Effects of Aspirin, Ace-Inhibitors/Sartans and Statins on Neoplastic and Pre-Neoplastic Lesions of the Pancreas

PhD Candidate: Dott. Roberto Valente, MD, Gastroenterologist

Main Supervisor: Dott. Gabriele Capurso, MD, Gastroenterologist, PhD

PhD Course Director:

Prof. Bruno Annibale, MD Gastroenterologist, Professor of Gastroenterology and Hepatology

Chief of Gastroenterology Unit, Sant'Andrea University Hospital, Sapienza University of Rome, Rome, Italy

Residency Director of Gastroenterology and Hepatology

Background:

Pancreatic cancer is expected to become the third cause of cancer related death within 2030.(1) In the last decades, the spread use of cross-sectional imaging and the progressive quality improvement of abdominal imaging, has led to a more frequent detection of pancreatic cystic neoplasm, whose actual prevalence is estimated to be around 45%. (2, 3)

Intrapapillary mucinous neoplasms (IPMNs) represent circa half of these lesions and are increasingly considered as possible precursor lesions of pancreatic cancer. (4) According to the isolated, combined or absent cystic involvement of the main pancreatic duct are divided into main duct (MD), mixed type(MT) and branch duct (BD) IPMNs. The increasing of pancreatic IPMNs detection, will probably represent in the next future, the only possibility to address pancreas cancer at early or ideally pre-invasive stage. (5)

If from an oncological point of view, MD-IPMNs and MT-IPMNs are the most challenging, harboring a risk of pancreatic cancer respectively in up to 91% (6-8) and 70% (9)of cases, from a decisional point of view, they are not. In fact, whenever the patient is fit for surgery, he/she encounter a surgical indication.(2, 5)

The situation is more complex for BD-IPMNs, since they display 15% of progression at 3-5 years follow-up(2) and 8-10% risk of pancreas cancer during lifetime. (10)

For BD-IPMNs, which represent by far, the majority of IPMN lesions in the general population, a more conservative approach has been suggested, though lifelong follow up with MRI/MRCP and/or EUS until the patient is fit for surgery. (3, 4)

The economic counterpart of such strategy, will unavoidably imply an excess health care costs that has still not been clearly quantified, but that probably will be unrealistically economical sustainable.

In the next future therefore, two possible strategies can overcome the problem: an improvement in detection of the lesions at risk for progression, that will allow optimizing of resources, by continuing/intensifying follow up in a more targeted way and application of a chemoprevention, able to slow down and ideally stop the progression on IPMNs.

While the first aspect has been addressed by several authors and guidelines and is still largely matter of debate, the second one has poorly been investigated.

So far, no effective chemoprevention is available, to slow or prevent the progression of BD-IPMNs.

In addition, IPMNs harboring high-grade dysplasia/cancer represent a target for surgery but long term prognosis is still impacted by rates of post-surgical locoregional and distant recurrences.(2)

Although several authors have identified possible risk factors for recurrence after surgery such as family history for pancreas cancer and the grade of dysplasia in the resected specimen, (11, 12) even in this case, no specific study investigated the role of a possible chemoprevention able to prevent or slow down recurrence of pancreas cancer in operated patients.

Beside the progressive increasing in incidence of pancreatic exocrine tumors, in the last decades even incidence of endocrine tumors has been continuously rising. Pancreatic neuroendocrine tumors (PNENs) are generally considered rare neoplasm, although represent 10% of pancreatic masses as prevalence.(13, 14) Few studies, with heterogenous results, have specifically investigated potential risk and protective factors for their occurrence, including the effect of possible pharmacological exposures.(14)

Aspirin (ASA), Ace Inibitors/Sartans (ACEI/ARB) and Statins (STAT) are among the most used drugs for primary and secondary cardiovascular prevention have shown to inhibit cell growth in several cancers, including some clinical and preclinical models of pancreatic cancer. (15-20)

Aspirin, is a selective and irreversibly acetylator of prostaglandin (PG)G/H-synthases (COX-1 and COX-2), that blocks the catalytic reaction that converts arachidonic acid into PGG2 and PGH2 and therefore thromboxane (TX)A2 resulting in anti-inflammatory, analgesic, antipyretic and antiplatelet effects. (21) COX-2 expression and platelets activation seems to contribute to development, progression and metastatic potential of cancer, through locoregional release of a variety of cell growth, pro angiogenic and pro inflammatory mediators, while PGE2 seems to be implied into immunological escapes from Interferon and/or T-cell-dependent cancer killing.(22) Beside the well-known cardiovascular protective effect (23), aspirin has been recently proved to play a major role in inhibit cancerogenesis, acting on several pathways (mTOR, EGFR, 15-PGDH) (24) Observational studies support the idea that aspirin use is associated with a significant decrease in the risk of overall and site-specific cancers.(25)

Angiotensin I-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) are widely diffused antihypertensive drugs for their property to inhibit the activation of systemic renin–angiotensin system (RAS).(26) Angiotensin II, which represents a cornerstone in such a cascade, has shown to harbor proangiogenic properties by acting on the vascular endothelial growth factor (VEGF), MAP Kinases and G-protein cascades with potential major implications on cancer management, as suggested for lung, breast and pancreas cancer.(26-28)

Statins, are inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and used as cholesterol lowering agents in the setting of both primary and secondary cardiovascular prophylaxis. Statins seem to act a regulation on several intracellular pathways such as Ras, MEK, mTOR, BCL-2 and Rho kinases, that also play a major role in cancer development. Recently their possible role in decreasing the incidence of cancer has been suggested in epidemiological studies and meta-analysis. (29-31)

As a matter of facts, most meta-analysis on the possible role of aspirin and statins on pancreas cancer were based on cohort and case-control studies suffering from high heterogeneity.(32)

In addition, aspirin and statins are often co-prescribed, mostly for cardiovascular disease prevention or treatment. It is therefore difficult to exclude possible overlap phenomena. As studies on the possible chemo-preventive activity of both aspirin and statins on PDAC gathered heterogeneous results, the analysis of their potential combined or confounding effect in this cancer type seems particularly important. So far, no study examined the association of both these two drugs at the same time with PDAC risk.

In summary, during the last decades the incidence of pancreatic endocrine and exocrine tumors has been progressively increasing. The underlining reason is unknown, as it is unclear whether it might be the result of the exposure to common risk factors. Aspirin, ace inhibitors/sartans and statins, that have been suggested to act on molecular pathways involved in cancer in several types of tumor.

Currently, no data is available on the possible effect that such drugs might have, on the progression of BD-IPMN, increasingly considered precursor lesions of pancreas cancer. So far it is also unknown whether aspirin ace inhibitors/sartans and statins might improve prognosis or prevent recurrences and cancer related death in operated IPMNs patients.

Therefore, the current work has been divided into 4 papers with the aim of shed light on such aspects:

Paper I: Effect of Aspirin, ACE Inhibitors/Sartans and Statins use on the progression of BD-IPMN in follow up: a multicenter study (**main paper of PhD**)

Paper II: Clinical and pharmacological factors associated with cancer related death in operated IPMN patients

Paper III: Risk and protective factors for the occurrence of sporadic pancreatic endocrine neoplasm

Paper IV: Exclusive and Combined Use of Statins and Aspirin and the Risk of Pancreatic Cancer: a Case-Control Study

Aims:

Paper I: to evaluate the possible effect of ASA, ACEI/ARB and STAT used for cardiovascular prevention on the progression of BD-IPMN in follow-up. (**main paper**)

Paper II: to evaluate the effect of aspirin, ace inhibitors/sartans and statins alone or in association, on cancer related death in a cohort of operated IPMNs patients

Paper III: to investigate the association between potential risk and protective factors, including pharmacological exposures, and the occurrence of sporadic PNENs across a European population from several institutions

Paper IV: the primary aim of the study was to examine the association between overall and exclusive aspirin and statin use and their combined use with the risk of pancreas cancer. The secondary aim was to explore whether the association was stronger in specific subgroups.

Materials and Methods:

Paper I (main paper)

Study design:

Multicenter, retrospective cohort study. Patients were collected at Sant' Andrea University Hospital, Sapienza University of Rome, Rome Italy, Karolinska University Hospital in Stockholm Sweden and San Raffaele Hospital, Vita Salute University, Milan Italy between 2002 and 2017.

Data collection: Prospectively collected data about medical history, family history of cancer and exposures were extracted by a specific created retrospective database at Sant 'Andrea Hospital and San Raffaele Hospital. Eventual missing data were filled as possible by re-contacting patients. A specific IT database (®*Take Care)* with in real time updated inpatient and outpatient clinic charts was consulted for data collection in Sweden.

Patients and outcomes:

Inclusion Criteria: BD-IPMNs patients without "*ab initio"* surgical indications and undergoing radiological follow-up according to current guidelines at the participating centers. Clinical- and radiological characteristics as well as exposure to the target drugs were collected and analyzed in relation to the progression of BD-IPMN.

Exclusion criteria at diagnosis:

- presence of mural nodules
- main pancreatic duct (MPD) diameter ≥5 mm
- cyst diameter ≥40 mm

*Exposures:*The exposure to several potential risk factors for the development of pancreas cancer were considered:

Smoking habit: participants were considered as being ever smokers when reporting to have smoked for a period above 6 months or a quantity superior to 100 cigarettes.

Alcohol drinking: the cut-off to be considered a regular ever drinker was a daily intake of at least 12.5 g of alcohol for at least one year. Such quantity is approximately equal to either one glass of wine, one shot of hard liquor or one pint of beer. Participants were then classified as ever drinkers or nondrinkers.

General risk factors for pancreas cancer: A 1st degree family history of pancreatic cancer, the presence of symptoms, the cyst's mean diameter and a diagnosis of diabetes were specifically recorded. Recent onset diabetes was defined as that which occurrence was in the 12 months prior to diagnosis of BD-IPMN.

Target drugs exposure: The previous use of drugs such as aspirin (ASA), ace inhibitors AND/OR Sartans (ACEI/ARB), exclusive ace inhibitors (ACEI), exclusive Sartans (ARB), statins (STAT), insulin and metformin has been recorded and analyzed. For STAT, ACEI and ARB a further distinct analysis was performed sub-dividing different drugs within the same class. For ASA a distinction between low and high dose was considered. Considering that many of these drugs are often prescribed together in patients displaying high cardiovascular risk profile, beside the evaluation of single class effect, we also considered the potential summative effect resulting from the simultaneous treatment with more than one drug. We therefore analyzed several possible combinations of the studied drugs.

Definition of outcomes: progression during follow up defined as:

-dimensional progression: increasing of cyst maximum diameter of at least 2 mm between two follow-up imaging

-clinical significant progression: appearance of mural nodules and/or increasing in MPD diameter ≥ 5 mm and/or the occurrence of cancer and/or increasing of cyst maximum diameter above 40 mm.

-any-progression: indiscriminate appearance of either dimensional and/or clinical significant progression and increased number of cystic lesions.

***Statistical analysis*:**

Patients exposed and non-exposed to the target drugs or displaying possible risk factors for pancreas cancer were compared by chi-square test and fisher test with Yates correction for categorical variables; Student's t test or long Rank test for continuous ones. Significant variables were further analyzed by sex and age adjusted univariable and multivariable logistic regression analysis. The effect of the target drugs over the time was evaluated through univariable and multivariable Cox hazard regression analysis (by enter selection procedure). The 95% confidence interval (95% CI) was calculated where possible and all p-values were two-sided and considered statistically significant when $p<0.05$. A dedicated statistical software (MedCald Mariakerke, Belgium) was used.

Results:

A total of 594 patients with BD-IPMN without mural nodules, suspect of main duct involvement and cyst dimension above 40 mm were recruited at the three participating centers (62.28% at Karolinska University Hospital, 23.73% at Sant 'Andrea Hospital and 13.9% at San Raffaele Hospital). The mean age 64.84 years (63.95-65.73; 95% CI), 38.38% were male. During a mean follow-up of 44.78 months (42.01-47.54; 95% CI), 46.86% of patients displayed progression (any), 41.32% displayed dimensional progression (mean size increasing: 7.77 mm), 7.74% displayed a clinically significant progression. Thirty-two-point forty-six percent of patients had a previous use of ASA, 31.9% of STAT and 43.39% of ACEI/ARB. Among this last group, 30.86% of patients were exclusive users of ACEI, while 45.67% were

exclusive users of ARB. Some of the patients were exposed to multiple drugs belonging to the same class and some to association of the different classes of drugs. Patients characteristics and a more detailed report of drug use are summarized in **Table 1**.

Past medical history:

Multifocal of BD IPMN (73.3% vs 56.8% p=0.03), a history of diabetes (31.8% vs 16.5%; p=0.01) and especially newly onset diabetes (18.2% vs 4.8% p=0.06) were all factors significantly associated to higher rates of clinically relevant progression **Table 2**.

Drug exposures:

Insulin use was significantly associated to higher rates of both dimensional and clinically significant progression (respectively: 12.3% vs 6.8%, p=0.02 and 22.7% vs 7.9%, p=0.0009). At sex and age adjusted univariable Cox proportional hazard regression analysis insulin was confirmed to be associated to an increased risk of clinically significant progression: HR= 3.07 (95%CI: 1.49 to 6.30; p=0.002).

ACEI/ARB use was significantly associated to higher rates of both dimensional and clinically significant progression (62.8% vs 41.7%; p=0.007) **Figure 1a**.

Exclusive use of ACEI and ARB were also associated with significantly higher rates of any progression (respectively 16.6% vs 23.6%; p=0.04 and 32.4% vs 22.6%; p=0.01). Additionally, ARB use was associated with higher rates of dimensional and clinically significant progression (respectively 33.6% vs 22.4%; p=0.0039 and 44.7% vs 25.7%; p=0.01). Among the same class of drugs Losartan use displayed an association to higher rates of (any) progression and dimensional progression (respectively: 44.3% vs 18.8%; p=0.0001 and 44.9% vs 19.3%; p=0.0001) **Table 2**.

At sex and age adjusted univariable Cox proportional hazard regression analysis the exclusive use of ACEI was confirmed to be associated to a decreased risk of dimensional progression: HR= 0.65 (0.45-

0.94; 95%CI; p=0.02) and any progression: HR= 0.64 (0.45-0.90; 95%CI; p= 0.01). The exclusive use of ARB was instead confirmed to be associated to an increased risk of clinically significant progression: HR= 1.94 (95%CI: 1.00-3.76; p=0.04) **Figure 1b**.

The use of statins and, particularly simvastatin, were significantly associated to higher rates of clinically relevant progressions (respectively: 46.5% vs 30.6%, p=0.03 and 34.1% vs 19.4%, p=0.02). At sex and age adjusted univariable Cox proportional hazard regression analysis the use of statins was significantly associated to decreased risk of dimensional progression (HR= 0.72; 0.54-0.97 95%CI; p=0.03) **Table 2**.

We performed several models of sex and age adjusted multivariable Cox proportional hazard regression analysis. In a first model the use of ACEI (HR=0.70; 95% CI 048.1.01; p=0.06) and STAT (HR= 0.67; 95%CI 0.49-0.93; p=0.01) was associated to a decreased risk to display dimensional progression, while the use of Insulin was associated to a statistically significant higher risk of dimensional progression (HR 1.65; 95% CI 1.09-2.51; p=0.01.

In a second model, the use of ACEI (HR=0,6937; 95%CI 0,4879 to 0,9862, p=0.04) and STAT (HR= 0,7209=0,5316 to 0,9776; 95% CI, p=0.03) was associated to a decreased risk of (any) progression, while the use of Insulin was associated to an increased risk of (any) progression Insulin (HR= 1.49; 95% CI 0.99-2-24; p=0.05).

In a third model the use of Insulin (HR=2.97; 95% CI 1.34-6.55; p=0.006) but not ARB (HR 1.41; 95% CI 0.69-2.87; p=0.34) was statistically associated to clinically relevant progression **Figure 1c**.

Table 1 Characteristics of BD-IPMN patients in follow up. *__ASA__: Aspirin users (ever);** **ACE**: Ace Inhibitors only users (ever);§ **ARB**: Sartans only users (ever);§§ **STAT**: Statins users (ever);# **ACEI/ARB**: Ace Inhibitors AND/OR Sartans users (ever);## **plus** : concomitant use

Characteristic	**N (%)- (95% CI)**
Patients	594
Number of male	228/594 (38.38)
Mean Age (years)	64.84 (63.95–65.73; 95% CI)
Mean follow-up (months)	44.78 (42.01–47.54; 95% CI)
Smoking	156/579 (26.94)
1st degree family history of PDAC	27/582 (4.63)
Diabetes	102/579 (17.61)
Alcohol (ever)	136/579 (23.48)
Recent onset diabetes	10/102 (9.80)
Mean Cyst Diameter (mm)	15.1 (14.42-15.85; 95% CI)
Multifocal Disease	344/592 (58.10)
Symptomatic	104/592 (17.56)
Progression (any)	277/591 (46.86)
Dimensional Progression	243/588 (41.32)
Mean dimensional increase (mm)	7.77 (6.82–8.72; 95% CI)
Clinically significant progression	46/594 (7.74)
ASA	186/573 (32.46)
Low dose (<160mg/die)	170/186 (91.39)
ACEI/ARB:	243/560 (43.39)
ACEI:	75/243 (30.86)
-Enalapril	70/110 (63.63)
-Perindopril	4/110 (3.63)
-Captopril	1/110 (0.90)
-Lisinopril	3/110 (2.72)
-Ramipril	30/110 (27.27)
- Others	2/110 (1.81)
ARB:	111/243 (45.67)
-Telmisartan	6/156 (3–84)
-Olmesartan	6/156 (3.84)
-Combisartan	2/156 (1.28)
-Valsartan	6/156 (3.84)
-Candesartan	56/156 (35.89)
-Irbesartan	16/156 (10.25)
-Losartan	64/156 (41.02)
UNSPECIFIED:	22/243 (9.05)
Multiple ARB	10/243 (4.11)
ACE plus ARB	35/243 (14.40)
ASA plus ACEI/ARB plus STAT	82/558 (14.69)
ASA plus ACEI/ARB	122/559 (21.82)
ASA plus STAT	114/571 (19.96)
ASA plus ARB plus STAT	47/536 (8.76)
ASA plus ARB	71/537 (13.22)
ARB plus STAT	81/537 (15.08)
STAT	183/594 (30.80)
Simvastatin	112/208 (53.84)
Atorvastatin	56/208 (26.92)
Pravastatin	5/208(2.40)
Rosuvastatin	17/208 (8.17)
Multiple	25/208 (12.01)
Unknown	18/208 (8.65)

Features	Progression (any): Yes/No	Progression (dimension): Yes/No	Significant progression:Yes/No
Acute pancreatitis	13 (4.7) vs 14 (4.5) p=0.88	8 (3.3) vs 19 (5.5) p=0.21	2 (4.4) vs 25 (4.6) p=1.0
1st degree FH PDAC	16 (5.9) vs 11 (3.6) p=0.20	15 (6.2) vs 12 (3.6) p=0.13	1 (2.3) vs 26 (4.8) p=0.71
Multifocal	166 (60.4) vs 176 (56.2) p=0.31	151 (62.4) vs 188 (54.8) p=0.06	**33 (73.3) vs 310 (56.8) p=0.03**
Smoking	67 (24.8) vs 88 (28.9) p=0.27	58 (24.2) vs 97 (29.0) p=0.22	13 (30.2) vs 142 (26.5) p=0.59
Alcohol	59 (21.9) vs 76 (24.9) p=0.38	51 (21.4) vs 84 (25.1) p=0.31	11 (25.6) vs 124 (23.2) p=0.72
Diabetes	49 (17.9) vs 52 (17.3) p=0.84	44 (18.2) vs 57 (17.2) p=0.76	**14 (31.8) vs 88 (16.5) p=0.01**
New Onset Diabetes	6 (7.9) vs 4 (4.0) p=0.26	5 (7.7) vs 5 (4.5) p=0.50	**2 (18.2) vs 8 (4.8) p=0.06**
Insulin	30 (11.2) vs 21 (7.1) p=0.09	**29 (12.3) vs 22 (6.8) p=0.02**	**10 (22.7) vs 41 (7.9) p=0.0009**
Metformin	25 (9.3) vs 37 (12.5) p=0.21	22 (9.3) vs 41 (12.6) p=0.21	3 (6.8) vs 60 (11.5) p=0.34
ASA	86 (32.2) vs 98 (32.5) p=0.95	75 (32.1) vs 108 (32.5) p=0.90	17 (39.5) vs 168 (31.8) p=0.29
Low dose ASA	78 (92.9) vs 90 (96.8) p=0.23	68 (91.9) vs 100 (97.1) p=0.12	16 (100.0) vs 153 (94.4) p=0.33
ACEI /ARB	116 (44.4) vs 124 (42.0) p=0.56	103 (44.8) vs 136 (42.0) p=0.51	**27 (62.8) vs 215 (41.7) p=0.007**
ACEI	**41 (16.6) vs 68 (23.6) p=0.04**	37 (16.8) vs 72 (23.0) p=0.08	7 (18.4) vs 103 (20.6) p=0.74
Enalapril	29 (29.0) vs 41 (36.0) p=0.27	26 (28.6) vs 44 (36.1) p=0.25	4 (19.0) vs 66 (34.0) p=0.16
Perindopril	0 (0) vs 4 (3.4) p=0.12	0 (0) vs 4 (3.2) p=0.13	0 (0) vs 4 (2.0) p=1.0
Captopril	1 (1.0) vs 0 (0) p=0.46	1 (1.1) vs 0 (0) p=0.42	0 (0) vs 1 (0.5) p=1.0
Lisinopril	1(1.0) vs 3 (2.7) p=0.62	1 (1.1) vs 3 (2.5) p=0.63	0 (0) vs 4 (2.1) p=1.0
Ramipril	10 (10.0) vs 19 (16.5) p=0.22	9 (9.9) vs 20 (16.3) p=0.17	2 (9.5) vs 28 (14.4) p=0.74
ARB	**80 (32.4) vs 65 (22.6) p=0.01**	**74 (33.6) vs 70 (22.4) p=0.0039**	**17 (44.7) vs 128 (25.7) p=0.01**
Telmisartan	4 (3.9) vs 2 (1.7) p=0.42	3 (3.2) vs 3 (2.4) p=0.70	0 (0) vs 6 (3.0) p=1.0
Olmesartan	1 (1.0) vs 5 (4.3) p=0.21	1 (1.1) vs 5 (4.0) p=0.24	0 (0) vs 6 (3.0) p=1.0
Combisartan	2 (2.0) vs 0 (0) p=0.21	2 (2.2) vs 0 (0) p=0.17	0 (0) vs 2 (1.0) p=1.0
Valsartan	5 (5.0) vs 1 (0.9) p= 0.10	**5 (5.4) vs 1 (0.8) p=0.08**	1 (4.8) vs 5 (2.6) p=0.46
Candesartan	24 (24.0) vs 31 (27.7) p=0.54	23 (25.3) vs 32 (26.7) p=0.82	4 (19.0) vs 51 (26.6) p=0.60
Irbesartan	10 (10.1) vs 6 (5.3) p=0.18	9 (10.0) vs 7 (5.8) p=0.25	5 (23.8) vs 11 (5.7) p=0.01
Losartan	**43 (44.3)vs 21 (18.8);p=0.0001**	**40 (44.9) vs 23 (19.3) p=0.0001**	9 (42.9) vs 55 (29.1) p=0.19
STAT	81 (30.3) vs 101 (33.4) p=0.42	67 (28.6) vs 114 (34.2) p=0.15	**20 (46.5) vs 162 (30.6) p=0.03**
Simvastatine	54 (21.3) vs 57 (19.9) p=0.68	44 (19.7) vs 66 (21.0) p=0.71	**14 (34.1) vs 97 (19.4) p=0.02**

Atorvastatine	19 (7.5) vs 36 (12.6) p=0.05	17 (7.6) vs 37 (11.8) p=0.11	5 (12.2) vs 50 (10.0) p=0.59
Pravastatine	4 (1.6) vs 1 (0.3) p=0.13	3 (1.3) vs 2 (0.6) p=0.65	1 (2.4) vs 4 (0.8) p=0.32
Rosuvastatine	6 (2.3) vs 11 (3.8) p=0.33	6 (2.6) vs 11 (3.4) p=0.60	1 (2.4) vs 16 (3.1) p=1.0
ASA plus ACEI/ARB	57 (21.8) vs 63 (21.4) p=0.90	52 (22.6) vs 68 (21.1) p=0.66	14 (32.6) vs 107 (20.8) p=0.07
ASA plus STAT	46 (17.2) vs 67 (22.3) p=0.12	41 (17.5) vs 72 (21.8) p=0.21	13 (30.2) vs 100 (19.0) p=0.07
ACEI/ARB plus STAT	61 (23.4) vs 66 (22.4) p=0.79	53 (23.0) vs 73 (22.6) p=0.90	**17 (39.5) vs 110 (21.4) p=0.006**
ASA plus ACEI/ARB plus STAT	35 (13.4) vs 46 (15.7) p=0.44	32 (13.9) vs 49 (15.2) p=0.66	**11 (25.6) vs 70 (13.6) p=0.03**
ASA plus ARB plus STAT	24 (9.7) vs 22 (7.7) p=0.40	22 (10) vs 24 (7.7) p=0.35	**7 (18.4) vs 39 (7.8) p=0.02**
ASA plus ARB	**39 (15.8) vs 31 (10.8) p=0.08**	**36 (16.4) vs 34 (10.9) p=0.06**	**9 (23.7) vs 61 (12.2) p=0.04**
ARB plus STAT	**45 (18.2) vs 35 (12.2) p=0.05**	**40 (18.2) vs 39 (12.5) p=0.06**	**12 (31.6) vs 68 (13.7) p=0.002**

Table 2: Association between patient's features/exposure and the progression of the IPMN at Chi-square/ Fisher test

Figure 1 a) Sex and age adjusted univariable Cox proportional hazard regression analysis for drug exposure

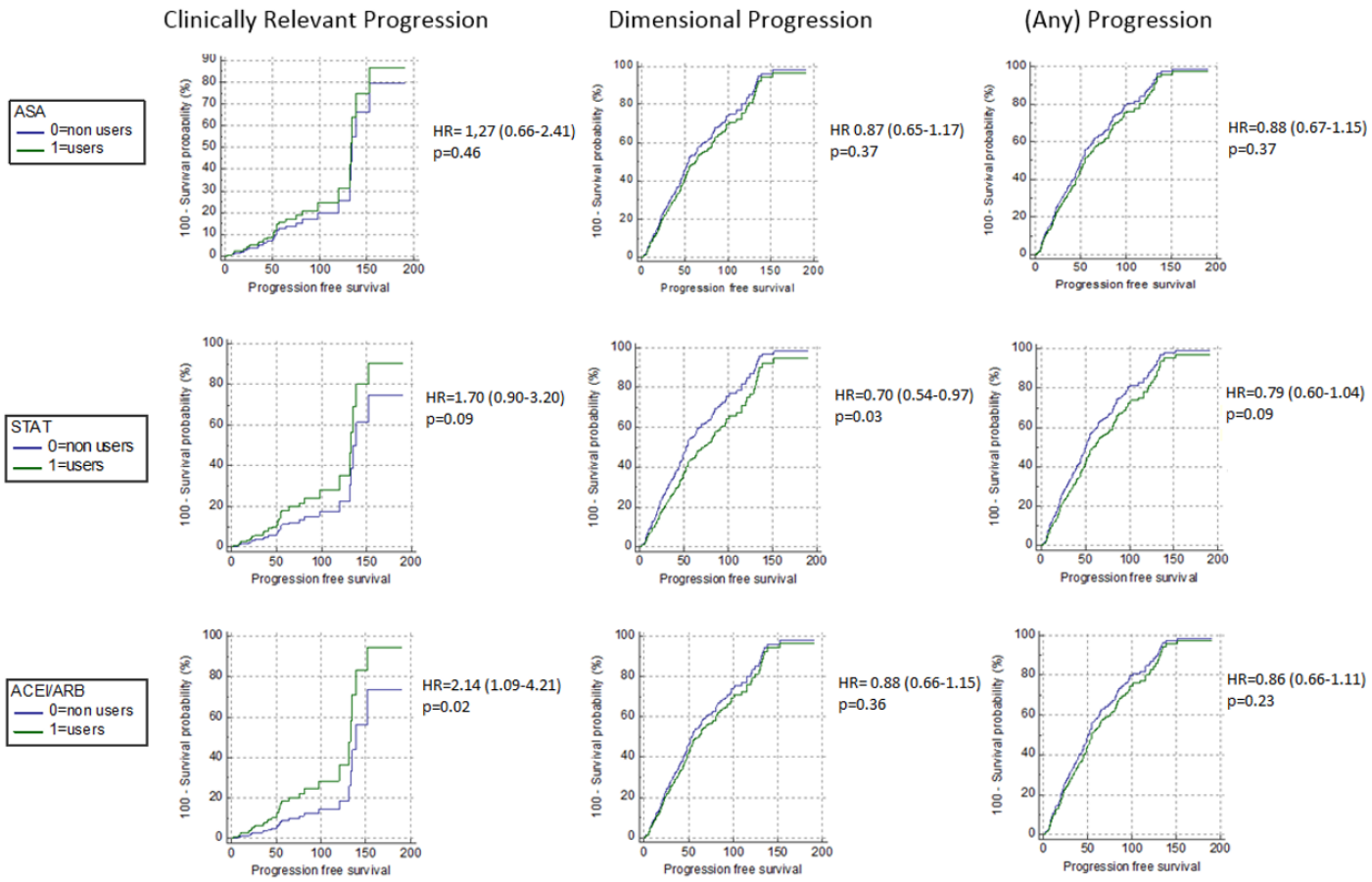


Figure 1 b) Sex and age adjusted univariable Cox proportional hazard regression analysis for the exclusive use of ACEI and ARB

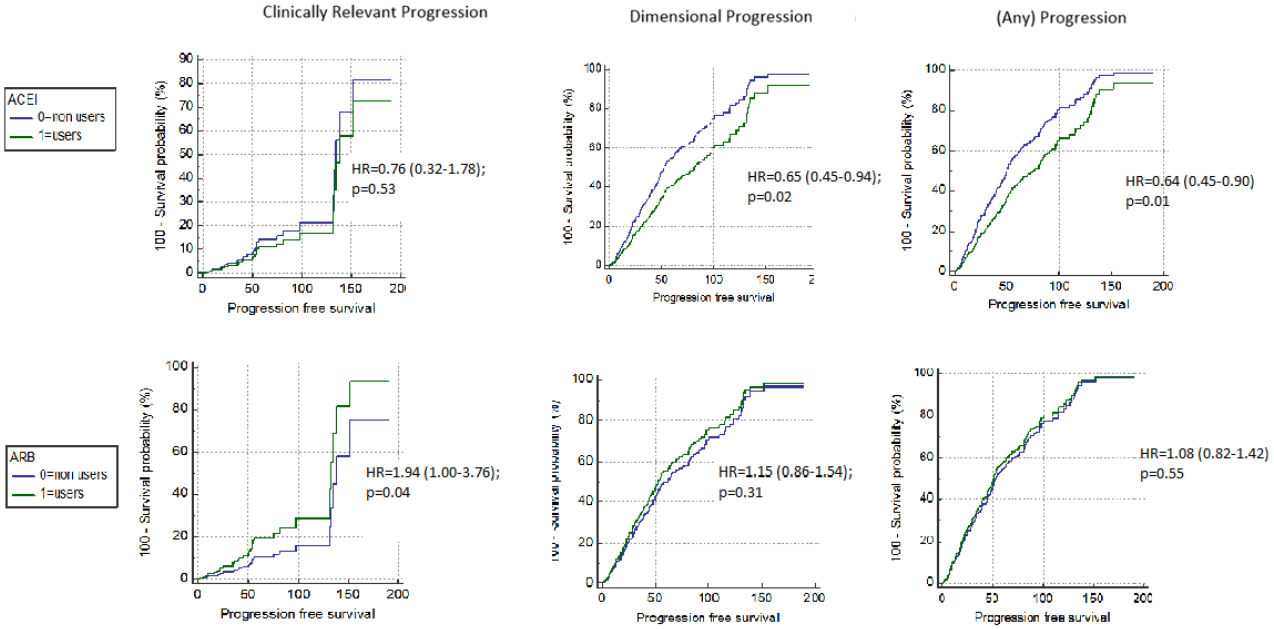
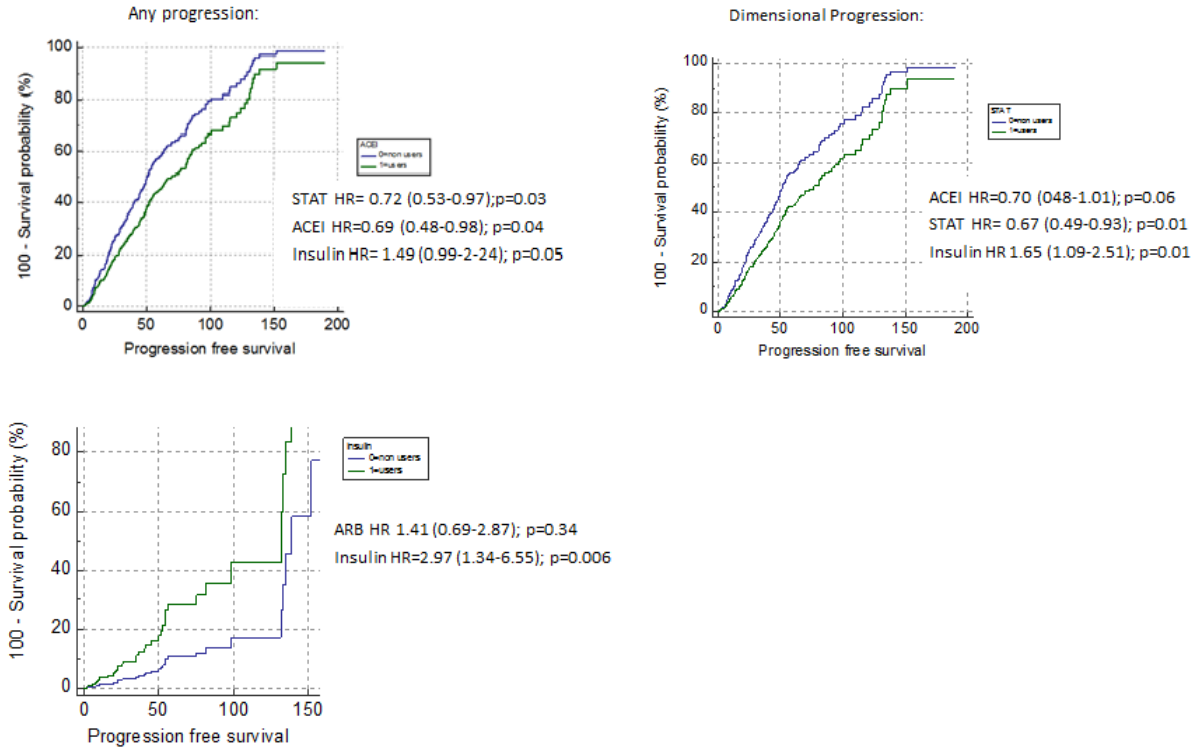


Figure 2 a) Sex and age adjusted multivariable Cox proportional hazard regression analysis for pharmacological exposure



Paper II

Study design

A single-center hospital-based retrospective cohort study on prospectively collected patients surgically resected for pancreatic IPMNs. Patients were enrolled between 2007 and 2017 at Pancreatic Disease Unit, Karolinska University Hospital, Stockholm, Sweden. Ethical committee approval was obtained (EPN 2015/1544-31/4). Demographics, hereditary factors, clinical and pharmacological history were collected and analyzed.

