorter turnaround times, resulting in improved user-friendliness when compared to conventional techniques.[12,14,15] We hypothesize that the advantages of PCR over conventional DFT make GPs more prone to routine use of DFT and could, therefore, increase inappropriate requests of DFT, consequently lowering CPG adherence. Various studies indicate that physicians

encounter multiple barriers for adherence to CPGs and show that non-adherence is often intentional and supported by valid reasons,[16,17] but do not elaborate on the effect of novel diagnostic techniques on CPG adherence.

Here, we quantify the adherence of Dutch GPs to the Dutch primary care CPG on acute diarrhea when requesting DFT and the effect of PCR introduction on adherence.

METHODS

Patients and setting

Patient data were gathered from the Utrecht General Practice Network (UGPN), a large dynamic primary care cohort containing routine care electronic medical records (EMRs) of 290,000 patients listed with 225 GPs in metropolitan area Utrecht in the Netherlands (reference date: 31 July 2012). Patients' laboratory records were gathered from Saltro Diagnostic Center. Approximately 96% of the UGPN GPs use the laboratory facilities of Saltro Diagnostic Center for their microbiological requests. Saltro Diagnostic Center replaced conventional enteropathogen DFT by molecular PCR DFT in April 2012.

All patients in the UGPN database consulting with an episode of GE between 2010 and 2013 were identified. GE was defined as a contact coded with International Classification of Primary Care (ICPC) D11 (diarrhea), D70 (gastrointestinal infection) or D73 (suspected infectious GE). UGPN data of patients with a GE episode were linked to the laboratory data from Saltro using a pseudonymization procedure through a 'trusted third party'. We excluded all episodes in the year 2012 to allow for an adaptation period for GPs to PCR DFT. We also disregarded all episodes for which the full EMR consultation text was unavailable for technical reasons. For the assessment of CPG adherence, two random samples of GE episodes with DFT were drawn; a sample with 500 episodes from the period 2010–11 (when conventional DFT was performed) and a sample with 500 episodes from the period in 2013 (when PCR DFT was performed).

Data collection

For each episode (defined as the period of all ICPC coded contacts for GE with a maximum of 60 days between two contacts), we collected the patient's age, gender, ICPC coded comorbidities (Supplement 9), Anatomical Therapeutic Chemical (ATC) coded medication prescriptions (Supplement 9), immune status (defined as presence of ICPC B72, B73, B74, B76, B90, U88, U99,

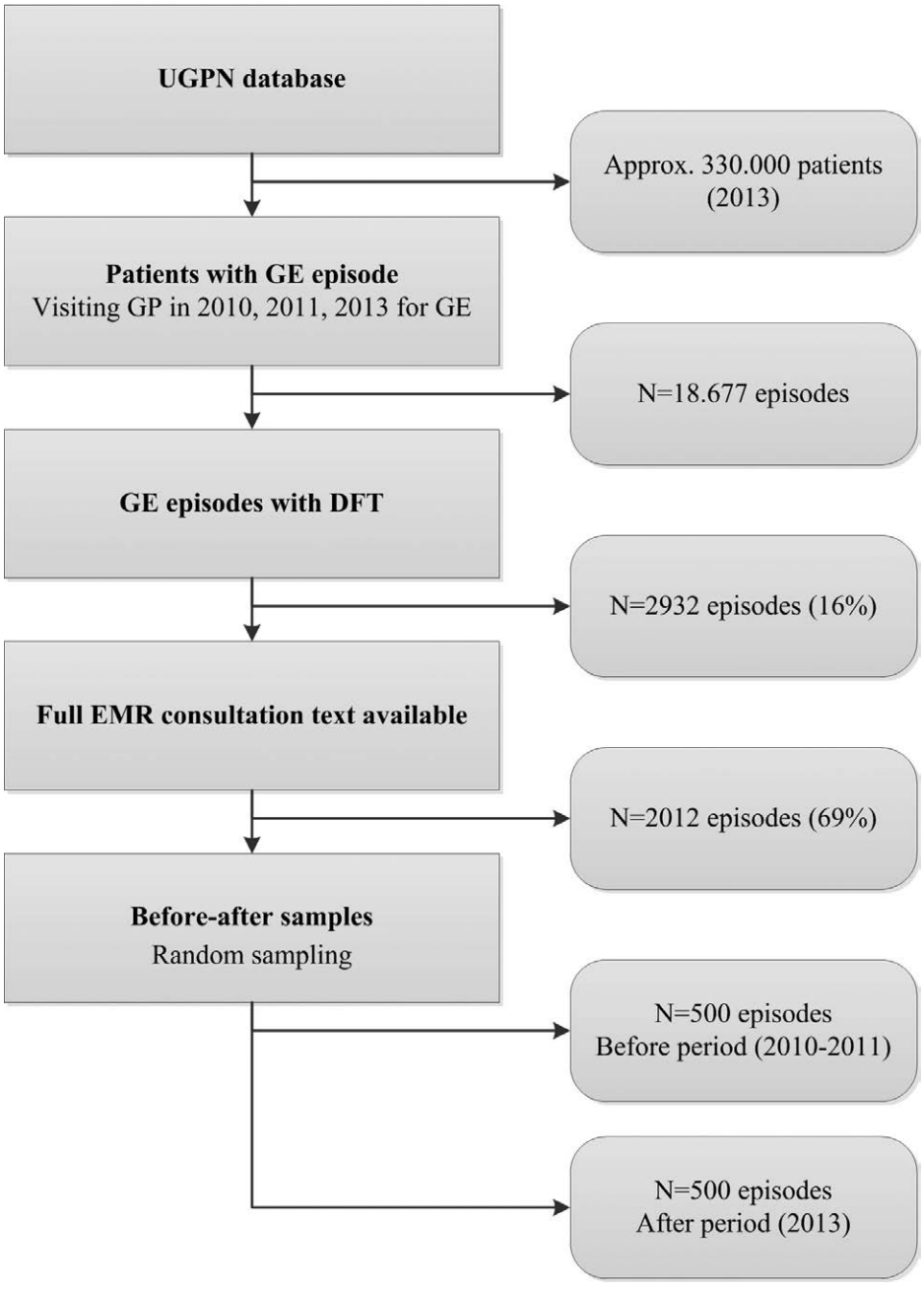
T99, D97 and ATC H02X, L01X, L04X), number of GP contacts per disease episode, number of feces test performed during the episode and the full consultation texts of the EMR.

Analysis

Patient characteristics of continuous variables were expressed as mean (including SD) and as proportions for dichotomous variables per period and overall. Patient characteristics between both periods were compared using the chi-square test (or the Fisher's exact test when cells contained less than 5 episodes) for dichotomous variables and the Mann–Whitney *U*-test for continuous and categorical variables. Presenting symptoms related to the disease episode were identified in the full EMR consultation text (Supplement 14) and expressed as proportions.

To quantify CPG adherence for DFT, an assessment tool was developed, based on the indications stated in the 2007 Dutch primary care CPG on 'acute diarrhea'.[6] Indicators of CPG adherence were 'severely ill patients', 'patients with compromised immunity' and 'patients with increased transmission risk'. Each indicator was assessed by making use of the full consultation texts, and relevant ICPC coded diseases and ATC coded drug prescriptions (see Supplement 15). Adherence for DFT was scored positive when one or more indicators were present. Adherence proportions were calculated overall and for both the before and after samples. To ensure consistency, the primary assessment of the consultation texts was performed by one assessor (MB) and in accordance with the predefined set of characteristics (Supplement 14) and indicators for CPG adherence (Supplement 15). A second assessor (AS) performed random checks on the first assessor. Disagreement was solved by discussion and led to the final recording of CPG adherence indicators and presenting symptoms. The relation between testing modality (independent variable) and CPG adherence for DFT (dependent variable) was estimated as odds ratio (OR) with 95% confidence intervals (CIs) using a multivariable logistic regression model. To correct the effect estimate for potential differences between both periods (i.e. testing modality), we identified epidemiological risk factors for (consulting with) GE, such as age and gender,[18] factors that may influence clinical decision-making, such as number of contacts per episode, intestinal comorbidities, cardiovascular comorbidities, and factors that may influence disease initiation or course, such as diabetes mellitus, chronic obstructive pulmonary disease or asthma and antacid use. All confounding factors were entered into the model. For this analysis, we aimed to detect a minimally important difference of 10%. Assuming a pre-PCR adherence rate of 60% and the inclusion of 1000 patients, the minimally important difference could be detected with a power of 88%. Data analysis were performed using SPSS (v21 for Windows) and Microsoft Excel (2010 for Windows).

Figure 1. Flow chart describing the selection of the study population



RESULTS

Patient characteristics

In total 18,677 episodes of GE were identified: 5,541 episodes in 2010, 5,654 in 2011 and 6,810 in 2013. DFT was performed in 15% (*n* = 1,712 over 2 years) and 18% (*n* = 1,220) of the episodes in the periods before and after PCR introduction, respectively. The complete EMR consultation details were available for 69% of the episodes (*n* = 2012). In the period before PCR introduction, the mean age of patients with GE was 36 years and 59% was female. In the period after PCR introduction, the mean age was 33 years and 55% was female (Table 1). Patients in the PCR period significantly were younger, had less often asthma and had more contacts per episode, when compared to before period with conventional DFT. Figure 1 shows the selection of the study population.

Clinical presentation

Specific presenting symptoms related to GE were identified in 88% of the patients with DFT (Table 2). GPs recorded frequent watery stool (58%), illness duration >10 days (40%), a changed defecation pattern (29%), recent visit to (sub)tropics (22%), blood/mucus in stool (17%), abdominal discomfort (12%) and fever (11%) as most frequent clinical symptoms in patients receiving DFT. Concerns or a request of a patient was identified as the reason for DFT in 8% of the patients.

Guideline adherence

Overall, in 17% of the GE episodes with DFT, the indication was considered in accordance with the CPG. During the periods before and after PCR introduction, in 6% and, respectively, 7% ($\chi^2 = 0.15$, $P = 0.70$) of the episodes the patient was considered severely ill, in 10% and, respectively, 9% ($\chi^2 = 0.11$, $P = 0.74$) immunocompromised, and 2% and, respectively, 4% ($\chi^2 = 2.88$, $P = 0.09$) as having an increased transmission risk. After PCR introduction, the adherence rate did not significantly change, with a rate of 16% in the period before PCR introduction and 18% ($\chi^2 = 0.85$, $P = 0.36$) in the period after PCR introduction (Table 1). Also, after adjustment for potential confounders (Table 3), the use of PCR-based DFT in primary care was not associated with CPG adherence (adjusted OR 1.2, 95% CI 0.9–1.7).

Table 1. Patient characteristics and CPG adherence for diagnostic feces testing (n = 1000) before (2010–11) and after (2013) PCR introduction.

Characteristics	2010–11 (before PCR introduction)	2013 (after PCR introduction)	Total	*P*-value
n	500	500	1000	–
Age, mean (SD)[a]	35.9 (23.4)	33 (25.6)	34.5 (24.6)	0.03
Gender, female[a]	59%	55%	57%	0.28
Number of contacts in episode, mean (SD)[a]	3.3 (2.5)	3.5 (2.5)	3.4 (2.5)	0.03
Number of tests per DFT request[b], mean (SD)	2.2 (1.4)	2 (0.8)	2.1 (1.2)	0.50
Comorbidities[a]				
Intestinal	11%	11%	11%	0.92
Cardiovascular	6%	6%	6%	0.90
Diabetes mellitus	5%	6%	6%	0.34
COPD	4%	3%	4%	0.73
Asthma	11%	7%	9%	0.02
Acid-related disorder drug use[c]	27%	25%	26%	0.68
CPG adherence for DFT				
Overall	16%	18%	17%	0.36
Severely ill[d]	6%	7%	6%	0.70
Immunocompromised patient[d]	10%	9%	9%	0.74
Increased transmission risk[d]	2%	4%	3%	0.09

COPD, chronic obstructive pulmonary disease; DFT, diagnostic feces testing.
[a]All characteristics were included in the multivariable regression analysis (see also Supplement 9).
[b]Test for, for example, bacteria, parasites or viruses.
[c]Indicated on a prescription of an ATC A02 class drug.
[d]Criteria A–C scored using a predefined algorithm; multiple criteria per disease episode are possible (Supplement 15).

Table 2. Clinical presentation (>5%) in gastroenteritis patients with diagnostic feces testing (n = 1000), multiple characteristics per patients are possible.

Presenting characteristics	Percentage
Frequent watery stool	58
Illness duration >10 days	40
Changed defecation pattern	29
Recent visit to (sub)tropics	22
Blood/mucus in stool	17
Abdominal discomfort	12
Fever	11
Severe illness	7
GE case in close environment	5
Recent hospital admission/antibiotic use	5
Concerns or request patient	8
Other	11

Table 3. Results from the multivariable logistic regression analysis on the effect of testing modality on CPG adherence for DFT estimated as odds ratio with 95% confidence intervals (CI).

Variables	Odds ratio	95% CI odds ratio	
		Lower	Upper
Testing modality, PCR	1.2	0.85	1.70
Confounding factors			
Age	1.0*	1.00	1.02
Gender, female	1.3	0.89	1.84
Number of contacts in episode	1.1*	1.00	1.14
Intestinal comorbidity[a]	1.8*	1.13	2.94
Cardiovascular comorbidity[a]	1.4	0.71	2.58
Chronic obstructive pulmonary disease[a]	3.9*	1.87	8.17
Diabetes mellitus[a]	1.8	0.98	3.50
Acid-related disorder drug use[a]	1.4	0.90	2.05

[a]See Supplement 9 for included ICPC/ATC codes.
*Significant at *P*-value < 0.05.

DISCUSSION

Summary

In patients in which DFT was requested for an episode of GE, the most common presenting symptoms were frequent watery stools (58%), illness duration >10 days (40%), changed defecation pattern (29%) and a recent visit to (sub)tropics (22%). CPG adherence when requesting DFT in primary care patients with GE was 17% and not influenced by the introduction of PCR-based DFT in 2012.

Comparison with existing literature

Two studies on CPG adherence of Dutch GPs reported that around 60% of the decisions were in accordance with the guideline.[10,11] The adherence rate for DFT of 17% in our study is comparatively low. However, whereas adequate clinical reasoning allows for deviation from professional guidelines, it cannot be concluded that the low level of CPG adherence should be judged as bad clinical practice in all cases.[17] Several factors could explain the relatively low adherence rate in our study. First, as highlighted in the Introduction section, most CPGs on GE define criteria for DFT primarily on pragmatism and consensus, as solid scientific evidence is lacking. Possible ambiguity or inconsistency in these CPGs may partly explain the low adherence, as GPs may perceive the lack of evidence as a legitimation for a less strict interpretation of the indications.[19,20] Secondly, perceived patient pressure is another important factor that influences CPG adherence. In an observational study in the UK, GPs were three times more likely to request diagnostic investigations when perceiving patient pressure (OR 3.18, 95% CI 1.31–7.70). Also, GPs only saw sufficient medical need in half (54%) of the patients who received diagnostic investigations.[21] We think that, in our study, patient pressure can be equally influential on CPG adherence for DFT, which may translate in the lack of a clear indication in the patient records for requesting DFT. Finally, whereas the aim of our study was to quantify adherence only in GE patients with DFT, it is probable that the assessment of DFT indications in all GE patients would have resulted in a higher degree of overall adherence. However, as DFT is not seen as routine care for primary care patients with GE, it does not seem appropriate to assess adherence criteria for episodes in which the GP refrained from testing.

The main conclusion from our study is that the introduction of the more accurate and user-friendly PCR DFT does not change CPG adherence. This is remarkable, as one might expect that the availability of a faster and easier DFT procedure would increase the number of DFT request and subsequently reduce CPG adherence. Obviously, it is not only the low efficiency of the conventional procedure that results in low adherence.

Strengths and limitations

To our knowledge, this is the first study formally evaluating CPG adherence for DFT in primary care. We included multiple years and an adaptation period to PCR after its introduction. We analyzed a large random sample from a dynamic primary care cohort with 225 GPs to assess CPG adherence, ensuring representativeness of the data.

