BMJ Open

Multicentric randomized study of H. pylori eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-016999
Article Type:	Protocol
Date Submitted by the Author:	31-Mar-2017
Complete List of Authors:	Leja, Marcis; Latvijas Universitate, Institute of Clinical and Preventive Medicine Park, Jin Young; International Agency for Research on Cancer, Prevention and Implementation Group Murillo, Raul; International Agency for Research on Cancer, Prevention and Implementation Group Liepniece-Karele, Inta; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Austrumu kliniska universitates slimnica, Department of Research Isajevs, Sergejs; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Austrumu kliniska universitates slimnica, Department of Research Kikuste, Ilze; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Digestive Diseases Centre GASTRO Rudzite, Dace; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Austrumu kliniska universitates slimnica, Department of Research Krike, Petra; Latvijas Universitate, Institute of Clinical and Preventive Medicine Parshutin, Sergei; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Tehniska universitate, Institute of Information Technology Polaka, Inese; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Tehniska universitate, Institute of Information Technology Kirsners, Arnis; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Tehniska universitate, Institute of Information Technology Santare, Daiga; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Austrumu kliniska universitates slimnica, Department of Research Folkmanis, Valdis; Latvijas Universitate, Faculty of Medicine Daugule, Ilva; Latvijas Universitate, Institute of Clinical and Preventive Medicine Plummer, Martyn; International Agency for Research on Cancer, Herrero, Rolando; IARC, Early Detection and Prevention
Primary Subject Heading:	Public health
Secondary Subject Heading:	Gastroenterology and hepatology, Oncology, Epidemiology
Keywords:	Gastric cancer, prevention, H. pylori, eradication, pepsinogen, randomised

	study

Title page

Multicentric randomized study of *H. pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study

Marcis Leja[1-3], Jin Young Park[4], Raul Murillo[4], Inta Liepniece-Karele[1, 2 ,5], Sergejs Isajevs[1 ,2, 5], Ilze Kikuste[1, 3], Dace Rudzite[1, 2], Petra Krike[1], Sergei Parshutin[1, 6], Inese Polaka[1, 6], Arnis Kirsners[1, 6], Daiga Santare[1, 2], Valdis Folkmanis[1], Ilva Daugule[1], Martyn Plummer[7], Rolando Herrero[4]

1- Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia, Riga, Latvia
2- Department of Research, Riga East University hospital, Riga, Latvia
3- Digestive Diseases Centre GASTRO, Riga, Latvia
4- Prevention and Implementation Group, International Agency for Research on Cancer, Lyon, France
5- Academic Histology Laboratory, Riga, Latvia
6- Institute of Information Technology, Riga Technical University, Riga, Latvia
7- Infection and Cancer Epidemiology Group, International Agency for Research on Cancer, Lyon, France

Contact details of the authors:

Marcis Leja, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: cei@latnet.lv

Jin Young Park, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: ParkJY@iarc.fr

Raul Murillo, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: MurilloR@iarc.fr

Inta Liepniece-Karele, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: intaliepniecekarele@inbox.lv

Sergejs Isajevs, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: sergisajevs@inbox.lv

Ilze Kikuste, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: ikikuste@gmail.com

Dace Rudzite, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: dacerudzite2008@inbox.lv

Petra Krike, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: petra.krike@gmail.com

Sergei Parshutin, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: Sergejs.Parsutins@rtu.lv

Inese Polaka, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: inese.polaka@gmail.com

Arnis Kirsners, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: arnis.kirsners@rtu.lv

Daiga Santare, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: daiga.santare@inbox.lv

Valdis Folkmanis, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: valdis.folkmanis@lu.lv

Ilva Daugule, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: ilva_daugule@hotmail.com

Martyn Plummer, Infection and Cancer Epidemiology Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: plummerm@iarc.fr

Rolando Herrero, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: HerreroR@iarc.fr

Corresponding author:

Prof. Marcis Leja: Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia, Riga, Latvia; Address: 19 Raina boulv., Riga LV1586; Tel.: +371-29497500; Fax: +371-67040248, E-mail: cei@latnet.lv

Abstract

Introduction: Population-based eradication of *H. pylori* has been suggested to be cost-effective and is recommended by international guidelines. However, the potential adverse effects of widespread antibiotic use this would entail have not been sufficiently studied. An alternative way to decrease gastric cancer mortality is by non-invasive search for precancerous lesions, in particular gastric atrophy; pepsinogen tests are the best currently available alternative.

Methods and analysis: The primary objective of GISTAR is to determine whether *H. pylori* eradication combined with non-invasive screening of precancerous lesions by pepsinogen tests reduces mortality from gastric cancer among 40-64 year old individuals. The secondary objectives include evaluation of *H. pylori* eradication effectiveness in gastric cancer prevention in patients already carrying precancerous lesions and evaluation of the potential adverse events (including effects on microbiome).

Individuals are recruited from general population (50% men), and undergo detailed lifestyle and medical history questionnaire before being randomly allocated to intervention or control groups. The intervention group undergoes *H. pylori* testing, and is offered eradication therapy if positive; in addition, pepsinogen levels are detected in plasma, and those with decreased levels are referred for upper endoscopy. Effectiveness of eradication and the spectrum of adverse events are evaluated in study subpopulations. A 35% difference in gastric cancer mortality between the groups is expected to be detectable at 90% power after 15 years if 30,000 individuals are recruited. Biological materials (e.g. serum, plasma, DNA, breath sample, material for microbiota analysis) are biobanked for the main and ancillary studies. The study procedure and assumptions will be tested during the pilot phase.

Ethics and dissemination: The study was approved by the Ethics Committees of the involved institutions. An independent Data Safety and Monitoring Board has been established. The findings will be published in peer-reviewed journals and presented at scientific meetings.

Trial registration number: NCT02047994

Strengths and limitations of this study

- This is currently the only study in Europe addressing population-based eradication of *H. pylori* to prevent gastric cancer as recommended by international guidelines (Maastricht V, Kyoto Global Consensus, EU Joint Action Cancer prevention project CanCon)
- Gastric cancer mortality is used as an end-point which corresponds to the requirements of a cancer screening program
- The strategy of combining population-based *H. pylori* eradication to pepsinogen detection with endoscopic surveillance of participants in whom precancerous lesions have been detected, has not been evaluated before
- The study biorepository with a wide range of biospecimen collection will be a great resource to conduct a number of unique ancillary studies
- However, the large sample size with a long follow-up required to demonstrate a statistical difference in mortality reduction between the two groups is a challenge to the study, with the possibility to increase the sample size even further in case of lower prevalence of *H. pylori* infection, higher number of women in the study group and/or lower acceptance rate for the intervention

Keywords

Gastric cancer, prevention, *H. pylori*, eradication, pepsinogen, randomised study

Introduction

Although gastric cancer remains a major cause of death among malignant diseases, its prevention has been neglected in the Western world for decades.[1] Most countries show declining gastric cancer incidence trends, but the total number of cases is not expected to decrease in the next decades due to demographic changes.[2]

There are considerable geographical variations in the incidence of gastric cancer, with some of the lowest rates seen in North America and Western Europe and the highest in Eastern Asia, Eastern Europe, and South America.[3] According to recent estimates from Europe, high rates have been observed in Central and Eastern European regions including Belarus, Ukraine and the Russian Federation and the Baltic States in the Northern Europe, including Latvia. For example, gastric cancer incidence rates are the highest in Belarus (age-standardised rate (ASR) of 42.1/100,000 in men and 17.2/100,000 in women) among 40 European countries.[4 5] These estimates are over three-fold higher than those in France or Switzerland.[4 5] The rates in the majority of the former Soviet Union regions remain high.

Infection with *Helicobacter pylori* (*H. pylori*) is the major etiologic factor responsible for developing gastric cancer.[6 7] It is estimated that 89% of non-cardia gastric cancers are attributable to this infection.[8]

Searching for and eradicating *H. pylori* in healthy asymptomatic adults (the "*search-and treat*" strategy) has been suggested to be cost-effective by considering the reduction of gastric cancer burden as well as other diseases related to this microorganism.[9-11] The recent global Kyoto conference[12] encouraged the broad application of *search-and-treat*, particularly in high-risk areas. This has been further endorsed by the Maastricht European consensus group.[13] However, due to the limited data available on target groups, feasibility and population impact of the intervention, a working group convened by the International Agency for Research on Cancer (IARC) in 2013 proposed implementation of the strategy via well-designed implementation studies.[1]

H. pylori eradication in the general population would lead to high antibiotic consumption, particularly in areas of high prevalence of the infection. Widespread use of the same antibiotics used to treat common diseases, some of them life-threatening, may lead to increased antibiotic resistance of microorganisms other than *H. pylori*.[14] An inverse association observed between the occurrence of gastric and oesophageal cancers may suggest potential opposing effects of the related environmental factors, including *H. pylori*.[15]

The potential risks of these effects in community settings were not considered in the above mentioned cost-effectiveness analyses[9-11], and knowledge about the potential adverse effects of *H. pylori* eradication on the gut microbiome is scant.

