PROTOCOL
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"PRE-PDAC: Evaluation of **P**olygenic **R**isk scor**E** for **P**ancreatic **D**uctal **A**deno**C**arcinoma risk prediction: a case-control study"

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ACRONYM: PRE-PDAC (**P**olygenic **R**isk scor**E** for **P**ancreatic **D**uctal **A**deno**C**arcinoma)

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Background

Pancreatic ductal adenocarcinoma (PDAC) is the most common histological type of pancreatic cancer, accounting for 85%-95% of all solid pancreatic tumors [1]. Originating from the epithelium of the pancreatic ductal tree, PDAC is characterized by its remarkable biological aggressiveness and its high resistance to current therapeutic options, including chemotherapy and radiotherapy [2]. Despite advancements in clinical management, PDAC remains one of the deadliest malignancies, with a 5-year survival rate of less than 10% [3]. These data highlight the urgent need for more effective approaches to combat this disease.

A major challenge in managing PDAC is late diagnosis, largely due to the asymptomatic nature of the disease in its early stages [4]. Consequently, approximately 50%-55% of patients are diagnosed at a metastatic stage and 30%-35% at a locally advanced stage, significantly reducing the availability of curative treatment options [5,6]. Surgical resection, for instance, is feasible in only about 20% of cases, further exacerbating the prognosis [7,8]. This scenario underscores the critical need for novel strategies aimed at improving both early diagnosis and prevention.

From an epidemiological perspective, the incidence of PDAC in the general population is relatively low, rendering mass screening an ineffective and economically unsustainable solution [9,10]. Moreover, the absence of non-invasive, reliable, and scalable screening tests, combined with the risk of false positives, limits the feasibility and efficacy of such approaches [11]. As a result, primary prevention plays a pivotal role in reducing PDAC incidence.

Primary prevention efforts primarily target modifiable risk factors known to contribute to PDAC development. These include smoking, obesity, diabetes, excessive alcohol consumption, and genetic predisposition [12,13]. Promoting healthy lifestyle choices and addressing high-risk behaviors are essential components of preventive strategies against PDAC.

In recent years, scientific evidence has suggested that combining traditional risk factors, blood biomarkers, and genetic information could enhance the identification of high-risk populations [14]. A promising approach in this context is the polygenic risk score (PRS). The PRS offers a quantitative estimate of an individual's

genetic predisposition to a specific disease by combining the effects of multiple genetic variants, known as single-nucleotide variants (SNVs), identified through genome-wide association studies (GWAS). While the contribution of each individual variant is relatively small, their combined effect enables a moderate yet cumulative estimate of genetic risk [15,16]. The PRS is calculated by weighting each SNV according to its association with the disease of interest, resulting in a single score that represents the cumulative genetic burden [17].

In the context of PDAC, recent studies have highlighted the potential of the PRS as a predictive tool. Research has shown that the PRS is significantly associated with an increased risk of PDAC, and the incorporation of genetically predicted ABO blood groups into the PRS further strengthens its association with PDAC risk, thereby enhancing the predictive accuracy of the model [18]. The integration of the PRS with clinical risk factors, such as smoking and diabetes, has further enhanced the predictive accuracy of models, making them more effective in identifying high-risk populations [19].

These findings suggest that the PRS is a promising tool for risk stratification within the general population, allowing for the identification of high-risk subgroups who may benefit from targeted preventive interventions [20]. However, the scientific literature in this field is still evolving, and further studies are necessary to validate the effectiveness of the PRS in clinical practice.

The aim of our study is to evaluate the effectiveness of the PRS in predicting PDAC risk, contributing to the development of more accurate predictive models that could improve clinical management and the prevention of PDAC.

Objectives

Primary objective

Evaluation of the association between the PRS, derived from the combination of known risk-associated SNPs, including ABO alleles, and the risk of developing PDAC in a case-control study.

Secondary objectives

i. In the case-control study, we will calculate a multifactorial risk score by integrating the weighted PRS PDAC with two well-established risk factors (smoking and diabetes);

ii. In the PDAC patient cohort, we will evaluate the association of the PRS PDAC with the following variables:

1. Clinical and pathological characteristics;
2. Serological levels of exploratory biomarkers (CA19-9 and IL-6);
3. Cancer management (including surgery and medical treatments).

ENDPOINTS

Primary endpoints

- Odds of developing PDAC by different PRS percentiles.

Secondary endpoints

i. Odds of developing PDAC by different multifactorial risk score percentiles;

ii. According to PRS percentiles:

1. Frequency distribution of age, sex, stage, and primary localization (head versus body-tail);
2. Frequency distribution of Ca19-9 levels and Il-6 levels;
3. Frequency of surgery, chemotherapy, radiotherapy, EUS-guided ablative therapy.