Despite all efforts taken, some potential limitations need to be pointed out. Firstly, we were unable to gather all EMR records for all patients. However, we think that it is unlikely that this has influenced the evaluation of CPG adherence, as the selection was dependent on the type of EMR software, and not to patient or GP characteristics. Secondly, our assessment of guideline adherence was based on the physician reported characteristics of the disease episode, together with ICPC and ATC coded immunocompromising disease and immunomodulating drug use, and the extent to which these related to the CPG recommended indications for DFT (Supplement 15). This definition may not represent CPG adherence of the GP in all cases, as symptoms or risk factors may not have been recorded. Although this definition may have led to an underestimation of the overall guideline adherence, we assume that its influence is similar in the before and after PCR introduction. In other words, the finding that the adherence did not change was not confounded by our definition. Furthermore, whereas we identified GE-related presenting symptoms in 88% of the episodes, the EMR records completeness was quite high. Nevertheless, without actually enquiring the GP, his or her motivations that were actually decisive in requesting DFT remain undetermined. However, such a qualitative study design is prone to induce the 'Hawthorne effect' and can lead to a transient increase in adherence during the study period due to observation of the GP.[8,9] Finally, although we adjusted for various potential confounding factors when estimating the relation between testing modality and CPG adherence for DFT, residual confounding may still have affected our results. However, factors that are related to the periods that were compared (i.e. test modality), as well as whether DFT was performed in concordance with the guideline, are not plentiful and in our view largely included in the analysis or part of the assessment of CPG adherence.

Implications for research and practice

To increase CPG adherence, awareness should be raised in GPs on the indications for DFT. Most CPGs provide clear guidance and can be easily employed in daily practice. For example, short in-service training programs on GE in children have demonstrated to be effective to improve knowledge of CPGs and adherence.[8,9] Such training programs could be easily adapted to be used for a wider population and in other countries. Furthermore, because CPG adherence increases when a solid empirical basis exists and when higher effectiveness of the CPG is

expected,[19,22,23] we aim to investigate the cost-effectiveness of DFT strategies and risk factors for unfavorable disease course. We anticipate that these studies can help improve the diagnostic confidence of GPs in episodes with GE and limit the number of patients unnecessarily tested. Consequently, this will potentially increase the efficacy of DFT and lower healthcare-associated costs, without compromising the quality of clinical care.

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CHAPTER

Clinical benefit of broad-panel diagnostic stool testing of patients with gastroenteritis in primary care

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ABSTRACT

Background

Diagnostic feces testing (DFT) in patients with intestinal symptoms in primary care is typically aimed at the identification of specific pathogens (bacteria, parasites or viruses) in individual patients (selective DFT). Introduction of PCR testing allows for testing all intestinal pathogens in a single stool specimen (broad-panel DFT). We compared the results of selective DFT and broad-panel DFT and assessed the potential clinical benefits of broad-panel DFT.

Methods

All stool samples received in 2014 for DFT from patients in the Julius General Practitioners Network were tested as requested by GPs (selective DFT) and – post hoc – for a panel of 16 pathogens (broad-panel DFT). Pathogen detection rates were determined for selective and broad-panel DFT, and appropriateness of prescribed antibiotic therapy was assessed based upon broad-panel DFT results and patient medical records.

Results

Of 2,029 patients tested, GPs requested selective DFT for parasites in 1,750 (86%), bacteria in 1,160 (57%) and viruses in 150 (7%). Detection rates for selective DFT were 4.5% (78 of 1,750), 11.4% (132 of 1,160) and 3.3% for (5 of 150) for bacteria, parasites and virus, respectively. *D. fragilis* or *Blastocystis* spp. were identified in 40.3% (705 of 1,750) of patients with DFT for parasites. Compared with selective DFT, 20%, 6%, 10% and 98% of the bacteria, parasites, *D. fragilis* or *Blastocystis* spp., and viruses, respectively, were additionally identified using broad-panel DFT. The numbers needed to test to identify one additional patient with a bacteria, parasites, *D. fragilis* or *Blastocystis* spp., or viruses was 26, 54, 3 and 9, respectively. In 171 (8.4%) patients broad-panel DFT could have altered antibiotic treatment; 82 patients (4%) would have been eligible for antibiotic treatment and in 89 (4.4%) prescribed antibiotics were deemed inappropriate (20% of patients with antibiotics).

Conclusions

Selective DFT in primary care patients with GE is associated with 6-20% underdetection of clinically relevant gastrointestinal bacteria and parasites. Broad-panel DFT could change clinical decision making with regard to antibiotic treatment in 1 in 12 patients.

INTRODUCTION

In the Netherlands, restrictive use of microbiological diagnostic feces testing (DFT) in primary care is advocated. DFT is performed in 12% of the patients who consult their general practitioner (GP) with gastroenteritis (GE).[1] According to current guidelines on the use of DFT in primary care, it is recommended to test selectively for infectious agents (bacteria, parasites or viruses) guided by the patient's clinical presentation and medical history ('selective DFT'). However, as the signs and symptoms of diarrhea are generally not specific to particular infectious agents, defining which tests should be selected in which patient can be challenging.[2–7]

The recent implementation of molecular polymerase chain reaction (PCR) based DFT facilitates an alternative approach, where fecal samples are tested for all relevant infectious agents simultaneously (so-called 'broad-panel DFT'). So far, the diagnostic yield of broad-panel DFT, compared to the current practice of selective DFT is unknown. The use of broad-panel DFT may increase the detection of clinically relevant infections that remain untreated by selective DFT. It could also help avoid antibiotic treatment in patients with an identified viral infection or a non-pathogenic parasite, like *Blastocystis spp.* and *Dientamoeba fragilis*.[8–10] In order to evaluate the potential clinical benefit of broad-panel DFT in primary care, we performed a post-hoc comparison of the diagnostic yield of current practice targeted DFT – guided by the clinical judgment of the GP – with that of broad-panel DFT in primary care patients.

METHODS

Patients

The study was conducted within the coverage area of the Julius General Practitioners Network (JGPN) and the main regional primary care laboratory (Saltro Diagnostic Center, Utrecht, The Netherlands). The JGPN database consists of pseudonymized healthcare data of 370,000 patients from 45 primary care practices with 160 GPs.[11] Patient records contain information from all GP contacts during office hours. Diagnoses and medication prescriptions during these contacts are coded according to the International Classification of Primary Care (ICPC)[12] and Anatomical Therapeutic Chemical (ATC) classification.[13] GPs working in participating practices are trained in the correct use of ICPC coding and have on average 10 years' experience in the systematic coding of disease episodes.[14] Saltro Diagnostic Center provides diagnostic services (including microbiological testing) to approximately 96% of the primary care professionals

in the central region of the Netherlands. JGPN and Saltro databases were linked using a standardized, General Data Protection Regulation (GDPR) proof pseudonymization procedure.[11]

We selected patients from the JGPN cohort for whom the GP requested DFT because of gastroenteritis between January 1st, 2014 and January 31st, 2015. We collected data on patient's age, gender, and on relevant comorbidity (Supplement 6). To evaluate the antibiotic treatment of infectious agents during each episode of GE (using a timeframe of 60 days around the date of the DFT request), we identified ATC coded prescriptions for all antimicrobial drugs that are recommended by the Dutch clinical practice guideline.[15,16] These included "intestinal anti-infectives" (paromomycin and vancomycin), "antibacterials for systemic use" (co-trimoxazole, erythromycin, azithromycin, ciprofloxacin, teicoplanin, fidaxomicin) and "antiprotozoal agents" (metronidazole and clioquinol). More elaborate information on the study design and population can be found in the study design statement.[17]

DFT testing

In agreement with routine practice, stool samples were tested for bacterial, parasitic or viral causes of diarrhea, as was requested by GPs ('selective DFT'). The bacterial PCR panel included five bacteria (*Campylobacter spp., Salmonella spp., Shigella spp., Yersinia spp.* and *Plesiomonas spp.*) and the parasitic panel five parasites (*Cryptosporidium spp., Entamoeba histolytica, Giardia lamblia, Blastocystis hominis,* and *Dientamoeba fragilis)*. Individual microbiological tests included *Clostridium difficile*, Adenovirus, Norovirus and Rotavirus (all immunochromatographic rapid strip tests). GPs could order each of these tests as a single test or in combination (for details see Supplement 16). Further details on de molecular techniques can be found in our design statement.[17] Subsequently, samples were stored for broad-panel testing, which consisted of both the abovementioned bacteria and parasite PCR panels as well as a PCR panel for Adenovirus 40/41, Astrovirus, Norovirus, Rotavirus, Sapovirus and *Clostridium difficile* ('broad-panel DFT'). All broad-panel stool tests were run after the inclusion of patients was completed. These results were therefore not reported to the requesting GP.

Analysis

Characteristics of patients who had a stool sample tested were described as percentages for dichotomous variables and as means for continuous and categorical variables. We determined the total number of samples testing positive by selective and broad-panel DFT for individual infectious agents and for pathogen groups. The pathogen detection rate was calculated as the number of positive tests divided by the number of tests performed. We calculated separate detection rates for selective and broad-panel DFT. The proportion of infections detected and

undetected by selective DFT was calculated per infectious agent and visualized in a stacked bar plot. To indicate the number of additional tests needed for identification of an additional infectious agent under broad-panel DFT, we calculated the needed to test (NNT) by dividing 1 with the pathogens detection rate among additionally tested samples. To assess the potential clinical benefit of broad-panel over selective DFT, we quantified: a) *overtreatment* as the number of patients that initially received empirical antibiotic treatment, but for whom post hoc broad-panel testing revealed no infectious agents, a viral infection, or *Dientamoeba fragilis* and/or *Blastocystis spp.*, and b) *undertreatment* as the number of bacterial and parasitic infections, or *D. fragilis* and *Blastocystis* spp. undetected by selective DFT, but potentially qualifying for antibiotic treatment. Statistical analysis was conducted in R 3.3.2, using the *tableone* and *dplyr* packages.[18–20]

RESULTS

Patients characteristics

In total, stool samples of 2,029 patients were received and tested during the 13 months period. The mean age of patients was 34 (SD±23) years and 59% was female. Relevant comorbidity was identified in 27% of the patients, most frequently intestinal comorbidity (15%) such as peptic ulcers, diverticular disease, and irritable bowel syndrome, while 19% of the patients were treated with antacids. During follow-up, antibiotics were prescribed in 22% of GE episodes and 5% of patients were referred (Table 1).

Selective DFT

GPs requested selective DFT for parasites in 1,750 (86%) patients, for bacteria in 1,160 (57%) patients and for viruses in 150 (7%) patients (Table 2). In 116 (6%) samples, all three pathogen groups were ordered (therefore resembling the broad-panel testing scenario). The overall detection rate of selective DFT was 37.3% (757 of 2,029 patients), 11.4% (132 of the 1,160 requested tests) for bacterial DFT, 4.5% (78 of 1,750) for parasite DFT (excluding *D. fragilis* and *Blastocystis* spp.), and 3.3% for virus DFT (5 of 150). *D. fragilis* or *Blastocystis* spp. were identified in 40.3% (705 of 1,750) of the requests for parasite DFT. Next to these protozoa, *Campylobacter* (n=92; 7.9%), Norovirus (n=5; 4.9%) and *Giardia* (n=72; 4.1%) were the most frequently identified infectious agents in selective DFT requests, with the remaining infectious agents being detected in 0-1.4%.

Table 1. Patient characteristic and comorbidity. Numbers are counts (%) unless otherwise specified.

Characteristics	N (%)
n	2,029
Age (mean [SD])	33.67 (22.83)
Gender, Female	994 (58.7)
Comorbidity (ICPC)[1]	
Any	553 (27.3)
Intestinal	299 (14.7)
Malignancy	33 (1.6)
Immunocompromising disease	52 (2.6)
DM	83 (4.1)
COPD	39 (1.9)
Asthma	186 (9.2)
Chronic medication (ATC)[1]	
Systemic corticosteroids (H02)	61 (3.0)
Chemotherapy (L01)	2 (0.1)
Immunosuppressants (L04)	5 (0.2)
Acid-suppressive drug (A02)	375 (18.5)
Prescribed antibiotics	454 (22.4)
Referrals	
Internist/gastroenterologist	91 (4.5)
Pediatrician	30 (1.5)
Endoscopy	8 (0.4)

ATC=Anatomical Therapeutic Chemical Classification, DFT= Diagnostic Feces Testing, ICPC=International Classification of Primary Care.
[1]See Supplement 6.

Broad panel DFT

The additional yield of broad-panel DFT in 2,029 samples was: 34 bacteria, 5 parasites, and 205 viruses, and an additional 77 samples were positive for *D. fragilis* or *Blastocystis* spp. (Supplement 17). In total, 301 patients were additionally found positive for any of the microorganisms included in broad-panel DFT. The number needed to test (NNT) to identifying an additional infectious agent was lowest for the detection of *Dientamoeba fragilis* and/or *Blastocystis* spp. (NNT=4) and viruses (NNT=9), followed by bacterial (NNT=26) and parasite (NNT=56) testing. For 12 out of 16 microorganisms, the number of positive samples increased with broad-panel compared to selective DFT. Compared with selective DFT, broad-panel DFT additionally identified 20%, 6%, 10%, and 98% of the previously undetected bacteria, parasites,

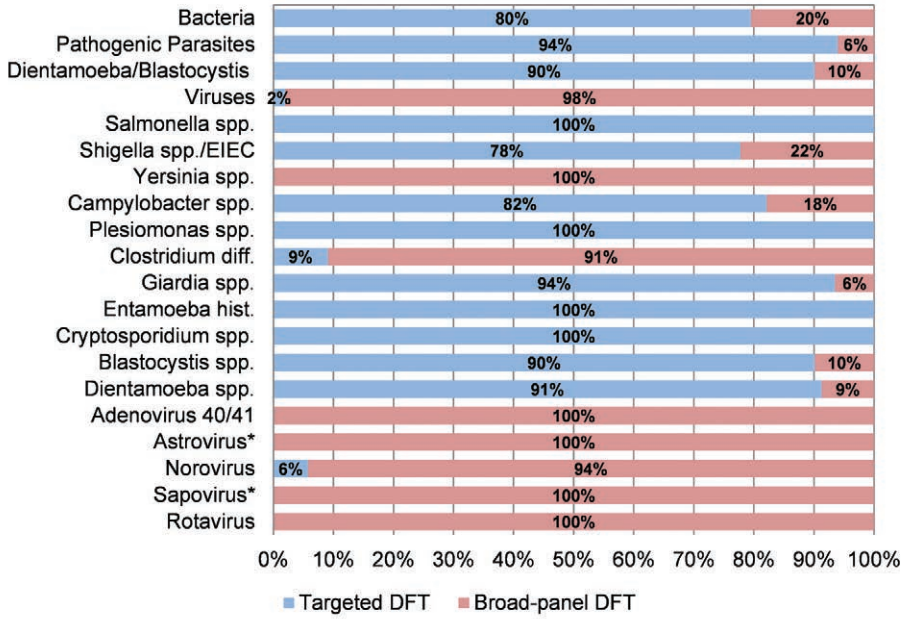
D. fragilis or *Blastocystis* spp., and viruses, respectively (Figure 1). Notably, a large proportion of the *Clostridium difficile* (91%, n=20) and Norovirus (94%, n=82) infections were additionally captured with broad-panel DFT.

Table 2. Test results for current practice selective DFT and overall broad-panel DFT.

