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VAlidation of an 8-item-questionnaire predictive for a positive CaLprotectin tEst and Real-life implemenTation in primary care to reduce diagnostic delay in inflammatory bowel disease (ALERT): a prospective diagnostic observational study

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ABSTRACT

Introduction

Diagnosis of inflammatory bowel disease (IBD) in primary health care is challenging and often associated with a considerable diagnostic delay. This delay is associated with worse disease progression and outcomes. Although testing for fecal Calprotectin is a useful screening tool to identify patients who need endoscopy for IBD, the widespread use may not be appropriate due to the low prevalence of patients with IBD among all patients attending a general practitioner (GP) with stomach ache. To increase the appropriate application of the fecal Calprotectin test, an 8-item-questionnaire, the CalproQuest, has been developed to increase pretest-probability for a positive test result.

Methods and analysis

This is a prospective diagnostic observational trial. The study consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patients included in part A are referred to the gastroenterologist for any endoscopic evaluation. Patients included in part B present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

CalproQuest consists of 4 main and 4 secondary questions specific for IBD it is considered positive, if ≥ 2 main criteria are answered positively or 1 main criterion and 2 secondary criteria are answered positively.

In part A the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis will be investigated. In part B the feasibility of CalproQuest in daily primary health care practice will be assessed.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Kanton Zurich (reference KEK-ZH-number 2013-0516). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration number

The study protocol is registered at ISRCTN66310845

INTRODUCTION

IBD versus IBS

Crohn's Disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC) represent the three subtypes of inflammatory bowel disease (IBD) [1]. Estimated prevalence rates of IBD in Swiss population are about 205.6 cases per 100'000 [2].

Meanwhile, the prevalence of irritable bowel syndrome (IBS) in Europe and North America is estimated to be 10 – 15% [3].

Physicians are often faced with the diagnostic challenge of differentiating patients with IBD from functional disorders such as irritable bowel syndrome (IBS). Indeed, symptoms similar to IBS are frequently reported in patients before IBD is diagnosed [4]. The gold diagnostic standard is endoscopy; however, not every patient with the symptoms overlapping with those of IBS can be investigated by the invasive endoscopic exam. Therefore, many different, non-invasive markers have been investigated. Several studies have shown that fecal Calprotectin accurately reflects intestinal inflammation in patients with known IBD [5].

It has also been shown to consistently differentiate IBD from IBS due to its excellent negative predictive value. It can therefore be used ruling out IBD in undiagnosed, symptomatic patients [6].

Calprotectin, a S100 protein

Calprotectin is a complex of two calcium-binding proteins that belong to the S100 protein family [7]. It is abundant in the cytosolic fraction of neutrophils. High levels of Calprotectin have been found in extracellular fluid during various inflammatory conditions, such as rheumatoid arthritis, cystic fibrosis and abscesses. Calprotectin released from neutrophils has growth-inhibitory and apoptosis-inducing activities against various cell types including tumor cells and normal fibroblasts [7]. This suggests that Calprotectin has regulatory activities during inflammatory processes through its effect on the survival or growth states of cells participating in the inflammatory reaction. Furthermore, Calprotectin inhibits microbial growth through competition for zinc [8].

Calprotectin has been shown to be stable in feces during storage for 7 days at room temperature, which is very important for its value in evaluating mucosal wall inflammation [9].

Stool Calprotectin levels as marker of intestinal inflammation

Data correlated to fecal Calprotectin showed a sensitivity and specificity of Calprotectin (using a cut-off value of 10mg/l) for organic disease of 89% and 79%, respectively [10]. Studies using cut-off value of 50 µg/g showed a sensitivity and specificity of 86.8% and 95.7% [11] and 83% and 100% [12] discriminating IBD versus IBS. Fecal Calprotectin levels correlate significantly with histological and endoscopic assessment of disease activity in

ulcerative colitis (UC) [13-15] as well as with fecal alpha-1-antitrypsin levels and fecal excretion of 111indium-labeled white blood cells in patients with Crohn's disease (CD) [16 17]. Schoepfer and colleagues showed that stool Calprotectin levels correlate well with endoscopic indices both in ulcerative colitis and in Crohn's disease [18 19].

Diagnostic delay in IBD is predictive for worse disease progression and outcomes

Difficulties in differentiation early IBD from IBS, especially in a primary health care, is leading to a considerable diagnostic delay in IBD [1]. This delay has important clinical impact, as there is increasing evidence demonstrating that treatment success is increased in early disease [20-23]. Vavricka and colleagues suggest, that diagnostic delay is sub-dived into two intervals, where interval 1 is defined as time from first symptoms to physician visit, and interval 2 the time from first physician visit to IBD diagnosis [1]. The study by Vavricka et al. estimates that 25% of all CD and UC patients experienced more than 24 and 12 months, respectively, from first onset of symptoms until an accurate IBD diagnosis [1]. Most importantly Schoepfer and colleagues recently showed, that the length of diagnostic delay is correlated with an increased risk of bowel stenosis and CD-related intestinal surgery, concluding that efforts should be undertaken to shorten diagnostic delay [24].

Testing Calprotectin in Switzerland

Most analytical laboratories in Switzerland offer Calprotectin testing, which is reimbursed by health insurances.

Fecal Calprotectin testing in primary health care versus tertiary healthcare

Although Calprotectin tests are easily accessible and reimbursed in Switzerland, this diagnostic test is not routinely performed in primary health care. However, the low prevalence of IBD in the primary health care must be taken into account: Analysing the reasons for encounter in primary health care, the group of digestive disorders is not one of the predominant diagnostic groups. Scandinavian studies show frequencies between 5-7% [25 26]. Focusing on this diagnostic group, IBS is much more common in primary health care (population-based prevalence of 10-15% of IBS compared with 0.2% of IBD). Therefore, a new tool has been developed to narrow the patient collective in which Calprotectin testing may lead to the correct diagnosis of IBD in an earlier stadium.

Hypothesis and goal

This study pursuits two main aims A and B, which are investigated independently:

A. Prospective validation and evaluation of sensitivity and specificity of an 8-item IBD-questionnaire (CalproQuest; see Table 1) for 1) a positive Calprotectin test result ≥ 50 µg/g feces and for 2) a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis, respectively, in tertiary healthcare

B. Prospective implementation of CalproQuest in primary health care to investigate feasibility in daily practice.

METHODS AND ANALYSIS

Study design

The study is a prospective diagnostic observational trial. It consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and general practitioners (GPs) (B), respectively.

Patients included in part A of the study are referred for endoscopic evaluation to gastroenterologists, specialised for IBD. Patients included in part B of the study present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

The study design and procedure are summarized in Figure 1.

Inclusion and exclusion criteria

Patients will be eligible if they

- Are ≥18 years old (part A, B)
- Are referred to their gastroenterologist for any endoscopic examination (part A)
- Visit their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks (part B)
- Underwent no earlier diagnostic procedures (endoscopy) for the current episode (part B)

Patients are not eligible, if they

- Are younger than 18 years (part A, B)
- Have known further/other abdominal pathologies as e.g. cancer (part A, B)
- Had previous abdominal surgeries (part B)
- Have been treated with steroids (topical and/or oral) and/or aminosalicylates within 30 days prior inclusion into this study (part B)
- Underwent endoscopic examination within 3 years prior screening (part B)

Primary and secondary outcomes

Primary outcomes:

A. 1. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces

2. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis.

B. Feasibility of CalproQuest in daily primary health care practice.

Secondary outcomes:

A. Patient-reported diagnostic delay.

B. Patient-reported acceptance of stool sampling.

Procedure of the study

In brief, the study will be divided in two independent parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patient data will be encoded.

A. Investigation of the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis

Patients referred to the gastroenterologist for endoscopic examination are subjected to CalproQuest and Calprotectin stool testing prior endoscopy. At baseline T0, patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens from outpatients will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. At T2, endoscopic examination will be performed to obtain a diagnosis. Eventually, patients diagnosed with IBD will be asked to complete a questionnaire at T3 investigating duration of first onset of symptoms to IBD diagnosis (diagnostic delay).

B. Investigation of feasibility of CalproQuest in daily primary health care practice

Patients with on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for more than two weeks presenting at the GP will be included into the study if all inclusion criteria are met and informed patient consent is obtained.

At baseline (T0), patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. According to the current standard of care it is recommended to refer patients with Calprotectin levels ≥ 50 µg/g to a gastroenterologist for endoscopic examination at T2; results of the endoscopy are communicated back to the GP. Patients will be asked at T3 to complete a questionnaire on acceptance of stool sampling, and physicians will complete the questionnaire on feasibility of CalproQuest in daily practice.

CalproQuest

CalproQuest is an 8-item IBD-questionnaire consisting of 4 main and 4 secondary questions specific for IBD (see Table 1). CalproQuest was pre-validated by IBD-experts trough an international Delphi-process. CalproQuest is considered positive, if ≥ 2 main criteria or 1 main criterion and 2 secondary criteria are answered positively.

We assume that a positive CalproQuest result may predict Calprotectin levels ≥ 50 µg/g. Calprotectin levels above 50 µg/g are indicative for on-going intestinal inflammation and call for further endoscopic examination.

Table 1: CalproQuest (8-item IBD questionnaire)

Type	Criteria	Yes (1)	No (0)	Comment
Major	Does the patient suffer from abdominal pain at least 3 times a week for at least 4 weeks?			
	Does the patient suffer from diarrhoea (more than 3 bowel movements daily) for 7 consecutive days?			
	Does the patient have diarrhoea at night-time/Does the patient awake from sleep because of abdominal pain or diarrhoea?			
	Does the patient report bloody stool?			
Minor	Does the patient report mucus in stool for more than 4 weeks?			
	Does the patient report unwanted weight loss (5% of normal body weight over 6 months)?			
	Does the patient present with fever or report fever over the last 4 weeks (Temp > 38°C)?			
	Does the patient report fatigue over the last 4 weeks?			

Fecal Calprotectin

Fecal Calprotectin levels will be measured at the University Hospital Zurich by a novel ELISA-based Calprotectin test named EliA Caprotectin (Thermo Scientific, for product description see http://www.phadia.com/PageFiles/29347/Product%20information%20EliA%20Calprotectin.pdf).

Patient questionnaire on diagnostic delay

Three relevant time intervals of diagnostic delay will be assessed in a patient questionnaire. The time intervals are defined as follows (see Figure 2):

1. Interval 1: Time from first IBD related symptoms to first consultation with the GP: This interval represents the time span between the first manifestations of IBD-related symptoms (patient-reported) and a consultation with the GP specifically due to these

IBD-related complaints. The length of this period is mainly dependent on the patient herself/himself.