METODHS

Study design

We will conduct a case-control multicenter study**.**

Study population

The population of the case-control study is composed as follow:

- Cases: consecutive patients with histologically confirmed PDAC, enrolled at the Ospedale San Raffaele in Milan, with samples stored at the hospital's biobank;
- Controls: consecutive people with no personal history of PDAC, enrolled at the Fondazione Policlinico Universitario A. Gemelli IRCCS, with samples stored at the Hygiene Section of the Università Cattolica del Sacro Cuore. In addition, we will also recruit patients at the Complex Operational Unit of Rheumatology, Fondazione Policlinico Universitario A. Gemelli IRCCS, from whom a blood sample will be collected.

The population of the cohort study (cases) is composed as follow:

- Consecutive patients with histologically confirmed PDAC, enrolled at the Ospedale San Raffaele.

Inclusion criteria

For cases:

- Histologically confirmed PDAC;
- First diagnosis;
- Age ≥ 18 years;
- Written informed consent;
- Available of a blood sample.

For controls:

- Individuals without a diagnosis of PDAC in the previous 10 years;
- Age ≥ 18 years;
- Written informed consent;

- Available blood sample.

A formal matching between cases and controls will not be applied in order to ensure cases have a comparable age distribution to the controls. However, the selection of controls from the archives of the Hygiene Section of the Università Cattolica del Sacro Cuore (Protocol N. 2580, in which participants were enrolled during a routine visit, upon informed consent, between February 2019 and February 2021) will be based on the age distribution observed in the context of a biobank/exposome observational registry study, approved by the Institutional Review Board (BIO-PANCREAS protocol 96/INT/2021, previously named BIOGASTRO/2011) performed within the Pancreas Translational and Clinical Research Centre of Ospedale San Raffaele. In more detail, PDAC cases were enrolled at the time of diagnosis, upon informed consent, between April 2016 and September 2024. The observed age distribution is as follows:

- <40 yrs: 1% of cases;
- [40-50) yrs: 7% of cases;
- [50-60) yrs: 19% of cases;
- [60-70) yrs: 32% of cases;
- [70-80) yrs: 35% of cases;
- >=80 yrs: 6% of cases.

Exclusion Criteria

For cases at least one of the following:

- Previous diagnosis of PDAC;
- DNA sample not available or insufficient quality for analysis.

For controls at least one of the following:

- Personal history of PDAC or other pancreatic diseases;
- Presence of concomitant malignancies;
- DNA sample not available or insufficient quality for analysis.

PROCEDURE

Informed consent and participant contact

Both cases and controls will be contacted to obtain written informed consent for the molecular analyses and the use of data collected through questionnaires. Specifically, participants will be informed about the purpose of the study, the type of analyses to be performed, and their right to withdraw at any time.

For prospectively enrolled controls, written informed consent will be obtained at recruitment, and a blood sample will be collected.

Type of data collected

For eligible participants the following data were collected:

- Sex;
- Age at enrollement;
- Smoking;
- Alcohol consumption;
- BMI (Body Mass Index);
- Personal and family history of cancer (particularly pancreas and breast cancer);
- Personal history of pancreatic diseases, diabetes, hypertension, periodontitis, ulcers (gastric or duodenal), Helicobacter pylori infection;
- Previous surgical interventions (such as gastrectomy and cholecystectomy);
- Pharmacological history (use of aspirin, NSAIDs, statins, PPIs, ACE inhibitors, ARBs):
- PRS.

For cases the following additional information were required for:

- Diagnosis: age at diagnosis, primary localization (head versus body-tail);
- Surgery: type of surgery;

- Histology: stage (defined according to NCCN as resectable, borderline resectable, locally advanced, or metastatic [21])
- Treatment: chemotherapy, radiotherapy, EUS-guided ablative therapy.

Medical form

At the time of diagnosis of cases, the physician completed the questionnaire provided in **Appendix A**, which includes both the medical history (physiological, familial, pathological, and pharmacological) and the diagnostic-therapeutic pathway, with the latter being updated progressively.

For controls, during a routine visit, the physician completed the questionnaire provided in **Appendix B**, which focuses exclusively on questions related to the medical history (physiological, familial, pathological, and pharmacological) relevant to PDAC.

For prospectively enrolled controls, during a routine visit, the physician will complete the questionnaire provided in **Appendix B**, which focuses exclusively on questions related to the medical history (physiological, familial, pathological, and pharmacological) relevant to PDAC.

The questionnaires included in Appendix A and B are the same as those used in previous studies (Protocol No. 2580 and the BIO-PANCREAS protocol 96/INT/2021) and were developed based on clinical expertise and relevant scientific literature.

Molecular analysis

The PRS analysis will be conducted using blood samples collected at the time of diagnosis for cases and at the time of enrollment during a routine visit for controls. A minimum of 3 mL of blood will be required to extract sufficient DNA quantity (100–200 ng).