Infectious agents	**Selective DFT**		**Broad-panel DFT**			
Groups	**Samples tested (proportion of all samples)**	**Positive (%)**	**Samples tested**	**Positive (%)**	**Additionally tested samples (proportion of all samples)**	**NNT**
Any positive	2,029 (100)	757 (37.3)	2,029	1058 (52.1)	-	-
Bacteria	1,160 (57)	132 (11.4)	2,029	166 (8.2)	869 (43)	26
Pathogenic parasites	1,750 (86)	78 (4.5)	2,029	83 (4.1)	269 (14)	56
Dientamoeba/ Blastocystis	1,750 (86)	705 (40.3)	2,029	782 (38.5)	269 (14)	4
Viruses	150 (7)	4 (3.9)	2,029	207 (10.2)	1,869 (95)	9
Bacteria						
Salmonella	1,160 (57)	16 (1.4)	2,029	16 (0.8)	869 (43)	-
Shigella/EIEC	1,160 (57)	14 (1.2)	2,029	18 (0.9)	869 (43)	217
Yersinia	1,160 (57)	0 (0)	2,029	3 (0.1)	869 (43)	290
Campylobacter	1,160 (57)	92 (7.9)	2,029	112 (5.5)	869 (43)	43
Plesiomonas	1,160 (57)	1 (0.1)	2,029	1 (0)	869 (43)	-
C. difficile	218 (11)	2 (0.9)	2,029	22 (1.1)	1,811 (89)	91
Parasites						
Giardia	1,750 (86)	72 (4.1)	2,029	77 (3.8)	269 (14)	56
Entamoeba	1,750 (86)	2 (0.1)	2,029	2 (0.1)	269 (14)	-
Cryptosporidium	1,750 (86)	5 (0.3)	2,029	5 (0.2)	269 (14)	-
Blastocystis	1,750 (86)	475 (27.1)	2,029	527 (26)	269 (14)	5
Dientamoeba	1,750 (86)	450 (25.7)	2,029	493 (24.3)	269 (14)	6
Viruses						
Adenovirus 40/41	48 (2)	0 (0)	2,029	22 (1.1)	1,981 (98)	90
Astrovirus	0 (0)	-	2,029	31 (1.5)	2,029 (100)	65
Norovirus	103 (5)	5 (4.9)	2,029	87 (4.3)	1,926 (95)	23
Sapovirus	0 (0)	-	2,029	82 (4)	2,029 (100)	25
Rotavirus	47 (2)	0 (0)	2,029	20 (1)	1,982 (98)	99

EIEC=Enteroinvasive Escherichia coli, DFT=diagnostic feces testing, NNT=number needed to test.

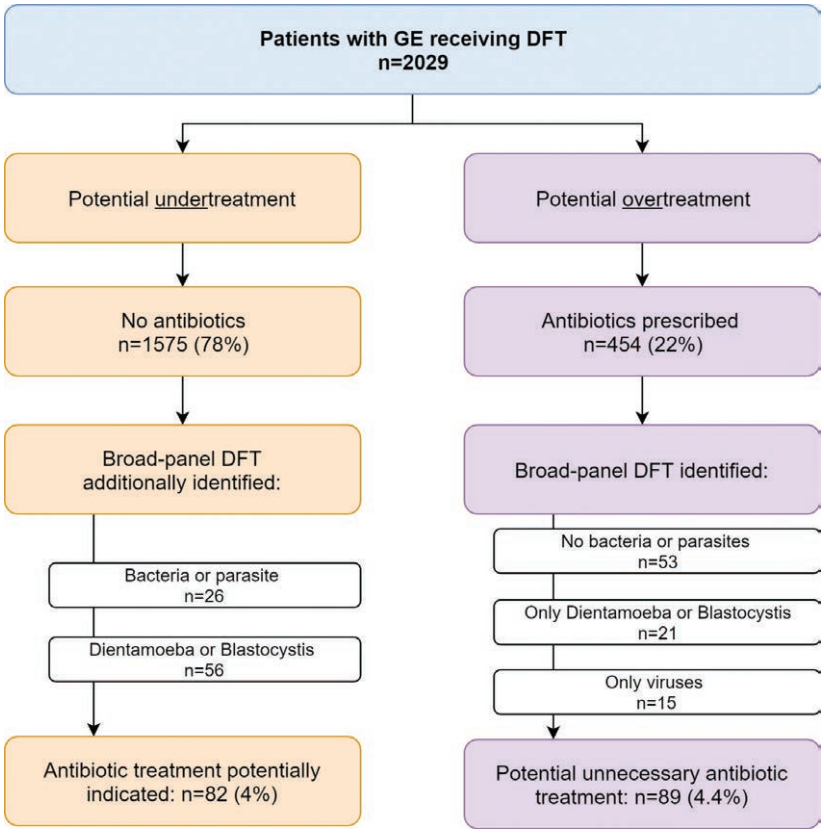
Figure 1. Detection of infectious agents with selective DFT and broad-panel DFT (n=2029). The proportion of infectious agents detected (blue) and undetected (red) by selective DFT, as compared to broad panel DFT. *Sapovirus and Astrovirus were not yet routinely available for selective DFT to the GP during the study period.



Potential clinical benefit of broad-panel DFT

The clinical benefit of broad-panel DFT was assessed through quantification of potential over- and undertreatment with selective DFT (Figure 2). Prescribed antibiotic treatment was assessed as unnecessary in 89 patients (4.4% of the total number of patients and 20% of patients with antibiotics), because of absence of any detected microorganism in broad-panel DFT (n=53), the detection of virus only (n=15), or of *Dientamoeba fragilis* or *Blastocystis* spp. only (n=21) in broad-panel DFT. Potential undertreatment occurred in 82 patients (4% of the total number of patients and 5.2% of patients without antibiotics), since broad-panel DFT yielded previously undiagnosed bacteria or parasites (excl. *D. fragilis* and *Blastocystis* spp.) that were not already treated with empirical antibiotics (n=26), or because previously undiagnosed and untreated *D. fragilis* or *Blastocystis* spp. were identified (n=56). Taken together, broad-panel DFT could have changed clinical decision making regarding antibiotic treatment in 171 (8.4%) patients.

Figure 2. Flow-chart describing the potential under- and overtreatment following selective DFT.



DISCUSSION

Main findings

When compared with selective DFT, 20%, 6%, 10% and 98% of the bacteria, parasites, *D. fragilis* or *Blastocystis* spp., and viruses, respectively, were additionally identified using broad-panel DFT. The numbers needed to test to identify one additional bacteria, parasites, *D. fragilis* or *Blastocystis* spp., or viruses was 26, 54, 3 and 9, respectively. In our study population, the use of broad-panel DFT could have changed clinical decision making regarding antibiotic treatment in 1 in every 12 patients, which included patients not receiving antibiotics for a clinically relevant bacterial or parasitic infection and patients inappropriately treated with antibiotics.

Strengths and limitations

We included a large consecutive sample of primary care patients referred for DFT following routine clinical practice. All samples were tested for a comprehensive panel of infectious agents, enabling accurate assessment of potential underdetection of selective DFT.

The main limitation of our study is that we did not have access to data regarding the indication for DFT, the disease severity and disease course of the patient. However, in the assessment of the potential clinical relevance of the undiagnosed infectious agents, we primarily focused on subgroups for which treatment is generally considered useful (e.g. bacteria) or not useful (e.g. antibiotic treatment of patients with a viral mono-infection). Moreover, the observed underdiagnosis seems clinical relevant when taking into account that confirmed bacterial or parasitic infections are treated with antibiotics in 36% and 60% of the patients, respectively, whereas less than 5% of the patients with GE receive empirical treatment.[21] Still, whether broad-panel DFT would indeed change clinical decision-making and whether patients had legitimate indications for DFT requires further study.

Interpretation of results

We assessed the potential clinical benefit of broad-panel DFT for primary care use, compared to current practice selective DFT, which is guided by the clinical presentation and medical history of the patient. We observed underdetection for 12 out of 16 pathogens when using selective DFT, confirming that adequate selection of pathogens for DFT based on the clinical presentation of patients is challenging for GPs.[22]

Overall, we found high detection rates for both *Dientamoeba fragilis* and *Blastocystis* spp. Comparable high detection rates have been reported before,[23,24] but their etiologic role in gastrointestinal symptoms and clinical relevance remains uncertain.[8–10] The relevance of diagnostic testing for these protozoa in routine parasite DFT panels remains to be determined. We also found considerable under detection of *C. difficile* and Norovirus, which infections are relevant when patients are admitted to healthcare settings. The numbers needed to test for these microorganisms were 91 for *C. difficile* and 23 for Norovirus. GPs treat 88% of the patients with *C. difficile* with metronidazole (80%) or vancomycin (20%),[21] although asymptomatic carriage with uncertain clinical relevance may occur.[25]

The identification of enteric viruses is not routinely recommended in guidelines, as it does not have direct clinical consequences for individual patients.[16] However, identification may be important from a public health perspective, such as in patients working in healthcare or food

industry. For example, Norovirus was additionally identified in 82 patients. As this virus is a frequent cause of outbreaks, timely diagnosis can be vital. In total, broad-panel DFT identified enteric viruses in 207 of 2,029 samples, of which 98% had remained undiagnosed with selective DFT. As GPs prescribed empirical antibiotics in 15 of these patients, broad-panel DFT could have prevented these episodes of antibiotic treatment for viral gastrointestinal infections.

Taken together, broad-panel DFT could have changed clinical decision making regarding antibiotic treatment in 8.4% of the patients; half of these might have benefited from antibiotic treatment and the other half received unnecessary antibiotics. These findings suggest a clinical benefit from broad-panel DFT in patients with GE. However, this benefit is conditional on the compliance of GPs with guideline recommendations for DFT and antibiotic treatment.

CONCLUSIONS

Selective DFT in primary care patients with GE is associated with 6-20% underdetection of clinically relevant gastrointestinal bacteria and parasites when compared to broad-panel DFT. In our study, broad-panel DFT could have changed clinical decision making regarding antibiotic treatment in 1 in 12 patients.

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CHAPTER

General discussion

Main conclusions of this thesis

Regarding the effects of the introduction of PCR-based diagnostic feces testing in general practice for patients with gastroenteritis, it can be concluded that:

Effects on clinical management

- The overall use of diagnostic feces testing in patients with gastroenteritis did increase during the study period;
- The adherence of GPs to clinical practice guideline indications for stool testing was not affected by the introduction of PCR;
- The increase in diagnostic feces testing for parasites was accompanied by a marked increase in the detection and antibiotic treatment of *Blastocystis* spp. and *Dientamoeba fragilis;*

Effects on healthcare costs

- After the introduction of PCR testing, the healthcare-associated costs increased with 22% per GE episode, of which 79% was attributable to healthcare resources consumption and 21% to PCR-based bacterial testing costs.

Regarding the current management of gastroenteritis by general practitioners, it can be concluded that:

Guideline adherence

- The performance of diagnostic feces testing by general practitioners in patients with gastroenteritis is in accordance with clinical practice guidelines in only a minority of patients;
- In general, only a few patients with gastroenteritis in general practice are treated with antibiotics
- Most patients with gastroenteritis in general practice that receive antibiotics are treated empirically (i.e. without diagnostic feces testing) and with antibiotics that are not recommended in the guidelines;
- Antibiotic treatment initiated after diagnostic feces testing (targeted antibiotic treatment) follows clinical practice guideline recommendations in almost all patients;

Selective versus broad-panel stool testing

- The traditional practice of selective stool testing, in which general practitioners request stool tests that specifically target (individual) bacteria, parasites, and viruses, is associated with underdetection of almost all gastrointestinal infectious agents, and results in 20% and 6% underdetection of clinically relevant gastrointestinal bacteria and parasites, respectively, and a 98% underdetection of viruses;
- Using broad-panel DFT, the numbers needed to test to identify one additional bacteria, parasites, *D. fragilis* or *Blastocystis* spp., or viruses was 26, 54, 3 and 9, respectively.
- The use of broad-panel stool testing, in which a comprehensive panel of bacteria, parasites, and viruses is tested with PCR, may augment clinical decision making regarding antibiotic treatment in 1 in every 12 tested patients;

Taken together, current general practice management of GE – including PCR-based DFT – demonstrates poor adherence to guideline indications for empirical antibiotic treatment and DFT, and is associated with an increase in the use of DFT, costs per episode, detection and treatment of non-pathogenic protozoa, when compared to traditional stool testing. However, these findings need to be interpreted with care as we were not able to quantify the potential clinical benefits of PCR testing over conventional DFT. In this chapter, we interpret these finding and put them into context. Subsequently, directions for future guideline development, research, and implementations strategies are provided.

Developments in diagnostic testing in general practice

In the last decade, the use of diagnostic tests in general practice has increased substantially.[1] This partly results from advances in diagnostic possibilities and increased access to more technologically advanced tests, such as point-of-care tests to measure hemoglobin levels for the diagnosis of anemia and CRP to diagnose pneumonia or appendicitis, and PCR testing to diagnose infectious diseases. Another contributing factor is that many health services have been diverted from secondary to general practice.[2,3] More responsibility rests on the GPs now that they treat more complex patients that were previously managed in secondary care. GPs will, therefore, more often rely on the available diagnostic tests. Additionally, a change in the patient-physician relationship may contribute to the increase in tests use by GPs, where patients increasingly request diagnostic testing.[4] Also, both physicians and patients seem to underestimate the harms and overestimate the benefits of diagnostic tests.[5,6] These factors all contribute to increased use of diagnostic tests in general practice.[7]

The development of PCR has substantially improved the utility of microbiological diagnostic tests. Initially used for the diagnosis of sexually transmitted diseases,[8] PCR has become available as a diagnostic tool in general practice for other infectious diseases, including gastroenteritis. Introduction of these novel techniques may improve diagnostic performance, but may also have intended and unintended effects on clinical management and health care costs. A recent example is the implementation of CRP point-of-care testing (POCT) for the detection of pneumonia in both adults and children, with the primary aim of reducing antibiotic prescriptions. CRP POCT demonstrated additional diagnostic value over signs and symptoms alone and showed a reduction in antibiotic use in experimental clinical studies.[9–11] However, after implementation of CRP POCT in daily clinical practice antibiotic prescribing in adults did not decline, and the indication for testing was found to be in accordance with guidelines in only 40% of the patients.[12] The use of CRP POCT in children with acute cough also failed to demonstrate an effect on antibiotic prescribing in an experimental study.[13] In another example, the introduction of molecular-based testing for *Chlamydia* in general practice did improve the diagnostic process by increasing accuracy and patient-friendliness (as there is no need for a ureteral swab and testing only requires a urine sample),[14] but was also associated with substantial additional costs per diagnosis. Therefore, the introduction of novel diagnostic tests in general practice requires adequate monitoring and evaluation of the potential consequences on management, health outcomes and costs.

How to evaluate novel diagnostic tests?

In contrast to novel pharmacological treatments, which are in general thoroughly investigated with regard to their clinical effectiveness and safety, advances in medical technology, including diagnostic devices, are usually less well evaluated.[15] Diagnostic devices directly influence clinical management, which may lead to both benefit and harm to the patient. It is therefore essential that newly developed diagnostic devices demonstrate their clinical benefit, as well as their safety, in prospective controlled research.[15] Presently, these types of studies are rare as they are not compulsory for market entry of the diagnostic device (in contrast to novel pharmacological agents)[16] and costly to perform. Fortunately, new legislation will tighten the rules for market access and hopefully will stimulate more thorough investigations into the clinical benefit of novel diagnostic tests.[17] For the evaluation of PCR-based DFT in general practice, we decided to perform a comparative before and after study. This was mainly because PCR was already implemented and the potential changes in testing practice had already occurred in daily primary care practice. A prospective cluster randomized approach was therefore no longer feasible.

Evaluation of PCR-based diagnostic feces testing

Based on our before and after evaluation, we conclude that the use of PCR-based DFT in patients with GE does have potential benefits and drawbacks.

Potential benefits of PCR

Theoretically, PCR has clear advantages over conventional DFT. For example, the shorter time-to-diagnosis and higher sensitivity will enable earlier and better targeted antibiotic therapy when necessary, potentially preventing *undertreatment* of patients. Furthermore, PCR testing can also prevent *overtreatment*, as it will provide a more confident diagnosis to physician and patient, as compared to conventional DFT. This can assist GPs to reassure patients that they do not need antibiotic treatment after a negative test. However, if these advantages also clinically benefit the patient needs thorough investigation. Hypothetically, earlier targeted antibiotic treatment could shorten the disease duration and decrease the chance of infecting others, this, in turn, will minimize productivity loss for patients or for parents of young patients that visit nursery school.