2. Interval 2: Time from the first GP visit to referral to a gastroenterologist: This represents the time span between the IBD symptom-related consultation of the GP and the time of referral to a gastroenterologist for further examination. The length of this period is mainly dependent on the treating GP.
3. Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2): This interval is calculated by the addition of interval 1 and 2 and is defined as diagnostic delay. Diagnostic delay is defined as the time span (in weeks) from first symptoms to IBD diagnosis.

The following items in the patient questionnaires are assessed for the purpose of this study: ‘‘Before the IBD diagnosis, how long did you experience symptoms that are now attributed to IBD?’’ and ‘‘How long was the time interval between first symptoms and the first visit to your GP?’’ and “How long were you treated by your GP before referral to a gastroenterologist?” and ‘‘What was the time span from the first physician visit (due to these complaints) until IBD diagnosis was established?’’ Additionally, patients will answer questions regarding smoking habits, intake of non-steroidal anti-inflammatory drugs (NSAIDs), or oral contraception at the time of diagnosis.

Physician questionnaire on feasibility and acceptance of CalproQuest in primary health care

Goal of the feasibility questionnaire is to investigate feasibility and acceptance of CalproQuest in daily primary health care practice.

The questionnaire is based on an even-point Likert scale consisting of seven items.

Patient questionnaire on acceptance of stool sampling

Aim of the patient’s acceptance questionnaire is to investigate patients’ physical and mental ability to handle stool sampling at home.

The questionnaire is based on an even-point Likert scale consisting of four Likert items.

Administration of patient records

Physicians will be supplied with a master data list providing patient codes that can be assigned to the patient. All documents containing patient data will carry the respective patient code assigned by the physician. Encoded documents will be sent to the Institute of Primary Care, University of Zurich, Switzerland and stored for 10 years. Only physicians have access to the patient codes. Data entry is performed continuously at the Institute of Primary Care, University of Zurich, and if data is missing, a research assistant will investigate to obtain all information as required.

Sample size calculation

In part A, sample size was calculated according to Flahault et al.[27]. Assuming a 0.05 two-sided significance level, n=162 would have 90 % power to detect a sensitivity and specificity of 90% of CalproQuest for a Calprotectin level ≥ 50 µg/g feces, or for a Calprotectin level ≥ 50 µg/g feces and a positive IBD diagnosis. For the purpose of this calculation, expected sensitivity and specificity are 90% with a lower acceptable limit of sensitivity of 70%. Assumed prevalence of IBD within the sample is 20%. A $p<0.05$ is considered statistically significant. 80 patients were assumed to be necessary for the feasibility testing in part B.

Statistical data evaluation

We provide sensitivity and specificity calculation of CalproQuest based on confidence intervals (see Figure 3). For the other primary or secondary outcomes parametric or non-parametric tests are used where appropriate.

Physician recruitment

Physician recruitment is ongoing, to achieve the target number of 162 patients about 12-15 gastroenterologists and IBD-centres will be recruited in part A and 30 GPs in part B. Country of recruitment: Switzerland. Physicians will receive a financial incentive.

Patient recruitment

Physicians from part A and B are asked to approach consecutively patients eligible for the trial. Patients don't receive a financial incentive, the stool sampling material and the fecal Calprotectin test will be provided for free.

Patient informed consent

Prior to study participation patients receive written and oral information about the consent and extent of the planned study. In case of acceptance they sign the informed consent form. In case of study discontinuation all material will be destroyed or the patient will be asked if he/she accepts that the existing material can be used for the study.

Time frame of the study

Recruitment of gastroenterologists and GP's started in October 2014. Recruitment time of the eligible patients will be 12 months. Data analysis will be performed 12 months after recruiting the last study centre (part A: gastroenterologists, part B: GPs) or earlier, when the target number of patients (part A: 162, part B: 80) has been achieved.

Description of risks

In part A an endoscopy will be performed, however these patients are already referred for endoscopic evaluation to gastroenterologists. Therefore an additional risk is not expected.

Confidentiality

The patient names and all other confidential information fall under medical confidentiality rules and are treated according to appropriate Federal Data Security Laws. The results of the patient questionnaires are not accessible to the GPs. Questionnaires are directly mailed to the study centre by the patient. All study related data and documents are stored on a protected central server of the University of Zurich. Only direct members of the internal study team can access the respective files. Intermediate and final reports are stored in the office of the Institute of Primary Care at the Zurich University Hospital.

DISCUSSION

An early diagnosis of IBD is associated with a better outcome. In primary health care the prevalence of IBD is much lower than in tertiary healthcare (gastroenterologist, or even in an IBD-centre), where patients are already preselected by GPs. The emphasis in tertiary healthcare is usually on “ruling in”: increasing the probability of IBD to carry out more expensive, time consuming, and invasive procedures; establish a firm diagnosis; and start appropriate treatment. At tertiary healthcare level a diagnostic test with a high positive likelihood ratio is preferred. In primary health care, the emphasis is on “ruling out”: lowering the probability of the target disease to provide reassurance, or to adopt a “watchful waiting” strategy. In these instances tests with a low negative likelihood ratio are preferred [6]. Increasing the sensitivity and specificity of fecal Calprotectin test by the CalproQuest questionnaire, which is feasible in the primary health care, we could provide a simple, convenient tool lowering the diagnostic delay in patients with IBD.

Limitations and strength

No conclusions can be made about the sensitivity or specificity of the test in primary health care, as the sample size is too low (n= 80 patients in part B). From ongoing other studies with diseases, which are more prevalent, we know that recruitment of patients in primary health care is very difficult since participation is based on GPs free choice and implementation of research in the daily routine of a general practice is time-consuming. We therefore chose the design of 2 different parts in two different settings. If CalproQuest is validated in tertiary healthcare, further studies will be needed to evaluate the accuracy of the test in primary health care.

In primary health care (part B), patients present themselves with symptoms, not with a presumed diagnosis. This is a difference between the tertiary healthcare (part A), where almost every patient might already have had the first endoscopic investigations as well as treatment and is referred because they don’t response on the established therapy or unclearness of the diagnosis. As different habits or conditions interfere with the value of

Calprotectin, the exclusion criteria in some studies are strict (regular intake of aspirin and/or non-steroid anti-inflammatory drugs, urinary incontinence as fecal Calprotectin is not water proof, infectious enterocolitis, colorectal cancer etc.) [19]. As we want to determine the feasibility in primary health care population, we do not exclude patients presenting with these conditions, because we want to represent the “real life” in the daily routine of a GP.

TRIAL STATUS

Patient recruitment had started in November 2014.

LIST OF ABBREVIATIONS

CD Crohn’s Disease
GP general practitioner
IBD inflammatory bowel disease
IBS irritable bowel syndrome
IC indeterminate colitis
UC Ulcerative Colitis

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

TR, SV and GR were the initiators for this study. TR is the trial sponsor. TR, SV, GR and NZ developed the questionnaires. NZ, SV and GR organized the recruitment of the gastroenterologists. SH, RT and TR organized the recruitment of the practices. SH wrote and revised the final manuscript and all authors read and approved it.

FIGURES

Figure 1: Study design

Figure 2: Interval 1-3 in diagnostic delay, adapted from [1]

Interval 1: Time from first IBD symptoms to consultation with the GP

Interval 2: Time from GP visit to referral to a gastroenterologist

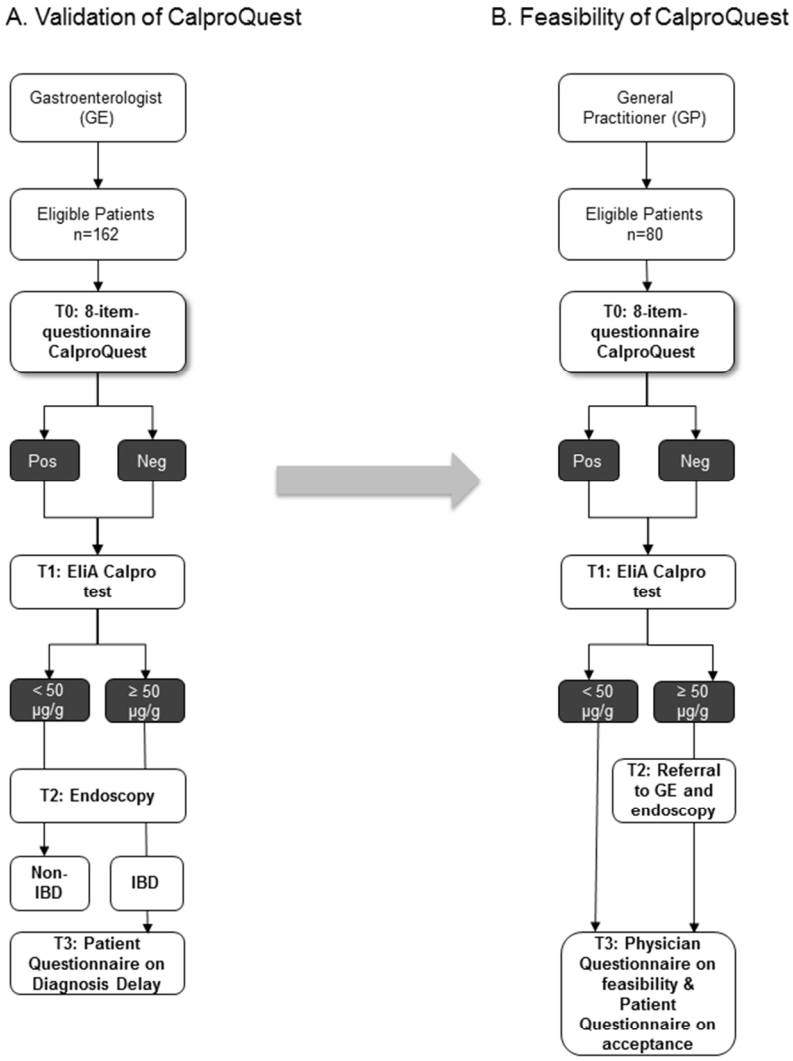
Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2)

