Efficacy of chemopreventive agents on overall survival in patients with pancreatic ductal adenocarcinoma

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A mio fratello,

l'amico ritrovato che tanto mancava!

A Marco,

per avermi insegnato ad amare me stesso!

Summary

Aims of the projects

Project I: To investigate the effect of chemopreventive agents on overall survival in patients with pancreatic ductal adenocarcinoma with a retrospective analysis of patients with pancreatic adenocarcinoma, divided by stage, in therapy with statin and/or metformin and /or NSAIDs.

Project II: Statin use improves survival in patients with pancreatic ductal adenocarcinoma: A Meta-analysis

Project III: To investigate the effect of chemopreventive agents on overall survival in patients with pancreatic ductal adenocarcinoma with an observational prospective analysis of patients with pancreatic adenocarcinoma

Project I

Type of study: Retrospective study

Department: Pancreatic Surgery Unit San Raffaele Hospital, Milan, Italy

State of art: Manuscript submission December 2019.

Background

Pancreatic ductal adenocarcinoma is currently the fourth leading cause of cancer-related death in the United States with a 5-year survival rate of 6.7% [1]. Surgical resection of early-stage disease remains the only opportunity for potential cure. Despite advances in therapy, pancreatic cancer continues to have a poor prognosis and up to 80–85% of patients undergoing resection experience disease recurrence [2,3]. The main reason for this poor prognosis is the propensity of pancreatic cancers to invade adjacent tissues and to metastasize. Median survival following resection is 24–25 months even in the setting of adjuvant or neoadjuvant chemotherapy [4]. In this setting cancer chemoprevention with the use of natural or synthetic substances to inhibit, retard or reverse the carcinogenesis has been recently investigated by several authors. A wealth of evidence from preclinical studies have convincingly demonstrated the cancer preventive efficacy of various agents in different animal models. However, the data from observational, case–control, cohort studies, and randomized trials in humans have overall demonstrated different results. Statins, metformin and nonsteroidal antiinflammatory drugs (NSAIDs), have been reported to be potential cancer chemopreventive agents. Several authors have shown that pancreatic adenocarcinoma is often associated with overexpression of a variety of mitogenic growth factors, including epidermal growth factor (EGF), and of growth factor receptors [5]. Recently Kusama et al. [6] showed that HMG-CoA reductase inhibitors, fluvastatin and lovastatin, markedly attenuated EGF-induced translocation of RhoA from the cytosol to the membrane fraction and the in vitro invasive capacity of human pancreatic cancer cell lines. Jeon et al. [7] have found that statin use after cancer diagnosis was associated with survival in those with no exposure to statin prior to cancer diagnosis, but not in those with prior statin exposure. For this reason statin treatment after cancer diagnosis may have a greater impact on statin-naïve tumors that are sensitive to the molecular effects of statin, whereas tumors that arose in patients already receiving statins may have been selected for statin resistance before diagnosis.

Also other authors have demonstrated the cancer preventive effects of NSAIDs, especially in colorectal cancer, despite the relative high dose required for the observed chemopreventive effect in human trials may discourage the singular use of NSAIDs on a long-term basis for cancer prevention because of possibly increased risk for serious gastrointestinal side effects [8,9].

In a pooled analysis of 25,570 patients in eight trials, Rothwell et al. [10] recently reported that daily aspirin use reduced deaths due to several common cancers, including significant reductions in colorectal and pancreatic cancer deaths, with most benefit seen after 5 years of the scheduled trial treatment.

Tan et al. [11] also showed that metformin treatment may inhibit pancreatic tumorigenesis in the LSL-KrasG12D/+;Trp53F2-10 mice by modulating multiple molecular targets in signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NFκB) inflammatory pathways.

Metformin and aspirin can inhibit the mTOR signaling pathway through both AMPK-dependent and AMPK-independent mechanisms. Given that persistent low-grade inflammation is an important factor for the development of pancreatic cancer, it is worth noting that two major inflammatory mediators, STAT3 and NFκB, also can be suppressed by metformin and aspirin [10,11].

These investigations suggest that both metformin and aspirin might have preventive effects against the development of pancreatic cancer.

Reni et al. [12] recently published a randomized phase II trial of 60 patients with metastatic pancreatic cancer treated with cisplatin, epirubicin, capecitabine, and gemcitabine (PEXG) randomly assigned to addition of metformin (n = 31) or without metformin (n = 29). Unfortunately the study was ended for futility. They concluded that addition of metformin at the dose commonly used in diabetes did not improve outcome in patients with metastatic pancreatic cancer treated with standard systemic therapy.

The survival outcome was also investigated by Ambe et al. [13] in metformin users patients with resected pancreatic cancer. They showed that metformin users had a better median survival than

non-users, but the difference was not statistically significant (35.3 versus 20.2 months; P = 0.3875). The potential benefit of metformin should be investigated in adequately powered prospective studies.

Materials and methods

The present study was a retrospective cohort study conducted at San Raffaele Scientific Institute, Milan, Italy following the STrengthening the Reporting of OBservational studies in Epidemiology statement (STROBE) guidelines [14]. All patients who underwent pancreatic resections between January 2015 and September 2018, were retrospectively reviewed. All the procedures were carried out at the Department of Pancreatic Surgery at San Raffaele Hospital in Milan. Inclusion criteria were as follows: age ≥ 18 years, pancreatic ductal adenocarcinoma (PDAC) at histology, absence of distant metastasis at preoperative imaging, use of statin and/or metformin and/or ACE-inibitors and/or B-Blockers and/or aspirin (chemopreventive agents) at least 6 months before diagnosis.

Patients

Demographic variables, perioperative and postoperative variables, and follow-up records were retrospectively reviewed from an electronic database. Data on clinical outcomes, including overall survival (OS), progression free survival (PFS), and the comorbidities of DM, hypertension, and hyperlipidemia were retrospectively extracted. The "chemopreventive agents" considered for the analysis were statins, ACE inibitors, B-blockers, metformin and aspirin. Patients were considered eligible for the study only in case of regular assumption of at least one of the "chemopreventive agents". Preoperative chemotherapy was administered in presence of borderline resectable pancreatic cancer [15] or in presence of Ca 19.9 >200 U/L [16]. A preoperative contrast enhancement computed tomography (CECT) scan, pancreas protocol, was performed within 30 days before surgery to assess the resectability. Postoperative chemotherapy was proposed to all patients, during multidisciplinary team meeting (MDT) meeting, according to NCCN guidelines [17]. All patients were followed up in the outpatients clinic every 4 months with a CECT scan of the chest and abdomen and laboratory tests including tumoral markers. The disease recurrence was suspected in presence of hypodense tissue narrowing the vascular structures in the CECT scan or presence of distant metastasis and /or increase of Ca 19.9 over the upper limit. Histologically

confirmation of the recurrence was not routinely performed. Postoperative mortality was defined as death occurred within 90th day after surgery or any in-hospital death. The primary end point of this study was OS and DFS within the two groups (patients on regular treatment with one or more target drugs and patients who were not).

Statistical Analysis

Overall survival and PFS were estimated using the Kaplan-Meier method and compared using the log-rank test. The W2 test or the Fisher exact test was used to compare categorical variables. The independent t test or Mann-Whitney U test was used to compare continuous variables as appropriate. Univariate and multivariate analyses of prognostic factors for OS were performed. All reported P values were the result of 2-sided tests, with P G 0.05 considered statistically significant. Data were described using number and percentage for categorical variables and median and range for continuous variables. Statistical analysis were performed by using the SPSS software.

Results

A total of 477 patients underwent pancreatic resections for PDAC from January 2015 to September 2018 at the department of Pancreatic Surgery at San Raffaele Hospital in Milan. The clinical features and preoperative characteristics of the entire population are shown in **Table 1**. The median age was 68 years and 48% (n=230) were female. Only 5% of patients (n=24) had a body mass index (BMI) > 30 Kg/m^2. Neoadjuvant chemotherapy was administered to 39% of patients (n=188) and 338 patients (71%) received a postoperative chemotherapy. Taking into account the preoperative comorbidities, an ASA >3 was found in 200 patients (42%). In the target population, aspirin was taken routinely in 23% (n= 107) of patients, ACE-inhibitors in 22% (n=101), B-Blockers in 30% (n=140), statins in 23% (n=106) and metformin in 15% (n=70). Overall, 219 (46%) of patients were on regular therapy with one or more drugs.

Operative details, histology and post-operative outcomes

Almost 70% of patients (n=327) underwent pancreaticoduodenectomy, a distal pancreatectomy and splenectomy was performed in 106 patients (22%) and 44 patients (9%) required a total pancreatectomy and splenectomy. Perineural and lymphovascular invasion was found in 305 (64%) and in 333(70%) respectively. Overall 92% (n=442) of patients had a T1-T2 tumor stage. The rate of lymph-nodes metastases was 72% (n= 342). An R0 resection was achieved in 27% of patients (n= 130). Only 1% of patients (n=6) had a G1 tumor. Overall, 90-day mortality after surgery was 3% (n= 13). The operative, perioperative details and histology outcomes are shown in **Table 2**.

Survival

The median follow-up time was 29 months (IQR: 19-43 months). The median DFS and OS of the entire population was 21 months (95% CI: 17-24 months) and 34 (95% CI: 30-38) respectively. The median DFS of patients, on regular treatment with one or more than one drug, compared with patients who were not on therapy, did not show any difference (**Figure 1**). ASA and metformin use

demonstrated a protective effect on DFS. The association of ASA and/or metformin showed an increased DFS for these patients if compared with patients who were not on regular treatment (**p=0.003**) (**Figure 2**). The **Figure 3** shows the OS of patients treated with one or more drug, compared with patients not on regular treatment. The regular treatment with ACE-inhibitors is associated with a poor prognosis and lower survival (**p=0.02**).

On multivariable analysis factors associated with DFS were: pT3/pT4 (HR: 2.5; p=0.001); N2 (HR:2.5; p<0.0001); No adjuvant treatment (HR:2.0; p<0.0001); No assumption of metformin and or ASA (HR: 2.0; p=0.01). On multivariable analysis factors associated with OS were: pT3/pT4 (HR: 2.6; p=0.001); N2 (HR: 4.9; p<0.0001); No adjuvant treatment (HR:1.6; p<0.0001); G3 (HR: 1.5; p=0.01); ACE-inhibitors (HR: 1.7; p=0.009); ASA >3 (HR:1.4; p=0.02)

Discussion

The present study demonstrates that the routinely assumption of metformin and/or aspirin is associated with an increased DFS among patients with PDAC. The regular treatment, with one or more target drugs, in the previous 6 months before the diagnosis of PDAC did not improve the OS. Several authors have investigated the role of the aspirin in the chemopreventive setting. Two studies one in colon cancer and one in breast cancer, have shown prevention of recurrence with aspirin use the use of aspirin [18,19]. In addition, a protective effect has been demonstrated in the prevention of other cancers including gastric cancer, esophageal cancer, leukemia, breast cancer, ovarian cancer, endometrial cancer, and prostate cancer [20-28]. Several authors have also shown a protective effect of aspirin in pancreatic cancer prevention [29]. However in literature data about aspirin as chemopreventive agent in patients with pancreatic cancer are not yet published. This is, as far as we know, the largest series, triyng to explore the role of the aspirin in patients already diagnosed and treated for pancreatic cancer. A protective effect was also demonstrated in a recent systematic review and meta-analysis of different cancers, but not pancreatic cancer that showed reductions in metastatic spread and a decrease in overall mortality by about 15% [30]. A possible mechanism by which aspirin might have an anticancer effect is the capability to inhibit platelet upregulation of c-MYC which stimulates cancer cell proliferation and to inhibit survivin, a protein that reduces cell apoptosis that is overly expressed in pancreatic cancer [31-33].