Potential drawbacks of PCR

PCR testing may lead to *overdiagnosis* as the clinical significance of identified microorganisms may not be clear. PCR cannot discriminate between viable and non-viable microorganisms, or between microorganisms with pathogenic and non-pathogenic behavior in a certain patient. This can be a substantial problem, given the high incidence of asymptomatic carriers of infectious agents like for example *Salmonella* and *Clostridium difficile*.[18–21] *Overtreatment* will occur if GPs prescribe antibiotic therapy for these patients. An indication of overdiagnosis and subsequent overtreatment was seen in chapter 3, where we investigated the replacement of conventional triple feces test (TFT) with protozoal PCR panel. PCR introduction resulted in a sizable increase in protozoal stool testing, and an increased detection and antibiotic treatment of *Blastocystis* spp. and *Dientamoeba fragilis*. This increase was most likely caused by the enhanced sensitivity of PCR-based DFT over TFT. The major concern here is that there is no conclusive evidence from randomized placebo-controlled trials that antibiotic therapy of *D. fragilis* or Blastocystis spp. infections does provide clinical benefit.[22–28] Until the clinical significance of these protozoa has been determined it is important that GPs follow current clinical practice guidelines. This means that antibiotics are only indicated in patients with chronic abdominal discomfort and diarrhea, and only after ruling out other causes.[29,30] Furthermore, patients should not be re-tested after treatment, whereas test result with PCR can remain positive for a prolonged period of time. Furthermore, due to the various benefits of PCR, we anticipated a high uptake of this new test. In chapter 4, we, therefore, investigated

the effects on healthcare consumption and associated costs. Both increased after the introduction of PCR, translating to a total cost increase of 22.4%, of which 79% (€13.5 per GE episode) was attributable to the increase in healthcare resources consumption and 21% (€3.6 per GE episode) to the higher costs of PCR-based bacterial testing. Extrapolated to the Dutch population, these incremental costs amount to € 7.4 million annually. Although a more timely and accurate diagnosis through PCR-based DFT in general practice may provide health gain to individual patients and result in more cost-effective management of GE, these topics could not be evaluated in this thesis. From previous research, it is known that PCR-based DFT can have beneficial effects on both antibiotic prescribing and costs. For example, two hospital-based studies demonstrated a decrease in costs associated with patient isolation for severe GE cases and an increase in targeted rather than empirical therapy.[31–33] However, these results have limited generalizability to general practice. Therefore, future research is needed to provide insight into the potential individual health gain from PCR-based DFT in general practice.

Methodological considerations

Several methodological issues need consideration. First, as mentioned earlier we were not able to fully investigate all potential clinical effects of PCR-based DFT. This was because reliable data on downstream clinical outcomes, such as sick leave, hospitalization and mortality, and information on the clinical status during the disease episode of patients were lacking in the cohort that we used. This also meant that we were not able to quantify the potential benefit of PCR in minimizing productivity loss for patients or for parents of children that visit nursery school. Furthermore, it was not possible to estimate the incremental cost-effectiveness ratio (ICER) for the available outcome, since the healthcare costs and the use of most healthcare resources both increased after PCR introduction (i.e. conventional DFT dominated PCR-based testing). This means that based on the available costing and clinical outcome data, PCR-based DFT seems to be more expensive and less "effective" (defined as decreased resource use) compared to conventional DFT. Finally, the applied design of our study (before and after comparison) is considered suboptimal for the determination of the clinical utility of new diagnostic interventions, as it is more prone to introduce confounding bias. To resolve these issues, a cluster randomized controlled trial would have been the optimal design. Such a study would ideally also include downstream (in-hospital) healthcare costs and costs from productivity-loss. As discussed previously, such an approach was not feasible, and in the situation where PCR-based DFT was already implemented in practice (and effects of this introduction already effectuated) also not desirable. Despite these limitations, we think the current thesis provides several directions for clinical practice.

Current management of GE in general practice

It is commonly accepted that for the majority of patients with GE diagnostic testing will have no benefit, because of the favorable disease course.[29,34] GE has a viral etiology in most patients and usually disappears within a few days. The Dutch general practice CPG on acute diarrhea recommends to consider DFT only when patients have a) severe illness with fever, frequent watery stools, or bloody or mucosal stools, b) comprised immunity, c) increased risk of disease transmission, as is the case for healthcare workers or food handlers.[29,35] These recommendations correspond with those in the United Kingdom and the United States.[30,34,36] Provided that DFT is merely performed in patients with one of the above-described characteristics, subsequent identification of a clinically plausible organism will generally be followed by targeted or modified empirical (initiated before test results are known) antibiotic therapy. From this, it becomes apparent that the (potential) clinical utility and cost-effectiveness of PCR-based testing for GE are largely contingent on the adherence of GPs to the CPG. Although we did not observe a change in the adherence to CPG indications for DFT after implementation of PCR, overall adherence to DFT indications was lower than 20%. Subsequently, we also demonstrated that over half (55%) of antibiotic treatment was empirical and in more than half of these patients with metronidazole or ciprofloxacin. Both antibiotics are not recommended as first-line empirical treatment. These results were unexpected as CPGs discourage the prescription of empirical antibiotics and recommend azithromycin as empirical treatment for suspected bacterial GE.[29,37] In previous studies, around 60% of clinical decisions made by the general practitioner were in accordance with guidelines. Obviously, guideline adherence for GE management is substantially lower.[38,39]

Several factors could explain the low guideline adherence in our study. First, as highlighted above, the CPGs criteria for DFT are primarily based on pragmatism and consensus, as solid scientific evidence is lacking. Possible ambiguity or inconsistency in these CPGs may partly explain the low adherence, as GPs may perceive the lack of evidence as a legitimation for a less strict interpretation of the indications.[40,41] Secondly, perceived patient pressure is another important factor that influences CPG adherence. In an observational study in the UK, GPs were three times more likely to request diagnostic investigations when perceiving patient pressure. Also, in one study GPs indicated an adequate medical reason in only half (54%) of the patients they sent for diagnostic investigations.[42] Although not studied, one can imagine that patient pressure may also have influenced CPG adherence for DFT in our study. Inappropriate prescription of antibiotics for other infectious diseases is well documented.[43–45] With most infectious diseases (e.g. otitis media, sinusitis, acute cough, and sore throat), diagnostic testing is less often linked to potential antibiotic prescription.

Recommendations for clinical practice

Based on the findings of the studies in this thesis, it becomes apparent that improving adherence to clinical practice guidelines is pivotal to improve clinical management of GE in general practice. This does require updating of existing guidelines using all available (and new) evidence, raising awareness among GPs on optimal diagnostic and therapeutic management and providing reliable information to patients. When updating the current guideline on gastroenteritis, the position of PCR-based DFT will need to (more) explicitly embedded, describing the different types of indications within the diagnostic workup (e.g. public health or patient's health perspectives) and the potential drawbacks and benefits for clinical management and patients. Furhermore, several more specific opportunities are worth mentioning:

- ***Indication-specific PCR panels***
  One of the main advantages of PCR is that it enables for the detection of multiple pathogens at the same time, including bacterial, parasitic and viral targets. Depending on the platform, up to 20 or more targets can be detected simultaneously.[36] It may be useful to develop disease- or indication-specific panels for example for patients with severe illness, immune deficiency, travelers' diarrhea, and for those working in healthcare or food-industry. "Syndromic panels" to for example screen patients presenting with influenza-like illness for respiratory viruses have gained popularity.[46] Several panels have been developed for screening patient with gastroenteritis,[46] but these can be further tailored for specific subgroups;

- ***Broad-panel DFT***
  Current practice includes targeted testing of suspected individual pathogens or groups of pathogens. The selection is made by the GP based on clinical presentation. Since broad-panel DFT (including a wide array of bacterial, parasitic and viral targets) has demonstrated potential benefit in clinical decision-making, broad panel DFT may be useful for some patients or for some indications (e.g. immunocompromised), but this needs to be verified in practice. Broad-panel DFT could especially be useful for patients working in healthcare or in the food industry when a viral etiology needs to be ruled out;

- ***Restricted testing for D. fragilis*** *and* ***Blastocystis*** *spp.*
  When using PCR testing high incidences of both *D. fragilis* and *Blastocystis* spp. were demonstrated, but the benefit of treatment is not established. As our study demonstrated that PCR testing was associated with an increase in antibiotic treatment after identification

of these protozoa, a more stringent policy for testing and treatment of protozoa seems necessary. A simple modification would be to only perform DFT for *D. fragilis* and *Blastocystis* spp. on clinical indication, with clear indications for treatment when a sample tests positive;

- ***Strengthening the association between testing and treating***
  It is essential to stress the association between testing and treating. DFT should be reserved for patients with a CPG indication and antibiotic treatment should be considered only after a clinically plausible organism has been identified. Conversely, the absence of an indication for DFT also provides sufficient ground to refrain from antibiotic treatment. Although the current guideline does address this issue, the results of the studies in this thesis demonstrate that the non-compliance with the current guideline is associated with empirical antibiotic therapy and the request of DFT. A more strict linkage between testing and treating will improve the confidence of GPs when managing patients with an episode of GE and limit the number of patients unnecessarily tested or treated. Consequently, this will increase the efficacy of DFT and reduce healthcare-associated costs, without compromising the quality of clinical care;

- ***Adding costs to the equation***
  DFT methods are relatively costly when compared to other routine tests used in general practice. Broad-panel PCR-based DFT will generally cost between €300-600 euro per patient, and further increase when additional antibiotic susceptibility testing of identified bacteria is needed. Adding this information in the decision-making process will help GPs to better weigh the cost against the benefits of testing. For patients, more transparency on test costs could lead to more understanding when GPs refrain from testing, especially when patients are faced with these costs when they have to be paid for via they healthcare insurance deductibles;

- ***Improving the guideline***
  Because adherence to a guideline increases when a solid evidence base exists, it is worthwhile to optimize the empirical basis of the CPG for GE.[40,47] One of the major challenges in this process will be the limited amount of evidence available on the clinical utility of diagnostic and antibiotic interventions in patients presenting to general practice with a clinical presentation that fits with infectious GE. However, for the recommendations in the current guideline, it can be made explicit to what extent they are grounded on scientific evidence or based on pragmatism and consensus, which in turn will translate

into the strength of each recommendation. The Infectious Diseases Society of America (IDSA) and the American College of Gastroenterology (ACG) have recently published systematic evidence based guidelines which do provide the strength and direction of each recommendation[34,36];

- ***Improving guideline awareness through training and feedback***
  It is difficult to change physicians' behavior and most interventions aiming to do so only yielded modest behavioral effects.[48,49] Monitoring and feedback of key indicators of performance will help to improve adherence. For instance, peer group discussion of indicators will help GP's to benchmark their individual diagnostic performance with colleagues. Such an approach recently demonstrated effective in reducing vitamin D and B_{12} tests in general practice.[50] Additionally, short in-service training programs could which appeared effective for GE management in children, could be provided to improve knowledge of CPGs and guideline adherence.[51,52] Such training programs could be easily adopted. A more elaborate approach would entail a multifaceted approach, including a wider variety of sequential interventions.[53]

Future research

Besides updating the current guidelines and improving adherence, several topics have emerged that require further research:

- As mentioned, there is a pressing need to elucidate the clinical significance of detecting *Dientamoeba fragilis and Blastocystis* spp. in feces of patients with clinical symptoms of GE. Future studies would ideally randomize patients (especially adults) in general practice to antibiotic therapy or placebo;
- GPs often deviate from the guideline recommendations for testing and treating GE. Further exploration of the motives of GPs for these deviations through qualitative research might benefit guideline development and implementation of strategies. Furthermore, it might be useful to explore the patient-related aspects of diagnostic testing in general practice;
- Empirical antibiotic treatment occurred more frequently than expected and also included non-recommended antibiotics in half of the cases. It would be worthwhile to investigate the effectiveness of this practice.

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Supplementary material

Supplement 1. Secondary objectives of the PROUD study

Two secondary objectives are studied within the PROUD study: i) guideline adherence; ii) role of IID in Irritable Bowel Syndrome (IBS) development.

Guideline adherence

Objective: To determine the proportion of patient referred for feces testing in compliance with the guideline "acute diarrhea" from the Dutch College of General Practitioners from 2007 before and after PCR introduction.

Measurements: For patients with a microbiological feces test, the EMR charts of the related IID episode are extracted together with the clinical patient data (as outlined in the "measurements" section).

Outcome measure: As defined by the guideline "acute diarrhea" from the Dutch College of General Practitioners,[1] compliance is defined for bacterial testing when a patient suspected of IID is either (a) severely ill; (b) immunocompromised; (c) at increased risk for transmission; (d) diarrhea duration of 10 or more days. Feces testing for parasitic enteropathogens is only recommended in case diarrhea duration of 10 or more days and testing for enteropathogenic viruses is not recommended.

Analysis: The difference in the proportion of patients that are tested according to the Dutch GP's guideline is compared in both the before and after periods ($p \leq 0.05$, two-tailed). Subsequently, an interrupted time series analysis is performed and adjustment made for other variables.

Role of IID in Irritable Bowel Syndrome (IBS) development

Objective: To determine the influence of relevant comorbidities and specific enteropathogens on the development of IBS after an episode of infectious intestinal disease.

Measurements: For all patients in the microbiological study novel diagnosis of IBS (ICPC D93) are extracted from the EMR one year after consultation of the GP with suspected IID. Detected relevant enteropathogens (bacterial, parasitic and viral) are detected by PCR testing (table 1). Clinical patient data (as outlined in the "measurements" section) and known risk factors (e.g. psychosocial) for IBS development are gathered from the UGPN.

Outcome measure: Novel diagnosis of physician-diagnosed IBS one year after infection with a relevant enteropathogen.

Analysis: Multivariable odds ratios are calculated for the contribution of infection with an enteropathogen (viral, bacterial and parasitic) additionally to known risk factors for the development of IBS using logistic regression.

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Supplement 2. Measurements of basic patient characteristics and comorbidities coded with International Classification of Primary Care (ICPC)

Variable	Variable type	ICPC/ATC
Patient characteristics		
Age	-	Date of birth
Gender	-	M/F
Socio-economic status	-	Zip code
Comorbidities		
Malignant neoplasm bronchus / lung	Comorb. General	R84
Other malignant neoplasm tr. resp.	Comorb. General	R85
Chronic bronchitis / bronchiectasis	Comorb. General	R91
Emphysema / COPD	Comorb. General	R95
Asthma	Comorb. General	R96
Angina pectoris	Comorb. General	K74
Acute myocardial infarction	Comorb. General	K75
Other / chronic ischemic heart disease	Comorb. General	K76
Congestive heart failure	Comorb. General	K77
Atrial fibrillation	Comorb. General	K78
Cor pulmonale	Comorb. General	K82
Non- rheumatic valve disease	Comorb. General	K83
Other heart diseases	Comorb. General	K84
Diabetes mellitus	Comorb. General	T90
Dementia	Comorb. General	P70
CVA	Comorb. General	K90
Chronic alcohol abuse	Comorb. General	P15
Irritable bowel syndrome	Comorb. General Intestinal	D93
Chronic enteritis/colitis ulcerosa	Comorb. General Intestinal	D94
Diverticulosis/diverticulitis	Comorb. General Intestinal	D92
Other disease digestive organs	Comorb. General Intestinal	D99
Hodgkin's lymphoma and other malignant lymphomas	Comorb. Immunocompromised	B72
Leukemia	Comorb. Immunocompromised	B73
Other malignant tumors in blood or blood supplying organs	Comorb. Immunocompromised	B74
Traumatic spleen rupture	Comorb. Immunocompromised	B76
HIV-infection/AIDS/ARC	Comorb. Immunocompromised	B90
Glomerulonephritis/nephrosis	Comorb. Immunocompromised	U88
Other diseases of the urinary tract	Comorb. Immunocompromised	U99

Variable	Variable type	ICPC/ATC
Renal function disorder/renal insufficiency	Comorb. Immunocompromised	U99.01
Nephrosclerosis/hypoplastic kidney e.c.i.	Comorb. Immunocompromised	U99.02
Obstructive and reflux-uropathy/ hydronephrosis	Comorb. Immunocompromised	U99.03
Other endocrine/metabolic/food related diseases	Comorb. Immunocompromised	T99
Immunodeficiency	Comorb. Immunocompromised	T99.01
Thyroiditis	Comorb. Immunocompromised	T99.02
Cushing's syndrome	Comorb. Immunocompromised	T99.08
Addison's syndrome	Comorb. Immunocompromised	T99.09
Cystic fibrosis	Comorb. Immunocompromised	T99.10
Liver cirrhosis /other liver disease	Comorb. Immunocompromised	D97
Fatigue	Various	A04
Feeling ill	Various	A05
Risk factors		
Drugs for acid related disorders	Risk factors diarrhea	A02 (ATC)
Corticosteroids	Immune compromising medications	H02 (ATC)
Chemotherapy	Immune compromising medications	L01 (ATC)
Immunosuppressants	Immune compromising medications	L04 (ATC)
MTX	Immune compromising medications	L04AX03 (ATC)

