Study Protocol and Statistical Analysis Plan:

The impact of endoscopic surveillance of atrophic gastritis with and without intestinal metaplasia on a high-risk population for gastric cancer in Latin America: The ECHOS cohort study

NCT: not assigned

July 10, 2023

INTRODUCTION

Gastric cancer is the 5th most common cancer and the 4th leading cause of cancer-related deaths worldwide.[1] Chronic atrophic gastritis (CAG) is part of a sequential histopathological cascade that precedes the development of gastric adenocarcinoma (GA).[2] Chronic *Helicobacter pylori* (*H. pylori*) infection is the primary trigger of this cascade and leads to chronic inflammatory changes in the gastric mucosa which can lead to CAG, intestinal metaplasia (IM), dysplasia and eventually GA.[3,4]

Multiple strategies have been suggested for GA prevention.[5-8] Primary prevention is based on the recognition and treatment of *H. pylori* as the main risk factor for GA.[9-12] Secondary prevention relies on endoscopic surveillance of high-risk patients for the detection of early-stage GA with potentially curative resection. CAG and IM can be accurately identified and graded through gastric mapping biopsies obtained according to the updated Sydney System biopsy protocol (USSBP).[13] Staging of preneoplastic conditions with Operative Link on Gastritis Assessment (OLGA) or Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM) scales[14] has demonstrated to accurately predict the risk of progression to GA in some populations;[15-18] however, the value of these systems for risk stratification has not been comprehensively evaluated in a high-risk Latin American population.

In Chile, gastric cancer is the leading cause of cancer-related deaths in men and the 3rd in females, with mortality rates of 25 and 12.2 per 100,000 inhabitants, respectively. Risk stratification of patients with CAG/IM would help with prioritization of EGDs for those with CAG/IM at highest risk of progression and minimizing endoscopic burden. However, there are limited data in Latin America regarding risk factors associated with progression of gastric preneoplasia, and specifically, the performance of OLG/OLGIM staging for risk stratification.[24]

Accordingly, our objective was to evaluate the risk factors associated with incident high grade dysplasia (HGD)/GA in patients with CAG with or without IM who are enrolled in endoscopic surveillance, as well as to compare HGD/GA incidence according to the OLGA and OLGIM

scales. As an exploratory analysis, we also evaluated the stage of GA among those enrolled in endoscopic surveillance, given that most GA in Chile are diagnosed in the advanced stage.

METHODS

Study design and settings

The Endoscopic Cohort and Histological OLGA staging (ECHOS) study is a retrospective longitudinal cohort developed in the Pontificia Universidad Católica de Chile health care system, an open health care system with 3 endoscopic units in Santiago, Chile. Patients 18 years or older were eligible for the study if they had at least one non-urgent outpatient EGD with gastric biopsies collected following the USSBP between June 2015 and June 2021. The baseline EGD was defined as the earliest examination conducted between this time frame that met inclusion criteria. Participants were included in the cohort if they underwent a subsequent "surveillance" EGD. In order for the subsequent EGD to qualify as surveillance, it needed to occur at least 6 months after the baseline EGD and include gastric biopsies collected according to the USSBP; one exception to the USSBP requirement was if HGD or GA was detected on the surveillance (i.e. non-baseline) EGD. Patients with prior history of HGD or any type of gastric malignancy, gastrectomy or non-gastric active malignancy were excluded. Also, patients with HGD or GA on the baseline EGD were excluded. Patients with indefinite (IND) or low-grade dysplasia (LGD) on the baseline EGD were considered for the cohort (see below).

Demographic variables, medical comorbidities, and first-degree family history of gastric cancer were recorded from clinical records. The baseline and follow-up endoscopic findings were abstracted from the procedure reports, including the presence of any overt lesions or abnormalities. The baseline and follow-up histological findings, including *H. pylori* status (as defined by histologic findings), CAG and IM anatomical extent, IM histologic type, and OLGA/OLGIM stage (detailed below) were recorded. Although EGD surveillance schedules were determined by the endoscopist, these generally followed the published Chilean guideline recommendations for CAG and IM endoscopic surveillance—namely, surveillance annually for OLGA III/IV, IND, or LGD, every 3 years for OLGA I/II and every 5 years if OLGA 0 with persistent *H. pylori* infection.[21]

Endoscopic follow-up of all individuals with their baseline EGD between June 2015-2021 was captured through the end of the study period, March 6, 2023. The composite primary outcome was defined as incident HGD or GA, as confirmed based on histology. Secondary outcomes were incident GA alone, incident LGD, global histopathological progression (defined as a progression from one global stage in the precancerous cascade to another, e.g. from chronic superficial gastritis (CSG) to CAG or from CAG without IM to IM), progression or regression of OLGA and OLGIM stages (defined as an increase or decrease of at least one point in the scales, e.g. OLGA II to III). We also captured the stage of GA. A flow chart of the study is illustrated in **Supplementary Figure 1**.

