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Necrosis as a strong independent prognostic factor required in the implementation of pathological reporting for pancreatic adenocarcinoma resection specimens.

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Abstract:

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a major public health issue with an incidence/mortality ratio reaching 98%. Only 15 to 20% of patients with PDAC can undergo surgery. Following PDAC surgical resection, 80% of patients will experience local or metastatic recurrence of this disease. pTNM staging is the gold standard for risk stratification but is not sufficient to recapitulate the prognosis. Several prognostic factors are known to impact survival after surgery when uncovered during pathological examination. However, necrosis has been poorly studied in pancreatic adenocarcinoma.

Materials & Methods: We retrieved clinical data and reviewed all tumor slides from patients who had a pancreatic surgery between January 2004 and December 2017, in the Hospices Civils de Lyon, to assess the presence of histopathological prognosis factors associated with poor prognosis.

Results: 514 patients with complete clinico-pathological description were included. Necrosis was found in 231 PDAC (44.9%) and had an important impact on overall survival with a double risk of death when present in tumor samples (HR: 1.871, 95% CI [1.523; 2.299], $p < 0.001$). When integrated in the multivariate model, necrosis is the only morphological aggressive feature to retain high statistical significance associated with the TNM staging but independently of it. This effect is independent of the preoperative treatment.

Conclusions: Despite improvement in treatment of PDAC, mortality rates remain relatively stable amongst the last years. There is a desperate need to better stratify patients. Here, we report the strong and prognostic impact of necrosis in surgical PDAC samples and encourage pathologists to report its presence in the future.

Keywords: pancreatic cancer, adenocarcinoma, prognostic factor, necrosis, overall survival

Introduction:

Pancreatic ductal adenocarcinoma's (PDAC) 5-year survival rate is around 5 to 9% and seems to be stable in many countries despite improvement and standardization of care with mortality/incidence ratio of 94% [1–3]. Besides PDAC incidence is expected to double with a stable mortality within 10 years, ranking PDAC as the 2nd most frequent cause of cancer-related deaths in 2030 [4]. It is commonly noted that only 15 to 20% of patients diagnosed for PDAC undergo surgery [5]. The number of patients undergoing surgery is expected to increase in the next years due to neoadjuvant chemotherapy administered in patients with borderline tumors which helps to locally control PDAC and gives patients a chance to undergo surgery following tumor size reduction [6]. After PDAC surgical resection, 80% of patients will experience local or metastatic recurrence of the disease [7], highlighting the importance to stratify patients, a responsibility of the pathologists. In the era of personalized medicine, pathological reports help to predict each patient's recurrence risk leading to delivery of the most appropriate treatment.

Initially, prognostic evaluation of surgical specimen to pathologists nowadays means Union for International Cancer Control staging using the pTNM system [8]. Between 2017 (the year of the release of its 8th edition) and 2019, only few studies have tried to evaluate the input of this new staging system, mostly based for the T staging on tumor size with controversial results [9, 10]. Hopefully, UICC staging is not the only source of prognostic information. Recently, a consensus set a 1 mm clearance from inked margins to determine a R0 resection [6, 11, 12]. When this consensual criterion is applied, the quality of resection is strongly associated with long-term outcome and local recurrence [13–15]. Other conventional morphological prognosis criteria such as perineural invasion, differentiation grade, lymphovascular invasion, superior mesenteric vein/portal vein histopathologic mural invasion, duodenal wall invasion and high grade budding remain strong indicators of PDAC aggressiveness, in terms of overall or disease-free survival (OS and DFS) and local recurrence. The same criteria applies even after neoadjuvant chemotherapy for perineural invasion [16–24]. The final pathological prognosis factor described is the loss of SMAD4 in immunohistochemistry. It is now accepted to be associated with a decrease of the OS and the presence of lymph node metastasis and lymphovascular invasion [25–27].