The effect of metformin on patients with pancreatic cancer is still debated. Ambe et al. [13] In their series failed to demonstrate a significant trend toward improved survival with metformin use in patients with resectable pancreatic cancer. The results published by Ambe et al. [13] showed a median OS of 10.4 months which was longer in patients who took metformin than in those who did not. In addition, the 5-year survival rate was 34 % and 14% in the metformin group than in the non-metformin group. These results were consistent with results from a previous study. In this paper Sadeghi et al. [34] have shown that the OS was 4.1 months longer and the 1-year survival rate was

18.8% higher in patients treated with metformin than in those not treated with metformin. Despite there was a protective effect in all stages, the results were statistically significant only in patients with non metastatic disease. In fact, metformin use was associated with a 32% reduction in the risk of death. In our series the regular use of metformin and/or aspirin was associated with an increased DFS which was statistically significant and was found to be an independent predictor of late-recurrence.

In previous reported series statin use was significantly associated with a lower mortality using a time-dependent Cox model (HR: 0.78; 95%CI, 0.62–0.99) [7,35]. Jian-Yu et al. [36] have shown that statin use was significantly associated with improved overall survival [HR, 0.94; 95% CI, 0.90-0.98], and survival was more pronounced in post-diagnosis statin users (HR, 0.69; 95% CI, 0.56-0.86). In the present series statin use was not associated with better survival or lower recurrence rate. However, a subgroup analysis within different statins type's might be helpful to show different results. At present this analysis was not performed due to unavailable data.

In the multivariable analysis of factors associated with OS, the ACE-inhibitors were found to be independent predictors of poor prognosis. Nakay et al. [37] previously reported that the inhibition of renin-angiotensin system was associated with better clinical outcomes in patients receiving gemcitabine monotherapy. In another phase 1 trial of gemcitabine and candesartan Nakay et al. [38] showed that the inhibition of renin-angiotensin system (RAS) in advanced pancreatic cancer might improve clinical outcomes. A possible explanation regarding different results of the present series compared with previous reported results might be that patients on ACE-inhibitors are more fragile due to cardiovascular comorbidities. For this reason in case of relapse these patients are not fit for other treatments such as chemotherapy and/or chemoradiation.

This study has several strengths, this is the largest series, as far as we know, aiming to investigate the role of different drugs on survival of patients with resected pancreatic cancer. Another important aspect of the study is the length of the follow-up. In this series the median follow-up was 29 months

and this time is long enough to detect variations in the OS and DFS among patients on treatment with chemopreventive agents and patients who were not.

However several limitations inherent with our data must be addressed. First, the study is limited by its retrospective design and is not a randomized clinical trial. Second, due to the retrospective nature it was impossible to obtain from clinical records data about subtypes of statins and/or B-blockers and/or ACE-inhibitors. In this setting a prospective trial is needed to better assess the role of these drugs in patients with pancreatic cancer.

Table 1. The clinical features and preoperative characteristics of 477 patients who underwent pancreatic resection for PDAC.

Gender	
Male	247 (52)
Female	230 (48)
Age	
≤ 70 years	272 (57)
>70 years	205 (43)
BMI	
≤ 30 (kg/m^2)	453 (95)
> 30 (kg/m^2)	24 (5)
Neoadjuvant treatment	
No	289 (61)
Yes	188 (39)
ASA ≥3	
No	277 (58)
Yes	200 (42)
Adjuvant chemotherapy	
No	139 (29)
Yes	338 (71)
Aspirin	
No	370 (77)
Yes	107 (23)
ACE-inhibitors	
No	376 (78)
Yes	101 (22)
B-Blockers	
No	337 (70)
Yes	140 (30)
Statins	
No	371 (77)
Yes	106 (23)
Metformin	
No	407 (85)
Yes	70 (15)
At least 1 drug	
No	258 (54)
Yes	219 (46)

BMI: Body Mass Index

Table 2. The operative, perioperative details and histology of 477 patients who underwent pancreatic resection for PDAC.

Type of Surgery	
Pancreaticoduodenectomy	327 (69)
Distal pancreatectomy and splenectomy	106 (22)
Total pancreatectomy	44 (9)
Grading	
G1	6 (1)
G2	252 (53)
G3	219 (46).
T stage	
Tis/T1	155 (32)
T2	287 (60)
T3/T4	35 (8)
N stage	
N0	135 (28)
N1	194 (41)
N2	148 (31)
Resection margin	
R0	130 (27)
R1	347 (73)
Perineural invasion	
No	172 (36)
Yes	305 (64)
Lymphovascular invasion	
No	144 (30)
Yes	333 (70)
90-days mortality	
No	464 (97)
Yes	13 (3)

Figure 1: DFS of patients on regular treatment with 1 drug and more than 1 drug

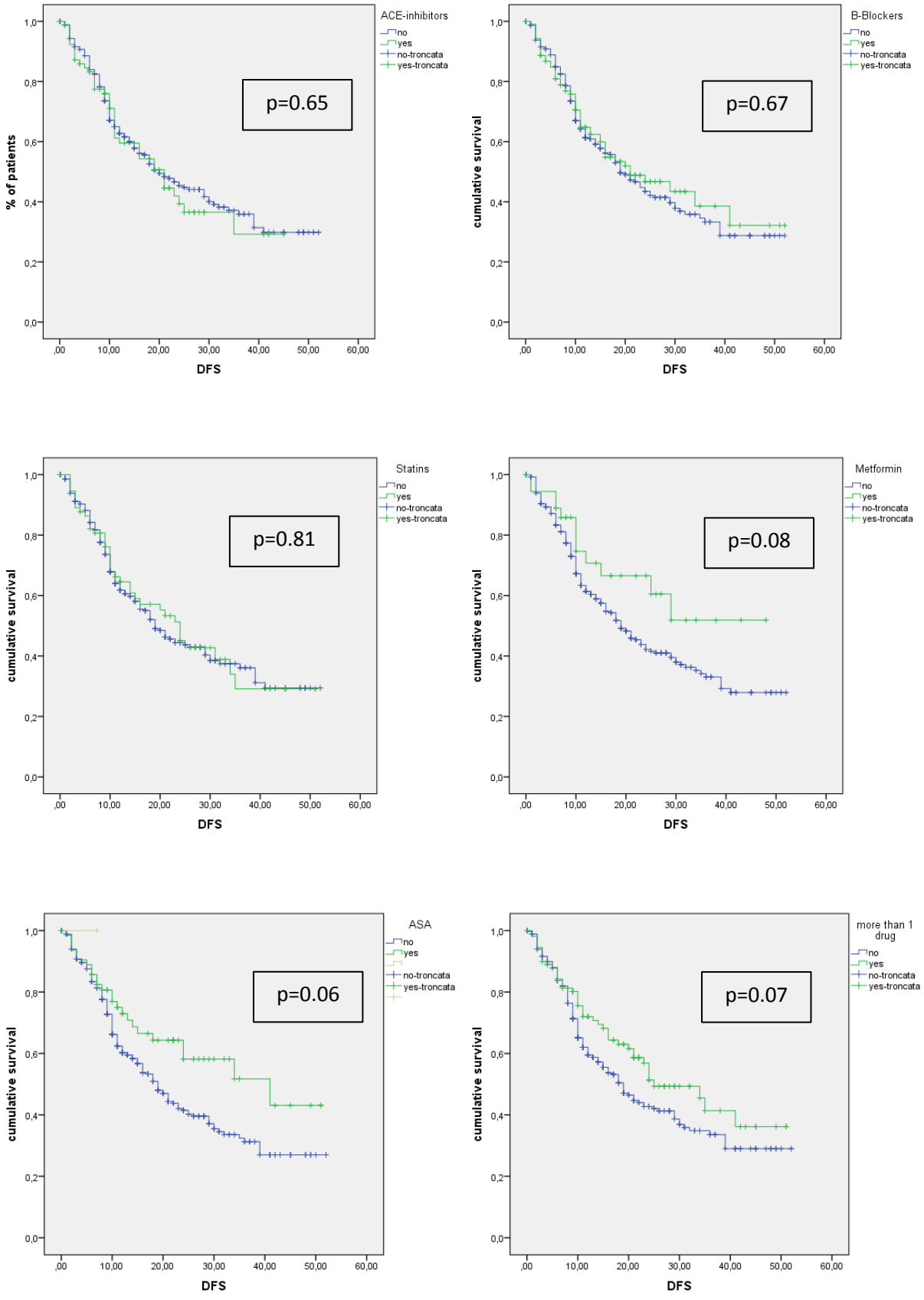


Figure 2: DFS of patients on regular treatment with Metformin and/or ASA

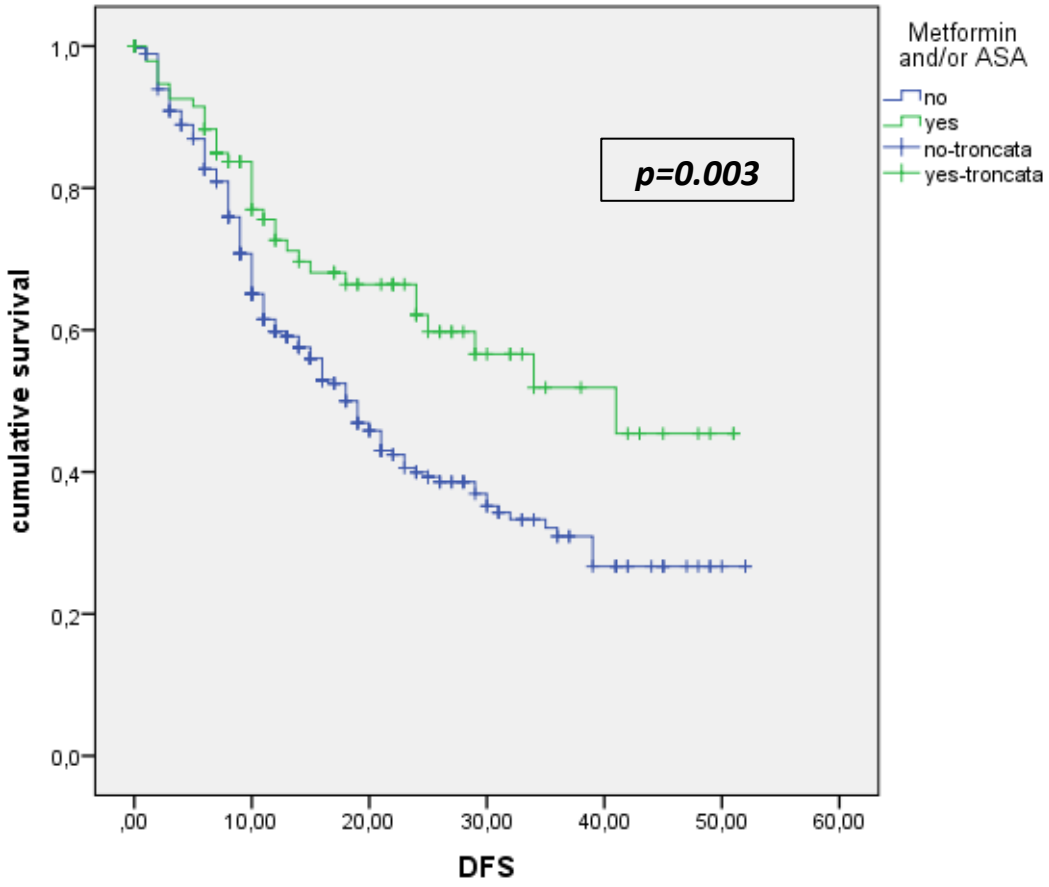
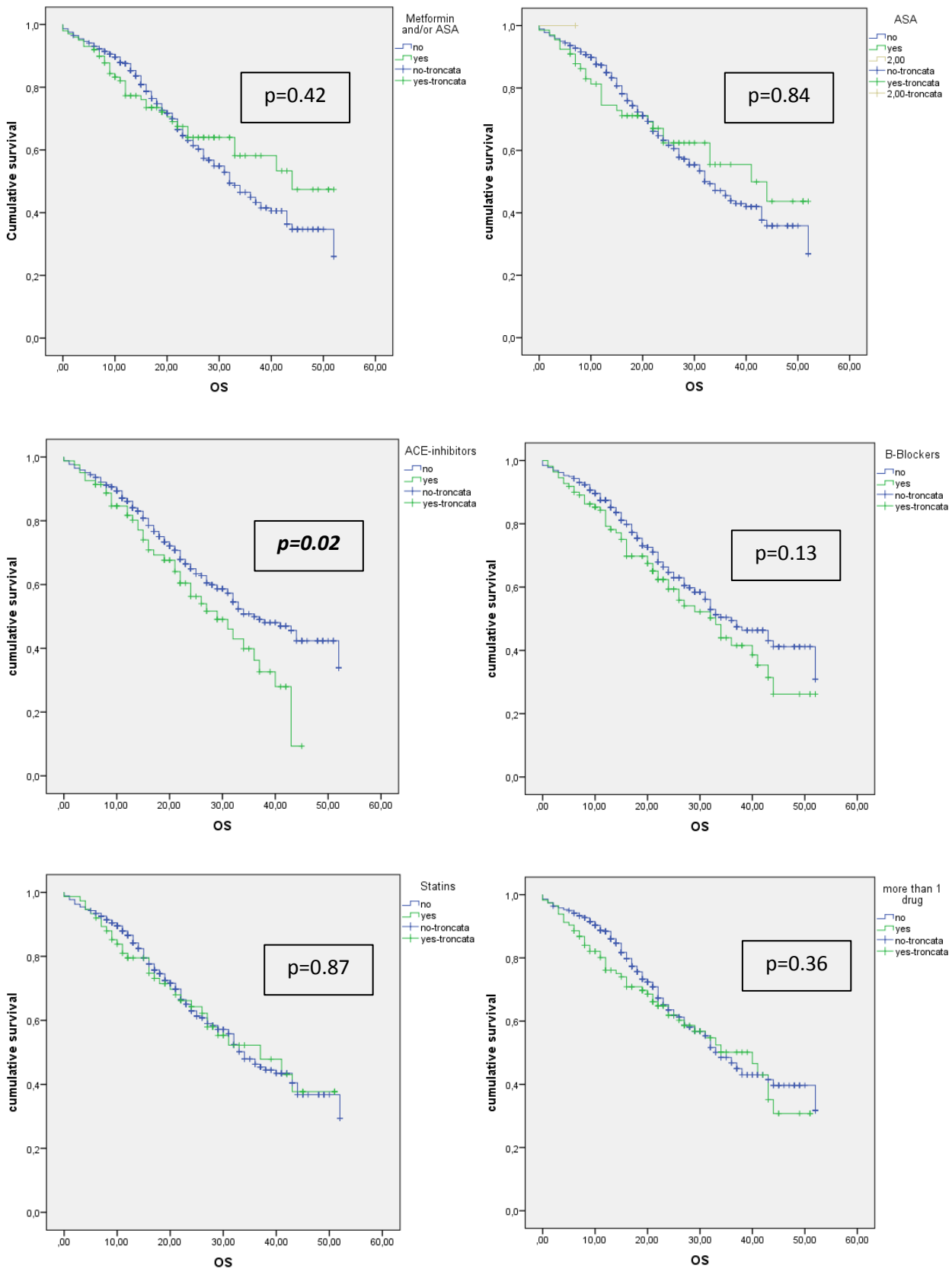