Cohort and Population's Characteristics

Consecutive cases of histologically verified IPMNs who had undergone surgery for radiological suspect of high grade IPMNs according to the European Guidelines for the management of cystic tumors of the pancreas (from October 2011). The date of surgery was considered as date of diagnosis. All patients were discussed in a multidisciplinary conference before surgery.

Definition of the exposure: Patients were investigated for ever use of aspirin, ace inhibitors, sartans and statins used alone or in combination as well as for exposure to known risk factors for pancreas cancer. Data regarding demographics, known risk factors for pancreatic cancer (hereditary, smoking, alcohol, overweight/obesity) as well as final pathological assessment (degree of dysplasia, presence of cancer, TNM, specific histological phenotype), and exposure to the target drugs was retrospectively collected. A specific Swedish electronic database (®*Take Care)* with in time updated inpatient and outpatient clinic charts was consulted for data collection.

Patients and outcomes:

Outcome definition: Overall survival was defined as the interval of time between surgery and the date of analysis if patient was alive or between diagnosis and cancer related death in case he/she died during follow-up due to metastatic disease or locoregional recurrence.

All patients in which death was not related to cancer, were excluded from survival analysis as a censored data, to specifically analyze the possible effect of investigated factors/drugs on cancer related survival.

Inclusions criteria:

Consecutive patients who have undergone surgery at *Karolinska University Hospital* because suspect malignified IPMN according to the European Guidelines for the Management of Pancreatic Cystic Neoplasms.

Exclusion criteria:

- The presence of a synchronous pancreatic cancer, cholangiocarcinoma or high grade neuroendocrine tumor that might influence the prognosis.
- The presence of surgical margins at final histology showing cancer or high-grade dysplasia (**Figure 1**)

***Statistical analysis*:**

Categorical variables were analyzed through chi-square, continuous variable through Students t-test. Statistically significant variables were further analyzed in sex and age adjusted univariate and multivariate logistic regression analysis. Sex and age adjusted univariable and multivariable logistic regression analysis was used to evaluate the possible association between cancer related death and known risk factors for pancreas cancer and sex and age adjusted univariable and multivariable cox hazard regression analysis was used to identify associations between pharmacological exposures and cancer related death. The 95% confidence interval (CI) was calculated. All p-values were two-sided and a $p<0.05$ was considered statistically significant. A statistical software package was used for data analysis (MedCalc Mariakerke, Belgium).

***Results*:**

Between 2008 and 2017, 274 IPMN patients were operated at Pancreatic Disease Unit, Karolinska University Hospital for suspect malignified IPMN. Two hundred ten, mean age 70.55 years (67.60-73.50; 95% CI), 47.61% male, were included in the final analysis. Patients' characteristics are summarized in **Table1**

Clinical known risk factors for pancreas cancer:

Fifty patients (23.80%) were smokers and among them 66% displayed active smoking. First-degree family history of pancreatic cancer was present in 3.8% of patients. Diabetes was present in 19.52% of patients and in 2.43% diabetes was of early onset (diagnosed within the latest 12 months before surgery). Multifocal disease was present in 43.8% of patients, preoperatively increased levels of Ca19.9 in 32.66%, while 34.05% of patients had a mean diameter of mean cyst above 40 mm. Mean MPD diameter was 7.64 (6.88-8.39; 95% CI). Significantly higher percentage of patients with preoperative jaundice, increased Ca19.9 and cyst diameter above 40 mm died for cancer (respectively: 36.4 vs 9.8%, p=0.0005; 76.2% vs 27.6%, p<0.0001; 62.5% vs 30.0%, p=0.05). **Table 2**

At sex and age adjusted univariable logistic regression analysis preoperative jaundice and preoperative increased level of Ca19.9 were associated with increased risk of cancer related death (respectively OR =4.99, 95% CI 1.80-13.87, p=0.02 and OR= 8.75, 95% CI 2.96-25.87, p=0.001. **Table 3a**

At sex and age adjusted multivariable logistic regression analysis, preoperative increased level of Ca19.9 remained consistent with increased risk of cancer related death OR=7.31, 95% CI 2.40-22.21, p=0.0004. **Table 3b**

A previous or current use (ever use) of aspirin, ace inhibitors/sartans and statins was present respectively in 29.18%, 51.90% and 36.84% of cases. ASA ever users displayed significantly higher rates of cancer related death 50.0% vs 27.4% p= 0.03. Sex and age adjusted univariable cox hazard regression analysis ASA users displayed a borderline significantly higher risk of cancer related death HR 2.11 (0.90-

4.94, 95% CI, p=0.08). The risk of cancer related death was not statistically significant different among exclusive STAT and exclusive ACEI/ARB users (respectively HR= 0.77, 95% CI 0.32-1.89, p=0.58 and HR= 1.18, 95% CI 0.49-2.82, p=0.69) or their combinations. (**Table and Figure 2a)**

At multivariable cox hazard regression analysis adjusted for sex, age, clinical factors associated with both pancreas cancer and cardiovascular risk (such as smoking, diabetes, first degree family history, overweight/obesity and elevated Ca19.9) ASA users were confirmed to be associated with increased risk of cancer related death (HR=2.70, 95% CI 1.10-6.59, p=0.02). **Figure 2b**

Figure 1: Patients' inclusion flow chart

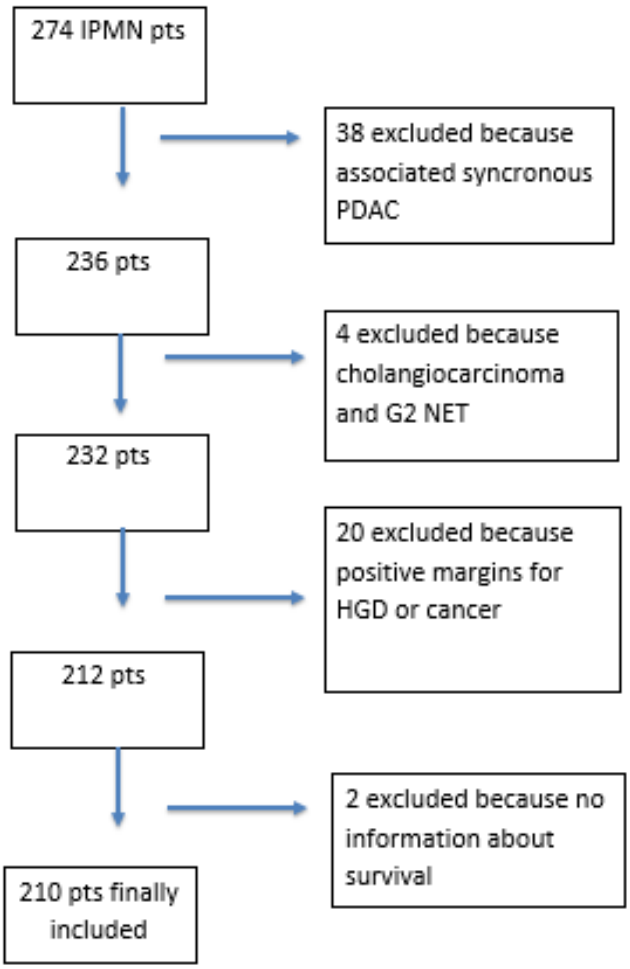


Table 1 Patients demographic, disease morphology and operative characteristics

Characteristic	**N/mean (%; 95%CI)**
Patients	210
Male	100/210 (47.61)
Age	70.55 (67.60-73.50; 95% CI)
Mean OS (months)	37.60 (33.76-41.43; 95%CI)
Dead Cancer related death	51/210 (24.24) 22/210 (10.47)
Mean BMI	25.73 (25.12-26.38; 95% CI)
Obesity	18/210 (8.57)
Overweight	41/210 (19.52)
Smokers	50/210 (23.80)
Current smokers	33/50 (66.0)
1st degree FH PDAC	8/210(3.80)
Diabetes	41/210 (19.52)
Recent Onset Diabetes	1/41 (2.43)
Jaundice	26/210 (12.38)
Abdominal pain	42/210 (20.0)
Weight loss	32/210 (15.23)
Acute pancreatitis	23/210 (10.95)
Incidental diagnosis	100/209 (47.84)
Multifocal	92/210 (43.80)
Mean MPD diameter (mm)	7.64 (6.88-8.39; 95% CI)
MPD diameter 5-9.9 mm	89/210 (42.38)

MPD diameter ≥10 mm	50/210 (23.80)
Max Cystic Size (mm)	33.93 (30.23-37.62; 95%CI)
Cyst Size ≥4 cm in BD-IPMNs	47/138 (34.05)
Mural nodules	16/209 (7.65)
Increased Ca19.9	65/199 (32.66)
Pancreaticoduodenectomy	97/210 (46.19)
Distal Pancreatectomy	66/210 (31.42)
Total Pancreatectomy	39/210 (18.57)
Atypical resections	8/210 (3.80)
Margin status: No dysplasia Low grade of dysplasia NA (total pancreatectomy)	 64/171 (37.42) 107/171 (62.57) 39/210 (18.57)
ASA ACEI/ARB STAT ASA plus ACEI/ARB ASA plus STAT ACEI/ARB plus STAT ASA plus ACEI/ARB plus STAT	61/209 (29.18) 109/210 (51.90) 77/209 (36.84) 46/209 (22.00) 42/208 (20.19) 58/209 (27.75) 32/208 (15.38)
Final histology: Cancer IPMN with high grade dysplasia IPMN with low grade dysplasia	 60/210 (28.57) 44/210 (20.95) 106/210 (50.47)

Table 2 IPMNs features and patient's exposure possibly associated to cancer relater death at fisher or chi square test

Features/exposures	**Cancer related death vs alive patient**	**p value**
Smoking	5 (22.7) vs 37 (22.6)	0.98
1st degree FH PDAC	0 (0) vs 8 (4.9)	0.29
Diabetes	4 (18.2) vs 32 (19.5)	0.88
Preoperative Jaundice	**8 (36.4) vs 16 (9.8)**	**0.0005**
Abdominal pain	4 (18.2) vs 35 (21.3)	0.73
Weight loss	5 (22.7) vs 23 (14.0)	0.28
Incidental diagnosis	7 (31.8) vs 79 (48.5)	0.14
Head location	10 (45.5) vs 48 (29.3)	0.12
Preoperative Increased Ca19.9 (≥37 UI/L)	**16 (76.2) vs 43 (27.6)**	**<0.0001**
Multifocal IPMNs	6 (27.3) vs 76 (46.3)	0.09
Positive Margin for dysplasia	12 (85.70) vs 87 (63.00)	0.09
Preoperative presence of mural nodules	1 (4.5) vs 14 (8.6)	0.51
maximum diameter 5-9.9 mm	10 (45.5) vs 71 (43.3)	0.84
maximum diameter ≥10 mm	6 (27.3) vs 37 (22.6)	0.62
Preoperative Cyst Size ≥4 cm	**5 (62.5) vs 33 (30.0)**	**0.05**
ASA use (ever)	**11 (50.0) vs 45 (27.4)**	**0.03**
STAT use (ever)	8 (36.4) vs 62 (38.0)	0.87
ACEI use (ever)	7 (31.8) vs 33 (20.1)	0.21
ARB use (ever)	4 (18.2) vs 41 (25.0)	0.48
ACE/ARB use (ever)	13 (59.1) vs 82 (50.0)	0.42
ASA plus STAT use (ever)	6 (27.3) vs 34 (20.9)	0.49
ASA plus ACEI/ARB use (ever)	6 (27.3) vs 36 (22.0)	0.57
ASA plus ACEI/ARB plus STAT use (ever)	5 (22.7) vs 26 (16.0)	0.42
ACEI/ARB plus STAT use (ever)	6 (27.3) vs 47 (28.8)	0.87

Table 3 Sex and age adjusted univariate and multivariable logistic regression analysis for the assessment of possible associations between clinical features and the occurrence of cancer related death.

Clinical features	**Univariable OR (95%CI)**	**Multivariable OR (95%CI)**
Jaundice	**4.99 (1.80-13.87); p=0.02**	2.57 (0.82-8.04); p=0.10
Increased Ca19.9 (≥37 UI/L)	**8.75 (2.96-25.87); p=0.001**	**7.31 (2.40-22.21); p=0.0004**
Preoperative Cyst Size ≥4 cm	4.43 (0.96-20.30); p=0.05	

Figure 2a Sex and age adjusted univariable cox hazard regression analysis for the assessment of possible associations between pharmacological exposure and the occurrence of cancer related death

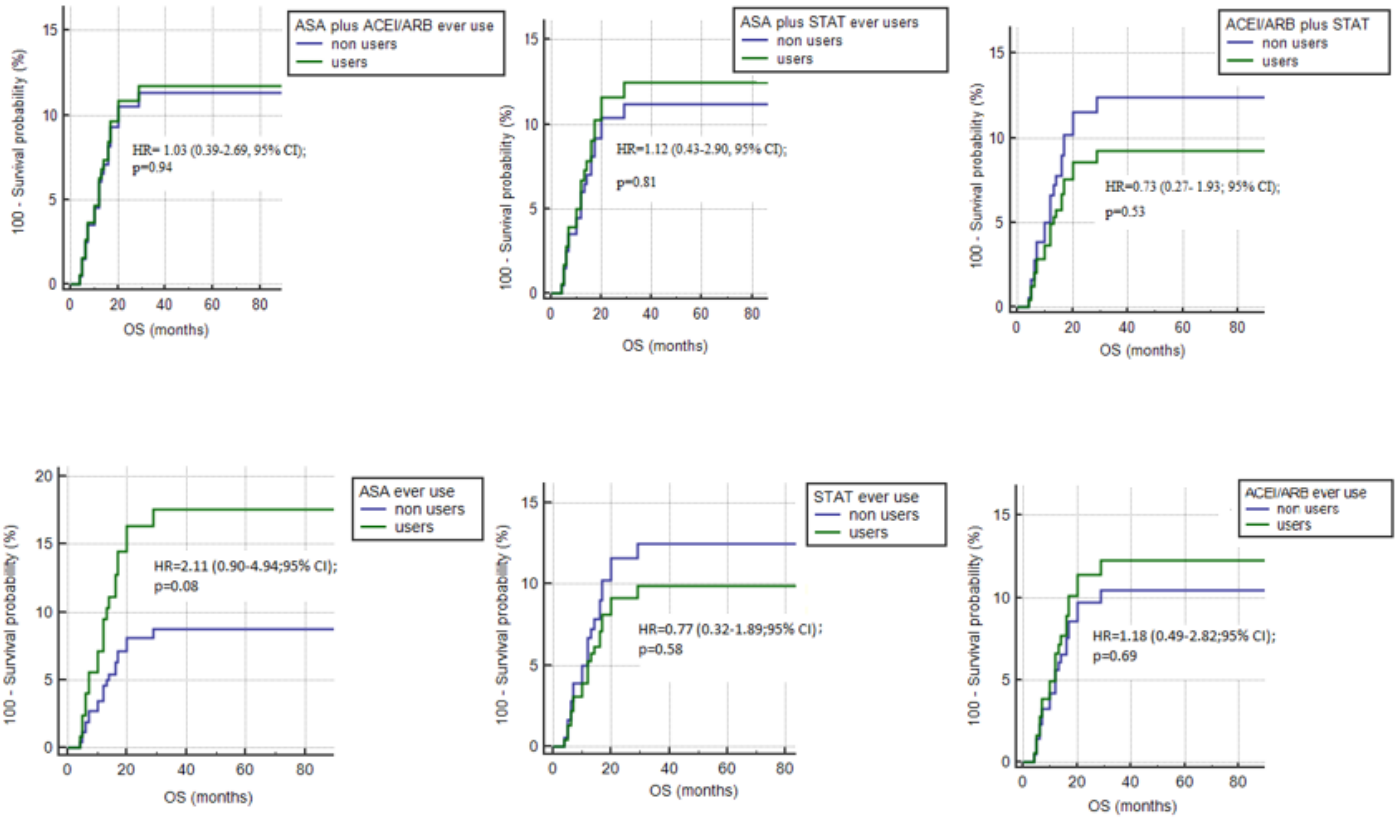
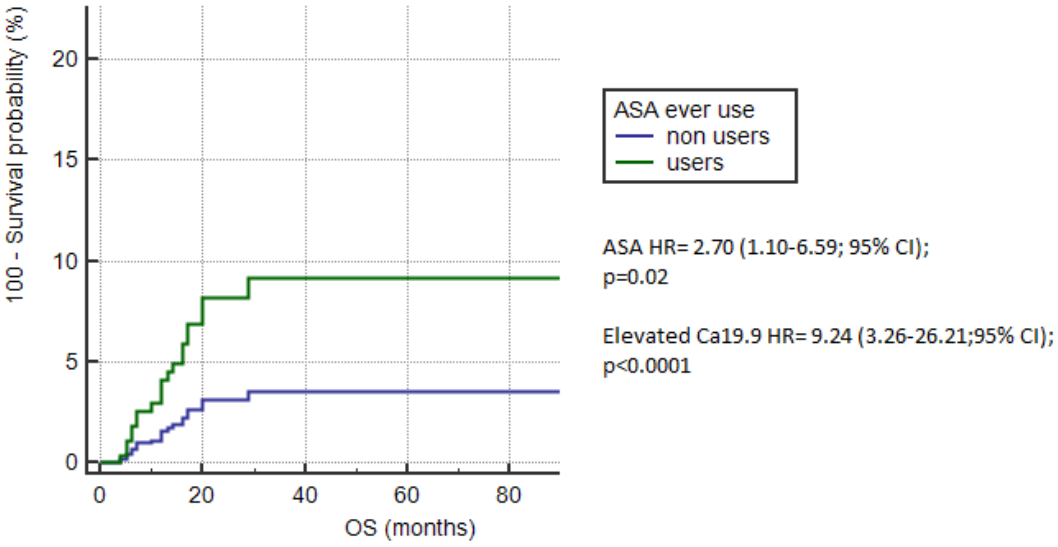


Figure 2b Multivariable cox hazard regression analysis for the assessment of possible associations between aspirin exposure and the occurrence of cancer related death, adjusted for sex, age, clinical factors associated with pancreas cancer (smoking, diabetes, first degree family history, overweight/obesity, elevated Ca19.9)



Paper III

Study design

A collaborative multicenter hospital-based case-control study was conducted in six European countries: Italy, Norway, Sweden, Slovenia, United Kingdom and Germany as part of the "Pancreas 2000" educational project (www.pancreas2000.org), upon local hospital ethical committee approval.

The cases were prevalent sporadic PNEN patients diagnosed within 24 months from the beginning of the study (January 2013) and new incident cases of sporadic PNENs diagnosed from January 2013 to December 2015 that were recruited at the participating centers. A standardized questionnaire, investigating demographics and potential risk factors, such as family history of cancer, environmental factors, previous use of drugs such aspirin and other medical history features was administered to patients, after gaining participant consent, by a trained medical doctor. The cases were prevalent sporadic PNEN patients diagnosed within 24 months from the beginning of the study and new incident cases of sporadic PNENs diagnosed afterwards and recruited at the participating centers.

Patients and outcomes

The inclusion criterion: histological or cytological diagnosis of PNEN. The date of the confirmatory pathological report was accepted as the date of diagnosis.

Exclusion criteria:

-inherited form of PNEN such as those associated with multiple endocrine neoplasia-type 1 (MEN1), von Hipple-Lindau disease (VHL), neurofibromatosis type 1 (NF-1), tuberous sclerosis (TSC)

-inability to participate, such as dementia.

According to the absence or presence of a clinical syndrome due to hormonal hypersecretion, cases were classified as non-functioning or functioning tumors (gastrinoma, insulinoma, glucagonoma).

Cases were classified according with the European Neuroendocrine Tumor Society (ENETs) and the 2010 World Health Organization classifications.

Eligible controls were either individuals seen in the participating hospitals' outpatient clinic for a non-specific, non-organic gastrointestinal disorder (bloating, aspecyfic dyspeptic symptoms, eructation) or visitors attending the same network of referring hospitals, matched by country, sex and age (+/- 5 years). Visitors and hospital outpatients' clinic belonged to the same catchment area of cases.

Specific exclusion criteria for controls were the presence of:

1) Genetic syndromes associated with the occurrence of PNENs

2) History of active cancer (diagnosed within 5 years)

3) any biological relation of a participating PNEN case in this study

4) Previous medical history of any chronic inflammatory condition (e.g. chronic obstructive pulmonary disease, liver cirrhosis, inflammatory bowel disease, end stage kidney disease)

5) Familial cancer syndromes

Controls were included in the same country and interviewed within 6 months of the inclusion of the matched corresponding case.

Exposure definitions

Subjects were questioned about risk factors that were present at least 12 months before diagnosis or presentation of symptoms, to avoid potential bias due to lifestyle modifications, cancer symptoms or cancer treatments.

Ever smokers were considered as subjects with cumulative lifetime smoking history greater than 6 months or 100 cigarettes smoked. A quantification of the smoking habit was performed considering the number of pack-years (pack-year = number of packs per day x years of smoking), with 20 pack-years being the lower limit to qualify a participant a heavy smoker.

A daily intake of at least 12.5 g of alcohol, equivalent to one glass of wine, one pint of beer or one shot of hard liquor, for at least one year, was considered the cut-off to be a regular ever alcohol drinker.(33)Because of possible different drinking habits within different European countries, the weekly alcohol amount was also sub-analyzed according to low (1-7 weekly units assumption), medium (8-14 weekly units), medium-high (14-20 weekly units) and heavy alcohol consumption (≥21 weekly units).

Coffee drinking was also recorded as ever drinking (at least one cup per day) or heavy coffee drinking (>5 cups per day).

Height and weight were recorded from which body mass index (BMI) (kg/m2) was calculated. A medical history of chronic pancreatitis, acute pancreatitis, peptic ulcer disease, biliary stones and previous surgery were specifically recorded as well as diagnosis of diabetes that was further subdivided for type and onset. For cases, recent onset diabetes was defined as that which was diagnosed in the 12 months prior to the PNEN diagnosis, and for controls a diagnosis of diabetes 12 months prior to the date of recruitment was required. Sensitivity analysis were also performed for different intervals of the onset of diabetes, (respectively inferior to 1 year, between 1 and 3 years, between 3 and 5 years and above 5 years). Another sensitivity analysis was also conducted considering incident/prevalent cases and hospitals controls/visitors controls compared to respective cases.

As atopy and allergy have been associated with a reduced risk of pancreatic cancer, cases and controls were interrogated about a history of allergy, with specific enquiry for eczema, hay fever and asthma.

Subjects were interrogated about 1st and 2nd degree family history of cancer, and the total number of siblings and children was recorded.

The use of drug exposures such as aspirin, proton pump inhibitors, metformin and insulin were recorded.

Statistical Analysis

An a priori power calculation was performed. We estimated sample size based on the differences reported in the frequencies of exposure in cases and controls according to a previous study(34), and considering a ratio 1:3, with a statistical power equal to 80% and an alpha error equal to 0.05. According to the previous study (34), the reported prevalence in cases and controls was respectively 53% vs. 32% for 1st degree family history of cancer, 10% vs. 2% for diabetes and 14% vs. 3% for heavy alcohol consumption. Therefore, the sample size's estimate of cases and controls, to show true differences whether existing, were respectively 64 cases and 191 controls for 1st degree family history of cancer, 117 cases and 350 controls for diabetes, 85 cases and 253 controls for heavy alcohol consumption. We therefore estimated that a total of 200 cases and 600 controls across participating centers, to be an adequate sample size to reveal differences in the prevalence of most risk factors analyzed among cases and controls, where these exist. Characteristics of cases and controls were compared by chi-square test for categorical variables or Student's t test for continuous variables. Significant variables were analyzed by logistic regression analysis adjusted for sex, age and enrolment center. A multivariable logistic regression analysis, adjusted for sex, age and enrolment center was also performed with an enter selection procedure for statistically significant risk factors. A dedicated statistical software package (MedCald Mariakerke, Belgium) was used for data analysis. The 95 % confidence interval (CI 95) was calculated where possible. All p-values were two-sided and $p<0.05$ was considered statistically significant.

Results

Patients characteristics

A total of 201 cases and 603 sex and age matched controls were enrolled among the six centers (Italy, Norway, Sweden, United Kingdom, Slovenia and Germany) as shown in table 1. The mean age was 59.6 years in cases (CI 57.7-61.4) and 59.5 years in controls (CI 58.4- 203 60.55), and 51% of cases and controls were male. Amongst the 201 PNEN cases, 154 (76.6%) had a non-functioning tumor. Of the 47 functioning PNENs, 26 (55.3%) had an insulinoma, 9 (19.1%) a gastrinoma, 7 (14.9%) a glucagonoma and 5 (10.6%) other functioning tumors. The majority were G1 (44.8%) or G2 (43.3%) PNENs and were equally distributed among different disease stages (Table 1). Of the 201 PNEN cases, 80 (38%) cases were incident and 121 (62%) prevalent. Of the 603 controls, 422 (70%) were hospital outpatients controls and 181 (30%) visitors.

Risk factors for the occurrence of PNEN

The proportion of subjects who had a 1st degree family history of cancer was similar in cases and in controls (respectively 51.1% vs. 45.3% p=0.17), while 2nd degree family history was slightly more prevalent in cases (36.8% vs. 30.2% p=0.09). A 1st degree family history of specific cancer sites was also not significantly different (Table 2). No cases or controls reported a family history of neuro-endocrine tumor (NET). At multiple regression analysis 2nd degree family history of any cancer was, however associated with an increased risk of PNEN (see Table 2 and 3).

Body Mass Index

Mean BMI was not significantly different amongst cases and controls (26.8 kg/m2 223 (CI 26.0- 27.5) and 26.4 kg/m2 (CI 26.4-26.8), p=0.10. Similarly, whilst the overall prevalence of obesity was more frequent in cases than controls (25.5% vs. 18.2%) this was not significant (p=0.44). At regression analysis after adjustment for matching variables, there remained no significant association with obesity (OR 1.36, 95% CI 0.88-2.08, p=0.15) (Table 3).

Cigarette smoking, alcohol intake and coffee drinking

The proportion of smokers (55.5% vs. 53.5%, p=0.59), heavy smokers (25.5% vs. 24.0%, 231 p=0.59), alcohol drinkers (75.6% vs. 70.8%, p=0.33), heavy alcohol drinkers (3.4% vs. 4.2%, 232 p=0.33), coffee drinkers (84.8% vs. 87.8%, p=0.24) and heavy coffee drinkers (18.8% vs. 233 19.2%, p=0.24) did not significantly differ between cases and controls (Table 2).

History of diabetes mellitus

A history of diabetes mellitus was more prevalent in cases than in controls (18.4% vs. 12.3%, 237 p=0.03). This difference was greater on analysis of non-recent onset diabetes, defined as diabetes diagnosed more than 12 months before the diagnosis of PNEN in cases, or 12 months prior to the interview for controls (17.4% vs. 9.7%, p=0.004) (Table 2). After adjustment for the matching variables, non-recent onset diabetes was confirmed to be consistent with the occurrence of PNEN (OR 1.89, 95% CI 1.17-3.05, p=0.008). At multivariable analysis the association with non-recent onset diabetes remained statistically significant (OR 2.09, 95% CI 243 1.27-3.45, p=0.003) (Table3). At sensitivity analysis, a history of diabetes with an onset between 1-3 year and between 3- 5 years, was increasingly prevalent in PNEN compared to controls (respectively 3.0% vs 1.1% p=0.07 and 4% vs 0.9% p=0.004). At univariable logistic regression analysis this difference remained significant (respectively OR 2.56, 95% C.I 0.83- 247 7.91 and OR 4.31, 95% C.I 1.43-12.98). For intervals of occurrence of diabetes superior to 5 years no statistically significant difference was found between cases and controls (respectively 10.7% vs 7.4%, p=0.16) (Table 2). We also performed a separate analysis for “late onset diabetes” using as controls either only the 422 hospital controls or only the 181 visitors controls. In the first case, the OR resulted to be 2.52 (95%CI 1.08-5.86; p=0.03), while in the second one the OR was 1.7 (95%CI 0.95-3; p=0.07), most likely due to the lower number of controls reducing the power of the analysis. Neither the use of metformin (7.3% vs. 5.1%, p=0.3), nor insulin (4.1% vs. 1.6%, p=0.1), or their association together (5.2% vs 3.4%, p=0.4) were statistically different between cases and controls.

Past medical history

With regards to past medical history, the prevalence of acute pancreatitis (3.5% vs. 2.4%, p=0.60), peptic ulcer disease (12.3% vs. 10.6%, p=0.59), cholecystectomy (9.0% vs. 8.5%, 260 p=0.92) and gastrectomy (1.0% vs. 0.7%, p=0.99) were similar in cases and controls. None of the participants reported a medical history of chronic pancreatitis (Table 2). A higher proportion of cases reported a history of gallstone disease than controls (19.2% vs. 13.3%) but this did not reach the significance threshold (p=0.06). After adjustment for age, sex and enrolment center at multivariable analysis, this latter association remained borderline significant (OR 1.52, 95% CI 0.95-2.44, p=0.08) (Table 3).A history of allergies was not different in cases and in controls (28.9% vs. 25.0%, p=0.32). Specifically, neither asthma (12.1% vs. 8.6%, p=0.19), eczema (11.1% vs. 7.6%, p=0.17) nor hay fever (15.6% vs. 14.5%, p=0.79) were more prevalent in PNEN patients than in controls (Table 2).

Non-diabetic medications

The use of proton pump inhibitors (PPI) (39.2% vs. 39.6%, p=0.97) and aspirin (22.5% vs. 26.5%, p=0.29) did not differ among cases and controls respectively (Table 2).

Risk factors for the advanced grades and stages of PNEN

When stratifying cases for the TNM stage at diagnosis and for the tumor grade, diabetes mellitus was statistically more prevalent in patients with G3 tumors (pancreatic neuro endocrine carcinoma; PNEC) than with G1 or G2 tumors (40.9% vs. 15.8%, p=0.01). Amongst cases, non-recent onset diabetes was associated with a more advanced stage at diagnosis (TNM III-IV vs TNM I-II respectively 23.3% vs. 11.8%, p=0.05) and with a G3 vs G1-2 tumor (respectively 40.9% vs. 14.9%, p=0.006). The use of metformin in combination with insulin was more prevalent in patients with G3 than G1-G2 tumors (respectively 23.5% vs. 3.2%, 285 p=0.003) (Table 4). Asthma was more prevalent in G3 cases than in G1-2 (30.0% vs. 10.2% 286 p=0.02), and eczema was also more prevalent in G3 cases than in G1-2 but

without reaching statistical significance (25.0% vs. 9.6%, p=0.08). Coffee drinking was more prevalent in localized disease (TNM 1-2) at diagnosis compared with advanced stage (TNM 3-4) (92.3% vs. 75.9%, p=0.003).

Table 1 Characteristics of PNENs cases

		Cases N (%)
Total		**201**
Referral center		
	Italy	62 (30.8)
	Norway	44 (21.9)
	Sweden	40 (19.9)
	Slovenia	24 (11.9)
	United Kingdom	20 (10.0)
	Germany	11 (5.5)
Sex	**Male**	103 (51.2)
Age (years)	**Mean ±**	59.6 ± 13.1
SD		
Race		196 (97.5)
Caucasians		
Tumor type		
	Functioning	47 (23.3)
	Non functioning	154 (76.7)
Functioning only (n=47)		
	Insulinomas	26 (55.3)
	Gastrinomas	9 (19.1)
	Glucagonomas	7 (14.9)
	Other	5 (10.6)
Tumor grade		
	G1	90 (44.8)
	G2	87 (43.3)
	G3	22 (10.9)
	Unknown	2 (1.0)
Tumor stage		
	Stage I	51 (25.4)
	Stage II	53 (26.4)
	Stage III	47 (23.4)
	Stage IV	43 (21.4)
	Unknown	7 (3.5)
Tumor site		
	Head	75 (37.3)
	Body or tail	120 (59.7)
	Unknown	6 (3.0)

Table 2 Risk factors for PNENs

	Cases N (%)	Controls N (%)	p value	OR* (95% CI)
Total	**201**	**603**		
Family history*				
1st degree FH of any cancer	103 (51.1)	272 (45.3)	0.17	1.32 (0.94-1.83)
2nd degree FH of any cancer	74 (36.8)	181 (30.2)	0.09	1.51 (1.05-2.17)
1st degree FH pancreatic cancer	6 (3.0)	13 (2.2)	0.69	1.42 (0.52-3.84)
1st degree FH esophageal	2 (1.0)	4 (0.7)	0.99	1.58 (0.28-8.79)
1st degree FH gastric cancer	7 (3.5)	26 (4.3)	0.75	0.77 (0.32-1.85)
1st degree FH colorectal cancer	18 (9.0)	57 (9.5)	0.92	0.99 (0.56-1.75)
1st degree FH breast cancer	20 (10.0)	51 (8.3)	0.62	1.25 (0.72-2.18)
1st degree FH lung cancer	17 (8.5)	48 (8.0)	0.95	1.12 (0.62-2.00)
1st degree FH NETs	-	-		
1st degree FH hematological cancer	12 (6.0)	30 (5.0)	0.72	1.28 (0.63-2.57)
1st degree FH hepatobiliary cancer	4 (2.0)	17 (2.8)	0.69	0.45 (0.12-1.66)
1st degree FH sarcoma	-	3 (0.5)	0.73	-
Number of siblings mean ± SD	2.1 ± 1.7	2.5 ± 2.0	0.05	
BMI				
Underweight	5 (2.5)	10 (1.6)		1.68 (0.55-5.04)
Normal weight	82 (41.0)	243 (40.2)	p-trend**	1.00 (0.71-1.39)
Overweight	63 (31.5)	240 (39.8)	0.44	0.75 (0.52-1.10)
Obese	51 (25.5)	110 (18.2)		1.36(0.88-2.08)
Smoking				
Never smoke	89 (44.5)	279 (46.5)		0.86 (0.62-1.20)
<20 pack-years	60 (30.0)	176 (29.3)	P-trend	1.07 (0.75-1.53)
≥20 pack-years	51 (25.5)	144 (24.0)	0.59	1.15 (0.78-1.69)
Unknown	1 (0.5)	3 (0.5)		1.00
Alcohol intake				
Never drink	49 (24.4)	173 (29.2)		0.77 (0.53-1.12)
<21 units/week	145 (72.1)	394 (66.6)	P-trend**	1.29 (0.90-1.85)
≥21 units/week	7 (3.4)	25 (4.2)	0.33	0.88 (0.37-2.11)
Coffee				
Never drink	30 (15.2)	73 (12.2)		1.37 (0.83-2.14)
≤4 cups/day	135 (68.2)	411 (68.7)	P-trend**	0.94 (0.66-1.34)
>4 cups/day	33 (18.8)	114 (19.2)	0.24	0.86 (0.55-1.35)
Unknown	3 (1.4)	5 (0.8)		1.00
Diabetes				
No	164 (81.6)	529 (87.7)	0.03	1.00
Recent onset (<1 year)	1 (0.5)	10 (1.6)	0.4	0.24 (0.03-1.88)
No-recent onset (≥1 year)	35 (17.4)	58 (9.7)	0.004	1.89 (1.17-3.05)
Unknown	1 (0.5)	6 (1.0)		1.00
Diabetes(≥1 year, <3 years)	6 (3.0)	7 (1.1)	0.07	2.56 (0.83-7.91)
Diabetes(≥3 year, <5 years)	8 (4.0)	6 (0.9)	0.0045	4.31 (1.43-12.98)
Diabetes(≥5 years)	21(10.7)	45 (7.4)	0.16	1.47 (0.84-2.56)
Diabetes treatment				
Metformin (no insulin)	13(7.3)	29 (5.1)	0.3	1.35 (0.68-2.66)
Insulin	7(4.1)	9 (1.6)	0.1	1.63 (0.86-3.08)
Metformin and insulin	9 (5.2)	19 (3.4)	0.4	1.48 (0.65-3.35)
Past medical history				
Acute pancreatitis	7 (3.5)	15 (2.5)	0.60	1.42(0.56-3.60)
Chronic pancreatitis	-	-	-	
Peptic ulcer	24 (12.3)	64 (10.6)	0.59	1.25 (0.75-2.07)
Cholecystectomy	18 (9.0)	51 (8.5)	0.92	1.07 (0.61-1.90)
Gastrectomy	2 (1.0)	4 (0.7)	0.99	1.56 (0.28-8.65)
Gallstone disease	34 (19.2)	77 (13.3)	0.06	1.53 (0.97-2.42)
Asthma	24 (12.1)	52 (8.6)	0.19	1.47 (0.87-2.47)
Eczema	22 (11.1)	46 (7.6)	0.17	1.52 (0.88-2.62)
Hay fever	31 (15.6)	87 (14.5)	0.79	1.17 (0.74-1.84)
Any allergy	58 (28.9)	151 (25.0)	0.32	1.26 (0.88-1.82)
Use of aspirin	45 (22.5)	160 (26.5)	0.29	0.79 (0.53-1.18)
Use of proton pump inhibitors	78 (39.2)	239 (39.6)	0.97	1.04 (0.74-1.45)

FH= family history; NET = neuroendocrine tumor.* OR adjusted for sex, age and center of enrolment; ** P-value based on the Mantel Haenszel test for trend excluding missing category

Table 3 Risk Factors for the occurrence of PNENs at the Logistic regression analysis

Risk factor	Univariate* OR (95% CI)	p value	Multivariable* OR (95% CI)	p value
Diabetes				
No	1.00		1.00	
Early onset (≤1 year)	0.24 (0.03-1.88)	0.17		
Late onset (>1 year)	1.89 (1.17-3.05)	0.008	2.09 (1.27-3.45)	0.003
2nd degree FH of any cancer	1.51 (1.05-2.17)	0.02	1.53 (1.03-2.27)	0.03
Gallstone Disease				
No	1.00			
Yes	1.48 (0.93-2.35)	0.08	1.52 (0.95-2.44)	0.08

adjusted for sex, age, underweight, 1st degree family history for esophageal cancer and centre of enrolment

Table 4 Factors associated with TNM stage and tumor grade in PNEN patients

	TNM Stage 1-2 N (%)	TNM Stage 3-4 N (%)	p value	OR (95% CI)
Late onset diabetes (≥1 year)	12 (11.8)	21 (23.3)	0.05	1.98 (0.83-4.74)
Coffee drinking	96 (92.3)	66 (75.9)	0.003	0.14 (0.05-0.4)
	G1-G2 N (%)	**G3 N (%)**	**p value**	**OR (95% CI)**
Diabetes	28 (15.8)	9 (40.9)	0.01	4.28 (1.41-12.93)
Late onset diabetes (≥1 year)	26 (14.9)	9 (40.9)	0.006	5.43 (1.66-17.72)
Metformin plus insulin use	5 (3.2)	4 (23.5)	0.003	9.71 (1.86-50.74)
Allergic Factors				
Asthma	18 (10.2)	6 (30.0)	0.02	3.96 (1.91-13.21)
Eczema	17 (9.6)	5 (25.0)	0.08	3.06 (0.92-10.19)

Paper IV

Study design, patients and outcomes

A single-center case-control study between January 2006 to February 2016.