Therefore, the recently published European Guide on Quality Improvement in Comprehensive Cancer Control emphasised the need for additional clinical studies to clarify whether and how to implement population-based *H. pylori* screening and eradication programmes for gastric cancer prevention.[16]

In addition to the population-based eradication of *H. pylori*, detection and treatment of precancerous lesions or early gastric cancer have been proposed as a means to reduce gastric cancer mortality and some countries in the Western Pacific region have introduced nationwide gastric cancer screening programmes.[17] Pepsinogen testing is currently the best available non-invasive option to identify individuals with precancerous lesions (in particular, gastric atrophy)[13] who are at increased risk of gastric cancer. However, a recent meta-analysis[18] concluded that pepsinogens exhibit only a moderate diagnostic yield in gastric cancer detection; thus large-scale and well-designed prospective studies are encouraged, particularly in East, Central and part of Northern Europe and Latin American countries where gastric cancer burden is relatively high and prevention effort is scarce.

Here, we present the design of a clinical trial aimed at investigating the role of *H. pylori* eradication combined with non-invasive screening for precancerous lesions in the reduction of gastric cancer mortality in a predominantly Caucasian population in Northern and Eastern Europe (GISTAR).

Methods and analysis

The **aim** of the study is to search for new intervention strategies to decrease mortality from gastric cancer in high-risk areas in the Baltic States and Eastern Europe. The main study site is Latvia, where the estimated ASR (world) per 100,000 for gastric cancer mortality was 16.2 in men and 6.4 in women in 2011. Other potential sites include the Russian Federation with gastric cancer mortality of 20.8 in men and 8.5 in women, Belarus with 20.2 in men and 7.8 in women, and Ukraine with 17.4 in men and 6.6 in women in 2011.[19]

The **primary objective** is to determine if *H. pylori* eradication combined with non-invasive screening and follow-up of precancerous lesions (atrophic gastritis or higher) reduces mortality from gastric cancer in a high risk population among 40-64 years old subjects.

In addition to the above, **secondary objectives** include analyses of success-rates of *H. pylori* eradication therapy, resistance rates of *H. pylori* to the key antibiotics used in standard therapies (in subgroups), potential adverse effects of population-based eradication (including effects on gut microbiome), optimisation of follow-up strategies as well as search for new biomarkers, including volatile markers.

The **key hypotheses** of the study are: 1) *H. pylori* eradication in middle aged individuals in a high risk population with endoscopic follow-up of those with evidence of atrophic gastritis prevents gastric cancer mortality; 2) *H. pylori* eradication is effective in preventing gastric cancer mortality even after the development of gastric mucosal atrophy; 3) Certain population subgroups can derive more benefit from *H. pylori* eradication, and therefore could be targeted if general population eradication is not feasible; 4) A combination of biomarker screening and upper endoscopy is an appropriate strategy to prevent mortality from gastric cancer in high incidence areas.

The study protocol (version 4.5, revised on 07 September, 2015) was approved by the Ethics Committee of the International Agency for Research on Cancer (IEC 12-36) as well as the national Ethics Committees in Latvia; the Ethics Committee of Riga East University Support Foundation (No.14-A/13) and the Central Medical Ethics Committee (No. 01-29.1/11). The protocol is registered in the clinicaltrials.gov database (NCT02047994). It needs to be noted that the study initiation date recorded on the Clinical Trials Registry reflects the commencement of the pilot phase of the study which was conducted between October 2013 and December 2015 while the main GISTAR trial that we present here was initiated on 13 March 2016.

Participants

The study aims to enrol men and women at equal proportions at the risk- age (40-64 years at inclusion) for developing gastric cancer. The recruitment centres are planned in high gastric cancer risk areas, and predominantly Caucasian origin populations in Europe will be enrolled. The initial enrolment is planned in Latvia (Caucasian population); more genetically diverse populations would be enrolled when the study is expanded to other sites.

All participants must sign an informed consent and they should be in good health at enrolment, as determined by medical history and physical examination performed by a study physician.

Individuals will be excluded from the trial if they have any of the following: personal history of gastric cancer prior to enrolment; gastric resections due to benign disease (participants with ulcer suturing and vagotomy are eligible); *H. pylori* eradication therapy within 12 months prior to inclusion (irrespective of the treatment result); presence of alarm symptoms for digestive or any other diseases; pathological findings at physical investigation suggestive of a serious disease requiring immediate management; factors otherwise limiting the participation according to the protocol; serious psychological conditions/psychiatric disease limiting the possibilities to understand the requirements for diagnostic and/or medical interventions; or low expectations on the compliance for the diagnostic work-up, treatment or follow-up.

Interventions

The general study design is illustrated in Figure 1.

After being provided detailed information on the study by the study personnel and signing a consent form, individuals with alarm and exclusion symptoms will be identified by a study physician. The remaining participants will complete a detailed lifestyle and medical history questionnaire and then will be randomised online into two groups (50% in the intervention group, 50% in the control group) via central data management system. Randomisation will be stratified by gender, age group and recruitment site.

The intervention group will be tested for pepsinogens (Pg) I and II (Eiken Chemical Co., Tokyo, Japan) and for *H. pylori* infection which will be detected by IgG group antibodies by ELISA (Biohit, Plc., Finland), or alternative methods including ^{13}C-urea breath test (UBT). The choice of the methods will be decided based on the results of the pilot study (see below). For participants undergoing endoscopy, histological confirmation will be required for *H. pylori* positivity.

Those with pepsinogen PgI/PgII≤2 and PgI≤30 ng/ml will be referred for upper endoscopy with a detailed biopsy work-up according to the updated Sydney system.[20]

All *H. pylori* positive participants will be re-invited and offered *H. pylori* eradication treatment. The treatment will be chosen according to the Maastricht guidelines,[13] based on the resistance patterns to clarithromycin in the particular recruitment site as well as the clinical effectiveness of the particular regimen whenever data are available. In low resistance areas (<15-20%) including Latvia, the first choice of eradication treatment will be standard

triple therapy for 10 days: esomeprazole 40 mg, clarithromycin 500 mg, amoxicillin 1000 mg, each administered twice a day. Second-line treatment will not be offered within the study; however, study participants requiring it will be referred to their general practitioners with relevant recommendations. All individuals diagnosed with precancerous lesions during upper endoscopy will be followed up according to the Management of precancerous conditions and lesions in the stomach (MAPS) guidelines.[21]

The control group will receive standard care and will not be systematically investigated for *H. pylori* or precancerous lesions. As an incentive for participation, both groups will be offered faecal occult blood testing by a laboratory-based immunochemical test (FIT) OC-Sensor (Eiken Chemical Co., Tokyo, Japan), and whenever positive, referred for colonoscopy. Biological materials (e.g. serum, plasma, DNA, breath sample, materials for microbiota analysis) will be collected for biobanking; the materials will provide the unique opportunity to perform ancillary studies including, but not limited to the following: searching for new biomarkers; and analysing the impact of wide antibiotic use and presence of precancerous lesions on gut microbiome.

The effectiveness of *H. pylori* eradication will be verified in a subgroup of participants by using UBT 6-24 months after the treatment. The treatment adherence as well as presence or absence, frequency and severity of adverse events potentially related to the eradication therapy will be actively assessed by telephone interview 45-60 days following the delivery of drugs, and adverse events will be recorded throughout the study. The susceptibility of *H. pylori* to commonly used antibiotics in the eradication therapies will be investigated in approximately 200 referrals for upper endoscopy with evidence of *H. pylori* in antral biopsies.

The groups will be followed at 5-year intervals by direct or telephone contact or alternative means of communication until the study end-points are reached. A record linkage will also be made to the national Cancer and Mortality Registry database to ascertain cases of and deaths from gastric cancer.

Data-capture system and centralized biorepository

A centralised multiple-language web-based electronic data-capture system and data management facilities have been developed for the study. The questionnaire and investigation data are recorded in a standardised way and the system provides the primary data source. The system is built using the DotNetNuke content management platform providing the required conditions for personalized data security. The collected data are

stored in a MicroSoft SQL Server database. Initially three languages are being used: English, Latvian, and Russian. The publicly available information can be viewed at https://www.gistar.eu.

A centralized biorepository will be run by the University of Latvia and supervised by IARC. Pathology services and archiving of formalin- fixed and paraffin-embedded (FFPA) material will be handled by the Academic Histology Laboratory in Riga, Latvia.

Trial endpoints and statistical analyses

The primary end-point of the study is mortality difference from gastric cancer between the intervention and control groups at 15 years or when enough cases accumulate to demonstrate a statistically significant difference between the groups. Secondary end-points are the difference in gastric cancer incidence, and all-cause mortality between the two groups. The proportion of gastric cancer cases arising in the subgroup with biomarkers indicating high risk (e.g. low PgI/PgII ratio and low PgI levels) will be compared to the group with normal biomarkers at inclusion. Additional estimates will be made on the incidence and stages of cancers comparing participants under endoscopic surveillance and without it as well as comparing participants having undergone *H. pylori* eradication versus those having refused.

The sample size of the study is estimated based on the primary variable of interest, gastric cancer mortality. Estimates of the age- and sex-specific mortality rates from gastric cancer were taken from the GLOBOCAN 2008 estimates for Belarus.[22] Estimates of the number of deaths from gastric cancer were calculated for 5, 10, 15, and 20 years of follow-up. Censoring due to mortality from other causes was taken into account using mortality rates available on the WHO mortality database for Belarus in the years 2007-2009.[19] In addition, a loss to follow-up of 1% per year was included in the calculations to account for migration and other reasons not related to mortality that may prevent the assessment of the primary outcome.

Based on a significance level of 5% and a target power of 90%, with given number of 30,000 participants, 112 deaths from gastric cancer are expected in the control group, and a 35% reduction in gastric cancer mortality is detectable, corresponding to 73 cases in the intervention group at 15 years of follow-up. The study size may need to be increased if lower prevalence of *H. pylori* infection, higher number of women included in the study and/or lower acceptance/compliance to *H. pylori* eradication therapy are observed.

