Study Protocol

Title: Multigene Panel Test in Gastric Cancer Patients in Portugal

NCT:

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Authors:

1. Mourato B., MD
    a. Affiliation: Unidade Local de Saúde do Norte Aletenjano;
    b. Contacts: email: beatriz.mourato@ulsna.min-saude.pt; Address: Hospital Doutor José Maria Grande, Avenida de Santo António, 7300-853, Portalegre;
    c. Contributions: Main author; Study designer.
2. Areia M., PhD
    a. Affiliation
        i. Instituto Português de Oncologia de Coimbra;
        ii. RISE@CI-IPO (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)
    b. Contacts: email: miguel.areia75@gmail.com ; Address: Avenida Bissaya Barreto 98, 3000-075 Coimbra.
    c. Contributions: Intellectual contributor; Reviewer.
3. Fronteira, I., PhD
    a. Affiliation:
    b. Contacts: ines.fronteira@ensp.unl.pt; Address: Avenida Padre Cruz, 1600-560 Lisboa.
    c. Contributions: Study designer; Intellectual contributor; Reviewer.
4. Dinis, R., PhD
    a. Affiliation: Hospital do Espírito Santo de Évora,
    b. Contacts: email: rui.p.dinis@ulsna.min-saude.pt; Address: Largo do Sr. Da Probreza, 7000-811 Évora;
    c. Contributions: Study designer; Study collaborator; Reviewer.

Abstract

Abstract: Gastric cancer (GC) is the fifth with the highest incidence and mortality worldwide (1). It is a multifactorial and heterogeneous disease, where both environmental and genetic factors are involved. Three hereditary syndromes primarily affect the stomach: Hereditary Diffuse Gastric Cancer (HDGC), Gastric Adenocarcinoma and Stomach Proximal Polyposis (GAPPS), and Familial Intestinal Gastric Cancer (FIGC) (2), but in addition to these, many other syndromes and genetic mutations are associated with an increased risk of GC (3–5). As GC is a neoplasm with numerous somatic and hereditary mutations involved in its carcinogenesis, the use of the Multigene Germline Panel Test (MGPT) may be crucial, as it allows the analysis of hereditary mutations in a significant number of genes simultaneously (6,7). This observational, retrospective study aims to quantify and characterise the pathogenic variants (PVs) found in the MGPT of GC patients in Alto Alentejo (Portugal).

Introduction

Background/ Rationale

According to data from the Global Cancer Observatory, gastric cancer (GC) ranks fifth in terms of incidence and mortality worldwide (1).

It is a multifactorial and heterogeneous disease involving both environmental and genetic factors. Although the majority of cases are sporadic, caused by environmental exposures, up to 20% of patients have a familial aggregation and of these, 3 to 5% are classified as hereditary with an identified mutation (8,9).

Three hereditary syndromes primarily affect the stomach: Hereditary Diffuse Gastric Cancer (HDGC); Gastric Adenocarcinoma and Stomach Proximal Polyposis (GAPPS); and Familial Intestinal Gastric Cancer (FIGC) (2). HDGC is an autosomal dominant syndrome, predominantly caused by germline mutations in the CDH1 gene, which encodes the e-cadherin protein with important functions in cell aggregation, and is characterised by a high risk of early-onset diffuse GC and invasive lobular breast cancer (10). GAPPS is caused by germline mutations in APC promoter 1B and is characterised by the presence of fundic gland polyposis with focal dysplasia and intestinal or mixed adenocarcinoma at the level of the gastric fundus and sparing the antrum (4,11). Finally, FIGC is an autosomal dominant syndrome, still poorly characterized genetically, which is associated with an increased risk of intestinal GC (10).

Other genetic syndromes are also associated with an increased risk of GC. Some of the genes involved include MLH1, MSH2, MSH6, and PMS2 (Lynch Syndrome - LS), TP53 (Li-Fraumeni Syndrome), APC (PAF and ADC / proximal polyposis of the stomach) and MUTYH (Polyposis Associated MUTYH), BMPR1A and SMAD4 (Juvenile Polyposis Syndrome), STK11 (Peutz-Jegher Syndrome) and PTEN (Cowden Syndrome) (3–5).

As GC is a neoplasm with numerous somatic and hereditary mutations involved in its carcinogenesis, the use of MGPT can be crucial, as it performs the analysis of hereditary mutations in a significant number of genes simultaneously (depending on the genes included in the panel) (6,7). In addition to the described mutations, MGPT has allowed the identification of new genetic mutations associated with GC risk, including in the BRCA 1/2, PALB2, ATM and RAD51C genes (typically associated with hereditary breast and ovarian cancer), MAP3K6,

MYD88, among others (9,12–17). However, the use of MGPT, especially in unselected GC patients, is not without risks, such as the unexpected detection of PV in genes not strictly associated with the phenotype (secondary findings) or, more commonly, the high rate of variants of unknown significance (VUS), which can raise important questions regarding the uncertainty of how to approach these patients (18). Therefore, some studies advocate that MGPT or single gene testing (SGT) should be offered only to selected patients, especially those with suspected HDCG (5,19–21). On the other hand, the evidence of many other germline mutations associated with GC and the lack of genetic characterisation of FIGC justifies the growing interest in performing MGPT in unselected patients with GC (10,18,22,23). Although these studies suggest abenefit in the use of MGPT in unselected patients with GC, this test is not yet widely used.