Incident cases: prospectively recruited patients with histological proved PDAC

Controls consisted of hospital non-patient visitors not genetically related to cases, or Gastroenterology Unit outpatients and inpatients. Both cases and controls demonstrated the will and ability to participate providing personal data, clinical and cancer history.Patients enrolled in either group provided written informed consent for interviews. The study received local IRB approval at Sant'Andrea Hospital. Methods were performed in accordance with the relevant guidelines and regulations.

Exclusion criteria for controls: (a) personal history of IBD, chronic kidney disease or liver cirrhosis, (b) referral to our center for family history (FH) of gastrointestinal cancer, (c) referral for NSAID-induced ulcer disease, (d) history of neoplasia within 5 years (e)inability to participate or providing personal data, clinical and cancer history. For each case enrolled, the first two eligible controls matched for sex and age (±2 years) were enrolled and interviewed within 30 days.

Data collection and exposure definition

A trained physician through direct patient interview collected data on a standardized questionnaire. Clinical, epidemiological, therapeutic and morphological parameters were collected such as sex, age, race, tobacco and alcohol intake, body mass index (BMI), family history (FH) of cancer, previous pancreatic diseases, history of diabetes, aspirin and statin use, length, type and dosage of their use. During the interview, a list of brand and generic medication names for aspirin and statins was provided to help facilitate recall. All cases were interviewed within 1 month from diagnosis and data pertaining the disease were also recorded.

Smoking (ever) was considered a consumption of at least 100 cigarettes or >6 months of smoking. The total amount of smoking was evaluated as pack-years, defined as the product of packs smoked per day and the total years of smoking. Twenty pack-years was set as cut-off of to define heavy smokers.

Current smoker was considered as a person currently smoking or who had quit less than 1 year in the past while to be considered ex-smokers patient should had quit at least 1 year before the diagnosis or the time of interview for controls.

A consumption of at least 12.5 g (1 unit) of alcohol/month was needed to be considered a drinker. One glass of wine, 1 pint of beer, one shot of hard liquor was considered equal to 12.5 g amount of alcohol. Heavy drinkers were considered as individuals drinking more than 21 units/week (262.5 g of alcohol).(33)

BMI was calculated as usual adult weight/height^2 (kg/m^2) with obesity considered as BMI >30 kg/m^2. Diabetes was recorded as a potential risk factor when diagnosed >1 year before the diagnosis of cancer or its first symptoms for cases or before the interview for controls.

Participants were considered as aspirin or statin users when reporting ever use of the medication for at least 3 consecutive months and were subsequently categorized as “high dosage users” (≥300 mg) and “low dosage users” (≤160 mg) for aspirin and “low-dosage users” (<20 mg) or “moderate/high dosage users” (≥20 mg) for statins. Aspirin or statin users were categorized into different length of duration (<60 months and ≥60 months for aspirin, <48 months and ≥48 months for statins) based on median value. To avoid possible bias due to cancer symptoms, subjects were asked about risk factors present 12 months before diagnosis or presentation symptom.

Statistical analysis

A preliminary power calculation was performed. Considering an exposure of ~20% for aspirin or statins as previously recorded in controls, 395 cases and 790 controls were needed to have a 80% power of identifying an odds ratio (OR) ≤ 0.62 as single effect of aspirin or statins. This would also allow to detect an OR ≤ 0.50 for the combined used of aspirin and statins, based on a 10% combined exposure among controls.

Case-control comparisons were made using Chi-square and Fisher's exact tests for categorical variables and Student's t-test for continuous variables. Univariate logistic regression analysis was used to calculate ORs and their 95% confidence intervals (CI). Multivariable logistic regression models were adjusted for potential confounders. All statistical analysis was performed using MedCalc version 13 (MedCalc Software, Belgium). All reported *P* values were 2-sided and considered statistically significant when $p < 0.05$.

***Results*:**

Of 421 PDAC cases seen at the Gastroenterology Department during the study period, 9 (2.1%; 4 males and 5 females, mean age 74) were not histologically confirmed. Of the remaining 412, 2 (0.5%) refused to participate and 2 (0.5%) were too ill to take part; of the 893 controls recruited in the same timeframe, 51 (5.7%) were excluded for matching exclusion criteria, 8 (0.9%) refused to be interviewed for privacy concerns, 18 (2.0%) were too ill to take part. This led to a participation rate of 99% for cases and 91% for controls; the analysis was therefore conducted on a final population of 408 cases and 816 matched controls. Respectively 48.7%, 13.4% and 37.9% of the controls, consisted of visitors, inpatients and outpatients. Most of the inpatients were hospitalized for diverticulitis or gastrointestinal infections; outpatients were visiting for either gastro-esophageal reflux disease, irritable bowel syndrome, chronic constipation or dyspepsia. The median age of cases and matched controls was 68 years (range 35 to 99); 51.2% were men.

Almost all cases and controls were Caucasians.

Risk factors for pancreatic cancer

Compared with controls, cases had higher mean BMI value, higher proportion of obesity, 1st degree FH of PDAC, previous history of diabetes, previous chronic pancreatitis, and were more frequently smokers, heavy smokers and heavy drinkers (**see Table 1**).

Overall use of aspirin and statins and risk of pancreatic cancer

Seventy-eight cases (19.1%) and 191 controls (23.4%) reported ever use of aspirin, which was associated with a statistically borderline significant reduced PDAC risk (age- and sex-adjusted OR, 0.74; 95% CI, 0.54–1.02; Table 2). Only 2 of the 78 cases and 5 of the 191 controls (0.5% of both cases and controls) were on high-dose aspirin. The median duration of aspirin use was 60 months for both cases

and controls, with 20 patients (4.9%) among cases and 33 patients (4.0%) among controls not recalling the exact duration of use. A borderline significant protective association was recorded for shorter duration of exposure.

Seventy-four cases (18.1%) and 203 controls (24.9%) reported ever use of statins, which was associated with a statistically significant reduced PDAC risk (age- and sex-adjusted OR, 0.64; 95% CI, 0.48–0.88; **Table 2**).

The median dosage of statins was 20 mg and the median duration of use was 48 months for both cases and controls. A higher dosage of statins (for ≥20 mg, OR, 0.43; 95% CI, 0.27–0.71) was associated with a stronger protective effect. The most commonly used statin was atorvastatin. Of the 74 cases and the 203 controls, respectively 11 (2.7%) and 46 (5.6%) could not recall the type of drug, 38 (9.3%) and 69 (8.5%) could not recall the dosage, and 27 (6.6%) and 48 (5.9%) could not recall the duration of use.

At multivariable analysis, statins (OR 0.61; 95% CI, 0.43–0.88) but not aspirin (OR, 0.77; 95% CI, 0.53–1.11) use was associated to a reduced PDAC risk **(Table 2).**

To avoid possible bias due to controls selection, a sensitive analysis for control type (visitors or hospital patients) was performed. At multivariable analysis, statin use was associated to a reduced risk of PDAC both with sensitive analysis restricted to visitors (OR 0.60; 95% CI, 0.40–0.89) or to hospital controls (OR 0.59; 95% CI 0.40–0.87), while the use of aspirin was not associated to PDAC risk for either visitors (OR, 0.82; 95% CI, 0.55–1.23) nor hospital controls (OR 0.67; 95% CI 0.45–1.01).

Exclusive and Combined use of Aspirin and Statins and risk of pancreatic cancer

In order to evaluate the exclusive or combined effect of the two drugs and avoid possible confounding effects, we analyzed the use of aspirin excluding patients reporting also the use of statins and *vice versa* **(Table 3).** An exclusive aspirin use was recorded in 39 (9.6%) of all cases and 95 (11.6%) of all controls, and an exclusive statin use was recorded in 35 (8.6%) of cases and 107 (13.1%) of controls. In

an age- and sex- adjusted analysis the exclusive use of statins was associated with a stronger risk reduction (OR, 0.54; 95% CI, 0.36–0.82) than the exclusive use of aspirin (OR, 0.67; 95% CI, 0.45–1.02). The concomitant use of statins and aspirin was reported in 39 cases (9.6%) and 96 controls (11.8%). This combined use did not further reduce the risk (OR, 0.67; 95% CI, 0.44–1.01) compared with the use of statins alone.

At logistic regression multivariable analysis adjusted for other potential confounding factors, statin use (OR, 0.51; 95% CI, 0.32–0.80) was associated to a reduced risk of PDAC occurrence, while the association for aspirin use was of borderline significance (OR, 0.64; 95% CI, 0.40–1.01). The combined use of the two drugs did no further reduce the risk compared to the use of statins alone at multivariable logistic regression analysis (OR, 0.54; 95% CI, 0.34–0.87). No evidence of interaction between statin and aspirin was found when adding an interaction term to the main effects of aspirin and statin in a multivariable model adjusted for other potential confounding variables (p = 0.17).

Association between use of aspirin and statins and risk of pancreatic cancer in subgroups

To evaluate a potential specific association between the use of the two drugs and the risk of PDAC among different subgroups, separate multivariable subgroup analyses were conducted for the exclusive use of aspirin and statin according to: gender, smoking habit, obesity, history of diabetes and age ≥ or <70 years. Results are shown in **Fig. 1.**

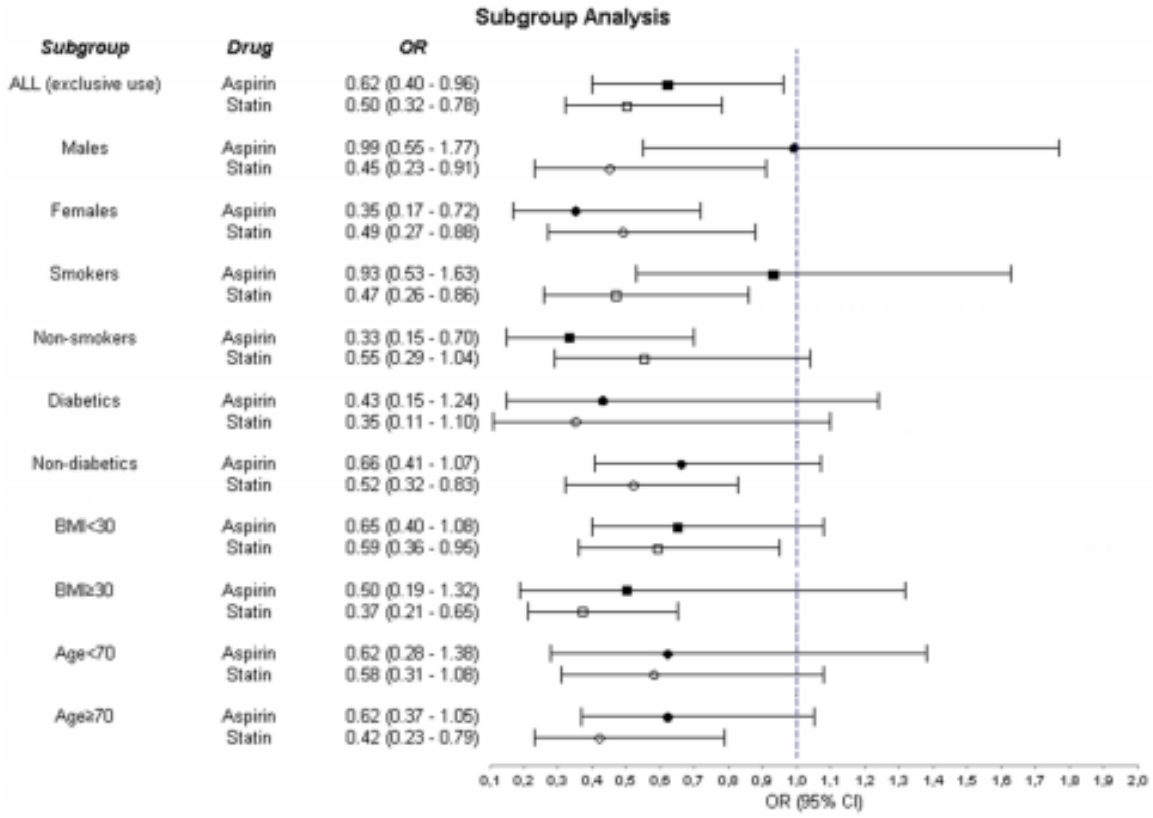


Figure 1. Subgroup analysis of the association between the exclusive use of either statins or aspirin and pancreatic cancer risk. Subgroup estimates are adjusted for age, sex, body mass index, first degree family history of pancreatic cancer, history of diabetes >1 year and smoking habit. OR: Odds Ratio, CI: Confidence Interval.

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Age	68.1 ± 11.6	67.9 ± 11.9	1.00 (0.99–1.01)	0.67	1.00 (0.99–1.02)	0.48
Male gender	209 (51.2%)	418 (51.2%)	1.00 (0.79–1.27)	0.99	0.77 (0.54–1.10)	0.16
1st degree FH of any cancer	218 (53.4%)	403 (49.4%)	1.22 (0.95–1.57)	0.12	—	
2nd degree FH of any cancer	30 (7.4%)	54 (6.6%)	1.29 (0.79–2.11)	0.31	—	
1st degree FH of PDAC	32 (7.8%)	23 (2.8%)	3.02 (1.73–5.26)	0.0001	3.26 (1.79–5.92)	0.0001
2nd degree FH of PDAC	5 (1.2%)	6 (0.7%)	1.83 (0.55–6.09)	0.33	2.17 (0.59–7.97)	0.24
BMI (mean ± Std.dev.)	26.8 ± 4.9	25.9 ± 4.1	1.05 (1.02–1.08)	0.001	1.04 (1.01–1.08)	0.009
BMI >30	79 (19.4%)	120 (14.7%)	1.47 (1.07–2.02)	0.02	—	
History of diabetes	72 (17.6%)	79 (9.7%)	2.03 (1.43–2.88)	<0.0001	1.84 (1.25–2.71)	0.002
Chronic pancreatitis	15 (3.7%)	2 (0.2%)	15.9 (3.60–70.0)	<0.0001	14.7 (3.18–67.6)	0.0006
Cigarette smoking[3]						
Never smoker	155 (38.0%)	416 (51.0%)	1.00		1.00	
Ever smoker	253 (62.0%)	400 (49.0%)	1.80 (1.39–2.32)	<0.0001	—	
<20 Pack-years	75 (18.4%)	171 (21.0%)	1.24 (0.89–1.74)	0.21	1.27 (0.88–1.84)	0.19
≥20 Pack-years	153 (37.5%)	229 (28.1%)	1.92 (1.43–2.57)	0.0001	1.93 (1.40–2.66)	<0.0001
Alcohol drinking[4,5]						
Never drinker	203 (49.8%)	446 (54.7%)	1.00		1.00	
Ever drinker	173 (42.4%)	370 (45.3%)	0.99 (0.77–1.29)	0.96	—	
<21 alcohol units/week	116 (28.4%)	312 (38.2%)	0.81 (0.61–1.07)	0.14	0.90 (0.66–1.21)	0.47
≥21 alcohol units/week	49 (12.0%)	57 (7.0%)	1.81 (1.17–2.80)	0.008	1.55 (0.96–2.49)	0.07

Table 1. Characteristics of pancreatic cancer cases and controls by selected variables of family history, chronic conditions, and lifestyle. FH: Family History, PDAC: Pancreatic Ductal AdenoCarcinoma, BMI: Body Mass Index, OR: Odds Ratio, CI: Confidence Intervals. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits. [3]Exact amount and duration not recalled by 25 (6.1%) cases and 0 controls. [4]Exact amount and duration not recalled by 32 (7.8%) cases and 0 controls. [5]Unknown units/week for 8 (2.0%) cases and 1 (0.1%) control.

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Aspirin use[3,4]						
Never	330 (80.9%)	625 (76.6%)	1.00			
Ever	78 (19.1%)	191 (23.4%)	0.74 (0.54–1.02)	0.06	0.77 (0.53–1.11)	0.16
Low-dose (≤160 mg)	68 (16.7%)	154 (18.9%)	0.80 (0.58–1.12)	0.20		
High-dose (≥300 mg)	2 (0.5%)	5 (0.6%)	0.72 (0.14–3.76)	0.70		
<60 months	25 (6.1%)	73 (9.0%)	0.62 (0.38–1.01)	0.05		
≥60 months	33 (8.1%)	85 (10.4%)	0.70 (0.45–1.09)	0.11		
Statin use[5–7]						
Never	334 (81.9%)	613 (75.1%)	1.00			
Ever	74 (18.1%)	203 (24.9%)	0.64 (0.48–0.88)	0.005	0.61 (0.43–0.88)	0.007
Atorvastatin	29 (7.1%)	85 (10.4%)	0.60 (0.38–0.94)	0.03		
Simvastatin	23 (5.6%)	45 (5.5%)	0.92 (0.54–1.56)	0.76		
Other forms*	11 (2.7%)	27 (3.3%)	0.72 (0.35–1.48)	0.37		
<20 mg	14 (3.4%)	44 (5.4%)	0.56 (0.30–1.05)	0.07		
≥20 mg	22 (5.4%)	90 (11.0%)	0.43 (0.27–0.71)	0.0008		
<48 months	25 (6.1%)	84 (10.3%)	0.53 (0.33–0.84)	0.008		
≥48 months	22 (5.4%)	71 (8.7%)	0.55 (0.33–0.90)	0.02		

Table 2. Overall aspirin and statin use among pancreatic cancer cases and controls. OR: Odds Ratio, CI: Confidence Interval. *Fluvastatin, Lovastatin, Pravastatin, Rosuvastatin. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits. [3]Unknown dose for 8 (2%) cases and 32 (3.9%) controls. [4]Unknown duration for 20 (4.9%) cases and 33 (4%) controls. [5]Unknown type for 11 (2.7%) cases and 46 (5.6%) controls. [6]Unknown dose for 38 (9.3%) cases and 69 (8.5%) controls. [7]Unknown duration for 27 (6.6%) cases and 48 (5.9%) controls.

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Exclusive or combined use						
Neither aspirin nor statins	295 (72.3%)	518 (63.5%)	1.00		1.00	
Aspirin only	39 (9.6%)	95 (11.6%)	0.67 (0.45–1.02)	0.06	0.64 (0.40–1.01)	0.06
Statins only	35 (8.6%)	107 (13.1%)	0.54 (0.36–0.82)	0.004	0.51 (0.32–0.80)	0.004
Aspirin and Statins	39 (9.6%)	96 (11.8%)	0.67 (0.44–1.01)	0.06	0.54 (0.34–0.87)	0.01

Table 3. Exclusive and combined aspirin and statin use among pancreatic cancer cases and controls. OR: Odds Ratio, CI: Confidence Interval. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits.

Discussion:

Paper I has shown as the use of ACEI and STAT was associated with lower rates of dimensional progression and overall progression (any) of BD-IPMNs in follow-up. Such effect remained significant even at sex, age and insulin adjusted multivariable cox hazard regression analysis. Interestingly the lowering of dimensional and any progression in STAT users was in line with the risk reduction of pancreas cancer elsewhere described.(19) Anyway, no statistically significant effect has been shown on clinically relevant progression.

The use of ARB use was associated with an increased risk for clinically significant progression at sex and age adjusted univariable cox hazard regression analysis, but the data was not confirmed in the multivariate model after correction for possible risk factors. Thus, such association has still to be confirmed even because the absence of statistical significance, might be related to the absence of statistical power for such outcome.

In opposition to several observational studies on pancreas cancer in the current study, ASA use was not significantly associated with a slower progression. On the other hand, recently questioning have been risen regarding the real effect of ASA on cancer, since a large trial has shown a higher incidence of cancer in patients treated with aspirin arm compared to controls group. (35)

Takasaki et al. in the only paper addressing such aspect in IPMNs, investigated the effect of aspirin in a cohort of BD-IPMNs in follow up, suggesting after a median 5.5 years follow up a possible effect of low dose aspirin in slowing MPD dilation over the time (4.8% vs 12.7%, p=0.02). In such study, no specific investigation was performed regarding the use of STAT or ACEI/ARB nor their possible associations.(36)

As a matter of facts, it is also still unclear whether the potential anti neoplastic effect of ASA could be a long-term rather than immediate effect. (37) As previously suggested by some authors the effect of

aspirin might be balanced by confounding drugs such as statins, since often these two drugs are co-prescribed. (31)

So far, **Paper I** represents the first paper investigating the effects of ASA, ACEI/ARB and STAT on the progression os BD-IPMNs. Although several papers have already suggested an active role of ACEI/ARB in inhibiting cancer initiation and progression in several cancer models, none of them investigated the exclusive effect of the two classes of drugs, nor the effect of different molecules among the same class of drug.(26, 38)

The main breakthrough of **Paper I** is the identification of different clinical effects for the exclusive use of ACEI and ARB. In fact, in many papers they are generally pooled together, although acting on different points of the renin-angiotensin-aldosterone cascade.

ACEI act on angiotensin I converting enzyme (ACE1), which is an enzyme upstream the entire cascade converting circulating angiotensin I into angiotensin II (AngII).

Ang II is the main effector of the cascade, acting either on Ang II receptor 1 (AT1), which is the target of ARB, and on receptor II (AT2), which is not. In addition, Ang II through multiple intermediate mediators, such as ang III, ang IV, ang 1-7, acts even on other different pathways, through different receptors such as AT2, AT4, MAS. Therefore, while the blocking of ACE1 by ACEI might inhibit the entire cascade, the selective blocking of AT1-2 might instead imply escape mechanisms with retrograde shifting of the cascade towards other mediators and receptors. The exact activity of such other receptors is still matter of debate and has not be fully elucidated, but some of them are implied in the regulation of the neovascularization after myocardium infarction through a VEGF pathway, with possible activity even on different intracellular kinases. These pathways are important mediators in neoplastic initiation and progression and might be at least one of the molecular mechanism justifying of our results **(Figure3)**.(39)

Beside some limitations, such as the absence of a preliminary power calculation, the retrospective design and the absence on correlation with final histology, **Paper I** displays some strengths such as the relatively large sample size, the inclusions of patients from high volume centers assuring high quality of data, the investigation of several pharmacological previously uninvestigated factors on a high prevalence disease, with potential major implications for health care policies.

Despite a possible heterogeneity in modalities of data collection among Italian and Swedish cohort, in two third of patients the extraction of data from a specific IT system, constantly updated, gave clinicians direct access to inpatient's/outpatient's clinical charts and prescriptions, therefore minimizing possible recall biases.

Paper II is the first paper analyzing the effect of potentially chemo-preventive agents on cancer related death in a cohort of operated IPMNs. In the past years, huge body of literature has been focusing on the role of different clinical features in predicting cancer or invasive IPMNs “recurrence” in the remnant pancreas.(11, 12, 40)

In the current study, heterogeneity of our cohort in terms of resected specimen (ranging from absence of dysplasia to invasive IPMNs), is largely counterbalanced by the strict inclusion of patients undergone surgery with curative intent. For that purpose, all patients with positive margins for high grade dysplasia and/or cancer, synchronous pancreas cancer and/or periampullary tumors, potentially influencing prognosis, were excluded from our analysis.

On the other hand, we do think that such heterogeneity of resected specimens, might be more representative of “real world” population undergoing surgery for suspect malignified IPMN. It does therefore specifically served our aim of investigating the effect of such drugs on definitive prognosis of patients undergone surgery. In the current study, STAT use was associated with 23% reduction of cancer related death. Although not statistically significant, it is interesting to note how the data is in line with what reported in other epidemiological studies and meta-analysis (19, 31)

Surprisingly and in contradiction to several observational studies, that have previously shown a potential anti-cancer effect, in the current study, ASA was associated to an increased risk of cancer related death.(21, 37) The exact molecular mechanism behind that has not been clarified yet and deserves further investigations. ASA use maintained its association with cancer related death even when corrected for the presence of possible known risk factors for pancreas cancer, such as smoking, diabetes overweight/obesity, who might also increase cardiovascular risk and therefore need primary or secondary cardiovascular prevention with ASA. Further studies are needed to specifically investigate whether such association display

a real pathophysiological background or whether it's the result of the exposure to a common risk factor for both pancreas cancer and cardiovascular disease.

Paper II displays several strengths as well as limitations. Inherent with its design, our study displays as main limitation the absence of a preliminary power calculation. The strength of the study is represented by the relatively large sample size, the investigation of several clinical factors possibly implicated in progression of IPMNs after surgery and, for the first time, the evaluation of pharmacological exposures possibly influencing cancer occurrence and prognosis. The inclusions criteria were clearly defined and aiming at identifying prognostic factors in a population treated with curative intent. Data were extracted by IT system, constantly updated and giving clinicians access to inpatient's/outpatient's clinical charts and prescriptions, therefore minimizing possible recall biases.

Paper III is this largest multicenter study investigating possible risk and protective factors for the occurrence of pancreatic neuro endocrine tumors. To the best of our knowledge, there have been six published studies to have investigated risk factors for PNEN with heterogeneos results. (34, 41-45) Due to the low incidence of such tumors, which would make a longitudinal cohort study highly problematic, it is not unexpected that these were all case–control studies. In the current study, diabetes mellitus was associated with an increased risk of PNEN occurrence, however it is noteworthy that we identify the significance of non-recent onset diabetes as a risk factor. Non-recent onset diabetes was confirmed to be increasingly consistent with the occurrence of PNEN for intervals superior to 1 year and up to 5 years. For intervals of onset of diabetes superior to 5 years this association was not anymore statistically significant. This might be interpreted on the base of a lack of power of the study when considering small subgroups or, alternatively, it could be biologically explained by the trophic influence that diabetes plays on cancer. On the other hand, one should also consider that PNEN display a slower growing rate compared to PDAC and therefore it might justify a major latency of occurrence of symptom diabetes. Differently from what elsewhere suggested(34), no specific difference in cancer occurrence was noted between cases and controls with regards to family history of cancer, past medical history (other than diabetes) and exposure to environmental factors such as alcohol and smoking.

As the potential role of pharmacological exposures has been reported to possible influence pancreatic carcinogenesis (32, 46) we specifically investigated also the role of aspirin, metformin and insulin. Although the prevalent use insulin alone was more frequent among cases than in controls (4.1% vs 1.6%), this difference was not significant as the study was underpowered to assess it. No statistically significant differences were detected for the use of aspirin (22.5% vs 26.5, p=0.29), possibly reflecting intrinsic biological differences between endocrine and exocrine neoplasia of the pancreas.

Paper III displays several strengths as well as limitations. The strengths of the study are represented by the relatively large sample size keeping in mind the low incidence of this tumor type, the European

multicenter setting (6 countries involved), the preliminary power calculation, the investigation for the first time of a large set of factors possibly associated with the risk of PNENs and the conduct of the study by face-to-face interview with a standardized questionnaire. The inclusion criteria were clearly defined, controls were well matched for age and gender with a 1:3 ratio and all questionnaires were administered by trained medical doctors fluent in the local language, who evaluated exposures present 12 months before diagnosis, to minimize bias due to cancer symptoms. Inherent with a multi-national case-control design, our study displays some limitations such as potential recall bias and heterogeneity in data from different countries, although the analysis was corrected for center of enrollment. Furthermore, the analysis might have been underpowered for some of the investigated factors and additional studies might be important to confirm the lack of significant association. Another important matter of concern, as for any case-control study, regards the choice of the control population. We opted for a mixed control group that we believed to represent the same population as the case group, as living in the same catchment area of the corresponding cases, to limit possible bias that could have been specific of either hospital controls or visitors.

Paper IV

This is the first study evaluating the possible association between overall, exclusive and combined use of both aspirin and statins and PDAC risk at the same time. This is a relevant issue, as both drugs together are very frequently co-prescribed. Therefore, one might hypothesize the presence of both a possible confounding or a synergistic effect due to the prescription of both drugs. In the present study, PDAC risk is inversely associated with the overall statin use, with a dosage-dependent effect while the overall aspirin use is not associated to a statistically significant reduced PDAC risk. Statin use displayed a risk reduction of 49%, higher than that of aspirin, which was only borderline statistically significant. The concomitant use of the two drugs was also associated to a 46% reduced PDAC risk, without conferring a stronger risk reduction compared to the use of statins alone. Furthermore, no synergistic effect was shown through the analysis of interaction. Two previous cohort and case-control studies evaluated the use of statins and the risk of PDAC. The first study on a female population adjusted the analysis for the use of aspirin and NSAIDs, and the second also analysed the use of aspirin, but both did not evaluate the association of the two drugs or their exclusive use. (47, 48) As a difference with previous case-control studies evaluating the association between statin use and PDAC risk, **Paper IV** had a preliminary power calculation and the investigation of possible confounding factors such as BMI or dosage and duration of drug use.(49, 50) Our results shown a more consistent association between the exclusive use of statins and PDAC risk reduction. The effect was similar for both genders, but limited to smokers, elderly subjects, obese and non-diabetic patients.

Statins might exert a specific protective effect on cigarette-related carcinogenesis, because their possible effect of AKT and ERK pathways .(51)

The reasons for a more important protective effect of statins in non-diabetics are unclear. The association between statin use and reduced PDAC risk in subjects aged ≥70 years is probably due to

the increased PDAC risk in older subjects that might also have a prolonged drug exposure. **Paper IV** has some strengths:

1) it is the first specifically aimed at evaluating the association between overall, exclusive and combined use of both aspirin and statins

2) *a priori* power calculation

3) the investigations of possible known risk factors, allowing the identification of possible selection biases among our population

4) statin results are consistent with most Bradford Hill criteria for causation.(52)

As every case control study, even the current one might display as limitation the possible presence of recall biases. To reduce the risk of confounding factors, the questionnaire was carefully conducted by a trained physician with expertise on pancreatic disorders, asking information regarding risk factors exposure as present 1 year before the interview.

As in any case-control study the choice of the control population is a possible matter of concern. We opted for a control group that we believed to represent the same population as the case group, derived from the same catchment area seeking medical attention for similar symptoms but with a final diagnosis unrelated with the disease of interest, and by hospital non-patient visitors to minimize possible selection biases.

Conclusions:

Paper I: Results of this large multi institutional cohort study suggest a possible effect of ACEI and STAT on the progression of BD-IPMNs. Such effect has not been confirmed for ASA and ARB. Further studies are needed to validate such association and shed light on the possible underlining pathophysiological mechanism.

Paper II: Results of this large single institution cohort study suggest a possible association between aspirin use and cancer related death after surgical resection for pancreatic IPMNs. On the contrary no specific influence on cancer related death has been reported for statins and ace inhibitors/sartans use. Further studies are needed to investigate if this association is the result of an underling pathophysiologic mechanism or the result to a common and yet unknown risk factors exposure.

Paper III: Findings of this large multicenter case-control study suggest that non-recent onset diabetes was associated with an increased risk of PNENs occurrence. Our results do not support the view of a strict similarity with factors affecting the risk of pancreatic adenocarcinoma such as the use of insulin and aspirin.

Paper IV: Results of the present study suggests that statin use, rather than that of aspirin, particularly at higher dosages is associated to a reduced PDAC risk. These findings support a chemo-preventive action of statins on PDAC and no apparent synergistic activity of the two medications.