Interestingly, during the era of -omics technology, simple morphological criterion such as necrosis, commonly associated with poor survival outcome in many cancers, has been poorly studied in PDAC [28–32]. Evidently, only three studies were previously performed to assess the impact of necrosis on surgical specimen as a prognostic factor in PDAC after surgery [33–35]. Hiraoka et al found that necrosis was associated with a shorter DFS and disease-specific survival (DSS) in multivariate analysis even if this analysis did not include pTNM staging. In 2005, Mitsunaga et al reported the same results in multivariate analysis for DFS but also for OS. They included the then-current TNM staging in their analysis. The more recent work of Kudo et al, also found an association between DFS and necrosis in multivariate analysis.

Nevertheless in the multivariate analysis, the current pTNM staging was not found to be associated with DFS whereas several other studies state the opposite [3, 9, 36].

Here we aim to define the clinical relevance of necrosis in term of OS, and its potential prognostic role compared to other known and well-described histopathological aggressive features including the current 2017 pTNM staging system in a large surgical patient cohort of PDAC.

Material and methods:

Human PDAC cohort and clinical data retrieval

Samples from patients who underwent surgery in one of the two expert centers for pancreatic surgery (Edouard Herriot and Croix Rousse Hospital Digestive surgery unit) of the Hospices Civils de Lyon between the 01st of January 2004 and the 31th of December 2017 for pancreatic ductal adenocarcinoma were included in the cohort. The exclusion criteria included samples with absence of well conserved material left after diagnosis, samples with the presence of an adenocarcinoma with no evidence of pancreatic origin especially for duodeno-pancreatectomy specimens and relapsing tumors. Clinical data was retrieved from the shared computerized medical files of each patient (age at diagnosis, gender, date of diagnosis, date of the latest news or date of death or recurrence).

Morphological analysis

All pathological reports were reviewed and the pTNM staging (according to the 2010 classification) used at the time of diagnosis were recorded. This included the number of examined nodes, the number of invaded nodes, the size of the tumor after being fixed in 4% formaldehyde and its localization in the pancreas (head, body tail).

Whole tumors were sectioned with a 0.5cm thickness using a standardized protocol that has been implemented from 2004 to 2021 following the different versions of the dataset for the histopathological reporting of carcinomas of the pancreas published by the Royal College of Pathologists (last version : [37]). The samples were fixed with formalin, and paraffine embedded blocks in our center. All the histopathological tumor slides for each patient were reviewed at the same time by two pathologists who specialize in pancreatic pathology that were blindfolded to the clinical and immunohistochemical data, to assess the presence of several criteria. One of the pathologists was an expert in digestive pathology with a more than 20 years of experience in this field whereas the second was a general pathologist with more than 5 years of experience in digestive pathology. Morphological variants and histological types of PDAC were recorded following the WHO classification of tumors of the pancreas criteria as well as the histological differentiation grade [3]. All the recognized prognostic factor definitions were taken from the WHO classification of Digestive Tumors [3]. Perineural invasion was defined by the presence of tumor cells inside nerves or around more of 30% of nerve perimeter. We retained vascular invasion when at least one tumor embolus was seen in a vascular structure lumen with recognizable vessel wall. The quality of resection was attested (R0/R1) with a cut off clearance of 1 mm between tumor structures and inked surgical section. Finally, the presence of necrosis was certified, as in other works [33–35], by the existence of either coagulation necrosis (ghostly nuclei with conserved cell outlines) or tumor necrosis (degraded and ruptured epithelial structures with nuclear debris/karyorexis) areas. To detect necrosis, the authors first screened all

the slides of each tumor at x200 magnification. When no necrosis was seen, all the slides were screened a second time at x400 magnification and the presence of necrosis was accepted when a foci of at least two necrotic glands was found. Considering Hiraoka *et al* described micronecrosis and massive necrosis, they lacked the use of it in their statistical survival analysis and they stated that it can be hard to differentiate both with mixed cases. Moreover, the size of necrosis used by Kudo *et al* was not based on micronecrosis versus massive but based on an arbitrary threshold of 5mm to distinguish small versus large necrosis area. They also declared that both micronecrosis and massive necrosis was found in both the small and large necrosis groups. In the present study based on a practical and possibly daily based analysis, the authors decided not to analyze the size but rather the presence or absence of necrosis. To avoid any misinterpretation in necrosis presence which can be difficult, the evaluators were not recording necrosis in the areas of chronic pancreatitis or in areas with suppurative changes (defined by the presence of a massive neutrophil infiltration with altered inflammatory cells). Finally, intraluminal tumor cell shedding without any gland rupture or without extension to the stroma was not considered as necrosis.