In Portugal, the incidence and mortality rates of GC have been increasing over the last few decades. It is therefore important to better understand the genetic characteristics of GC in this country in order to develop better strategies for managing this disease.

Objectives

The **main objective** of this research is to quantify and characterize the PV found in MGPT of GC patients in Alto Alentejo (Portugal).

The **specific objectives** are:

- Determine the global rate of PVs in GC patients of Alto Alentejo;
- Determine which PVs are most common in our GC population;
- Correlate the PVs found with clinical factors: Age at diagnosis; Sex; Weight; Height; Presence of other diseases (Diabetes; Arterial Hypertension; Dyslipidaemia; Stroke; Heart attack); Date of diagnosis; Stage at diagnosis; Histologic subtype; Surgery for GC; Personal history of other cancers; Familial history of GC and other cancers;
- Correlate the PVs found with prognostic factors: 12-month survival rate; mortality.

Methods

Study design

Observational, retrospective study

Despite this design, there was uniformity in the data recorded for each participant who agreed to be submitted to MGPT, minimizing the variability between records or the missing clinical data.

Setting

This study takes place at the Unidade Local de Saúde do Alto Alentejo (ULSAALE). The MGPT was always performed in the same laboratory (Germano de Sousa – Centro de Genética Laboratorial) using peripheral blood samples from patients with GC.

Two types of MGPT were used:

- MGPT 4005 (15 genes): APC, ATM, BLM, BMPR1A, CDH1, CTNNA1, EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN, SMAD4, STK11TP53.

- MGPT 4013 (30 genes): ATM, BARD1, BLM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FANCC, MLH1, MRE11A, MSH2, MSH6, MUTYH,

NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, RECQL4, RINT1, SLX4, SMARCA4, STK11, TP53, XRCC2.

The majority of patients with GC were tested with MGPT 4005. Patients with neoplasms other than GC or with a family history of other neoplasms were tested with MGPT 4013. The decision to perform MGPT and the choice of panel type were made in multidisciplinary consultation.

The recruitment process takes place between January 2023 and December 2024. All data will be collected and entered in a database until September 2025

The manuscript for publication will be written between October and November 2025.

Participants

Eligibility criteria:

- Adult patients (over 18 years old) with a histologically proven diagnosis of gastric adenocarcinoma that have done MGPT between 2023 and 2024;
- Ability to understand and speak Portuguese and/or English language;
- Ability to reliably provide informed consent for inclusion in the study.

There are no matched or randomized groups.

Variables / Measurement

Table 2

Data sources

The variables related to the patient's clinical history and personal data will be collected through a questionnaire administered during a personal interview and supplemented by consultation of each patient's digital medical record in order to obtain more detailed information on the variables characterising the disease and the results of the MGPT.

Bias

Efforts to address potential sources of bias:
Clear Inclusion and Exclusion Criteria: The inclusion criteria are clear to select patients that are representative of the target population (patients with gastric adenocarcinoma) and that there is no bias in participant selection.
Control of Confounding Factors: There are some confounding factors that may influence the study results, such as age, sex, Weight; Height; Body mass index; Presence of other diseases: Diabetes; Arterial Hypertension; Dyslipidaemia; Stroke; Heart attack). This will be achieved through appropriate statistical techniques or data stratification during analysis.
Uniformity in data records: There was uniformity in the data recorded for each participant who agreed to be submitted to MGPT, minimizing the variability between records or the missing clinical data.
Consistent and Accurate Data Collection: This will be achieved through adequate training of interviewers or data collection personnel and the use of standardized data collection protocols.

Blind Data Assessment: After collection, the data will be pseudo-anonymized so that the researcher who analyses the data will not have access to the patient's identifying data.
Time-Sensitive Analysis: Data analysis takes into account the timing of data collection relative to relevant clinical events.
Sensitivity Analysis: It will be performed a sensitivity analyses to assess the robustness of the results across different results of MGPT and clinical factors.

Study size

The sample calculation takes into account the number of annual cases of GC recorded in Alto Alentejo (65 cases in 2020, data from National Oncologic Registry). Therefore, the sample calculation suggests that it should include 56 patients with GC, with a confidence level of 95% and a margin of error of 5%.

Quantitative variables

For quantitative variables we will do the descriptive statistics analyses with mean, mode, median, standard deviation, interquartile variation, maximum and minimum.
Subgroup analysis will be carried out, for each quantitative and qualitative variable, dividing patients who have positive or negative MGPT. We will analyse the effect measures of the difference between the two groups, with confidence interval (IC) 95%, and if possible, inferential statistical analysis will be conducted.