Figure 3: Sensitivity and specificity calculation of CalproQuest

REFERENCES

1. Vavricka SR, Spigaglia SM, Rogler G, et al. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. Inflammatory bowel diseases 2012;**18**(3):496-505 doi: 10.1002/ibd.21719[published Online First: Epub Date]|.
2. Juillerat P, Pittet V, Bulliard JL, et al. Prevalence of Inflammatory Bowel Disease in the Canton of Vaud (Switzerland): A population-based cohort study. Journal of Crohn's & colitis 2008;**2**(2):131-41 doi: 10.1016/j.crohns.2007.10.006[published Online First: Epub Date]|.
3. Guideline WGOG. Irritable bowel syndrome: a global perspective http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/20_irritable_bowel_syndrome.pdf. 2009
4. Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? Gastroenterology clinics of North America 2005;**34**(2):235-45, vi-vii doi: 10.1016/j.gtc.2005.02.007[published Online First: Epub Date]|.
5. Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflammatory bowel diseases 2014;**20**(8):1407-15 doi: 10.1097/MIB.0000000000000057[published Online First: Epub Date]|.
6. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. Bmj 2010;**341**:c3369 doi: 10.1136/bmj.c3369[published Online First: Epub Date]|.
7. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biological & pharmaceutical bulletin 2003;**26**(6):753-60
8. Loomans HJ, Hahn BL, Li QQ, et al. Histidine-based zinc-binding sequences and the antimicrobial activity of calprotectin. The Journal of infectious diseases 1998;**177**(3):812-4
9. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scandinavian journal of gastroenterology 1992;**27**(9):793-8
10. Tibble JA, Sigthorsson G, Foster R, et al. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 2002;**123**(2):450-60
11. Otten CM, Kok L, Witteman BJ, et al. Diagnostic performance of rapid tests for detection of fecal calprotectin and lactoferrin and their ability to discriminate inflammatory from irritable bowel syndrome. Clinical chemistry and laboratory medicine : CCLM / FESCC 2008;**46**(9):1275-80 doi: 10.1515/CCLM.2008.246[published Online First: Epub Date]|.
12. Schoepfer AM, Trummler M, Seeholzer P, et al. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflammatory bowel diseases 2008;**14**(1):32-9 doi: 10.1002/ibd.20275[published Online First: Epub Date]|.
13. Limburg PJ, Ahlquist DA, Sandborn WJ, et al. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. The American journal of gastroenterology 2000;**95**(10):2831-7 doi: 10.1111/j.1572-0241.2000.03194.x[published Online First: Epub Date]|.
14. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;**58**(2):176-80
15. Costa F, Mumolo MG, Ceccarelli L, et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;**54**(3):364-8 doi: 10.1136/gut.2004.043406[published Online First: Epub Date]|.
16. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;**47**(4):506-13

17. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scandinavian journal of gastroenterology 1999;**34**(1):50-4
18. Schoepfer AM, Beglinger C, Straumann A, et al. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflammatory bowel diseases 2009;**15**(12):1851-8 doi: 10.1002/ibd.20986[published Online First: Epub Date]|.
19. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. The American journal of gastroenterology 2010;**105**(1):162-9 doi: 10.1038/ajg.2009.545[published Online First: Epub Date]|.
20. Markowitz J. Early inflammatory bowel disease: different treatment response to specific or all medications? Digestive diseases 2009;**27**(3):358-65 doi: 10.1159/000228574[published Online First: Epub Date]|.
21. Etchevers MJ, Aceituno M, Sans M. Are we giving azathioprine too late? The case for early immunomodulation in inflammatory bowel disease. World journal of gastroenterology : WJG 2008;**14**(36):5512-8
22. Punati J, Markowitz J, Lerer T, et al. Effect of early immunomodulator use in moderate to severe pediatric Crohn disease. Inflammatory bowel diseases 2008;**14**(7):949-54 doi: 10.1002/ibd.20412[published Online First: Epub Date]|.
23. Gupta N, Bostrom AG, Kirschner BS, et al. Presentation and disease course in early- compared to later-onset pediatric Crohn's disease. The American journal of gastroenterology 2008;**103**(8):2092-8 doi: 10.1111/j.1572-0241.2008.02000.x[published Online First: Epub Date]|.
24. Schoepfer AM, Dehlavi MA, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. The American journal of gastroenterology 2013;**108**(11):1744-53; quiz 54 doi: 10.1038/ajg.2013.248[published Online First: Epub Date]|.
25. Mansson J, Nilsson G, Strender LE, et al. Reasons for encounters, investigations, referrals, diagnoses and treatments in general practice in Sweden--a multicentre pilot study using electronic patient records. The European journal of general practice 2011;**17**(2):87-94 doi: 10.3109/13814788.2010.538675[published Online First: Epub Date]|.
26. Moth G, Olesen F, Vedsted P. Reasons for encounter and disease patterns in Danish primary care: changes over 16 years. Scandinavian journal of primary health care 2012;**30**(2):70-5 doi: 10.3109/02813432.2012.679230[published Online First: Epub Date]|.
27. Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. Journal of clinical epidemiology 2005;**58**(8):859-62 doi: 10.1016/j.jclinepi.2004.12.009[published Online First: Epub Date]|.



Study design
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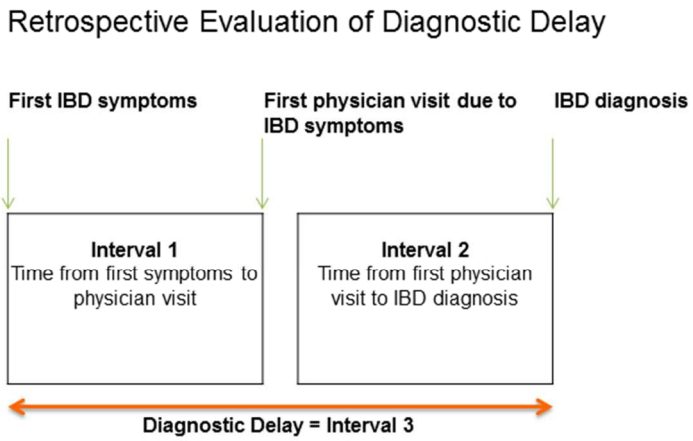

Interval 1-3 in diagnostic delay, adapted from [1]
Interval 1: Time from first IBD symptoms to consultation with the GP
Interval 2: Time from GP visit to referral to a gastroenterologist
Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2)

190x254mm (96 x 96 DPI)

Evaluation of sensitivity/specificity and PPV/NPV of CalproQuest

Is CalproQuest sensitive/specific for Calpro ≥ 50 µg/g?		Patients referred to GE for endoscopic examination		
		Calprotectin positive (≥ 50 µg/g)	Calprotectin negative (< 50 µg/g)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

Is CalproQuest sensitive/specific for IBD?		Patients with Calprotectin ≥ 50 µg/g		
		IBD (confirmed by endoscopy)	Non-IBD (confirmed by endoscopy)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

TP	true positive	TN	true negative
FP	false positive	FN	false negative

Sensitivity and specificity calculation of CalproQuest
153x186mm (96 x 96 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	1-14
Funding	4	Sources and types of financial, material, and other support	7,11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	11
	5b	Name and contact information for the trial sponsor	1, 11
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4	
	6b	Explanation for choice of comparators	-	(no RCT)
Objectives	7	Specific objectives or hypotheses	4	
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5	
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9	
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5	
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6	
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5	
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5, 6	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9	

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	no RCT
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	no RCT
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	no RCT
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	no RCT
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	no RCT
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-8
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6, 8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8, 10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9, 11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	8, 10
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in English
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

VAlidation of an 8-item-questionnaire predictive for a positive CaLprotectin tEst and Real-life implemenTation in primary care to reduce diagnostic delay in inflammatory bowel disease (ALERT): protocol for a prospective diagnostic study

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Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Gastroenterology and hepatology, General practice / Family practice, Health services research
Keywords:	Inflammatory bowel disease < GASTROENTEROLOGY, sensitivity, specificity, early diagnosis, feasibility

VAlidation of an 8-item-questionnaire predictive for a positive CaLprotectin tEst and Real-life implemenTation in primary care to reduce diagnostic delay in inflammatory bowel disease (ALERT): protocol for a prospective diagnostic study

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Word count

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ABSTRACT

Introduction

Diagnosis of inflammatory bowel disease (IBD) in primary health care is challenging and often associated with a considerable diagnostic delay. This delay is associated with worse disease progression and outcomes. Although testing for fecal Calprotectin is a useful screening tool to identify patients who need endoscopy for IBD, the widespread use may not be appropriate due to the low prevalence of patients with IBD among all patients attending a general practitioner (GP) with gastrointestinal symptoms. To increase the appropriate application of the fecal Calprotectin test, an 8-item-questionnaire, the CalproQuest, has been developed to increase pretest-probability for a positive test result.

Methods and analysis

This is a prospective diagnostic observational trial. The study consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patients included in part A are referred to the gastroenterologist for any endoscopic evaluation. Patients included in part B present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

CalproQuest consists of 4 main and 4 secondary questions specific for IBD it is considered positive, if ≥ 2 main criteria are answered positively or 1 main criterion and 2 secondary criteria are answered positively.

In part A the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis will be investigated. In part B the feasibility of CalproQuest in daily primary health care practice will be assessed.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Kanton Zurich (reference KEK-ZH-number 2013-0516). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration number

The study protocol is registered at ISRCTN66310845

INTRODUCTION

IBD versus IBS

Crohn's Disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC) represent the three subtypes of inflammatory bowel disease (IBD) [1]. Estimated prevalence rates of IBD in Swiss population are about 205.6 cases per 100'000 [2].

Meanwhile, the prevalence of irritable bowel syndrome (IBS) in Europe and North America is estimated to be 10 – 15% [3].

Physicians are often faced with the diagnostic challenge of differentiating patients with IBD from functional disorders such as irritable bowel syndrome (IBS). Indeed, symptoms similar to IBS are frequently reported in patients before IBD is diagnosed [4]. The gold diagnostic standard is endoscopy; however, not every patient with the symptoms overlapping with those of IBS can be investigated by the invasive endoscopic exam. Therefore, many different, non-invasive markers have been investigated. Several studies have shown that fecal Calprotectin accurately reflects intestinal inflammation in patients with known IBD [5].

It has also been shown to consistently differentiate IBD from IBS due to its excellent negative predictive value. It can therefore be used ruling out IBD in undiagnosed, symptomatic patients [6].