References:

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer research. 2014;74(11):2913-21.
2. European evidence-based guidelines on pancreatic cystic neoplasms. Gut. 2018;67(5):789-804.
3. Crippa S, Pezzilli R, Bissolati M, Capurso G, Romano L, Brunori MP, et al. Active Surveillance Beyond 5 Years Is Required for Presumed Branch-Duct Intraductal Papillary Mucinous Neoplasms Undergoing Non-Operative Management. The American journal of gastroenterology. 2017;112(7):1153-61.
4. Del Chiaro M, Ateeb Z, Hansson MR, Rangelova E, Segersvard R, Kartalis N, et al. Survival Analysis and Risk for Progression of Intraductal Papillary Mucinous Neoplasia of the Pancreas (IPMN) Under Surveillance: A Single-Institution Experience. Annals of surgical oncology. 2017;24(4):1120-6.
5. Tanaka M, Fernandez-Del Castillo C, Kamisawa T, Jang JY, Levy P, Ohtsuka T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2017;17(5):738-53.
6. Dortch JD, Stauffer JA, Asbun HJ. Pancreatic Resection for Side-Branch Intraductal Papillary Mucinous Neoplasm (SB-IPMN): a Contemporary Single-Institution Experience. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2015;19(9):1603-9.
7. Robles EP, Maire F, Cros J, Vullierme MP, Rebours V, Sauvanet A, et al. Accuracy of 2012 International Consensus Guidelines for the prediction of malignancy of branch-duct intraductal papillary mucinous neoplasms of the pancreas. United European gastroenterology journal. 2016;4(4):580-6.
8. Seo N, Byun JH, Kim JH, Kim HJ, Lee SS, Song KB, et al. Validation of the 2012 International Consensus Guidelines Using Computed Tomography and Magnetic Resonance Imaging: Branch Duct and Main Duct Intraductal Papillary Mucinous Neoplasms of the Pancreas. Annals of surgery. 2016;263(3):557-64.
9. Sahora K, Fernandez-del Castillo C, Dong F, Marchegiani G, Thayer SP, Ferrone CR, et al. Not all mixed-type intraductal papillary mucinous neoplasms behave like main-duct lesions: implications of minimal involvement of the main pancreatic duct. Surgery. 2014;156(3):611-21.
10. Pergolini I, Sahora K, Ferrone CR, Morales-Oyarvide V, Wolpin BM, Mucci LA, et al. Long-term Risk of Pancreatic Malignancy in Patients With Branch Duct Intraductal Papillary Mucinous Neoplasm in a Referral Center. Gastroenterology. 2017;153(5):1284-94.e1.
11. Marchegiani G, Mino-Kenudson M, Ferrone CR, Morales-Oyarvide V, Warshaw AL, Lillemoe KD, et al. Patterns of Recurrence After Resection of IPMN: Who, When, and How? Annals of surgery. 2015;262(6):1108-14.
12. Passot G, Lebeau R, Hervieu V, Ponchon T, Pilleul F, Adham M. Recurrences after surgical resection of intraductal papillary mucinous neoplasm of the pancreas: a single-center study of recurrence predictive factors. Pancreas. 2012;41(1):137-41.
13. Fitzgerald TL, Hickner ZJ, Schmitz M, Kort EJ. Changing incidence of pancreatic neoplasms: a 16-year review of statewide tumor registry. Pancreas. 2008;37(2):134-8.
14. Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2008;26(18):3063-72.

15. Arafat HA, Gong Q, Chipitsyna G, Rizvi A, Saa CT, Yeo CJ. Antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. Journal of the American College of Surgeons. 2007;204(5):996-1005; discussion -6.
16. Anandanadesan R, Gong Q, Chipitsyna G, Witkiewicz A, Yeo CJ, Arafat HA. Angiotensin II induces vascular endothelial growth factor in pancreatic cancer cells through an angiotensin II type 1 receptor and ERK1/2 signaling. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2008;12(1):57-66.
17. Bang UC, Watanabe T, Bendtsen F. The relationship between the use of statins and mortality, severity, and pancreatic cancer in Danish patients with chronic pancreatitis. European journal of gastroenterology & hepatology. 2018;30(3):346-51.
18. Lee HS, Lee SH, Lee HJ, Chung MJ, Park JY, Park SW, et al. Statin Use and Its Impact on Survival in Pancreatic Cancer Patients. Medicine. 2016;95(19):e3607.
19. Archibugi L, Arcidiacono PG, Capurso G. Statin use is associated to a reduced risk of pancreatic cancer: A meta-analysis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2018.
20. Jankowski JAZ, de Caestecker J, Love SB, Reilly G, Watson P, Sanders S, et al. Esomeprazole and aspirin in Barrett's oesophagus (AspECT): a randomised factorial trial. Lancet (London, England). 2018;392(10145):400-8.
21. Patrignani P, Patrono C. Aspirin, platelet inhibition and cancer prevention. Platelets. 2018:1-7.
22. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. Cell. 2015;162(6):1257-70.
23. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). European heart journal. 2016;37(3):267-315.
24. Patrignani P, Sacco A, Sostres C, Bruno A, Dovizio M, Piazuelo E, et al. Low-Dose Aspirin Acetylates Cyclooxygenase-1 in Human Colorectal Mucosa: Implications for the Chemoprevention of Colorectal Cancer. Clinical pharmacology and therapeutics. 2017;102(1):52-61.
25. Qiao Y, Yang T, Gan Y, Li W, Wang C, Gong Y, et al. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. BMC cancer. 2018;18(1):288.
26. Nakai Y, Isayama H, Ijichi H, Sasaki T, Sasahira N, Hirano K, et al. Inhibition of renin-angiotensin system affects prognosis of advanced pancreatic cancer receiving gemcitabine. British journal of cancer. 2010;103(11):1644-8.
27. Wilop S, von Hobe S, Crysandt M, Esser A, Osieka R, Jost E. Impact of angiotensin I converting enzyme inhibitors and angiotensin II type 1 receptor blockers on survival in patients with advanced non-small-cell lung cancer undergoing first-line platinum-based chemotherapy. Journal of cancer research and clinical oncology. 2009;135(10):1429-35.
28. Chae YK, Valsecchi ME, Kim J, Bianchi AL, Khemasuwan D, Desai A, et al. Reduced risk of breast cancer recurrence in patients using ACE inhibitors, ARBs, and/or statins. Cancer investigation. 2011;29(9):585-93.
29. Laezza C, Malfitano AM, Proto MC, Esposito I, Gazzerro P, Formisano P, et al. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and of Ras farnesylation mediate antitumor effects of anandamide in human breast cancer cells. Endocrine-related cancer. 2010;17(2):495-503.
30. Duncan RE, El-Sohemy A, Archer MC. Statins and cancer development. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(8):1897-8.

31. Archibugi L, Piciucchi M, Stigliano S, Valente R, Zerboni G, Barucca V, et al. Exclusive and Combined Use of Statins and Aspirin and the Risk of Pancreatic Cancer: a Case-Control Study. Scientific reports. 2017;7(1):13024.
32. Zhang YP, Wan YD, Sun YL, Li J, Zhu RT. Aspirin might reduce the incidence of pancreatic cancer: A meta-analysis of observational studies. Scientific reports. 2015;5:15460.
33. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, et al. Light alcohol drinking and cancer: a meta-analysis. Annals of oncology : official journal of the European Society for Medical Oncology. 2013;24(2):301-8.
34. Capurso G, Falconi M, Panzuto F, Rinzivillo M, Boninsegna L, Bettini R, et al. Risk factors for sporadic pancreatic endocrine tumors: a case-control study of prospectively evaluated patients. The American journal of gastroenterology. 2009;104(12):3034-41.
35. McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, et al. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. The New England journal of medicine. 2018;379(16):1519-28.
36. Takasaki Y, Nagata N, Imbe K, Hisada Y, Sekine K, Tajima T, et al. Effect of low-dose aspirin use on pancreatic cancer development and morphological changes on imaging in IPMN: A long-term cohort study. United European gastroenterology journal. 2017;5(7):1030-6.
37. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DS, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Annals of internal medicine. 2016;164(12):814-25.
38. Mandilaras V, Bouganim N, Yin H, Asselah J, Azoulay L. The use of drugs acting on the renin-angiotensin system and the incidence of pancreatic cancer. British journal of cancer. 2017;116(1):103-8.
39. Karnik SS, Unal H, Kemp JR, Tirupula KC, Eguchi S, Vanderheyden PM, et al. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin Receptors: Interpreters of Pathophysiological Angiotensinergic Stimuli [corrected]. Pharmacological reviews. 2015;67(4):754-819.
40. Dhar VK, Merchant NB, Patel SH, Edwards MJ, Wima K, Imbus J, et al. Does Surgical Margin Impact Recurrence in Noninvasive Intraductal Papillary Mucinous Neoplasms?: A Multi-institutional Study. Annals of surgery. 2018;268(3):469-78.
41. Ben Q, Zhong J, Fei J, Chen H, Yv L, Tan J, et al. Risk Factors for Sporadic Pancreatic Neuroendocrine Tumors: A Case-Control Study. Scientific reports. 2016;6:36073.
42. Halfdanarson TR, Bamlet WR, McWilliams RR, Hobday TJ, Burch PA, Rabe KG, et al. Risk factors for pancreatic neuroendocrine tumors: a clinic-based case-control study. Pancreas. 2014;43(8):1219-22.
43. Hassan MM, Phan A, Li D, Dagohoy CG, Leary C, Yao JC. Family history of cancer and associated risk of developing neuroendocrine tumors: a case-control study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008;17(4):959-65.
44. Hassan MM, Phan A, Li D, Dagohoy CG, Leary C, Yao JC. Risk factors associated with neuroendocrine tumors: A U.S.-based case-control study. International journal of cancer. 2008;123(4):867-73.
45. Zhan HX, Cong L, Zhao YP, Zhang TP, Chen G. Risk factors for the occurrence of insulinoma: a case-control study. Hepatobiliary & pancreatic diseases international : HBPD INT. 2013;12(3):324-8.
46. De Souza A, Khawaja KI, Masud F, Saif MW. Metformin and pancreatic cancer: Is there a role? Cancer chemotherapy and pharmacology. 2016;77(2):235-42.
47. Simon MS, Desai P, Wallace R, Wu C, Howard BV, Martin LW, et al. Prospective analysis of association between statins and pancreatic cancer risk in the Women's Health Initiative. Cancer causes & control : CCC. 2016;27(3):415-23.

48. Kho PF, Fawcett J, Fritschi L, Risch H, Webb PM, Whiteman DC, et al. Nonsteroidal anti-inflammatory drugs, statins, and pancreatic cancer risk: a population-based case-control study. 2016;27(12):1457-64.
49. Walker EJ, Ko AH, Holly EA, Bracci PM. Statin use and risk of pancreatic cancer: results from a large, clinic-based case-control study. Cancer. 2015;121(8):1287-94.
50. Carey FJ, Little MW, Pugh TF, Ndokera R, Ing H, Clark A, et al. The differential effects of statins on the risk of developing pancreatic cancer: a case-control study in two centres in the United Kingdom. Digestive diseases and sciences. 2013;58(11):3308-12.
51. Hermann PC, Sancho P, Canamero M, Martinelli P, Madriles F, Michl P, et al. Nicotine promotes initiation and progression of KRAS-induced pancreatic cancer via Gata6-dependent dedifferentiation of acinar cells in mice. Gastroenterology. 2014;147(5):1119-33.e4.
52. Hill AB. THE ENVIRONMENT AND DISEASE: ASSOCIATION OR CAUSATION? Proceedings of the Royal Society of Medicine. 1965;58:295-300.

Study 1: Presented as oral presentation at UEGW2018

Effect of Aspirin, ACE Inhibitors/Sartans and Statins use on the progression of BD-IPMN in follow up: a multicenter study

Roberto Valente[1,2], Stefano Crippa[3], Urban Arnelo[3], Giuseppe Vanella[2], Giulia Zerboni[2], Laura Zarantonello[1],Alessandro Fogliati[3], Massimo Falconi[3], Gabriele Capurso*[2] and Marco del Chiaro*[1]

Pancreatic Disease Unit, Karolinska University Hospital, CLINTEC, Stockholm, Sweden

Digestive and Liver Disease Unit, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy

Pancreatic Surgery Unit, Pancreas Translational and Clinical Research Centre, San Raffaele Scientific Institute, 'Vita-Salute' University, Milan Italy

**authors contribute equally to the writing of manuscript*

Corresponding author:

Marco Del Chiaro, MD, PhD, FACS
Professor of Surgery
Chief of Surgical Oncology
Director of the Hepato-Pancreato-Biliary Program
University of Colorado Anschutz Medical Campus | Department of Surgery
12631 E. 17th Avenue, C-313 | Aurora, CO 80045

Email: marco.delchiaro@ucdenver.edu

Introduction:

Pancreatic cancer is expected to become the third cause of cancer related death within 2030.(1) In the last decades, the spread use of cross-sectional imaging and the progressive quality improvement of abdominal imaging, has led to a more frequent detection of pancreatic cystic neoplasm, whose actual prevalence is estimated to be around 45%. (2, 3) Intrapapillary mucinous neoplasms (IPMNs) represent circa half of these lesions and are increasingly considered as possible precursor lesions of pancreatic cancer. (4)

According to the isolated, combined or absent cystic involvement of the main pancreatic duct are divided into main duct (MD), mixed type(MT) and branch duct (BD) IPMNs. (5)

If patient is fit for surgery, the presence of mixed type or main duct IPMN represents a clear surgical indication. A more selective approach has been proposed instead for BD IPMNs, which harbor a lower, despite not inexistent, lifetime risk of pancreas cancer occurrence (approximately 8-10%). (3, 6, 7)

Several authors and different guidelines have suggested a lifelong follow-up with MRI/MRCP and/or EUS for all BD-IPMNs patients "potentially fit for surgery".(2-5) Considering the high prevalence of BD-IPMNs (around 20%), the relative low incidence of pancreas cancer and the need for a life-long follow-up, questions have been risen on cost-effectiveness of such a strategy for health care systems.

The long-term follow-up of BD-IPMNs will probably represent in the upcoming future, from a logistic and economic point of view, one of the main challenges for the development of wide scale screening programs for the early detection and treatment of pancreas cancer.

So far, no effective chemoprevention is available to slow or prevent the progression of BD-IPMNs. Aspirin (ASA), Ace Inibitors/Sartans (ACEI/ARB) and Statins (STAT) are among the most used drugs for primary and secondary cardiovascular prevention. Aspirin, is a selective and irreversibly acetylator of prostaglandin (PG)G/H-synthases (COX-1 and COX-2), that blocks the catalytic reaction that converts arachidonic acid into PGG2 and PGH2 and therefore thromboxane (TX)A2 resulting in anti-

inflammatory, analgesic, antipyretic and antiplatelet effects. (8) COX-2 expression and platelets activation seems to contribute to development, progression and metastatic potential of cancer, through locoregional release of a variety of cell growth, pro-angiogenic and pro inflammatory mediators, while PGE2 seems to be implied into immunological escapes from Interferon and/or T-cell-dependent cancer killing.(9) Beside the well-known cardiovascular protective effect (10), aspirin has been recently proved to play a major role in inhibit cancerogenesis, acting on several pathways (mTOR, EGFR, 15-PGDH) (11) Observational studies support the idea that aspirin use is associated with a significant decrease in the risk of overall and site-specific cancers.(12)

Angiotensin I-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are widely diffused antihypertensive drugs for their property to inhibit the activation of systemic renin–angiotensin system (RAS).(13) Angiotensin II, which represents a cornerstone in such a cascade, has shown to harbor proangiogenic properties by acting on the vascular endothelial growth factor (VEGF), MAP Kinases and G-protein cascades with potential major implications on cancer management, as suggested for lung, breast and pancreas cancer.(13-15)

Statins, are inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase and used as cholesterol lowering agents in the setting of both primary and secondary cardiovascular prophylaxis. Statins seem to act a regulation on several intracellular pathways such as Ras, MEK, mTOR, BCL-2 and Rho kinases, that also play a major role in cancer development. Recently their possible role in decreasing the incidence of cancer has been suggested in epidemiological studies and meta-analysis. (16-18)

Therefore despite Aspirin (ASA), Ace Inibitors/Sartans (ACEI/ARB) and Statins (STAT) have shown to inhibit tumoral growth in several clinical and preclinical models, including pancreatic cancer (PDAC),(19-24) their possible role in IPMNs has never been investigated. (25)

Aim: to evaluate the possible effect of ASA, ACEI/ARB and STAT used for cardiovascular prevention on the progression of BD-IPMN in follow-up.

Materials and method: Multicenter, retrospective cohort study. Patients were collected at Karolinska University Hospital in Stockholm Sweden, Sant' Andrea Hospital, Sapienza University of Rome, Rome Italy and San Raffaele Vita Salute Hospital, Milan Italy.

Inclusion Criteria: BD-IPMNs patients without "*ab initio"* surgical indications and undergoing radiological follow-up according to current guidelines at the participating centers. Clinical- and radiological characteristics as well as exposure to the target drugs were collected and analyzed in relation to the progression of BD-IPMN.

Data collection: Prospectively collected data about medical history, family history of cancer and exposures were extracted by a specific created retrospective database at Sant 'Andrea Hospital and San Raffaele Hospital. Eventual missing data were filled as possible by re-contacting patients. A specific IT database (®*Take Care)* with in real time updated inpatient and outpatient clinic charts was consulted for data collection in Sweden.

Exclusion criteria at diagnosis:

- presence of mural nodules
- main pancreatic duct (MPD) diameter ≥5 mm
- cyst diameter ≥40 mm

Exposures:

The exposure to several potential risk factors for the development of pancreas cancer were considered:

Smoking habit: participants were considered as being ever smokers when reporting to have smoked for a period above 6 months or a quantity superior to 100 cigarettes.

Alcohol drinking: the cut-off to be considered a regular ever drinker was a daily intake of at least 12.5 g of alcohol for at least one year. Such quantity is approximately equal to either one glass of wine, one shot of hard liquor or one pint of beer. Participants were then classified as ever drinkers or nondrinkers.

General risk factors for pancreas cancer: A 1st degree family history of pancreatic cancer, the presence of symptoms, the cyst's mean diameter and a diagnosis of diabetes were specifically recorded. Recent onset diabetes was defined as that which occurrence was in the 12 months prior to diagnosis of BD-IPMN.

Target drugs exposure: The previous use of drugs such as aspirin (ASA), ace inhibitors AND/OR Sartans (ACEI/ARB), exclusive ace inhibitors (ACEI), exclusive Sartans (ARB), statins (STAT), insulin and metformin has been recorded and analyzed. For STAT, ACEI and ARB a further distinct analysis was performed sub-dividing different drugs within the same class. For ASA a distinction between low and high dose was considered. Considering that many of these drugs are often prescribed together in patients displaying high cardiovascular risk profile, beside the evaluation of single class effect, we also considered the potential summative effect resulting from the simultaneous treatment with more than one drug. We therefore analyzed several possible combinations of the studied drugs.

Definition of outcomes:

Definition of outcomes: progression during follow up defined as:

-dimensional progression: increasing of cyst maximum diameter of at least 2 mm between two follow-up imaging

-clinical significant progression: appearance of mural nodules and/or increasing in MPD diameter ≥ 5 mm and/or the occurrence of cancer and/or increasing of cyst maximum diameter above 40 mm.

-any-progression: indiscriminate appearance of either dimensional and/or clinical significant progression and increased number of cystic lesions.

Statistical Analysis: Patients exposed and non-exposed to the target drugs or displaying possible risk factors for pancreas cancer were compared by chi-square test and fisher test with Yates correction for categorical variables; Student's t test or long Rank test for continuous ones. Significant variables were further analyzed by sex and age adjusted univariable and multivariable logistic regression analysis. The effect of the target drugs over the time was evaluated through univariable and multivariable Cox hazard regression analysis (by enter selection procedure). The 95% confidence interval (95% CI) was calculated where possible and all p-values were two-sided and considered statistically significant when $p<0.05$. A dedicated statistical software (MedCald Mariakerke, Belgium) was used.

Results

Patients' Characteristics

A total of 594 patients with BD-IPMN without mural nodules, suspect of main duct involvement and cyst dimension above 40 mm were recruited at the three participating centers (62.28% at Karolinska University Hospital, 23.73% at Sant 'Andrea Hospital and 13.9% at San Raffaele Hospital). The mean age 64.84 years (63.95-65.73; 95% CI), 38.38% were male. During a mean follow-up of 44.78 months (42.01-47.54; 95% CI), 46.86% of patients displayed progression (any), 41.32% displayed dimensional progression (mean size increasing: 7.77 mm), 7.74% displayed a clinically significant progression.

Thirty-two-point forty-six percent of patients had a previous use of ASA, 31.9% of STAT and 43.39% of ACEI/ARB. Among this last group, 30.86% of patients were exclusive users of ACEI, while 45.67% were exclusive users of ARB. Some of the patients were exposed to multiple drugs belonging to the same class and some to association of the different classes of drugs. Patients characteristics and a more detailed report of drug use are summarized in **Table 1**.

Past medical history:

Multifocal of BD IPMN (73.3% vs 56.8% p=0.03), a history of diabetes (31.8% vs 16.5%; p=0.01) and especially newly onset diabetes (18.2% vs 4.8% p=0.06) were all factors significantly associated to higher rates of clinically relevant progression **Table 2**.

Drug exposures:

Insulin use was significantly associated to higher rates of both dimensional and clinically significant progression (respectively: 12.3% vs 6.8%, p=0.02 and 22.7% vs 7.9%, p=0.0009). At sex and age adjusted univariable Cox proportional hazard regression analysis insulin was confirmed to be associated to an increased risk of clinically significant progression: HR= 3.07 (95%CI: 1.49 to 6.30; p=0.002).

At sex and age adjusted univariable Cox proportional hazard regression analysis the exclusive use of ACEI was confirmed to be associated to a decreased risk of dimensional progression: HR= 0.65 (0.45-0.94; 95%CI; p=0.02) and any progression: HR= 0.64 (0.45-0.90; 95%CI; p= 0.01). The exclusive use of ARB was instead confirmed to be associated to an increased risk of clinically significant progression: HR= 1.94 (95%CI: 1.00-3.76; p=0.04) **Figure 1b**.

At sex and age adjusted univariable Cox proportional hazard regression analysis the use of statins was significantly associated to decreased risk of dimensional progression (HR= 0.72; 0.54-0.97 95%CI; p=0.03) **Table 2**.

We performed several models of sex and age adjusted multivariable Cox proportional hazard regression analysis. In a first model the use of ACEI (HR=0.70; 95% CI 048.1.01; p=0.06) and STAT (HR= 0.67; 95%CI 0.49-0.93; p=0.01) was associated to a decreased risk to display dimensional progression, while the use of Insulin was associated to a statistically significant higher risk of dimensional progression (HR 1.65; 95% CI 1.09-2.51; p=0.01.

In a second model, the use of ACEI (HR=0.69; 95%CI 0.48-0.98, p=0.04) and STAT (HR= 0.72=0.53- 0.76; 95% CI, p=0.03) was associated to a decreased risk of (any) progression, while the use of Insulin was associated to an increased risk of (any) progression Insulin (HR= 1.49; 95% CI 0.99-2-24; p=0.05).

In a third model the use of Insulin (HR=2.97; 95% CI 1.34-6.55; p=0.006) but not ARB (HR 1.41; 95% CI 0.69-2.87; p=0.34) was statistically associated to clinically relevant progression **Figure 1c**.

Discussion:

The increasing of pancreatic IPMNs detection will probably represent in the next future the only possibility to address pancreas cancer at early or ideally pre-invasive stage. Technical improving and spreading of cross sectional imaging has led to increased detection of such lesions. If, from an oncological point of view, MD-IPMNs and MT-IPMNs are the most challenging, because have a risk of pancreatic cancer respectively in up to 91% (26-28) and 70% (29)of cases, from a decisional point of view, they are not. In fact, whenever the patient is fit for surgery, he/she encounter a surgical indication.(2, 5) The situation is more complex for BD-IPMNs that display 15% risk of progression at 3-5 years (2) and harbor 8-10% lifetime risk for pancreas cancer. (7) In BD-IPMNs, which represent by far, the majority of IPMN lesions in the general population, a more conservative approach has been

suggested though lifelong follow up with MRI/MRCP and/or EUS until the patient is fit for surgery. (3, 4) The economic counterpart of such strategy will unavoidably imply an excess of costs for health care systems that will probably be economically not sustainable. In the next future, two possible strategies might overcome the problem: on one hand a better detection of the lesions at risk for progression. This will allow the optimizing of resources by continuing/intensifying follow up in a more targeted way. The other possible strategy will be the application of possible chemoprevention, able to slow down and, ideally, to stop the progression on IPMNs. While the first strategy has been addressed by several authors and guidelines and is still largely matter of debate, the second aspect has poorly been investigated so far.

Takasaki et al. in the only paper on the issue, investigated the effect of aspirin in a cohort of BD-IPMNs in follow up suggesting ,after a median 5.5 years follow-up, a possible effect of low dose aspirin in slowing MPD dilation over the time (4.8% vs 12.7%, p=0.02). In such study, no specific investigation was performed regarding the use of STAT or ACEI/ARB nor their possible associations.(25)

So far, the current study represents the first paper investigating such pharmacological exposures on BD-IPMNs. In addition, although several papers suggested an active role of ACEI/ARB in inhibiting cancer initiation and progression in several cancer models, none of them investigated the exclusive effect of the two classes of drugs, nor the effect of different molecules among the same class of drug.(13, 30)

In the current study, the use of ACEI and STAT was associated with lower rates of dimensional progression and overall progression (any). Such effect remained significant even after correction for other possible risk factors for pancreas cancer (such as diabetes, smoking, first degree family history of pancreas cancer), some of which in possible overlap as risk factors also for cardiovascular disease. Interestingly the lowering of dimensional and any progression in STAT users was in line with the risk reduction of pancreas cancer elsewhere described.(23) Anyway no significantly effect has been shown on clinically relevant progression.

The use of ARB use was associated with an increased risk for clinically significant progression at sex and age adjusted cox hazard regression analysis, but the data was not confirmed in the multivariate model after correction for possible risk factors. Thus, such association has still to be confirmed even because the absence of statistical significance, might be related to the absence of statistical power for such outcome.

The main breakthrough of such work is the identification of different clinical effects for the exclusive use of ACEI and ARB, generally pooled together when considering their clinical effect, although acting on different point of the renin-angiotensin-aldosterone cascade.

In fact, ACEI act on angiotensin I converting enzyme (ACE1), which is an enzyme upstream the entire cascade and converting circulating angiotensin I into angiotensin II (AngII).

Ang II is then the main effector of the cascade, acting either on Ang II receptor 1 (AT1), which is the target of ARB, and on receptor II (AT2), which is not. In addition, AngII through multiple intermediate mediators, such as ang III, ang IV, ang 1-7 acts even on other different pathways, through different receptors such as AT2, AT4, MAS. Therefore, while the blocking of ACE1 by ACEI might inhibit the entire cascade, the selective blocking of AT1, might instead imply escape mechanisms with retrograde shifting of the cascade towards other mediators and receptors. The exact activity of such other receptors is still matter of debate and has not be fully elucidated, but some of them are implied in the regulation of the neovascularization of myocardium infarction through a VEGF pathway, with possible activity even on different intracellular kinases. These pathways are important mediators in neoplastic initiation and progression and might be at least one of the molecular mechanism justifying of our results **(Figure3)**.(31)

In opposition to several observational studies on pancreas cancer, in the current study ASA use was not significantly associated with a slower progression. On the other hand, questioning have been risen regarding the real effect of ASA on cancer, since recently a large trial shown a higher incidence of cancer in patients treated with aspirin arm compared to controls group. (32)

It is also unclear whether the potential anti neoplastic effect of ASA could be a long-term rather than immediate effect. (33)As previously suggested by some authors the effect of aspirin might be balanced by confounding drugs such as statins, since often these two drugs are co-prescribed. (18)

Beside some limitations, such as the absence of a preliminary power calculation, the retrospective design and the absence on correlation with final histology, this study displays some strengths such as the relatively large sample size, the inclusions of patients from high volume centers assuring high quality of data, the investigation of several pharmacological, previously uninvestigated, factors on a high prevalence disease, with potential major implications for health care policies.

Despite a possible heterogeneity in modalities of data collection among Italian and Swedish cohort, in two third of patients the extraction of data from a specific IT system, constantly updated, gave clinicians direct access to inpatient's/outpatient's clinical charts and prescriptions, therefore minimizing possible recall biases.

In conclusion the results of this large multi institutional cohort study suggest a possible effect of ACEI and STAT on the progression of BD-IPMNs. Such effect has not been confirmed for ASA and ARB. Further studies are needed to validate such association and shed light on the possible underlining pathophysiological mechanism.

Acknowledgements

The present study was supported by Cancerfonden, Sweden. (CAN 2014/634 and CAN 2014/621 and ALF Medicine 2016 #20150113)

Table 1 Characteristics of BD-IPMN patients in follow up. ***ASA**: Aspirin users (ever);** **ACE**: Ace Inhibitors only users (ever);§ **ARB**: Sartans only users (ever);§§ **STAT**: Statins users (ever);# **ACEI/ARB**: Ace Inhibitors AND/OR Sartans users (ever);## **plus** : concomitant use

Characteristic	**N (%)- (95% CI)**
Patients	594
Number of male	228/594 (38.38)
Mean Age (years)	64.84 (63.95–65.73; 95% CI)
Mean follow-up (months)	44.78 (42.01–47.54; 95% CI)
Smoking	156/579 (26.94)
1st degree family history of PDAC	27/582 (4.63)
Diabetes	102/579 (17.61)
Alcohol (ever)	136/579 (23.48)
Recent onset diabetes	10/102 (9.80)
Mean Cyst Diameter (mm)	15.1 (14.42-15.85; 95% CI)
Multifocal Disease	344/592 (58.10)
Symptomatic	104/592 (17.56)
Progression (any)	277/591 (46.86)
Dimensional Progression	243/588 (41.32)
Mean dimensional increase (mm)	7.77 (6.82–8.72; 95% CI)
Clinically significant progression	46/594 (7.74)
ASA Low dose (<160mg/die)	186/573 (32.46) 170/186 (91.39)
ACEI/ARB: ACEI: -Enalapril -Perindopril -Captopril -Lisinopril -Ramipril - Others ARB: -Telmisartan -Olmesartan -Combisartan -Valsartan -Candesartan -Irbesartan -Losartan UNSPECIFIED: Multiple ARB ACE plus ARB	243/560 (43.39) 75/243 (30.86) 70/110 (63.63) 4/110 (3.63) 1/110 (0.90) 3/110 (2.72) 30/110 (27.27) 2/110 (1.81) 111/243 (45.67) 6/156 (3–84) 6/156 (3.84) 2/156 (1.28) 6/156 (3.84) 56/156 (35.89) 16/156 (10.25) 64/156 (41.02) 22/243 (9.05) 10/243 (4.11) 35/243 (14.40)
ASA plus ACEI/ARB plus STAT ASA plus ACEI/ARB ASA plus STAT ASA plus ARB plus STAT ASA plus ARB ARB plus STAT	82/558 (14.69) 122/559 (21.82) 114/571 (19.96) 47/536 (8.76) 71/537 (13.22) 81/537 (15.08)
STAT Simvastatin Atorvastatin Pravastatin Rosuvastatin Multiple Unknown	183/594 (30.80) 112/208 (53.84) 56/208 (26.92) 5/208(2.40) 17/208 (8.17) 25/208 (12.01) 18/208 (8.65)

Features	Progression (any): Yes/No	Progression (dimension): Yes/No	Significant progression:Yes/No
Acute pancreatitis	13 (4.7) vs 14 (4.5) p=0.88	8 (3.3) vs 19 (5.5) p=0.21	2 (4.4) vs 25 (4.6) p=1.0
1st degree FH PDAC	16 (5.9) vs 11 (3.6) p=0.20	15 (6.2) vs 12 (3.6) p=0.13	1 (2.3) vs 26 (4.8) p=0.71
Multifocal	166 (60.4) vs 176 (56.2) p=0.31	151 (62.4) vs 188 (54.8) p=0.06	**33 (73.3) vs 310 (56.8) p=0.03**
Smoking	67 (24.8) vs 88 (28.9) p=0.27	58 (24.2) vs 97 (29.0) p=0.22	13 (30.2) vs 142 (26.5) p=0.59
Alcohol	59 (21.9) vs 76 (24.9) p=0.38	51 (21.4) vs 84 (25.1) p=0.31	11 (25.6) vs 124 (23.2) p=0.72
Diabetes	49 (17.9) vs 52 (17.3) p=0.84	44 (18.2) vs 57 (17.2) p=0.76	**14 (31.8) vs 88 (16.5) p=0.01**
New Onset Diabetes	6 (7.9) vs 4 (4.0) p=0.26	5 (7.7) vs 5 (4.5) p=0.50	**2 (18.2) vs 8 (4.8) p=0.06**
Insulin	30 (11.2) vs 21 (7.1) p=0.09	**29 (12.3) vs 22 (6.8) p=0.02**	**10 (22.7) vs 41 (7.9) p=0.0009**
Metformin	25 (9.3) vs 37 (12.5) p=0.21	22 (9.3) vs 41 (12.6) p=0.21	3 (6.8) vs 60 (11.5) p=0.34
ASA	86 (32.2) vs 98 (32.5) p=0.95	75 (32.1) vs 108 (32.5) p=0.90	17 (39.5) vs 168 (31.8) p=0.29
Low dose ASA	78 (92.9) vs 90 (96.8) p=0.23	68 (91.9) vs 100 (97.1) p=0.12	16 (100.0) vs 153 (94.4) p=0.33
ACEI /ARB	116 (44.4) vs 124 (42.0) p=0.56	103 (44.8) vs 136 (42.0) p=0.51	**27 (62.8) vs 215 (41.7) p=0.007**
ACEI	**41 (16.6) vs 68 (23.6) p=0.04**	37 (16.8) vs 72 (23.0) p=0.08	7 (18.4) vs 103 (20.6) p=0.74
Enalapril	29 (29.0) vs 41 (36.0) p=0.27	26 (28.6) vs 44 (36.1) p=0.25	4 (19.0) vs 66 (34.0) p=0.16
Perindopril	0 (0) vs 4 (3.4) p=0.12	0 (0) vs 4 (3.2) p=0.13	0 (0) vs 4 (2.0) p=1.0
Captopril	1 (1.0) vs 0 (0) p=0.46	1 (1.1) vs 0 (0) p=0.42	0 (0) vs 1 (0.5) p=1.0
Lisinopril	1(1.0) vs 3 (2.7) p=0.62	1 (1.1) vs 3 (2.5) p=0.63	0 (0) vs 4 (2.1) p=1.0
Ramipril	10 (10.0) vs 19 (16.5) p=0.22	9 (9.9) vs 20 (16.3) p=0.17	2 (9.5) vs 28 (14.4) p=0.74
ARB	**80 (32.4) vs 65 (22.6) p=0.01**	**74 (33.6) vs 70 (22.4) p=0.0039**	**17 (44.7) vs 128 (25.7) p=0.01**
Telmisartan	4 (3.9) vs 2 (1.7) p=0.42	3 (3.2) vs 3 (2.4) p=0.70	0 (0) vs 6 (3.0) p=1.0
Olmesartan	1 (1.0) vs 5 (4.3) p=0.21	1 (1.1) vs 5 (4.0) p=0.24	0 (0) vs 6 (3.0) p=1.0
Combisartan	2 (2.0) vs 0 (0) p=0.21	2 (2.2) vs 0 (0) p=0.17	0 (0) vs 2 (1.0) p=1.0
Valsartan	5 (5.0) vs 1 (0.9) p= 0.10	**5 (5.4) vs 1 (0.8) p=0.08**	1 (4.8) vs 5 (2.6) p=0.46
Candesartan	24 (24.0) vs 31 (27.7) p=0.54	23 (25.3) vs 32 (26.7) p=0.82	4 (19.0) vs 51 (26.6) p=0.60
Irbesartan	10 (10.1) vs 6 (5.3) p=0.18	9 (10.0) vs 7 (5.8) p=0.25	5 (23.8) vs 11 (5.7) p=0.01
Losartan	**43 (44.3)vs 21 (18.8);p=0.0001**	**40 (44.9) vs 23 (19.3) p=0.0001**	9 (42.9) vs 55 (29.1) p=0.19
STAT	81 (30.3) vs 101 (33.4) p=0.42	67 (28.6) vs 114 (34.2) p=0.15	**20 (46.5) vs 162 (30.6) p=0.03**
Simvastatine	54 (21.3) vs 57 (19.9) p=0.68	44 (19.7) vs 66 (21.0) p=0.71	**14 (34.1) vs 97 (19.4) p=0.02**