Supplement 3. Inclusion list of medication coded with Anatomical Therapeutic Chemical (ATC) classification system

ATC Description	Medication category	ATC code
Intestinal antispasmodics	Symptomatic medication	A03A
Anti-emetics	Symptomatic medication	A04A
Propulsives	Symptomatic medication	A03F
Probiotic antidiarrheals	Symptomatic medication	A07F
Antipropulsives	Symptomatic medication	A07D
Intestinal adsorbents	Symptomatic medication	A07B
Other drugs for acid-related disorders	Symptomatic medication	A02X
Intestinal anti-infectives	Symptomatic medication	A07A
Drugs for acid-related disorders	Symptomatic medication	A02
ORS	Symptomatic medication	A07CA
Loperamide	Symptomatic medication	A07DA03, A07DA05, A07DA53
Carbo adsorbens	Symptomatic medication	A07BA01, A07BA51
Bismutsubsalicylaat	Symptomatic medication	A02BX05, A02BD08
Azitromycine	Curative medication	J01FA10
Ciprofloxacine	Curative medication	J01MA02
Cotrimoxazol	Curative medication	J01EE01
Metronidazol	Curative medication	A01AB17, J01XD01, P01AB01
Erythromycin	Curative medication	J01FA01
TMP-SMZ	Curative medication	J01EE01
Doxycycline	Curative medication	A01AB22, J01AA02
Vancomycine	Curative medication	A07AA09, J01XA01
Teicoplanin	Curative medication	J01XA02
Fidaxomicine	Curative medication	A07AA12
Clioquinol	Curative medication	P01AA52, P01AA02
Paromomycine	Curative medication	A07AA06

Supplement 4. PCR laboratory procedures in the microbiological study

Viral enteropathogens

Fecal specimens (approximately 100mg or 100ul of fluid feces) are added to 1ml of STAR buffer (Roche Diagnostics, Penzberg, Germany), and vortexed for complete homogenization. Thereafter, 100ul of Chloroform is added and the sample is vortex prior to a 1-minute centrifugation at 17,000 rpm. Subsequently, 200ul supernatant, spiked with Phocine Herpes Virus and Murine Encephalomyocarditis Virus as an Internal Control (IC) for DNA and RNA extraction and PCR inhibition respectively, is used for automated total nucleic acid extraction using the MagnaPure LC instrument and the Total Nucleic Acid Extraction kit (Roche, Penzeberg, Germany). Purified nucleic acid is eluted in 100ul elution buffer. Diagnostic Real-time PCR assays are performed for pan-Adenovirus and for Adenovirus type 40/41 specifically, as well as for the RNA viruses of interest (Rotavirus, Norovirus GI and GII, Astrovirus), using an ABI 7500 real-time PCR system (Lifetechnology, Foster City USA). Positive and negative controls are included in each run. Samples are considered positive for a specific pathogen if the Cycle Threshold value (Ct) was <45. Samples are considered negative if the Ct value equaled 45 and the Internal Control meets the pre-set acceptance criteria for optimal amplification.

Bacterial and parasitic enteropathogens

DNA isolation for PCR based detection of bacterial and protozoan pathogens approximately 100mg of each fecal sample are added to 1ml of lysis buffer (Cobas PCR Female Swab Sample Kit, Roche Molecular Systems Inc., Branchburg, USA). After homogenization (vortexing for approximately 1 minute) the solution is frozen at -80C° for a minimum of 2 hours. Just prior to DNA isolation frozen samples are heated at 95C° for 15 minutes in order to release the DNA and to inactivate DNAses. Samples are spiked with Phocine Herpes Virus as an Internal Process Control to allow checking for DNA extraction and PCR inhibition. DNA is then extracted from a 100ul aliquot of this sample on MagNA Pure96 (Roche), using the Roche MagNA Pure96 and Viral NA Small Volume Kit with the Viral NA Universal SV extraction protocol according to the manufacturer's instructions. Final DNA elution is in 100ul, and 2ul of these elutes is used as input for all bacterial and protozoan pathogen PCR reactions. Positive and negative controls are included in each PCR run. PCRs are performed using a LightCycler 480 II (Roche) and are run 45 cycles. LightCycler 480 Probes Master (Roche) is used for the bacterial and protozoan pathogens specific PCR and reactions are performed in a total reaction volume of 15ul in 384 well plates on the LightCycler 480. PCR reactions are performed as multiplex PCR reactions using the LightMix Modular Gastro Parasites and Gastro Bacteria according to the manufacturer's conditions for multiplex PCR (TIB Molbiol, GmbH Berlin, Germany). Samples are considered positive for a specific pathogen if the Cycle Threshold value (Ct) was <45. Samples are considered negative

if the Ct value > 45 and the Internal Process Control meets the pre-set acceptance criteria for optimal amplification. All PCR curves and the database are subsequently double-read to prevent interpretation and data-entry errors.

Supplement 5. Expected 95% CIs for enteropathogen proportions in a 1-year microbiological study

Microorganism	Reported proportion (%)	95% CI width* for min/max expected sample size** (%)
Campylobacter spp.	10.4[1]	±2.5 / ±1.6
Clostridium difficile	2.1[2]	±1.2 / ±0.8
Salmonella spp.	3.9[1]	±1.6 / ±1.0
Shigella spp.	0.1[1]	±0.3 / ±0.2
Yersinia spp.	0.7[1]	±0.7 / ±0.4
Adenovirus	2.2[1]	±1.2 / ±0.8
Norovirus	5.1[1]	±1.8 / ±1.2
Rotavirus	5.3[1]	±1.9 / ±1.2
Astrovirus	1.5[1]	±1.0 / ±0.6
Cryptosporidium spp.	2.1[1]	±1.2 / ±0.8
Entabamoeba spp.	0.9[1]	±0.8 / ±0.5
Giardia Lamblia	5.4[1]	±1.9 / ±1.2
Dientamoeba fragilis	10.3[1]	±2.5 / ±1.6
Blastocystis hominis	21.7[1]	±3.4 / ±2.2

*Expected precision of enteropathogen proportions in the microbiological study, measured as the 95% confidence interval (CI) widths of reported enteropathogen proportions.[1,2]
** Expected minimum (n=554) and maximum (n=1,385) samples size of a 1-year microbiological study.[3,4]

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Supplement 6. International Classification of Primary Care (ICPC) and Anatomical Therapeutic Chemical (ATC) codes extracted form patient records.

Comorbidities	ICPC codes (or indicated else)	
Intestinal disorders	D72	Viral hepatitis
	D81	Congenital anomaly digestive system
	D84	Congenital anomaly digestive system
	D85	Duodenal ulcer
	D86	Peptic ulcer other
	D92	Diverticular disease
	D93	Irritable bowel syndrome
	D94	Chronic enteritis/ulcerative colitis
	D97	Liver disease NOS
	D98	Cholecystitis/cholelithiasis
	D99	Disease digestive system, other
Malignancies	A79	Malignancy, NOS
	B72	Hodgkin's disease/lymphoma
	B73	Leukaemia
	B74	Malignant neoplasm blood other
	B90	HIV infection/AIDS
	D74	Malignant neoplasm stomach
	D75	Malignant neoplasm colon/rectum
	D76	Malignant neoplasm pancreas
	D77	Malignant neoplasm digestive other/NOS
	F74	Neoplasm of eye/adnexa
	K72	Neoplasm, cardiovascular
	L71	Malignant neoplasm, musculoskeletal
	N74	Malignant neoplasm nervous system
	N76	Neoplasm nervous system, unspecified
	R84	Malignant neoplasm bronchus, lung
	R85	Malignant neoplasm respiratory, other
	S77	Malignant neoplasm of skin
	T71	Malignant neoplasm thyroid
	T73	Neoplasm endocrine other/uncertain
	U75	Malignant neoplasm kidney
	U76	Malignant neoplasm bladder
	U77	Malignant neoplasm, urinary, other
	W72	Malignant neoplasm related to fertility
	X75	Malignant neoplasm cervix
	X76	Malignant neoplasm breast female
	X77	Malignant neoplasm genital female other
	Y77	Malignant neoplasm prostate
	Y78	Malignant neoplasm male genital, other
Chemotherapy	L01 (ATC)	Chemotherapy

Comorbidities	ICPC codes (or indicated else)	
Immunocompromising disorders (45,46)	B72	Hodgkin and other malignant lymphoma's
	B73	leukemia
	B74	Other malignant tumor in blood or blood supplying organs
	B76	Traumatic spleen rupture
	B90	HIV-infection/AIDS/ARC
	D97	Liver cirrhosis /other liver disease
	T99	Other endocrine/metabolic/food related diseases
	U88	Glomerulonephritis/nefrosis
	U99	Other diseases of the urinary tract
Immunosuppressive therapy	H02 (ATC)	Corticosteroids
	L04 (ATC)	Immunosuppressants
	L04AX03 (ATC) MTX	
Diabetes mellitus	T89	Diabetes, insulin dependent
	T90	Diabetes, non-insulin dependent
COPD	R95	Chronic obstructive pulmonary disease
Asthma	R96	Asthma
Acid-suppressive medication	A04 (ATC) Drugs for acid-related disorders	

Supplement 7. Baseline characteristics of primary care patients presenting with GE in the period before (2010-2011) and after (2013-2014) PCR introduction.

Episode characteristics	Episodes by period								
	All available data			Complete cases			Episodes with imputed data		
	Before	*After*	*p-value*	Before	After	p-value	Before	After	p-value
n	10,947	13,271		8,929	10,790		2,018	2,481	
Age, mean (SD)	33.04 (27.5)	32.28 (27.1)	0.030	32.84 (27.6)	31.47 (26.8)	<0.001	33.94 (27.2)	35.81 (28.0)	0.024
Gender, Female	6,266 (57.2)	7,199 (54.2)	<0.001	5,072 (56.8)	5,806 (53.8)	<0.001	1,194 (59.2)	1,393 (56.1)	0.045
Patient comorbidity[1]									
Intestinal	1,255 (11.5)	1,639 (12.4)	0.036	1,046 (11.7)	1,387 (12.9)	0.016	209 (10.4)	252 (10.2)	0.865
Malignancy	267 (2.4)	353 (2.7)	0.297	223 (2.5)	303 (2.8)	0.192	44 (2.2)	50 (2.0)	0.779
Immunocompromising disease	338 (3.1)	505 (3.8)	0.003	295 (3.3)	418 (3.9)	0.036	43 (2.1)	87 (3.5)	0.008
DM	846 (7.7)	996 (7.5)	0.530	720 (8.1)	840 (7.8)	0.487	126 (6.2)	156 (6.3)	1.000
COPD	403 (3.7)	467 (3.5)	0.521	345 (3.9)	385 (3.6)	0.291	58 (2.9)	82 (3.3)	0.458
Asthma	923 (8.4)	1,397 (10.5)	<0.001	818 (9.2)	1,202 (11.1)	<0.001	105 (5.2)	195 (7.9)	<0.001
Chronic medication use[1]									
Systemic corticosteroids	480 (4.4)	713 (5.4)	<0.001	440 (4.9)	584 (5.4)	0.135	40 (2.0)	129 (5.2)	<0.001
Chemotherapy	25 (0.2)	51 (0.4)	0.041	23 (0.3)	42 (0.4)	0.139	2 (0.1)	9 (0.4)	0.140
Immunosuppressants	51 (0.5)	76 (0.6)	0.291	47 (0.5)	58 (0.5)	0.993	4 (0.2)	18 (0.7)	0.021
Acid suppressive drug	2,090 (19.1)	3,016 (22.7)	<0.001	2,047 (22.9)	2,462 (22.8)	0.871	43 (2.1)	554 (22.3)	<0.001
DFT orders			<0.001			<0.001			<0.001
None	9,483 (86.6)	10,842 (81.7)		7,710 (86.3)	8,822 (81.8)		1773 (87.9)	2,020 (81.4)	
1 order	1,263 (11.5)	2,219 (16.7)		1,049 (11.7)	1,791 (16.6)		214 (10.6)	428 (17.3)	
2 orders	173 (1.6)	186 (1.4)		145 (1.6)	156 (1.4)		28 (1.4)	30 (1.2)	
3 orders	28 (0.3)	22 (0.2)		25 (0.3)	19 (0.2)		3 (0.1)	3 (0.1)	
4 orders	0 (0.0)	2 (0.0)		0 (0.0)	2 (0.0)				
Bacteria	1,053 (9.6)	1,871 (14.1)	<0.001	879 (9.8)	1,496 (13.9)	<0.001	174 (8.6)	375 (15.1)	<0.001

Episode characteristics	Episodes by period								
	All available data			Complete cases			Episodes with imputed data		
	Before	*After*	*p-value*	Before	After	p-value	Before	After	p-value
Bacteria (positive) with AST	140 (13.3)	284 (15.2)	0.182	117 (13.3)	229 (15.3)	0.203	23 (13.2)	55 (14.7)	0.748
Parasites	1,042 (9.5)	2,073 (15.6)	<0.001	865 (9.7)	1,665 (15.4)	<0.001	177 (8.8)	408 (16.4)	<0.001
Viruses	142 (1.3)	306 (2.3)	<0.001	126 (1.4)	263 (2.4)	<0.001	16 (0.8)	43 (1.7)	0.009
Consultations									
Mean per episode (SD)	1.75 (1.72)	1.93 (1.98)	<0.001	1.89 (1.84)	2.03 (2.13)	<0.001	1.36 (1.06)	1.49 (0.98)	<0.001
In-office	1.09 (1.13)	1.14 (1.25)	0.006	1.15 (1.18)	1.19 (1.31)	0.018	0.88 (0.98)	0.91 (0.88)	0.373
Home visits	0.12 (0.52)	0.09 (0.49)	<0.001	0.12 (0.52)	0.08 (0.47)	<0.001	0.14 (0.53)	0.13 (0.57)	0.531
Telephone	0.57 (1.08)	0.71 (1.26)	<0.001	0.62 (1.15)	0.76 (1.35)	<0.001	0.34 (0.57)	0.46 (0.67)	<0.001
Antibiotic prescriptions									
Any	791 (7.2)	1,316 (9.9)	<0.001	763 (8.5)	1,099 (10.2)	<0.001	28 (1.4)	216 (8.7)	<0.001
Intestinal anti-infectives	40 (0.4)	41 (0.3)	0.519	32 (0.4)	35 (0.3)	0.775	8 (0.4)	5 (0.2)	0.351
Antibacterials for systemic use	530 (4.8)	624 (4.7)	0.633	519 (5.8)	539 (5.0)	0.012	10 (0.5)	85 (3.4)	<0.001
Antiprotozoal agents	240 (2.2)	687 (5.2)	<0.001	229 (2.6)	556 (5.2)	<0.001	11 (0.5)	131 (5.3)	<0.001
Specialist referrals									
Any	286 (3.2)	660 (6.1)	<0.001	274 (3.1)	660 (6.1)	<0.001	111 (5.5)	144 (5.8)	0.709
Internal medicine	173 (1.9)	406 (3.8)	<0.001	165 (1.8)	406 (3.8)	<0.001	28 (1.4)	87 (3.5)	<0.001
Pediatrician	108 (1.2)	226 (2.1)	<0.001	104 (1.2)	226 (2.1)	<0.001	81 (4.0)	57 (2.3)	0.001

AST=Antibiotic Susceptibility Testing; DFT=Diagnostic Feces Testing; PCR=Polymerase Chain Reaction;
*Numbers represent counts (%), unless indicated otherwise, and are based on non-imputed data;
[1]See Supplementary Table S2 for the specification of comorbidity and medication groups;
Variables containing any missing data are highlighted in grey.