Gastric cancer mortality will be compared between intervention and control groups using a log-rank test. The survival curves will also be compared with use of the Kaplan-Meier life-table method and the Cox proportional-hazards model. The stratified randomisation process should ensure that groups are balanced with respect to age and gender. In addition, a multivariate Cox proportional hazards model will be used to account for confounding factors. The effect of confounding factors on the endpoints will be evaluated using univariate models in the first place. These analyses will be repeated for gastric cancer incidence difference between the two groups.

The study subject recruitment to the pilot phase has just been completed to test assumptions defined for the study including acceptability and adherence to the intervention, and *H. pylori* prevalence, and to test the appropriateness of the chosen tools and infrastructure for the study. In addition, in this phase the accuracy of biomarkers for detecting atrophy will be evaluated by comparing different alternatives (e.g. different manufacturer tests, different cut-off values) against histology.

Discussion

H. pylori gastritis has been defined as an infectious disease according to the Kyoto Global Consensus Conference,[12] and once-per-lifetime eradication treatment with antibiotics seems to be a rational and cost-effective approach to prevent gastric cancer as well as other *H. pylori*-related diseases, including peptic ulcer and functional dyspepsia.[12 23] In high-risk countries for gastric cancer this would mean giving antibiotic treatment to the majority of the population, as is the case for Latvia where *H. pylori* prevalence is around 80%.[24] The risk of adverse events and increased antibiotic resistance are major concerns; the magnitude of these risks has not been sufficiently investigated in well-controlled studies, and no country has implemented a population-based *search-and-treat* strategy for *H. pylori*.[17]

Pepsinogens are markers for atrophy of the stomach mucosa;[25] decreased pepsinogen values have been demonstrated to correlate with increased risk of gastric cancer;[26-28] furthermore, a combination of pepsinogen testing and *H. pylori* detection has been suggested to be the best available non-invasive option for gastric cancer risk stratification.[13 29] However, the accuracy of pepsinogen tests to identify gastric cancer and even atrophy is imperfect.[18] There is still a lack of evidence from randomised control trials of combining once-per-lifetime eradication of *H. pylori* and screening for high-risk conditions with blood markers such as pepsinogens for reducing gastric cancer mortality.

Pepsinogen tests to identify atrophy has demonstrated a wide range of sensitivity in various studies,[18] indicating that several factors may influence pepsinogen levels in different populations. The GISTAR study will allow us to investigate the role of *H. pylori* infection and participants' characteristics on the performance of biomarkers for identifying individuals at high risk of gastric cancer.

A few limitations of the study design should be mentioned. Serological detection of *H. pylori* was selected for the pilot study as the most feasible method in population-based settings. However, we acknowledge that *H. pylori* antibodies may remain positive following successful elimination of the bacteria.[30] In order to avoid misclassification in the current study, participants having received eradication within the last 12 months prior to enrolment will be not included; however, this cannot exclude the chance of getting a false positive serology test result completely. Under the circumstances that unacceptably low specificity of the serological test is observed in the pilot phase, an alternative method to detect *H. pylori* (e.g. UBT) or a combination of several tests will be applied in the main study. Excluding any participants having reported a previous eradication could be another alternative.

The effect of the intervention would be influenced by participation rates of the target population and acceptance rate of the *H. pylori* eradication treatment; in addition, participation in and acceptance of endoscopic examinations would affect the yield of endoscopic follow-up. The inclusion of colorectal cancer screening as an incentive may encourage participation and adherence; however, the general participation may be affected by the fact that only half of the participants are offered *H. pylori* eradication and screening for precancerous lesions. One of the main limitations of the study is the long term follow-up that is required to achieve its objectives. We will make multiple efforts to assure compliance and retention within the study, including periodic phone calls and interim visits.

While the randomisation process should ensure that groups are balanced with respect to age and gender, adjustment of proportion between genders might be required if a substantially higher proportion of women or men is recruited into the intervention group. To prevent this, we will make an extra effort to balance the gender ratio by actively inviting men or women required to obtain proper balance.

As described, the study design and the organization of the field work have taken into account not only the scientific background but also contextual conditions for a successful implementation and execution of the trial. If new sites outside Latvia are to be included,

study design will be adapted to local conditions for better acceptance and affordability without compromising the scientific objectives.

In conclusion, the study would have major public health implications by providing leads for prevention activities in populations with elevated rates of gastric cancer, particularly in Baltic and Eastern European regions where the public health burden from the disease is substantial.

Figure 1. General study design

Declarations

List of abbreviations

FIT – laboratory-based faecal immunochemical test

H. pylori – Helicobacter pylori

IARC - International Agency for Research on Cancer

FFPA - formalin-fixed and paraffin- embedded material

MAPS guidelines - Management of precancerous conditions and lesions in the stomach

Pg - pepsinogen

UBT - ^{13}C-urea breath test

Ethics, data safety and dissemination

The Ethics Committee of IARC has approved the study protocol 26/03/2013 and the relevant protocol updates 02/10/2015. reg. No. IEC 12-36; the Ethics Committee of Riga East University Hospital Support foundation has approved the protocol 03/10/2013, reg. No. 14-A/13, and the Central Medical Ethics Committee in Latvia has approved the protocol 09/12/2013, reg. No. 01-29.1/11.

All the study participants are required to provide signed consent prior the enrolment.

An independent Data Safety and Monitoring Board (DSMB) has been established for the GISTAR study which involves experts in epidemiology, statistics, clinical trials,

gastroenterology and pathology to safeguard the interests of study participants and to ensure the scientific validity of the study.

The findings will be published in peer-reviewed journals and presented at scientific meetings. We anticipate that study results will provide necessary information to be considered in further updates of the European and international guidelines for gastric cancer prevention and *H. pylori* management.

Availability of data and material

Not applicable since the current manuscript does not contain the results of the study. Development of study-specific biorepository and data capture system has been described within the main text.

Competing interests statement

ML is a partner in institutions involved in realization of the project – Digestive Diseases Centre GASTRO and Academic Histology laboratory. ILK and SI are employees of Academic Histology laboratory, IK – of Digestive Diseases Centre GASTRO. Otherwise, the authors declare that they have no competing interests.

Funding statement

The study has been supported in part by various funding sources in the University of Latvia; this includes the funding schemes from the European Regional Development Fund (ERDF) and the National Program for Research in Latvia: Biomedicine 2014-2017. The protocol development was supported in part by the project of the European Social Fund No. 009/0220/1DP/1.1.1.2.0/09/APIA/VIAA/016 'Multidisciplinary research group for early cancer detection and cancer prevention'.

Authors' contributions

ML, JYP, MP, RH have been involved in initial design of the protocol, JYP, SP, ILK, SI, IK, DR, AK, DS, ID, VF committed to developing particular specialized parts of the protocol; RM, IP, RH committed to adjusted version of the initial protocol, MP, JYP, RM – to the statistical evaluations and study sample size estimates, ML, JYP and RM wrote the manuscript, all co-authors – have participated in improvements to the manuscript and acceptance of it.

Data Sharing Statement

Data from the pilot study are currently being analysed to be presented in further publications. These data are available to the principal investigators, study statistician and DSMB members. The results will be disseminated during international and national conferences and congresses, published in peer-reviewed papers.

Acknowledgements

We acknowledge the contribution of the DSMB members: Iris Lansdorp-Vogelaar, Jack Cuzick, Fatima Carneiro, Ernst Kuipers, Jose Machado, Peter Malfertheiner, Colm O'Morain and Mangesh Thorat. Special thanks to Ann Zauber and Olof Nyren for the valuable input to the protocol design as well as to Hossam Haick, Lars Engstrand and other members to the protocol workshop. Particular acknowledgements to administrative-managing staff of the study - Aiga Rudule, Inga Upmace, Janis Kotlers.

The study would not have been possible without the involvement of medical professional organizations, in particular Digestive Diseases Centre GASTRO, Academic Histology laboratory, Insights-A as well as municipalities and their healthcare institutions in Cesis, Aluksne, Ludza, Saldus and Tukums regions of Latvia. We appreciate the support from industry for the special conditions in supplying with reagents, equipment, medication and/or professional advice – BIOHIT, Oyi., Eiken Chemical Co., Meridian Bioscience, Inc., KRKA, Fujfilm Europe.

The study has been endorsed by European Helicobacter and Microbiota Study Group (EHMSG), Healthy Stomach Initiative (HIS) and International Digestive Cancer alliance (IDCA). Support has been provided by the Riga East University hospital Support Foundation. We acknowledge also the support from the Ministry of Health of Latvia.