Statistical analysis

For survival curves, the Kaplan Meyer method was used, and the mean OS was given with a 95% confidence interval.

For survival univariate and multivariate analysis, the Cox model was used. The effect of each tested factor on the OS was assessed with death the hazard ratio with a 95% confidence interval. A p value <0.05 was considered as significant. Only the significant variables in univariate analysis were used to construct the multivariate analysis model.

For association analysis between the different histopathological aggressive features, the Chi² test was used. A p value <0.05 was considered as significant.

Ethics

The human data was obtained from the database declared (MR004 certification n°19-088) and has received a favorable opinion from the Committee for the protection of persons (CPP) of the Hospices Civils de Lyon (n°19-109).

Results:

Cohort description

Based on the inclusion criteria, 573 patients were reviewed, however only statistical analysis was performed on 514 patients with complete histopathological and clinical descriptions. Patients were excluded that lacked the certainty of the pancreatic origin of adenocarcinoma, especially for periampullary tumors, recurrent tumors, or where the material left was too much altered to be analyzed (Supplemental Data, Figure 1).

The clinical characteristics of the patients are described in Table I. As expected, the mean age of diagnosis for pancreatic ductal adenocarcinoma (PDAC) is 66 years with a sex ratio of 1.05. Surprisingly, median aftercare time reached 6.7 years. However only few patients are still alive and followed up after 12 years, defining time of censorship. Almost 92.8% of the patients underwent surgery as the first line of treatment for their cancer whereas only 7.2% have neoadjuvant chemotherapy performed prior to surgery (N=37). These characteristics are also presented in the Table 1. Most patients at time of diagnosis presented with 2 to 4 centimeters PDAC (median size of 3 centimeters) and metastatic involvement of 1 to 3 lymph node (respectively n=332, 64.6% and n=219, 42.6%) resulting in 43,4% of stage II PDAC. 73.9% of patients had lymph node metastasis in regional nodes with a median ratio of invaded nodes of 10.17%. Metastatic disease is a surgery contraindication, yet 3.5% of patients are diagnosed with pM1 status after pathological examination. Metastases are mostly located in the inter-aorto-caval lymph nodes, in spite of systematical fresh examination and per operative frozen section analysis. Concerning the staging data, the neoadjuvant treatment seems to be mostly associated with a decrease of the regional extension as highlighted by the decrease of lymph node positivity.

Histopathological aggressive feature frequencies are recapitulated in Table 1. Perineural invasion was the most frequent, found in more than 80% of PDAC. Vascular invasion and SMAD4 loss are found in approximatively half of the cases. The quality of resection, assessed on per operative inked specimen, is curative (1-millimeter clearance from ink) for 58.9% of patients. The latest known risk factor is the differentiation grade as defined by Klöppel et al (24) in 1985. In our cohort, 316 PDAC (61.5%) are moderately differentiated, and the poor and well differentiated tumors represent almost 20% each. In regard to neoadjuvant treatment, we observed a decrease in the frequencies of all the histopathological aggressive features.

Overall survival of PDAC patients and association with pTNM staging

By the end of data retrieval period, 373 of all patients (72.6%) passed away, 117 are alive and 24 were lost during the follow up. The follow up range from 0 to 174 months after surgery. The median OS after surgery for PDAC patients is 2.0 years [1.8, 2.4] (minimum of 0 year and maximum of 20 years) (Table 1). In line

with this result, the 5 years OS rate is 25% (95% IC [21.1; 29.5]), which is very low considering that surgery is the only curative treatment.