Calprotectin, a S100 protein

Calprotectin is a complex of two calcium-binding proteins that belong to the S100 protein family [7]. It is abundant in the cytosolic fraction of neutrophils. High levels of Calprotectin have been found in extracellular fluid during various inflammatory conditions, such as rheumatoid arthritis, cystic fibrosis and abscesses. Calprotectin released from neutrophils has growth-inhibitory and apoptosis-inducing activities against various cell types including tumor cells and normal fibroblasts [7]. This suggests that Calprotectin has regulatory activities during inflammatory processes through its effect on the survival or growth states of cells participating in the inflammatory reaction. Furthermore, Calprotectin inhibits microbial growth through competition for zinc [8].

Calprotectin has been shown to be stable in feces during storage for 7 days at room temperature, which is very important for its value in evaluating mucosal wall inflammation [9].

Stool Calprotectin levels as marker of intestinal inflammation

Data correlated to fecal Calprotectin showed a sensitivity and specificity of Calprotectin (using a cut-off value of 10mg/l) for organic disease of 89% and 79%, respectively [10]. Studies using cut-off value of 50 µg/g showed a sensitivity and specificity of 86.8% and 95.7% [11] and 83% and 100% [12] discriminating IBD versus IBS. Fecal Calprotectin levels correlate significantly with histological and endoscopic assessment of disease activity in ulcerative

colitis (UC) [13-15] as well as with fecal alpha-1-antitrypsin levels and fecal excretion of 111indium-labeled white blood cells in patients with Crohn's disease (CD) [16 17]. Previous studies showed that stool Calprotectin levels correlate well with endoscopic indices both in ulcerative colitis and in Crohn's disease [18-20].

Diagnostic delay in IBD is predictive for worse disease progression and outcomes

Difficulties in differentiation early IBD from IBS, especially in a primary health care, is leading to a considerable diagnostic delay in IBD [1]. This delay has important clinical impact, as there is increasing evidence demonstrating that treatment success is increased in early disease [21-24]. Vavricka and colleagues suggest, that diagnostic delay is sub-dived into two intervals, where interval 1 is defined as time from first symptoms to physician visit, and interval 2 the time from first physician visit to IBD diagnosis [1]. The study by Vavricka et al. estimates that 25% of all CD and UC patients experienced more than 24 and 12 months, respectively, from first onset of symptoms until an accurate IBD diagnosis [1]. Most importantly Schoepfer and colleagues recently showed, that the length of diagnostic delay is correlated with an increased risk of bowel stenosis and CD-related intestinal surgery, concluding that efforts should be undertaken to shorten diagnostic delay [25].

Testing Calprotectin in Switzerland

Most analytical laboratories in Switzerland offer Calprotectin testing, which is reimbursed by health insurances.

Fecal Calprotectin testing in primary health care versus tertiary healthcare

Although Calprotectin tests are easily accessible and reimbursed in Switzerland, this diagnostic test is not routinely performed in primary health care. However, the low prevalence of IBD in the primary health care must be taken into account: Analysing the reasons for encounter in primary health care, the group of digestive disorders is not one of the predominant diagnostic groups. Scandinavian studies show frequencies between 5-7% [26 27]. Focusing on this diagnostic group, IBS is much more common in primary health care (population-based prevalence of 10-15% of IBS compared with 0.2% of IBD). Therefore, a new tool has been developed to narrow the patient collective in which Calprotectin testing may lead to the correct diagnosis of IBD in an earlier stadium.

Hypothesis and goal

This study pursuits two main aims A and B, which are investigated independently:

A. Prospective validation and evaluation of sensitivity and specificity of an 8-item IBD-questionnaire (CalproQuest; see Table 1) for 1) a positive Calprotectin test result ≥ 50 µg/g feces and for 2) a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis, respectively, in tertiary healthcare

B. Prospective implementation of CalproQuest in primary health care to investigate feasibility in daily practice.

METHODS AND ANALYSIS

Study design

The study is a prospective diagnostic observational trial. It consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and general practitioners (GPs) (B), respectively.

Patients included in part A of the study are referred for endoscopic evaluation to gastroenterologists. Patients included in part B of the study present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

The study design and procedure are summarized in Figure 1.

Inclusion and exclusion criteria

Patients will be eligible if they

- Are ≥18 years old (part A, B)
- Are referred to their gastroenterologist for any endoscopic examination (part A)
- Visit their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks (part B)
- Underwent no earlier diagnostic procedures (endoscopy) for the current episode (part B)

Patients are not eligible, if they

- Are younger than 18 years (part A, B)
- Have known further/other abdominal pathologies as e.g. cancer (part A, B)
- Had previous abdominal surgeries (part B)
- Have been treated with steroids (topical and/or oral) and/or aminosalicylates within 30 days prior inclusion into this study (part B)
- Underwent endoscopic examination within 3 years prior screening (part B)

Primary and secondary outcomes

Primary outcomes:

A. 1. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces

2. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis.

B. Feasibility of CalproQuest in daily primary health care practice.

Secondary outcomes:

A. Patient-reported diagnostic delay.

B. Patient-reported acceptance of stool sampling.

Procedure of the study

In brief, the study will be divided in two independent parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patient data will be encoded.

A. Investigation of the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis

Patients referred to the gastroenterologist for endoscopic examination are subjected to CalproQuest and Calprotectin stool testing prior endoscopy. At baseline T0, patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens from outpatients will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. At T2, endoscopic examination will be performed to obtain a diagnosis. Eventually, patients diagnosed with IBD will be asked to complete a questionnaire at T3 investigating duration of first onset of symptoms to IBD diagnosis (diagnostic delay).

B. Investigation of feasibility of CalproQuest in daily primary health care practice

Patients with on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for more than two weeks presenting at the GP will be included into the study if all inclusion criteria are met and informed patient consent is obtained.

At baseline (T0), patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. According to the current standard of care it is recommended to refer patients with Calprotectin levels ≥ 50 µg/g to a gastroenterologist for endoscopic examination at T2; results of the endoscopy are communicated back to the GP. Patients will be asked at T3 to complete a questionnaire on acceptance of stool sampling, and physicians will complete the questionnaire on feasibility of CalproQuest in daily practice.

CalproQuest

CalproQuest is an 8-item IBD-questionnaire consisting of 4 main and 4 secondary questions specific for IBD (see Table 1). CalproQuest was pre-validated by IBD-experts trough an international Delphi-process. CalproQuest is considered positive, if ≥ 2 main criteria or 1 main criterion and 2 secondary criteria are answered positively.

We assume that a positive CalproQuest result may predict Calprotectin levels ≥ 50 µg/g. Calprotectin levels above 50 µg/g are indicative for on-going intestinal inflammation and call for further endoscopic examination.

Table 1: CalproQuest (8-item IBD questionnaire)

Type	Criteria	Yes (1)	No (0)	Comment
Major	Does the patient suffer from abdominal pain at least 3 times a week for at least 4 weeks?			
	Does the patient suffer from diarrhoea (more than 3 bowel movements daily) for 7 consecutive days?			
	Does the patient have diarrhoea at night-time/Does the patient awake from sleep because of abdominal pain or diarrhoea?			
	Does the patient report bloody stool?			
Minor	Does the patient report mucus in stool for more than 4 weeks?			
	Does the patient report unwanted weight loss (5% of normal body weight over 6 months)?			
	Does the patient present with fever or report fever over the last 4 weeks (Temp > 38°C)?			
	Does the patient report fatigue over the last 4 weeks?			

Fecal Calprotectin

Fecal Calprotectin levels will be measured at the University Hospital Zurich by a novel ELISA-based Calprotectin test named EliA Caprotectin (Thermo Scientific, for product description see http://www.phadia.com/PageFiles/29347/Product%20information%20EliA%20Calprotectin.pdf).

Patient questionnaire on diagnostic delay

Three relevant time intervals of diagnostic delay will be assessed in a patient questionnaire. The time intervals are defined as follows (see Figure 2):

1. Interval 1: Time from first IBD related symptoms to first consultation with the GP: This interval represents the time span between the first manifestations of IBD-related symptoms (patient-reported) and a consultation with the GP specifically due to these

IBD-related complaints. The length of this period is mainly dependent on the patient herself/himself.

2. Interval 2: Time from the first GP visit to referral to a gastroenterologist: This represents the time span between the IBD symptom-related consultation of the GP and the time of referral to a gastroenterologist for further examination. The length of this period is mainly dependent on the treating GP.
3. Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2): This interval is calculated by the addition of interval 1 and 2 and is defined as diagnostic delay. Diagnostic delay is defined as the time span (in weeks) from first symptoms to IBD diagnosis.

The following items in the patient questionnaires are assessed for the purpose of this study: ‘‘Before the IBD diagnosis, how long did you experience symptoms that are now attributed to IBD?’’ and ‘‘How long was the time interval between first symptoms and the first visit to your GP?’’ and “How long were you treated by your GP before referral to a gastroenterologist?” and ‘‘What was the time span from the first physician visit (due to these complaints) until IBD diagnosis was established?’’ Additionally, patients will answer questions regarding smoking habits, intake of non-steroidal anti-inflammatory drugs (NSAIDs), or oral contraception at the time of diagnosis.

Physician questionnaire on feasibility and acceptance of CalproQuest in primary health care

Goal of the feasibility questionnaire is to investigate feasibility and acceptance of CalproQuest in daily primary health care practice.

The questionnaire is based on an even-point Likert scale consisting of seven items.

Patient questionnaire on acceptance of stool sampling

Aim of the patient’s acceptance questionnaire is to investigate patients’ physical and mental ability to handle stool sampling at home.

The questionnaire is based on an even-point Likert scale consisting of four Likert items.

Administration of patient records

Physicians will be supplied with a master data list providing patient codes that can be assigned to the patient. All documents containing patient data will carry the respective patient code assigned by the physician. Encoded documents will be sent to the Institute of Primary Care, University of Zurich, Switzerland and stored for 10 years. Only physicians have access to the patient codes. Data entry is performed continuously at the Institute of Primary Care, University of Zurich, and if data is missing, a research assistant will investigate to obtain all information as required.

Sample size calculation

In part A, sample size was calculated according to Flahault et al.[28]. Assuming a 0.05 two-sided significance level, n=162 would have 90 % power to detect a sensitivity and specificity of 90% of CalproQuest for a Calprotectin level ≥ 50 µg/g feces, or for a Calprotectin level ≥ 50 µg/g feces and a positive IBD diagnosis. For the purpose of this calculation, expected sensitivity and specificity are 90% with a lower acceptable limit of sensitivity of 70%. Assumed prevalence of IBD within the sample is 20%. A $p<0.05$ is considered statistically significant. 80 patients were assumed to be necessary for the feasibility testing in part B.