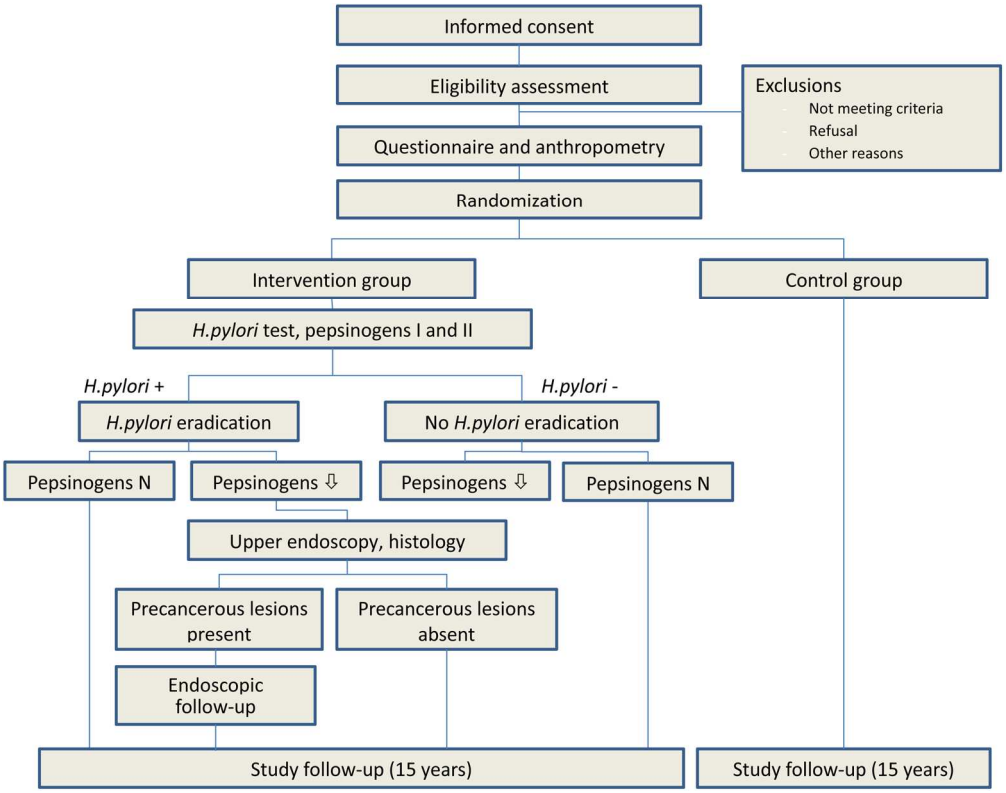
REFERENCES

1. Herrero R, Parsonnet J, Greenberg ER. Prevention of gastric cancer. *JAMA* 2014;312(12):1197-8.
2. Pasechnikov V, Chukov S, Fedorov E*, et al.* Gastric cancer: Prevention, screening and early diagnosis. *World J Gastroenterol* 2014;20(38):13842-62.
3. Jemal A, Bray F, Center MM*, et al.* Global cancer statistics. *CA: A Cancer Journal for Clinicians* 2011;61(2):69-90.
4. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *European journal of cancer (Oxford, England : 1990)* 2010;46(4):765-81.
5. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J*, et al.* Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013;49(6):1374-403.
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and Helicobacter pylori *IARC Monogr Eval Carcinog Risks Hum* 1994;61:1-241.
7. IARC. Monographs on the evaluation of carcinogenic risks to humans, volume 100. A review of carcinogen - Part B: biological agents. Lyon: International Agency for Research on Cancer 2011.
8. Plummer M, Franceschi S, Vignat J*, et al.* Global burden of gastric cancer attributable to Helicobacter pylori. *Int J Cancer* 2015;136(2):487-90.
9. Lansdorp-Vogelaar I, Sharp L. Cost-effectiveness of screening and treating Helicobacter pylori for gastric cancer prevention. *Best Pract Res Clin Gastroenterol* 2013;27(6):933-47.
10. Areia M, Carvalho R, Cadime AT*, et al.* Screening for gastric cancer and surveillance of premalignant lesions: a systematic review of cost-effectiveness studies. *Helicobacter* 2013;18(5):325-37.
11. Moayyedi P. Feasibility and cost effectiveness of population-based H. pylori eradication. In: IARC Helicobacter pylori Working Group Helicobacter pylori Eradication as a Strategy for Preventing Gastric Cancer Lyon, France: International Agency for Research on Cancer (IARC Working Group Reports, No 8); pp 174-180 Available from: http://wwwiarcfr/en/publications/pdfs-online/wrk/wrk8/indexphp2014.
12. Sugano K, Tack J, Kuipers EJ*, et al.* Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015;64(9):1353-67.

13. Malfertheiner P, Megraud F, O'Morain CA*, et al.* Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017;66(1):6-30.
14. Malhotra-Kumar S, Lammens C, Coenen S*, et al.* Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet* 2007;369(9560):482-90.
15. Derakhshan MH, Arnold M, Brewster DH*, et al.* Worldwide Inverse Association between Gastric Cancer and Esophageal Adenocarcinoma Suggesting a Common Environmental Factor Exerting Opposing Effects. *Am J Gastroenterol* 2016;111(2):228-39.
16. Lonnberg S, Sekerija M, Malila N*, et al.* Cancer screening: policy recommendations on governance, organization and evaluation of cancer screening. In: European Guide on Quality Improvement in Comprehensive Cancer Control Available from: http://wwwcancercontroleu/guide-landing-page/. Belgium: National Institute of Public Health, Slovenia, Scientific Institute of Public Health, Belgium 2017:39-76.
17. Leja M, You W, Camargo MC*, et al.* Implementation of gastric cancer screening - The global experience. *Best practice & research Clinical gastroenterology* 2014;28(6):1093-106.
18. Huang YK, Yu JC, Kang WM*, et al.* Significance of Serum Pepsinogens as a Biomarker for Gastric Cancer and Atrophic Gastritis Screening: A Systematic Review and Meta-Analysis. *PLoS One* 2015;10(11):e0142080.
19. IARC. World Health Organization Cancer mortality database, available from http://www-dep.iarc.fr/WHOdb/WHOdb.htm. Accessed on 29/02/2016. 2015
20. Dixon MF, Genta RM, Yardley JH*, et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20(10):1161-81.
21. Dinis-Ribeiro M, Areia M, de Vries AC*, et al.* Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 2012;44(1):74-94.
22. Ferlay J, Shin HR, Bray F*, et al.* GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. *Lyon, France: International Agency*

for Research on Cancer Available from: http://globocaniarcfr, accessed on 22/12/2011 2010

23. Ford AC, Forman D, Hunt RH*, et al.* Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2014;348:g3174.
24. Leja M, Cine E, Rudzite D*, et al.* Prevalence of Helicobacter pylori infection and atrophic gastritis in Latvia. *Eur J Gastroenterol Hepatol* 2012;24(12):1410-7.
25. Agreus L, Kuipers EJ, Kupcinskas L*, et al.* Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. *Scand J Gastroenterol* 2012;47(2):136-47.
26. Oishi Y, Kiyohara Y, Kubo M*, et al.* The serum pepsinogen test as a predictor of gastric cancer: the Hisayama study. *Am J Epidemiol* 2006;163(7):629-37.
27. Yanaoka K, Oka M, Mukoubayashi C*, et al.* Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2008;17(4):838-45.
28. Kurilovich S, Belkovets A, Reshetnikov O*, et al.* Stomach-specific Biomarkers (GastroPanel) Can Predict the Development of Gastric Cancer in a Caucasian Population: A Longitudinal Nested Case-Control Study in Siberia. *Anticancer research* 2016;36(1):247-53.
29. Miki K. Gastric cancer screening by combined assay for serum anti-Helicobacter pylori IgG antibody and serum pepsinogen levels - "ABC method". *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87(7):405-14.
30. Atkinson NS, Braden B. Helicobacter Pylori Infection: Diagnostic Strategies in Primary Diagnosis and After Therapy. *Digestive diseases and sciences* 2016;61(1):19-24.



173x136mm (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	3, 7
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	7
Funding	4	Sources and types of financial, material, and other support	14, 15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2
	5b	Name and contact information for the trial sponsor	2, 14
	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	14
	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)	14, 15

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	5-7
	6b	Explanation for choice of comparators	5-6
Objectives	7	Specific objectives or hypotheses	6-7
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)	8
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	7-8
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)	7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
	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)	8-9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
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	10, 11
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)	10

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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	7, 9
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	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7, 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
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	9, 10
	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	12

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	9, 10
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)	9
	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)	NA
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	13
	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	13
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	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
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)	13

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7, 9
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	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
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	13-15
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	NA
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	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	13
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
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	9, 10

*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

Multicentric randomized study of H. pylori eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-016999.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Jun-2017
Complete List of Authors:	Leja, Marcis; Latvijas Universitate, Institute of Clinical and Preventive Medicine Park, Jin Young; International Agency for Research on Cancer, Prevention and Implementation Group Murillo, Raul; International Agency for Research on Cancer, Prevention and Implementation Group Liepniece-Karele, Inta; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Austrumu kliniska universitates slimnica, Department of Research Isajevs, Sergejs; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Austrumu kliniska universitates slimnica, Department of Research Kikuste, Ilze; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Digestive Diseases Centre GASTRO Rudzite, Dace; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Austrumu kliniska universitates slimnica, Department of Research Krike, Petra; Latvijas Universitate, Institute of Clinical and Preventive Medicine Parshutin, Sergei; Latvijas Universitate, Institute of Clinical and Preventive Medicine ; Rigas Tehniska universitate, Institute of Information Technology Polaka, Inese; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Tehniska universitate, Institute of Information Technology Kirsners, Arnis; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Tehniska universitate, Institute of Information Technology Santare, Daiga; Latvijas Universitate, Institute of Clinical and Preventive Medicine; Rigas Austrumu kliniska universitates slimnica, Department of Research Folkmanis, Valdis; Latvijas Universitate, Faculty of Medicine Daugule, Ilva; Latvijas Universitate, Institute of Clinical and Preventive Medicine Plummer, Martyn; International Agency for Research on Cancer, Herrero, Rolando; IARC, Early Detection and Prevention
Primary Subject Heading:	Public health
Secondary Subject Heading:	Gastroenterology and hepatology, Oncology, Epidemiology
Keywords:	Gastric cancer, prevention, H. pylori, eradication, pepsinogen, randomised

	study

Title page

Multicentric randomized study of *H. pylori* eradication and pepsinogen testing for prevention of gastric cancer mortality: the GISTAR study