Atorvastatine	19 (7.5) vs 36 (12.6) p=0.05	17 (7.6) vs 37 (11.8) p=0.11	5 (12.2) vs 50 (10.0) p=0.59
Pravastatine	4 (1.6) vs 1 (0.3) p=0.13	3 (1.3) vs 2 (0.6) p=0.65	1 (2.4) vs 4 (0.8) p=0.32
Rosuvastatine	6 (2.3) vs 11 (3.8) p=0.33	6 (2.6) vs 11 (3.4) p=0.60	1 (2.4) vs 16 (3.1) p=1.0
ASA plus ACEI/ARB	57 (21.8) vs 63 (21.4) p=0.90	52 (22.6) vs 68 (21.1) p=0.66	14 (32.6) vs 107 (20.8) p=0.07
ASA plus STAT	46 (17.2) vs 67 (22.3) p=0.12	41 (17.5) vs 72 (21.8) p=0.21	13 (30.2) vs 100 (19.0) p=0.07
ACEI/ARB plus STAT	61 (23.4) vs 66 (22.4) p=0.79	53 (23.0) vs 73 (22.6) p=0.90	**17 (39.5) vs 110 (21.4) p=0.006**
ASA plus ACEI/ARB plus STAT	35 (13.4) vs 46 (15.7) p=0.44	32 (13.9) vs 49 (15.2) p=0.66	**11 (25.6) vs 70 (13.6) p=0.03**
ASA plus ARB plus STAT	24 (9.7) vs 22 (7.7) p=0.40	22 (10) vs 24 (7.7) p=0.35	**7 (18.4) vs 39 (7.8) p=0.02**
ASA plus ARB	**39 (15.8) vs 31 (10.8) p=0.08**	**36 (16.4) vs 34 (10.9) p=0.06**	**9 (23.7) vs 61 (12.2) p=0.04**
ARB plus STAT	**45 (18.2) vs 35 (12.2) p=0.05**	**40 (18.2) vs 39 (12.5) p=0.06**	**12 (31.6) vs 68 (13.7) p=0.002**

Table 2: Association between patient's features/exposure and the progression of the IPMN at Chi-square/ Fisher test

Figure 1 a) Sex and age adjusted univariable Cox proportional hazard regression analysis for drug exposure

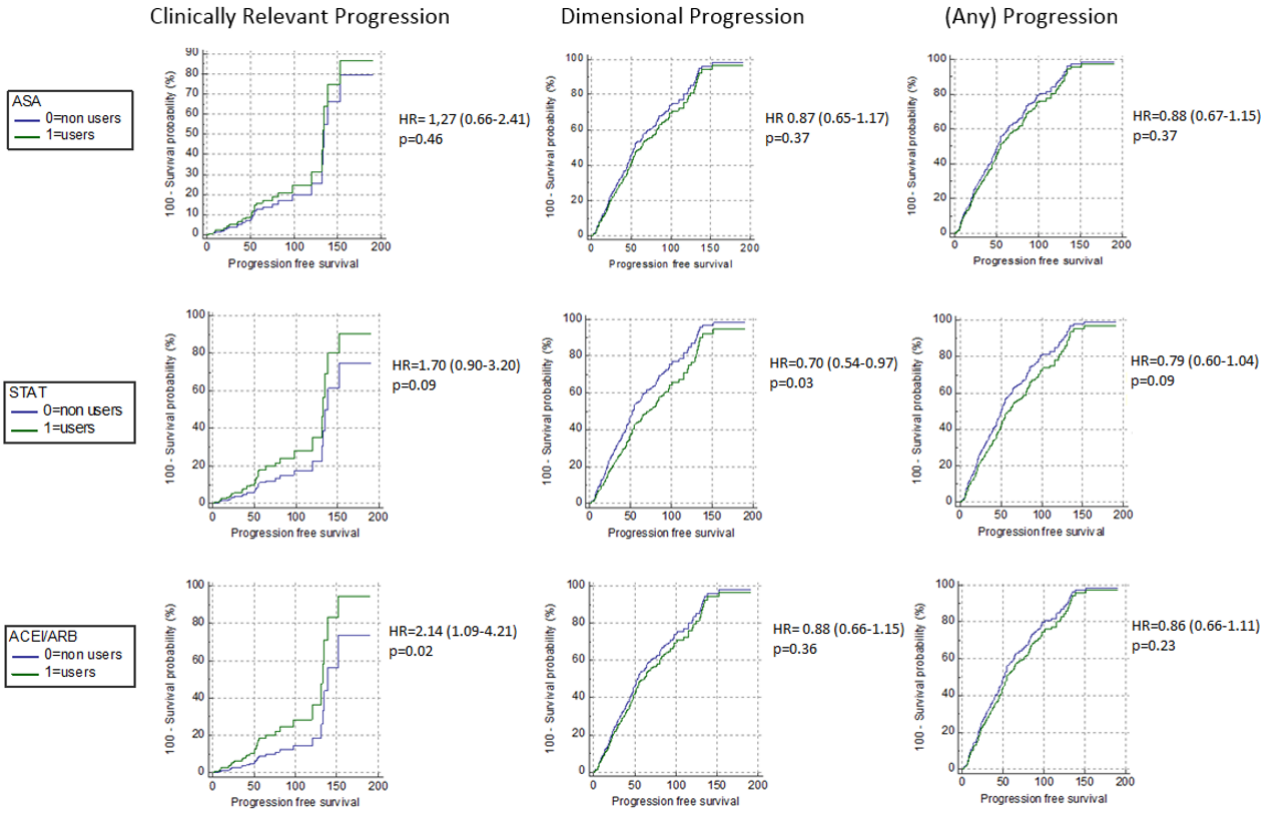


Figure 1 b) Sex and age adjusted univariable Cox proportional hazard regression analysis for the exclusive use of ACEI and ARB

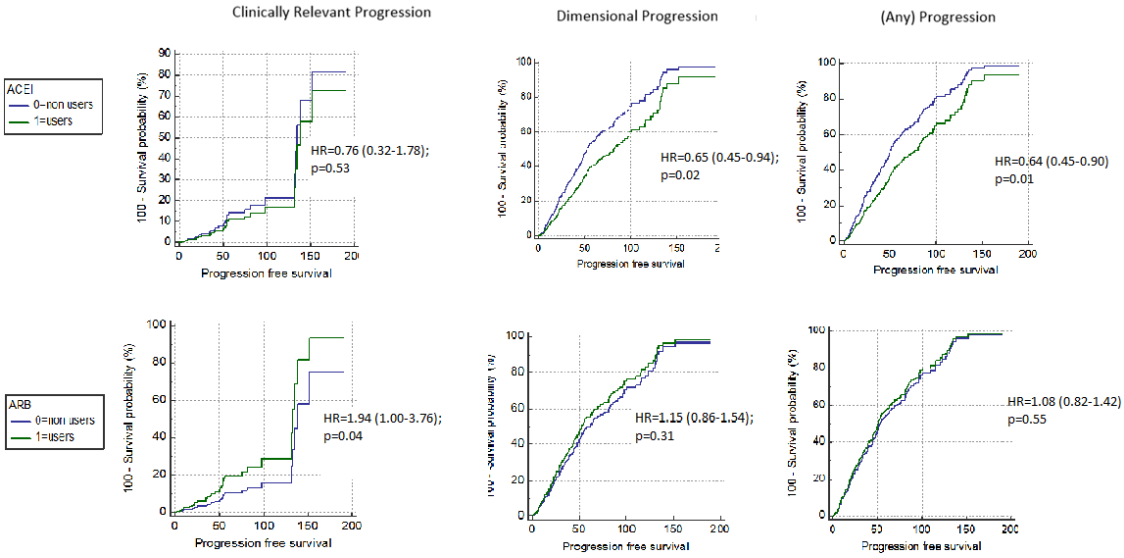
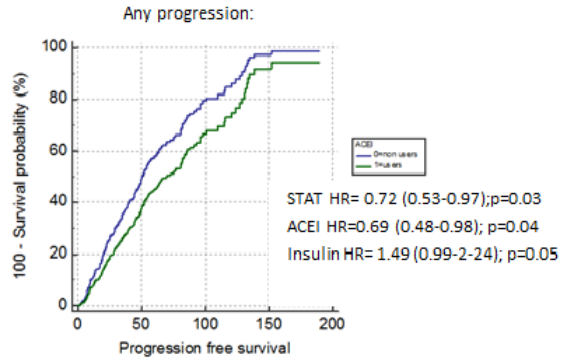
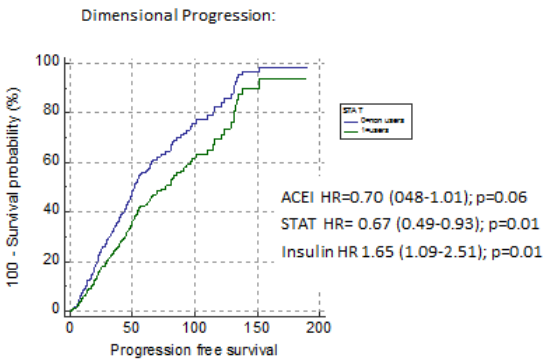


Figure 2 a) Sex and age adjusted multivariable Cox proportional hazard regression analysis for pharmacological exposure





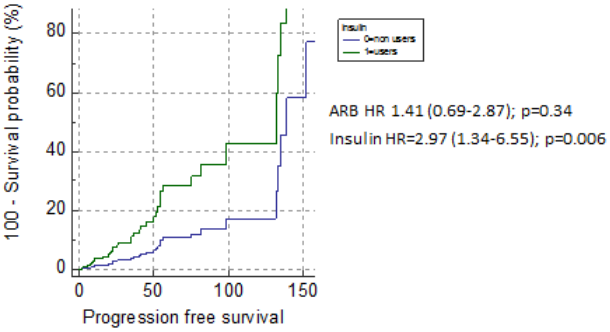
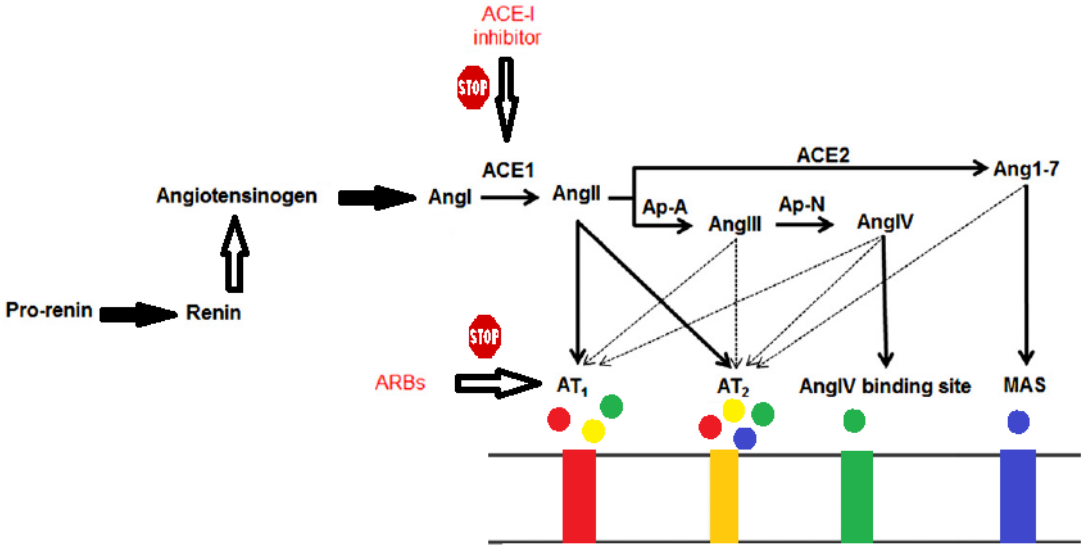


Figure 3: The renin angiotensin aldosterone axis and its blockers.



Bibliography

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer research. 2014;74(11):2913-21.
2. European evidence-based guidelines on pancreatic cystic neoplasms. Gut. 2018;67(5):789-804.
3. Crippa S, Pezzilli R, Bissolati M, Capurso G, Romano L, Brunori MP, et al. Active Surveillance Beyond 5 Years Is Required for Presumed Branch-Duct Intraductal Papillary Mucinous Neoplasms Undergoing Non-Operative Management. The American journal of gastroenterology. 2017;112(7):1153-61.
4. Del Chiaro M, Ateeb Z, Hansson MR, Rangelova E, Segersvard R, Kartalis N, et al. Survival Analysis and Risk for Progression of Intraductal Papillary Mucinous Neoplasia of the Pancreas (IPMN) Under Surveillance: A Single-Institution Experience. Annals of surgical oncology. 2017;24(4):1120-6.
5. Tanaka M, Fernandez-Del Castillo C, Kamisawa T, Jang JY, Levy P, Ohtsuka T, et al. Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2017;17(5):738-53.
6. Morales-Oyarvide V, Mino-Kenudson M, Ferrone CR, Warshaw AL, Lillemoe KD, Sahani DV, et al. Intraductal Papillary Mucinous Neoplasm of the Pancreas in Young Patients: Tumor Biology, Clinical Features, and Survival Outcomes. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2018;22(2):226-34.
7. Pergolini I, Sahora K, Ferrone CR, Morales-Oyarvide V, Wolpin BM, Mucci LA, et al. Long-term Risk of Pancreatic Malignancy in Patients With Branch Duct Intraductal Papillary Mucinous Neoplasm in a Referral Center. Gastroenterology. 2017;153(5):1284-94.e1.
8. Patrignani P, Patrono C. Aspirin, platelet inhibition and cancer prevention. Platelets. 2018:1-7.
9. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-Dependent Tumor Growth through Evasion of Immunity. Cell. 2015;162(6):1257-70.
10. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). European heart journal. 2016;37(3):267-315.
11. Patrignani P, Sacco A, Sostres C, Bruno A, Dovizio M, Piazuelo E, et al. Low-Dose Aspirin Acetylates Cyclooxygenase-1 in Human Colorectal Mucosa: Implications for the Chemoprevention of Colorectal Cancer. Clinical pharmacology and therapeutics. 2017;102(1):52-61.
12. Qiao Y, Yang T, Gan Y, Li W, Wang C, Gong Y, et al. Associations between aspirin use and the risk of cancers: a meta-analysis of observational studies. BMC cancer. 2018;18(1):288.
13. Nakai Y, Isayama H, Ijichi H, Sasaki T, Sasahira N, Hirano K, et al. Inhibition of renin-angiotensin system affects prognosis of advanced pancreatic cancer receiving gemcitabine. British journal of cancer. 2010;103(11):1644-8.
14. Wilop S, von Hobe S, Crysandt M, Esser A, Osieka R, Jost E. Impact of angiotensin I converting enzyme inhibitors and angiotensin II type 1 receptor blockers on survival in patients with advanced non-small-cell lung cancer undergoing first-line platinum-based chemotherapy. Journal of cancer research and clinical oncology. 2009;135(10):1429-35.
15. Chae YK, Valsecchi ME, Kim J, Bianchi AL, Khemasuwan D, Desai A, et al. Reduced risk of breast cancer recurrence in patients using ACE inhibitors, ARBs, and/or statins. Cancer investigation. 2011;29(9):585-93.

16. Laezza C, Malfitano AM, Proto MC, Esposito I, Gazzerro P, Formisano P, et al. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and of Ras farnesylation mediate antitumor effects of anandamide in human breast cancer cells. Endocrine-related cancer. 2010;17(2):495-503.
17. Duncan RE, El-Sohemy A, Archer MC. Statins and cancer development. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005;14(8):1897-8.
18. Archibugi L, Piciucchi M, Stigliano S, Valente R, Zerboni G, Barucca V, et al. Exclusive and Combined Use of Statins and Aspirin and the Risk of Pancreatic Cancer: a Case-Control Study. Scientific reports. 2017;7(1):13024.
19. Arafat HA, Gong Q, Chipitsyna G, Rizvi A, Saa CT, Yeo CJ. Antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. Journal of the American College of Surgeons. 2007;204(5):996-1005; discussion -6.
20. Anandanadesan R, Gong Q, Chipitsyna G, Witkiewicz A, Yeo CJ, Arafat HA. Angiotensin II induces vascular endothelial growth factor in pancreatic cancer cells through an angiotensin II type 1 receptor and ERK1/2 signaling. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2008;12(1):57-66.
21. Bang UC, Watanabe T, Bendtsen F. The relationship between the use of statins and mortality, severity, and pancreatic cancer in Danish patients with chronic pancreatitis. European journal of gastroenterology & hepatology. 2018;30(3):346-51.
22. Lee HS, Lee SH, Lee HJ, Chung MJ, Park JY, Park SW, et al. Statin Use and Its Impact on Survival in Pancreatic Cancer Patients. Medicine. 2016;95(19):e3607.
23. Archibugi L, Arcidiacono PG, Capurso G. Statin use is associated to a reduced risk of pancreatic cancer: A meta-analysis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2018.
24. Jankowski JAZ, de Caestecker J, Love SB, Reilly G, Watson P, Sanders S, et al. Esomeprazole and aspirin in Barrett's oesophagus (AspECT): a randomised factorial trial. Lancet (London, England). 2018;392(10145):400-8.
25. Takasaki Y, Nagata N, Imbe K, Hisada Y, Sekine K, Tajima T, et al. Effect of low-dose aspirin use on pancreatic cancer development and morphological changes on imaging in IPMN: A long-term cohort study. United European gastroenterology journal. 2017;5(7):1030-6.
26. Dortch JD, Stauffer JA, Asbun HJ. Pancreatic Resection for Side-Branch Intraductal Papillary Mucinous Neoplasm (SB-IPMN): a Contemporary Single-Institution Experience. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract. 2015;19(9):1603-9.
27. Robles EP, Maire F, Cros J, Vullierme MP, Rebours V, Sauvanet A, et al. Accuracy of 2012 International Consensus Guidelines for the prediction of malignancy of branch-duct intraductal papillary mucinous neoplasms of the pancreas. United European gastroenterology journal. 2016;4(4):580-6.
28. Seo N, Byun JH, Kim JH, Kim HJ, Lee SS, Song KB, et al. Validation of the 2012 International Consensus Guidelines Using Computed Tomography and Magnetic Resonance Imaging: Branch Duct and Main Duct Intraductal Papillary Mucinous Neoplasms of the Pancreas. Annals of surgery. 2016;263(3):557-64.
29. Sahora K, Fernandez-del Castillo C, Dong F, Marchegiani G, Thayer SP, Ferrone CR, et al. Not all mixed-type intraductal papillary mucinous neoplasms behave like main-duct lesions: implications of minimal involvement of the main pancreatic duct. Surgery. 2014;156(3):611-21.
30. Mandilaras V, Bouganim N, Yin H, Asselah J, Azoulay L. The use of drugs acting on the renin-angiotensin system and the incidence of pancreatic cancer. British journal of cancer. 2017;116(1):103-8.
31. Karnik SS, Unal H, Kemp JR, Tirupula KC, Eguchi S, Vanderheyden PM, et al. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin Receptors: Interpreters of

Pathophysiological Angiotensinergic Stimuli [corrected]. Pharmacological reviews. 2015;67(4):754-819.
32. McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, et al. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. The New England journal of medicine. 2018;379(16):1519-28.
33. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DS, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Annals of internal medicine. 2016;164(12):814-25.

Study 2: unpublished data

Clinical and pharmacological factors associated with cancer related death in operated IPMN patients

Roberto Valente[1,2], Zeeshan Ateeb[1], Gabriele Capurso[2], Chiara Maria Scandavini[1], Francesca Vespasiano[1], Giuseppe Anzillotti[1], Johannes Matthias Löhr[1], Urban Arnelo[1] and Marco Del Chiaro[3]

[1] Pancreatic Disease Unit, Karolinska University Hospital, Stockholm, Sweden.

[2] Sapienza University of Rome, Rome, Italy.

[3] Department of Surgery, University of Colorado Anschutz Medical Campus, Denver, USA

Short Title: Factors associated with cancer recurrence in operated IPMN patients

Corresponding Author

Marco Del Chiaro, MD, PhD, FACS

Professor of Surgery

Chief of Surgical Oncology

Director of the Hepato-Pancreato-Biliary Program

University of Colorado Anschutz Medical Campus | Department of Surgery

12631 E. 17th Avenue, C-313 | Aurora, CO 80045

Email: marco.delchiaro@ucdenver.edu

Specific authors contribution

Zeeshan Ateeb collected the data and contributed to write the manuscript, Roberto Valente contributed to write the manuscript and performed the statistical analysis. Chiara Maria Scandavini, Francesca Vespasiano, Giuseppe Anzillotti, Johannes Matthias Löhr and Urban Arnelo critically revised the manuscript. Roberto Valente, Gabriele Capurso and Marco Del Chiaro were responsible of the study design and contributed to write the paper.

Financial support

The present study was supported by Cancerfonden, Sweden. (CAN 2014/634 and CAN 2014/621 and ALF Medicine 2016 #20150113)

Potential competing interest

None

Introduction: pancreas cancer is expected to become the 2nd leading cause of cancer related death within 2030.(1) Its precursor lesions, intrapapillary mucinous neoplasms (IPMNs), are increasingly recognized because of the spread use of cross sectional imaging and have been reported to be as prevalent as 20% in general population.(2, 3)

IPMNs harboring high-grade dysplasia/cancer represent a target for surgery but the long term their post-surgical prognosis is still largely impacted by rates of locoregional and distant recurrences.(3)

Several authors have identified possible risk factors for recurrence after surgery in IPMNs such as family history for pancreas cancer and the grade of dysplasia in the resected specimen. (4-6) So far, no specific chemoprevention has been shown to prevent or slow down post-surgical recurrence and therefore cancer related death in operated IPMNs patients.

Ace inhibitors/sartans (ACEI/ARB), statins (STAT) and Aspirin (ASA), widely used in the setting of primary and secondary cardiovascular prevention, seem also to play a role in modulating incidence and prognosis of different type of tumors.(7-9)

Angiotensin I-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are inhibitors at different levels of the renin–angiotensin aldosterone cascade displaying powerful anti-hypertensive properties. (9, 10) Beside its cardiovascular action, angiotensin II has been suggested to possibly influence the activity of important pro-angiogenetic regulators such as vascular endothelial growth factor (VEGF), MAP Kinases and several intracellular G-protein. This might therefore eventually result even in potential secondary effect on cancer growth, as suggested for different clinical models in lung, breast and pancreas cancer. (8-14)
The inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, also known as statins (STAT) and employed as cholesterol lowering agents, have been associated

to a possible decreased incidence of cancer in several epidemiological studies and meta-analysis, possibly because their ability to act on intracellular kinase pathways (Ras, MEK, mTOR, BCL-2 and Rho). (8)

Aspirin, is a selective and irreversible inhibitor of COX-1 and COX-2. Beside its well-known anti-inflammatory, analgesic, antipyretic and antiplatelet effects, aspirin seems also to inhibit cancer spread and progression by modulating cell growth, angiogenesis and inflammation, especially in colorectal cancer. (15, 16) Several intracellular molecular pathways, such as mTOR, EGFR, 15-PGDH, have been suggested to explain aspirin pleiotropic effects (17). In several observational studies aspirin seems to be associated with an overall lower risk of cancer occurrence and mortality(12). As a matter of facts, anyway recent evidences questioned about the existence of a real chemo-preventive effect in the first 10 year of treatment, suggesting that aspirin might display its chemo preventive effect on colorectal cancer from 10 to 19 years after the initiation of therapy. (12) In addition, a recent trial from the ASPREE group published on the New England Journal of Medicine, has highlighted as aspirin use is associated to overall increased risk of cancer mortality. Such increase in cancer related mortality was anyway not confined to any specific cancer location or pathologic type.(18)

Although the exact mechanism has not been elucidated so far, clinical and preclinical models have increasingly suggested a possible action of ACEI/ARB and STAT in inhibiting cancer development and spreading while the real chemo preventive effect of ASA, that used to be given for granted, has recently questioned and deserves further studies to be confirmed.

Interestingly, although several studies have been focusing on pancreas cancer(8, 10, 19, 20), none of them has specifically investigated the effect of such drugs on the prognosis of IPMNs

considered its precursor lesions. More specifically no study has investigated possible effects of such drugs to influence the rate of cancer related death after surgery with curative intent.

Aim: to evaluate the effect of aspirin, ace inhibitors/sartans and statins alone or in association, on cancer related death in a cohort of operated IPMNs patients

Materials and Methods:

Study design

A single-center hospital-based retrospective cohort study on prospectively collected patients with surgically resected IPMNs of the pancreas. Ethical committee approval was obtained (EPN 2015/1544-31/4). Demographics, hereditary factors, clinical and pharmacological history were collected and analyzed.

Cohort and Population's Characteristics

Consecutive cases of histologically verified IPMNs who had undergone surgery for radiological suspect of high grade IPMNs according to the European Guidelines for the management of cystic tumors of the pancreas (from October 2011).(2) The date of surgery was considered as date of diagnosis. All patients were discussed in a multidisciplinary conference before surgery.

Definition of the exposure: Patients were investigated for ever use of aspirin ace inhibitors, sartans and statins used alone or in combination as well as for exposure to known risk factors for pancreas cancer. Data regarding demographics, known risk factors for pancreatic cancer (hereditary, smoking, alcohol, overweight/obesity) as well as final pathological assessment (degree of dysplasia, presence of cancer, TNM, specific histological phenotype), and exposure to the target drugs was retrospectively collected. A specific Swedish electronic database

(®*Take Care)* with in time updated inpatient and outpatient clinic charts was consulted for data collection.

Outcome definition: Overall survival was defined as the interval of time between surgery and the date of analysis if patient was alive or between diagnosis and cancer related death in case he/she died during follow-up due to metastatic disease or locoregional recurrence.

All patients in which death was not related to cancer, were excluded from survival analysis as a censored data, to specifically analyze the possible effect of investigated factors/drugs on cancer related survival.

Inclusions criteria:

Consecutive patients who have undergone surgery at *Karolinska University Hospital* because suspect malignified IPMN according to the European Guidelines for the Management of Pancreatic Cystic Neoplasms.

Exclusion criteria:

- The presence of a synchronous pancreatic cancer, cholangiocarcinoma or high grade neuroendocrine tumor which could influence the final prognosis.
- The presence of surgical margins at final histology showing cancer or high-grade dysplasia (**Figure 1**)

Statistical analysis

Categorical variables were analyzed through chi-square, continuous variable through Students t-test. Statistically significant variables were further analyzed in sex and age adjusted univariate and multivariate logistic regression analysis. Sex and age adjusted univariable and

multivariable logistic regression analysis was used to evaluate the possible association between cancer related death and known risk factors for pancreas cancer and sex and age adjusted univariable and multivariable cox hazard regression analysis was used to identify associations between pharmacological exposures and cancer related death. The 95% confidence interval (CI) was calculated. All p-values were two-sided and a $p<0.05$ was considered statistically significant. A statistical software package was used for data analysis (MedCalc Mariakerke, Belgium).

Results:

Patient characteristics

Between 2008 and 2017, 274 IPMN patients were operated at Pancreatic Disease Unit, Karolinska University Hospital for suspect malignified IPMN. Two hundred ten, mean age 70.55 years (67.60-73.50; 95% CI), 47.61% male, were included in the final analysis. Inclusion criteria are summarized in **Figure1** and patients' characteristics are summarized in **Table 1**.

Clinical known risk factors for pancreas cancer:

Fifty patients (23.80%) were smokers and among them 66% displayed active smoking. First-degree family history of pancreatic cancer was present in 3.8% of patients. Diabetes was present in 19.52% of patients and in 2.43% diabetes was of early onset (diagnosed within the latest 12 months before surgery). Multifocal disease was present in 43.8% of patients, preoperatively increased levels of Ca19.9 in 32.66%, while 34.05% of patients had a mean diameter of mean cyst above 40 mm. Mean MPD diameter was 7.64 (6.88-8.39; 95% CI).

Significantly higher percentage of patients with preoperative jaundice, increased Ca19.9 and cyst diameter above 40 mm died for cancer (respectively: 36.4 vs 9.8%, p=0.0005; 76.2% vs 27.6%, p<0.0001; 62.5% vs 30.0%, p=0.05). **Table 2**

At sex and age adjusted univariable logistic regression analysis preoperative jaundice and preoperative increased level of Ca19.9 were associated with increased risk of cancer related death (respectively OR =4.99, 95% CI 1.80-13.87, p=0.02 and OR= 8.75, 95% CI 2.96-25.87, p=0.001. **Table 3a**

At sex and age adjusted multivariable logistic regression analysis, preoperative increased level of Ca19.9 remained consistent with increased risk of cancer related death OR=7.31, 95% CI 2.40-22.21, p=0.0004. **Table 3b**

A previous or current use (ever use) of aspirin, ace inhibitors/sartans and statins was present respectively in 29.18%, 51.90% and 36.84% of cases.

ASA ever users displayed significantly higher rates of cancer related death 50.0% vs 27.4% p= 0.03. Sex and age adjusted univariable cox hazard regression analysis ASA users displayed a borderline significantly higher risk of cancer related death HR 2.11 (0.90-4.94, 95% CI, p=0.08). The risk of cancer related death was not statistically significant different among exclusive STAT and exclusive ACEI/ARB users (respectively HR= 0.77, 95% CI 0.32-1.89, p=0.58 and HR= 1.18, 95% CI 0.49-2.82, p=0.69) or their combinations. (**Table and Figure 2a)**

At multivariable cox hazard regression analysis adjusted for sex, age, clinical factors associated with both pancreas cancer and cardiovascular risk (such as smoking, diabetes, first degree family history, overweight/obesity and elevated Ca19.9) ASA users were confirmed to have higher risk of cancer related death (HR=2.70, 95% CI 1.10-6.59, p=0.02). **Figure 2b**

Discussion:

This is the first paper analyzing the effect of potentially chemo-preventive agents on cancer related death in a cohort of operated IPMNs. In the past years, huge body of literature has been focusing on the role of different clinical features in predicting cancer or invasive IPMNs "recurrence" in the remnant pancreas.(4-6, 21) A first interesting point to underline is the choice of the main outcome, which in our case was cancer related death instead of "IPMN recurrence".

In fact, being IPMNs a field defect in which by definition the whole gland is involved, when considering post-surgical outcomes, it does seem more appropriate to use as a primary outcome cancer related death instead of "recurrence", since the latter would imply post-surgical absence of residual disease, which is namely incorrect in the case of IPMNs. (21)

This also in the light that pancreas cancer recurrence is associated to cancer death in virtually almost 100% of cases. In the current study, heterogeneity of our cohort in terms of resected specimen (ranging from absence of dysplasia to invasive IPMNs), is largely counterbalanced by the strict inclusion of patients undergone surgery with curative intent. For that purpose, all patients with positive margins for high grade dysplasia and/or cancer, synchronous pancreas cancer and/or periampullary tumors, potentially influencing prognosis, were excluded from our analysis.

On the other hand, we do think that such heterogeneity of resected specimens, might be more representative of "real world" population undergoing surgery for suspect malignified IPMN. Therefore, it did specifically serve our aim of investigating the effect of such drugs on definitive prognosis of patients undergone surgery. In the current study, STAT use was associated with 23% reduction of cancer related death. Although not statistically significant, it is interesting to

note how the data is in line with what reported in other epidemiological studies and meta-analysis. (8, 19) In addition, the absence of statistically significance, might be related to the absence of statistical power for such outcome.

The current paper shows no statistically significant effect of ACEI/ARB in influencing cancer related death, both when pooling together the use of ACEI and/or ARB and when considered it separately, as exclusive use of ACEI or ARB (data not shown).

Surprisingly, and in contradiction to several observational studies, that have previously shown a potential anti-cancer effect(7), in the current study, ASA was associated to an increased risk of cancer related death. On the other hand, a recent trial has questioned these previously ascertained knowledges, by showing in a large cohort of patients undergone circa 5 years follow up, an increased incidence of cancer in aspirin arm compared to controls group. (18)

In addition, during the last years, several papers suggested that the potential benefits of ASA might represent a long-term effect, visible after at least 10 years of exposure to the drug.(12)

In a large case control study, Archibugi et al.(19) reported have shown that the combined use of ASA and STAT was not superior to the exclusive use of STAT in reducing the risk of pancreatic cancer. Therefore, the association between ASA use and pancreas cancer occurrence‘s reduction, might also be secondary to overlaps with other drugs such as STAT which seldom have been investigated separately as potential protective/risk factor.

In this respect, in the present study, the association between ASA use and STAT use was not significantly associated with higher risk of cancer related death as shown in **table 2a**.

The exact molecular mechanism behind that has not been clarified yet and deserves further investigations. ASA use maintained its association with cancer related death, even when

corrected for the presence of possible known risk factors for pancreas cancer, such as smoking, diabetes overweight/obesity, who might also increase cardiovascular risk and therefore need primary or secondary cardiovascular prevention with ASA. Further studies are needed to specifically investigate whether such association display a real pathophysiological background or whether it's the result of the exposure to a common risk factor for both pancreas cancer and cardiovascular disease.

In the current study the presence of a pre-operative elevated value of Ca19.9 was the only factor in sex and age adjusted logistic and cox hazard regression multivariable analysis associated with an increased risk of cancer related death.

This is not surprising and is in line with what recommended in the European evidence based guidelines for the management of cystic tumors, which identified in increased Ca19.9 levels a possible predictor of cancer occurrence.(3)

The present study displays several strengths as well as limitations. Inherent with its design, our study displays as main limitation the absence of a preliminary power calculation. The strength of the study is represented by the relatively large sample size, the investigation of several clinical factors possibly implicated in progression of IPMNs after surgery and, for the first time, the evaluation of pharmacological exposures possibly influencing cancer occurrence and prognosis. The inclusions criteria were clearly defined and aiming at identifying prognostic factors in a population treated with curative intent. Data were extracted by IT system, constantly updated and giving clinicians access to inpatient's/outpatient's clinical charts and prescriptions, therefore minimizing possible recall biases.

In conclusion the results of this large single institution cohort study suggest a possible association between aspirin use and cancer related death after surgical resection for

pancreatic IPMNs. On the contrary no specific influence on cancer related death has been reported for statins and ace inhibitors/sartans use. Further studies are needed to investigate if this association is the result of an underling pathophysiologic mechanism or the result to a common and yet unknown risk factors exposure.