Figure 3: OS of patients on regular treatment with 1 drug and more than 1 drug



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Project II

Type of study: Statin use improves survival in patients with pancreatic ductal adenocarcinoma: A Meta-analysis

Department: Pancreatic Surgery Unit San Raffaele Hospital, Milan, Italy

State of art: Manuscript submission October 2019

Background

Pancreatic adenocarcinoma (PDAC) is a highly lethal cancer, showing a dismal prognosis with a 5-year survival rate of less than 5%. At present it represents the fourth leading cause of cancer death in the United States ,but is estimated to become the 2nd cause of cancer related death within 2030 [1,2]. Almost 80% of patients at diagnosis have metastatic or locally advanced disease and, for this reason, these patients are not eligible for surgical resection [3]. Chemotherapy and radiotherapy, for patients with advanced disease, remain the only possible therapy that might improve survival [4]. In this setting, several authors have recently investigated cancer chemoprevention, with the use of natural or synthetic substances to inhibit, retard or reverse the carcinogenesis [5]. However, the data from observational, case–control, cohort studies, and randomized trials in humans have overall demonstrated different results. Statins are cholesterol-lowering agents that have been widely prescribed to prevent and manage cardiovascular disease and act as inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzymeA (HMG CoA) reductase [6]. Statins also have an immunomodulatory and anti-inflammatory activity that seem to induce tumor apoptosis, inhibit angiogenesis, and suppress tumor metastasis, which can all be related to their antineoplastic effect [7-11]. Although a meta-analysis of 27 randomized controlled trials [12] failed to show an effect of statin use on overall cancer incidence or mortality, observational studies support that statins may decrease the risk of certain cancer types as well as cancer-related mortality. Among patients with pancreatic cancer, retrospective cohort studies have suggested a survival benefit associated with regular statin use, both in resectable and unresectable pancreatic cancer. Nevertheless, their role as chemopreventive agents is still controversial [13-15]. However, in the past few years, many other large studies conducted specifically on the topic have been published, most of which suggested a protective effect [16,17]. Therefore, we conducted this meta-analysis

trying to develop a new perspective and focusing on the relationship between the usage of statin and PDAC's mortality.

Materials and methods

Search Strategy

A computerized literature search of MEDLINE and the Cochrane Database of Systematic Reviews for prior systematic reviews and meta-analyses on association between the use of statins and the outcome of PDAC did not reveal previous meta-analyses on this topic. In the search for original studies, a MEDLINE search was run from inception until March 2019. Specific search terms were defined and are detailed in **Appendix 1**. The titles of all identified articles were screened to evaluate their relevance and the abstracts and/or full texts of selected, potentially relevant papers were further evaluated. With a snowball method, additional articles were searched by hand-searching reference lists of all the articles retrieved to identify potentially relevant studies. Searchers were physicians with experience in pancreatic disorders.

Inclusion and Exclusion Criteria

Studies related to our research question were included if they were either randomized controlled trials (RCTs), cohort (C) or case-control (CC) studies with available data for a quantitative synthesis; studies had, therefore, to include the relative risk (RR) or odds ratio (OR) for the outcome of PDAC associated with the exposure to statin versus placebo or no treatment, or sufficient information necessary for their estimate. Thus, included studies had to: (a) evaluate exposure to statin in a cohort or population of PDAC patients; (b) evaluate the outcome in terms of overall survival (OS) and/or disease-specific survival (DSS) and/or progression-free survival (PFS), and (c) report the RR or OR with 95% CI or original raw data sufficient to evaluate the hypothesized effect. In case of report of adjusted and unadjusted OR or RR, the adjusted one was selected. No language filters were applied. In the event of duplicate publications, the most recent or more complete publication was used. Two

independent reviewers (D.T. and G.C.) completed study identification and selection, and disagreements were discussed with another reviewer (SC). Excluded studies and reasons for exclusion were recorded.

Data Extraction and Quality Assessment

From the studies that met the eligibility criteria, the following data were extracted into a Microsoft Excel spreadsheet (2016 Edition; Microsoft Corp., Redmond, Washington, USA): (a) study – first author, year of publication, study design, Country, study accrual period; (b) cases – definition (i.e. clinical charts, histological diagnosis, or other means), number, gender, and age, stage of disease (c) type of exposure – definition, type of statin used, dosage, and length of exposure if available, and (f) main study outcome, type of outcome measures and eventual adjustment to the analysis.

Quality of each study included in the quantitative synthesis was assessed by two independent reviewers (D.T. and G.C.) using specific quality appraisal tools developed for cohort studies [18] and randomized controlled studies [19]. Disagreements were discussed with a third reviewer (SC).

Statistical Analysis

A meta-analysis of all eligible studies identified was planned using the software package Comprehensive Meta-Analysis (Biostat, Englewood, N.J., USA) with calculation of the pooled estimates (OR and 95% CI) using the DerSimonian-Laird method and a random-effects model. Random-effects models were used as they consider both sampling variance within the different studies and the variation in the underlying effect across studies. The assumption of variation in the underlying effect seems plausible given the different populations, study designs, drugs type, and exposure assessment methods used in the original studies. The quantity of heterogeneity was assessed by means of the I2 value and Cochran's Q

statistics[20-22]. An I2 value of ≤ 25% was considered as trivial heterogeneity and an I2 value of ≥ 75% as important heterogeneity. Publication bias was assessed using the Begg and Mazumdar test. A p-value<0.05 was accepted as statistically significant. We developed the following a priori hypotheses that would explain heterogeneity and planned sensitivity analyses for a) Area of origin (i.e. Asia, or Europe or Americas); b) type of statins c) tumor stage (i.e. resected, locally advanced, all stages). The methodology was developed and reviewed with the Preferred Reporting Items for Systematic Reviews and MetaAnalyses (PRISMA) statement [23] and the Meta-analyses Of Observational Studies in Epidemiology (MOOSE) [24] and the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) [25] checklist were checked for items that should be included in the report.

Results

A total of 92 references were identified by the MEDLINE and hand search (**Figure 1**). After the evaluation of titles and abstracts, 77 records were removed as not related to the study topic. Thus, the remaining 15 studies were examined in detail, leaving 14 as potentially appropriate for inclusion into the meta-analysis [26-39].

There was absolute agreement amongst the reviewers for the assessment of eligibility and selection of studies.

Study Characteristics

A summary of relevant studies, listing the population characteristics, exposures, and outcome is shown in **Tables 1 and 2.**

Concerning geographic area of enrollment, 8 studies came from North America, 1 from Europe, 4 from Asia, 1 included a worldwide population. Accrual periods ranged between 1976 and 2014; 3 studies recruited prospectively, 11 retrospectively. The diagnosis of PDAC was based on histological confirmation, exposure to statins was assessed in 1 study through direct patient interview or obtained by health registries for prescriptions in 13 studies. In the RCT statins were administered as part of study design. For cohort studies, mean follow-up was 13.5 months (range 3-36). Most of the studies included all types of statins, except for four which evaluated only some specific statin type. Two studies included patients with resectable PDAC, four studies included only patients with advanced disease (stage III and IV), the other eight studies did not perform a subgroup analysis within stages.

Association between the use of statins and the outcome of pancreatic cancer

The overall rate of statin use in the studies providing this information was 42.8% (14210/33137 patients). **Figure 2** shows the estimated HR for the association between the use

of any statin and OS for any PDAC stage in all the 14 studies providing this information. The pooled estimated HR was 0.871 (95% CI: 0.819-0.927; p=0.0001)) suggesting a protective effect. There was considerable heterogeneity for this global analysis with an I2 value of 62.3% and Q value being 34.5. Four studies provided information on PFS. The pooled estimated HR was 0.740 (95% CI: 0.624-0.878; p=0.001) (**Figure 3**).

Sensitivity Analyses

In order to evaluate reasons for heterogeneity and investigate which patients would benefit the most of a possible action of statins or which statin was the most effective, further pre-planned sensitivity analyses were performed.

In keeping with our a priori hypotheses for heterogeneity, we also performed a sensitivity analysis for Geographic Area of origin (**Figure 4**). Notably, while a protective effect of statins was confirmed in studies conducted in Asia (HR 0.79; 95% CI: 0.66-0.95; p=0.012).

Regarding the stage of the disease, we performed a sensitivity analysis divided by stages. As shown in **Figure 5** there was a protective effect in patients who underwent surgical resection (HR 0.50; 95% CI: 0.32-0.76; p=0.001).

The type of statin used was reported only in 4 studies. The sensitivity analysis shows a protective effect of Rosuvastatin. The pooled estimated HR was 0.88 (95% CI: 0.81-0.96; p=0.004) (**Figure 6**).