Marcis Leja[1-3], Jin Young Park[4], Raul Murillo[4], Inta Liepniece-Karele[1, 2 ,5], Sergejs Isajevs[1 ,2, 5], Ilze Kikuste[1, 3], Dace Rudzite[1, 2], Petra Krike[1], Sergei Parshutin[1, 6], Inese Polaka[1, 6], Arnis Kirsners[1, 6], Daiga Santare[1, 2], Valdis Folkmanis[1], Ilva Daugule[1], Martyn Plummer[7], Rolando Herrero[4]

1- Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia, Riga, Latvia
2- Department of Research, Riga East University hospital, Riga, Latvia
3- Digestive Diseases Centre GASTRO, Riga, Latvia
4- Prevention and Implementation Group, International Agency for Research on Cancer, Lyon, France
5- Academic Histology Laboratory, Riga, Latvia
6- Institute of Information Technology, Riga Technical University, Riga, Latvia
7- Infection and Cancer Epidemiology Group, International Agency for Research on Cancer, Lyon, France

Contact details of the authors:

Marcis Leja, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: cei@latnet.lv

Jin Young Park, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: ParkJY@iarc.fr

Raul Murillo, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: MurilloR@iarc.fr

Inta Liepniece-Karele, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: intaliepniecekarele@inbox.lv

Sergejs Isajevs, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: sergisajevs@inbox.lv

Ilze Kikuste, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: ikikuste@gmail.com

Dace Rudzite, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: dacerudzite2008@inbox.lv

Petra Krike, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: petra.krike@gmail.com

Sergei Parshutin, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: Sergejs.Parsutins@rtu.lv

Inese Polaka, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: inese.polaka@gmail.com

Arnis Kirsners, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: arnis.kirsners@rtu.lv

Daiga Santare, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: daiga.santare@inbox.lv

Valdis Folkmanis, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: valdis.folkmanis@lu.lv

Ilva Daugule, Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia; Address: 19 Raina boulv., LV1586 Riga, Latvia; e-mail: ilva_daugule@hotmail.com

Martyn Plummer, Infection and Cancer Epidemiology Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: plummerm@iarc.fr

Rolando Herrero, Prevention and Implementation Group, International Agency for Research on Cancer, Address: 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France; e-mail: HerreroR@iarc.fr

Corresponding author:

Prof. Marcis Leja: Institute of Clinical and Preventive Medicine & Faculty of Medicine, University of Latvia, Riga, Latvia; Address: 19 Raina boulv., Riga LV1586; Tel.: +371-29497500; Fax: +371-67040248, E-mail: cei@latnet.lv

Abstract

Introduction: Population-based eradication of *H.pylori* has been suggested to be cost-effective and is recommended by international guidelines. However, the potential adverse effects of widespread antibiotic use this would entail have not been sufficiently studied. An alternative way to decrease gastric cancer mortality is by non-invasive search for precancerous lesions, in particular gastric atrophy; pepsinogen tests are the best currently available alternative. The primary objective of GISTAR is to determine whether *H.pylori* eradication combined with pepsinogen testing reduces mortality from gastric cancer among 40-64 year old individuals. The secondary objectives include evaluation of *H.pylori* eradication effectiveness in gastric cancer prevention in patients with precancerous lesions and evaluation of the potential adverse events, including effects on microbiome.

Methods and analysis: Individuals are recruited from general population (50% men) in areas with high gastric cancer risk in Europe, and undergo detailed lifestyle and medical history questionnaire before being randomly allocated to intervention or control groups. The intervention group undergoes *H. pylori* testing, and is offered eradication therapy if positive; in addition, pepsinogen levels are detected in plasma, and those with decreased levels are referred for upper endoscopy. All participants are offered faecal occult blood testing as an incentive for study participation. Effectiveness of eradication and the spectrum of adverse events are evaluated in study subpopulations. A 35% difference in gastric cancer mortality between the groups is expected to be detectable at 90% power after 15 years if 30,000 individuals are recruited. Biological materials are biobanked for the main and ancillary studies. The study procedure and assumptions will be tested during the pilot phase.

Ethics and dissemination: The study was approved by the respective Ethics Committees. An independent Data Safety and Monitoring Board has been established. The findings will be published in peer-reviewed journals and presented at scientific meetings.

Trial registration number: NCT02047994

Strengths and limitations of this study

- This is currently the only study in Europe addressing population-based eradication of *H. pylori* to prevent gastric cancer as recommended by international guidelines (Maastricht V, Kyoto Global Consensus, EU Joint Action Cancer prevention project CanCon)
- Gastric cancer mortality is used as an end-point which corresponds to the requirements of a cancer screening program
- The strategy of combining population-based *H. pylori* eradication to pepsinogen detection with endoscopic surveillance of participants in whom precancerous lesions have been detected, has not been evaluated before
- The study biorepository with a wide range of biospecimen collection will be a great resource to conduct a number of unique ancillary studies
- However, the large sample size with a long follow-up required to demonstrate a statistical difference in mortality reduction between the two groups is a challenge to the study, with the possibility to increase the sample size even further in case of lower prevalence of *H. pylori* infection, higher number of women in the study group and/or lower acceptance rate for the intervention

Keywords

Gastric cancer, prevention, *H. pylori*, eradication, pepsinogen, randomised study

Introduction

Although gastric cancer remains a major cause of death among malignant diseases, its prevention has been neglected in the Western world for decades.[1] Most countries show declining trends in age-specific gastric cancer incidence, but the total number of cases in the world is not expected to decrease in the next decades due to demographic changes including population growth and aging.[2]

There are considerable geographical variations in the incidence of gastric cancer, with some of the lowest rates seen in North America and Western Europe and the highest in Eastern Asia, Eastern Europe, and South America.[3] According to recent estimates from Europe, high rates have been observed in Central and Eastern European regions including Belarus, Ukraine and the Russian Federation and the Baltic States in Northern Europe, including Latvia. For example, gastric cancer incidence rates are the highest in Belarus (age-standardised rate (ASR) of 42.1/100,000 in men and 17.2/100,000 in women) among 40 European countries.[4 5] These estimates are over three-fold higher than those in France or Switzerland.[4 5] The rates in the majority of the former Soviet Union regions remain high.

Infection with *Helicobacter pylori* (*H. pylori*) is the major etiologic factor responsible for developing gastric cancer.[6 7] It is estimated that 89% of non-cardia gastric cancers are attributable to this infection.[8]

Searching for and eradicating *H. pylori* in healthy asymptomatic adults (the "*search-and treat*" strategy) has been suggested to be cost-effective by considering the reduction of gastric cancer burden as well as other diseases related to this microorganism.[9-11] The recent global Kyoto conference[12] encouraged the broad application of *search-and-treat*, particularly in high-risk areas. This has been further endorsed by the Maastricht European consensus group.[13] However, due to the limited data available on target groups, feasibility and population impact of the intervention, a working group convened by the International Agency for Research on Cancer (IARC) in 2013 proposed implementation of the strategy via well-designed implementation studies.[1]

H. pylori eradication in the general population would lead to high antibiotic consumption, particularly in areas of high prevalence of the infection. Widespread use of the same antibiotics used to treat common diseases, some of them life-threatening, may lead to increased antibiotic resistance of microorganisms other than *H. pylori*.[14] An inverse association observed between the occurrence of gastric and oesophageal cancers may suggest potential opposing effects of the related environmental factors, including *H. pylori*.[15]

The potential risks of these effects in community settings were not considered in the above mentioned cost-effectiveness analyses[9-11], and knowledge about the potential adverse effects of *H. pylori* eradication on the gut microbiome is scant.

Therefore, the recently published European Guide on Quality Improvement in Comprehensive Cancer Control emphasised the need for additional clinical studies to clarify whether and how to implement population-based *H. pylori* screening and eradication programmes for gastric cancer prevention.[16]

In addition to the population-based eradication of *H. pylori*, detection and treatment of precancerous lesions or early gastric cancer has been proposed as a means to reduce gastric cancer mortality and some countries in the Western Pacific region have introduced nationwide gastric cancer screening programmes.[17] Pepsinogen testing is currently the best available non-invasive option to identify individuals with precancerous lesions (in particular, gastric atrophy)[13] who are at increased risk of gastric cancer. However, a recent meta-analysis[18] concluded that pepsinogens exhibit only a moderate diagnostic yield in gastric cancer detection; thus large-scale and well-designed prospective studies are encouraged, particularly in East, Central and part of Northern Europe and Latin American countries where gastric cancer burden is relatively high and prevention effort is scarce.

Here, we present the design of a clinical trial aimed at investigating the role of *H. pylori* eradication combined with non-invasive screening for precancerous lesions in the reduction of gastric cancer mortality in a predominantly Caucasian population in Northern and Eastern Europe (GISTAR).

Methods and analysis

The **aim** of the study is to search for new intervention strategies to decrease mortality from gastric cancer in high-risk areas in the Baltic States and Eastern Europe. The main study site is Latvia, where the estimated ASR (world) per 100,000 for gastric cancer mortality was 16.2 in men and 6.4 in women in 2011. Other potential sites include the Russian Federation with gastric cancer mortality of 20.8 in men and 8.5 in women, Belarus with 20.2 in men and 7.8 in women, and Ukraine with 17.4 in men and 6.6 in women in 2011.[19]

The **primary objective** is to determine if *H. pylori* eradication combined with non-invasive screening and follow-up of precancerous lesions (atrophic gastritis or higher) reduces mortality from gastric cancer in a high risk population among 40-64 years old subjects.