Acknowledgements

The present study was supported by Cancerfonden, Sweden. (CAN 2014/634 and CAN 2014/621 and ALF Medicine 2016 #20150113)

Figure 1: Patients' inclusion flow chart

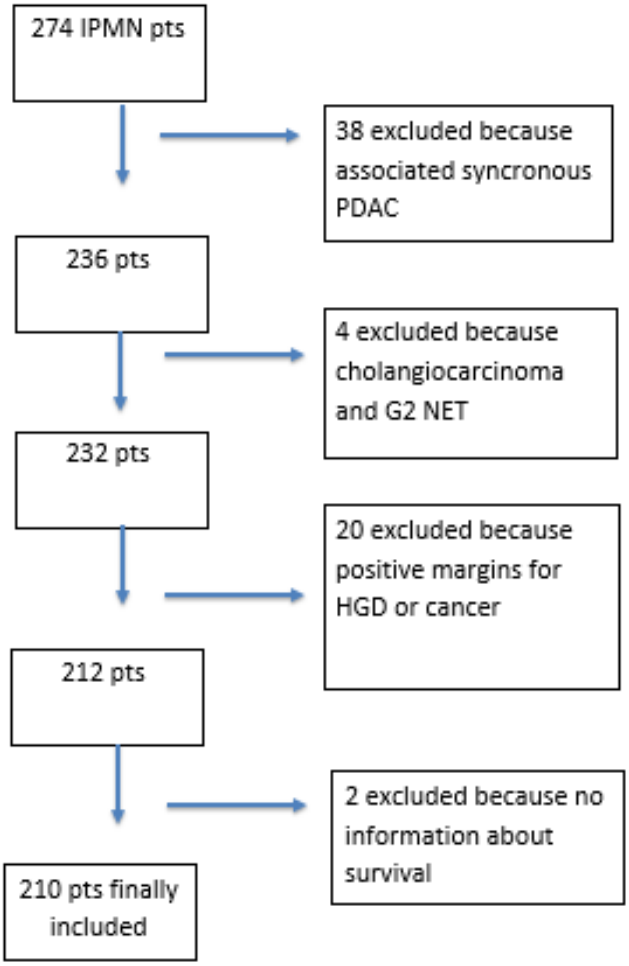


Table 1 Patients demographic, disease morphology and operative characteristics

Characteristic	**N/mean (%; 95%CI)**
Patients	210
Male	100/210 (47.61)
Age	70.55 (67.60-73.50; 95% CI)
Mean OS (months)	37.60 (33.76-41.43; 95%CI)
Dead	51/210 (24.24)
Cancer related death	22/210 (10.47)
Mean BMI	25.73 (25.12-26.38; 95% CI)
Obesity	18/210 (8.57)
Overweight	41/210 (19.52)
Smokers	50/210 (23.80)
Current smokers	33/50 (66.0)
1st degree FH PDAC	8/210(3.80)
Diabetes	41/210 (19.52)
Recent Onset Diabetes	1/41 (2.43)
Jaundice	26/210 (12.38)
Abdominal pain	42/210 (20.0)
Weight loss	32/210 (15.23)
Acute pancreatitis	23/210 (10.95)
Incidental diagnosis	100/209 (47.84)
Multifocal	92/210 (43.80)
Mean MPD diameter (mm)	7.64 (6.88-8.39; 95% CI)
MPD diameter 5-9.9 mm	89/210 (42.38)
MPD diameter ≥10 mm	50/210 (23.80)
Max Cystic Size (mm)	33.93 (30.23-37.62; 95%CI)
Cyst Size ≥4 cm in BD-IPMNs	47/138 (34.05)
Mural nodules	16/209 (7.65)
Increased Ca19.9	65/199 (32.66)
Pancreaticoduodenectomy	97/210 (46.19)
Distal Pancreatectomy	66/210 (31.42)
Total Pancreatectomy	39/210 (18.57)
Atypical resections	8/210 (3.80)
Margin status:	
No dysplasia	64/171 (37.42)
Low grade of dysplasia	107/171 (62.57)
NA (total pancreatectomy)	39/210 (18.57)
ASA	61/209 (29.18)
ACEI/ARB	109/210 (51.90)
STAT	77/209 (36.84)
ASA plus ACEI/ARB	46/209 (22.00)
ASA plus STAT	42/208 (20.19)
ACEI/ARB plus STAT	58/209 (27.75)
ASA plus ACEI/ARB plus STAT	32/208 (15.38)
Final histology:	
Cancer	60/210 (28.57)
IPMN with high grade dysplasia	44/210 (20.95)
IPMN with low grade dysplasia	106/210 (50.47)

Table 2 IPMNs features and patient's exposure possibly associated to cancer relater death at fisher or chi square test

Features/exposures	**Cancer related death vs alive patient**	**p value**
Smoking	5 (22.7) vs 37 (22.6)	0.98
1st degree FH PDAC	0 (0) vs 8 (4.9)	0.29
Diabetes	4 (18.2) vs 32 (19.5)	0.88
Preoperative Jaundice	**8 (36.4) vs 16 (9.8)**	**0.0005**
Abdominal pain	4 (18.2) vs 35 (21.3)	0.73
Weight loss	5 (22.7) vs 23 (14.0)	0.28
Incidental diagnosis	7 (31.8) vs 79 (48.5)	0.14
Head location	10 (45.5) vs 48 (29.3)	0.12
Preoperative Increased Ca19.9 (≥37 UI/L)	**16 (76.2) vs 43 (27.6)**	**<0.0001**
Multifocal IPMNs	6 (27.3) vs 76 (46.3)	0.09
Positive Margin for dysplasia	12 (85.70) vs 87 (63.00)	0.09
Preoperative presence of mural nodules	1 (4.5) vs 14 (8.6)	0.51
maximum diameter 5-9.9 mm	10 (45.5) vs 71 (43.3)	0.84
maximum diameter ≥10 mm	6 (27.3) vs 37 (22.6)	0.62
Preoperative Cyst Size ≥4 cm	**5 (62.5) vs 33 (30.0)**	**0.05**
ASA use (ever)	**11 (50.0) vs 45 (27.4)**	**0.03**
STAT use (ever)	8 (36.4) vs 62 (38.0)	0.87
ACEI use (ever)	7 (31.8) vs 33 (20.1)	0.21
ARB use (ever)	4 (18.2) vs 41 (25.0)	0.48
ACE/ARB use (ever)	13 (59.1) vs 82 (50.0)	0.42
ASA plus STAT use (ever)	6 (27.3) vs 34 (20.9)	0.49
ASA plus ACEI/ARB use (ever)	6 (27.3) vs 36 (22.0)	0.57
ASA plus ACEI/ARB plus STAT use (ever)	5 (22.7) vs 26 (16.0)	0.42
ACEI/ARB plus STAT use (ever)	6 (27.3) vs 47 (28.8)	0.87

Table 3 Sex and age adjusted univariate and multivariable logistic regression analysis for the assessment of possible associations between clinical features and the occurrence of cancer related death.

Clinical features	**Univariable OR (95%CI)**	**Multivariable OR (95%CI)**
Jaundice	**4.99 (1.80-13.87); p=0.02**	2.57 (0.82-8.04); p=0.10
Increased Ca19.9 (≥37 UI/L)	**8.75 (2.96-25.87); p=0.001**	**7.31 (2.40-22.21); p=0.0004**
Preoperative Cyst Size ≥4 cm	4.43 (0.96-20.30); p=0.05	

Figure 2a Sex and age adjusted univariable cox hazard regression analysis for the assessment of possible associations between pharmacological exposure and the occurrence of cancer related death

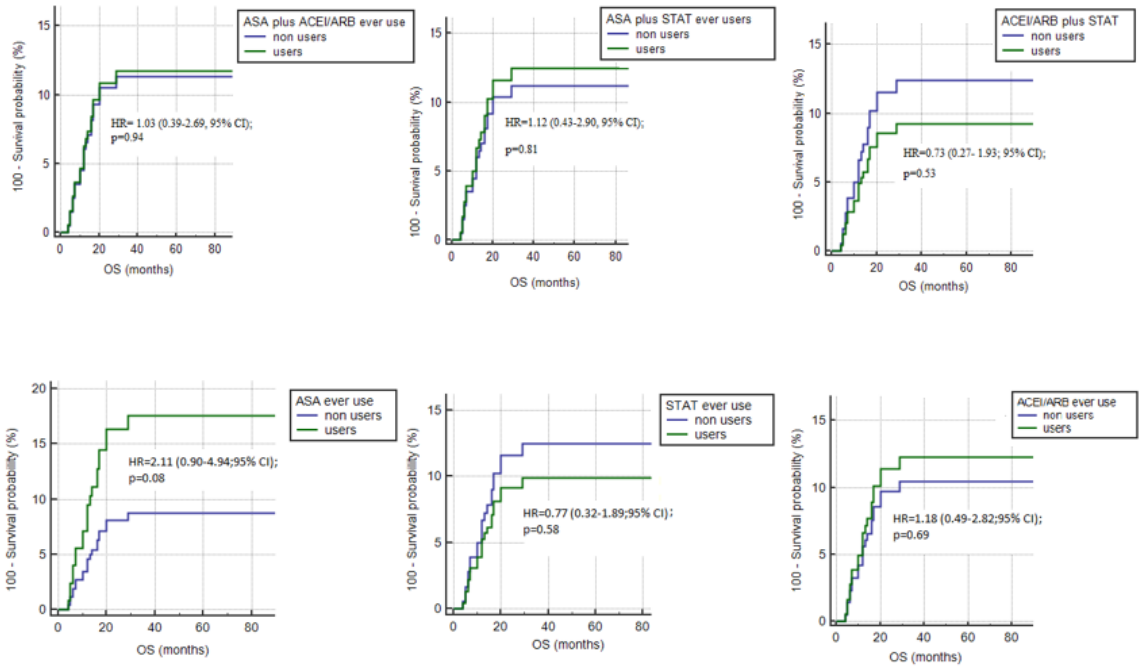
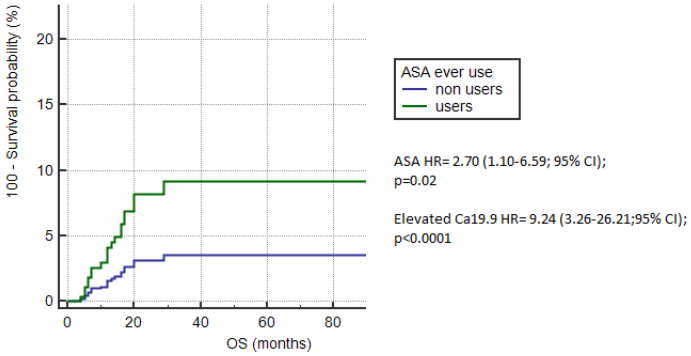


Figure 2b Multivariable cox hazard regression analysis for the assessment of possible associations between aspirin exposure and the occurrence of cancer related death, adjusted for sex, age, clinical factors associated with pancreas cancer (smoking, diabetes, first degree family history, overweight/obesity, elevated Ca19.9)



References

1. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer research. 2014;74(11):2913-21.

2. Del Chiaro M, Verbeke C, Salvia R, Kloppel G, Werner J, McKay C, et al. European experts consensus statement on cystic tumours of the pancreas. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2013;45(9):703-11.

3. European evidence-based guidelines on pancreatic cystic neoplasms. Gut. 2018;67(5):789-804.

4. Marchegiani G, Mino-Kenudson M, Ferrone CR, Morales-Oyarvide V, Warshaw AL, Lillemoe KD, et al. Patterns of Recurrence After Resection of IPMN: Who, When, and How? Annals of surgery. 2015;262(6):1108-14.

5. Tamura K, Ohtsuka T, Ideno N, Aso T, Shindo K, Aishima S, et al. Treatment strategy for main duct intraductal papillary mucinous neoplasms of the pancreas based on the assessment of recurrence in the remnant pancreas after resection: a retrospective review. Annals of surgery. 2014;259(2):360-8.

6. Passot G, Lebeau R, Hervieu V, Ponchon T, Pilleul F, Adham M. Recurrences after surgical resection of intraductal papillary mucinous neoplasm of the pancreas: a single-center study of recurrence predictive factors. Pancreas. 2012;41(1):137-41.

7. Streicher SA, Yu H, Lu L, Kidd MS, Risch HA. Case-control study of aspirin use and risk of pancreatic cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2014;23(7):1254-63.

8. Archibugi L, Arcidiacono PG, Capurso G. Statin use is associated to a reduced risk of pancreatic cancer: A meta-analysis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2018.

9. Arafat HA, Gong Q, Chipitsyna G, Rizvi A, Saa CT, Yeo CJ. Antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. Journal of the American College of Surgeons. 2007;204(5):996-1005; discussion -6.

10. Nakai Y, Isayama H, Ijichi H, Sasaki T, Sasahira N, Hirano K, et al. Inhibition of renin-angiotensin system affects prognosis of advanced pancreatic cancer receiving gemcitabine. British journal of cancer. 2010;103(11):1644-8.

11. Mandilaras V, Bouganim N, Yin H, Asselah J, Azoulay L. The use of drugs acting on the renin-angiotensin system and the incidence of pancreatic cancer. British journal of cancer. 2017;116(1):103-8.

12. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DS, et al. Aspirin for the Prevention of Cancer Incidence and Mortality: Systematic Evidence Reviews for the U.S. Preventive Services Task Force. Annals of internal medicine. 2016;164(12):814-25.

13. Chae YK, Valsecchi ME, Kim J, Bianchi AL, Khemasuwan D, Desai A, et al. Reduced risk of breast cancer recurrence in patients using ACE inhibitors, ARBs, and/or statins. Cancer investigation. 2011;29(9):585-93.

14. Wilop S, von Hobe S, Crysandt M, Esser A, Osieka R, Jost E. Impact of angiotensin I converting enzyme inhibitors and angiotensin II type 1 receptor blockers on survival in patients with advanced non-small-cell lung cancer undergoing first-line platinum-based chemotherapy. Journal of cancer research and clinical oncology. 2009;135(10):1429-35.

15. Patrignani P, Patrono C. Aspirin, platelet inhibition and cancer prevention. Platelets. 2018:1-7.

16. Patrono C. Low-dose aspirin in primary prevention: cardioprotection, chemoprevention, both, or neither? European heart journal. 2013;34(44):3403-11.

17. Guillem-Llobat P, Dovizio M, Bruno A, Ricciotti E, Cufino V, Sacco A, et al. Aspirin prevents colorectal cancer metastasis in mice by splitting the crosstalk between platelets and tumor cells. Oncotarget. 2016;7(22):32462-77.

18. McNeil JJ, Nelson MR, Woods RL, Lockery JE, Wolfe R, Reid CM, et al. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. The New England journal of medicine. 2018;379(16):1519-28.

19. Archibugi L, Piciucchi M, Stigliano S, Valente R, Zerboni G, Barucca V, et al. Exclusive and Combined Use of Statins and Aspirin and the Risk of Pancreatic Cancer: a Case-Control Study. Scientific reports. 2017;7(1):13024.

20. Nakai Y, Isayama H, Sasaki T, Mizuno S, Sasahira N, Kogure H, et al. Clinical outcomes of chemotherapy for diabetic and nondiabetic patients with pancreatic cancer: better prognosis with statin use in diabetic patients. Pancreas. 2013;42(2):202-8.

21. Dhar VK, Merchant NB, Patel SH, Edwards MJ, Wima K, Imbus J, et al. Does Surgical Margin Impact Recurrence in Noninvasive Intraductal Papillary Mucinous Neoplasms?: A Multi-institutional Study. Annals of surgery. 2018;268(3):469-78.

Risk and protective factors for the occurrence of sporadic pancreatic endocrine neoplasms

Roberto Valente[1], Alastair J Hayes[2], Sven-Petter Haugvik[3], Per Hedenström[4], Darko Siuka[5], Emilie Korsæth[3], Daniel Kämmerer[6], Stuart M Robinson[7], Patrick Maisonneuve[8], Gianfranco Delle Fave[1], Bjorn Lindkvist[4] and Gabriele Capurso[1]

[1]Digestive and Liver Disease Unit, Sant' Andrea Hospital, Sapienza University of Rome, Rome, Italy
[2]Department of General Surgery, Royal Infirmary of Edinburgh, Edinburgh, UK
[3]Department of Hepato-Pancreato-Biliary Surgery, Oslo University Hospital, Oslo, Norway
[4]Unit of Gastroenterology, Department of Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
[5]Department of Gastroenterology, University Medical Centre Ljubljana, Ljubljana, Slovenia
[6]Department of General and Visceral Surgery, Zentralklinik Bad Berka, Bad Berka, Germany
[7]Department of Hepatopancreatobiliary and Transplantation Surgery, The Freeman Hospital, Newcastle upon Tyne, UK
[8]Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy

Correspondence should be addressed to G Capurso
Email
gabriele.capurso@gmail.com

Abstract

Pancreatic neuroendocrine neoplasms (PNENs) represent 10% of all pancreatic tumors by prevalence. Their incidence has reportedly increased over recent decades in parallel with that of pancreatic adenocarcinoma. PNENs are relatively rare, and of the few institutions that have published potential risk factors, findings have been heterogeneous. Our objective was to investigate the association between potential risk and protective factors for the occurrence of sporadic PNENs across a European population from several institutions. A multinational European case–control study was conducted to examine the association of selected environmental, family and medical exposure factors using a standardized questionnaire in face-to-face interviews. A ratio of 1:3 cases to controls were sex and age matched at each study site. Adjusted univariate and multivariate logistic regression analysis were performed for statistically significant factors. The following results were obtained: In 201 cases and 603 controls, non-recent onset diabetes (OR 2.09, CI 1.27–3.46) was associated with an increased occurrence of PNENs. The prevalence of non-recent onset diabetes was higher both in cases with metastatic disease (TNM stage III–IV) or advanced grade (G3) at the time of diagnosis. The use of metformin in combination with insulin was also associated with a more aggressive phenotype. Drinking coffee was more frequent in cases with localized disease at diagnosis. Our study concluded that non-recent onset diabetes was associated with an increased occurrence of PNENs and the combination of metformin and insulin was consistent with a more aggressive PNEN phenotype. In contrast to previous studies, smoking, alcohol and first-degree family history of cancer were not associated with PNEN occurrence.

Key Words
- pancreas
- neuroendocrine neoplasms
- insulinomas
- gastrinomas
- risk factors

Endocrine-Related Cancer (2017) **24**, 405–414

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

Introduction

Pancreatic neuroendocrine neoplasms (PNENs) are a group of tumors which originate from endocrine cells within the pancreas gland. PNENs have heterogeneous clinical behavior owing to their hormone functional status, cellular characteristics and the extent of metastatic disease. While representing only 1–2% of pancreatic neoplasms by incidence (Fitzgerald *et al*. 2008, Yao *et al*. 2008), PNENs may account for as much as 10% by prevalence (Yao *et al*. 2008). Such discrepancy is due in part to the relatively indolent clinical course of many PNENs compared to pancreatic adenocarcinoma. PNENs are rare tumors but their incidence has reportedly increased in recent decades, particularly that of non-functioning tumors (Lepage *et al*. 2004, Fitzgerald *et al*. 2008, Yao *et al*. 2008). To a lesser degree, investigators have also identified a modest increase in the incidence of pancreatic adenocarcinoma (Fitzgerald *et al*. 2008).

The increased reported incidence of both endocrine and exocrine pancreatic tumors is likely due to rising population lifespan and the wider availability of high-resolution cross-sectional imaging (Del Chiaro *et al*. 2013, Ellison *et al*. 2014), but additionally raises the possibility of changing exposure to factors which may alter the risk of pancreatic neoplasia.

There are a small number of case–control studies that have investigated potential risk factors for the occurrence of PNENs (Hassan *et al*. 2008*b*, Capurso *et al*. 2009, Zhan *et al*. 2013, Halfdanarson *et al*. 2014, Ben *et al*. 2016). These studies recruited participants from a single institution or geographical region, and were recently summarized in a meta-analysis that found personal history of diabetes mellitus and family history of cancer to be associated with an increased risk of PNEN (Haugvik *et al*. 2015). The association of PNENs with smoking and alcohol drinking was less clear, and only heavy smoking and heavy alcohol consumption reached statistical significance. The included studies differed in their design and population definitions, with a considerable heterogeneity limiting the significance of the meta-analysis. Moreover, these studies had some specific methodological limitations, such as the absence of a power calculation and exposures were often recorded at the time of diagnosis or treatment, rather than considering the exposure history prior to diagnosis. The latter raises the possibility of a bias due to cancer symptoms (e.g. weight loss, new onset diabetes) or lifestyle modifications such as changes in smoking behavior and alcohol consumption.

It appears that there is overlap in risk factors, such as smoking and alcohol, for the occurrence of PNENs and pancreatic adenocarcinoma; however, a number of other factors that have been associated with the occurrence of pancreatic cancer have not, to our knowledge, been investigated for PNENs. For example, the use of medications such as aspirin, and the association with allergy and atopy that have been reported to be protective against pancreatic adenocarcinoma occurrence (Streicher *et al*. 2014, Gomez-Rubio *et al*. 2017).

For these reasons, we conducted a multicenter European study aimed at assessing the association between a large number of potential risk or protective factors for the development of sporadic PNENs.

Materials and methods

Study design and population

A collaborative multicenter hospital-based case–control study was conducted in six European countries: Italy, Norway, Sweden, Slovenia, United Kingdom and Germany as part of the 'Pancreas 2000' educational project (www.pancreas2000.org), upon local hospital ethical committee approval.

A standardized questionnaire, including questions about demographics and potential risk factors, such as family history of cancer, environmental factors, previous use of drugs and other medical history features was administered to patients by a trained medical doctor. Each questionnaire took ~15 min to be completed during a face-to-face interview, after gaining participant consent.

The cases were prevalent sporadic PNEN patients diagnosed within 24 months from the beginning of the study (January 2013) and new incident cases of sporadic PNENs diagnosed from January 2013 to December 2015 that were recruited at the participating centers.

The inclusion criterion was to have a histological or cytological diagnosis of PNEN. The date of the confirmatory pathological report was accepted as the date of diagnosis.

Exclusion criteria were the presence of an inherited form of PNEN such as those associated with multiple endocrine neoplasia-type 1 (MEN1), von Hipple-Lindau disease (VHL), neurofibromatosis type 1 (NF1), tuberous sclerosis (TSC) or an inability to participate, such as dementia.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

According to the absence or presence of a clinical syndrome due to hormonal hypersecretion, cases were classified as non-functioning or functioning tumors (gastrinoma, insulinoma, glucagonoma). For example, the Zollinger Ellison Syndrome was clinically suspected in the presence of a PNEN associated with peptic disease and its complications and diarrhea, insulinomas in the presence of severe hypoglycemia with associated neurological symptoms (varying from confusion to coma), glucagonoma in the presence of rash, glucose intolerance and weight loss. In any case, the syndrome was confirmed with a specific laboratory workup according to guidelines (Jensen *et al*. 2012, Falconi *et al*. 2016).

Cases were classified according to the European Neuroendocrine Tumor Society (ENETs) and the 2010 World Health Organization classifications (Rindi *et al*. 2010, Solcia *et al*. 2002, Falconi *et al*. 2016).

Eligible controls were either individuals seen in the participating hospitals' outpatient clinic for a non-specific, non-organic gastrointestinal disorder (bloating, aspecific dyspeptic symptoms, eructation) or visitors attending the same network of referring hospitals, matched by country, sex and age (+/− 5 years). Visitors and hospital outpatients' clinic belonged to the same catchment area of cases. Specific exclusion criteria for controls were the following: (1) the presence of any genetic syndrome associated with the occurrence of PNENs; (2) a history of active cancer (diagnosed within 5 years); (3) any biological relation of a participating PNEN case in this study; (4) a history of any chronic inflammatory condition (e.g. chronic obstructive pulmonary disease, liver cirrhosis, inflammatory bowel disease, end stage kidney disease); and (5) undergoing evaluation of a possible familial cancer syndrome. Controls were included in the same country and interviewed within 6 months of the inclusion of the matched corresponding case.

Exposure definitions

Subjects were questioned about risk factors that were present at least 12 months before diagnosis or presentation of symptoms, in order to avoid potential bias due to lifestyle modifications, cancer symptoms or cancer treatments.

Subjects were considered ever smokers if they reported a cumulative lifetime smoking history greater than 6 months or 100 cigarettes smoked. A quantification of the smoking habit for cases and controls was performed considering the number of pack-years (pack-year = number of packs per day × years of smoking), with 20 pack-years being the lower limit to qualify a participant a heavy smoker.

A daily intake of at least 12.5 g of alcohol, equivalent to one glass of wine, one pint of beer or one shot of hard liquor, for at least one year, was considered the cut-off to be a regular ever alcohol drinker. Because of possible different drinking habits within different European countries, the weekly alcohol amount was also sub-analyzed according to low (1–7 weekly units assumption), medium (8–14 weekly units), medium-high (14–20 weekly units) and heavy alcohol consumption (≥21 weekly units). Coffee drinking was also recorded as ever drinking (at least one cup per day) or heavy coffee drinking (>5 cups per day).

Height and weight were recorded from which body mass index (BMI) (kg/m^2) was calculated. A history of chronic pancreatitis, acute pancreatitis, peptic ulcer disease, biliary stones and previous surgery were specifically recorded. Additionally, a diagnosis of diabetes was documented and subdivided for type and onset. For cases, recent onset diabetes was defined as that which was diagnosed in 12 months prior to the PNEN diagnosis, and for controls, a diagnosis of diabetes 12 months prior to the date of recruitment was required. Sensitivity analysis was also performed for different intervals of the onset of diabetes, (inferior to 1 year, between 1 and 3 years, between 3 and 5 years and above 5 years, respectively). Another sensitivity analysis was also conducted considering incident/prevalent cases and hospitals controls/visitors controls compared to respective cases.

As atopy and allergy have been associated with a reduced risk of pancreatic cancer (Gomez-Rubio *et al*. 2017), cases and controls were interrogated about a history of allergy, with specific enquiry for eczema, hay fever and asthma. The use of aspirin, proton pump inhibitors, metformin and insulin were recorded. Subjects were interrogated about 1st and 2nd degree family history of cancer, and the total number of siblings and children was recorded.

Statistical analysis

An a priori power calculation was performed. We estimated sample size based on the differences reported in the frequencies of exposure in cases and controls according to a previous study (Capurso *et al*. 2009), and considering a ratio 1:3, with a statistical power equal to 80% and an alpha error equal to 0.05.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

According to Capurso and coworkers (Capurso *et al.* 2009) the reported prevalence in cases and controls was, respectively, 53% vs 32% for 1st degree family history of cancer, 10% vs 2% for diabetes and 14% vs 3% for heavy alcohol consumption. Therefore, the sample size's estimate of cases and controls, to show true differences whether existing, were, respectively, 64 cases and 191 controls for 1st degree family history of cancer, 117 cases and 350 controls for diabetes, and 85 cases and 253 controls for heavy alcohol consumption.

We therefore estimated that a total of 200 cases and 600 controls across participating centers to be an adequate sample size to reveal differences in the prevalence of most risk factors analyzed among cases and controls, where these exist.

Characteristics of cases and controls were compared by chi-square test for categorical variables or Student's *t*-test for continuous variables. Significant variables were analyzed by logistic regression analysis adjusted for sex, age and enrollment center. A multivariable logistic regression analysis, adjusted for sex, age and enrollment center was also performed with an enter selection procedure for statistically significant risk factors. A dedicated statistical software package (MedCalc, Mariakerke, Belgium) was used for data analysis. Ninety-five percent confidence interval (95%) was calculated where possible. All *P* values were two sided, and a $P<0.05$ was considered statistically significant.

Results

Characteristics of cases and controls

A total of 201 cases and 603 sex- and age-matched controls were enrolled among the six centers (Italy, Norway, Sweden, United Kingdom, Slovenia and Germany) as shown in Table 1. The mean age was 59.6 years in cases (CI 57.7–61.4) and 59.5 years in controls (CI 58.4–60.55), and 51% of cases and controls were male.

Among the 201 PNEN cases, 154 (76.6%) had a non-functioning tumor. Of the 47 functioning PNENs, 26 (55.3%) had an insulinoma, 9 (19.1%) a gastrinoma, 7 (14.9%) a glucagonoma and 5 (10.6%) other functioning tumors. The majority were G1 (44.8%) or G2 (43.3%) PNENs, and were equally distributed among different disease stages (Table 1). Of the 201 PNEN cases, 80 (38%) cases were incident and 121 (62%) prevalent. Of the 603 controls, 422 (70%) were hospital outpatients controls and 181 (30%) visitors.

Table 1 Characteristics of PNENs cases.

	Cases *N* (%)
Total	201
Referral center	
Italy	62 (30.8)
Norway	44 (21.9)
Sweden	40 (19.9)
Slovenia	24 (11.9)
United Kingdom	20 (10.0)
Germany	11 (5.5)
Sex	
Male	103 (51.2)
Age (years)	
Mean ± S.D.	59.6 ± 13.1
Race Caucasians	196 (97.5)
Tumor type	
Functioning	47 (23.3)
Non-functioning	154 (76.7)
Functioning only ($n=47$)	
Insulinomas	26 (55.3)
Gastrinomas	9 (19.1)
Glucagonomas	7 (14.9)
Other	5 (10.6)
Tumor grade	
G1	90 (44.8)
G2	87 (43.3)
G3	22 (10.9)
Unknown	2 (1.0)
Tumor stage	
Stage I	51 (25.4)
Stage II	53 (26.4)
Stage III	47 (23.4)
Stage IV	43 (21.4)
Unknown	7 (3.5)
Tumor site	
Head	75 (37.3)
Body or tail	120 (59.7)
Unknown	6 (3.0)

Risk factors for the occurrence of PNEN

Family history of cancer The proportion of subjects who had a 1st degree family history of cancer was similar both in cases and in controls (51.1% vs 45.3% $P=0.17$, respectively), while 2nd degree family history was slightly more prevalent in cases (36.8% vs 30.2% $P=0.09$). A 1st degree family history of specific cancer sites was also not significantly different (Table 2). No cases or controls reported a family history of neuroendocrine tumor (NET). At multiple regression analysis, 2nd degree family history of any cancer was, however, associated with an increased risk of PNEN (Tables 2 and 3).

Body mass index Mean BMI was not significantly different among cases and controls (26.8 kg/m² (CI 26.0–27.5) and 26.4 kg/m² (CI 26.4–26.8), $P=0.10$. Similarly, while the overall prevalence of obesity was more frequent

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

Table 2 Risk factors for PNENs.

	Cases *N* (%)	Controls *N* (%)	*P* value	OR* (95% CI)
Total	201	603		
Family history*				
1st degree FH of any cancer	103 (51.1)	272 (45.3)	0.17	1.32 (0.94–1.83)
2nd degree FH of any cancer	74 (36.8)	181 (30.2)	0.09	1.51 (1.05–2.17)
1st degree FH pancreatic cancer	6 (3.0)	13 (2.2)	0.69	1.42 (0.52–3.84)
1st degree FH esophageal	2 (1.0)	4 (0.7)	0.99	1.58 (0.28–8.79)
1st degree FH gastric cancer	7 (3.5)	26 (4.3)	0.75	0.77 (0.32–1.85)
1st degree FH colorectal cancer	18 (9.0)	57 (9.5)	0.92	0.99 (0.56–1.75)
1st degree FH breast cancer	20 (10.0)	51 (8.3)	0.62	1.25 (0.72–2.18)
1st degree FH lung cancer	17 (8.5)	48 (8.0)	0.95	1.12 (0.62–2.00)
1st degree FH NETs	–	–		
1st degree FH hematological cancer	12 (6.0)	30 (5.0)	0.72	1.28 (0.63–2.57)
1st degree FH hepatobiliary cancer	4 (2.0)	17 (2.8)	0.69	0.45 (0.12–1.66)
1st degree FH sarcoma	–	3 (0.5)	0.73	–
Number of siblings				
Mean ± S.D.	2.1 ± 1.7	2.5 ± 2.0	0.05	
BMI				
Underweight	5 (2.5)	10 (1.6)		1.68 (0.55–5.04)
Normal weight	82 (41.0)	243 (40.2)	P-trend**	1.00 (0.71–1.39)
Overweight	63 (31.5)	240 (39.8)	0.44	0.75 (0.52–1.10)
Obese	51 (25.5)	110 (18.2)		1.36 (0.88–2.08)
Smoking				
Never smoke	89 (44.5)	279 (46.5)		0.86 (0.62–1.20)
<20 pack-years	60 (30.0)	176 (29.3)	P-trend	1.07 (0.75–1.53)
≥20 pack-years	51 (25.5)	144 (24.0)	0.59	1.15 (0.78–1.69)
Unknown	1 (0.5)	3 (0.5)		1.00
Alcohol intake				
Never drink	49 (24.4)	173 (29.2)		0.77 (0.53–1.12)
<21 units/week	145 (72.1)	394 (66.6)	P-trend**	1.29 (0.90–1.85)
≥21 units/week	7 (3.4)	25 (4.2)	0.33	0.88 (0.37–2.11)
Coffee				
Never drink	30 (15.2)	73 (12.2)		1.37 (0.83–2.14)
≤4 cups/day	135 (68.2)	411 (68.7)	P-trend**	0.94 (0.66–1.34)
>4 cups/day	33 (18.8)	114 (19.2)	0.24	0.86 (0.55–1.35)
Unknown	3 (1.4)	5 (0.8)		1.00
Diabetes				
No	164 (81.6)	529 (87.7)	0.03	1.00
Recent onset (<1 year)	1 (0.5)	10 (1.6)	0.4	0.24 (0.03–1.88)
No-recent onset (≥1 year)	35 (17.4)	58 (9.7)	0.004	1.89 (1.17–3.05)
Unknown	1 (0.5)	6 (1.0)		1.00
Diabetes (≥1 year, <3 years)	6 (3.0)	7 (1.1)	0.07	2.56 (0.83–7.91)
Diabetes (≥3 year, <5 years)	8 (4.0)	6 (0.9)	0.0045	4.31 (1.43–12.98)
Diabetes (≥5 years)	21 (10.7)	45 (7.4)	0.16	1.47 (0.84–2.56)
Diabetes treatment				
Metformin (no insulin)	13 (7.3)	29 (5.1)	0.3	1.35 (0.68–2.66)
Insulin	7 (4.1)	9 (1.6)	0.1	1.63 (0.86–3.08)
Metformin and insulin	9 (5.2)	19 (3.4)	0.4	1.48 (0.65–3.35)
Past medical history				
Acute pancreatitis	7 (3.5)	15 (2.5)	0.60	1.42 (0.56–3.60)
Chronic pancreatitis	–	–	–	
Peptic ulcer	24 (12.3)	64 (10.6)	0.59	1.25 (0.75–2.07)
Cholecystectomy	18 (9.0)	51 (8.5)	0.92	1.07 (0.61–1.90)
Gastrectomy	2 (1.0)	4 (0.7)	0.99	1.56 (0.28–8.65)
Gallstone disease	34 (19.2)	77 (13.3)	0.06	1.53 (0.97–2.42)
Asthma	24 (12.1)	52 (8.6)	0.19	1.47 (0.87–2.47)
Eczema	22 (11.1)	46 (7.6)	0.17	1.52 (0.88–2.62)
Hay fever	31 (15.6)	87 (14.5)	0.79	1.17 (0.74–1.84)
Any allergy	58 (28.9)	151 (25.0)	0.32	1.26 (0.88–1.82)
Use of aspirin	45 (22.5)	160 (26.5)	0.29	0.79 (0.53–1.18)
Use of proton pump inhibitors	78 (39.2)	239 (39.6)	0.97	1.04 (0.74–1.45)

*OR adjusted for sex, age and center of enrollment; ***P* value based on the Mantel–Haenszel test for trend excluding missing category.
FH, family history; NET, neuroendocrine tumor.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

Table 3 Risk factors for the occurrence of PNENs at the Logistic regression analysis.