The association with pTNM was the first to be analyzed. Indeed, pTNM staging is built up to summarize the major aggressive features to stratify patients. The new 2017 pTNM staging was applied. It confirmed that pT stage and pN stage correlate with OS ($p < 0.001$ for both features) (Table 2). The survival curves show a clear-cut stratification even if the number of stage IV was not sufficient enough to perform analysis on this subgroup alone (Figure 1A and 1B). Besides, we found that the risk of death is higher for each T stage with a respective twofold, threefold, and fourfold risk of death for pT2, pT3 and pT4 PDAC compared to pT1 tumors (respectively HR: 1.927, 95% CI [1.399; 2.653], HR: 2.718, 95% CI [1.882; 3.927], HR: 4.325, 95% CI [1.340; 13.958], $p < 0.001$) (Table 2). For pN stages the same results are observed with 50% and 100% increased risk of death for pN1 and pN2 lesions (HR: 1.409, 95% CI [1.081; 1.837] and HR: 1.951, 95% CI [1.478; 2.575], $p < 0.001$) (Table 2). Parallel results are obtained when looking at the ratio of invaded nodes with a three-tiered system ($p < 0.001$) (Table 2 and Figure 1E). Given the small number of pM1 PDAC cases, it is not surprising that the association with OS is borderline with statistical significance ($p = 0.067$) (Table 2 and Figure 1C). The global staging (from I to IV) is significantly associated with OS ($p < 0.001$) and allows a relevant stratification of patients as seen on the survival curves (Figure 1D). Finally, it is possible that the low number of patients who received a neoadjuvant treatment contributed to this condition not being significantly associated with a change in OS (HR: 0,7174, 95% CI [0.442; 1.095], $p = 0.149$) (Table 2, Figure 1F).

Impact of the known histopathological aggressive features on survival of patients with PDAC

We set out to confirm the known histopathological poor prognostic factors by univariate analysis to validate our morphological review. We show a moderate effect of perineural invasion on OS in PDAC (HR : 1. 581, 95% CI [1.173 ; 2.130], $p = 0.001$) as well as that of vascular invasion (HR : 1.437, 95% CI [1.170 ; 1.764], $p < 0.001$), incomplete resection (R1 with 1 millimeter clearance, HR : 1.449, 95% CI [1.180 ; 1.779], $p = 0.001$), and poor differentiation (HR : 1.478, 95% CI [1.137 ; 1.922], $p < 0.001$), with an approximate 50% death risk when these features are respectively present (Table 2). The size of tumors is not analyzed as a separate histopathological feature because the new pTNM staging is largely based on the size of tumors for pT assessment.

In the multivariate analysis, including these known factors of poor prognosis in PDAC, all retain their significant association with poorer OS (p range from 0.001 to 0.04) except for the vascular invasion (HR: 1.197, 95% IC [0.968; 1.480] versus HR : 1.437, 95% CI [1.170 ; 1.764] in univariate analysis) (Table 3).

Impact of necrosis on overall survival

Our first aim was to better characterize the presence of necrosis in human PDAC and its prognostic potential role. In our cohort, necrosis is found in 231 PDAC (44.9%) (Figure 2A and 2B, Table 1). The univariate analysis suggests an important effect of necrosis on OS with an almost double risk of death when necrosis is found in tumor samples (HR: 1.871, 95% CI [1.523; 2.299], $p=0.001$) (Table 2 and Figure 2C). This seems to be the strongest histopathological aggressive feature as it has the higher HR amongst them, and it could be as important as the node invasion (respectively HR: 1.871, 95% CI [1.523; 2.299] for necrosis and HR : 1.951, 95% CI [1.478 ; 2.575] for pN2 status). Moreover, the presence of necrosis is associated with a reduction of OS of 1.3 years or more than 15 months (median survival of 2.8 years, 95% CI [2.4, 3.6] versus 1.5 years, 95% CI [1.3, 1.7]) (Table 3). When integrating necrosis in a global multivariate model, it is the only prognostic factor to retain a high statistical significance facing the pTNM staging (respectively HR:1.734, 95% CI [1.403, 2.143], $p<0.001$ and HR: 1.911, 95% CI [1.416, 2.580], $p<0.001$) (Table 3). Moreover, it seems more important than the other known prognostic factors (HR:1.734 versus HR:1.172 to 1.463).