Discussion

In this meta-analysis, we observed that the use of statins was significantly associated with reduced mortality risk among patients with pancreatic cancer. In the last decade several authors have tried to investigate the role of statins in the chemopreventive setting among patients with pancreatic and colorectal cancer [16]. Most of papers published, have shown a protective effects of statins in patients with pancreatic cancer, regardless the stage of disease, type and duration of statin use [40,41]. However it is still difficult to assess the real effect of statins in these patients because most of papers, published in literature, are based on data extracted from retrospective database. After the search strategy only 14 studies, were considered eligible for the inclusion [26-39]. Only one RCT was considered in the analysis [37], all the others, were retrospective studies. The extraction data from Non-RCTs is a debated topic in the field of meta-analysis. However, data from well-designed Non-RCTs may be reliable and helpful for meta-analyses. The results of funnel plots and egger's test indicated there might not be a publication bias. However, there was also heterogeneity caused by the different number of patients enrolled in the included series and the differences in definitions and measurements of the outcomes of interest. In addition, most studies did not report the dosage as well as cumulative effects of statin use which might also increase the heterogeneity. All the fourteen studies, have investigated the association between statins and OS, the meta-analysis confirmed a positive effect of statins in the OS of patients with PC. Only 4 studies have also analyzed the PFS which was also increased in patients in therapy with statins. After stratifying by individual statins, we found that rosuvastatin, was significantly associated with decreased risk of mortality in PC patients. Most of the studies did not report the details of statin categories which might explain the heterogeneity of results. However this result is in line with recent reports that show a protective effect of hydrophilic statin. In literature there are several in vivo and in vitro evidences, which reported anti-tumor effects of statins. In vivo

evidences demonstrated that statins inhibited tumor progression and increase survival in mice models of PC [42-45]. More than 90% of PCs presented the K-Ras oncogene mutation which indicated that inhibition of protein prenylation was important [46]. The results were supported by Gbelcova et al [47] who demonstrated that different individual statins expressed large differences in gene transcription profiles of PC cells. High potency statins including simvastatin, atorvastatin and rosuvastatin were demonstrated to be more effective in modulating gene expressions, suppressing cell proliferation and promoting apoptosis [48-49]. In the present study, statins are associated with an increased OS in all stages. However, the main protective effect of statin use has been reached in patients with localized disease, who underwent surgical resection. The possible explanations of this result might be that in the advanced and metastatic disease there is a loss of control in the tumor cell replacing. In this setting also chemotherapy has low chances to control and reduce the burden of the disease. In the localized lesion the burden of the disease is localized in the pancreatic nodule and the statin, in association with chemotherapy, have an higher possibility to control and reduce the aggressive beahviour of the disease. The present study has several methodological strengths, such as the Egger test and funnel plot were conducted to provided quantitative evidence for publication bias; Compared with previous studies this work represents an appraisal of the literature including different papers that were not considered in the previous meta-analysis with the similar aim; the analysis was also conducted in different subgroups in order to explore different sources of heterogeneity; The authors also tried to explore the role of different statin types and the real effect of statins within different stages of disease. However, this study also has some limitations. Most of the paper considered for the analysis derive from retrospective and cohort studies; most studies did not evaluate the dose-response effect on relationship between statins and mortality of PC patients; Only four included studies conducted subgroup analysis on association between different statins and PC mortality which

reduced the power of the analyses. In conclusion the current evidences suggested that the use of statin was associated with lower risk of mortality in patients with PC. However, substantial heterogeneity of the results cannot be ignored. In subgroup analysis, we observed hydrophilic and high potency statin use may lower PC mortality, especially rosuvastatin. Further high quality RCTs and population-based cohort studies with sufficient follow-up time and information such as statin type etc should be conducted to examine the potential association between specific statin use and PC mortality.

Appendix 1

(statin OR statins OR simvastatin OR cerivastatin OR rosuvastatin OR pravastatin OR fluvastatin OR atorvastatin OR lovastatin OR Hydroxymethylglutaryl CoA Reductase Inhibitors OR HMG-CoA reductase inhibitors) AND (pancreatic cancer OR pancreatic neoplasms OR pancreatic neoplasm OR pancreatic adenocarcinoma OR pancreatic malignancy OR pancreas) AND (survival OR recurrence OR OS OR DFS OR disease free survival OR outcome)

Figure 1: flow chart of search strategy and study identification.

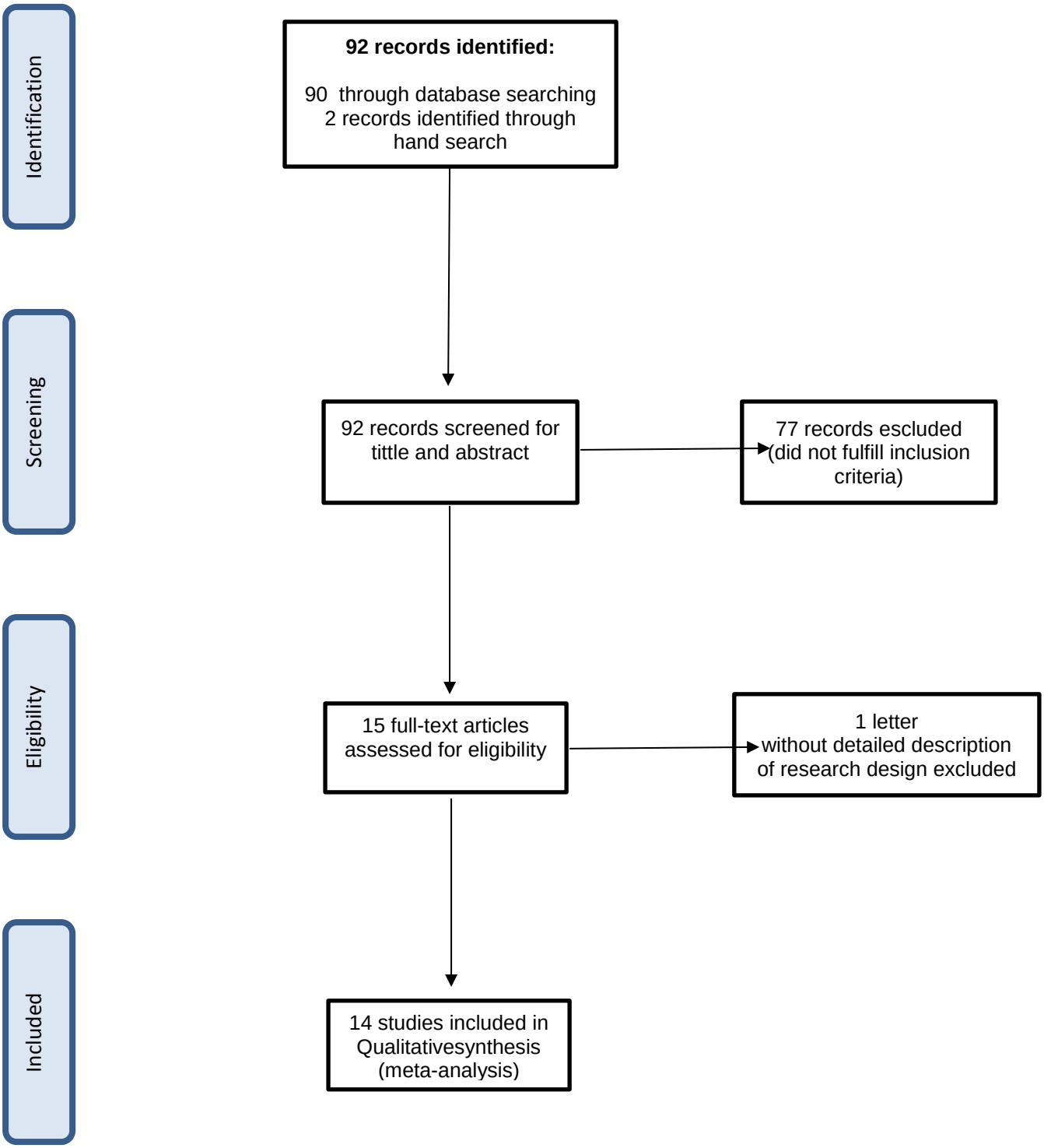


Table 1: Characteristics of the included studies in terms of Country, study accrual period, study design, case definition and matching design if appropriate, sex and age of included individuals, follow-up length and outcome definition.

Reference (year)	Country	Study accrual period	Study Setting and Design	Study Cases	Gender	Median age/ range	FU median (months)	Definition of outcome
Nakai (2013)	Japan	2001-2011	Unicentre CC	Patients with Stage III and IV PDAC on chemotherapy	103 F 147 M	66 (39-89)	9.9	OS
Hong JY (2014)	Japan	2008-2012	Multicentre RCT	Patients with Stage III and IV PDAC on chemotherapy randomized to Gemcitabine + Simvastatin and Gemcitabine + placebo.	45 F 69 M	60; 56 (25-80)	NR	TTP
Jeon CY (2015)	USA	2007-2009	Multicentre C	Patients with pancreatic cancer (all stages) >65yo	4601 F 3212 M	65-85	3.1	OS
Wu BU (2015)	USA	2005-2011	Multicentre C	Patients with stage I-IIb PDAC underwent pancreatic resection with curative intent	102 F 124 M	57-73	NR	OS and PFS
Moon DC (2016)	Korea	2006-2014	Unicentre C	Patients with unresectable or recurrent PDAC	70 F 110 M	65 (18-81)	NR	Long term response (stability after 6 monhts of chemo) and OS/PFS
Lee HS (2016)	Korea	2006-2014	Unicentre C	Patients with PDAC all stages	726 F 1036 M	62.5	NR	OS
Kozak MM (2016)	USA	1998-2013	Unicentre C	Patients with resectable PDAC	69 F 102 M	67 (37-86)	11.23	OS and PFS
Haukka J (2017)	Europe	1997-2010	Multicentre C	Patients with PDAC all stages	1059 F 1078 M	<50 >70	NR	Death after cancer diagnosis
Huang BZ (2017)	USA	2006-2014	Multicentre C	Patients with PDAC all stages. Data from regional integrated healthcare system	1053F 1089M	69.2	>12mo	OS
Jian-Yu E (2017)	USA	2008-2011	Multicentre C	Patients with PDAC all stages	2587F 1919M	76	3.8	OS
Beg MS (2018)	USA	2006-2009	Multicentre C	Data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare–linked database with PDAC all stages	NA	76	36	OS
Iarrobino	USA	2004-2014	Unicentre	Patients with PDAC all stages	151 F	70	18.4	OS

(2018)			C		152 M	(33-90)		
Abdel-Rahman O (208)	Worldwide	2009-2012	Multicentre C	Patients with stage IV PDAC	292F 419M	61-66	NR	OS and PFS
Hamada T (2018)	USA	1976-1986	Multicentre C	Nurses' health study and health professionals follow-up study	121.700 F 51.529 M	75.9	NA	OS

USA=United States of America; RCT=Randomized Controlled Trial; C=cohort; CC=case-control; GP=General Practitioner; NHS=National Health Service; PDAC=Pancreatic Ductal Adenocarcinoma; M=Males; F=Females; FU=Follow-Up; OS= overall survival; PFS= progression free survival; TTP= time to progression; NR=not reported; NA= not applicable;

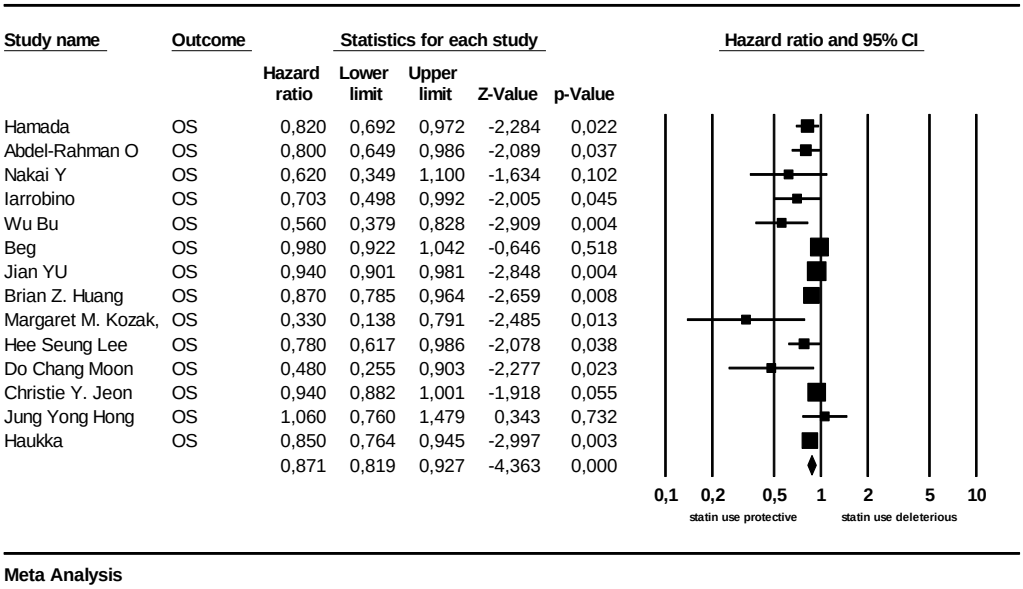
Table 2. Characteristics of the included studies in terms of type of statin, length of sue, dosage, type of outcome measures and patients on treatment.