Procedures were performed according to ethical standards established in the Helsinki Declaration. The study protocol was approved by the Ethics Committee of Hospital Clínico Universidad Católica de Chile (ID 16-341). All patients signed written informed consent for the EGD prior to the procedure.

EGD and gastric biopsies sampling method

All baseline and surveillance EGDs were performed by experienced endoscopists using the same equipment across the 3 endoscopic units (Olympus (GIF-H190/GIF-H170 or Fujinon EC-600ZW). Gastric biopsies were obtained following the USSBP as described in supplementary methods and additional gastric biopsies were collected and stored in a different jar if there were any additional concerning findings during the EGD.

Histological classification of gastric biopsies

Gastric samples were evaluated at one centralized pathology laboratory by two independent experienced pathologists (JT and JCR). Biopsies were hematoxylin and eosin (H&E) and Giemsa stained. Evaluation of CAG was performed according to the OLGA scale (stages 0 to IV).[13] IM was classified for clinical purposes by the anatomical extent (antrum-restricted *vs.* corpus-extended) and the histological subtype (complete type *vs.* incomplete type) based on H&E. All slides were retrospectively reviewed by the pathologists (JT and JCR) to confirm the baseline

presence or absence of CAG/IM and to provide a corresponding OLGIM stage.[25] Agreement between the pathologists for OLGA groups, OLGIM groups and IM were Kappa 0.73, 0.78 and 0.86, respectively. In all discrepant cases, an agreement was reached between the pathologists with no need for a third pathologist as an arbiter. Gastric dysplasia was categorized as IND, LGD or HGD according to the Vienna classification.[26] The most advanced discrete histology observed in the set of biopsies was used as the global diagnosis for analysis purposes. Antrum-restricted IM was defined as IM located only in the antrum or incisura angularis without evidence of corpus involvement, while corpus-extended IM was defined as IM located in the corpus irrespective of the findings in the antrum or incisura angularis.

Active *H. pylori* infection was determined based on Giemsa staining in any of the gastric samples. Presence of *H. pylori* at both baseline and surveillance EGD was considered as persistent *H. pylori* infection; whereas positive *H. pylori* at baseline and negative at follow-up was considered *H. pylori* eradication, as long as treatment occurred in between. Autoimmune gastritis (AIG) histologic features were also assessed and recorded (further described in supplementary methods).[27]

Statistical Analysis Plan

Patients were categorized based on OLGA and OLGIM stage as low-risk (OLGA/OLGIM 0-I), intermediate-risk (OLGA/OLGIM II) and high-risk (OLGA/OLGIM III-IV).[15-18] Demographic and clinical characteristics were compared between the OLGA/OLGIM groups. Categorical variables were expressed as percentages (%) and continuous variables were expressed as mean and 95% confidence interval (95%CI) or median and interquartile range (IQR) as appropriate. The Chi-square test was used to compare categorical variables and differences in continuous variables were evaluated with t-test or Mann-Whitney U test, as appropriate.

For the primary analysis, we analyzed the rate of progression to HGD/GA in each of the defined OLGA/OLGIM groups, expressed as incidence rate per 100 person-year with 95%CI. Also, given that OLGA/OLGIM II is potentially an intermediate-risk category,[15-18] we conducted a subgroup analysis evaluating factors associated with progression of OLGA II vs. OLGA 0/I. For the models

evaluating cumulative HGD/GA risk, follow-up time started at the baseline EGD and continued until the earliest of the following: last available surveillance EGD, occurrence of HGD/GA or death. Cumulative risk of the composite primary outcome was estimated from Kaplan-Meier functions.

Univariate and multivariable Cox proportional hazards models were used to assess factors associated with HGD/GA, expressed as hazard ratio (HR) and 95%CI. Given the expected low number of primary outcome events (HGD/GA), Poisson regression models were used as a sensitivity analysis to estimate the adjusted incidence rate ratio (aIRR) by OLGA and OLGIM staging. Separate models for OLGA and OLGIM staging were performed, given the high correlation and collinearity of this variables. Models were adjusted for age, sex, active *H. pylori* infection, and the number of surveillances EGDs during the study period.

Secondary analyses included 1) differences in progression to HGD/GA based on anatomic extent and IM subtype, according to the following subgroups: antral-restricted complete-type IM, antral-restricted incomplete-type IM, corpus-extended complete-type IM and corpus-extended incomplete-type IM, using the log-rank test for equality survival function. Patients with unavailable IM histological subtype were excluded from these analyses (i.e. "IM unavailable, 10.9%"); 2) change in a global histological category with respect to either histopathological progression or regression; 3) changes in OLGA and OLGIM stages during the follow-up. Multivariable logistic regression analyses, adjusted for age, sex and *H. pylori* infection, were used to evaluate factors associated with histopathological or OLGA/OLGIM progression and regression (binary outcome), and expressed as adjusted odds ratios (aOR) and 95%CI. We also evaluated the stage of GA among patients enrolled in endoscopic surveillance.