Association of necrosis with others aggressive histopathological features

Finally in the multivariate analysis, the introduction of necrosis in the model induces a decrease of both the histological grade and the perineural invasion effects and significances, indicating a link between both and necrosis. There seems to be a dominant effect of necrosis on histological grade and perineural invasion as the HR decreased for both histological grade and perineural invasion after necrosis introduction in the multivariate analysis (HR: 1.681, 95% CI [1.297; 2.179], $p<0.001$ versus HR: 1.463, 95% CI [1.124; 1.905], $p=0.005$ for histological grade, HR: 1.450, 95% CI [1.068; 1.970], $p=0.017$ versus HR: 1.349, 95% CI [0.993; 1.832], $p=0.055$ for perineural invasion respectively in the multivariate analysis without and with necrosis). The link between these factors is confirmed by the association of both the differentiation grade and the perineural invasion with necrosis ($p<0.001$ and $p=0.008$) (Supplemental Data, Table 1). On the contrary, the prognostic role of resection quality is not associated with that of the necrosis (HR:1.251, 95% CI [1.010, 1.549], $p=0.040$ versus HR:1.285, 95% CI [1.038, 1.590], $p=0.021$). It is important to note that the presence of necrosis is less frequent in patients who received a neoadjuvant treatment compared to the others ($p=0.0031$, Supplemental Data, Table 1). The poor OS associated with necrosis is consequently not due to neoadjuvant treatment induced changes and is supported by the survival curves in the two groups with and without neoadjuvant treatment (Figure 2D and 2E). All of these results suggest necrosis could be one of the most important histopathological aggressive features, independent from the TNM staging, that needs to be implemented in pathological report.

Discussion:

Concerning PDAC is the second most frequent cancer of digestive tract, it has a pivotal role in the area of oncology and especially in the surgical field [4]. Indeed, the cure of PDAC can only be obtained through complete surgical resection. 5 years survival rate of 25% [21.1; 29.5] after surgery in our cohort is in line with the statement that even in the optimal conditions of curative surgery, 80% of patients will experience local or metastatic recurrence of the disease [2, 3]. Moreover median survival after surgery of 2.0 years [1.8, 2.4] is equal to other reported data [7]. However, no predictive algorithm can anticipate the good responders following surgery from those patients that will present recurrent tumor or metastasis. This emphasizes the urgency for pathologists to report all the poor prognostic factors present to help discriminate the different patients' profiles.

The most important way of stratifying patients is through the use of pTNM staging. A new classification has been published in 2017 and already been reported to be perfectible [8–10]. It was important that our work also describe the impact of the 2017 pTNM staging on survival. It appears that the switch towards size as a major criterion of the pT staging is associated with a stronger effect on survival leading to a better stratification than the extra pancreatic tissue invasion. Additionally, the size seems to be more robust and reproducible than extra pancreatic invasion [38]. Concerning the pN stage, the implementation of two levels for positive lymph node staging seems to be relevant as the median survival is significantly different in the pN0, pN1 and pN2 subgroups (3.1, 2.0 and 1.5 years respectively). These results have been produced in two other publications which were probably at the base of the new pN sub classification [39, 40]. The combination on pT, pN and pM in a four tiered system shows clinical relevance as is shown in our results and is consistent with others studies [36, 38].

As expected, our study is in accordance with the data of poor prognosis being associated with perineural and vascular invasion, R1 resection quality and poor histological differentiation. [14, 17, 20, 41]. This is an important result as it validates the quality of the pathological review and the morphological assessment of these criteria. However, it seems that after introduction of other prognostic factors in the multivariate statistical model, vascular invasion shows a slight decrease in its effect. This may be in line with studies that suggest that vascular invasion prognostic role is mostly important in pN0 tumors and not in the whole PDAC population [42].