The selection of genotyped SNPs for inclusion in the PRS will be guided by the findings of a study that developed a 30-SNP PRS, integrating SNPs associated with PDAC risk and those necessary for inferring ABO blood groups from genotypes to incorporate blood group data into the score calculation [18].

DNA extraction and PRS testing will be performed in the Section of Hygiene at UCSC (blood samples from cases will be shipped from San Raffaele to UCSC to this end). PRS testing will be performed using the Gene Titan Thermo Fisher system and the Axiom PMDA (Precision Medicine Diversity Array) kit. The Axiom™

analysis workflow includes the use of Axiom™ Analysis Suite, Applied Biosystems™ Analysis Power Tools, and the SNPolisher™ package to perform quality control (QC) for samples and plates, SNP filtering prior to downstream analyses, and advanced genotyping methods [22].

Blood samples from PDAC patients will also be analyzed using the INFINITE 200PRO® CON i-CONTROL™, in order to measure the concentration of CA19-9, a glycoprotein currently used as biomarker of gastrointestinal cancers, including PDAC, and interleukin-6 (IL-6), a proinflammatory cytokine implicated in the biological processes of tumor progression [23]. Although these biomarkers are primarily validated for diagnostic and prognostic purposes in clinical settings [24], in this study they will be explored for their potential association with genetic risk profiles within the PDAC patient cohort.

Sample size calculation

For the case-control study, the sample size was calculated based on the findings reported by Galeotti et al. [18] which reported an association between the PRS and an increased risk of PDAC with an odds ratio (OR) of 2.70 (95% CI 1.99-3.68) for the highest quintile compared to the lowest quintile of the weighted PRS. A sample size of 570 subjects in the control group and 570 subjects in the case group provides more than 80% power to detect a difference between the PRS distributions in controls and cases, assuming a significance level (alpha) of 0.05.

Statistical analysis

All analyses will perform using *Stata software* (STATA/BE 17.0 for Windows, StataCorp LP, College Station, TX 77845, USA), with a statistical significance level set at $p<0.05$. Patients' characteristics will be described as absolute frequencies and percentages for categorical variables and as medians with ranges or means with standard deviations for continuous variables, as appropriate. Differences between groups (cases and controls) will be assessed using the chi-square test or Fisher's exact test for categorical variables, while numerical variables will be tested for normality using the Shapiro-Francia test, followed by Student's t-test or the Mann-Whitney U test, based on the normality assessment.

Logistic regression will be employed to assess the association between individual SNPs and the outcome (PDAC: yes/no). The relationship between ABO blood groups derived from genotypes and PDAC risk will also be evaluated using logistic regression, with blood group O serving as the reference category.

Two types of PRS will be calculated: an unweighted PRS and a weighted PRS. Both will be assessed as continuous and categorical variables. The unweighted PRS will be computed by summing the total number of risk alleles for each individual (assigning a value of 1 to each risk allele) and incorporating ABO blood group values, with 0 assigned for group OO, 1 for OA/OB, and 2 for group AB. The weighted PRS will be calculated as the sum of an individual's risk alleles, weighted by the odds ratios (ORs) derived from GWAS data on PDAC, with similar weighting applied for ABO blood groups.

The PRS will be categorized into the following percentiles based on its distribution in the control group: [0,5%), [5%,10%), [10%,20%), [20%,40%), [40%,60%), [60%,80%), [80%,90%), [90%,95%), and [95%,100%]. The lowest 5% ([0,5%]) will represent the lowest PRS group, while the highest 5% ([95%,100%]) will represent the highest PRS group. The middle percentile range ([40%,60%]) will be used as the reference category.

Multifactorial risk scores will also be calculated by integrating the weighted PRS with tobacco smoking and diabetes variables. The calculated scores will be analyzed for their association with PDAC risk using logistic regression.

All analyses will adjust for the following variables: age, sex, primary tumor localization, stage, and family history of cancer.

In addition, given the different recruitment periods between cases and controls, potential biases may arise due to temporal changes in clinical practice, lifestyle factors, or exposure prevalence. To address this, we will include adjustment for the recruitment period in the regression models where applicable, and perform sensitivity analyses to evaluate whether the association between PRS and PDAC risk is affected by recruitment timing.

Finally, the frequency of the following variables will be calculated based on PRS percentiles: age, sex, stage, Ca19-9 and IL-6 levels, primary localization, surgery, chemotherapy, radiotherapy, and EUS-guided ablative therapy. Statistical tests will then be performed to explore any potential associations.

Data collection and management

A customized eCRF will be developed to collect clinical data for participants included in the study. Pseudo-anonymized data will be collected and managed using REDCap electronic data capture tools hosted at https://redcap-irccs.policlinicogemelli.it/. REDCap (Research Electronic Data Capture) [25] is a secure, web-based software platform designed to support data capture for research studies. It is fully compliant with 21 CFR Part 11 and GDPR, offering:

1. An intuitive interface for accurate data acquisition;
2. Audit trail to track data management and export activities.;
3. Automated export functions for compatibility with common statistical software;
4. Procedures to integrate and synchronize data with external sources [26].