In addition to the above, **secondary objectives** include analyses of success-rates of *H. pylori* eradication therapy, resistance rates of *H. pylori* to the key antibiotics used in standard therapies (in subgroups), potential adverse effects of population-based eradication (including effects on gut microbiome), optimisation of follow-up strategies as well as search for new biomarkers, including volatile markers.

The **key hypotheses** of the study are: 1) *H. pylori* eradication in middle aged individuals in a high risk population with endoscopic follow-up of those with evidence of atrophic gastritis prevents gastric cancer mortality; 2) *H. pylori* eradication is effective in preventing gastric cancer mortality even after the development of gastric mucosal atrophy; 3) Certain population subgroups can derive more benefit from *H. pylori* eradication, and therefore could be targeted if general population eradication is not feasible; 4) A combination of biomarker screening and upper endoscopy is an appropriate strategy to prevent mortality from gastric cancer in high incidence areas.

The study protocol (version 4.5, revised on 07 September, 2015) was approved by the Ethics Committee of the International Agency for Research on Cancer (IEC 12-36) as well as the national Ethics Committees in Latvia; the Ethics Committee of Riga East University Support Foundation (No.14-A/13) and the Central Medical Ethics Committee (No. 01-29.1/11). The protocol is registered in the clinicaltrials.gov database (NCT02047994).

Participants

The study aims to enrol men and women at equal proportions at the risk- age (40-64 years at inclusion) for developing gastric cancer. The recruitment centres are planned in high gastric cancer risk areas, and predominantly Caucasian origin populations in Europe will be enrolled. The enrolment has been initiated in three study centers in Latvia: Tukums, Dobele and Rezekne (Caucasian population), with the potential expansion to other locations; more genetically diverse populations would be enrolled when the study is expanded to other sites.

Recruitment centres will be set up reflecting the study requirements. One recruitment centre is expected to randomise 3000 study participants, although in locations with smaller number of inhabitants, fewer than 3000 participants are acceptable. Based on the sample size calculation (see below) at least 10 centres, each recruiting 3000 study participants would be required. The study participants will be contacted by phone and invitation mails through lists that we obtain from the general practitioners (GPs), local primary care medical centres, and national medical registration databases, as appropriate, in different locations of

the potential recruitment centres. We will pay particular attention to keep the gender balance during recruitment, ensuring at least 50% of the participants are men. To achieve this, we will invite men in priority by direct telephone calls and invitation mails while accept participation of women in case they are the family members of the invited men or express their interest in participating in the study by contacting the study team.

All participants must sign an informed consent and they should be in good health at enrolment, as determined by medical history and physical examination performed by a study physician.

Individuals will be excluded from the trial if they have any of the following: personal history of gastric cancer prior to enrolment; gastric resections due to benign disease (participants with ulcer suturing and vagotomy are eligible); *H. pylori* eradication therapy within 12 months prior to inclusion (irrespective of the treatment result); presence of alarm symptoms for digestive or any other diseases; pathological findings at physical investigation suggestive of a serious disease requiring immediate management; factors otherwise limiting the participation according to the protocol; serious psychological conditions/psychiatric disease limiting the possibilities to understand the requirements for diagnostic and/or medical interventions; or low expectations on the compliance for the diagnostic work-up, treatment or follow-up.

Interventions

The general study design is illustrated in Figure.

After being provided detailed information on the study by the study personnel and signing a consent form, individuals with alarm and exclusion symptoms will be identified by a study physician. The remaining participants will complete a detailed lifestyle and medical history questionnaire and then will be randomised online into two groups (50% in the intervention group, 50% in the control group) via central data management system. Randomisation will be stratified by gender, age group and recruitment site.

The intervention group will be tested for pepsinogens (Pg) I and II by a latex-agglutination test-system (Eiken Chemical Co., Tokyo, Japan). For *H. pylori* infection testing IgG group antibodies by ELISA (Biohit, Plc., Finland) was initially planned, however based on the preliminary result from the pilot study which indicated false positivity of serology, ^{13}C-urea

breath test (UBT) is decided to be used for confirmation of the infection. For participants undergoing endoscopy, histological confirmation will be required for *H. pylori* positivity.

The selected cutoff values for pepsinogens characteristic for gastric mucosal atrophy is based on our previous research; [20] those with pepsinogen PgI/PgII≤2 and PgI≤30 ng/ml will be referred for upper endoscopy with a detailed biopsy work-up according to the updated Sydney system.[21] Histological assessment of the biopsies collected from the stomach will be independently performed by two experienced pathologists; in the case of discrepant results, the particular slides will be reviewed together to reach consensus.

All *H. pylori* positive participants will be re-invited and offered *H. pylori* eradication treatment. The treatment will be chosen according to the Maastricht guidelines,[13] based on the resistance patterns to clarithromycin in the particular recruitment site as well as the clinical effectiveness of the particular regimen whenever data are available. In low resistance areas (<15-20%) including Latvia[22], the first choice of eradication treatment will be standard triple therapy for 10 days: esomeprazole 40 mg, clarithromycin 500 mg, amoxicillin 1000 mg, each administered twice a day. Second-line treatment will not be offered within the study; however, study participants requiring it will be referred to their general practitioners with relevant recommendations. All individuals diagnosed with precancerous lesions during upper endoscopy will be followed up according to the Management of precancerous conditions and lesions in the stomach (MAPS) guidelines.[23]

The control group will receive standard care and will not be systematically investigated for *H. pylori* or precancerous lesions. As an incentive for participation, both groups will be offered faecal occult blood testing by a laboratory-based immunochemical test (FIT) OC-Sensor (Eiken Chemical Co., Tokyo, Japan), and whenever positive (cut-off at 10 µg/g faeces from a single faecal sample), referred for colonoscopy. Any additional rounds of colorectal screening will be provided within the respective national colorectal cancer screening programs.

Biological materials including serum, plasma, DNA, as well as stool and biopsies for microbiota analysis will be collected from different groups of participants for biobanking. Plasma/serum samples will be processed immediately after being obtained, stored and transported at -70°C temperature. These materials will provide the unique opportunity to perform ancillary studies including, but not limited to the following: searching for new biomarkers; and analysing the impact of wide antibiotic use and presence of precancerous lesions on gut microbiome.

The effectiveness of *H. pylori* eradication will be verified in a subgroup of participants (n=100-150) from the study centres in Latvia and other centres where resistance patterns are expected to be different based on the available epidemiological data, by using UBT 6-24 months after the treatment. The treatment adherence as well as presence or absence, frequency and severity of adverse events potentially related to the eradication therapy will be actively assessed by telephone interview 45-60 days following the delivery of drugs, and adverse events will be recorded throughout the study. The susceptibility of *H. pylori* to commonly used antibiotics in the eradication therapies will be investigated using the pilot study data from approximately 200 upper endoscopy referrals with evidence of *H. pylori* in antral biopsies (proportion of individuals with altered biomarker results, and another proportion with normal biomarkers).

The groups will be followed at 5-year intervals by direct or telephone contact or alternative means of communication until the study end-points are reached. Particular attention will be given to collect detailed information on potentially *H. pylori* related morbidity and mortality. Whenever possible, we will invite the participants to the study centres to obtain follow-up data including demographic information, socio-economic status, physical examination as well as biological samples (plasma, serum and stool samples and biopsies for microbiome testing). The new protocol will be developed to update the follow-up data collection. A record linkage will also be made to the national Cancer and Mortality Registry database to ascertain cases of and deaths from gastric cancer.

Data-capture system and centralized biorepository

A centralised multiple-language web-based electronic data-capture system and data management facilities has been developed for the study. The questionnaire and investigation data are recorded in a standardised way and the system provides the primary data source. The system is built using the DotNetNuke content management platform providing the required conditions for personalized data security. The collected data are stored in a Microsoft SQL Server database. Initially three languages are being used: English, Latvian, and Russian. The publicly available information can be viewed at https://www.gistar.eu.

A centralized biorepository will be run by the University of Latvia and supervised by IARC. Pathology services and archiving of formalin-fixed and paraffin-embedded (FFPA) material will be handled by the Academic Histology Laboratory in Riga, Latvia.

Trial endpoints and statistical analyses

The primary end-point of the study is mortality difference from gastric cancer between the intervention and control groups at 15 years or when enough cases accumulate to demonstrate a statistically significant difference between the groups. Secondary end-points are the difference in gastric cancer incidence, and all-cause mortality between the two groups. The proportion of gastric cancer cases arising in the subgroup with biomarkers indicating high risk (e.g. low PgI/PgII ratio and low PgI levels) will be compared to the group with normal biomarkers at inclusion. Additional estimates will be made on the incidence and stages of cancers comparing participants under endoscopic surveillance and without it as well as comparing participants having undergone *H. pylori* eradication versus those having refused.

The sample size of the study is estimated based on the primary variable of interest, gastric cancer mortality. Estimates of the age- and sex-specific mortality rates from gastric cancer were taken from the GLOBOCAN 2008 estimates for Belarus.[24] Estimates of the number of deaths from gastric cancer were calculated for 5, 10, 15, and 20 years of follow-up. Censoring due to mortality from other causes was taken into account using mortality rates available on the WHO mortality database for Belarus in the years 2007-2009.[19] In addition, a loss to follow-up of 1% per year was included in the calculations to account for migration and other reasons not related to mortality that may prevent the assessment of the primary outcome.