Reference	Type of statin	Length of use	Dosage	Outcome measure	Possibility of sub-analysis within statins	Patients on statins Yes/No
Nakai (2013)	All	NR	NR	HR	No	30/250
Hong JY (2014)	Simvastatin	After diagnosis	40 mg daily	HR	Simvastatin	58/56
Jeon CY (2015)	All	NR	NR	HR	No	2456/5357
Wu BU (2015)	Simvastatin and Lovastatin	90 days before surgery	≤ 10 mg; 10-40 mg; >40 mg	HR	No	98/128
Moon DC (2016)	Atorvastatin, rosuvastatin, simvastatin, and pitavastatin	At least for 30 days consecutively	30 mg of simvastatin was used as a reference	HR	No	17/163
Lee HS (2016)	Simvastatin, atorvastatin, rosuvastatin, pravastatin, and fluvastatin	At least 30 days before cancer diagnosis	30 mg of simvastatin was used as a reference	HR	Simvastatin, atorvastatin, rosuvastatin, pravastatin, and fluvastatin	118/1643
Kozak MM (2016)	Atorvastatin and simvastatin	Regular	< 40 mg daily > 40 mg daily	HR	No	34/137
Haukka J (2017)	All	Regular	NR	RR	No	488/1649
Huang BZ (2017)	Simvastatin, lovastatin, atorvastatin, pravastatin, and rosuvastatin	< 6 mo; 6-9 mo; 9-12 mo	NR	HR	Simvastatin, lovastatin , atorvastatin, pravastatin	1155/987
Jian-Yu E (2017)	Atorvastatin fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin	At least 12 months before diagnosis	NR	HR	Atorvastatin fluvastatin, lovastatin, pravastatin, rosuvastatin, simvastatin	4506/12570
Beg MS (2018)	All	At least 12 months before diagnosis	NR	HR	No	4720/8982
Iarrobino (2018)	All	Before and after diagnosis	NR	HR	No	130/173
Abdel-Rahman O (208)	All	Before diagnosis	NR	HR	No	156/641

Hamada T (2018)	All	Before diagnosis	NR	HR	No	247/401

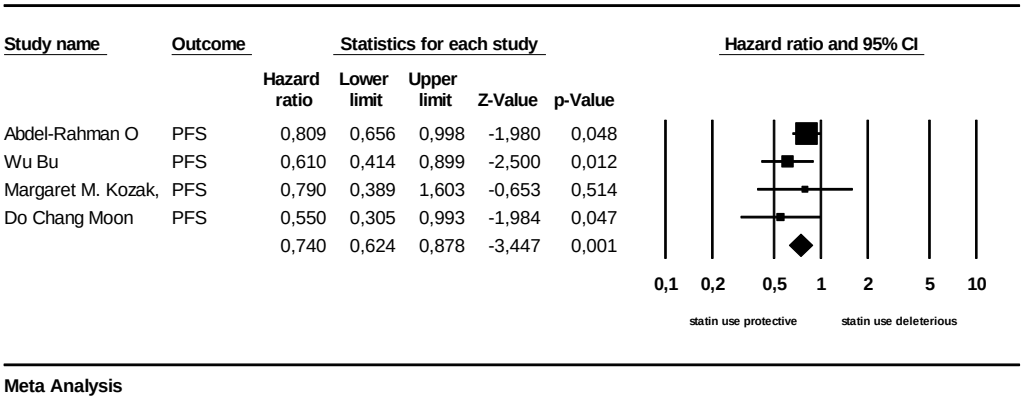
HR=Hazard Ratio; RR=relative risk; NR=not reported;

Figure 2: Statin use and overall survival (OS)



Q value=34.5 I2=62.3

Figure 3: Statin use and Progression free survival (PFS)



Q value=2.65 I2=0

Figure 4: Statin use in Asia VS West countries

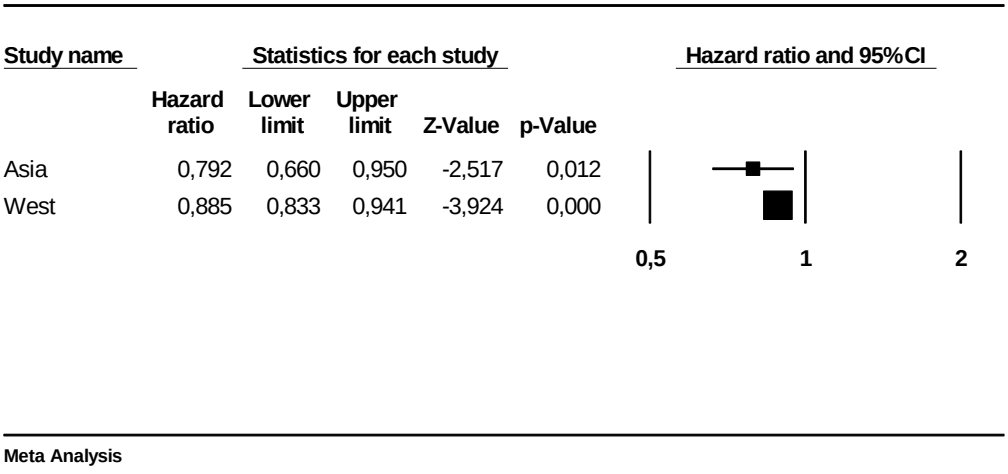


Figure 5: Statin use among different stages of pancreatic ductal adenocarcinoma

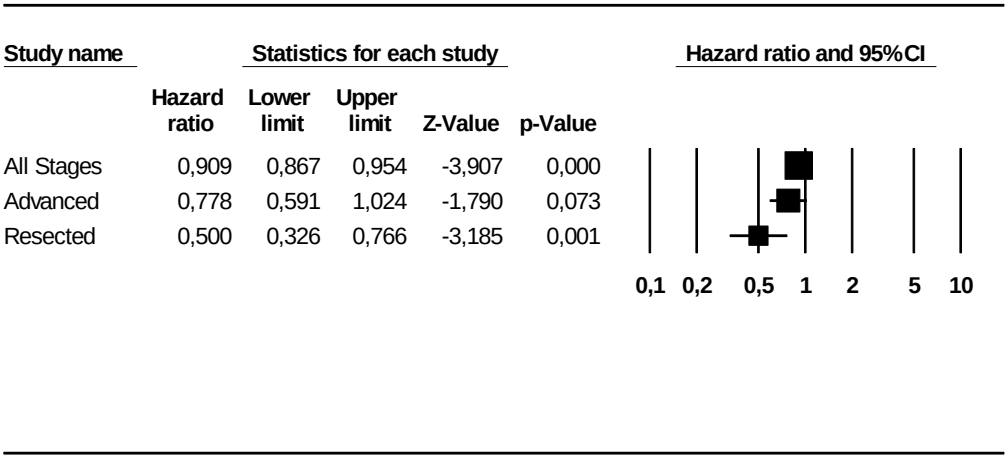
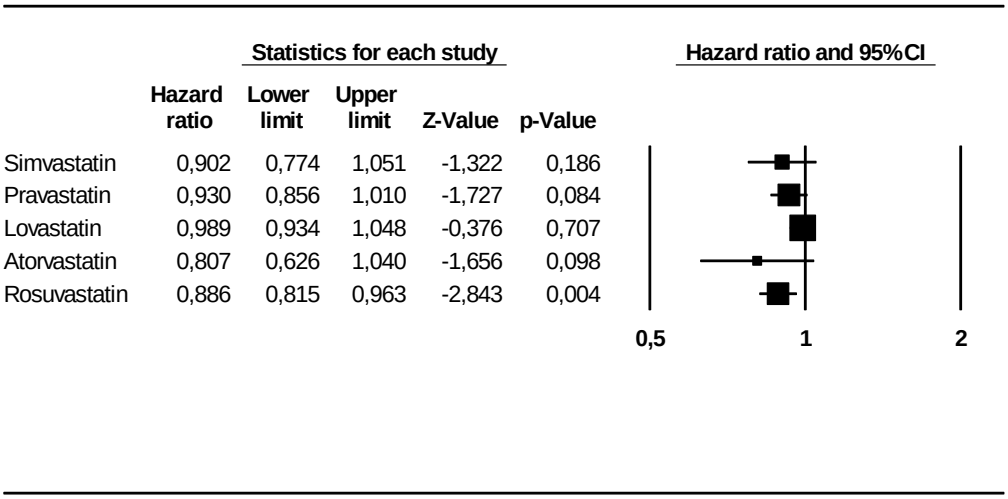


Figure 6: Sensitivity analysis of different types of statins



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Project III

Type of study: Prospective observational study on Disease-free and Overall Survival in patients with pancreatic ductal adenocarcinoma: **The CAOS study**

Department: Pancreatic Surgery Unit San Raffaele Hospital, Milan, Italy

State of art: Data collection still on-going; **Ethical committee approved the study on March 2019.** March 2019-September 2019: 321 patients recruited.

Study Chief Investigator:

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Study management group:

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1. Background and literature review

Pancreatic ductal adenocarcinoma is currently the fourth leading cause of cancer-related death in the United States with a 5-year survival rate of 6.7% [1].

Surgical resection of early-stage disease remains the only opportunity for potential cure. Despite advances in therapy, pancreatic cancer continues to have a poor prognosis and up to 80–85% of patients undergoing resection experience disease recurrence. The main reason for this poor prognosis is the propensity of pancreatic cancers to invade adjacent tissues and to metastasize [2]. Median survival following resection is 24–25 months even in the setting of adjuvant or neoadjuvant chemotherapy. In this setting cancer chemoprevention with the use of natural or synthetic substances to inhibit, retard or reverse the carcinogenesis has been recently investigated by several authors. A wealth of evidence from preclinical studies have convincingly demonstrated the cancer preventive efficacy of various agents in different animal models. However, the data from observational, case–control, cohort studies, and randomized trials in humans have overall demonstrated different results. Statins, metformin and nonsteroidal antiinflammatory drugs (NSAIDs), have been reported to be potential cancer chemopreventive agents. Several authors have shown that pancreatic adenocarcinoma is often associated with overexpression of a variety of mitogenic growth factors, including epidermal growth factor (EGF), and of growth factor receptors. Kusama et al. showed that HMG-CoA reductase inhibitors, fluvastatin and lovastatin, markedly attenuated EGF-induced translocation of RhoA from the cytosol to the membrane fraction and the in vitro invasive capacity of human pancreatic cancer cell lines [3]. Yeon et al. have found that statin use after cancer diagnosis was associated with survival in those with no exposure to statin prior to cancer diagnosis, but not in those with prior statin exposure [4]. For this reason statin treatment after cancer diagnosis may have a greater impact on statin-naïve tumors that are

sensitive to the molecular effects of statin, whereas tumors that arose in patients already receiving statins may have been selected for statin resistance before diagnosis.

Also other authors have demonstrated the cancer preventive effects of NSAIDs, especially in colorectal cancer, despite the relative high dose required for the observed chemopreventive effect in human trials may discourage the singular use of NSAIDs on a long-term basis for cancer prevention because of possibly increased risk for serious gastrointestinal side effects.

In a pooled analysis of 25,570 patients in eight trials, Rothwell et al. recently reported that daily aspirin use reduced deaths due to several common cancers, including significant reductions in colorectal and pancreatic cancer deaths, with most benefit seen after 5 years of the scheduled trial treatment [5].

Tan et al. also showed that metformin treatment may inhibit pancreatic tumorigenesis in the LSL-KrasG12D/+;Trp53F2-10 mice by modulating multiple molecular targets in signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa B (NFκB) inflammatory pathways.

Metformin and aspirin can inhibit the mTOR signaling pathway through both AMPK-dependent and AMPK-independent mechanisms. Given that persistent low-grade inflammation is an important factor for the development of pancreatic cancer, it is worth noting that two major inflammatory mediators, STAT3 and NFκB, also can be suppressed by metformin and aspirin. [6]

These investigations suggest that both metformin and aspirin might have preventive effects against the development of pancreatic cancer.