Supplement 8. Medication costs in euro (€) per unit (2016 list prices from Medicijnkosten.nl)

ATC code	Strength	Average cost per unit, €
A02AA02	500MG	0.04
A02AA02	500mg	0.04
A02AA02	NA	0.04
A02AA03	500mg	0.04
A02AA03	NA	0.04
A02AA04	724MG	0.04
A02AA04	724mg	0.04
A02AA04	NA	0.04
A02AD01	200/400MG	0.18
A02AD01	40/20MG/ML	0.02
A02AD01	40/20mg/ml	0.02
A02AD01	680/80/25MG	0.27
A02AD01	680/80MG	0.125
A02AD01	90/60MG/ML	0.03
A02AD01	90/60mg/ml	0.03
A02AD01	NA	0.096429
A02AF	NA	0.23
A02AH	NA	0.48
A02BA01	400mg	0.085
A02BA01	NA	0.085
A02BA02	150MG	0.075
A02BA02	150mg	0.075
A02BA02	15MG/ML	0.04
A02BA02	15mg/ml	0.04
A02BA02	300MG	0.135
A02BA02	300mg	0.135
A02BA02	75MG	0.6
A02BA02	75mg	0.6
A02BA02	NA	0.2125
A02BA03	40mg	0.44
A02BA03	NA	0.44
A02BB01	NA	1.02
A02BC01	10MG	0.165
A02BC01	10mg	0.165
A02BC01	20MG	0.51
A02BC01	20mg	0.51
A02BC01	2MG/ML	0.29
A02BC01	40MG	0.575
A02BC01	40mg	0.575
A02BC01	NA	0.398571
A02BC02	20MG	0.515
A02BC02	20mg	0.515
A02BC02	40MG	0.145
A02BC02	40mg	0.145
A02BC02	NA	0.33
A02BC03	15MG	0.055
A02BC03	30MG	0.16
A02BC03	30mg	0.16
A02BC03	NA	0.125
A02BC04	10MG	0.135
A02BC04	10mg	0.135
A02BC04	20MG	0.295
A02BC04	20mg	0.295
A02BC04	NA	0.215
A02BC05	10MG	0.9
A02BC05	20MG	0.525
A02BC05	20mg	0.525
A02BC05	40MG	0.26
A02BC05	40mg	0.26
A02BC05	NA	0.494
A02BD04	1000/500/40MG	1.66
A02BD04	1000/500/40mg	1.66
A02BD04	NA	1.66
A02BX02	1G	0.235
A02BX02	1g	0.235
A02BX02	200mg/ml	0.215

ATC code	Strength	Average cost per unit, €
A02BX02	200MG/ML	0.215
A02BX02	NA	0.225
A02BX13	250/133,5/80mg	0.21
A02BX13	50/26,7mg/ml	0.04
A02BX13	NA	0.125
A03AA04	200MG	0.2
A03AA04	200mg	0.2
A03AA04	NA	0.2
A03AB02	NA	0.415
A03AX13	NA	0.19
A03FA01	10MG	0.095
A03FA01	10mg	0.095
A03FA01	1MG/ML	0.02
A03FA01	20MG	0.19
A03FA01	20mg	0.19
A03FA01	5mg/ml	0.67
A03FA01	NA	0.21
A03FA03	10MG	0.08
A03FA03	10mg	0.08
A03FA03	1MG/ML	0.035
A03FA03	1mg/ml	0.035
A03FA03	30MG	0.24
A03FA03	30mg	0.09
A03FA03	60MG	0.48
A03FA03	60mg	0.48
A03FA03	NA	0.19
A04AA01	0,8MG/ML	1.03
A04AA01	16MG	14.815
A04AA01	4MG	1.99
A04AA01	4mg	1.99
A04AA01	8MG	3.21
A04AA01	NA	4.607
A04AA02	NA	6.81
A04AD12	125+80MG	21.65
A04AD12	NA	21.65
A04AD	125+80MG	21.65
A04AD	NA	21.65
A07AA02	100.000E/ML	0.11
A07AA02	100.000e/ml	0.11
A07AA02	NA	0.11
A07AA06	250MG	0.6
A07AA06	NA	0.6
A07AA07	100MG/ML	0.11
A07AA07	NA	0.11
A07AA09	250MG	10.5
A07AA09	250mg	10.5
A07AA09	NA	10.5
A07AC01	20MG/G	0.08
A07AC01	20mg/g	0.08
A07AC01	NA	0.08
A07BA01	200MG	0.23
A07BA01	NA	0.23
A07CA1	NA	0.715
A07CA2	NA	0.715
A07CA	NA	0.715
A07DA02	NA	0.001
A07DA03	0,2MG/ML	0.065
A07DA03	0,2mg/ml	0.065
A07DA03	2MG	0.345
A07DA03	2mg	0.345
A07DA03	NA	0.205
A07DA05	1MG	0.245
A07DA05	1mg	0.245
A07DA05	NA	0.245
J01AA02	100MG	0.355
J01AA02	100mg	0.355
J01AA02	NA	0.355
J01EE01	160/800MG	0.18
J01EE01	160/800mg	0.18
J01EE01	8/40MG/ML	0.03
J01EE01	8/40mg/ml	0.03
J01EE01	80/400MG	0.12

ATC code	Strength	Average cost per unit, €
J01EE01	80/400mg	0.12
J01EE01	NA	0.11
J01FA01	1G	0.66
J01FA01	1g	0.66
J01FA01	250mg	0.15
J01FA01	25MG/ML	0.09
J01FA01	500MG	0.335
J01FA01	500mg	0.335
J01FA01	50mg/ml	0.07
J01FA01	NA	0.328571
J01FA10	250MG	0.685
J01FA10	250mg	0.685
J01FA10	40MG/ML	0.275
J01FA10	40mg/ml	0.275
J01FA10	500MG	1
J01FA10	500mg	1
J01FA10	NA	0.653333
J01MA02	250MG	0.085
J01MA02	250mg	0.085
J01MA02	500MG	0.135
J01MA02	500mg	0.135
J01MA02	50MG/ML	0.29
J01MA02	50mg/ml	0.29
J01MA02	750MG	0.295
J01MA02	NA	0.187857
J01XA01	NA	10.05
J01XD01	250MG	0.155
J01XD01	500MG	0.235
J01XD01	40MG/ML	0.15
J01XD01	NA	0.18
P01AA02	100MG/ML	0.235
P01AA02	100mg/ml	0.235
P01AA02	250MG	1.31
P01AA02	NA	0.593333
P01AB01	250MG	0.155
P01AB01	250mg	0.155
P01AB01	40MG/ML	0.15
P01AB01	40mg/ml	0.15
P01AB01	500MG	0.235
P01AB01	500mg	0.235
P01AB01	NA	0.18

Supplement 9. International Classification of Primary Care (ICPC) and Anatomical Therapeutic Chemical (ATC) codes extracted form patient records.

Intestinal Comorbidities

ICPC code	Description
D92	Diverticular disease
D93	Irritable bowel syndrome
D94	Chronic enteritis/ulcerative colitis
D99	Disease digestive system, other

Cardiovascular Comorbidities

ICPC code	Description
K74	Ischemic heart disease with angina
K75	Acute myocardial infarction
K76	Ischemic heart disease without angina
K77	Heart failure
K78	Atrial fibrillation/flutter
K82	Pulmonary heart disease
K83	Heart valve disease NOS
K84	Heart disease, other

Immunocompromising comorbidities

ICPC code	Description[1,2]
Malignancy	
B72	Hodgkin's disease/lymphoma
B73	Leukaemia
B74	Malignant neoplasm blood other
Immunocompromising disease	
B76	Spleen rupture
B90	HIV infection/AIDS
U88	Glomerulonephritis/nephrosis
U99	Urinary disease, other
T99	Endocrine/metabolic/nutritional disease, other
D97	Liver disease NOS
ATC code	
H02	Corticosteroids for systemic use
L01	Chemotherapy
L04	Immunosuppressants

Other Comorbidities

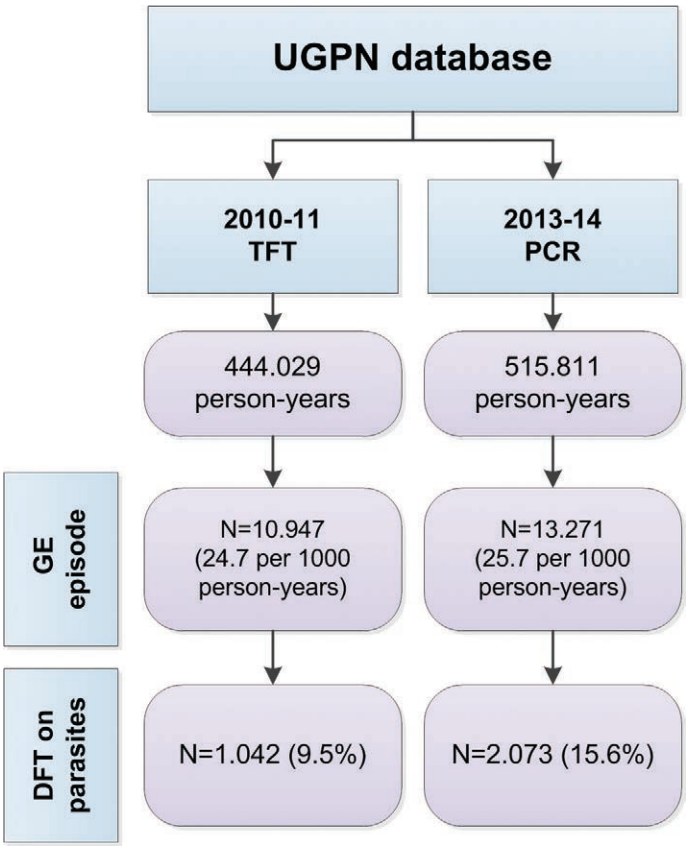
ICPC code	Description
T90	DM
R95	COPD
R96	Asthma
ATC codes	
A02	Drugs for acid related disorders

References

1. Jansen B et al. Monitoring vaccinatiegraad Nationaal Programma Grieppreventie 2012. RIVM. 2012;

2. Van Deursen AMM et al. Epidemiol Infect. 2012;140(05):823–34.

Supplement 10. Flow chart describing the patient selection process from the UGPN cohort.



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2. Heins M, Hooiveld M, Veen P ten, et al. Monitor Vaccinatiegraad Nationaal Programma Grieppreventie. Utrecht; 2015.

3. Schierenberg A, Nipshagen MD, Broekhuizen BDL, et al. Design of the PROUD study: PCR faeces testing in outpatients with diarrhoea. BMC Infect. Dis. 2016;16:39.

Supplement 11. Baseline characteristics of GE episodes with parasite stool testing performed, stratified by stool test method (n = 3,115). Numbers are counts (%)unless specified otherwise.

	Total	TFT	PCR	p-value
	N= 3,115	N=1,042	N=2,073	
Patient Characteristics				
Median age (IQR)	32 (12, 52)	33 (21, 52)	32 (9, 52)	0.060
Female gender (%)	1,754 (56.3)	620 (59.5)	1,134 (54.7)	0.012
Comorbidities[1]				
Intestinal (%)	407 (13.1)	138 (13.2)	269 (13.0)	0.879
Malignancy (%)	65 (2.1)	22 (2.1)	43 (2.1)	1.000
Immunocompromising disease (%)	105 (3.4)	36 (3.5)	69 (3.3)	0.937
Asthma (%)	286 (9.2)	87 (8.3)	199 (9.6)	0.283
DM (%)	206 (6.6)	64 (6.1)	142 (6.8)	0.500
COPD (%)	89 (2.9)	30 (2.9)	59 (2.8)	1.000
Medication use (ATC)				
Corticosteroids (H02) (%)	146 (4.7)	47 (4.5)	99 (4.8)	0.810
Chemotherapy (L01) (%)	5 (0.2)	1 (0.1)	4 (0.2)	0.870
Immunosuppressants (L04) (%)	16 (0.5)	7 (0.7)	9 (0.4)	0.542
Acid suppressive drug (A02) (%)	678 (21.8)	213 (20.4)	465 (22.4)	0.221
Consultations per episode				
Total consultations (median [IQR]) [2]	2 [2, 4]	2 [1, 4]	3 [2, 4]	0.001
Additional DFT performed[3]				
Bacteria	2,255 (72.4)	664 (63.7)	1,591 (76.7)	<0.001
Viruses	290 (9.3)	77 (7.4)	213 (10.3)	0.011

ATC: Anatomical Therapeutic Chemical Classification;
[1] See Supplementary Table S1;
[2] In-office consultations, home visits, and telephone consultations;
[3] See design statement for the specification of the tests.[3]

Supplement 12. Proportions of cases with empirical and targeted treatment, stratified by gender. Numbers are counts (%).

Empirical antibiotics*	**Male**	**Female**	**p-value**
N (GE)	10,753	13,465	-
Metronidazole	181 (1.7)	232 (1.7)	0.851
Clioquinol	20 (0.2)	21 (0.2)	0.684
Targeted antibiotics#			
N (DFT)	1,361	1,754	-
Metronidazole	132 (9.7)	179 (10.2)	0.684
Clioquinol	12 (0.9)	15 (0.9)	1.000

*Treatment in GE episodes with either negative or no DFT.
#Treatment for a microbiologically confirmed infection with Dientamoeba and/or Blastocystis without other pathogenic parasitic co-infection (i.e. Giardia, Entamoeba, Cryptosporidium).

Supplement 13. First- and second-choice CPG recommended antibiotics for the treatment of infectious GE (1, 2).

Pathogen	Antibiotic[1]							
	Paromomycin	Vancomycin	Co-trimoxazole	Erythromycin	Azithromycin	Ciprofloxacin	Clioquinol	Metronidazole
Salmonella			2nd		ET	1st		
Shigella			2nd		2nd	1st		
Yersinia			2nd		ET	1st		
Campylobacter				2nd	1st			
Plesiomonas			2nd *		ET	1st*		
C. difficile		2nd			ET			1st
G. lamblia					ET			1st
E. histolytica	2nd				ET		2nd	1st
Cryptosporidium					ET			
B. hominis					ET			1st
D. fragilis					ET		2nd	1st

ET=Empirical treatment;
[1]Teicoplanin and Fidaxomicin are the 3rd and 4th options for treatment of C. difficile, but not prescribed during this study
*No antibiotic treatment was recommended in Dutch CPG,[3,4] taken from: https://www.uptodate.com/contents/plesiomonas-shigelloides-infections

References

1. Stichting Werkgroep Antibioticabeleid (SWAB). SWAB richtlijn antimicrobiële therapie voor acute infectieuze diarree. Nijmegen; 2013 May.
2. Belo J, Bos M, Brühl P, Lemmen W, Pijpers M, Van der Donk M, et al. NHG-Standaard Acute diarree. 2014;57(9):462–71.

Supplement 14. List of clinical presenting characteristics for patients with gastroenteritis, extracted from patient records.

- Recent visit (sub)tropics, defined as a recent visit (within months) to all countries except for North America and West-European countries.
- Duration of acute diarrhea of ≥ 10 days.
- Changed defecation pattern, including all described defecation patterns which did not meet 'Frequently watery stool'.
- Recent hospital admission or use of antibiotics.
- ≥ 1 case of acute diarrhea in the work environment or ≥ 2 cases of acute diarrhea in the private environment (family, friends).
- Pregnancy.
- Suspicion of presence of Helicobacter Pylori.
- Abdominal cramps or abdominal ache.
- Impaired cognition or impaired communication, for instance by confusion of patient or linguistic problems.
- Weight loss.
- Concern of patient or parent(s) of patient.
- On request of the patient or parent(s) of the patient.
- Other factors, including, but not exclusively: decreased growth of baby, a worm seen in the stool, a pet with Giardiasis, patient history of stomach-operation, advice of a doctor in alternative medicine.
- Individual guideline factors.

Supplement 15. Assessment tool for scoring clinical practice guideline adherence for diagnostic feces testing according to the 2007 Dutch primary care guideline for acute diarrhea[1].

The presence of one or more of the following criteria (A-C) in the consultation texts was considered as guideline adherence:

A. Electronic medical record (EMR) implicating 'severely ill patient' (written down by general practitioner or judged upon by reviewers), including either:
   1. Fever, either anamnestic within the current episode, or measured by a general practitioner (if specified T ≥ 38,0°);
   2. Blood and/or mucus in the stool;
   3. Frequent watery stool (if specified ≥3 watery stools in <24 hours).