Based on a significance level of 5% and a target power of 90%, with given number of 30,000 participants, 112 deaths from gastric cancer are expected in the control group, and a 35% reduction in gastric cancer mortality is detectable, corresponding to 73 cases in the intervention group at 15 years of follow-up. The study size may need to be increased if lower prevalence of *H. pylori* infection, higher number of women included in the study and/or lower acceptance/compliance to *H. pylori* eradication therapy are observed.

Gastric cancer mortality will be compared between intervention and control groups using a log-rank test. The survival curves will also be compared with use of the Kaplan-Meier life-table method and the Cox proportional-hazards model. The stratified randomisation process should ensure that groups are balanced with respect to age and gender. In addition, a multivariate Cox proportional hazards model will be used to account for confounding factors. The effect of confounding factors on the endpoints will be evaluated using

univariate models in the first place. These analyses will be repeated for gastric cancer incidence difference between the two groups.

It is expected that the obtained data will allow running cost-effectiveness ancillary studies on mass-eradication of *H. pylori* by considering the costs of the adverse effects as well as on endoscopic surveillance of patients with gastric precancerous lesions in European countries with a relatively high-risk.

The study subject recruitment to the pilot phase has just been completed to test assumptions defined for the study including acceptability and adherence to the intervention, and *H. pylori* prevalence, and to test the appropriateness of the chosen tools and infrastructure for the study. In addition, in this phase the accuracy of biomarkers for detecting atrophy will be evaluated by comparing different alternatives (e.g. different manufacturer tests, different cut-off values) against histology.

Discussion

H. pylori gastritis has been defined as an infectious disease according to the Kyoto Global Consensus Conference,[12] and once-per-lifetime eradication treatment with antibiotics seems to be a rational and cost-effective approach to prevent gastric cancer as well as other *H. pylori*-related diseases, including peptic ulcer and functional dyspepsia.[12 25] In high-risk countries for gastric cancer this would mean giving antibiotic treatment to the majority of the population, as is the case for Latvia where *H. pylori* prevalence is around 80%.[26] The risk of adverse events and increased antibiotic resistance are major concerns; the magnitude of these risks has not been sufficiently investigated in well-controlled studies, and no country has implemented a population-based *search-and-treat* strategy for *H. pylori*.[17]

Pepsinogens are markers for atrophy of the stomach mucosa;[27] decreased pepsinogen values have been demonstrated to correlate with increased risk of gastric cancer;[28-30] furthermore, a combination of pepsinogen testing and *H. pylori* detection has been suggested to be the best available non-invasive option for gastric cancer risk stratification.[13 31] However, the accuracy of pepsinogen tests to identify gastric cancer and even atrophy is imperfect.[18]

The current European MAPS guidelines being referred to above, are recommending surveillance of patients with precancerous lesions to enable detection of those progressing to high-risk lesions or cancer as a strategy of decreasing gastric cancer related mortality.[23]

However, there is still a lack of evidence from randomised control trials of combining once-per-lifetime eradication of *H. pylori* and screening for high-risk conditions with blood markers such as pepsinogens for reducing gastric cancer mortality. To the best of our knowledge, this is the first study evaluating the yield of the above combination, i.e. mass-eradication of *H. pylori* and surveillance of pepsinogen-detected precancerous lesions as a strategy to reduce gastric cancer mortality.

Pepsinogen tests to identify atrophy have demonstrated a wide range of sensitivity in various studies,[18] indicating that several factors may influence pepsinogen levels in different populations. The GISTAR study will allow us to investigate the role of *H. pylori* infection and participants' characteristics on the performance of biomarkers for identifying individuals at high risk of gastric cancer.

A few limitations of the study design should be mentioned. While the randomisation process should ensure that groups are balanced with respect to age and gender, adjustment of proportion between genders might be required if a substantially higher proportion of women or men is recruited into the intervention group. To prevent this, we will make an extra effort to balance the gender ratio by actively inviting men or women required to obtain a balance. However we acknowledge that our extra effort to balance the male and female ratio to ensure sufficient study power to answer the research questions may influence the generalisability of the study results.

The inclusion of colorectal cancer screening in both groups as an incentive may encourage participation and adherence; however, the general participation may be affected by the fact that only half of the participants are offered *H. pylori* eradication and screening for precancerous lesions. Furthermore, we acknowledge that the effect of the intervention would be influenced by participation rates of the target population and acceptance rate of the *H. pylori* eradication treatment while participation in and acceptance of endoscopic examinations would affect the yield of endoscopic follow-up. Another limitation of the study is the long term follow-up that is required to achieve its objectives. We will make multiple efforts to assure compliance and retention within the study, including periodic phone calls and interim visits.

As described, the study design and the organization of the field work have taken into account not only the scientific background but also contextual conditions for a successful implementation and execution of the trial. If new sites outside Latvia are to be included,

study design will be adapted to local conditions for better acceptance and affordability without compromising the scientific objectives.

In conclusion, the study would have major public health implications by providing leads for prevention activities in populations with elevated rates of gastric cancer, particularly in Baltic and Eastern European regions where the public health burden from the disease is substantial.

Figure. GISTAR general study design

Declarations

List of abbreviations

FIT – laboratory-based faecal immunochemical test

H. pylori – Helicobacter pylori

IARC - International Agency for Research on Cancer

FFPA - formalin-fixed and paraffin- embedded material

MAPS guidelines - Management of precancerous conditions and lesions in the stomach

Pg - pepsinogen

UBT - ^{13}C-urea breath test

Ethics, data safety and dissemination

The Ethics Committee of IARC has approved the study protocol 26/03/2013 and the relevant protocol updates 02/10/2015. reg. No. IEC 12-36; the Ethics Committee of Riga East University Hospital Support foundation has approved the protocol 03/10/2013, reg. No. 14-A/13, and the Central Medical Ethics Committee in Latvia has approved the protocol 09/12/2013, reg. No. 01-29.1/11.

All the study participants are required to provide signed consent prior the enrolment.

An independent Data Safety and Monitoring Board (DSMB) has been established for the GISTAR study which involves experts in epidemiology, statistics, clinical trials,

gastroenterology and pathology to safeguard the interests of study participants and to ensure the scientific validity of the study.

The findings will be published in peer-reviewed journals and presented at scientific meetings. We anticipate that study results will provide necessary information to be considered in further updates of the European and international guidelines for gastric cancer prevention and *H. pylori* management.

Availability of data and material

Not applicable since the current manuscript does not contain the results of the study. Development of study-specific biorepository and data capture system has been described within the main text.

Competing interests statement

ML is a partner in institutions involved in realization of the project – Digestive Diseases Centre GASTRO and Academic Histology laboratory. ILK and SI are employees of Academic Histology laboratory, IK – of Digestive Diseases Centre GASTRO. Otherwise, the authors declare that they have no competing interests.

Funding statement

The study has been supported in part by various funding sources in the University of Latvia; this includes the funding schemes from the European Regional Development Fund (ERDF) and the National Program for Research in Latvia: Biomedicine 2014-2017. The protocol development was supported in part by the project of the European Social Fund No. 009/0220/1DP/1.1.1.2.0/09/APIA/VIAA/016 'Multidisciplinary research group for early cancer detection and cancer prevention'.

Authors' contributions

ML, JYP, MP, RH have been involved in initial design of the protocol, JYP, SP, ILK, SI, IK, DR, AK, DS, ID, VF committed to developing particular specialized parts of the protocol; RM, IP, RH committed to adjusted version of the initial protocol, MP, JYP, RM – to the statistical evaluations and study sample size estimates, ML, JYP and RM wrote the manuscript, all co-authors – have participated in improvements to the manuscript and acceptance of it.

Data Sharing Statement

Data from the pilot study are currently being analysed to be presented in further publications. These data are available to the principal investigators, study statistician and DSMB members. The results will be disseminated during international and national conferences and congresses, published in peer-reviewed papers.

Acknowledgements

We acknowledge the contribution of the DSMB members: Iris Lansdorp-Vogelaar, Jack Cuzick, Fatima Carneiro, Ernst Kuipers, Jose Machado, Peter Malfertheiner, Colm O'Morain and Mangesh Thorat. Special thanks to Ann Zauber and Olof Nyren for the valuable input to the protocol design as well as to Hossam Haick, Lars Engstrand and other members to the protocol workshop. Particular acknowledgements to administrative-managing staff of the study - Aiga Rudule, Inga Upmace, Janis Kotlers.

The study would not have been possible without the involvement of medical professional organizations, in particular Digestive Diseases Centre GASTRO, Academic Histology laboratory, Insights-A as well as municipalities and their healthcare institutions in Cesis, Aluksne, Ludza, Saldus and Tukums regions of Latvia. We appreciate the support from industry for the special conditions in supplying with reagents, equipment, medication and/or professional advice – BIOHIT, Oyi., Eiken Chemical Co., Meridian Bioscience, Inc., KRKA, Fujfilm Europe.

The study has been endorsed by European Helicobacter and Microbiota Study Group (EHMSG), Healthy Stomach Initiative (HIS) and International Digestive Cancer alliance (IDCA). Support has been provided by the Riga East University hospital Support Foundation. We acknowledge also the support from the Ministry of Health of Latvia.