Statistical data evaluation

We provide sensitivity and specificity calculation of CalproQuest based on confidence intervals (see Figure 3). For the other primary or secondary outcomes parametric or non-parametric tests are used where appropriate.

Physician recruitment

Physician recruitment is ongoing, to achieve the target number of 162 patients about 12-15 gastroenterologists and IBD-centres will be recruited in part A and 30 GPs in part B. Country of recruitment: Switzerland. Physicians will receive a financial incentive.

Patient recruitment

Physicians from part A and B are asked to approach consecutively patients eligible for the trial. Patients don't receive a financial incentive, the stool sampling material and the fecal Calprotectin test will be provided for free.

Patient informed consent

Prior to study participation patients receive written and oral information about the consent and extent of the planned study. In case of acceptance they sign the informed consent form. In case of study discontinuation all material will be destroyed or the patient will be asked if he/she accepts that the existing material can be used for the study.

Time frame of the study

Recruitment of gastroenterologists and GP's started in October 2014. Recruitment time of the eligible patients will be 12 months. Data analysis will be performed 12 months after recruiting the last study centre (part A: gastroenterologists, part B: GPs) or earlier, when the target number of patients (part A: 162, part B: 80) has been achieved.

Description of risks

In part A an endoscopy will be performed, however these patients are already referred for endoscopic evaluation to gastroenterologists. Therefore an additional risk is not expected.

Confidentiality

The patient names and all other confidential information fall under medical confidentiality rules and are treated according to appropriate Federal Data Security Laws. The results of the patient questionnaires are not accessible to the GPs. Questionnaires are directly mailed to the study centre by the patient. All study related data and documents are stored on a protected central server of the University of Zurich. Only direct members of the internal study team can access the respective files. Intermediate and final reports are stored in the office of the Institute of Primary Care at the Zurich University Hospital.

DISCUSSION

An early diagnosis of IBD is associated with a better outcome. In primary health care the prevalence of IBD is much lower than in tertiary healthcare (gastroenterologist, or even in an IBD-centre), where patients are already preselected by GPs. The emphasis in tertiary healthcare is usually on “ruling in”: increasing the probability of IBD to carry out more expensive, time consuming, and invasive procedures; establish a firm diagnosis; and start appropriate treatment. At tertiary healthcare level a diagnostic test with a high positive likelihood ratio is preferred. In primary health care, the emphasis is on “ruling out”: lowering the probability of the target disease to provide reassurance, or to adopt a “watchful waiting” strategy. In these instances tests with a low negative likelihood ratio are preferred [6].
Increasing the sensitivity and specificity of fecal Calprotectin test by the CalproQuest questionnaire, which is feasible in the primary health care, we could provide a simple, convenient tool lowering the diagnostic delay in patients with IBD.

Limitations and strength

No conclusions can be made about the sensitivity or specificity of the test in primary health care, as the sample size is too low (n= 80 patients in part B). From ongoing other studies with diseases, which are more prevalent, we know that recruitment of patients in primary health care is very difficult since participation is based on GPs free choice and implementation of research in the daily routine of a general practice is time-consuming. We therefore chose the design of 2 different parts in two different settings. If CalproQuest is validated in tertiary healthcare, further studies will be needed to evaluate the accuracy of the test in primary health care.
In primary health care (part B), patients present themselves with symptoms, not with a presumed diagnosis. This is a difference between the tertiary healthcare (part A), where almost every patient might already have had the first endoscopic investigations as well as treatment and is referred because they don’t response on the established therapy or unclearness of the diagnosis. As different habits or conditions interfere with the value of

Calprotectin, the exclusion criteria in some studies are strict (regular intake of aspirin and/or non-steroid anti-inflammatory drugs, urinary incontinence as fecal Calprotectin is not water proof, infectious enterocolitis, colorectal cancer etc.) [19]. As we want to determine the feasibility in primary health care population, we do not exclude patients presenting with these conditions, because we want to represent the “real life” in the daily routine of a GP.

TRIAL STATUS

Patient recruitment had started in November 2014.

LIST OF ABBREVIATIONS

CD	Crohn’s Disease
GP	general practitioner
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IC	indeterminate colitis
UC	Ulcerative Colitis

FUNDING: IBDNET

This project is supported by grants from the IBDnet, Swiss Research and Communication Network on Inflammatory Bowel Disease.

This funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

TR, SV and GR were the initiators for this study. TR is the trial sponsor. TR, SV, GR and NZ developed the questionnaires. NZ, SV and GR organized the recruitment of the gastroenterologists. SH, RT, SM and TR organized the recruitment of the practices. SH wrote and revised the final manuscript and all authors read and approved it.

FIGURES

Figure 1: Study design

Figure 2: Interval 1-3 in diagnostic delay, adapted from [1]

Interval 1: Time from first IBD symptoms to consultation with the GP

Interval 2: Time from GP visit to referral to a gastroenterologist

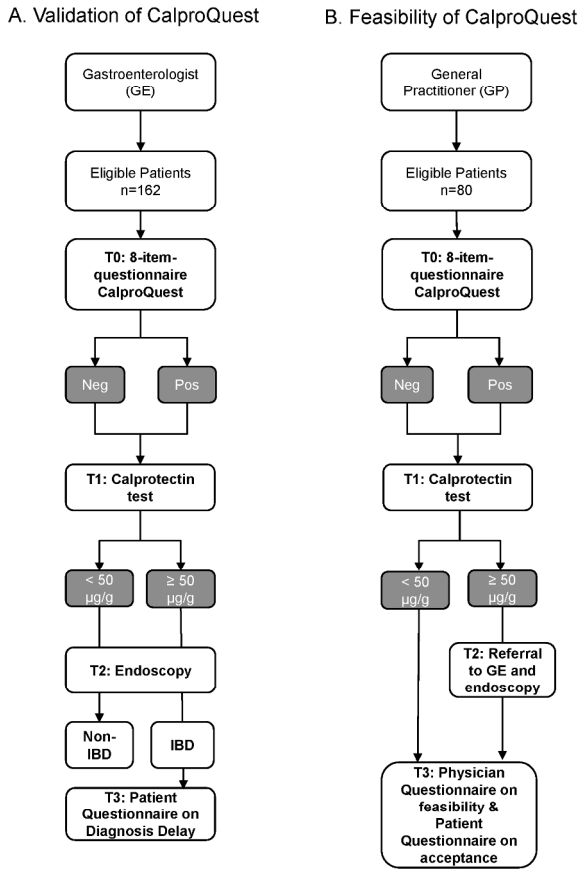
Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2)

Figure 3: Sensitivity and specificity calculation of CalproQuest

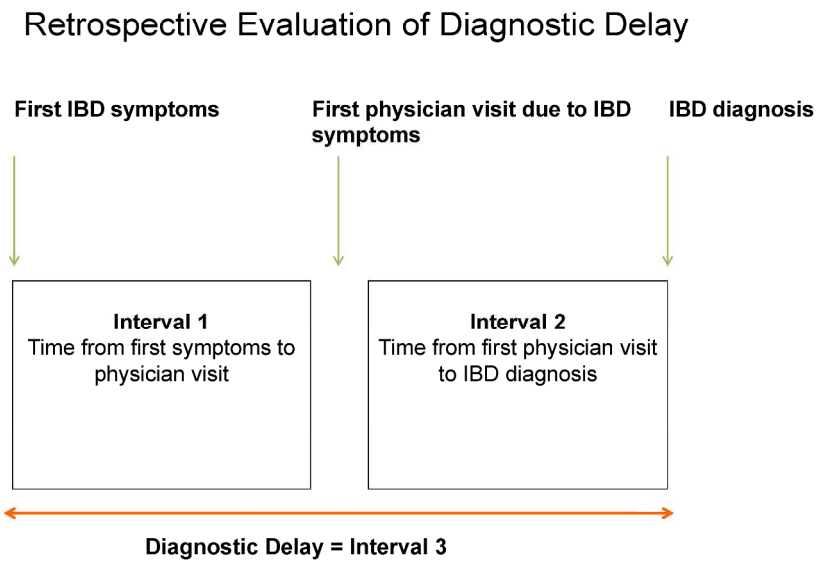
REFERENCES

1. Vavricka SR, Spigaglia SM, Rogler G, et al. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. Inflammatory bowel diseases 2012;**18**(3):496-505.
2. Juillerat P, Pittet V, Bulliard JL, et al. Prevalence of Inflammatory Bowel Disease in the Canton of Vaud (Switzerland): A population-based cohort study. Journal of Crohn's & colitis 2008;**2**(2):131-41.
3. Guideline WGOG. Irritable bowel syndrome: a global perspective http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/20_irritable_bowel_syndrome.pdf. 2009.
4. Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? Gastroenterology clinics of North America 2005;**34**(2):235-45, vi-vii.
5. Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflammatory bowel diseases 2014;**20**(8):1407-15.
6. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. Bmj 2010;**341**:c3369.
7. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biological & pharmaceutical bulletin 2003;**26**(6):753-60.
8. Loomans HJ, Hahn BL, Li QQ, et al. Histidine-based zinc-binding sequences and the antimicrobial activity of calprotectin. The Journal of infectious diseases 1998;**177**(3):812-4.
9. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scandinavian journal of gastroenterology 1992;**27**(9):793-8.
10. Tibble JA, Sigthorsson G, Foster R, et al. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 2002;**123**(2):450-60.
11. Otten CM, Kok L, Witteman BJ, et al. Diagnostic performance of rapid tests for detection of fecal calprotectin and lactoferrin and their ability to discriminate inflammatory from irritable bowel syndrome. Clinical chemistry and laboratory medicine : CCLM / FESCC 2008;**46**(9):1275-80.
12. Schoepfer AM, Trummler M, Seeholzer P, et al. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflammatory bowel diseases 2008;**14**(1):32-9.
13. Limburg PJ, Ahlquist DA, Sandborn WJ, et al. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. The American journal of gastroenterology 2000;**95**(10):2831-7.
14. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;**58**(2):176-80.
15. Costa F, Mumolo MG, Ceccarelli L, et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;**54**(3):364-8.
16. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;**47**(4):506-13.
17. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scandinavian journal of gastroenterology 1999;**34**(1):50-4.
18. Schoepfer AM, Beglinger C, Straumann A, et al. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflammatory bowel diseases 2009;**15**(12):1851-8.

19. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. The American journal of gastroenterology 2010;**105**(1):162-9.
20. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases 2012;**18**(12):2218-24.
21. Markowitz J. Early inflammatory bowel disease: different treatment response to specific or all medications? Digestive diseases 2009;**27**(3):358-65.
22. Etchevers MJ, Aceituno M, Sans M. Are we giving azathioprine too late? The case for early immunomodulation in inflammatory bowel disease. World journal of gastroenterology : WJG 2008;**14**(36):5512-8.
23. Punati J, Markowitz J, Lerer T, et al. Effect of early immunomodulator use in moderate to severe pediatric Crohn disease. Inflammatory bowel diseases 2008;**14**(7):949-54.
24. Gupta N, Bostrom AG, Kirschner BS, et al. Presentation and disease course in early- compared to later-onset pediatric Crohn's disease. The American journal of gastroenterology 2008;**103**(8):2092-8.
25. Schoepfer AM, Dehlavi MA, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. The American journal of gastroenterology 2013;**108**(11):1744-53; quiz 54.
26. Mansson J, Nilsson G, Strender LE, et al. Reasons for encounters, investigations, referrals, diagnoses and treatments in general practice in Sweden--a multicentre pilot study using electronic patient records. The European journal of general practice 2011;**17**(2):87-94.
27. Moth G, Olesen F, Vedsted P. Reasons for encounter and disease patterns in Danish primary care: changes over 16 years. Scandinavian journal of primary health care 2012;**30**(2):70-5.
28. Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. Journal of clinical epidemiology 2005;**58**(8):859-62.



Study design
210x297mm (300 x 300 DPI)



Interval 1-3 in diagnostic delay, adapted from Vavricka et al. [1]
297x210mm (300 x 300 DPI)

Evaluation of sensitivity/specificity and PPV/NPV of CalproQuest

Is CalproQuest sensitive/specific for Calpro ≥ 50 µg/g?		Patients referred to GE for endoscopic examination		
		Calprotectin positive (≥ 50 µg/g)	Calprotectin negative (< 50 µg/g)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

Is CalproQuest sensitive/specific for IBD?		Patients with Calprotectin ≥ 50 µg/g		
		IBD (confirmed by endoscopy)	Non-IBD (confirmed by endoscopy)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

TP true positive
FP false positive
TN true negative
FN false negative

Sensitivity and specificity calculation of CalproQuest
210x297mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	1-14
Funding	4	Sources and types of financial, material, and other support	7,11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	11
	5b	Name and contact information for the trial sponsor	1, 11
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4	
	6b	Explanation for choice of comparators	-	(no RCT)
Objectives	7	Specific objectives or hypotheses	4	
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5	
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9	
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5	
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6	
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5	
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5, 6	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9	

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	no RCT
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	no RCT
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	no RCT
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	no RCT
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	no RCT
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-8
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6, 8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8, 10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9, 11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	8, 10
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in English
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

VAlidation of an 8-item-questionnaire predictive for a positive CaLprotectin tEst and Real-life implemenTation in primary care to reduce diagnostic delay in inflammatory bowel disease (ALERT): protocol for a prospective diagnostic study

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Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Gastroenterology and hepatology, General practice / Family practice, Health services research
Keywords:	Inflammatory bowel disease < GASTROENTEROLOGY, sensitivity, specificity, early diagnosis, feasibility

VAlidation of an 8-item-questionnaire predictive for a positive CaLprotectin tEst and Real-life implemenTation in primary care to reduce diagnostic delay in inflammatory bowel disease (ALERT): protocol for a prospective diagnostic study

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Word count

3'059 (abstract 290)

ABSTRACT

Introduction

Diagnosis of inflammatory bowel disease (IBD) in primary health care is challenging and often associated with a considerable diagnostic delay. This delay is associated with worse disease progression and outcomes. Although testing for fecal Calprotectin is a useful screening tool to identify patients who need endoscopy for IBD, the widespread use may not be appropriate due to the low prevalence of patients with IBD among all patients attending a general practitioner (GP) with gastrointestinal symptoms. To increase the appropriate application of the fecal Calprotectin test, an 8-item-questionnaire, the CalproQuest, has been developed to increase pretest-probability for a positive test result.

Methods and analysis

This is a prospective diagnostic trial. The study consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patients included in part A are referred to the gastroenterologist for any endoscopic evaluation. Patients included in part B present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

CalproQuest consists of 4 main and 4 secondary questions specific for IBD it is considered positive, if ≥ 2 main criteria are answered positively or 1 main criterion and 2 secondary criteria are answered positively.

In part A the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis will be investigated. In part B the feasibility of CalproQuest in daily primary health care practice will be assessed.

Ethics and dissemination

The study protocol was approved by the Ethics Committee of the Kanton Zurich (reference KEK-ZH-number 2013-0516). The results will be published in a peer-reviewed journal and shared with the worldwide medical community.

Trial registration number

The study protocol is registered at ISRCTN66310845

INTRODUCTION

IBD versus IBS

Crohn's Disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC) represent the three subtypes of inflammatory bowel disease (IBD) [1]. Estimated prevalence rates of IBD in Swiss population are about 205.6 cases per 100'000 [2].

Meanwhile, the prevalence of irritable bowel syndrome (IBS) in Europe and North America is estimated to be 10 – 15% [3].

Physicians are often faced with the diagnostic challenge of differentiating patients with IBD from functional disorders such as irritable bowel syndrome (IBS). Indeed, symptoms similar to IBS are frequently reported in patients before IBD is diagnosed [4]. The gold diagnostic standard is endoscopy; however, not every patient with the symptoms overlapping with those of IBS can be investigated by the invasive endoscopic exam. Therefore, many different, non-invasive markers have been investigated. Several studies have shown that fecal Calprotectin accurately reflects intestinal inflammation in patients with known IBD [5].

It has also been shown to consistently differentiate IBD from IBS due to its excellent negative predictive value. It can therefore be used ruling out IBD in undiagnosed, symptomatic patients [6].

Calprotectin, a S100 protein

Calprotectin is a complex of two calcium-binding proteins that belong to the S100 protein family [7]. It is abundant in the cytosolic fraction of neutrophils. High levels of Calprotectin have been found in extracellular fluid during various inflammatory conditions, such as rheumatoid arthritis, cystic fibrosis and abscesses. Calprotectin released from neutrophils has growth-inhibitory and apoptosis-inducing activities against various cell types including tumor cells and normal fibroblasts [7]. This suggests that Calprotectin has regulatory activities during inflammatory processes through its effect on the survival or growth states of cells participating in the inflammatory reaction. Furthermore, Calprotectin inhibits microbial growth through competition for zinc [8].

Calprotectin has been shown to be stable in feces during storage for 7 days at room temperature, which is very important for its value in evaluating mucosal wall inflammation [9].

Stool Calprotectin levels as marker of intestinal inflammation

Data correlated to fecal Calprotectin showed a sensitivity and specificity of Calprotectin (using a cut-off value of 10mg/l) for organic disease of 89% and 79%, respectively [10]. Studies using cut-off value of 50 µg/g showed a sensitivity and specificity of 86.8% and 95.7% [11] and 83% and 100% [12] discriminating IBD versus IBS. Fecal Calprotectin levels correlate significantly with histological and endoscopic assessment of disease activity in ulcerative

colitis (UC) [13-15] as well as with fecal alpha-1-antitrypsin levels and fecal excretion of 111indium-labeled white blood cells in patients with Crohn's disease (CD) [16 17]. Previous studies showed that stool Calprotectin levels correlate well with endoscopic indices both in ulcerative colitis and in Crohn's disease [18-20].

Diagnostic delay in IBD is predictive for worse disease progression and outcomes

Difficulties in differentiation early IBD from IBS, especially in a primary health care, is leading to a considerable diagnostic delay in IBD [1]. This delay has important clinical impact, as there is increasing evidence demonstrating that treatment success is increased in early disease [21-24]. Vavricka and colleagues suggest, that diagnostic delay is sub-dived into two intervals, where interval 1 is defined as time from first symptoms to physician visit, and interval 2 the time from first physician visit to IBD diagnosis [1]. The study by Vavricka et al. estimates that 25% of all CD and UC patients experienced more than 24 and 12 months, respectively, from first onset of symptoms until an accurate IBD diagnosis [1]. Most importantly Schoepfer and colleagues recently showed, that the length of diagnostic delay is correlated with an increased risk of bowel stenosis and CD-related intestinal surgery, concluding that efforts should be undertaken to shorten diagnostic delay [25].

Testing Calprotectin in Switzerland

Most analytical laboratories in Switzerland offer Calprotectin testing, which is reimbursed by health insurances.

Fecal Calprotectin testing in primary health care versus tertiary healthcare

Although Calprotectin tests are easily accessible and reimbursed in Switzerland, this diagnostic test is not routinely performed in primary health care. However, the low prevalence of IBD in the primary health care must be taken into account: Analysing the reasons for encounter in primary health care, the group of digestive disorders is not one of the predominant diagnostic groups. Scandinavian studies show frequencies between 5-7% [26 27]. Focusing on this diagnostic group, IBS is much more common in primary health care (population-based prevalence of 10-15% of IBS compared with 0.2% of IBD). Therefore, a new tool has been developed to narrow the patient collective in which Calprotectin testing may lead to the correct diagnosis of IBD in an earlier stadium.

Hypothesis and goal

This study pursuits two main aims A and B, which are investigated independently:

A. Prospective validation and evaluation of sensitivity and specificity of an 8-item IBD-questionnaire (CalproQuest; see Table 1) for 1) a positive Calprotectin test result ≥ 50 µg/g feces and for 2) a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis, respectively, in tertiary healthcare

B. Prospective implementation of CalproQuest in primary health care to investigate feasibility in daily practice.

METHODS AND ANALYSIS

Study design

The study is a prospective diagnostic trial. It consists of two independent and consecutive parts A and B, conducted by gastroenterologists (A) and general practitioners (GPs) (B), respectively.

Patients included in part A of the study are referred for endoscopic evaluation to gastroenterologists. Patients included in part B of the study present at their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks.

The study design and procedure are summarized in Figure 1.

Inclusion and exclusion criteria

Patients will be eligible if they

- Are ≥18 years old (part A, B)
- Are referred to their gastroenterologist for any endoscopic examination (part A)
- Visit their GP because of on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for at least two weeks (part B)
- Underwent no earlier diagnostic procedures (endoscopy) for the current episode (part B)

Patients are not eligible, if they

- Are younger than 18 years (part A, B)
- Have known further/other abdominal pathologies as e.g. cancer (part A, B)
- Had previous abdominal surgeries (part B)
- Have been treated with steroids (topical and/or oral) and/or aminosalicylates within 30 days prior inclusion into this study (part B)
- Underwent endoscopic examination within 3 years prior screening (part B)

Primary and secondary outcomes

Primary outcomes:

A. 1. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces

2. Sensitivity and specificity of CalproQuest for a positive Calprotectin test result ≥ 50 µg/g feces and positive IBD-diagnosis.