All recorded information is confidential, and the database is privacy-protected, ensuring that no data can be traced back to individual patients in research reports. Access to the database is restricted, and only authorized individuals, officially registered as investigators or data managers, will receive user credentials. Access will be granted via multifactor authentication through the REDCap web platform, enabling secure data entry and management.

Data quality and standards

The eCRF will be developed in alignment with the study protocol, incorporating dataset configuration, validation, and programmed edit checks. The dataset will only be activated to accept data once it has been thoroughly reviewed and tested.

Investigators participating in the study are responsible for ensuring that the CRFs are accurately and comprehensively completed. Clinical data sources include the physician's patient records, hospital documentation, original laboratory reports, pharmacy records, imaging results, and other relevant materials. Data entry into the CRF must be truthful, precise, and completed in a timely manner. Each Investigator is

accountable for maintaining high data quality. Personal medical information may be reviewed to confirm patient safety and will always remain confidential.

During the data collection phase, remote monitoring and automated data quality checks will be implemented to address discrepancies and inconsistencies, triggering the generation of queries where necessary. The "Data Resolution Workflow" module will facilitate the documentation and resolution of data issues, including the creation, response, and closure of queries. User privileges will be assigned as needed, allowing appropriate control over who can view, initiate, respond to, or close data queries.

Ethical consideration

The study will adhere to the ethical principles outlined in the Declaration of Helsinki [27].

DNA samples of the patients will be stored in the Biobank of Fondazione Policlinico A. Gemelli-IRCCS, following signed informed consent. The processing of sensitive data will take place in compliance with Regulation (EU) No. 2016/679 (General Data Protection Regulation or GDPR) and Legislative Decree No. 196/2003 (as amended by Legislative Decree no. 10 August 2018 n. 101) on provisions for the adaptation of national legislation to the provisions of Regulation (EU) 2016/679. The Biobank has obtained the UNI EN ISO 9001:2015 certification (Certificate No. IT317572).

References

1. Schawkat K, Manning MA, Glickman JN, Mortele KJ. Pancreatic Ductal Adenocarcinoma and Its Variants: Pearls and Perils. Radiographics. 2020;40: 1219–1239. doi:10.1148/RG.2020190184

2. Karamitopoulou E. Molecular Pathology of Pancreatic Cancer. Cancers (Basel). 2022;14. doi:10.3390/CANCERS14061523

3. Barhli A, Cros J, Bartholin L, Neuzillet C. Prognostic stratification of resected pancreatic ductal adenocarcinoma: Past, present, and future. Dig Liver Dis. 2018;50: 979–990. doi:10.1016/J.DLD.2018.08.009

4. Mangge H, Niedrist T, Renner W, Lyer S, Alexiou C, Haybaeck J. New Diagnostic and Therapeutic Aspects of Pancreatic Ductal Adenocarcinoma. Curr Med Chem. 2017;24. doi:10.2174/0929867324666170510150124

5. Park W, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. JAMA. 2021;326: 851–862. doi:10.1001/JAMA.2021.13027

6. Wood LD, Canto MI, Jaffee EM, Simeone DM. Pancreatic Cancer: Pathogenesis, Screening, Diagnosis, and Treatment. Gastroenterology. 2022;163: 386-402.e1. doi:10.1053/J.GASTRO.2022.03.056

7. Słodkowski M, Wroński M, Karkocha D, Kraj L, Śmigielska K, Jachnis A. Current Approaches for the Curative-Intent Surgical Treatment of Pancreatic Ductal Adenocarcinoma. Cancers (Basel). 2023;15. doi:10.3390/CANCERS15092584

8. Qayyum A, Tamm EP, Kamel IR, Allen PJ, Arif-Tiwari H, Chernyak V, et al. ACR Appropriateness Criteria® Staging of Pancreatic Ductal Adenocarcinoma. J Am Coll Radiol. 2017;14: S560–S569. doi:10.1016/J.JACR.2017.08.050

9. Singhi AD, Koay EJ, Chari ST, Maitra A. Early Detection of Pancreatic Cancer: Opportunities and Challenges. Gastroenterology. 2019;156: 2024–2040. doi:10.1053/J.GASTRO.2019.01.259

10. Ngamruengphong S, Canto MI. Screening for Pancreatic Cancer. Surg Clin North Am. 2016;96: 1223–1233. doi:10.1016/J.SUC.2016.07.016

11. Caldwell KE, Conway AP, Hammill CW. Screening for Pancreatic Ductal Adenocarcinoma: Are We Asking the Impossible? Cancer Prev Res (Phila). 2021;14: 373–382. doi:10.1158/1940-6207.CAPR-20-0426