B. Patient EMR implicating 'compromised immunity', defined as any of the following:
   1. Agammaglobulinemia or hypogammaglobulinemia;
   2. Generalized malignant disease, implicating immunocompromising therapy;
   3. Radiotherapy;
   4. Splenectomy;
   5. Impaired immunity, including other specifically mentioned reasons for an impaired immune system and International Classification of Primary Care (ICPC) coded comorbidities (B72, B73, B74, B76, B90, U88, U99, T99, D97);
   6. Bone marrow transplant;
   7. Use of immune-modulating drugs (Anatomical Therapeutic Chemical (ATC) codes H02X, L01X, L04X).

C. Consultation text implicating an 'increased transmission risk', defined as any of the following:
   1. Job-related, defined by work in a nursing home or catering services;
   2. ≥ 2 cases of acute diarrhea in the work environment, defined as work at a nursery home or kindergarten.

References

1. Brühl P, Lamers H, Van Dongen A, Lemmen W, Graafmans D, Jamin R. NHG-Standaard Acute diarree. *Huisarts Wet*. 2007;(50):103-113.

Supplement 16. Selective and broad DFT as performed in the study.

With *selective* DFT, GPs were able to request individual *stool tests*. *Broad* DFT included all *stool tests* described below.

DFT type	Stool test	Pathogens included in DFT	DFT method
Selective			
	Bacterial PCR panel	*Campylobacter* spp., *Salmonella spp., Shigella spp., Plesiomonas spp.*, and *Yersinia spp.*	Multiplex PCR (TIB MOLBIOL LightMix® Modular Gastro Bacteria and R-Biopharm RIDA®GENE C. difficile Toxin A/B)
	Parasites PCR panel	*Cryptosporidium spp., Entamoeba histolytica, Giardia spp., Dientamoeba fragilis* and *Blastocystis hominis.*	Multiplex PCR (TIB MOLBIOL LightMix® Modular Gastro Parasites)
	Adenovirus 40/41	-	ICG rapid test
	Norovirus	-	ICG rapid test
	Rotavirus	-	ICG rapid test
	C. difficile	-	ICG rapid test
Broad			
	Bacterial PCR panel	*Campylobacter* spp., *Salmonella spp., Shigella spp.,Plesiomonas spp., Yersinia spp.* *C. difficile*	Multiplex PCR (TIB MOLBIOL LightMix® Modular Gastro Bacteria and R-Biopharm RIDA®GENE Clostridium difficile Toxin A/B)
	Parasites PCR panel	*Cryptosporidium spp., Entamoeba histolytica, Giardia spp., Dientamoeba fragilis, Blastocystis hominis.*	Multiplex PCR (TIB MOLBIOL LightMix® Modular Gastro Parasites)
	Viruses PCR panel	Adenovirus 40/41, Astrovirus, Norovirus, Rotavirus, Sapovirus.	Multiplex PCR panel (Laboratory Developed PCR Test

DFT=Diagnostic feces testing, ICG=immunochromatographic, PCR = Polymerase chain reaction.

Supplement 17. Test results for additional tested samples.

Infectious agent	Additional tested samples	
	Tests	Positive (%)
Groups		
Bacteria	869	34 (3.9)
Pathogenic parasites	279	5 (1.8)
Dientamoeba/ Blastocystis	279	77 (27.6)
Viruses	1,879	203 (10.5)
Bacteria		
Salmonella	869	0 (0)
Shigella/EIEC	869	4 (0.5)
Yersinia	869	3 (0.3)
Campylobacter	869	20 (2.3)
Plesiomonas	869	0 (0)
C. difficile	1,811	20 (1.1)
Parasites		
Giardia	279	5 (1.8)
Entamoeba	279	0 (0)
Cryptosporidium	279	0 (0)
Blastocystis	279	52 (18.6)
Dientamoeba	279	43 (15.4)
Viruses		
Adenovirus 40/41	1,981	22 (1.1)
Astrovirus	2,029	31 (1.5)
Norovirus	1,926	82 (4.3)
Sapovirus	2,029	82 (4)
Rotavirus	1,982	20 (1)

Abbreviations: EIEC=Enteroinvasive Escherichia coli; DFT=diagnostic feces testing.

SUMMARY

Gastroenteritis (GE) is one of the most frequently occurring infectious disease and has a relatively high consultation rate in general practice (**Chapter 1**). In the Netherlands, 5 to 12% of patients with GE consult their general practitioner (GP), amounting to 240 to 600 thousand consultations annually. Although the disease course of GE is in generally favorable and self-limiting, in some patients diagnostics feces testing (DFT) may be indicated to guide further clinical management. DFT and subsequent guided antibiotic treatment may especially be necessary in patients working in healthcare or the food-industry, and in high-risk patients, such as immune-compromised patients, frail elderly, and young children. Traditionally, general practitioners used microbiological culture and microscopy to identify bacteria and parasites causing gastroenteritis. In recent years, molecular-based techniques, such as polymerase chain reaction (PCR), have become available as an alternative to traditional diagnostic modalities. PCR allows for highly sensitive identification of DNA or RNA of multiple enteropathogens in a single stool sample, with shorter turnaround times, improved sampling convenience and diagnostic yield as compared to conventional techniques. While conventional DFT requires up to 4 days before results become available, PCR-based DFT generally takes less than 24 hours. Although these relevant differences between PCR and conventional DFT are likely to influence patient management, healthcare use, and associated costs, an evaluation of the introduction of PCR-based DFT in general practice has not yet been performed. We set out to evaluate the effect of the introduction of PCR-based DFT testing on the management of GE in general practice, as well as on GE-related costs.

These topics were investigated within the PROUD study (PCR diagnostics in Outpatients with Diarrhea). The study design (**Chapter 2**) involved a before-after cohort study, which included patients that consulted a GP within the Julius General Practitioner Network (JGPN) with complaints of GE. The study covered the period before PCR introduction (2010–2011), in which patients were tested using conventional DFT, and the period after PCR introduction (2013–2014). Routine care data including patient characteristics, consultation texts, healthcare consumption, test use and results were extracted from the electronic patient and laboratory records. We additionally tested all patients for whom DFT was requested in 2014 on a broad panel of 16 enteropathogens, in order to evaluate the clinical benefit and diagnostic yield of broad-panel DFT as compared to current practice were GPs perform targeted testing of specific microorganisms.

In **Chapter 3**, we studied the effect of the altered diagnostic approach on healthcare consumption in general practice and associated costs, by performing a cost analyses. All GE episodes within the JGPN from 2010-2011 ("pre-PCR period"; conventional DFT) and 2013-2014 ("post-PCR period"; PCR testing) were extracted. For each GE episode, we extracted data on DFT use, antibiotic prescriptions, consultations, and specialist referrals. Healthcare resource consumption and direct healthcare costs were calculated for each GE episode. We estimated the effect of the DFT method on healthcare resource consumption and calculated the incremental direct healthcare costs – attributable to use of PCR-based DFT – while adjusting for secular time trends, seasonality and differences in case-mix in both study periods. A total of 10,947 GE episodes were identified in the pre-PCR period and 13,276 in the post-PCR period. In the post-PCR period, patients were more frequently tested for bacteria (RR=1.40; [95%CI=1.30-1.52]) and parasites (1.66; [1.55-1.79]), and received more GE-related referrals for DFT (1.29; [1.22-1.38]), telephone consultations (1.23; [1.18-1.28]), prescription of antiprotozoal drugs (2.49; [2.14-2.90]), referrals to an internal medical specialist (1.97; [1.67-2.32]), but less GE-related home visits (0.71; [0.64-0.79]). Average direct healthcare costs per GE episode were €76.2 before and €93.4 (95%CI=€92.8-93.9) after PCR introduction, while adjusting for cohort differences. The estimated adjusted incremental direct healthcare costs – attributable to the change in DFT method – was €17.1 (95%CI=€16.6-17.6) per GE episode. This translated into a 22.4% (21.8-23.1%) increase in direct healthcare costs, of which 79% resulted from increased healthcare resources consumption and 21% from the increased costs of DFT. Extrapolated to Dutch population, the incremental direct healthcare costs of PCR-based DFT in general practice amounts to an estimated €7.4 million (95%CI=€7.2-€7.6 million) per year.

In **Chapter 4**, we evaluated the effects of replacing triple feces test (TFT) with more sensitive protozoal PCR on detection and antibiotic treatment of *D. fragilis* and *Blastocystis spp.* among GE patients in general practice. The motivation for this was that both protozoa have unproven clinical significance. For GE episodes in which any of these tests were requested, we collected protozoal stool testing results and prescriptions for metronidazole and clioquinol. The absolute difference (AD) in the detection of both protozoa and the prescription of targeted antibiotic treatment were calculated between 2010-11 (TFT) and 2013-14 (PCR testing). We included 10,947 GE episodes during 2010-11 and 13,271 episodes during 2013-14. The number of protozoal stool tests performed increased from 9.5% (n=1042) with TFT to 15.6% (n=2073) with PCR testing (AD=6.1%, 95%CI=5.3-6.9). D. fragilis or Blastocystis spp. was detected in 2.4% and 35.6% of the TFT and PCR stool tests, respectively (AD=33.2%, 31-35). Targeted antibiotic treatment with metronidazole for D. fragilis or Blastocystis spp. increased from 1.1% (n=11) to 14.5% (n=300) after PCR introduction (AD=13.4%, 95%CI=12-15) and with clioquinol from 0% to 1.3% (n=27,

95%CI=1-2%). From this, we can conclude that replacing conventional TFT stool testing by a protozoal PCR panel in general practice is associated with a significant increase in protozoal stool testing, and in the detection and antibiotic treatment of Blastocystis spp. and D. fragilis. These results stress the need for clarification of the clinical significance of these protozoa.

To get a better picture of current antibiotic prescribing practice for GE in general practice, we gathered data on DFT results, including antimicrobial resistance testing, and data on antibiotic prescribing for all patients with GE that consulted a GP form the JGPN (**Chapter 5**). We included 13,217 GE episodes in total. Antibiotic treatment was prescribed in 1163 (8.8%) episodes, most frequently with metronidazole (n = 646, 4.9%), azithromycin (n = 254, 1.9%) or ciprofloxacin (n = 184, 1.4%). Treatment was empirical for 641 (5%) GE episodes, of which 30% (n = 191) was in line with the antibiotic recommendation in the clinical practice guideline. Targeted treatment following DFT results was prescribed for 537 GE episodes (4%), of which 99% (n = 529) followed CPG recommendations. Resistance to first- or second-choice antibiotics was demonstrated in three Salmonella isolates (9%-13% of all isolates) and one Campylobacter isolate (1%). We concluded that antibiotic treatment of GE in general practice is relatively infrequent, with only 1 in 11 patients treated, and that empirical treatment is more frequent compared with targeted treatment; mostly using non-CPG-recommended antibiotics.

In **Chapter 6**, we quantified the adherence of GPs to the Dutch general practice CPG on acute diarrhea when requesting DFT and the effect of PCR introduction on adherence. Two random samples of each 500 patients with GE where DFT were drawn, one sample included the period before PCR introduction (2010–11) and one the period after PCR introduction (2013). For each patient, GE-related presenting symptoms were extracted from the patient's medical file. These data and relevant patient characteristics were used to assess adherence to CPG indications for DFT. The association between PCR introduction and adherence was estimated using multivariable regression analysis. In 88% of all episodes, GE-related presenting symptoms were reported, most often 'frequent watery stool' (58%) and 'illness duration >10 days' (40%). Overall, 17% of the DFT request were considered compliant with the CPG, 16% before PCR introduction and 18% after (adjusted odds ratio=1.2, 95% CI 0.9–1.7). From this, we conclude that overall adherence to CPG indications – when requesting DFT in general practice patients with GE – is low, regardless of the diagnostic modality used for DFT.

Finally, in **Chapter 7** we evaluated the potential clinical benefit of broad-panel DFT (including a DFT panel of 16 infectious agents) in comparison with current practice targeted DFT. This included first a comparison of the detection rates for targeted DFT and broad-panel DFT.

Subsequently, the number of unnecessary empirical antibiotic prescriptions and number of untreated clinically relevant infections were quantified. We included 2029 samples. For 12 of the included enteropathogens additional positives samples were identified using broad-panel DFT. Using targeted DFT, 20% (n=34) of bacteria, 6% (n=5) of parasites, 10% (n=77) of Dientamoeba fragilis or Blastocystis spp. isolates, and 98% (n=203) of viruses remained undetected, when compared to broad-panel DFT. For 171 (8.4%) patients the use broad-panel DFT could have altered the antibiotic treatment strategy, of these 82 (5.2% of all non-treated patients) were potentially in need of treatment and 89 (20% of all treated patients) were prescribed potentially unnecessary antibiotics. We concluded that targeted DFT among general practice patients with GE is associated with underdetection of most common gastrointestinal infectious agents. Although this underdetection primarily concerns microorganisms without a direct clinical consequence, broad-panel DFT can still change the clinical decision regarding antibiotic treatment in 1 in every 12 patient tested using current practice targeted DFT.

In summary, the current management of patients with GE in general practice – now comprising PCR-based DFT – poorly aligns with guideline indications for empirical antibiotic treatment and DFT. Furthermore, the introduction of PCR is associated with increased use of DFT, healthcare costs per GE episode, and detection and treatment of non-pathogenic protozoa (**Chapter 8**). Additionally, using broad-panel DFT instead of current practice targeted DFT can improve the clinical decision regarding antibiotic treatment in 1 in every 12 patient. These findings need to be interpreted with care, as we were not able to quantify the potential clinical benefits of PCR testing over conventional DFT. However, we conclude that improving adherence to clinical practice guidelines is pivotal to improve current clinical management of GE in general practice. This includes updating of existing guidelines using all available (and new) evidence, increasing awareness among GPs and patients about the proper use of diagnostic and therapeutic resources and stimulating the use of reliable information to patients. When updating the current guideline on gastroenteritis, the position of (broad-panel) PCR-based DFT needs to be more explicitly discussed, describing the different types of indications within the diagnostic workup (e.g. public health or patient's health perspectives for DFT) and the potential drawbacks and benefits of PCR testing for patients.

NEDERLANDSE SAMENVATTING (DUTCH SUMMARY)

Gastroenteritis (GE) is een van de meest frequent voorkomende infectieuze aandoeningen, met een relatief hoge consultatiegraad in de huisartspraktijk (**Hoofdstuk 1**). In Nederland consulteert 5 tot 10% van de patiënten met GE zijn huisarts, resulterend in 240 tot 600 duizend consultaties per jaar. Ondanks dat het ziektebeloop van GE in het algemeen mild en kortdurend is, kan diagnostisch onderzoek van de ontlasting (diagnostic feces testing, DFT) geïndiceerd zijn en worden gebruikt om het verdere klinisch beleid te bepalen. DFT en de daaropvolgende gerichte behandeling met antibiotica is met name nodig bij patiënten die werkzaam zijn in de zorg of voedselindustrie, en bij hoog risico patiënten, zoals immuungecompromitteerden, kwetsbare ouderen en jonge kinderen. Oorspronkelijk maakten huisartsen gebruik van microbiologische kweek en microscopie om bacteriën en parasieten te identificeren. Echter, zijn in de laatste jaren ook moleculaire diagnostische technieken, zoals polymerasekettingreactie (PCR), beschikbaar gekomen als alternatief voor conventionele diagnostische technieken. PCR maakt het mogelijk om met hogere sensitiviteit het DNA c.q. RNA van meerdere enteropathogenen in een enkel feces monster te detecteren, met een kortere doorlooptijd, vereenvoudigde bemonstering en hogere diagnostische opbrengst in vergelijking met conventioneel fecesonderzoek. Waar de resultaten van conventionele DFT pas na 4 dagen beschikbaar komen, is dit bij PCR in het algemeen binnen 24 uur. Gezien deze relevante verschillen tussen PCR en conventionele DFT is het waarschijnlijk dat de overstap naar PCR diagnostiek in de huisartspraktijk ook invloed kan hebben op het klinisch beleid, het zorggebruik en de daaraan gerelateerde kosten. Echter, is hiernaar tot op heden is geen evaluatie gedaan. Dit was aanleiding om de effecten van de introductie van PCR fecesdiagnostiek op het klinisch beleid van de huisarts en de zorgkosten van GE te evalueren en het huidige beleid bij patiënten met GE in de huisartspraktijk in beeld te brengen.