The most interesting result of this study is the description of necrosis as a poor prognosis factor in PDAC after surgery in a large cohort of patients. Indeed in other cancers, this factor has been described and used for the past 10 years [28–32]. Surprisingly, just three studies are found despite a deep research in pancreatic cancer [33–35]. All these studies are in line with our results and found that necrosis is associated with survival in multivariate analysis even if only one focused on the OS which we consider interesting for

patients with PDAC given the high mortality rate of this disease [35]. However, only two included the pTNM staging in their multivariate analysis, one in 2005 included the then-current pTNM staging which changed a lot in 2019. The other one used the 2019 pTNM staging but failed to prove its prognostic role which was stated by other studies [3, 9, 36]. Our work is the first one to confirm the associated and independent prognostic role of necrosis and pTNM staging which is of outmost importance. Moreover, the previous reports of necrosis were done on smaller cohorts (from 101 to 348 patients). In our work necrosis is associated with poor OS in our multivariate analysis with a 56% increase of death risk and a median decrease of survival of more than 15 months. This factor consists in a minimal item for pathological report in many tumors. Its recognition for unspecialized pathologists should be feasible with good, expected reproducibility as previously described [33], to the condition that we use a strict definition for necrosis evaluation. For all these reasons, the presence of necrosis should systematically be reported in pathological examination of every surgical PDAC specimen. However it is important to note that all previous works have commonly stated that the recognition of necrosis can be challenging. Therefore, in a practical approach, we recommend to evaluate the presence of necrosis based on a whole tumor analysis, after exclusion of the chronic pancreatitis and suppuration areas that can mislead the recognition of true tumor necrosis as well as coagulation necrosis. Even if in this work we did not stratify the impact of necrosis based on the surface, Kudo *et al.* have describe a strong association between the area of necrosis and the prognosis [34]. If future work can confirm this and given the difficulties establishing a reproducible estimation of the surface during a whole tumor examination, there exists a new opening for digital pathology algorithm and/or artificial intelligence to strengthen the necrosis analysis. Finally, in our cohort, we analyzed only 37 patients that benefit from neoadjuvant treatment. No previous data was available in the literature. Interestingly and probably in a counter-intuitive way, necrosis was found to be less frequent in that setting ($p=0,0031$), which could indicate at best that necrosis evaluation should not be further complexified after neoadjuvant treatment. Future analysis with an increased number of patients undergoing neoadjuvant treatment is required to better define the impact of necrosis on survival as well as the reproducibility of its recognition in an even more fibrotic and inflammatory context.

In conclusion, here we offer a morphological but high-power pathological analysis of known morphological prognostic factors as well as new data that strengthen the role of necrosis. We report a strong and pTNM independent prognostic factor in PDAC which is necrosis. Furthermore, we recommend pathologists to report necrosis presence in PDAC as it may become relevant in a few years in the stratification of patients after surgery.

Statements and Declarations

Compliance with Ethical Standards:

The human data come from a database that is declared (MR004 certification n°19-088) and has received a favorable opinion from the Committee for the protection of persons (CPP) of the Hospices Civils de Lyon (n°19-109).

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Conflict of Interest:

The authors have no relevant financial or non-financial interests to disclose.

Contributions:

TF and VH contributed to the study conception and design. Material preparation was performed by TF, PML, MR, MA, KM, JYM, TW and VH. Data collection and analysis were performed by TF and VH. The first draft of the manuscript was written by TF and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1: Clinical and morphological description of the cohort