12. Del Chiaro M, Segersvärd R, Löhr M, Verbeke C. Early detection and prevention of pancreatic cancer: is it really possible today? World J Gastroenterol. 2014;20: 12118–12131. doi:10.3748/WJG.V20.I34.12118

13. Vasen H, Ibrahim I, Robbers K, Van Mil AM, Potjer T, Bonsing BA, et al. Benefit of Surveillance for Pancreatic Cancer in High-Risk Individuals: Outcome of Long-Term Prospective Follow-Up Studies From Three European Expert Centers. J Clin Oncol. 2016;34: 2010–2019. doi:10.1200/JCO.2015.64.0730

14. Pang Y, Holmes M V., Chen Z, Kartsonaki C. A review of lifestyle, metabolic risk factors, and blood-based biomarkers for early diagnosis of pancreatic ductal adenocarcinoma. J Gastroenterol Hepatol. 2019;34: 330–345. doi:10.1111/JGH.14576

15. Grebe TA, Khushf G, Greally JM, Turley P, Foyouzi N, Rabin-Havt S, et al. Clinical utility of polygenic risk scores for embryo selection: A points to consider statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2024;26. doi:10.1016/J.GIM.2023.101052

16. Wilde AAM, Semsarian C, Márquez MF, Sepehri Shamloo A, Ackerman MJ, Ashley EA, et al. European Heart Rhythm Association (EHRA)/Heart Rhythm Society (HRS)/Asia Pacific Heart Rhythm Society (APHRS)/Latin American Heart Rhythm Society (LAHRS) Expert Consensus Statement on the State of Genetic Testing for Cardiac Diseases. Heart Rhythm. 2022;19: e1–e60. doi:10.1016/J.HRTHM.2022.03.1225

17. Adeyemo A, Balaconis MK, Darnes DR, Fatumo S, Granados Moreno P, Hodonsky CJ, et al. Responsible use of polygenic risk scores in the clinic: potential benefits, risks and gaps. Nat Med. 2021;27: 1876–1884. doi:10.1038/S41591-021-01549-6

18. Galeotti AA, Gentiluomo M, Rizzato C, Obazee O, Neoptolemos JP, Pasquali C, et al. Polygenic and multifactorial scores for pancreatic ductal adenocarcinoma risk prediction. J Med Genet. 2021;58: 369–377. doi:10.1136/JMEDGENET-2020-106961

19. Sharma S, Tapper WJ, Collins A, Hamady ZZR. Predicting Pancreatic Cancer in the UK Biobank Cohort Using Polygenic Risk Scores and Diabetes Mellitus. Gastroenterology. 2022;162: 1665-1674.e2. doi:10.1053/J.GASTRO.2022.01.016

20. Wang L, Grimshaw AA, Mezzacappa C, Larki NR, Yang YX, Justice AC. Do Polygenic Risk Scores Add to Clinical Data in Predicting Pancreatic Cancer? A Scoping Review. Cancer Epidemiol Biomarkers Prev. 2023;32: 1490–1497. doi:10.1158/1055-9965.EPI-23-0468

21. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Pancreatic Adenocarcinoma. [cited 9 Dec 2024]. Available: https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

22. AxiomTM Genotyping Solution Data Analysis USER GUIDE. [cited 6 Dec 2024]. Available: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/axiom_genotyping_solution_analysis_guide.pdf

23. Hosein AN, Brekken RA, Maitra A. Pancreatic cancer stroma: an update on therapeutic targeting strategies. Nature Reviews Gastroenterology and Hepatology. Nature Research; 2020. pp. 487–505. doi:10.1038/s41575-020-0300-1

24. Schultz NA, Christensen IJ, Werner J, Giese N, Jensen B V., Larsen O, et al. Diagnostic and Prognostic Impact of Circulating YKL-40, IL-6, and CA 19.9 in Patients with Pancreatic Cancer. PLoS One. 2013;8. doi:10.1371/journal.pone.0067059

25. REDCap. [cited 6 Dec 2024]. Available: https://project-redcap.org/

26. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42: 377–381. doi:10.1016/J.JBI.2008.08.010

27. WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants – WMA – The World Medical Association. [cited 6 Dec 2024]. Available: https://www.wma.net/policies-post/wma-declaration-of-helsinki/

Appendix A – Questionnaire for cases

ENROLLMENT FORM

Select the room: ☐ Room 5 ☐ Room 7 | Completed by: _____ | Date: _____

Tissue Biopsy for Research ☐ Yes ☐ No (If yes, tube number: _____)