Reni et al. recently published a randomized phase II trial of 60 patients with metastatic pancreatic cancer treated with cisplatin, epirubicin, capecitabine, and gemcitabine (PEXG)

randomly assigned to addition of metformin (n = 31) or without metformin (n = 29). Unfortunately the study was ended for futility. They concluded that addition of metformin at the dose commonly used in diabetes did not improve outcome in patients with metastatic pancreatic cancer treated with standard systemic therapy [7]

The survival outcome was also investigated by Ambe et al. in metformin users patients with resected pancreatic cancer. They showed that metformin users had a better median survival than non-users, but the difference was not statistically significant (35.3 versus 20.2 months; P = 0.3875). The potential benefit of metformin should be investigated in adequately powered prospective studies.[8]

The limited evidence on the value of aspirin, statins, metformin, beta-blocking ACE inhibitors agents as chemopreventive agents in patients with pancreatic ductal adenocarcinoma is an incentive to carry out a prospective observational study to investigate that issue in San Raffaele hospital

2. Objectives

Primary objective

This study aims to assess whether regular use of aspirin, statins, metformin, ACE-inhibitors and beta-blocking agents use, before diagnosis, after surgery and in neo-adjuvant treatment setting, can increase rate of disease-free survival (DFS) and overall survival (OS) in participants with pancreatic ductal adenocarcinoma

Secondary objectives

To evaluate if there is any difference in terms of "chemoprevention" between aspirin, statins, metformin and beta-blocking as chemopreventive agents, and if their prolonged daily use can positively influence the chemopreventive action.

3. Study design

Sample size and Population

This study is designed as a monocentric observational prospective study. In a recent study [9] authors found that the use of low-dose aspirin before and after a diagnosis of pancreatic cancer reduces of 32% the risk of recurrence (Hazard ratio HR=0.68, $p<0.01$). On the basis of this study and considering that we will study the effect of other drugs as chemopreventive agents, the estimate required sample size to achieve 90% power to detect at least 28% reduction in a hazard of the "drug users" group, by using a two-sided 0.05-level log-rank test, is 400. ***Therefore, from February 2019 to February 2022 we expect to enroll 400 patients with a diagnosis of pancreatic ductal adenocarcinoma at any stage meeting the following inclusion criteria. Median follow-up is estimated to be 24 months after the first disease diagnosis***.

Inclusion and exclusion criteria

Inclusion criteria are:

1) cytological or histological diagnosis of pancreatic ductal adenocarcinoma in any portion of the glande, with or without metastases in other sites

2) patient age between 18 and 90 years

3) any medicine or drug in the daily patient therapy

4) Patients undergone to primary chemoradiotherapy or surgical resection, followed by adjuvant therapy or preceded by neoadjuvant chemoradiotherapy, are included in the study

Exclusion criteria are:

1) age under 18 years

2) lack of cytological or histological diagnosis of pancreatic ductal adenocarcinoma

Data collection methods

Anamnestic, clinical and pathological data, included data on the aspirin, statins, metformin, ACE-inhibitors and beta-blocking agents assumption (details on variable to collect see the CRF, annex 1) will be collected during the first visit with the surgeon. A database managed by a dedicated data manager will be created to collect and analyse data. The PI will be responsible of the data security.

Statistical analysis

Association between variables will be assessed using the Chi Squared test (or Fisher's exact text where appropriate) for categorical variables and the Spearman's correlation for scale variables. . DFS and OS will be estimated using Kaplan-Mayer method and Log Rank tests will be used to evaluate the difference between survival curves. The impact of aspirin, statins, metformin, ACE-inhibitors and beta-blocking agents on the risk of recurrence will be estimated using Cox regression models. Variables resulting significant (p value <0.05) at univariate analysis or variables which are known prognostic/risk factors will be included in the multivariable regression models. A p value of <0.05 will be considered statistically significant. Statistical analysis will be conducted using SPSS v23 (IBM, Armonk, NY, USA) and R v3.3.0 (https://cran.r-project.org).

4. References

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8. Ambe CM, Mahipal A, Fulp J, Chen L, Malafa MP. Effect of Metformin Use on Survival in Resectable Pancreatic Cancer: A Single-Institution Experience and Review of the Literature PLoS One. 2016 Mar 11;11(3)

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5. Annex 1 - CRF

CAOS study

Codice paziente: Data Compilazione ____ /____/_____

Criteri di inclusione:

□ Diagnosi di adenocarcinoma duttale del pancreas (PDAC) con o senza metastasi in alter sedi

□ Età compresa tra 18 e 90 anni

□ Assunzione giornaliera e continua di farmaci

□ Pazienti candidate a chemio-radio terapia primaria o intervento e chemioterapia adiuvante o neoadiuvante.

□ Paziente in grado di firmare il consenso informato

Criteri di esclusione:

□ Età inferiore a 18 anni

□ mancanza di diagnosi istologica o citologica di PDAC

STADIO TNM ALLA DIAGNOSI:

Localizzazione primitivo: □ testa □ corpo-coda □ processo unc. □ disseminato

Dimensioni primitivo (mm):

Invasività locale:

□ Non localmente avanzato-resecabile

□ Inv. Vascolare: □ tronco cel. □ AMS □ VMS □ v. porta □ v. splenica □ altro:_____

□ Inv. organi contigui: □ duodeno □ stomaco □ milza □ colon □ altro: _____

□ Presenza di metastasi (specifica): □ epatiche □ polmonari □ ossee □ cerebrali □ altro: _____

SINTOMI ALLA DIAGNOSI

□ Nessuno

□ Calo Ponderale (quanti kg? In quanto tempo?) _____

□ Diabete_____

□ Dolore (specificare sede)_____

□ Diarrea _____

□ Ittero _____

□ Astenia _____

□ Inappetenza_____

□ Pancreatite acuta _____

□ Altro _____

ANAMNESI FAMILIARE (specificare familiare ed età d'insorgenza)

Neoplasia pancreas: NO □ SI □

Altre neoplasie: NO □ SI □

ANAMNESI PATOLOGICA REMOTA

Diabete: NO□ SI □

□tipo I □tipo II dal _____

Terapia: insulina NO□ SI □ (dal_____)

Pancreatite cronica NO□ SI □ dal _____ terapia: _____

Ipertensione arteriosa NO□ SI □ dal _____ terapia: _____

Gastroresezione NO□ SI □ anno_____ tipo _____ motivazione _____

□ Neoplasie pregresse: NO□ SI □ (specificare tipo, età insorgenza e terapia)

_____

ANAMNESI FARMACOLOGICA (barrare farmaco e dose)

Uso di **aspirina:**

□ NO □ SI

□ Cardioaspirina 100 mg

□ Cardirene 75 mg/100 mg/160 mg/300 mg

dal _____

Uso di **Statine:**

□ NO □ SI

□ Atorvastatina (torvast, totalip)

□ Fluvastatina (lescol, lipaxan, primesin)

□ Lovastatina (lovinacor, rextat, tavacor)

□ Pravastatina

□ Rosuvastatina (crestor, provisacor, simestat)

□ Simvastatina (liponorm, medipo, sindaco, sivastin, zocor)

□ Simvastatina + ezetimibe (inegy, goltor, vytorin)

10 mg/20 mg/40 mg/60 mg
80 mg/100 mg/120 mg

dal _____

Uso di **ACE Inibitori**:

□ NO □ SI

□ Lisinopril (zestril, alapril, prinivil) 5 mg/20 mg

□ Enalapril (enapren, converten, naprilene) 5 mg/20 mg

□ Ramipril (quark, triatec, unipari) 1.25 mg/2.5 mg/ 5 mg)

□ Quinapril (accuprin, acequin, quinazil) 5 mg/20 mg

□ Perindopril (coversyl, procaptan) 4 mg

□ Trandolapril (gopten) 0.5 mg/2 mg

□ Zofenopril (bifil, zantipress, zopranol) 7.5 mg/30 mg

□ Fosinopril (eliten, fosipres, tensogard) 10 mg/20 mg

dal _____

Uso di **beta-bloccanti** :

□ NO □ SI

□ Nebivololo	□ Bisoprololo
□ Metoprololo	□ Pindololo

dal _____

Uso di **Metformina**

□ NO □ SI dal _____

EVENTI AVVERSI

□ NO □ SI dal _____

Se sì, indicare quali:_____

ITER TERAPEUTICO DIAGNOSTICO PROPOSTO

INTERVENTO CHIRURGICO: NO□ SI □

TIPO INTERVENTO: □DCP □PD □SPD □ SPT □Enucleazione □Resezione vascolare

TRATTAMENTO NEOADIUVANTE: NO□ SI □

CHEMIOTERAPIA SISTEMICA/PALLIATIVA: NO□ SI □

Ca19-9 diagnosi: _____ Altri markers diag.: _____

Consent Form

Gentile signora/e,

In questo Istituto Le viene proposto di partecipare ad una ricerca sul ruolo che può avere l'assunzione pregressa e continua di farmaci quali ACE-inibitori, aspirina, statine, metformina e beta-bloccanti sul decorso e la prognosi della sua malattia.

1) Il titolo dello studio è: L'efficacia di agenti chemiopreventivi sulla sopravvivenza totale e libera da malattia dei pazienti affetti da adenocarcinoma duttale del pancreas.

Ciò significa che si andrà a valutare il potenziale effetto protettivo dell'assunzione dei suddetti farmaci sul rischio di sviluppare progressione o recidiva di malattia.

Per svolgere tale ricerca abbiamo bisogno della collaborazione e della disponibilità di persone che, come Lei, soddisfino i requisiti scientifici idonei alla valutazione che verrà eseguita. Le proponiamo, pertanto, di partecipare a questa ricerca sulla quale Lei ha già avuto informazioni dettagliate dal medico responsabile dott. Domenico Tamburrino.

Prima, però, che Lei prenda la decisione di accettare o rifiutare di partecipare, La preghiamo di leggere con attenzione, prendendo tutto il tempo che Le necessita, queste pagine e di chiedere chiarimenti qualora non avesse ben compreso o avesse bisogno di ulteriori precisazioni. Inoltre, qualora lo desiderasse, prima di decidere può chiedere un parere ai suoi familiari o ad un suo medico di fiducia.

2) L'obiettivo principale di questo studio è quello di **valutare l'eventuale effetto protettivo dell'assunzione di farmaci quali statine, aspirina, metformina, ACE-inibitori e beta-bloccanti sul rischio di sviluppare recidiva o progressione di malattia.**

3) Nel caso decida di partecipare, lo studio prevede una raccolta di dati relativi ai farmaci che di routine prende (di cui il medico responsabile le avrà dato dettagliate informazioni). Le informazioni verranno raccolte durante la visita ambulatoriale con il chirurgo o al ricovero per intervento e durante le visite di controllo, che saranno a cadenza semestrale.

Lo studio si svolgerà solo all'interno di questa struttura, durerà in totale 5 anni e prevede di arruolare 400 pazienti che saranno scelti tra tutti quelli che sono affetti dalla stessa malattia di cui Lei è affetto.

4) Dalla partecipazione a questo studio Lei non avrà un diretto beneficio, ma le informazioni ottenute potranno essere utili in futuro per conoscere meglio la malattia da cui è affetto.

5) La partecipazione allo studio non dovrebbe comportare rischi trattandosi di uno studio osservazionale che richiede solo una raccolta di informazioni.

6) Si segnala che lo studio non prevede una copertura assicurativa specifica, non comportando rischi per l'individuo.

7) Lei è libero/a di non partecipare allo studio. In questo caso riceverà le terapie standard previste per la patologia da cui Lei è affetto ed i medici continueranno a seguirla con la dovuta attenzione assistenziale, ma i suoi dati non saranno utilizzati per lo studio.

8) La sua adesione a questo programma di ricerca è completamente volontaria e **Lei si potrà ritirare dallo studio in qualsiasi momento**:

.

Qualora divengano disponibili dati che possano influenzare la decisione di continuare lo studio in oggetto, sarà tempestivamente informato/a.