Risk factor	Univariate*OR (95% CI)	*P* value	Multivariable* OR (95% CI)	*P* value
Diabetes				
No	1.00		1.00	
Early onset (≤1 year)	0.24 (0.03–1.88)	0.17		
Late onset (>1 year)	1.89 (1.17–3.05)	0.008	2.09 (1.27–3.45)	0.003
2nd degree FH of any cancer	1.51 (1.05–2.17)	0.02	1.53 (1.03–2.27)	0.03
Gallstone disease				
No	1.00			
Yes	1.48 (0.93–2.35)	0.08	1.52 (0.95–2.44)	0.08

Adjusted for sex, age, underweight, 1st degree family history for esophageal cancer and center of enrollment.

in cases than controls (25.5% vs 18.2%), this was not significant (P=0.44).

At regression analysis after adjustment for matching variables, there remained no significant association with obesity (OR 1.36, 95% CI 0.88–2.08, P=0.15) (Table 3).

Cigarette smoking, alcohol intake and coffee drinking The proportion of smokers (55.5% vs 53.5%, P=0.59), heavy smokers (25.5% vs 24.0%, P=0.59), alcohol drinkers (75.6% vs 70.8%, P=0.33), heavy alcohol drinkers (3.4% vs 4.2%, P=0.33), coffee drinkers (84.8% vs 87.8%, P=0.24) and heavy coffee drinkers (18.8% vs 19.2%, P=0.24) did not significantly differ between cases and controls (Table 2).

History of diabetes mellitus A history of diabetes mellitus was more prevalent in cases than in controls (18.4% vs 12.3%, P=0.03). This difference was greater on analysis of non-recent onset diabetes, defined as diabetes diagnosed more than 12 months before the diagnosis of PNEN in cases, or 12 months prior to the interview for controls (17.4% vs 9.7%, P=0.004) (Table 2). After adjustment for the matching variables, non-recent onset diabetes was confirmed to be consistent with the occurrence of PNEN (OR 1.89, 95% CI 1.17–3.05, P=0.008). At multivariable analysis, the association with non-recent onset diabetes remained statistically significant (OR 2.09, 95% CI 1.27–3.45, P=0.003) (Table 3). At sensitivity analysis, a history of diabetes with an onset between 1–3 year and 3–5 years was increasingly prevalent in PNEN compared to controls (3.0% vs 1.1% P=0.07 and 4% vs 0.9% P=0.004, respectively). At univariable logistic regression analysis, this difference remained significant (OR 2.56, 95% CI 0.83–7.91 and OR 4.31, 95% CI 1.43–12.98, respectively). For intervals of occurrence of diabetes superior to 5 years, no statistically significant difference was found between cases and controls (10.7% vs 7.4%, P=0.16, respectively) (Table 2). We also performed a separate analysis for 'late onset diabetes' using as controls either only the 422 hospital controls or only the 181 visitors controls. In the first case, the OR resulted to be 2.52 (95% CI 1.08–5.86; P=0.03), while in the second one, the OR was 1.7 (95% CI 0.95–3; P=0.07), most likely due to the lower number of controls reducing the power of the analysis.

The use of metformin (7.3% vs 5.1%, P=0.3) and insulin (4.1% vs 1.6%, P=0.1) or their association together (5.2% vs 3.4%, P=0.4) was not statistically different between cases and controls.

Past medical history With regard to past medical history, the prevalence of acute pancreatitis (3.5% vs 2.4%, P=0.60), peptic ulcer disease (12.3% vs 10.6%, P=0.59), cholecystectomy (9.0% vs 8.5%, P=0.92) and gastrectomy (1.0% vs 0.7%, P=0.99) were similar in cases and controls. None of the participants reported a medical history of chronic pancreatitis (Table 2). A higher proportion of cases reported a history of gallstone disease than controls (19.2% vs 13.3%) but this did not reach the significance threshold (P=0.06). After adjustment for age, sex and enrollment center at multivariable analysis, this latter association remained borderline significant (OR 1.52, 95% CI 0.95–2.44, P=0.08) (Table 3).

Non-diabetic medications The use of proton pump inhibitors (PPI) (39.2% vs 39.6%, P=0.97) and aspirin (22.5% vs 26.5%, P=0.29) did not differ among cases and controls, respectively (Table 2).

Allergies A history of allergies was not different in cases and in controls (28.9% vs 25.0%, P=0.32). Specifically, asthma (12.1% vs 8.6%, P=0.19), eczema (11.1% vs 7.6%, P=0.17) and hay fever (15.6% vs 14.5%,

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

Table 4 Factors associated with TNM stage and tumor grade in PNEN patients.

	TNM Stage 1–2 *N* (%)	TNM Stage 3–4 *N* (%)	*P* value	OR (95% CI)
Late-onset diabetes (≥1 year)	12 (11.8)	21 (23.3)	0.05	1.98 (0.83–4.74)
Coffee drinking	96 (92.3)	66 (75.9)	0.003	0.14 (0.05–0.4)
	G1–G2 *N* (%)	**G3 *N* (%)**	***P* value**	**OR (95% CI)**
Diabetes	28 (15.8)	9 (40.9)	0.01	4.28 (1.41–12.93)
Late-onset diabetes (≥1 year)	26 (14.9)	9 (40.9)	0.006	5.43 (1.66–17.72)
Metformin plus insulin use	5 (3.2)	4 (23.5)	0.003	9.71 (1.86–50.74)
Allergic factors				
Asthma	18 (10.2)	6 (30.0)	0.02	3.96 (1.91–13.21)
Eczema	17 (9.6)	5 (25.0)	0.08	3.06 (0.92–10.19)

$P=0.79$) were not more prevalent in PNEN patients than in controls (Table 2).

Risk factors for the advanced grades and stages of PNEN

When stratifying cases for the TNM stage at diagnosis and for the tumor grade, diabetes mellitus was statistically more prevalent in patients with G3 tumors (pancreatic neuroendocrine carcinoma; PNEC) than with G1 or G2 tumors (40.9% vs 15.8%, $P=0.01$). Among cases, non-recent onset diabetes was associated with a more advanced stage at diagnosis (TNM III–IV vs TNM I–II, respectively, 23.3% vs 11.8%, $P=0.05$) and with a G3 vs G1–2 tumor (40.9% vs 14.9%, $P=0.006$, respectively). The use of metformin in combination with insulin was more prevalent in patients with G3 than G1–G2 tumors (23.5% vs 3.2%, $P=0.003$, respectively) (Table 4). Asthma was more prevalent in G3 cases than in G1–2 (30.0% vs 10.2% $P=0.02$), and eczema was also more prevalent in G3 cases than in G1–2 but without reaching statistical significance (25.0% vs 9.6%, $P=0.08$). Coffee drinking was more prevalent in localized disease (TNM 1–2) at diagnosis compared with advanced stage (TNM 3–4) (92.3% vs 75.9%, $P=0.003$).

Discussion

The present study was designed to recruit cases of PNEN from multiple sites across Europe using a standardized questionnaire in a face-to-face interview setting. To further strengthen our method, we incorporated a preliminary power calculation based on results from a similar previous study. To the best of our knowledge, there have been six published studies to have investigated risk factors for PNEN. Due to the low incidence of such tumors, which would make a longitudinal cohort study highly problematic, it is not unexpected that these were all case–control studies. Three were conducted in the USA, one in Europe and two in China (Hassan *et al.* 2008*a*,*b* Capurso *et al.* 2009, Zhan *et al.* 2013, Halfdanarson *et al.* 2014, Ben *et al.* 2016). Collective analysis of these studies has been limited by the various data collection methods employed, the selection of investigated exposures (and differing definitions), in addition to the disparate population pools, all of which lead to substantial heterogeneity (Haugvik *et al.* 2015). One study from China exclusively investigated a cohort of insulinomas, with the exclusion of non-functioning endocrine tumors; the latter represent the majority of PNEN cases, accounting for 60 to 90% of cases (Falconi *et al.* 2016). Another study from China investigated a cohort of PNEN cases in which 63% had functioning tumors, and 84% had early-stage disease (Ben *et al.* 2016). The results of this study may have limited applicability to other populations, as the majority of PNENs arising in Western populations are non-functioning and would typically present with more advanced disease (Panzuto *et al.* 2011).

The two publications from the USA reported different risk factors in the same population, from a retrospective analysis of a large hospital database of NETs (Hassan *et al.* 2008*a*,*b*). None of the reported studies sought to investigate potentially protective factors against the occurrence of PNENs. Two meta-analyses have summarized the results of the previous primary studies and reached similar conclusions: Diabetes mellitus and family history of cancer are risk factors for the occurrence of PNENs, while the role of environmental factors was unclear and warranted further investigation (Haugvik *et al.* 2015, Leoncini *et al.* 2016).

Our study affirms an increased risk of PNEN occurrence with diabetes mellitus; however, it is noteworthy that we identify the significance of non-recent onset diabetes as a risk factor. Four studies previously identified an association between PNEN and diabetes (Hassan *et al.* 2008*b*, Capurso *et al.* 2009, Halfdanarson *et al.* 2014,

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

Ben *et al*. 2016); however, in contrast to the current study, this association was for recent onset diabetes, which can represent an epiphenomenon of the disease as suggested elsewhere for pancreatic adenocarcinoma (Pannala *et al*. 2008).

Given that beta cells typically express low levels of cytoprotective antioxidant enzymes (Tiedge *et al*. 1997) and because oxidative stress contributes to both the pathogenesis of diabetes (Rolo & Palmeira 2006) and can potentiate somatic mutations, it would not be unexpected for long-standing diabetes to have an association with oncogenic transformation of islet cells. Indeed, PNEN proliferation, tumor invasion and disease stage have been found to be associated with expression of mTOR (mechanistic/mammalian target of rapamycin) and its effectors (Capurso *et al*. 2015), a cytoplasmic kinase that is activated by both glucose and insulin (Blagosklonny 2013).

As the relation between diabetes and carcinogenesis is complex and still not clear, we sought to specifically investigate the timing of onset of diabetes in respect to the clinical presentation of the cancer. We therefore analyzed risk factors present at least 12 months before diagnosis, minimizing the overlap between risk factors and cancer-related symptoms, which could include cancer-induced endocrine insufficiency. With such premises, our results support the view that long-standing diabetes is a risk factor for PNEN rather than sign of disease. In order to further analyze possible overlaps between diabetes as a risk factors and diabetes as a cancer-related symptom, we performed a further sensitivity analysis, investigating several different intervals of time between the onset of diabetes and the diagnosis of cancer. Non-recent onset diabetes was confirmed to be increasingly consistent with the occurrence of PNEN for intervals superior to 1 year and up to 5 years. For intervals of onset of diabetes superior to 5 years, this association was not anymore statistically significant. This might be interpreted on the base of a lack of power of the study when considering small subgroups or, alternatively, it could be biologically explained by the trophic influence that diabetes plays on cancer. On the other hand, one should also take into account that PNEN displays a slower growing rate compared to PDAC, and therefore it might justify a major latency of occurrence of symptom diabetes.

As the potential role of anti-diabetic drugs (metformin and insulin) in influencing pancreatic carcinogenesis has been reported (De Souza *et al*. 2016), we also specifically investigated the role of such drugs in our multinational cohort. Although the prevalent use of insulin alone was more frequent among cases than in controls (4.1% vs 1.6%), this difference was not significant as the study was underpowered to assess it.

Another noteworthy finding of the present study was the increased proportion of gallstone disease among PNEN cases compared to controls. However, this did not reach statistical significance, and we therefore cannot conclude that this was anything more than a chance observation. The apparent proportional increase among cases, however, may reflect the universal use of abdominal imaging in those diagnosed with PNEN, as compared to occult gallstones in controls. It may be that our study was underpowered to detect a true association between PNEN and biliary calculi, as the latter have been found to be associated with 'malignant neoplasm of the pancreas' (ICD-Oncology C25.0–C25.9) as a single entity, using a large combined US cancer registry with population-based controls (Nogueira *et al*. 2014).

In the present study, the rate of family history of cancer was not different between cases and controls. This finding was in contrast with previous studies on this topic. However, study design issues and/or selection bias in previous studies may account for this difference. For example, three of these reports (Hassan *et al*. 2008*a*,*b*, Zhan *et al*. 2013) did not exclude PNEN cases with genetic syndromes (i.e. MEN1 or VHL). Of particular note, 25% of PNEN cases had a genetic syndrome in the study by Zhan and coworkers, and therefore a higher proportion of family history of any cancer would be expected (Zhan *et al*. 2013). Halfdanarson and coworkers studied only sporadic cases, but excluded insulinomas and poorly differenced PNECs which may have affected results in this regard (Halfdanarson *et al*. 2014). In the present study, controls had a significantly higher number of siblings compared with cases, potentially biasing the probability of cancer family history in the control study arm. On the other hand, we found an association between 2nd degree family history of cancer and risk of PNEN, thus suggesting that some kind of hereditary component might exist in these patients.

Environmental factors such as smoking and alcohol, even in high doses, did not increase the risk of developing a PNEN in our study. This result is in keeping with the study by Hassan and coworkers (Hassan *et al*. 2008*b*) but in contrast with others (Capurso 2009, Halfdanarson *et al*. 2014, Zhan *et al*. 2013). A recent meta-analysis highlighted that the role of smoking and alcohol might be less relevant in PNENs than in pancreatic

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-17-0040

Published by Bioscientifica Ltd.

adenocarcinoma (Haugvik *et al*. 2015). To explore potential environmental factors that might alter the risk of PNEN occurrence, we investigated for the first time a number of factors associated with a lower incidence of pancreatic adenocarcinoma, such as the use of aspirin and a personal history of allergies. No statistically significant differences were detected, possibly reflecting intrinsic biological differences between endocrine and exocrine neoplasia of the pancreas. Furthermore, as the power of the present study was based on risk factors for which there were previous data, and this was not the case for previously uninvestigated exposures, a type II error may have occurred.

Finally, we investigated the possible prognostic relevance of the investigated factors, analyzing their distribution in PNEN patients according to their stage of disease at diagnosis (TNM stages III or IV compared with I and II) or with their grade assessed by proliferative activity (G1 and G2 compared to G3). Interestingly, the prevalence of non-recent onset diabetes was higher both in cases with metastatic disease (TNM stage III–IV) and advanced grade (G3) at the time of diagnosis (Table 4). Drinking coffee was more frequent in cases with localized disease at diagnosis. The use of metformin in combination with insulin was also associated with a more aggressive phenotype. Therefore, diabetes and use of insulin might also exert a proliferative effect on tumor progression, as reported for other cancer types (Vigneri *et al*. 2016).

The present study displays several strengths as well as limitations. The strengths of the study are represented by the relatively large sample size keeping in mind the low incidence of this tumor type, the European multicenter setting (6 countries involved), the preliminary power calculation, the investigation for the first time of a large set of factors possibly associated with the risk of PNENs and the conduct of the study by face-to-face interview with a standardized questionnaire. The inclusion criteria were clearly defined: controls were well matched for age and gender with a 1:3 ratio, and all questionnaires were administered by trained medical doctors fluent in the local language, who evaluated exposures present 12 months before diagnosis, to minimize bias due to cancer symptoms. Inherent with a multinational case-control design, our study displays some limitations such as potential recall bias and heterogeneity in data from different countries, although the analysis was corrected for centere of enrollment. Furthermore, the analysis might have been underpowered for some of the investigated factors, and additional studies might be important to confirm the lack of significant association.

Another important matter of concern, as for any case-control study, regards the choice of the control population. We opted for a mixed control group that we believed to represent the same population as the case group, as living in the same catchment area of the corresponding cases, to limit possible bias that could have been specific of either hospital controls or visitors. Interestingly, 'late onset diabetes' seemed to be associated with an increased risk of PNEN with both kind of controls used in separate analyses.

In conclusion, the findings of this large multicenter case-control study suggest that non-recent onset diabetes was associated with an increased risk of PNENs occurrence. Our results do not support the view of a strict similarity with factors affecting the risk of pancreatic adenocarcinoma.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this research.

Funding
Gabriele Capurso was supported by AIRC grant IG 2015, Id 17177.

Author contribution statement
Gabriele Capurso was the guarantor of the article. All authors approved the final version.
Roberto Valente, Gabriele Capurso, Alastair Hayes. Analyzed the data: Roberto Valente, Gabriele Capurso, Patrick Maisonneuve wrote the paper. All authors contributed to the design, data collection, data interpretation and writing of the manuscript.

Acknowledgements
This study was conducted through *Pancreas 2000*, which is a European educational and scientific pancreatology program initiated by the *Karolinska Institutet* in Stockholm, Sweden, and the *European Pancreatic Club*. The authors wish to acknowledge the contributions from the following sites: Italy: Dr Livia Archibugi, Dr Maria Rinzivillo. United Kingdom: Dr Lucy Wall, Dr Karen M Hayes, Christos Skouras, Prof. Rowan W Parks, Prof. Mark W J Strachan (Edinburgh); Stuart M Robinson, Colin H Wilson (Newcastle); Tani Fasih, Cho Ee Ng (Gateshead). Norway: Prof. Ivar P Gladhaug, Kjerstin Skrede Mordal. Sweden: Dr Louise Bexander, Dr Isabel Sjöholm and Medical Student Anna Sjögren.

References

Ben Q, Zhong J, Fei J, Chen H, Yv L, Tan J & Yuan Y 2016 Risk factors for sporadic pancreatic neuroendocrine tumors: a case-control study. *Scientific Reports* **6** 36073. (doi:10.1038/srep36073)

Blagosklonny MV 2013 TOR-centric view on insulin resistance and diabetic complications: perspective for endocrinologists and gerontologists. *Cell Death Discovery* **4** e964.

Published by Bioscientifica Ltd.

Capurso G, Falconi M, Panzuto F, Rinzivillo M, Boninsegna L, Bettini R, Corleto V, Borgia P, Pederzoli P, Scarpa A, *et al*. 2009 Risk factors for sporadic pancreatic endocrine tumors: a case-control study of prospectively evaluated patients. *American Journal of Gastroenterology* **104** 3034–3041. (doi:10.1038/ajg.2009.466)

Capurso G, Archibugi L & Delle Fave G 2015 Molecular pathogenesis and targeted therapy of sporadic pancreatic neuroendocrine tumors. *Journal of Hepato-Biliary-Pancreatic Sciences* **22** 594–601. (doi:10.1002/jhbp.210)

De Souza A, Khawaja KI, Masud F & Saif MW 2016 Metformin and pancreatic cancer: is there a role? *Cancer Chemotherapy and Pharmacology* **77** 235–242. (doi:10.1007/s00280-015-2948-8)

Del Chiaro M, Verbeke C, Salvia R, Kloppel G, Werner J, McKay C, Friess H, Manfredi R, Van Cutsem E, Lohr M, *et al*. 2013 European experts consensus statement on cystic tumours of the pancreas. *Digestive and Liver Disease* **45** 703–711. (doi:10.1016/j.dld.2013.01.010)

Ellison TA, Wolfgang CL, Shi C, Cameron JL, Murakami P, Mun LJ, Singhi AD, Cornish TC, Olino K, Meriden Z, *et al*. 2014 A single institution's 26-year experience with nonfunctional pancreatic neuroendocrine tumors: a validation of current staging systems and a new prognostic nomogram. *Annals of Surgery* **259** 204–212. (doi:10.1097/SLA.0b013e31828f3174)

Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Kloppel G, *et al*. 2016 ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. *Neuroendocrinology* **103** 153–171. (doi:10.1159/000443171)

Fitzgerald TL, Hickner ZJ, Schmitz M & Kort EJ 2008 Changing incidence of pancreatic neoplasms: a 16-year review of statewide tumor registry. *Pancreas* **37** 134–138. (doi:10.1097/MPA.0b013e318163a329)

Gomez-Rubio P, Zock JP, Rava M, Marquez M, Sharp L, Hidalgo M, Carrato A, Ilzarbe L, Michalski C, Molero X, *et al*. 2017 Reduced risk of pancreatic cancer associated with asthma and nasal allergies. *Gut* **66** 314–322.

Halfdanarson TR, Bamlet WR, McWilliams RR, Hobday TJ, Burch PA, Rabe KG & Petersen GM 2014 Risk factors for pancreatic neuroendocrine tumors: a clinic-based case-control study. *Pancreas* **43** 1219–1222. (doi:10.1097/MPA.0000000000000234)

Hassan MM, Phan A, Li D, Dagohoy CG, Leary C & Yao JC 2008*a* Family history of cancer and associated risk of developing neuroendocrine tumors: a case-control study. *Cancer Epidemiology, Biomarkers and Prevention* **17** 959–965. (doi:10.1158/1055-9965.EPI-07-0750)

Hassan MM, Phan A, Li D, Dagohoy CG, Leary C & Yao JC 2008*b* Risk factors associated with neuroendocrine tumors: a U.S.-based case-control study. *International Journal of Cancer* **123** 867–873. (doi:10.1002/ijc.23529)

Haugvik SP, Hedenstrom P, Korsaeth E, Valente R, Hayes A, Siuka D, Maisonneuve P, Gladhaug IP, Lindkvist B & Capurso G 2015 Diabetes, smoking, alcohol use, and family history of cancer as risk factors for pancreatic neuroendocrine tumors: a systematic review and meta-analysis. *Neuroendocrinology* **101** 133–142. (doi:10.1159/000375164)

Jensen RT, Cadiot G, Brandi ML, de Herder WW, Kaltsas G, Komminoth P, Scoazec JY, Salazar R, Sauvanet A & Kianmanesh R 2012 ENETS consensus guidelines for the management of patients with digestive neuroendocrine neoplasms: functional pancreatic endocrine tumor syndromes. *Neuroendocrinology* **95** 98–119. (doi:10.1159/000335591)

Leoncini E, Carioli G, La Vecchia C, Boccia S & Rindi G 2016 Risk factors for neuroendocrine neoplasms: a systematic review and meta-analysis. *Annals of Oncology* **27** 68–81. (doi:10.1093/annonc/mdv505)

Lepage C, Bouvier AM, Phelip JM, Hatem C, Vernet C & Faivre J 2004 Incidence and management of malignant digestive endocrine tumours in a well defined French population. *Gut* **53** 549–553. (doi:10.1136/gut.2003.026401)

Nogueira L, Freedman ND, Engels EA, Warren JL, Castro F & Koshiol J 2014 Gallstones, cholecystectomy, and risk of digestive system cancers. *American Journal of Epidemiology* **179** 731–739. (doi:10.1093/aje/kwt322)

Pannala R, Leirness JB, Bamlet WR, Basu A, Petersen GM & Chari ST 2008 Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. *Gastroenterology* **134** 981–987. (doi:10.1053/j.gastro.2008.01.039)

Panzuto F, Boninsegna L, Fazio N, Campana D, Pia Brizzi M, Capurso G, Scarpa A, De Braud F, Dogliotti L, Tomassetti P, *et al*. 2011 Metastatic and locally advanced pancreatic endocrine carcinomas: analysis of factors associated with disease progression. *Journal of Clinical Oncology* **29** 2372–2377. (doi:10.1200/JCO.2010.33.0688)

Rindi GAR, Bosman F, Capella C, Klimstra D, Klöppel G, Komminoth P & Solcia E 2010 Nomenclature and classification of neuroendocrine neoplasms of the digestive system. In *WHO Classification of Tumours of the Digestive System*.

Rolo AP & Palmeira CM 2006 Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicology and Applied Pharmacology* **212** 167–178. (doi:10.1016/j.taap.2006.01.003)

Solcia E, Klöppel G, Sobin LH & World Health Organization 2002 Histological typing of endocrine tumors. In *Collaboration With 9 Pathologists From 4 Countries*, 2nd edn, pp 1–5. Berlin, Heidelberg, New York: Sprinter Verlag.

Streicher SA, Yu H, Lu L, Kidd MS & Risch HA 2014 Case-control study of aspirin use and risk of pancreatic cancer. *Cancer Epidemiology, Biomarkers and Prevention* **23** 1254–1263. (doi:10.1158/1055-9965.EPI-13-1284)

Tiedge M, Lortz S, Drinkgern J & Lenzen S 1997 Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes* **46** 1733–1742. (doi:10.2337/diab.46.11.1733)

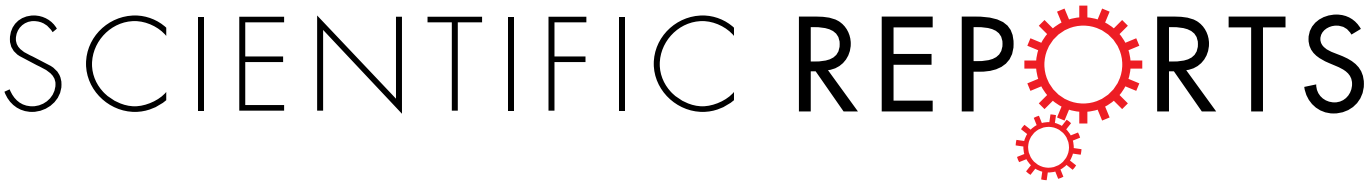
Vigneri R, Goldfine ID & Frittitta L 2016 Insulin, insulin receptors, and cancer. *Journal of Endocrinological Investigation* **39** 1365–1376. (doi:10.1007/s40618-016-0508-7)

Yao JC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, *et al*. 2008 One hundred years after 'carcinoid': epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *Journal of Clinical Oncology* **26** 3063–3072. (doi:10.1200/JCO.2007.15.4377)

Zhan HX, Cong L, Zhao YP, Zhang TP & Chen G 2013 Risk factors for the occurrence of insulinoma: a case-control study. *Hepatobiliary and Pancreatic Diseases International* **12** 324–328. (doi:10.1016/S1499-3872(13)60051-X)

Received in final form 12 May 2017
Accepted 30 May 2017
Accepted Preprint published online 31 May 2017

Published by Bioscientifica Ltd.



OPEN

Exclusive and Combined Use of Statins and Aspirin and the Risk of Pancreatic Cancer: a Case-Control Study

Received: 10 February 2017
Accepted: 25 September 2017
Published online: 12 October 2017

Livia Archibugi[1], Matteo Piciucchi[1], Serena Stigliano[1], Roberto Valente[1], Giulia Zerboni[1], Viola Barucca[1], Michele Milella[2], Patrick Maisonneuve[3], Gianfranco Delle Fave[1] & Gabriele Capurso[1]

Data on the association between aspirin and statin use and Pancreatic Ductal AdenoCarcinoma (PDAC) risk are conflicting. These drugs are often co-prescribed, but no studies evaluated the potential combined or confounding effect of the two at the same time. We aimed to investigate the association between aspirin and statin exclusive and combined use and PDAC occurrence. Data on environmental factors, family and medical history were screened in a case-control study. PDAC cases were matched to controls for age and gender. Power calculation performed ahead. Odds ratios (OR) and 95% confidence intervals(CI) were obtained from multivariable logistic regression analysis. In 408 PDAC patients and 816 matched controls, overall statin (OR 0.61; 95%CI,0.43–0.88), but not aspirin use was associated to reduced PDAC risk. Compared to non-users, exclusive statin (OR 0.51; 95%CI,0.32–0.80) and exclusive aspirin users (OR 0.64; 95%CI,0.40–1.01) had reduced PDAC risk. Concomitant statin and aspirin use did not further reduce the risk compared with statin use alone and no interaction was evident. Statin protective association was dose-dependent, and consistent in most subgroups, being stronger in smokers, elderly, obese and non-diabetic patients. The present study suggests that statin use is associated to reduced PDAC risk, supporting a chemopreventive action of statins on PDAC.

Pancreatic ductal adenocarcinoma (PDAC) is the 4th leading cause of cancer-related mortality[1] and is estimated to become the 2nd within 2030[2]. PDAC 5-year survival rate is only 6%[1] as only 20% of patients are eligible for surgery[3], and chemo/radiotherapy marginally improve survival in advanced disease[4]. Screening is advised only for high risk individuals in experimental settings[5].

Prevention might, therefore, play a key role in terms of modifiable lifestyle risk factors such as smoking and/or alcohol drinking, overweight, obesity and diet[6]. Evidence regarding potential chemopreventive drugs for PDAC is instead limited.

Aspirin is a nonsteroidal anti-inflammatory drug (NSAID), irreversible inhibitor of the cyclooxygenase 1/2, widely prescribed for its anti-inflammatory, antipyretic, analgesic and anti-platelet activities. Its chemopreventive role is well recognized for many cancer types[7] particularly colorectal cancer (CRC)[8,9].

Preclinical studies demonstrated that aspirin has a role in inhibiting different signaling pathways involved in the carcinogenesis of several cancers including PDAC, such as mTOR, NFkB and Wnt, resulting in enhanced DNA mismatch repair mechanisms, apoptosis, angiogenesis and tumour progression inhibition[10–12]. However, its role in clinical studies on PDAC is less clear, and the most recent meta-analysis on cohort and case-control studies, showed an overall protective effect of aspirin with an odds ratio (OR) of 0.77, although with high heterogeneity[13].

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors commonly prescribed for the treatment of hypercholesterolemia and for cardiovascular primary and secondary prevention[14]. The mevalonate pathway involved in their action has a role on multiple signaling cascades such as Ras, MEK, mTOR, BCL-2 and Rho kinases[15–17], that play role in carcinogenesis and tumour progression. Statin antineoplastic effect

[1]Digestive and Liver Disease Unit, S. Andrea Hospital, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy. [2]Medical Oncology Unit, Istituto Nazionale Tumori Regina Elena (IFO), Rome, Italy. [3]Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy. Correspondence and requests for materials should be addressed to G.C. (email: gabriele.capurso@gmail.com)

is also related with an immunomodulatory and anti-inflammatory activity and with angiogenesis inhibition[18,19]. Their clinical role as chemopreventive agents is still controversial, although a few meta-analyses showed a risk reduction associated with their use, mostly for gastrointestinal tract tumours[20–22]. The latest meta-analysis on the association between statin use and PDAC risk showed no pooled effect[23]. However, since then, other large case-control studies were published[24,25], recording a reduced PDAC risk associated with statin use.

As aspirin and statins are often co-prescribed, mostly for cardiovascular disease prevention or treatment, one might hypothesize that in studies investigating aspirin, at least a part of the observed effect might be due to the use of statins and *vice versa*. An investigation on the use of both these drugs, alone and in combination, has been conducted by Hoffmeister *et al*. in a case-control study on CRC. The authors reported a stronger risk reduction for statin exclusive use than for that of aspirin, while the combination of the two drugs did not further increase the effect of statins, unless for prolonged uses[26].

As studies on the possible chemopreventive activity of both aspirin and statins on PDAC gathered heterogeneous results, the analysis of their potential combined or confounding effect in this tumour type seems particularly important. However, there are no studies examining the association of both these two drugs at the same time with PDAC risk.

Therefore, the primary aim of the study was to examine the association between overall and exclusive aspirin and statin use and their combined use with the risk of PDAC. In addition, we conducted subanalyses to explore whether the association was stronger in specific subgroups.

Materials and Methods

Study design and population. A single-center case-control study was conducted from January 2006 to February 2016. Patients enrolled in either group provided written informed consent for interviews. The study received local IRB approval at Sant'Andrea Hospital. Methods were performed in accordance with the relevant guidelines and regulations.

Incident cases were prospectively recruited at the gastroenterology department and had PDAC histological diagnosis. Controls consisted of hospital non-patient visitors not genetically related to cases, as well as outpatients and inpatients from the gastroenterology department. Both cases and controls demonstrated the will and ability to participate providing personal data, clinical and cancer history. Exclusion criteria for controls in order to reduce the risk of recruitment bias were: (a) personal history of inflammatory bowel disease, chronic kidney disease or liver cirrhosis, (b) referral to our center for family history (FH) of gastrointestinal cancer, (c) referral to our center or hospitalization for NSAID-induced ulcer disease, (d) personal history of neoplasia in the last 5 years.

We used a frequency matching oversampling controls with a 2:1 ratio. For each case enrolled, the first two eligible controls of the same sex and age ($\pm$2 years) were enrolled and interviewed within 30 days.

Data collection and exposure definition. Data were recorded on a standardized form by a trained physician through direct patient interview; no proxies were interviewed.

The following clinical, epidemiological, therapeutic and morphological parameters were collected: sex, age, race, tobacco and alcohol intake, body mass index (BMI), FH of cancer, previous pancreatic diseases, history of diabetes, aspirin and statin use, length, type and dosage of their use. During the interview, a list of brand and generic medication names for aspirin and statins was provided to help facilitate recall. All cases were interviewed within 1 month from diagnosis and data pertaining the disease were also recorded.

For ever smokers, a consumption of at least 100 cigarettes or >6 months of smoking were needed to be considered a smoker. The total amount of smoking was evaluated as pack-years, defined as the product of packs smoked per day and the total years of smoking. A cut-off of 20 pack-years was set to define heavy smokers. Current smokers were considered as smokers who were currently smoking or who had quit less than 1 year in the past. Ex-smokers were classified as smokers who had quit at least 1 year before the diagnosis of the disease, or its first presentation symptom for cases, or the time of interview for controls.

For ever drinkers, a consumption of at least 12.5 g (1 unit) of alcohol/month was needed to be considered a drinker. One glass of wine, 1 pint or can of beer, one shot of hard liquor were each considered approximatively equal to 12.5 g of alcohol. A cut-off of 21 units/week (262.5 g of alcohol) was set to define heavy drinkers.

This cut-off was used because in a meta-analysis of 156 studies, drinking was reported as a risk factor for many cancers at a dose of both 25 and 50 g/day, and population studies in Italy suggest that 94% of subjects report either being teetotallers or consuming $\leq$4 alcoholic drinks daily[27,28].

BMI was calculated as usual adult weight/height^2 (kg/m^2) with obesity considered as BMI >30 kg/m^2. Diabetes was recorded as a potential risk factor when diagnosed >1 year before the diagnosis of cancer or its first symptoms for cases or before the interview for controls.

Aspirin or statin use was defined as the ever use of the medication for at least three consecutive months.

Aspirin users were categorized in "high dosage users" ($\geq$300 mg) and "low dosage users" ($\leq$160 mg)[29]. Statin users were categorized in "low-dosage users" (<20 mg) and "moderate/high dosage users" ($\geq$20 mg) based on median value.

Aspirin or statin users were categorized into different length of duration (<60 months and $\geq$60 months for aspirin, <48 months and $\geq$48 months for statins) based on median value.

To avoid possible bias due to cancer symptoms (i.e., weight loss, etc.) or subsequent cancer therapies, subjects were asked about risk factors that were present 12 months before diagnosis or presentation symptoms.