Date of Birth: _____

PHYSIOLOGICAL HISTORY

Weight 12 months before symptoms: ____ kg | Weight at diagnosis: ____ kg | Height: ____ cm
☐ Smoker ☐ No ☐ Ex (since ____ years) | Cigarettes/day: ____
☐ Alcohol consumption ☐ No ☐ Ex (since ____ years) | Units/day: ____
☐ Coffee consumption: ____ cups/day for ____ years | Menarche age: ____ years
Total pregnancies: ____ (age ____), Miscarriages: ____ | Children born: ____
☐ Breastfeeding ☐ No (Total months for all children: ____)
☐ Contraceptive pill ☐ No (for ____ years) | Menopause age: ____ years
☐ Post-menopausal hormone therapy ☐ No (for ____ years) | ☐ Irregular cycles ☐ No (for ____ years)
Other gynecological disorders: ____ | ☐ Previous gynecological surgery ☐ No (Hysterectomy, unilateral/bilateral oophorectomy, year: ____)

FAMILY HISTORY (*Specify relation and age at onset*)

☐ Pancreatic cancer ☐ No | Relation: ____ | Age: ____
☐ Breast cancer ☐ No | Relation: ____ | Age: ____
☐ Other cancers ☐ No | Relation: ____ | Organ: ____ | Age: ____

MEDICAL HISTORY - COMORBIDITIES AT DIAGNOSIS

☐ Diabetes ☐ No | Type (I/II): ____ | Since: ____ | Therapy: ☐ Insulin ☐ Metformin ☐ Other: ____
☐ Allergies ☐ No (*Specify: drugs, animals, insect bites, other:* ____)
☐ Periodontitis ☐ No | Since: ____ | Dentist visit: ☐ No (*since ____ years*)
☐ Tooth loss (*How many:* ____) | ☐ Oral surgery ☐ No (*Year:* ____)
Have you been diagnosed with periodontitis or pyorrhea? Do your gums bleed easily or recede? Any abscesses near teeth?
☐ Chronic pancreatitis ☐ No (*Since:* ____ *Therapy:* ____) | ☐ Hypertension ☐ No (*Since:* ____ *Therapy:* ____)
☐ Ulcer ☐ No | Gastric ☐ Duodenal ☐ | Diagnosis year: ____ | Therapy: ____
☐ H. Pylori infection ☐ No | Diagnosis year: ____ | ☐ Eradication therapy ☐ No (*Year:* ____) | Therapy: ____
☐ Gastrectomy ☐ No | Year: ____ | Type: ____ | Indication: ____
☐ Cholecystectomy ☐ No | Year: ____ | Indication: ____
☐ Previous cancers/surgeries ☐ No (*Specify: type, onset age, therapy:* ____)
☐ Other conditions/Previous surgeries ☐ No | Type: ____ | Year: ____ | Reason: ____ | Treatment: ___

PHARMACOLOGICAL HISTORY (Check drug and dose)

Pharmacological therapy at diagnosis:
☐ Aspirin use for prolonged periods:

☐Cardioaspirin 100 mg
☐Cardirene 75 mg / 100 mg / 160 mg / 300 mg
☐Aspirin 325 mg / 400 mg / 500 mg
Dose: ____ | Times/day: ____ | Total years: ____

☐ NSAID use: ☐ No | Drug: ____ | Dose: ____ | Times/day: ____ | Total years: ____
☐ Statins use: ☐ No | ☐ Atorvastatin | ☐ Fluvastatin | ☐ Lovastatin | ☐ Pravastatin | ☐ Rosuvastatin | ☐ Simvastatin | ☐ Simvastatin + Ezetimibe | Dose: ____ mg | Times/day: ____ | Total years: ____
☐ PPI use ☐ No | Drug: ____ | Dose: ____ | Times/day/week: ____ | Total years: ____

☐ACE Inhibitors:

☐Lisinopril (Zestril, Prinivil) 5 mg/20 mg
☐Enalapril (Enapren, Converten) 5 mg/20 mg
☐Ramipril (Triatec, Unipril) 1.25 mg/2.5 mg/5 mg
☐Quinapril (Accupril, Acequin) 5 mg/20 mg
☐Perindopril (Coversyl) 4 mg
☐Trandolapril (Gopten) 0.5 mg/2 mg
☐Zofenopril (Zantipress) 7.5 mg/30 mg
☐Fosinopril (Eliten, Fosipres) 10 mg/20 mg
Frequency: ____ times/day for ____ years

☐ARB (Angiotensin Receptor Blockers) use:

☐Valsartan (Tareg) 80 mg/160 mg
☐Telmisartan (Micardis) 20 mg/40 mg/80 mg
☐Losartan (Loartan) 12.5 mg/50 mg/100 mg
☐Irbesartan (Aprovel) 75 mg/150 mg/300 mg
☐Olmesartan (Olmetec) 20 mg/40 mg
☐Candesartan (Blopress) 8 mg/16 mg/32 mg
☐Eprosartan (Teveten) 600 mg
Frequency: ____ times/day for ____ years