As an exploratory analysis, we characterized the demographic and histopathological characteristics of those patients who had baseline EGD with USSBP but who did not undergo subsequent surveillance EGD (and thus did not meet inclusion criteria for the primary analytic cohort).

An *a priori* two-sided p-value threshold of 0.05 was established for statistically significant results. All statistical analyses were conducted using STATA v14.2 (Statacorp, College Station, TX, USA).

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021.
2. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res*. 1992;52:6735-6740.
3. International Agency for Research on Cancer. *Schistosomes, liver flukes and Helicobacter pylori*. Lyon, France: IARC, 1994.
4. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988;48:3554-60.
5. Gupta S, Li D, El Serag HB, et al. AGA Clinical Practice Guidelines on Management of Gastric Intestinal Metaplasia. Gastroenterology 2020; 158(3):693-702.
6. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, et al. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. *Endoscopy* 2019;51(4):365-388.
7. Banks M, Graham D, Jansen M, et al. British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma. Gut. 2019 Sep;68(9):1545-1575.
8. Matysiak-Budnik T, Camargo MC, Piazuelo MB, et al. Recent Guidelines on the Management of Patients with Gastric Atrophy: Common Points and Controversies. *Dig Dis Sci* 2020;65(7):1899-1903.
9. Yan L, Chen Y, Chen F, et al. Effect of *Helicobacter pylori* Eradication on Gastric Cancer Prevention: Updated Report From a Randomized Controlled Trial With 26.5 Years of Follow-up. Gastroenterology. 2022 Mar 29:S0016-5085(22)00338-9.
10. Choi IJ, Kook M-C, Kim Y-I, et al. *Helicobacter pylori* therapy for the prevention of metachronous gastric cancer. N Engl J Med 2018;378:1085-1095.

11. Choi IJ, Kim CG, Lee JY, et al. Family History of gastric cancer and *Helicobacter pylori* treatment. N Engl J Med 2020;382:427–436.
12. Chiang TH, Chang WJ, Chen SL, et al. Mass eradication of *Helicobacter pylori* to reduce gastric cancer incidence and mortality: a long-term cohort study on Matsu Islands. Gut. 2021 Feb;70(2):243-250.
13. 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.
14. Capelle LG, de Vries AC, Haringsma J, et al. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. Gastrointest Endosc. 2010 Jun;71(7):1150-8.
15. Rugge M, Genta RM, Fassan M, et al. OLGA Gastritis Staging for the Prediction of Gastric Cancer Risk: A Long-term Follow-up Study of 7436 Patients. *Am J Gastroenterol* 2018; 113(11):1621-1628.
16. Lee JWJ, Zhu F, Srivastava S, et al. Severity of gastric intestinal metaplasia predicts the risk of gastric cancer: a prospective multicentre cohort study (GCEP). Gut. 2021 May 11:gutjnl-2021-324057.
17. Yue H, Shan L, Bin L. The significance of OLGA and OLGIM staging systems in the risk assessment of gastric cancer: a systematic review and meta-analysis. Gastric Cancer. 2018 Jul;21(4):579-587.
18. den Hollander WJ, Holster IL, den Hoed CM, et al. Surveillance of premalignant gastric lesions: a multicentre prospective cohort study from low incidence regions. Gut. 2019 Apr;68(4):585-593.
19. Torres-Roman JS, Alvarez CS, Guerra-Canchari P, et al. Sex and age differences in mortality trends of gastric cancer among Hispanic/Latino populations in the United States, Latin America, and the Caribbean. Lancet Reg Health Am. 2022 Oct 7;16:100376.
20. Ministry of Health of Chile. Mortality statistics. In: Information. DoHSa, editor. [cited 2022 Jan 15]. Available from: https://deis.minsal.cl.
21. Rollán A, Cortés P, Calvo A, et al. Recommendations of the Chilean Association for Digestive Endoscopy for the management of gastric pre-malignant lesions. *Rev Med Chile* 2014; 142: 1181-92.