9) Il protocollo dello studio che Le è stato proposto è stato redatto in conformità alle Norme di Buona Pratica Clinica della Unione Europea e alla revisione corrente della Dichiarazione di Helsinki ed è stato approvato dal Comitato Etico di questa struttura al quale **Lei può segnalare qualsiasi fatto ritenga opportuno evidenziare, relativamente alla sperimentazione che La riguarda**, indirizzando la corrispondenza al Presidente del Comitato stesso: Presidente del Comitato Etico - Ospedale San Raffaele - Via Olgettina, 60, 20132 Milano.

Per **ulteriori informazioni** e comunicazioni durante lo studio potrà contattare il seguente personale:

Dr. Domenico Tamburrino, tamburrino.domenico@hsr.it (tel: 02 2643 2324)

Dr.ssa Giulia Maggi, maggi.giulia@hsr.it (tel: 02 2643 6591)

DICHIARAZIONE DI CONSENSO

Io sottoscritto ……

dichiaro di aver ricevuto dal dottor ……..

esaurienti spiegazioni in merito alla richiesta di partecipazione allo studio in oggetto, secondo quanto riportato nella scheda informativa qui allegata, copia della quale mi è stata consegnata con sufficiente anticipo.

Dichiaro altresì di aver potuto discutere tali spiegazioni, di aver posto tutte le domande che ho ritenuto necessarie e di aver ricevuto risposte soddisfacenti, come pure di aver avuto la possibilità di informarmi in merito ai particolari dello studio con persona di mia fiducia.

Accetto, dunque, liberamente di partecipare alla sperimentazione, avendo capito il significato della richiesta e avendo compreso i rischi e i benefici che sono implicati e acconsento a che il mio medico curante venga informato della mia partecipazione allo studio. Sono consapevole del mio diritto a recedere in ogni momento dalla sperimentazione.

Sono stato informato, inoltre, del mio diritto ad avere libero accesso alla documentazione relativa alla sperimentazione (assicurativa, clinico-scientifica, farmaco-terapeutica) ed alla valutazione espressa dal Comitato Etico.

Data…… Firma del paziente ……..

Data……Firma del medico che ha informato il paziente ……

……..

[*Se il paziente non è in grado di leggere o di firmare, un <u>testimone indipendente</u> dallo sperimentatore e dallo sponsor deve essere presente durante l'intera discussione relativa al consenso informato. Il testimone deve firmare e datare personalmente la dichiarazione di consenso informato dopo che il modulo stesso e qualsiasi altra informazione scritta siano stati letti e spiegati al soggetto e questi abbia espresso il consenso verbale alla partecipazione allo studio*].

In questo caso:

Io sottoscritto …. …….. testimonio che il dottor ……ha esaurientemente spiegato al sig. ……..

le caratteristiche dello studio in oggetto, secondo quanto riportato nella scheda informativa qui allegata, e che lo stesso, avendo avuto la possibilità di fare tutte le domande che ha ritenuto necessarie, ha accettato liberamente di aderire allo studio.

Data……. Firma del testimone indipendente ……..

Data……. Firma del medico che ha dato le informazioni al paziente …….

INFORMATIVA

AI SENSI DELL'ART. 13 DEL REGOLAMENTO GENERALE SULLA PROTEZIONE DEI DATI PERSONALI (UE) 2016/679 ("REGOLAMENTO" O "GDPR")

Parte 2

Titolare del trattamento

- **L' Istituto di Ricovero e Cura a Carattere Scientifico ("IRCCS") Ospedale San Raffaele, s.r.l.,** con sede in Milano, in via Olgettina n.60, quale "**Promotore**"), raggiungibile all'indirizzo di posta elettronica: falconi.massimo@hsr.it / tamburrino.domenico@hsr.it ("**Titolare**")

- Il **Promotore**, ha nominato responsabile per la protezione dei dati (d'ora innanzi, per brevità "Data Protection Officer" o **"DPO"**) raggiungibile all'indirizzo di posta elettronica **dpo@hsr.it**;

Descrizione e finalità dello studio

La Descrizione e le finalità dello studio sono state riportate nei paragrafi precedenti.

Finalità del Trattamento

L'**IRCCS Ospedale San Raffaele**, **s.r.l**., Promotore del progetto di ricerca che le è stato descritto, in accordo alle responsabilità previste dalle norme di buona pratica clinica (D.L. 211/2003)], è titolare delle operazioni di trattamento correlate all'effettuazione della ricerca scientifica e tratterà i Suoi dati personali comuni (nome, cognome, data di nascita, ecc) e particolari (dati relativi alla Sua salute, alla Sua origine, ai Suoi stili di vita, alla Sua vita sessuale, dati biologici), solo suo previo, specifico ed esplicito consenso esclusivamente per la realizzazione dello studio clinico e soltanto nella misura in cui siano indispensabili in relazione all'obiettivo dello studio stesso nonché ai fini di vigilanza.
A tal fine i dati indicati saranno raccolti dal Promotore

La base di legittimità per il trattamento dei suoi dati per suddetta finalità è il Suo consenso esplicito ai sensi degli artt 6.(1)(a) e 9(2)(a) GDPR. Il conferimento dei Suoi dati per tale finalità è facoltativo, tuttavia, essendo indispensabile allo svolgimento del progetto di ricerca, il suo eventuale rifiuto non Le consentirà di parteciparvi.
E' possibile revocare ex art. 7 del GDPR il consenso prestato per le suddette finalità in ogni momento senza fornire alcuna giustificazione;
Non saranno raccolti ulteriori dati che La riguardano ferma restando l'utilizzazione di quelli eventualmente già raccolti per determinare, senza alterarli, i risultati della ricerca.

Modalità di trattamento e natura dei dati

Il medico che La seguirà durante la sperimentazione La identificherà con un codice (ad esempio: ab0001) che non permette di risalire direttamente alla Sua identità: i dati che verranno raccolti nel corso dello Studio, ad eccezione del Suo nominativo, saranno registrati, elaborati e conservati unitamente a tale codice, alla Sua data di nascita, al sesso, al peso, alla statura e a tutti i dati clinici inerenti il Suo stato di salute.
I dati sopra indicati saranno raccolti, gestiti e custoditi, sia in formato cartaceo che elettronico e comunque trattati in ossequio alla normativa in materia di trattamento dei dati personali, compresi i provvedimenti e le autorizzazioni applicabili emanati dall'Autorità Garante Per la Protezione dei dati personali.

Maggiori informazioni sono disponibili presso il Titolare ovvero presso il DPO ai recapiti sopra indicati.

Ambito di circolazione dei dati

La Sua partecipazione allo studio implica che, in conformità alla normativa sulle sperimentazioni cliniche dei medicinali, il personale debitamente autorizzato dal Promotore ai sensi dell'art. 29 del GDPR,

il Comitato etico e le autorità sanitarie italiane e straniere, in qualità di autonomi titolari, potranno conoscere i dati che La riguardano, contenuti anche nella Sua documentazione clinica originale, con modalità tali da garantire la riservatezza della Sua identità.

Per quanto concerne l'eventuale trasferimento dei Dati verso Paesi Terzi, il Titolare rende noto che il trattamento avverrà comunque secondo una delle modalità consentite dalla legge vigente, quali ad esempio il Suo consenso, l'adozione di Clausole Standard approvate dalla Commissione Europea, la selezione di soggetti aderenti a programmi internazionali per la libera circolazione dei dati (es. EU-USA Privacy Shield) o operanti in Paesi considerati sicuri dalla Commissione Europea. Maggiori informazioni sono disponibili presso il Titolare ovvero presso il DPO ai recapiti sopra indicati.

I Suoi dati saranno diffusi solo in forma rigorosamente anonima in occasione di convegni scientifici o attraverso pubblicazioni scientifiche o statistiche.

Conservazione

I Dati Personali saranno conservati solo per il tempo necessario ai fini per cui sono raccolti, rispettando il principio di minimizzazione di cui all'articolo 5(1)(c) del GDPR nonché gli obblighi di legge cui è tenuto il Titolare.

Maggiori informazioni sono disponibili presso il Titolare ovvero presso il DPO ai recapiti sopra indicati.

Esercizio dei diritti privacy

Lei potrà, ai sensi e per gli effetti degli artt. 15 e ss. del GDPR, accedere ai Suoi dati personali, verificarne contenuto, origine, esattezza, ubicazione (anche in relazione ai Paesi Terzi ove i dati si trovino e/o ai soggetti cui i Dati possono essere comunicati), chiederne copia, integrazione, aggiornamento, rettificazione e, nei casi previsti dalla Legge vigente, cancellazione, trasformazione in forma anonima, la limitazione, la portabilità dei dati, la revoca del consenso prestato ex art. 7 del GDPR; nonché proporre reclamo all'autorità di controllo competente ex articolo 77 del GDPR (Garante per la Protezione dei Dati Personali).

La informiamo, inoltre, che lei potrà opporsi al trattamento dei suoi dati personali ai sensi dell'art. 21 del Regolamento.

La modifica dei dati originari può avere effetti significativi sui risultati dello studio, per cui in caso di esercizio di diritti che comportano variazione/integrazione dei dati registrati, le modifiche richieste potranno essere annotate e registrate a margine dei dati originari senza modificare questi ultimi.

Potrà interrompere in ogni momento e senza fornire alcuna giustificazione la Sua partecipazione allo studio.

Non saranno raccolti ulteriori dati che la riguardano ferma restando l'utilizzazione di quelli eventualmente già raccolti per determinare, senza alterarli, i risultati della ricerca.

Per esercitare i Suoi diritti privacy può contattare il DPO o il Titolare del trattamento ai recapiti sopra indicati.

Consenso

Il sottoscritto (nome e cognome) ……..…nato a …….. il ……

/……/……/… codice fiscale……. residente a (Comune)……. (Prov.) ……. via (indirizzo) ……

consapevole delle sanzioni penali previste dall'art. 76 del D.P.R. 445/2000 per le ipotesi di falsità in atti e dichiarazioni mendaci

☐ per sé

☐ oppure in qualità di Testimone indipendente*

[* Se il paziente non è in grado di leggere o di firmare, un testimone indipendente dallo sperimentatore e dallo sponsor deve essere presente durante l'intera discussione relativa al consenso informato. Il testimone deve firmare e datare personalmente la dichiarazione di consenso informato dopo che il modulo stesso e qualsiasi altra informazione scritta siano stati letti e spiegati al soggetto e questi abbia espresso il consenso verbale alla partecipazione allo studio].

Di (nome e cognome) ……..…nato a …….. il …… / …… / …… codice fiscale……. residente a (Comune)…….. (Prov.) ……. via (indirizzo) ……

Letta e compresa l'informativa di cui all'Art. 13 del Regolamento UE 2016/679 "Il Regolamento o GDPR" e consapevole del diritto di revocare il consenso in qualsiasi momento ai sensi dell'art. 7 del GDPR, ferma restando impregiudicata la liceità del trattamento basata sul consenso prima della revoca:

☐ Acconsento ☐ Non acconsento

al trattamento dei miei dati personali comuni e particolari per gli scopi di ricerca ma nei limiti e con le modalità indicate nell'informativa.

☐ Acconsento ☐ Non acconsento

all'eventuale utilizzo dei miei dati personali comuni e particolari anche al di fuori dell'Unione Europea per ulteriori ricerche scientifiche.

Data……. ; Luogo…… ;

Firma ……..

(Firma estesa e leggibile)

6. Interim analysis

The observational study on chemopreventive agents on disease-free and overall Survival in patients with pancreatic ductal adenocarcinoma (CAOS study) has been approved by ethical committee of San Raffalefe Hospital, Milan, Italy on the 2nd March 2019.

At the moment of submission of the study, we expected to enroll 400 patients, at any stage, from February 2019 to February 2022.

Surprisingly, from February 2019 to September 2019 we have already collected data on 321 patients, at any stage, with pancreatic ductal adenocarcinoma in a single high volume centre.

As stated in the protocol we would like to have at least a median follow-up of 24 months after diagnosis and at present is impossible to perform any survival analysis.

The characteristics of patients included until September 2019 are shown in **table 1 and 2.**

The data collection is still on-going and we submitted an appraisal to our ethical committee in order to reach 800 patients for this study.

Table 1: Distribution of patients on different therapies within different stages of PDAC.