	Total Patients	Patients with neoadjuvant treatment	Patients without neoadjuvant treatment
Clinical Data	N=514	N=37	N=477
Age at diagnosis (years)			
Mean (SD)	66.6 (10.7)	62.2 (9.6)	66.7 (10.7)
Median	67.7	62.3	67.7
Q1-Q3	59.3 – 74.3	54.9 – 70.1	59.8 – 74.4
Min-Max	27.3 – 91.5	37.0 – 78.3	27.3 – 91.3
Sex			
Male	263	20	250
Female	251	17	227
Follow up (months)			
Mean (SD)	32.3 (32.7)	31.5 (30.9)	32.3 (32.9)
Median	21.55	23.4	21.0
Q1-Q3	10.5 – 41.9	14.1 – 33.3	10.4 – 42.2
Min-Max	0 – 174.2	0.1 – 138.5	0 – 174.2
TNM Staging 2017	N=514	N=37	N=477
pT			
1	84 (16.3%)	7 (18.9%)	77 (16.1%)
2	332 (64.6%)	25 (67.6%)	307 (64.4%)
3	94 (18.3%)	3 (8.1%)	91 (19.1%)
4	4 (0.8%)	2 (5.4%)	2 (0.4%)
pN			
0	134 (26.1%)	15 (40.5%)	119 (24.9%)
1	219 (42.6%)	19 (51.4%)	200 (41.9%)
2	161 (31.3%)	3 (8.1%)	158 (33.1%)
pM			
0	496 (96.5%)	36 (97.3%)	460 (96.4%)
1	18 (3.5%)	1 (2.7%)	17 (3.6%)
Stage			
I	119 (23.2%)	13 (35.1%)	106 (22.2%)
II	223 (43.4%)	18 (48.6%)	205 (43.3%)
III/IV	172 (33.5%)	6 (16.2%)	166 (34.8%)
Invaded/examined lymph node ratio (%)			
Mean (SD)	15.69 (17.23)	7.38 (9.86)	16.34 (17.52)
Median	10.17	4.55	11.11
Q1-Q3	0.00 – 23.08	0.00 – 10.71	0.00 – 23.60
Min-Max	0.00 – 86.67	0.00 – 37.50	0.00 – 86.67
Tumor Size (mm)			
Mean (SD)	32.87 (15.05)	28,97 (12.56)	33.17 (15.20)
Median	30.00	27.00	30.00
Q1-Q3	25.00 – 39.75	22.00 – 35.00	25.00 – 40.00
Min-Max	2.00 – 120.00	2.00 – 70.00	2.00 – 120.00
Morphological aggressive features	N=514	N=37	N=477
Histological grade			
Well differentiated	101 (19.6%)	13 (35.13%)	88 (18.4%)
Moderately differentiated	316 (61.5%)	15 (40.5%)	301 (63.2%)
Poorly differentiated	97 (18.9%)	9 (24.3%)	88 (18.4%)
Resection quality			
R0	303 (58.9%)	26 (70.3%)	277 (58.1%)
R1	211 (41.1%)	11 (29.7%)	200 (41.9%)
Vascular invasion			
Absence	257 (50.0%)	24 (64.9%)	233 (48.8%)
Presence	257 (50.0%)	13 (35.1%)	244 (51.2%)
Perineural invasion			
Absence	86 (16.7%)	9 (24.3%)	77 (16.1%)
Presence	428 (83.3%)	28 (75.7%)	400 (83.9%)
Necrosis			

	Total Patients	Patients with neoadjuvant treatment	Patients without neoadjuvant treatment
Clinical Data	N=514	N=37	N=477
Absence	283 (55.1%)	29 (78.4%)	254 (53.2%)
Presence	231 (44.9%)	8 (21.6%)	223 (46.8%)

Table 2: Overall survival univariate analysis

Variable	Level	HR	95% CI	p-value
Neoadjuvant treatment	Presence	0,7174	[0.442; 1.095]	0.149
pT	2	1.927	[1.399; 2.653]	<0.001
	3	2.718	[1.882; 3.927]	
	4	4.325	[1.340; 13.958]	
pN	1	1.409	[1.081; 1.837]	<0.001
	2	1.951	[1.478; 2.575]	
pM	1	1.720	[1.006; 2.939]	0.067
Stage	II	1.570	[1.187; 2.078]	<0.001
	III/IV	2.225	[1.665; 2.973]	
Invaded/examined lymph node ratio	[4.76%-18.5%]	1.525	[1.175; 1.978]	<0.001
	[18.5%-86.7%]	1.977	[1.537; 2.543]	
Tumor Size	Millimeter	1.015	[1.009; 1.020]	<0.001
Histological grade	Well differentiated	0.752	[0.574; 0.984]	<0.001
	Poorly differentiated	1.478	[1.137; 1.922]	
Resection quality	R1	1.449	[1.180; 1.779]	<0.001
Vascular invasion	Presence	1.437	[1.170; 1.764]	<0.001
Perineural invasion	Presence	1.581	[1.173; 2.130]	0.001
Necrosis	Presence	1.871	[1.523; 2.299]	<0.001