Statistical methods

a) Statistical analysis will be carried out using SPSS software.

b) In inferential statistical analysis, the normality of the distribution of quantitative variables will be determined by the Kolmogorov-Smironov test. For variables with normal distribution, the T-sudent test will be used and for those that do not have normal distribution, the Mann-Whitney test will be performed, with the purpose of analysing the statistical significance of the difference between the groups with IC 95% (positive MGPT and negative MGPT). For qualitative variables, the statistical significance of the difference between groups, with 95% CI, will be calculated using the Chi-square test (if more than 10% of the cells in the 2x2 tables have frequencies ≤ 5) or the Exact Fisher Test.
We will later perform a multivariate analysis with logistic regression to control confounding variables.

c) Missing data: We addressed missing data by employing multiple strategies. Firstly, we conducted a thorough examination of patterns of missingness to discern if they followed a missing completely at random, missing at random, or missing not at random pattern. Subsequently, for cases with missing data, we utilized multiple imputation techniques to impute missing values based on observed data and variables that were predictive of missingness. Additionally, we performed sensitivity analyses to assess the robustness of our findings to different assumptions regarding the missing data mechanism. Overall, these

approaches allowed us to minimize bias and maintain the integrity of our analyses in the presence of missing data.

d) To conduct sensitivity analysis for this study, we will systematically vary key parameters or assumptions within our analytical framework to assess their impact on the study outcomes. This may involve testing different statistical models, criteria for participant inclusion/exclusion, handling of missing data, or adjustments for potential confounding variables. By exploring a range of scenarios, we aim to evaluate the robustness of our findings and assess the degree to which they are influenced by specific methodological choices or assumptions. Sensitivity analysis allows us to identify the potential sources of uncertainty and to provide a more comprehensive understanding of the reliability and generalizability of our study results.

Table 2 - Variables / Measurements

Type of Variable	Name of Variable	Measurements
MGPT Outcomes variables	MGPT	positive (1) or negative (0)
	PV	integer number
	Likely pathogenic variables (LP)	integer number
	Name of genes with PV	descriptive
	PV in each gene	integer number
	VUS	integer number
Potential confounders variables	Age at diagnosis	Years
	Sex	Female(1)/Male(0)
	Weight	Kg
	Height	cm
	Body mass index	Kg/m^2

	Presence of other diseases: Diabetes; Arterial Hypertension; Dyslipidaemia; Stroke; Heart attack);	Yes(1)/No(0) for each disease
	Stage at diagnosis (AJCC staging system);	0, I, IIa, IIb, III, IVa, IVb
	Histologic subtype;	Intestinal (1); Diffuse (2); Mixed (3)
"Exposures" variables	Personal history of other cancers	Yes (1)/No (0)
	Types of other cancer in GC patient	descriptive
	Familial history of GC	Yes (1)/No (0)
	Age of onset of each familial case of GC	integer number
	Familial history of other cancers (specifying)	descriptive
	Age of onset of each familial case of other cancers	Years
Prognostic related variables	12-months survival	Yes (1)/No(0)
	Death due to GC	Yes (1)/No(0)
	Date of death	mm/yyyy
Other variables:	Date of diagnosis	mm/yyyy
	Localization of tumor	Cardia / Fundos / Body / Antrum/pyloro
	Time since diagnosis until the current moment	months
	Surgery for GC	Yes (1)/No (0);
	Type of surgery	Total gastrectomy (1); Subtotal gastrectomy (0)
	Date of Surgery	mm/yyyy
	Histologic staging after surgery	0, I, IIa, IIb, III, IVa, IVb
	Underwent chemotherapy	Yes (1) / No (0)
	Type of chemotherapy	descriptive

	Cycles of chemotherapy	integer number
Positive MGPT is considered when it is found at least one PV in one gene of teste A comparative analysis will be made between the individual characteristics and tumor characteristics of positive MGPT cases with those with negative MGPT.		

Ethical Issues

This research has already been approved by the Ethics Committee and the Board of Directors of ULSAALE (Ata 06/2024, document 60 / 202400991) and it will be submitted to Ethics Committee of the Nova Medical School.

Participation in the study is voluntary, with informed consent and participants will be informed of the results before publication.

Risks and Benefits

The main benefits and expected outcomes of the study are the contribution to a better understanding of GC carcinogenesis, which could improve the diagnostic and therapeutic approach of GC patients and their relatives. In addition, this research may help to clarify whether the prevalence of hereditary GC is higher than that described in the literature (3-5%) (4,5), and may justify screening the population for this disease.

However, the use of MGPT is not without important risks, namely the identification of variants of undetermined significance (VUS), which requires careful integration of all clinical and genetic information for correct personal and family counselling. Whenever necessary, VUS carriers (as well as patients with identified PVs) will be referred to the Portuguese Institute of Oncology in Lisbon (IPOLFG) for family risk and genetic counselling. Other potential risks of this study include the psychological stress and anxiety caused by the survey and MGPT results, the need to travel to participate in the study, and the fact that the study is being conducted by non-geneticist researchers, which limits the immediate counselling that can be provided. In this way, we will have the collaboration of the psychologists of the Day Hospital, and our close collaboration with the Germano de Sousa Genetics Laboratory and the IPOLFG teams will allow an adequate response to technical questions about the MGPT. Finally, it should be noted that this observational study will only analyse the MGPT results of CG patients and will not propose any additional intervention for patients or their families.

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