DIAGNOSTIC SYMPTOMS or ☐ Incidental Diagnosis (Specify)

Diagnosis date (first imaging with mass detected): ____ | First symptom date: ____
Symptoms leading to diagnosis (Specify onset date and severity):
☐ Weight loss (____ kg in ____ time) |
☐ Diabetes /☐ Worsening diabetes
☐ Pain (*Location:* ____)
☐ Diarrhea
☐ Jaundice ☐ Fatigue (ASTHENIA) ☐ Appetite loss ☐ Acute pancreatitis ☐ Other: ____

DIAGNOSTIC-THERAPEUTIC PATHWAY

IMAGING at DIAGNOSIS (*Specify date and details or attach report*)
☐ Abdominal ultrasound ☐ No ☐ in OSR

☐ CT ☐ No _____☐ in OSR
☐ MRI ☐ No ☐ in OSR
☐ PET ☐ No☐ in OSR
☐ EUS ☐ No _____☐ in OSR
☐ EUS-FNA/FNB ☐ No _____☐ in OSR
Cytology/Histology report: _____
☐ Biopsy: ☐ Hepatic metastasis (*Date:*) | ☐ *Pancreatic (CT-guided, Date: ___)*
☐ **Laparoscopic (Date:*) | Histology: ____ (*in OSR ☐*)
☐ Clinical/Imaging diagnosis only ☐ No | TNM Stage: T N M

Primary Tumor Location: ☐ Head ☐ Body-Tail ☐ Uncinate Process ☐ Disseminated | Max Tumor Size (mm): CT EUS
☐ No local invasion ☐ Vascular invasion (*Specify:* ____)
CA19-9 at diagnosis: ____ u/ml (*During jaundice?* ☐ Yes ☐ No)
CEA at diagnosis: ____ | Other markers: ____
Lab Tests: Neutrophils: ____ /mm³ | Lymphocytes: ____ /mm³ | CRP: ____ g/L | Albumin: ____

☐ Biliary drainage performed? ☐ No Date: ____
☐ ERCP ☐ Choledochoduodenostomy ☐ Hepaticogastrostomy ☐ PTC

SURGERY WITH CURATIVE INTENT: ☐ Yes ☐ No Date: ____ ☐ Performed at OSR

TYPE OF SURGERY:
☐ DCP ☐ PD ☐ SPD ☐ Enucleation ☐ Vascular resection ☐ Other: _____

HISTOLOGY:

- Grade: G 1 ☐ 2 ☐ 3 ☐
- Resection margin: R 0 ☐ 1 ☐
- T: _____
- N1 ratio: ___
- N2 ratio: ___
- Vascular invasion: _____
- Perineural invasion: _____

Non-radical surgery performed? (Specify type) ☐ Yes ☐ No Date: _____

CHEMOTHERAPY (specify type, start and end date, duration)
☐ Yes ☐ No ☐ Performed at ORS ☐ Performed at _____

- Drug: _____ Period: _____
  ☐ Neoadjuvant/palliative ☐ Adjuvant post-surgery
- Drug: _____ Period: _____
  ☐ Neoadjuvant/palliative ☐ Adjuvant post-surgery

- Drug: _____ Period: _____
  ☐ Neoadjuvant/palliative ☐ Adjuvant post-surgery

RADIOTHERAPY (specify type, start and end date, duration)
☐ Yes ☐ No ☐ Performed at ORS ☐ Performed at _____

EUS-GUIDED ABLATIVE THERAPY ☐ Yes ☐ No Date: _____

PSYCHO-ONCOLOGICAL SUPPORT ☐ Yes (Performed at ORS) ☐ No

NUTRITIONAL SUPPORT ☐ Yes (Performed at ORS) ☐ No With: _____

FECAL ELASTASE AT DIAGNOSIS: _____

PERT: ☐ Yes ☐ No Start date: _____ Dosage: _____

Modifications or interruptions: _____

DEATH: ☐ Yes Date: _____ ☐ No Date of last contact: _____

Classification of Resectability of PDAC (Pancreatic Ductal Adenocarcinoma) According to NCCN Guidelines (Modified)

Vessel Involvement	Resectable	Borderline Resectable	Unresectable
Arterial vessels	No contact	Contact with common hepatic artery without contact to the celiac trunk or hepatic artery bifurcation (resection possible)	Distant metastasis or non-regional lymph node metastasis
Venous vessels	No contact without vessel alteration	Contact with SMA ≤ 180°, celiac trunk ≤ 180°, SMV/portal vein ≤ 180° (resection possible)	SMA or celiac trunk contact > 180°, aorta infiltration, non-reconstructable SMV or portal vein invasion

Appendix B. Questionnaire for subjects enrolled as controls.