Statistical analysis. A power calculation was performed ahead: considering an exposure of ~20% for aspirin or statins as previously recorded in 200 controls, to have a 80% power of identifying an odds ratio (OR) $\leq$ 0.62 as

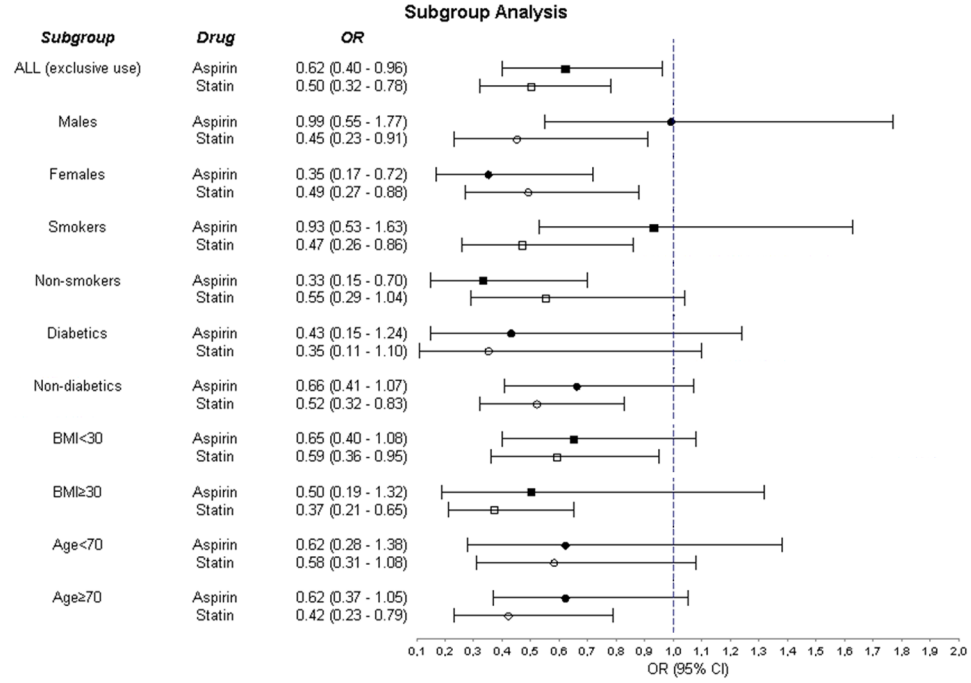


Figure 1. Subgroup analysis of the association between the exclusive use of either statins or aspirin and pancreatic cancer risk. Subgroup estimates are adjusted for age, sex, body mass index, first degree family history of pancreatic cancer, history of diabetes >1 year and smoking habit. OR: Odds Ratio, CI: Confidence Interval.

single effect of aspirin or statins, 395 cases and 790 controls were needed. This number would also allow to detect an OR $\leq$ 0.50 for the combined used of aspirin and statins, based on a 10% combined exposure among controls.

A descriptive analysis was conducted to show the characteristics of PDAC patients at time of diagnosis. Case-control comparisons were made using Chi-square and Fisher's exact tests where appropriate, for categorical variables and Student's t-test for continuous variables.

Logistic regression was used to calculate ORs and their 95% confidence intervals (CI). Multivariable logistic regression models were adjusted for potential confounders: age at diagnosis (for cases) or interview (for controls) (5-year age groups), gender, BMI, smoking history, drinking habits, diabetes history, chronic pancreatitis history, FH of PDAC.

All statistical analysis were performed using MedCalc version 13 (MedCalc Software, Belgium). All reported *P* values are 2-sided. *P* values < 0.05 were considered statistically significant.

The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) checklist for case-control studies was checked for items that should be included in the report.

Results

Of 421 PDAC cases seen at the Gastroenterology Department during the study period, 9 (2.1%; 4 males and 5 females, mean age 74) were not histologically confirmed. Of the remaining 412, 2 (0.5%) refused to participate and 2 (0.5%) were too ill to take part; of the 893 controls recruited in the same timeframe, 51 (5.7%) were excluded for matching exclusion criteria, 8 (0.9%) refused to be interviewed for privacy concerns, 18 (2.0%) were too ill to take part. This led to a participation rate of 99% for cases and 91% for controls; the analysis was therefore conducted on a final population of 408 cases and 816 matched controls. Respectively 48.7%, 13.4% and 37.9% of the controls, consisted of visitors, inpatients and outpatients. Most of the inpatients were hospitalized for diverticulitis or gastrointestinal infections; outpatients were visiting for either gastro-esophageal reflux disease, irritable bowel syndrome, chronic constipation or dyspepsia.

The median age of cases and matched controls was 68 years (range 35 to 99); 51.2% were men.

Almost all cases and controls were Caucasians.

Risk factors for pancreatic cancer. Compared with controls, cases had higher mean BMI value, higher proportion of obesity, 1st degree FH of PDAC, previous history of diabetes, previous chronic pancreatitis, and were more frequently smokers, heavy smokers and heavy drinkers (see Table 1).

Overall use of aspirin and statins and risk of pancreatic cancer. Seventy-eight cases (19.1%) and 191 controls (23.4%) reported ever use of aspirin, which was associated with a statistically borderline significant reduced PDAC risk (age- and sex-adjusted OR, 0.74; 95% CI, 0.54–1.02; Table 2). Only 2 of the 78 cases and 5 of the 191 controls (0.5% of both cases and controls) were on high-dose aspirin. The median duration of aspirin use

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Age	68.1 ± 11.6	67.9 ± 11.9	1.00 (0.99–1.01)	0.67	1.00 (0.99–1.02)	0.48
Male gender	209 (51.2%)	418 (51.2%)	1.00 (0.79–1.27)	0.99	0.77 (0.54–1.10)	0.16
1st degree FH of any cancer	218 (53.4%)	403 (49.4%)	1.22 (0.95–1.57)	0.12	—	
2nd degree FH of any cancer	30 (7.4%)	54 (6.6%)	1.29 (0.79–2.11)	0.31	—	
1st degree FH of PDAC	32 (7.8%)	23 (2.8%)	3.02 (1.73–5.26)	0.0001	3.26 (1.79–5.92)	0.0001
2nd degree FH of PDAC	5 (1.2%)	6 (0.7%)	1.83 (0.55–6.09)	0.33	2.17 (0.59–7.97)	0.24
BMI (mean ± Std.dev.)	26.8 ± 4.9	25.9 ± 4.1	1.05 (1.02–1.08)	0.001	1.04 (1.01–1.08)	0.009
BMI >30	79 (19.4%)	120 (14.7%)	1.47 (1.07–2.02)	0.02	—	
History of diabetes	72 (17.6%)	79 (9.7%)	2.03 (1.43–2.88)	<0.0001	1.84 (1.25–2.71)	0.002
Chronic pancreatitis	15 (3.7%)	2 (0.2%)	15.9 (3.60–70.0)	<0.0001	14.7 (3.18–67.6)	0.0006
Cigarette smoking[3]						
Never smoker	155 (38.0%)	416 (51.0%)	1.00		1.00	
Ever smoker	253 (62.0%)	400 (49.0%)	1.80 (1.39–2.32)	<0.0001	—	
<20 Pack-years	75 (18.4%)	171 (21.0%)	1.24 (0.89–1.74)	0.21	1.27 (0.88–1.84)	0.19
≥20 Pack-years	153 (37.5%)	229 (28.1%)	1.92 (1.43–2.57)	0.0001	1.93 (1.40–2.66)	<0.0001
Alcohol drinking[4,5]						
Never drinker	203 (49.8%)	446 (54.7%)	1.00		1.00	
Ever drinker	173 (42.4%)	370 (45.3%)	0.99 (0.77–1.29)	0.96	—	
<21 alcohol units/week	116 (28.4%)	312 (38.2%)	0.81 (0.61–1.07)	0.14	0.90 (0.66–1.21)	0.47
≥21 alcohol units/week	49 (12.0%)	57 (7.0%)	1.81 (1.17–2.80)	0.008	1.55 (0.96–2.49)	0.07

Table 1. Characteristics of pancreatic cancer cases and controls by selected variables of family history, chronic conditions, and lifestyle. FH: Family History, PDAC: Pancreatic Ductal AdenoCarcinoma, BMI: Body Mass Index, OR: Odds Ratio, CI: Confidence Intervals. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits. [3]Exact amount and duration not recalled by 25 (6.1%) cases and 0 controls. [4]Exact amount and duration not recalled by 32 (7.8%) cases and 0 controls. [5]Unknown units/week for 8 (2.0%) cases and 1 (0.1%) control.

was 60 months for both cases and controls, with 20 patients (4.9%) among cases and 33 patients (4.0%) among controls not recalling the exact duration of use. A borderline significant protective association was recorded for shorter duration of exposure.

Seventy-four cases (18.1%) and 203 controls (24.9%) reported ever use of statins, which was associated with a statistically significant reduced PDAC risk (age- and sex-adjusted OR, 0.64; 95% CI, 0.48–0.88; Table 2). The median dosage of statins was 20 mg and the median duration of use was 48 months for both cases and controls. A higher dosage of statins (for ≥20 mg, OR, 0.43; 95% CI, 0.27–0.71) was associated with a stronger protective effect. The most commonly used statin was atorvastatin. Of the 74 cases and the 203 controls, respectively 11 (2.7%) and 46 (5.6%) could not recall the type of drug, 38 (9.3%) and 69 (8.5%) could not recall the dosage, and 27 (6.6%) and 48 (5.9%) could not recall the duration of use.

At multivariable analysis, statins (OR 0.61; 95% CI, 0.43–0.88) but not aspirin (OR, 0.77; 95% CI, 0.53–1.11) use was associated to a reduced PDAC risk (Table 2). OR and 95% CI for all other risk factors for this first multivariable analysis model are reported in Supplementary Table 1.

In order to avoid possible bias due to controls selection, a sensitive analysis for control type (visitors or hospital patients) was performed. At multivariable analysis, statin use was associated to a reduced risk of PDAC both with sensitive analysis restricted to visitors (OR 0.60; 95% CI, 0.40–0.89) or to hospital controls (OR 0.59; 95% CI 0.40–0.87), while the use of aspirin was not associated to PDAC risk for either visitors (OR, 0.82; 95% CI, 0.55–1.23) nor hospital controls (OR 0.67; 95% CI 0.45–1.01).

Exclusive and Combined use of Aspirin and Statins and risk of pancreatic cancer. In order to evaluate the exclusive or combined effect of the two drugs and avoid possible confounding effects, we analyzed the use of aspirin excluding patients reporting also the use of statins and *vice versa* (Table 3). An exclusive aspirin use was recorded in 39 (9.6%) of all cases and 95 (11.6%) of all controls, and an exclusive statin use was recorded in 35 (8.6%) of cases and 107 (13.1%) of controls. In an age- and sex- adjusted analysis the exclusive use of statins was associated with a stronger risk reduction (OR, 0.54; 95% CI, 0.36–0.82) than the exclusive use of aspirin (OR, 0.67; 95% CI, 0.45–1.02). The concomitant use of statins and aspirin was reported in 39 cases (9.6%) and 96 controls (11.8%). This combined use did not further reduce the risk (OR, 0.67; 95% CI, 0.44–1.01) compared with the use of statins alone.

At multivariable analysis adjusted for other potential confounding factors, statin use (OR, 0.51; 95% CI, 0.32–0.80) was associated to a reduced risk of PDAC occurrence, while the association for aspirin use was of borderline significance (OR, 0.64; 95% CI, 0.40–1.01). Also at multivariable analysis the combined use of the two drugs did no further reduce the risk compared to the use of statins alone (OR, 0.54; 95% CI, 0.34–0.87). We found no evidence

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Aspirin use[3,4]						
Never	330 (80.9%)	625 (76.6%)	1.00			
Ever	78 (19.1%)	191 (23.4%)	0.74 (0.54–1.02)	0.06	0.77 (0.53–1.11)	0.16
Low-dose (≤160 mg)	68 (16.7%)	154 (18.9%)	0.80 (0.58–1.12)	0.20		
High-dose (≥300 mg)	2 (0.5%)	5 (0.6%)	0.72 (0.14–3.76)	0.70		
<60 months	25 (6.1%)	73 (9.0%)	0.62 (0.38–1.01)	0.05		
≥60 months	33 (8.1%)	85 (10.4%)	0.70 (0.45–1.09)	0.11		
Statin use[5–7]						
Never	334 (81.9%)	613 (75.1%)	1.00			
Ever	74 (18.1%)	203 (24.9%)	0.64 (0.48–0.88)	0.005	0.61 (0.43–0.88)	0.007
Atorvastatin	29 (7.1%)	85 (10.4%)	0.60 (0.38–0.94)	0.03		
Simvastatin	23 (5.6%)	45 (5.5%)	0.92 (0.54–1.56)	0.76		
Other forms*	11 (2.7%)	27 (3.3%)	0.72 (0.35–1.48)	0.37		
<20 mg	14 (3.4%)	44 (5.4%)	0.56 (0.30–1.05)	0.07		
≥20 mg	22 (5.4%)	90 (11.0%)	0.43 (0.27–0.71)	0.0008		
<48 months	25 (6.1%)	84 (10.3%)	0.53 (0.33–0.84)	0.008		
≥48 months	22 (5.4%)	71 (8.7%)	0.55 (0.33–0.90)	0.02		

Table 2. Overall aspirin and statin use among pancreatic cancer cases and controls. OR: Odds Ratio, CI: Confidence Interval. *Fluvastatin, Lovastatin, Pravastatin, Rosuvastatin. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits. [3]Unknown dose for 8 (2%) cases and 32 (3.9%) controls. [4]Unknown duration for 20 (4.9%) cases and 33 (4%) controls. [5]Unknown type for 11 (2.7%) cases and 46 (5.6%) controls. [6]Unknown dose for 38 (9.3%) cases and 69 (8.5%) controls. [7]Unknown duration for 27 (6.6%) cases and 48 (5.9%) controls.

	Cases (408)	Controls (816)	Age and sex adjusted[1] OR (95% CI)	*P* value	Multivariable analysis[2] OR (95% CI)	*P* value
Exclusive or combined use						
Neither aspirin nor statins	295 (72.3%)	518 (63.5%)	1.00		1.00	
Aspirin only	39 (9.6%)	95 (11.6%)	0.67 (0.45–1.02)	0.06	0.64 (0.40–1.01)	0.06
Statins only	35 (8.6%)	107 (13.1%)	0.54 (0.36–0.82)	0.004	0.51 (0.32–0.80)	0.004
Aspirin and Statins	39 (9.6%)	96 (11.8%)	0.67 (0.44–1.01)	0.06	0.54 (0.34–0.87)	0.01

Table 3. Exclusive and combined aspirin and statin use among pancreatic cancer cases and controls. OR: Odds Ratio, CI: Confidence Interval. [1]Odds Ratios adjusted for age (5-year age groups) and gender. [2]Odds ratios adjusted for age (5-year age groups), sex, body mass index (continuous scale), family history of pancreatic cancer (first and second degree relatives), history of chronic pancreatitis, history of diabetes >1 year, smoking and drinking habits.

of interaction between statin and aspirin (P = 0.17), when adding an interaction term to the main effects of aspirin and statin in a multivariable model adjusted for other potential confounding variables (Supplementary Table 2).

Association between use of aspirin and statins and risk of pancreatic cancer in subgroups. To evaluate a potential specific association between aspirin and statin use and the risk of PDAC among different subgroups, separate multivariable subgroup analyses were conducted for the exclusive use of the two drugs according to: gender, smoking habit, obesity, history of diabetes and age ≥ or <70 years. Results are shown in Fig. 1.

Discussion

This is the first study evaluating the possible association between overall, exclusive and combined use of both aspirin and statins and PDAC risk at the same time. This is a relevant issue, as both drugs together are very frequently prescribed in elderlies for cardiovascular prevention and treatment[30].

Therefore, one might hypothesize that both a confounding effect, for which only one of the two co-prescribed drugs is protective, or a synergistic effect, for which the activity of the combination of the two drugs is superior to their exclusive use, might occur.

In the present study, PDAC risk is inversely associated with the overall statin use, with a dosage-dependent effect. The overall aspirin use is not associated to a statistically significant reduced PDAC risk at multivariable

analysis (Table 2). Both exclusive aspirin and statin use were related to a reduced PDAC risk, with statin use showing a risk reduction of 49%, higher than that of aspirin, which was only borderline statistically significant. The concomitant use of the two drugs was also associated to a 46% reduced PDAC risk, without conferring a stronger risk reduction compared to the use of statins alone, therefore we might speculate that the main protective effect of the combined use is due to statins. Furthermore, the analysis of interaction did not suggest a synergistic effect (Supplementary Table 2).

A protective association with the use of statins has been described mostly in case-control studies and not in cohort studies before. Two recent cohort[31] and case-control studies[32] evaluated the use of statins and the risk of PDAC. The first study on a female population adjusted the analysis for the use of aspirin and NSAIDs, and the second also analysed the use of aspirin, but both did not evaluate the association of the two drugs or their exclusive use. Two other large case-control studies[24,25] evaluating the association between statin use and PDAC risk instead showed a risk reduction similar to that of the present study. However, in none of those previous publications, *a priori* power calculation was performed. Walker *et al.*[24] reported a reduced PDAC risk in overall statin users with OR = 0.66 and, in a subgroup analysis only in men and mostly for prolonged use. Carey *et al.*[25], described a reduced risk only in male smokers. However, in this latter study, OR were not adjusted for BMI and dosage and duration of drug use were not recorded. Compared to those previous studies, our results show a more consistent association between the exclusive use of statins and PDAC risk reduction, which was similar for both genders, but limited to smokers, elderly subjects, obese and non-diabetic patients (Fig. 1). Statins might exert a specific protective effect on cigarette-related carcinogenesis, as they have also been found to be protective against lung cancer[33]. Moreover, nicotine-mediated pancreatic carcinogenesis in animal models relies on the activation of AKT and ERK[34], and statins have been reported to negatively regulate such signaling pathways in PDAC models and in other cancers[35,36]. The reasons for a more important protective effect of statins in non-diabetics are unclear. Chen *et al.*, evaluated a cohort of diabetic patients, in whom statin use was associated to a reduced risk[37]. A more complex relation between statins and diabetes might be due to their pro-diabetogenic effect[38]. Moreover, although we considered only patients with a history of diabetes >1 year, in order to avoid PDAC causing, rather than being caused by diabetes, one cannot exclude that in some patients the onset of diabetes, although >1 year, might still be due to PDAC occurrence[39].

As far as regards the more significant association between statin use and reduced PDAC risk in subjects aged ≥70 years, this can be explained by the obvious increased PDAC risk in older subjects. Furthermore, an older age is usually associated to prolonged drug exposure.

There are many published studies on the preventive effect of aspirin on PDAC occurrence, with heterogeneous results[7,40–42] but, to our knowledge, no significant results were reported for specific subgroups. In our study aspirin was protective only in females and non-smokers. These results are in contrast with those of a previous large cohort study conducted on nurses by Schernhammer *et al.*[41], where aspirin intake was a risk factor for PDAC, directly related to the number of tablets taken. The results of our multivariable analyses (Tables 2 and 3) and of the subgroup analysis for the exclusive use of the two drugs (Fig. 1), suggest an inconsistent effect of aspirin, possibly supporting the hypothesis that at least a part of the previously reported association between aspirin use and reduced PDAC risk is due to concomitant statin use.

However, as in our population there was a very low proportion of high-dosage aspirin users, we cannot exclude a stronger protective effect for higher dosages of aspirin, although in previous studies also low-dose aspirin showed protective effect[40].

Among the different statin types, atorvastatin was the most frequently prescribed in the present study, and was associated to a reduced risk of PDAC. The chemopreventive effect of atorvastatin has already been described in *in vivo* studies[35,43], while previous cohort or case-control studies showed heterogeneous results between different statins[23,24,31]. This could therefore be only due to atorvastatin being the most frequently prescribed type of statins.

The present study has some strengths: 1) it is the first specifically aimed at evaluating the association between overall, exclusive and combined use of both aspirin and statins and PDAC risk, as they both seem to have chemopreventive properties and are very often prescribed together; 2) it is one of the few studies on this topic with an *a priori* power calculation; 3) as expected, an increased PDAC risk for patients with multiple 1st degree FH of neoplasia, 1st degree FH of PDAC, increased BMI, previous history of diabetes, chronic pancreatitis, smoking habit was seen, suggesting the absence of biases and the genuineness of our population; 4) statin results are consistent with most Bradford Hill criteria for causation[44] as the association is strong, a biological gradient is evident as higher dosages lower the risk and the association is plausible in terms of mechanisms and coherent with *in vitro* studies. A clear temporal relation was instead not evident. Of course, as this is a case-control study, causation of an effect cannot be observed and we can only report an association with risk, useful to generate hypotheses that need validation.

There are, however, some limitations. First, as for any case-control study, the risk of recall bias has to be taken into account, although when interviewed, patients were provided with a list of brand and dosages in order to reduce this risk. Furthermore, both the rate of non-participants and the rate of patients not recalling data about their drug use are similar among cases and controls. In order to reduce the risk of confounding factors, the questionnaire was carefully conducted by a trained physician with expertise on pancreatic disorders, asking information regarding risk factors exposure as present 1 year before the interview. As in any case-control study the choice of the control population is a possible matter of concern. We opted for a control group that we believed to represent the same population as the case group, derived from the same catchment area, and being composed both of patients seen for similar symptoms by the same doctors in the same clinics but with a final diagnosis unrelated with the disease of interest, and by hospital non-patient visitors; this, therefore, was unlikely to cause a specific bias[45]. To explore the possibility of a potential selection bias among hospital controls, who might have been less likely to use aspirin being selected in a Gastroenterology setting, we performed a sensitivity analysis comparing the effect

of aspirin and statins in the two different control groups (visitors and hospital controls) separately with findings suggesting a consistent effect of statins but not of aspirin.

Furthermore, our control group seems to be representative of the general Italian population in terms of environmental risk factors and exposure to the drugs of interest[46,47] and to populations used for other case-control studies on PDAC[48]. Also, as the study was not powered for the exclusive use analyses or subgroup analysis, these results have to be taken carefully into account as they might not be conclusive. For example, in a post-hoc calculation limited to the 653 smokers (253 cases and 400 controls), the statistical power of the study dropped to 67% for detecting an OR = 0.62 (as per protocol) considering the actual aspirin exposure (26%) measured among control smokers.

Furthermore, one cannot exclude that the observed reduced PDAC risk associated with statin use and to a lesser extent of aspirin, is a surrogate for other uninvestigated factors such as a healthier lifestyle. At any rate, the current analyses were corrected for most known risk factors associated with PDAC risk (see Table 1), but residual confounding due to other factors such as diet, antioxidants use, physical activity or different indication for statin use cannot be excluded. Notably, a recent meta-analysis showed no association between serum cholesterol levels and PDAC risk[49].

The biological mechanisms through which statins might prevent PDAC are not completely clarified. Interestingly, the importance of statins has been recently proved in retrospective studies also in terms of prolonging survival in operated PDAC patients[50–52], suggesting again an effect of this class of drugs on this tumour. In this context, the development of randomized controlled trials investigating the effect of statins in an adjuvant setting, in patients undergoing resection for PDAC could prove interesting. Moreover, as the statistical power of such studies might be limited by the very low rate of survival of PDAC patients, further studies on the possible chemopreventive effect of statins in individuals with an increased risk of developing PDAC, such as patients with genetic syndromes at high risk of PDAC[5], and patients diagnosed with pancreatic pre-neoplastic lesions such as Intraductal Papillary Mucinous Neoplasia (IPMNs)[53] might be of interest.

In conclusion, the present study suggests that statin use, rather than that of aspirin, in particular at higher dosages is associated to a reduced PDAC risk. These findings support a chemopreventive action of statins on PDAC and no apparent synergistic activity of the two medications.

References

1. Siegel, R., Naishadham, D. & Jemal, A. Cancer statistics, 2013. *CA: a cancer journal for clinicians* **63**, 11–30, https://doi.org/10.3322/caac.21166 (2013).
2. Rahib, L. *et al*. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer research* **74**, 2913–2921, https://doi.org/10.1158/0008-5472.CAN-14-0155 (2014).
3. Spanknebel, K. & Conlon, K. C. Advances in the surgical management of pancreatic cancer. *Cancer journal* **7**, 312–323 (2001).
4. Neuzillet, C. *et al*. State of the art and future directions of pancreatic ductal adenocarcinoma therapy. *Pharmacology & therapeutics* **155**, 80–104, https://doi.org/10.1016/j.pharmthera.2015.08.006 (2015).
5. Canto, M. I. *et al*. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. *Gastroenterology* **142**, 796–804; quiz e714–795, https://doi.org/10.1053/j.gastro.2012.01.005 (2012).
6. Maisonneuve, P. & Lowenfels, A. B. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. *International journal of epidemiology* **44**, 186–198, https://doi.org/10.1093/ije/dyu240 (2015).
7. Cao, Y. *et al*. Population-wide Impact of Long-term Use of Aspirin and the Risk for Cancer. *JAMA oncology* **2**, 762–769, https://doi.org/10.1001/jamaoncol.2015.6396 (2016).
8. Bibbins-Domingo, K. & Force, U. S. P. S. T. Aspirin Use for the Primary Prevention of Cardiovascular Disease and Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Annals of internal medicine* **164**, 836–845, https://doi.org/10.7326/M16-0577 (2016).
9. Emilsson, L. *et al*. Systematic review with meta-analysis: the comparative effectiveness of aspirin vs. screening for colorectal cancer prevention. *Alimentary pharmacology & therapeutics* **45**, 193–204, https://doi.org/10.1111/apt.13857 (2017).
10. Shen, X. *et al*. Aspirin: a potential therapeutic approach in pancreatic cancer. *Current medicinal chemistry* **20**, 4153–4162 (2013).
11. Din, F. V. *et al*. Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. *Gastroenterology* **142**, 1504–1515 e1503, https://doi.org/10.1053/j.gastro.2012.02.050 (2012).
12. Yue, W., Yang, C. S., DiPaola, R. S. & Tan, X. L. Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer prevention research* **7**, 388–397, https://doi.org/10.1158/1940-6207.CAPR-13-0337 (2014).
13. Zhang, Y. P., Wan, Y. D., Sun, Y. L., Li, J. & Zhu, R. T. Aspirin might reduce the incidence of pancreatic cancer: A meta-analysis of observational studies. *Scientific reports* **5**, 15460, https://doi.org/10.1038/srep15460 (2015).
14. Miller, P. E. & Martin, S. S. Approach to Statin Use in 2016: an Update. *Current atherosclerosis reports* **18**, 20, https://doi.org/10.1007/s11883-016-0578-1 (2016).
15. Laezza, C. *et al*. Inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and of Ras farnesylation mediate antitumor effects of anandamide in human breast cancer cells. *Endocrine-related cancer* **17**, 495–503, https://doi.org/10.1677/ERC-10-0009 (2010).
16. Duncan, R. E., El-Sohemy, A. & Archer, M. C. Statins and cancer development. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **14**, 1897–1898, https://doi.org/10.1158/1055-9965.EPI-05-0027 (2005).
17. Spampanato, C. *et al*. Simvastatin inhibits cancer cell growth by inducing apoptosis correlated to activation of Bax and down-regulation of BCL-2 gene expression. *International journal of oncology* **40**, 935–941, https://doi.org/10.3892/ijo.2011.1273 (2012).
18. Demierre, M. F., Higgins, P. D., Gruber, S. B., Hawk, E. & Lippman, S. M. Statins and cancer prevention. *Nature reviews. Cancer* **5**, 930–942, https://doi.org/10.1038/nrc1751 (2005).
19. Dulak, J. & Jozkowicz, A. Anti-angiogenic and anti-inflammatory effects of statins: relevance to anti-cancer therapy. *Current cancer drug targets* **5**, 579–594 (2005).
20. Bonovas, S. Statins: do they have a potential role in cancer prevention and modifying cancer-related outcomes? *Drugs* **74**, 1841–1848, https://doi.org/10.1007/s40265-014-0309-2 (2014).
21. Liu, Y. *et al*. Association between statin use and colorectal cancer risk: a meta-analysis of 42 studies. *Cancer causes & control: CCC* **25**, 237–249, https://doi.org/10.1007/s10552-013-0326-6 (2014).

22. Alexandre, L., Clark, A. B., Cheong, E., Lewis, M. P. & Hart, A. R. Systematic review: potential preventive effects of statins against oesophageal adenocarcinoma. *Alimentary pharmacology & therapeutics* **36**, 301–311, https://doi.org/10.1111/j.1365-2036.2012.05194.x (2012).
23. Cui, X. *et al*. Statin use and risk of pancreatic cancer: a meta-analysis. *Cancer causes & control: CCC* **23**, 1099–1111, https://doi.org/10.1007/s10552-012-9979-9 (2012).
24. Walker, E. J., Ko, A. H., Holly, E. A. & Bracci, P. M. Statin use and risk of pancreatic cancer: results from a large, clinic-based case-control study. *Cancer* **121**, 1287–1294, https://doi.org/10.1002/cncr.29256 (2015).
25. Carey, F. J. *et al*. The differential effects of statins on the risk of developing pancreatic cancer: a case-control study in two centres in the United Kingdom. *Digestive diseases and sciences* **58**, 3308–3312, https://doi.org/10.1007/s10620-013-2778-7 (2013).
26. Hoffmeister, M., Chang-Claude, J. & Brenner, H. Individual and joint use of statins and low-dose aspirin and risk of colorectal cancer: a population-based case-control study. *International journal of cancer* **121**, 1325–1330, https://doi.org/10.1002/ijc.22796 (2007).
27. Corrao, G., Bagnardi, V., Zambon, A. & La Vecchia, C. A meta-analysis of alcohol consumption and the risk of 15 diseases. *Preventive medicine* **38**, 613–619, https://doi.org/10.1016/j.ypmed.2003.11.027 (2004).
28. Loguercio, C. *et al*. Drinking habits and risk of altered liver enzymes in the general population of a rural area in Southern Italy. *Digestive and liver disease: official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver* **39**, 748–752, https://doi.org/10.1016/j.dld.2007.05.006 (2007).
29. Patrignani, P. & Patrono, C. Aspirin and Cancer. *Journal of the American College of Cardiology* **68**, 967–976, https://doi.org/10.1016/j.jacc.2016.05.083 (2016).
30. Lafeber, M. *et al*. The combined use of aspirin, a statin, and blood pressure-lowering agents (polypill components) and the risk of vascular morbidity and mortality in patients with coronary artery disease. *American heart journal* **166**, 282–289 e281, https://doi.org/10.1016/j.ahj.2013.04.011 (2013).
31. Simon, M. S. *et al*. Prospective analysis of association between statins and pancreatic cancer risk in the Women's Health Initiative. *Cancer causes & control: CCC* **27**, 415–423, https://doi.org/10.1007/s10552-016-0717-6 (2016).
32. Kho, P. F. *et al*. Nonsteroidal anti-inflammatory drugs, statins, and pancreatic cancer risk: a population-based case-control study. *Cancer causes & control: CCC* **27**, 1457–1464, https://doi.org/10.1007/s10552-016-0824-4 (2016).
33. Liu, J. C. *et al*. Statins dose-dependently exert a chemopreventive effect against lung cancer in COPD patients: a population-based cohort study. *Oncotarget*, https://doi.org/10.18632/oncotarget.11162 (2016).
34. Hermann, P. C. *et al*. Nicotine promotes initiation and progression of KRAS-induced pancreatic cancer via Gata6-dependent dedifferentiation of acinar cells in mice. *Gastroenterology* **147**, 1119–1133 e1114, https://doi.org/10.1053/j.gastro.2014.08.002 (2014).
35. Mohammed, A. *et al*. Atorvastatin delays progression of pancreatic lesions to carcinoma by regulating PI3/AKT signaling in p48Cre/+ LSL-KrasG12D/+ mice. *International journal of cancer* **131**, 1951–1962, https://doi.org/10.1002/ijc.27456 (2012).
36. Tsubaki, M. *et al*. Statins inhibited the MIP-1alpha expression via inhibition of Ras/ERK and Ras/Akt pathways in myeloma cells. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **78**, 23–29, https://doi.org/10.1016/j.biopha.2015.12.017 (2016).
37. Chen, M. J. *et al*. Statins and the risk of pancreatic cancer in Type 2 diabetic patients–A population-based cohort study. *International journal of cancer* **138**, 594–603, https://doi.org/10.1002/ijc.29813 (2016).
38. Backes, J. M., Kostoff, M. D., Gibson, C. A. & Ruisinger, J. F. Statin-Associated Diabetes Mellitus: Review and Clinical Guide. *Southern medical journal* **109**, 167–173, https://doi.org/10.14423/SMJ.0000000000000423 (2016).
39. Pannala, R., Basu, A., Petersen, G. M. & Chari, S. T. New-onset diabetes: a potential clue to the early diagnosis of pancreatic cancer. *The Lancet. Oncology* **10**, 88–95, https://doi.org/10.1016/S1470-2045(08)70337-1 (2009).
40. Streicher, S. A., Yu, H., Lu, L., Kidd, M. S. & Risch, H. A. Case-control study of aspirin use and risk of pancreatic cancer. *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **23**, 1254–1263, https://doi.org/10.1158/1055-9965.EPI-13-1284 (2014).
41. Schernhammer, E. S. *et al*. A prospective study of aspirin use and the risk of pancreatic cancer in women. *Journal of the National Cancer Institute* **96**, 22–28 (2004).
42. Capurso, G. *et al*. Meta-analysis: the use of non-steroidal anti-inflammatory drugs and pancreatic cancer risk for different exposure categories. *Alimentary pharmacology & therapeutics* **26**, 1089–1099, https://doi.org/10.1111/j.1365-2036.2007.03495.x (2007).
43. Liao, J. *et al*. Atorvastatin inhibits pancreatic carcinogenesis and increases survival in LSL-KrasG12D-LSL-Trp53R172H-Pdx1-Cre mice. *Molecular carcinogenesis* **52**, 739–750, https://doi.org/10.1002/mc.21916 (2013).
44. Hill, A. B. The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine* **58**, 295–300 (1965).
45. Lewallen, S. & Courtright, P. Epidemiology in practice: case-control studies. *Community eye health/International Centre for Eye Health* **11**, 57–58 (1998).
46. Ferrajolo, C. *et al*. Pattern of statin use in southern italian primary care: can prescription databases be used for monitoring long-term adherence to the treatment? *PloS one* **9**, e102146, https://doi.org/10.1371/journal.pone.0102146 (2014).
47. (ISTAT), I. N. d. S. http://dati.istat.it/Index.aspx?DataSetCode (Date of access:15/06/2017) (2016).
48. Hassan, M. M. *et al*. Risk factors for pancreatic cancer: case-control study. *The American journal of gastroenterology* **102**, 2696–2707, https://doi.org/10.1111/j.1572-0241.2007.01510.x (2007).
49. Wang, J., Wang, W. J., Zhai, L. & Zhang, D. F. Association of cholesterol with risk of pancreatic cancer: a meta-analysis. *World journal of gastroenterology* **21**, 3711–3719, https://doi.org/10.3748/wjg.v21.i12.3711 (2015).
50. Kozak, M. M. *et al*. Statin and Metformin Use Prolongs Survival in Patients With Resectable Pancreatic Cancer. *Pancreas* **45**, 64–70, https://doi.org/10.1097/MPA.0000000000000470 (2016).
51. Jeon, C. Y. *et al*. The association of statin use after cancer diagnosis with survival in pancreatic cancer patients: a SEER-medicare analysis. *PloS one* **10**, e0121783, https://doi.org/10.1371/journal.pone.0121783 (2015).
52. Wu, B. U. *et al*. Impact of statin use on survival in patients undergoing resection for early-stage pancreatic cancer. *The American journal of gastroenterology* **110**, 1233–1239, https://doi.org/10.1038/ajg.2015.217 (2015).
53. Distler, M., Aust, D., Weitz, J., Pilarsky, C. & Grutzmann, R. Precursor lesions for sporadic pancreatic cancer: PanIN, IPMN, and MCN. *BioMed research international* **2014**, 474905, https://doi.org/10.1155/2014/474905 (2014).

Acknowledgements

Livia Archibugi was granted by La Sapienza University "Borsa di Avvio alla Ricerca 2015" (Protocol number 0051276) and Gabriele Capurso was granted by AIRC IG Grant 2015, 17177.

Author Contributions

G.C. is the submission guarantor. L.A. and G.C. developed study concept and design, drafted the paper and acquired and interpreted the data; P.M. contributed to study design, interpretation of the data, statistical analysis and drafting of the paper, G.D.F. contributed to study design, interpretation of the data and drafting of the paper; M.P., R.V., S.S., G.Z., V.B., M.M. acquired data, contributed to drafting of the paper, performed critical revision of the manuscript for important intellectual content. All authors approved the final version of the article, including the author list.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-017-13430-z.

Competing Interests: The authors declare that they have no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.