Table 3: Overall survival multivariate analysis of histopronostic factors without and with necrosis

Multivariate analysis before necrosis introduction	HR	95% CI	P-value
Perineural invasion (Presence/Absence)	1.450	[1.068 ; 1.970]	0.017
Vascular invasion (Presence/Absence)	1.197	[0.968 ; 1.480]	0.097
Histological grade (Poorly/Moderately differentiated)	1.681	[1.297 ; 2.179]	<0.001
Resection quality (R1/R0)	1.251	[1.010 ; 1.549]	0.040
pTNM Stade II/I	1.414	[1.064 ; 1.878]	0.017
pTNM Stade III-IV/I	1.945	[1.440 ; 2.626]	<0.001
Multivariate analysis after necrosis introduction	HR	95% CI	P-value
Perineural invasion (Presence/Absence)	1.349	[0.993 ; 1.832]	0.055
Vascular invasion (Presence/Absence)	1.172	[0.948 ; 1.450]	0.142
Histological grade (Poorly/Moderately differentiated)	1.463	[1.124 ; 1.905]	0.005
Resection quality (R1/R0)	1.285	[1.038 ; 1.590]	0.021
pTNM Stade II/I	1.366	[1.028 ; 1.814]	0.032
pTNM Stade III-IV/I	1.911	[1.416 ; 2.580]	<0.001
Necrosis (Presence/Absence)	1.734	[1.403 ; 2.143]	<0.001

Supplemental Data Table 1: Association between Necrosis and other prognostic factors

Necrosis	Absence N = 283	Presence N = 231	p-value
Perineural invasion			
Absence	59 (20.8%)	27 (11.7%)	0.008 (Chi2)
Presence	224 (79.2%)	204 (88.3%)	
Histological grade (/)			
Well/Moderately differentiated	249 (88.0%)	168 (72.7%)	< 0.001 (Chi2)
Poorly	34 (12.0%)	63 (27.3%)	
Resection quality			
R0	167 (59.0%)	136 (58.9%)	1 (Chi2)
R1	116 (41.0%)	95 (41.1%)	
Neoadjuvant treatment			
Absence	254 (89.8%)	223 (96.5%)	0.0031 (Chi2)
Presence	29 (10.2%)	8 (3.5%)	

Table and Figure Legends

Figure 1: Survival analysis for pTNM staging and related features as well as for neoadjuvant treatment, with overall survival curves for pT stage (A), for pN stage (B), for pM stage (C), for global stage (D), invaded/examined lymph node ratio € and neoadjuvant treatment (F).

Figure 2: Necrosis: morphology, survival and statistics, **A.** PDAC with necrosis, bar scale represents 200µm. **B.** PDAC without necrosis, bar scale represents 200µm. **C.** Overall survival curve for necrosis. **D.** Overall survival curve for necrosis in the group without any neoadjuvant treatment. **E.** Overall survival curve for necrosis in the group with neoadjuvant treatment.

Table 1: Clinical and morphological description of the cohort

Table 2: Overall survival univariate analysis, using Cox model. p-values are considered as significant when <0.05 . (HR: hazard ratio, 95% CI: 95% confidence interval)

Table 3: Overall survival multivariate analysis of histopronostic factors without and with necrosis, using Cox model. p-values are significant when <0.05 . (HR: hazard ratio, 95% CI: 95% confidence interval)

Supplemental Data Figure 1: Patient Flow Chart

Supplemental Data Table 1: Association between Necrosis and other prognostic factors, using a Chi² square test. P-values are significant when <0.05 .