Deze onderwerpen zijn onderzocht binnen de PROUD studie (PCR diagnostics in Outpatients with Diarrhea). Het studieontwerp van de PROUD studie (**Hoofdstuk 2**) omvatte een voor-na cohortstudie, waarin patiënten werden geïncludeerd die binnen het Julius Huisartsen Netwerk (JHN) een huisarts consulteerde voor klachten van GE. Deze studie bestreek de jaren voor PCR introductie (2010-2011), waarin patiënten door middel van conventionele DFT werden getest, en de periode na PCR introductie (2013-2014). Routinematig verzamelde huisartsgegevens zoals patiënt karakteristieken, consultteksten (SOEP-regels), zorg- en testgebruik, en testresultaten werden geëxtraheerd van de elektronische patiënten- en laboratoriumdossiers In 2014 werden daarnaast alle patiënten voor wie de huisarts fecesonderzoek inzette, getest op een volledig panel van 16 enteropathogenen (breed-panel DFT). Dit werd gedaan, om het klinisch nut en

de aanvullende diagnostische opbrengst hiervan te bepalen, versus een strategie van selectief testen op gerichte pathogenen, zoals thans gebruikelijk in de huisartspraktijk.

In **Hoofdstuk 3** werd het effect van de verandering in diagnostische techniek op het zorggebruik in de huisartspraktijk en de bijbehorende zorgkosten onderzocht aan de hand van een kostenanalyse. Hiervoor werden alle episodes van GE die waren geregistreerd binnen de JHN database tussen 2010-2011("pre-PCR periode"; conventionele fecesonderzoek) en 2013-2014 ("post-PCR periode"; PCR fecesonderzoek) geëxtraheerd. Voor elke GE episode werden gegevens verzameld over DFT gebruik, antibiotica voorschriften, consultaties en verwijzingen naar een specialist. Het zorggebruik en de direct zorgkosten werden berekend voor elke GE episode. Het effect van de gebruikte methode van DFT op het zorggebruik werd vervolgens geschat en de incrementele directe zorgkosten – die toe te schrijven zijn aan het gebruik van PCR fecesonderzoek – berekend, waarbij werd gecorrigeerd voor seculiere tijdstrends, voor seizoen en voor verschillen in het cohortsamenstelling van beide periodes.

In totaal werden er 10.947 GE episodes geïdentificeerd in de pre-PCR periode en 13.276 in de post-PCR periode. In de post-PCR periode werd er vaker diagnostiek gedaan naar bacteriën (RR=1.40; [95% betrouwbaarheidsinterval (BI)=1.30-1.52]) and parasieten (1.66; [1.55-1.79]), en ontvingen patiënten per GE episode meer verwijzingen voor DFT (1.29; [1.22-1.38]), telefoon consultaties (1.23; [1.18-1.28]), medicatievoorschriften voor antiprotozoale middelen (2.49; [2.14-2.90]), verwijzingen naar de internist (1.97; [1.67-2.32]), maar minder GE-gerelateerde visites (0.71; [0.64-0.79]). De gemiddelde directe zorgkosten per GE episode bedroegen €76.2 vóór en (na correctie van eerder genoemde factoren) €93.4 (95%CI=€92,8-93,9) ná PCR introductie. De geschatte gecorrigeerde incrementele directe zorgkosten – die toe te schrijven zijn aan het gebruik van PCR fecesonderzoek – bedroegen €17,1 (95%CI=€16,6-17,6) per GE episode. Dit staat gelijk aan een toename in de directe zorgkosten van 22,4% (21,8-23,1%), waarvan 79% toe te schrijven is aan de toename in zorggebruik en 21% aan de toename in diagnostische kosten. Geëxtrapoleerd naar de gehele Nederlandse bevolking, leidt het gebruik van PCR fecesonderzoek in de huisartspraktijk tot een toename van naar schatting €7,4 miljoen (95%BI=€7,2-€7,6 miljoen) in de jaarlijkse zorgkosten.

In **Hoofdstuk 4** hebben we het effect bekeken van het vervangen van de triple feces test (TFT) met sensitievere parasitaire PCR op de detectie en antibiotische behandeling van *D. fragilis* and *Blastocystis spp.* bij patiënten in de huisartspraktijk met GE. De aanleiding hiervoor was dat voor beide protozoa onduidelijk is wat hun klinische relevantie is. Voor episodes waarin een aanvraag voor één van deze testen werd gedaan, verzamelden wij de resultaten van het

fecesonderzoek op protozoa en voorschriften voor metronidazol and clioquinol, antibiotica voor de gerichte behandeling van protozoa. Voorts, berekenden we het absolute verschil (AV) tussen de periodes 2010-11 (TFT) en 2013-14 (PCR) in aantallen gedetecteerde *D. fragilis* and *Blastocystis spp.* en gerelateerde behandelingen met antibiotica. Er waren 10.947 GE episodes in de periode 2010-11 (TFT) en 13.276 in de periode 2013-14. Het aandeel testen op protozoa steeg van 9,5% (n=1042) met TFT tot 15,6% (n=2073) met PCR (AV=6,1%, 95%BI=5,3-6,9). *D. fragilis* of *Blastocystis spp.* werden gedetecteerd in 2,4% en 35,6% van de respectievelijk TFT en PCR feces testen (AV=33,2%, 31-35). Behandeling van *D. fragilis* of *Blastocystis spp.* met metronidazol steeg van 1,1% (n=11) tot 14,5% (n=300) na PCR introductie (AV=13,4%, 95%BI=12-15) en met clioquinol van 0% tot 1,3% (n=27, 95%BI=1-2%). Hieruit kunnen we concluderen dat het vervangen van de TFT met een parasitair PCR panel in de huisartspraktijk geassocieerd is met een aanzienlijke toename in de detectie en antibiotische behandeling van *D. fragilis* and *Blastocystis spp.* Deze resultaten onderstrepen het belang van nadere opheldering over de klinische relevantie van deze protozoa.

Ten einde beter zicht te krijgen op het huidige antibiotisch beleid bij patiënten met GE in de huisartspraktijk, verzamelden wij voor alle patiënten met GE die tussen 2013 en 2014 een JHN huisarts bezochten de resultaten van het fecesonderzoek, inclusief de resultaten van het antimicrobieel resistentie-onderzoek, en gegevens van de antibiotica voorschriften (**Hoofdstuk 5**). Hierbij werden 13.217 GE episodes geïncludeerd. In 1163 (8.8%) episodes werd een antibioticum voorgeschreven, waarbij metronidazol (n = 646, 4.9%), azitromycine (n = 254, 1.9%) en ciprofloxacine (n = 184, 1.4%) het meest frequent werden voorgeschreven. Empirische antibiotische therapie werd in 641 (5%) van de GE episodes voorgeschreven, waarvan in 30% (n = 191) met een door de richtlijn aanbevolen antibioticum. DFT met daaropvolgende gerichte antibiotische therapie werd in 537 (4%) van de GE episodes voorgeschreven, waarvan 99% (n = 529) met een door de richtlijn aanbevolen antibioticum. Resistentie voor een eerstelijns- of tweedelijnsantibioticum werd gezien bij drie *Salmonella* isolaten (9%-13% van alle isolaten) en bij één *Campylobacter* isolaat (1%). Hieruit kunnen we concluderen dat in de huisartspraktijk slechts 1 op de 11 patiënten met GE wordt behandeld met antibiotica, dat deze behandeling vaker empirisch is dan op geleide van de resultaten van DFT, en dat daarbij meestal wordt gekozen voor een niet aanbevolen antibioticum.

In **Hoofdstuk 6** hebben we de naleving van de NHG-Standaard acute diarree door huisartsen onderzocht bij het aanvragen van DFT en vastgesteld of er een effect van de introductie van PCR was op de mate van naleving. In twee willekeurig genomen steekproeven met elk 500 patiënten met GE waarin de huisarts een aanvraag voor DFT deed, werden gegevens

over GE-gerelateerde symptomen verzameld vanuit de in het medisch dossier geregisterde consulttekst (SOEP-regels). Eén steekproef bevatte gegevens uit de periode 2010-2011("pre-PCR periode"; conventionele fecesonderzoek) en de andere uit 2013 ("post-PCR periode"; PCR fecesonderzoek). Richtlijn naleving van de indicaties voor DFT werd vervolgens beoordeeld aan de hand van deze gegevens en relevante patiëntkarakteristieken. De associatie tussen PCR introductie en richtlijn naleving werd geschat met behulp van multipele regressieanalyse. In 88% van de episodes werden er GE-gerelateerd symptomen gerapporteerd, het meest voorkomend waren 'frequente waterige ontlasting' (58%) en 'ziekteduur >10 dagen' (40%). Gemiddeld werd 17% van de DFT aanvragen beschouwd als conform de richtlijn, 16% vóór PCR introductie en 18% na PCR introductie (gecorrigeerde odds ratio=1.2, 95%BI 0.9–1.7). Hieruit concluderen we dat naleving van de professionele richtlijn ten aanzien van de indicaties voor DFT in de huisartspraktijk laag is, ongeacht de gebruikte techniek voor DFT.

Tenslotte, hebben we in **Hoofdstuk 7** het potentiele klinisch nut van breed-panel DFT (welke een DFT panel van 16 enteropathogenen bevat) ten opzichte van het huidige beleid met gerichte DFT bekeken. Als eerste hebben we de detectiegraad vergeleken tussen gerichte DFT en breed-panel DFT. Vervolgens is het aantal onnodige empirische antibiotische behandelingen en het aantal onbehandelde klinische relevante infecties gekwantificeerd. Onder de in totaal 2029 geteste monsters, werden er voor 12 verschillende enteropathogenen additioneel positieve monsters gevonden met breed-panel DFT. Bij gerichte DFT werd 20% (n=34) van de aanwezige bacteriën, 6% (n=5) van de parasieten, 10% (n=77) van de *Dientamoeba fragilis* of *Blastocystis spp.* isolaten, en 98% (n=203) van de virussen gemist. Voor 171 (8.4%) van de patiënten zou het inzetten van breed-panel DFT mogelijk consequenties voor het antibiotisch beleid hebben gehad. In totaal hadden 82 onbehandelde patiënten (5.2% van alle niet-behandelde patiënten) mogelijk baat bij antibiotische behandeling en 89 patiënten (20% van alle behandelde patiënten) kregen mogelijk ten onrechte antibiotica. Hieruit maken we op dat gebruik van gerichte DFT bij patiënten met GE in de huisartspraktijk thans leidt tot onderdetectie van veelvoorkomende gastrointestinale micro-organismen. Hoewel het in veel gevallen micro-organismen betreft die geen directe klinische consequentie hebben, zou bij 1 op de 12 patiënten het gebruik van breed-panel DFT toch aanleiding kunnen geven voor een ander antibiotisch beleid dan welke naar aanleiding van de gerichte DFT werd ingezet.

Samenvattend blijkt bij patiënten met GE het huidige beleid van huisartsen – waarvan PCR fecesonderzoek nu onderdeel is – slecht overeenstemt met de richtlijnindicaties voor empirische antibiotische behandeling en het aanvragen van DFT. Ook blijkt het in gebruik nemen van PCR geassocieerd met een toename in het gebruik van DFT, hogere zorgkosten

per episode en tot een toegenomen detectie en behandeling van non-pathogene protozoa in vergelijking met de voorliggende periode waarin conventionele DFT werd gebruikt (**Hoofdstuk 8**). Daarbij heeft het gebruik van breed-panel DFT ten opzichte van gerichte DFT een potentieel gunstige invloed op het antibiotische beleid bij 1 op de 12 patiënten. Omdat het niet mogelijk is gebleken de baten van PCR voor de patiënt te kwantificeren, moeten deze bevindingen wel met enige voorzichtigheid worden geïnterpreteerd. Desondanks is duidelijk geworden dat het stimuleren van het naleven van de richtlijn centraal moet staan bij het verbeteren van het beleid van de huisarts. Een dergelijk verbeterproces omvat onder andere het herzien van de NHG-Standaard acute diarree aan de hand van al het beschikbare (en nieuwe) wetenschappelijk bewijs, het verhogen van het bewustzijn onder huisartsen met betrekking tot gepast gebruik van diagnostische en therapeutische middelen en het stimuleren van het gebruik van betrouwbare patiënten informatie. Bij het herzien van de richtlijn zal de rol en de positionering van (breed-panel) PCR fecesonderzoek explicieter moeten worden besproken, waarbij de verschillende indicaties (i.e. een patiënten- of volksgezondheidsperspectief voor DFT) binnen het diagnostisch proces moeten worden verankerd, maar ook uitleg gegeven moet worden over potentiele nadelen en voordelen van de inzet van PCR voor de patiënt.

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Alle SUMMA's: wat een huzarenlichting zeg!

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ABOUT THE AUTHOR

Alwin Schierenberg was born on January 9th, 1986 in Amsterdam, The Netherlands. After graduating from secondary school in 2004 (St. Ignatiusgymnasium, Amsterdam), he studied Liberal Arts and Sciences at University College Utrecht. After obtaining his bachelor's degree in 2007, including a major in medical science and a minor in political science (University of Helsinki, Finland), he enrolled for a master's degree in medicine at Utrecht University (SUMMA). As part of this program, he joined the research group of Theo Verheij (Julius Center, UMC Utrecht), studying value of prediction models for pneumonia in general practice. This internship sparked his interest in epidemiology and laid the foundation for this thesis.

After obtaining his medical doctor's degree in 2013, Alwin decided to continue at the Julius Center for Health Sciences and Primary Care (University Medical Center Utrecht) as a PhD student, now with the supervision of prof. Niek de Wit and prof. Marc Bonten. During the course of his PhD, he followed the postgraduate master's program in epidemiology at the Utrecht University, specializing in clinical epidemiology. In between he also worked as a resident at the department for emergency psychiatry (Arkin, Amsterdam) for a year. In the summer of 2017, Alwin started working as a medical advisor for The National Health Care Institute in Diemen. To date, he advises on reimbursement issues, including advising on which types of health care are included in the basic care package and which are not. Meanwhile, he finished the remainder of his PhD project, which culminated in this doctoral thesis and his PhD defense on October 29th, 2019. Alwin lives together with his wife Michelle and son Ramses in Amsterdam, in joyful anticipation of a daughter.

LIST OF PUBLICATIONS

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Minnaard MC, de Groot JAH, Hopstaken RM, **Schierenberg A**, de Wit NJ, Reitsma JB, Broekhuizen BDL, van Vugt SF, Neven AK, Graffelman AW, Melbye H, Rainer TH, Steurer J, Holm A, Gonzales R, Dinant GJ, van de Pol AC, Verheij TJM. The added value of C-reactive protein measurement in diagnosing pneumonia in primary care: a meta-analysis of individual patient data. CMAJ. 2017 Jan 16;189(2):E56-E63.

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Schierenberg A, van Amsterdam J, van den Brink W, Goudriaan AE. Efficacy of contingency management for cocaine dependence treatment: a review of the evidence. Curr Drug Abuse Rev. 2012 Dec;5(4):320-31.

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Schierenberg A, Bruijning-Verhagen PCJ, Kusters JG, Schuurman R, van Delft S, Bonten MJM, de Wit NJ. Clinical benefit of broad-panel diagnostic stool testing of patients with gastroenteritis in primary care. Submitted.

Schierenberg A, Bruijning-Verhagen PCJ, van Delft S, Kusters JG, Bonten MJM, de Wit NJ. Substantially increased detection and treatment of Blastocystis and Dientamoeba in patients with gastroenteritis after the introduction of molecular stool testing. Submitted.

Book chapters

De Jongh TOH, **Schierenberg A.** (2018). Diarree. De Jongh TOH, de Vries H (Ed.), *Therapie van alledaagse klachten: Handreiking voor rationeel symptomatisch behandelen.* Houten: Bohn Stafleu van Loghum.

De Wit NJ, **Schierenberg A**, Witteman BJM. (2016). Diarrhee. De Jongh TOH, de Vries H, Grundmeijer HGLM, Knottnerus BJ (Ed.), *Diagnostiek van alledaagse klachten: Bouwstenen voor rationeel probleemoplossen.* Houten: Bohn Stafleu van Loghum.