B. Feasibility of CalproQuest in daily primary health care practice.

Secondary outcomes:

A. Patient-reported diagnostic delay.

B. Patient-reported acceptance of stool sampling.

Procedure of the study

In brief, the study will be divided in two independent parts A and B, conducted by gastroenterologists (A) and GPs (B), respectively. Patient data will be encoded.

A. Investigation of the sensitivity and specificity of CalproQuest for stool Calprotectin levels ≥ 50 µg/g feces and for positive IBD diagnosis

Patients referred to the gastroenterologist for endoscopic examination are subjected to CalproQuest and Calprotectin stool testing prior endoscopy. At baseline T0, patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens from outpatients will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. At T2, endoscopic examination will be performed to obtain a diagnosis. Eventually, patients diagnosed with IBD will be asked to complete a questionnaire at T3 investigating duration of first onset of symptoms to IBD diagnosis (diagnostic delay).

B. Investigation of feasibility of CalproQuest in daily primary health care practice

Patients with on-going unspecific gastrointestinal symptoms (abdominal pain, bloating, stool irregularities, diarrhea) for more than two weeks presenting at the GP will be included into the study if all inclusion criteria are met and informed patient consent is obtained.

At baseline (T0), patients will be subjected to CalproQuest. Subsequently, at T1 fecal samples will be obtained to measure Calprotectin levels. The patients themselves will perform collection of the fecal specimens. The fecal specimens will be shipped to the laboratory at the University Hospital Zurich by mail. After measurement, fecal samples will be disposed according to current guidelines. According to the current standard of care it is recommended to refer patients with Calprotectin levels ≥ 50 µg/g to a gastroenterologist for endoscopic examination at T2; results of the endoscopy are communicated back to the GP. Patients will be asked at T3 to complete a questionnaire on acceptance of stool sampling, and physicians will complete the questionnaire on feasibility of CalproQuest in daily practice.

CalproQuest

CalproQuest is an 8-item IBD-questionnaire consisting of 4 main and 4 secondary questions specific for IBD (see Table 1). CalproQuest was pre-validated by IBD-experts trough an international Delphi-process. CalproQuest is considered positive, if ≥ 2 main criteria or 1 main criterion and 2 secondary criteria are answered positively.

We assume that a positive CalproQuest result may predict Calprotectin levels ≥ 50 µg/g. Calprotectin levels above 50 µg/g are indicative for on-going intestinal inflammation and call for further endoscopic examination.

Table 1: CalproQuest (8-item IBD questionnaire)

Type	Criteria	Yes (1)	No (0)	Comment
Major	Does the patient suffer from abdominal pain at least 3 times a week for at least 4 weeks?			
	Does the patient suffer from diarrhoea (more than 3 bowel movements daily) for 7 consecutive days?			
	Does the patient have diarrhoea at night-time/Does the patient awake from sleep because of abdominal pain or diarrhoea?			
	Does the patient report bloody stool?			
Minor	Does the patient report mucus in stool for more than 4 weeks?			
	Does the patient report unwanted weight loss (5% of normal body weight over 6 months)?			
	Does the patient present with fever or report fever over the last 4 weeks (Temp > 38°C)?			
	Does the patient report fatigue over the last 4 weeks?			

Fecal Calprotectin

Fecal Calprotectin levels will be measured at the University Hospital Zurich by a novel ELISA-based Calprotectin test named EliA Caprotectin (Thermo Scientific, for product description see http://www.phadia.com/PageFiles/29347/Product%20information%20EliA%20Calprotectin.pdf).

Patient questionnaire on diagnostic delay

Three relevant time intervals of diagnostic delay will be assessed in a patient questionnaire. The time intervals are defined as follows (see Figure 2):

1. Interval 1: Time from first IBD related symptoms to first consultation with the GP: This interval represents the time span between the first manifestations of IBD-related symptoms (patient-reported) and a consultation with the GP specifically due to these

IBD-related complaints. The length of this period is mainly dependent on the patient herself/himself.

2. Interval 2: Time from the first GP visit to referral to a gastroenterologist: This represents the time span between the IBD symptom-related consultation of the GP and the time of referral to a gastroenterologist for further examination. The length of this period is mainly dependent on the treating GP.
3. Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2): This interval is calculated by the addition of interval 1 and 2 and is defined as diagnostic delay. Diagnostic delay is defined as the time span (in weeks) from first symptoms to IBD diagnosis.

The following items in the patient questionnaires are assessed for the purpose of this study: ‘‘Before the IBD diagnosis, how long did you experience symptoms that are now attributed to IBD?’’ and ‘‘How long was the time interval between first symptoms and the first visit to your GP?’’ and “How long were you treated by your GP before referral to a gastroenterologist?” and ‘‘What was the time span from the first physician visit (due to these complaints) until IBD diagnosis was established?’’ Additionally, patients will answer questions regarding smoking habits, intake of non-steroidal anti-inflammatory drugs (NSAIDs), or oral contraception at the time of diagnosis.

Physician questionnaire on feasibility and acceptance of CalproQuest in primary health care

Goal of the feasibility questionnaire is to investigate feasibility and acceptance of CalproQuest in daily primary health care practice.

The questionnaire is based on an even-point Likert scale consisting of seven items.

Patient questionnaire on acceptance of stool sampling

Aim of the patient’s acceptance questionnaire is to investigate patients’ physical and mental ability to handle stool sampling at home.

The questionnaire is based on an even-point Likert scale consisting of four Likert items.

Administration of patient records

Physicians will be supplied with a master data list providing patient codes that can be assigned to the patient. All documents containing patient data will carry the respective patient code assigned by the physician. Encoded documents will be sent to the Institute of Primary Care, University of Zurich, Switzerland and stored for 10 years. Only physicians have access to the patient codes. Data entry is performed continuously at the Institute of Primary Care, University of Zurich, and if data is missing, a research assistant will investigate to obtain all information as required.

Sample size calculation

In part A, sample size was calculated according to Flahault et al.[28]. Assuming a 0.05 two-sided significance level, n=162 would have 90 % power to detect a sensitivity and specificity of 90% of CalproQuest for a Calprotectin level ≥ 50 µg/g feces, or for a Calprotectin level ≥ 50 µg/g feces and a positive IBD diagnosis. For the purpose of this calculation, expected sensitivity and specificity are 90% with a lower acceptable limit of sensitivity of 70%. Assumed prevalence of IBD within the sample is 20%. A $p<0.05$ is considered statistically significant. 80 patients were assumed to be necessary for the feasibility testing in part B.

Statistical data evaluation

We provide sensitivity and specificity calculation of CalproQuest based on confidence intervals (see Figure 3). For the other primary or secondary outcomes parametric or non-parametric tests are used where appropriate.

Physician recruitment

Physician recruitment is ongoing, to achieve the target number of 162 patients about 12-15 gastroenterologists and IBD-centres will be recruited in part A and 30 GPs in part B. Country of recruitment: Switzerland. Physicians will receive a financial incentive.

Patient recruitment

Physicians from part A and B are asked to approach consecutively patients eligible for the trial. Patients don't receive a financial incentive, the stool sampling material and the fecal Calprotectin test will be provided for free.

Patient informed consent

Prior to study participation patients receive written and oral information about the consent and extent of the planned study. In case of acceptance they sign the informed consent form. In case of study discontinuation all material will be destroyed or the patient will be asked if he/she accepts that the existing material can be used for the study.

Time frame of the study

Recruitment of gastroenterologists and GP's started in October 2014. Recruitment time of the eligible patients will be 12 months. Data analysis will be performed 12 months after recruiting the last study centre (part A: gastroenterologists, part B: GPs) or earlier, when the target number of patients (part A: 162, part B: 80) has been achieved.

Description of risks

In part A an endoscopy will be performed, however these patients are already referred for endoscopic evaluation to gastroenterologists. Therefore an additional risk is not expected.

Confidentiality

The patient names and all other confidential information fall under medical confidentiality rules and are treated according to appropriate Federal Data Security Laws. The results of the patient questionnaires are not accessible to the GPs. Questionnaires are directly mailed to the study centre by the patient. All study related data and documents are stored on a protected central server of the University of Zurich. Only direct members of the internal study team can access the respective files. Intermediate and final reports are stored in the office of the Institute of Primary Care at the Zurich University Hospital.

DISCUSSION

An early diagnosis of IBD is associated with a better outcome. In primary health care the prevalence of IBD is much lower than in tertiary healthcare (gastroenterologist, or even in an IBD-centre), where patients are already preselected by GPs. The emphasis in tertiary healthcare is usually on “ruling in”: increasing the probability of IBD to carry out more expensive, time consuming, and invasive procedures; establish a firm diagnosis; and start appropriate treatment. At tertiary healthcare level a diagnostic test with a high positive likelihood ratio is preferred. In primary health care, the emphasis is on “ruling out”: lowering the probability of the target disease to provide reassurance, or to adopt a “watchful waiting” strategy. In these instances tests with a low negative likelihood ratio are preferred [6].
Increasing the sensitivity and specificity of fecal Calprotectin test by the CalproQuest questionnaire, which is feasible in the primary health care, we could provide a simple, convenient tool lowering the diagnostic delay in patients with IBD.

Limitations and strength

No conclusions can be made about the sensitivity or specificity of the test in primary health care, as the sample size is too low (n= 80 patients in part B). From ongoing other studies with diseases, which are more prevalent, we know that recruitment of patients in primary health care is very difficult since participation is based on GPs free choice and implementation of research in the daily routine of a general practice is time-consuming. We therefore chose the design of 2 different parts in two different settings. If CalproQuest is validated in tertiary healthcare, further studies will be needed to evaluate the accuracy of the test in primary health care.
In primary health care (part B), patients present themselves with symptoms, not with a presumed diagnosis. This is a difference between the tertiary healthcare (part A), where almost every patient might already have had the first endoscopic investigations as well as treatment and is referred because they don’t response on the established therapy or unclearness of the diagnosis. As different habits or conditions interfere with the value of

Calprotectin, the exclusion criteria in some studies are strict (regular intake of aspirin and/or non-steroid anti-inflammatory drugs, urinary incontinence as fecal Calprotectin is not water proof, infectious enterocolitis, colorectal cancer etc.) [19]. As we want to determine the feasibility in primary health care population, we do not exclude patients presenting with these conditions, because we want to represent the "real life" in the daily routine of a GP.