	Stage I	Stage II	Stage III	Stage IV
Aspirin	**13**	**22**	**3**	**6**
<1 year	1	1	0	0
2-5 years	3	11	1	1
6-10 years	4	5	1	4
>10 years	5	5	1	1
Beta Blockers	**18**	**16**	**7**	**11**
<1 year	2	1	2	0
2-5 years	6	4	2	1
6-10 years	5	5	1	6
>10 years	5	6	2	4
ACE inhibitors	**16**	**22**	**8**	**12**
<1 year	2	2	4	0
2-5 years	7	7	1	5
6-10 years	5	8	3	4
>10 years	2	5	0	3
Statins	**16**	**20**	**1**	**7**
<1 year	1	2	0	0
2-5 years	5	8	0	1
6-10 years	5	7	0	5
>10 years	5	3	1	1
Metformin	**7**	**15**	**10**	**6**
<1 year	2	4	3	2
2-5 years	2	6	2	2
6-10 years	1	2	2	1
>10 years	2	3	3	1

Stage I: 77

Stage II: 118

Stage III : 56

Stage IV: 70

Total : 321

Table 2: Principal comorbidities among 321 patients with PDAC

	Total	Hypertension	Diabetes	Previous gastric resection	Chronic pancreatitis	Previous neoplasms
STAGE I	77	40	18	0	3	12
STAGE II	118	48	31	1	1	26
STAGE III	56	20	19	0	0	10
STAGE IV	70	26	15	2	1	8

International project

In the last three years I have been selected for a post-graduated course, Pancreas 2000. This project is sponsored by *European Pancreatic Club*. The lengths of the course is three years and there are three annual meetings during which all the attending doctors discuss with the mentors about the research projects. I have been selected as **principal investigator** for one project on IPMN of the pancreas under surveillance.

As well known, branch-duct intraductal papillary mucinous neoplasms (BD-IPMN) are frequently detected as an incidental finding in clinical practice, and they have a more indolent behavior compared with mixed/main-duct IPMNs. In 2006 the International Association of Pancreatology (IAP) published consensus guidelines for the management of IPMNs (ref) suggesting a non-operative management for "low-risk" IPMNs (no symptoms, size < 30 mm, no nodules, no main-pancreatic duct dilatation (MPD), negative cytology). Several studies have supported the safety of this conservative approach. Since then, the IAP guidelines were updated in 2012 and in 2016 (ref) with the introduction of two categories of risk, namely worrisome features (WF) and high-risk stigmata (HRS). Moreover, other guidelines were published for the management of cystic lesions of the pancreas/IPMN, including the European guidelines (ref), the Italian guidelines (ref) and the American Gastroenterologic Association (AGA) guidelines (ref). These different guidelines recommend different follow-up strategies for patients with "low-risk" BD-IPMN (LR-BD-IPMN), but actual data regarding their clinical impact are lacking.

Aim of this study was to investigate the clinical impact of different guidelines for the surveillance of a large cohort of patients with a suspected LR-BD-IPMN; to analyze the adherence to follow-up strategies, proposed by several guidelines, in the "real –life" situation; to describe the natural history of a large cohort of patients with LR-BD-IPMN.

Results

The manuscript is not yet submitted but it has been shared with all the European authors. We are aiming to submit it to GUT. The results showed in the following tables are confidential and regarding unpublished data..

Overall 837 patients have been enrolled in the study. Over the study period, 168 patients (20%) developed a worrisome features (WF) or an high risk stigmata (HRS). The **table 1** shows the cumulative incidence of WF or HRS during the FU.

The independent factors associated with the development of WF or HRS are listed in **table 2.** Male gender, unifocal cyst, IPMN size >15 mm**,** Wirsung >3 mm and presence of symptoms are associated with an increased risk to develop WF or HRS.

Based on these information a risk score has been generated and it is shown in **Figure 1**.

The manuscript will be submitted after the approval of the other authors.

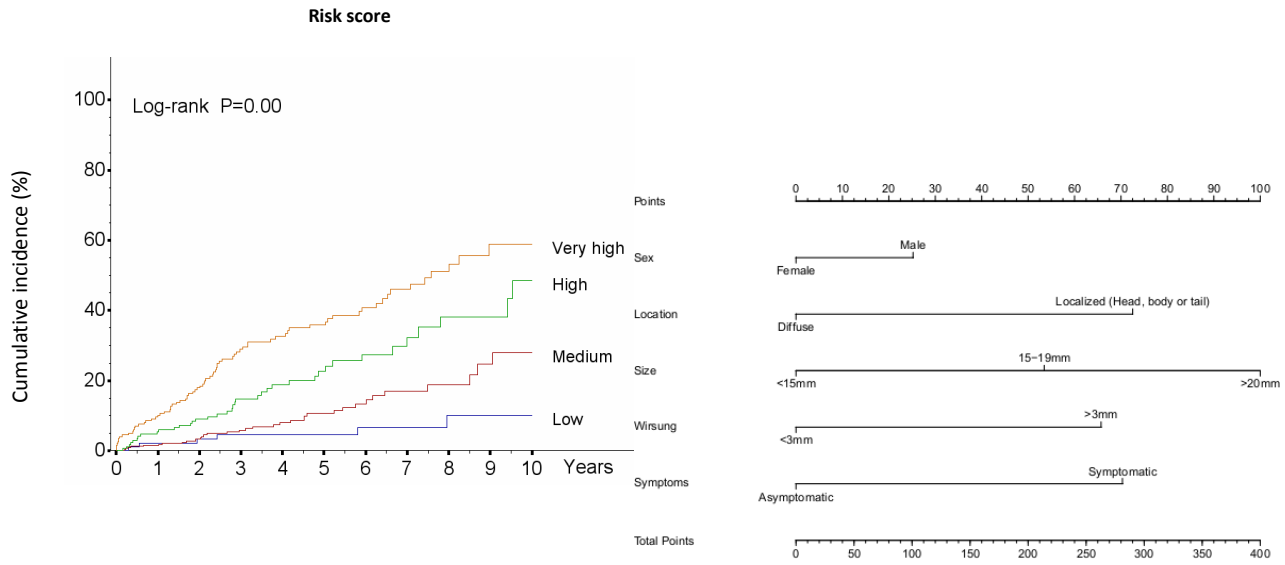
Table 1. Cumulative incidence of WF or HRS in the entire population (n=837 patients). (unpublished data)

	Cumulative incidence of WF or HRS (95% CI)
WF or HRS during surveillance	N=168
1-year	4.3% (3.1-5.9)
3-year	12.8% (10.6-15.4)
5-year	18.7% (15.8-22.0)
10-year.	37.3% (31.1-44.2)

Table 2. Factors associated with the development of WF or HRS at univariate and multivariable analysis. (Unpublished data)

		Univariate analysis		Multivariable analysis	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Gender	Females vs. Males	**0.68 (0.50-0.92)**	**0.01**	**0.69 (0.51-0.94)**	**0.02**
Age	50-59 vs. <50 years	1.23 (0.66-2.31)	0.52		
	60-69 vs. <50 years	1.30 (0.72-2.32)	0.38		
	70+ vs. <50 years	1.56 (0.87-2.79)	0.14		
BMI	Underweight vs. normal weight	0.91 (0.36-2.29)	0.84		
	Overweight vs. normal weight	1.01 (0.65-1.59)	0.95		
	Obese vs. normal weight	1.38 (0.69-2.73)	0.36		
Family history	Yes vs. No	0.79 (0.35-1.78)	0.57		
Smoking	Yes vs. No	**1.58 (1.09-2.27)**	**0.01**		
Alcohol	Yes vs. No	1.34 (0.93-1.93)	0.11		
Diabetes	Yes vs. No	1.33 (0.87-2.03)	0.19		
Focality	Multifocal vs. Unifocal	0.74 (0.54-1.02)	0.06		
Site	Head vs. Body tail	**1.53 (1.09-2.14)**	**0.01**		
	Diffuse vs. Body tail	0.63 (0.38-1.05)	0.07		
Site	Diffuse vs. localized	**0.49 (0.31-0.77)**	**0.002**	**0.54 (0.33-0.87)**	**0.01**
Size	15-19mm vs. <15mm	**1.93 (1.27-2.95)**	**0.002**	**1.91 (1.25-2.92)**	**0.003**
	≥20mm (<30) vs. <15mm	**3.47 (2.46-4.89)**	**<0.0001**	**3.66 (2.58-5.19)**	**<0.0001**
Wirsung	>3mm (<5) vs. <3mm	**1.84 (1.21-2.80)**	**0.004**	**2.04 (1.33-3.13)**	**0.001**
Symptoms	Yes vs. no	**2.28 (1.52-3.42)**	**<0.0001**	**1.76 (1.16-2.68)**	**0.008**
Pain	Yes vs. no	**2.57 (1.65-3.99)**	**<0.0001**		
Weight loss	Yes vs. no	2.37 (0.87-6.43)	0.09		
Steatorrhea	Yes vs. no	1.96 (0.48-7.93)	0.35		

Figure 1. Cumulative incidence of WF or HRS according to a risk score based on **gender, location, size, wirsung and symptoms.** (unpublished data)



Patients at risk

Low	93	87	59	40	27	13
Medium	333	314	155	81	36	13
High	168	147	71	44	21	7
Very high	198	153	84	53	24	10

Papers published in 2019

1. Decellularized Human Gut as a Natural 3D Platform for Research in Intestinal Fibrosis. Giuffrida P, Curti M, Al-Akkad W, Biel C, Crowley C, Frenguelli L, Telese A, Hall A, **Tamburrino D**, Spoletini G, Fusai G, Tinozzi FP, Pietrabissa A, Corazza GR, De Coppi P, Pinzani M, Di Sabatino A, Rombouts K, Mazza G. Inflamm Bowel Dis. 2019 Oct 18;25(11):1740-1750.
2. Is the Real Prevalence of Pancreatic Neuroendocrine Tumors Underestimated? A Retrospective Study on a Large Series of Pancreatic Specimens. Partelli S, Giannone F, Schiavo Lena M, Muffatti F, Andreasi V, Crippa S, **Tamburrino D**, Zamboni G, Rubini C, Doglioni C, Falconi M. Neuroendocrinology. 2019;109(2):165-170.
3. Management of small asymptomatic nonfunctioning pancreatic neuroendocrine tumors: Limitations to apply guidelines into real life. Partelli S, Mazza M, Andreasi V, Muffatti F, Crippa S, **Tamburrino D**, Falconi M Surgery. 2019 Aug;166(2):157-163.
4. Risk and Predictors of Postoperative Morbidity and Mortality After Pancreaticoduodenectomy for Pancreatic Neuroendocrine Neoplasms: A Comparative Study With Pancreatic Ductal Adenocarcinoma. Partelli S, **Tamburrino D**, Cherif R, Muffatti F, Moggia E, Gaujoux S, Sauvanet A, Falconi M, Fusai G. Pancreas. 2019 Apr;48(4):504-509.

Papers submitted and under review

1. The role of post-operative acute pancreatitis in the development of pancreatic fistula. **Domenico Tamburrino,** Stefano Partelli, Francesca Muffatti, Giulio Belfiori, Stefano Crippa, Renato Castoldi, Gianpaolo Balzano, Nicolò Pecorelli and Massimo Falconi
2. Portal Vein Resection during Pancreaticoduodenectomy for Pancreatic Neuroendocrine Tumours. An International Multicentre Comparative Study. Giuseppe Fusai, **Domenico Tamburrino**, Stefano Partelli, Francesca Di Salvo, T. Peter Kingham, Alain Sauvanet, Detlef Bartsch, Claudio Bassi, E.J.M. Nieveen van Dijkum, Sun Whe Kim, Marco Del Chiaro, Ugo Boggi, Mohamed Abu Hilal, Christofer Wolfgang ,Massimo Falconi
3. Time To Ca19-9 nadir: A clue for defining optimal treatment duration in patients with resectable pancreatic ductal adenocarcinoma. Giulia Orsi, Umberto Peretti, Paolo Giorgio Arcidiacono, Silvia Zanon, Marina Macchini, Elena Mazza, Gianpaolo Balzano, **Domenico Tamburrino,** Luca Gianni, Massimo Falconi and Michele Reni.