REFERENCES

1. Herrero R, Parsonnet J, Greenberg ER. Prevention of gastric cancer. *JAMA* 2014;312(12):1197-8.
2. Forman D, Sierra M, S. The current and projected global burden of gastric cancer. In: IARC Helicobacter pylori Working Group Helicobacter pylori Eradication as a Strategy for Preventing Gastric Cancer Lyon, France: International Agency for Research on Cancer (IARC Working Group Reports, No 8); pp 5-15 Available from: http://wwwiarcfr/en/publications/pdfs-online/wrk/wrk8/indexphp2014.
3. Jemal A, Bray F, Center MM*, et al.* Global cancer statistics. *CA: A Cancer Journal for Clinicians* 2011;61(2):69-90.
4. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *European journal of cancer (Oxford, England : 1990)* 2010;46(4):765-81.
5. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J*, et al.* Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013;49(6):1374-403.
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, liver flukes and Helicobacter pylori *IARC Monogr Eval Carcinog Risks Hum* 1994;61:1-241.
7. IARC. Monographs on the evaluation of carcinogenic risks to humans, volume 100. A review of carcinogen - Part B: biological agents. Lyon: International Agency for Research on Cancer 2011.
8. Plummer M, Franceschi S, Vignat J*, et al.* Global burden of gastric cancer attributable to Helicobacter pylori. *Int J Cancer* 2015;136(2):487-90.
9. Lansdorp-Vogelaar I, Sharp L. Cost-effectiveness of screening and treating Helicobacter pylori for gastric cancer prevention. *Best Pract Res Clin Gastroenterol* 2013;27(6):933-47.
10. Areia M, Carvalho R, Cadime AT*, et al.* Screening for gastric cancer and surveillance of premalignant lesions: a systematic review of cost-effectiveness studies. *Helicobacter* 2013;18(5):325-37.
11. Moayyedi P. Feasibility and cost effectiveness of population-based H. pylori eradication. In: IARC Helicobacter pylori Working Group Helicobacter pylori Eradication as a Strategy for Preventing Gastric Cancer Lyon, France: International Agency for

Research on Cancer (IARC Working Group Reports, No 8); pp 174-180 Available from: http://wwwiarcfr/en/publications/pdfs-online/wrk/wrk8/indexphp2014.

12. Sugano K, Tack J, Kuipers EJ*, et al.* Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015;64(9):1353-67.
13. Malfertheiner P, Megraud F, O'Morain CA*, et al.* Management of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. *Gut* 2017;66(1):6-30.
14. Malhotra-Kumar S, Lammens C, Coenen S*, et al.* Effect of azithromycin and clarithromycin therapy on pharyngeal carriage of macrolide-resistant streptococci in healthy volunteers: a randomised, double-blind, placebo-controlled study. *Lancet* 2007;369(9560):482-90.
15. Derakhshan MH, Arnold M, Brewster DH*, et al.* Worldwide Inverse Association between Gastric Cancer and Esophageal Adenocarcinoma Suggesting a Common Environmental Factor Exerting Opposing Effects. *Am J Gastroenterol* 2016;111(2):228-39.
16. Lonnberg S, Sekerija M, Malila N*, et al.* Cancer screening: policy recommendations on governance, organization and evaluation of cancer screening. In: European Guide on Quality Improvement in Comprehensive Cancer Control Available from: http://wwwcancercontroleu/guide-landing-page/. Belgium: National Institute of Public Health, Slovenia, Scientific Institute of Public Health, Belgium 2017:39-76.
17. Bornschein J, Leja M. The global challenge of a healthy stomach. *Best practice & research Clinical gastroenterology* 2014;28(6):949-51.
18. Huang YK, Yu JC, Kang WM*, et al.* Significance of Serum Pepsinogens as a Biomarker for Gastric Cancer and Atrophic Gastritis Screening: A Systematic Review and Meta-Analysis. *PLoS One* 2015;10(11):e0142080.
19. IARC. World Health Organization Cancer mortality database, available from http://www-dep.iarc.fr/WHOdb/WHOdb.htm. Accessed on 29/02/2016. 2015
20. Leja M, Camargo MC, Polaka I*, et al.* Detection of gastric atrophy by circulating pepsinogens: A comparison of three assays. *Helicobacter* 2017. *In Press*
21. Dixon MF, Genta RM, Yardley JH*, et al.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20(10):1161-81.
22. Kupcinskas J, Leja M. Management of Helicobacter pylori-related diseases in the Baltic States. *Dig Dis* 2014;32(3):295-301.

23. Dinis-Ribeiro M, Areia M, de Vries AC*, et al.* Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 2012;44(1):74-94.

24. Ferlay J, Shin HR, Bray F*, et al.* GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. *Lyon, France: International Agency for Research on Cancer Available from: http://globocaniarcfr, accessed on 22/12/2011* 2010

25. Ford AC, Forman D, Hunt RH*, et al.* Helicobacter pylori eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 2014;348:g3174.

26. Leja M, Cine E, Rudzite D*, et al.* Prevalence of Helicobacter pylori infection and atrophic gastritis in Latvia. *Eur J Gastroenterol Hepatol* 2012;24(12):1410-7.

27. Agreus L, Kuipers EJ, Kupcinskas L*, et al.* Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. *Scand J Gastroenterol* 2012;47(2):136-47.

28. Oishi Y, Kiyohara Y, Kubo M*, et al.* The serum pepsinogen test as a predictor of gastric cancer: the Hisayama study. *Am J Epidemiol* 2006;163(7):629-37.

29. Yanaoka K, Oka M, Mukoubayashi C*, et al.* Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2008;17(4):838-45.

30. Kurilovich S, Belkovets A, Reshetnikov O*, et al.* Stomach-specific Biomarkers (GastroPanel) Can Predict the Development of Gastric Cancer in a Caucasian Population: A Longitudinal Nested Case-Control Study in Siberia. *Anticancer research* 2016;36(1):247-53.

31. Miki K. Gastric cancer screening by combined assay for serum anti-Helicobacter pylori IgG antibody and serum pepsinogen levels - "ABC method". *Proc Jpn Acad Ser B Phys Biol Sci* 2011;87(7):405-14.

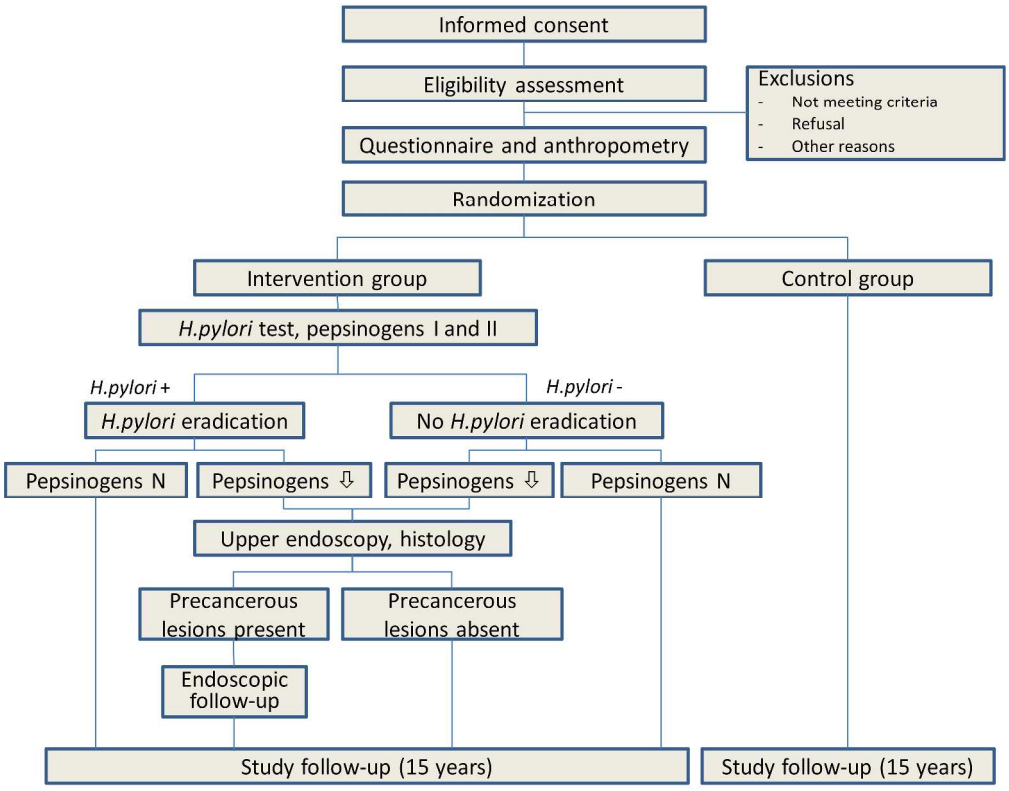


Figure. GISTAR general study design

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	3, 7
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	7
Funding	4	Sources and types of financial, material, and other support	14, 15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1,2
	5b	Name and contact information for the trial sponsor	2, 14
	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	14
	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)	14, 15

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	5-7
	6b	Explanation for choice of comparators	5-6
Objectives	7	Specific objectives or hypotheses	6-7
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)	8
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	7-8
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)	7-8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8-9
	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)	8-9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
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	10, 11
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)	10

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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7
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	7, 9
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	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7, 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
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	9, 10
	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	12

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	9, 10
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)	9
	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)	NA
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	13
	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	13
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	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
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)	13

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7, 9
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	9
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
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	13-15
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	NA
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	13
	31b	Authorship eligibility guidelines and any intended use of professional writers	13
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
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	9, 10

*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.