TRIAL STATUS

Patient recruitment had started in November 2014.

LIST OF ABBREVIATIONS

CD	Crohn's Disease
GP	general practitioner
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IC	indeterminate colitis
UC	Ulcerative Colitis

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The funding sources had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

TR, SV and GR were the initiators for this study. TR is the trial sponsor. TR, SV, GR and NZ developed the questionnaires. NZ, SV and GR organized the recruitment of the gastroenterologists. SH, RT, SM and TR organized the recruitment of the practices. SH wrote and revised the final manuscript and all authors read and approved it.

FIGURES

Figure 1: Study design

Figure 2: Interval 1-3 in diagnostic delay, adapted from [1]

Interval 1: Time from first IBD symptoms to consultation with the GP

Interval 2: Time from GP visit to referral to a gastroenterologist

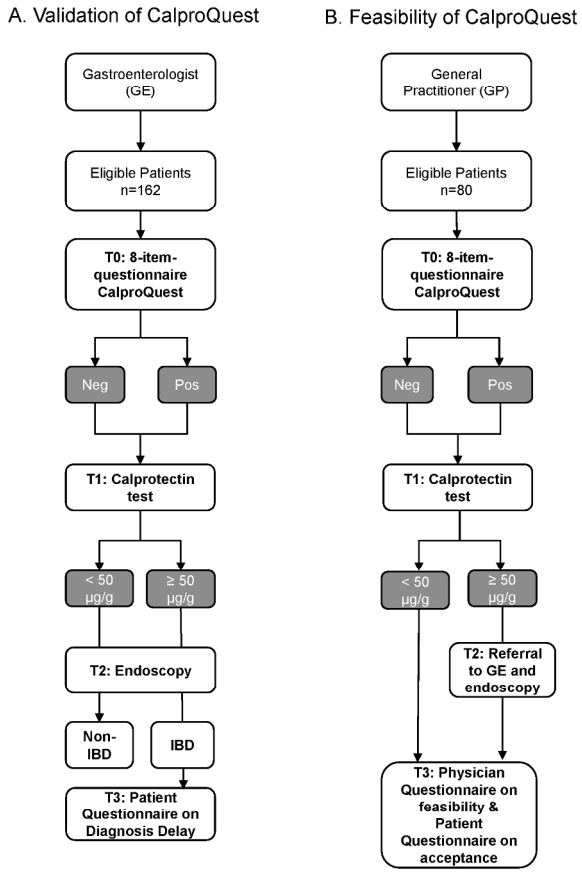
Interval 3: Time from first IBD symptoms to IBD diagnosis (interval 1+2)

Figure 3: Sensitivity and specificity calculation of CalproQuest

REFERENCES

1. Vavricka SR, Spigaglia SM, Rogler G, et al. Systematic evaluation of risk factors for diagnostic delay in inflammatory bowel disease. Inflammatory bowel diseases 2012;**18**(3):496-505.
2. Juillerat P, Pittet V, Bulliard JL, et al. Prevalence of Inflammatory Bowel Disease in the Canton of Vaud (Switzerland): A population-based cohort study. Journal of Crohn's & colitis 2008;**2**(2):131-41.
3. Guideline WGOG. Irritable bowel syndrome: a global perspective http://www.worldgastroenterology.org/assets/downloads/en/pdf/guidelines/20_irritable_bowel_syndrome.pdf. 2009.
4. Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? Gastroenterology clinics of North America 2005;**34**(2):235-45, vi-vii.
5. Lin JF, Chen JM, Zuo JH, et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflammatory bowel diseases 2014;**20**(8):1407-15.
6. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. Bmj 2010;**341**:c3369.
7. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biological & pharmaceutical bulletin 2003;**26**(6):753-60.
8. Loomans HJ, Hahn BL, Li QQ, et al. Histidine-based zinc-binding sequences and the antimicrobial activity of calprotectin. The Journal of infectious diseases 1998;**177**(3):812-4.
9. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scandinavian journal of gastroenterology 1992;**27**(9):793-8.
10. Tibble JA, Sigthorsson G, Foster R, et al. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 2002;**123**(2):450-60.
11. Otten CM, Kok L, Witteman BJ, et al. Diagnostic performance of rapid tests for detection of fecal calprotectin and lactoferrin and their ability to discriminate inflammatory from irritable bowel syndrome. Clinical chemistry and laboratory medicine : CCLM / FESCC 2008;**46**(9):1275-80.
12. Schoepfer AM, Trummler M, Seeholzer P, et al. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP, and IBD antibodies. Inflammatory bowel diseases 2008;**14**(1):32-9.
13. Limburg PJ, Ahlquist DA, Sandborn WJ, et al. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. The American journal of gastroenterology 2000;**95**(10):2831-7.
14. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;**58**(2):176-80.
15. Costa F, Mumolo MG, Ceccarelli L, et al. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005;**54**(3):364-8.
16. Tibble J, Teahon K, Thjodleifsson B, et al. A simple method for assessing intestinal inflammation in Crohn's disease. Gut 2000;**47**(4):506-13.
17. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scandinavian journal of gastroenterology 1999;**34**(1):50-4.
18. Schoepfer AM, Beglinger C, Straumann A, et al. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflammatory bowel diseases 2009;**15**(12):1851-8.

19. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. The American journal of gastroenterology 2010;**105**(1):162-9.
20. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflammatory bowel diseases 2012;**18**(12):2218-24.
21. Markowitz J. Early inflammatory bowel disease: different treatment response to specific or all medications? Digestive diseases 2009;**27**(3):358-65.
22. Etchevers MJ, Aceituno M, Sans M. Are we giving azathioprine too late? The case for early immunomodulation in inflammatory bowel disease. World journal of gastroenterology : WJG 2008;**14**(36):5512-8.
23. Punati J, Markowitz J, Lerer T, et al. Effect of early immunomodulator use in moderate to severe pediatric Crohn disease. Inflammatory bowel diseases 2008;**14**(7):949-54.
24. Gupta N, Bostrom AG, Kirschner BS, et al. Presentation and disease course in early- compared to later-onset pediatric Crohn's disease. The American journal of gastroenterology 2008;**103**(8):2092-8.
25. Schoepfer AM, Dehlavi MA, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. The American journal of gastroenterology 2013;**108**(11):1744-53; quiz 54.
26. Mansson J, Nilsson G, Strender LE, et al. Reasons for encounters, investigations, referrals, diagnoses and treatments in general practice in Sweden--a multicentre pilot study using electronic patient records. The European journal of general practice 2011;**17**(2):87-94.
27. Moth G, Olesen F, Vedsted P. Reasons for encounter and disease patterns in Danish primary care: changes over 16 years. Scandinavian journal of primary health care 2012;**30**(2):70-5.
28. Flahault A, Cadilhac M, Thomas G. Sample size calculation should be performed for design accuracy in diagnostic test studies. Journal of clinical epidemiology 2005;**58**(8):859-62.



Study design
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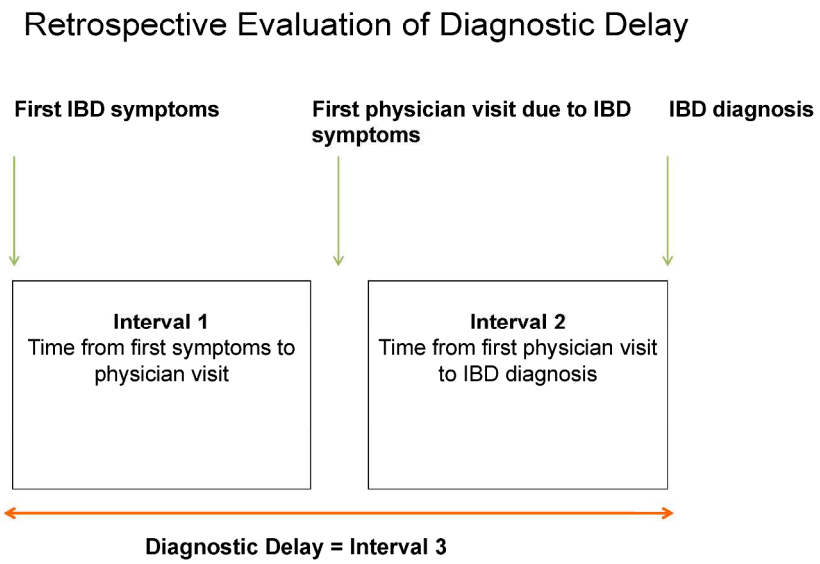

Interval 1-3 in diagnostic delay, adapted from Vavricka et al. [1]
297x210mm (300 x 300 DPI)

Evaluation of sensitivity/specificity and PPV/NPV of CalproQuest

Is CalproQuest sensitive/specific for Calpro ≥ 50 µg/g?		Patients referred to GE for endoscopic examination		
		Calprotectin positive (≥ 50 µg/g)	Calprotectin negative (< 50 µg/g)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

Is CalproQuest sensitive/specific for IBD?		Patients with Calprotectin ≥ 50 µg/g		
		IBD (confirmed by endoscopy)	Non-IBD (confirmed by endoscopy)	
CalproQuest	Positive (≥2 main criteria OR 1 main and 2 secondary criteria)	TP	FP	Positive Predictive Value (PPV) = TP / (TP+FP)
	Negative	FN	TN	Negative Predictive Value (NPV) = TN / (FN+TN)
		Sensitivity = TP / (TP+FN)	Specificity = TN / (FP+TN)	

TP true positive
FP false positive
TN true negative
FN false negative

Sensitivity and specificity calculation of CalproQuest
210x297mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	1-14
Funding	4	Sources and types of financial, material, and other support	7,11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	11
	5b	Name and contact information for the trial sponsor	1, 11
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	1
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

Introduction				
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3,4	
	6b	Explanation for choice of comparators	-	(no RCT)
Objectives	7	Specific objectives or hypotheses	4	
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5	
Methods: Participants, interventions, and outcomes				
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9	
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5	
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6	
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9	
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5	
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	5, 6	
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	9	

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	no RCT
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	no RCT
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	no RCT
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	no RCT
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	no RCT
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	6-8
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6, 8
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	-
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	-
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	-
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	9
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	-
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	2
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	-
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8, 10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9, 11
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	8, 10
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	-
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in English
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	-

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.