22. Latorre G, Álvarez J, Ivanovic-Zuvic D, et al. Coverage of diagnostic upper gastrointestinal endoscopy for the prevention of gastric cancer in Chile: results from the 2009-2010 National Health Survey. Rev Med Chil. 2015 Sep;143(9):1198-205.
23. González N, Parra-Blanco A, Arantes V, Mönkemüller. Current status of endoscopic submucosal dissection in Latin America. Acta Gastroenterol Latinoam 2016;46:213-219.
24. Piazuelo MB, Bravo LE, Mera RM, et al. The Colombian Chemoprevention Trial: 20-Year Follow-Up of a Cohort of Patients with Gastric Precancerous Lesions. Gastroenterology. 2021 Mar;160(4):1106-1117.e3.
25. Gawron AJ, Shah SC, et al. AGA Technical Review on Gastric Intestinal Metaplasia-Natural History and Clinical Outcomes. Gastroenterology. 2020 Feb;158(3):705-731.e5.
26. Dixon MF. Gastrointestinal epithelial neoplasia: Vienna revisited. Gut. 2002 Jul;51(1):130-1.
27. Lenti MV, Rugge M, Lahner E, et al. Autoimmune gastritis. Nat Rev Dis Primers 2020; 6(1):56.
28. Shichijo S, Hirata Y, Niikura R et al. Histologic intestinal metaplasia and endoscopic atrophy are predictors of gastric cancer development after Helicobacter pylori eradication. Gastrointest Endosc 2016; 84: 618 – 624.
29. Li D, Bautista MC, Jiang SF, et al. Risks and Predictors of Gastric Adenocarcinoma in Patients with Gastric Intestinal Metaplasia and Dysplasia: A Population-Based Study. Am J Gastroenterol. 2016 Aug;111(8):1104-13.
30. Reddy KM, Chang JI, Shi JM, Wu BU. Risk of Gastric Cancer Among Patients With Intestinal Metaplasia of the Stomach in a US Integrated Health Care System. Clin Gastroenterol Hepatol. 2016 Oct;14(10):1420-5.
31. Song H, Ekheden IG, Zheng Z, Ericsson J, Nyrén O, Ye W. Incidence of gastric cancer among patients with gastric precancerous lesions: observational cohort study in a low risk Western population. BMJ. 2015 Jul 27;351:h3867.
32. Matysiak-Budnik T, Camargo MC, Piazuelo MB, Leja M. Recent Guidelines on the Management of Patients with Gastric Atrophy: Common Points and Controversies. Dig Dis Sci. 2020 Jul;65(7):1899-1903.
33. Isajevs S, Liepniece-Karele I, Janciauskas D, et al. Gastritis staging: interobserver agreement by applying OLGA and OLGIM systems. *Virchows Arch*. 2014;464:403–407.

34. González CA, Sanz-Anquela JM, Companioni O, et al. Incomplete type of intestinal metaplasia has the highest risk to progress to gastric cancer: results of the Spanish follow-up multicenter study. J Gastroenterol Hepatol. 2016 May;31(5):953-8.
35. Whiting JL, Sigurdsson A, Rowlands DC, Hallissey MT, Fielding JW. The long term results of endoscopic surveillance of premalignant gastric lesions. Gut. 2002 Mar;50(3):378-81.
36. Sano T, Katai H, Sasako M, Maruyama K. The management of early gastric cancer. Surg Oncol. 2000 Jul;9(1):17-22.
37. Bollschweiler E, Berlth F, Baltin C, Mönig S, Hölscher AH. Treatment of early gastric cancer in the Western World. *World J Gastroenterol*. 2014;20(19):5672-5678.
38. Coppelli L, Díaz LA, Riquelme A, Waeger C, Rollán A, Bellolio E, et al. La derivación protocolizada a endoscopía asociada a la detección de Helicobacter pylori mediante antígeno en deposiciones disminuye lista de espera para endoscopía y optimiza la detección de lesiones premalignas y cáncer gástrico incipiente. Rev Med Chil. 2019;147:1382-9.
39. de Vries AC, van Grieken NC, Looman CW et al. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. Gastroenterology 2008; 134: 945 – 952.
40. Simone A, Casadei A, De Vergori E et al. Rescue endoscopy to identify site of gastric dysplasia or carcinoma found at random biopsies. Dig Liver Dis 2011; 43: 721 – 725.
41. Zhao G, Xue M, Hu Y, Lai S, Chen S, Wang L. How Commonly Is the Diagnosis of Gastric Low Grade Dysplasia Upgraded following Endoscopic Resection? A Meta-Analysis. PLoS One. 2015 Jul 16;10(7):e0132699.
42. Usui Y, Taniyama Y, Endo M, et al. *Helicobacter pylori*, Homologous-Recombination Genes, and Gastric Cancer. N Engl J Med. 2023 Mar 30;388(13):1181-1190.
43. Rugge M, Bricca L, Guzzinati S, Sacchi D, Pizzi M, Savarino E, Farinati F, Zorzi M, Fassan M, Dei Tos AP, Malfertheiner P, Genta RM, Graham DY. Autoimmune gastritis: long-term natural history in naïve *Helicobacter pylori*-negative patients. Gut. 2023 Jan;72(1):30-38.