PHYSIOLOGICAL HISTORY

1. Sex: _____
2. Age: _____
3. Weight (kg): _____
4. Height (cm): _____
5. BMI: _____
6. Smoking:
   o YES
   o NO
   o FORMER SMOKER
   If former, specify for how many years: ___
   If yes, indicate:
   - Cigarettes/day: _____
   - Years of smoking: _____
7. Alcohol Consumption:
   o YES
   o NO
   o FORMER DRINKER
   If former, specify for how many years: ___
   If yes, indicate:
   - Units/day: _____
   - Years of alcohol consumption: _____

FAMILY MEDICAL HISTORY ***(Specify relative and age of onset)***

1. Pancreatic Neoplasia:
   o YES
   o NO
   If yes, indicate:
   - Relation: _____
   - Age of onset: _____
   - Relation: _____
   - Age of onset: _____
2. Breast Neoplasia:
   o YES
   o NO
   If yes, indicate:
   - Relation: _____
   - Age of onset: _____
   - Relation: _____

- Age of onset: _____

3. Other Neoplasms:
   o YES
   o NO
   If yes, indicate:
   - Organ: _____
   - Relation: _____
   - Age of onset: _____
   - Organ: _____
   - Relation: _____
   - Age of onset: _____
   - Organ: _____
   - Relation: _____
   - Age of onset: _____

PAST MEDICAL HISTORY

4. Diabetes:
   o YES
   o NO
   If yes, indicate:
   - Type I
   - Type II
     Additionally, indicate type of therapy:
   - Insulin:
     o YES, for _____ years
     o NO
   - Metformin:
     o YES, for _____ years
     o NO

5. Periodontitis *(Have you ever been diagnosed with periodontitis or pyorrhea? Do your gums bleed easily, retract, or form abscesses near teeth?)*:
   o YES
   o NO
   o DO NOT VISIT DENTIST
   If yes, indicate:
   - For how many years: _____
   - Tooth loss (number): _____
   - Oral surgery (year): _____

6. Hypertension:
   o YES
   o NO
   If yes, indicate:
   - Since year: _____
   - Therapy: _____

7. Chronic Pancreatitis:
   o YES
   o NO
   If yes, indicate:
   - Since year: _____
   - Therapy: _____

8. Ulcer:
   o YES
   o NO
   If yes, indicate:
   - Ulcer type:
     o Gastric
     o Duodenal
   - Since year: _____
   - Therapy: _____

9. Helicobacter pylori (Hp) Infection:
   o YES
   o NO
   If yes, indicate:
   - Year of Hp diagnosis: _____
   - Eradication:
     o YES
     o NO
   - Year of eradication: _____
   - Therapy: _____

10. Gastroresection:
    o YES
    o NO
    If yes, indicate:
    - Since year: _____
    - Type: _____
    - Indication: _____

11. Cholecystectomy:
    o YES
    o NO
    If yes, indicate:
    - Since year: _____
    - Indication: _____

PHARMACOLOGICAL HISTORY ***(Check drug and dose)***

12. Prolonged Aspirin Use:
    o YES
    o NO
    If yes, indicate:
    - Which:
      o Cardioaspirin 100 mg – _____ times/day
      o Cardirene 75 mg/100 mg/160 mg/300 mg – _____ times/day
      o Aspirin 325 mg/400 mg/500 mg – _____ times/day
    - Total years: _____
13. Prolonged NSAID Use:
    o YES
    o NO
    If yes, indicate:
    - Drug type: _____
    - Dose: _____
    - Times/day or times/week: _____
    - Total years or within the last year: _____
14. Statin Use:
    o YES
    o NO
    If yes, indicate:
    - Which:
      o Atorvastatin (Torvast, Totalip)
      o Fluvastatin (Lescol, Lipaxan, Primesin)
      o Lovastatin (Lovinacor, Rextat, Tavacor)
      o Pravastatin (Aplactin, Prasterol, Pravaselect, Sanare, Selectin)
      o Rosuvastatin (Crestor, Provisacor, Simestat)
      o Simvastatin (Liponorm, Medipo, Sindaco, Sivastin, Zocor)
      o Simvastatin + Ezetimibe (Inegy, Goltor, Vytorin)
    - Dosage: 10 mg/20 mg/40 mg/60 mg/80 mg/100 mg/120 mg
    - Times/day: _____
    - Total years: _____

15. Prolonged PPI Use:
    o YES
    o NO
    If yes, indicate:
    - Drug type: _____
    - Dose: _____
    - Times/day or times/week: _____
    - Total years or within the last year: _____
16. Prolonged ACE Inhibitor Use:
    o YES
    o NO
    If yes, indicate:
    - Which:
      (List of ACE inhibitors as per original)
    - Times/day or times/week: _____
    - Total years or within the last year: _____
17. Prolonged ARB (Sartans) Use:
    o YES
    o NO
    If yes, indicate:
    - Which:
      (List of ARBs as per original)
    - Times/day or times/week: _____
    - Total years or within the last year: _____