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Randomized Clinical Trial

Effects of the superior mesenteric artery approach *versus* the no-touch approach during pancreatoduodenectomy on the mobilization of circulating tumour cells and clusters in pancreatic cancer (CETUPANC): randomized clinical trial

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Abstract

Background: Patients with pancreatic ductal adenocarcinoma present early postoperative systemic metastases, despite complete oncological resection. The aim of this study was to assess two pancreatoduodenectomy approaches with regard to intraoperative circulating tumour cells and cluster mobilization and their potential association with the development of distant metastasis.

Methods: Patients with periampullary tumours who underwent open pancreatoduodenectomy were randomly allocated to either the no-touch approach or the superior mesenteric artery approach. A total of four intraoperative portal vein samples (at the beginning of the intervention, after portal vein disconnection from the tumour, after tumour resection, and before abdominal closure) were collected to measure circulating tumour cells and cluster numbers. Primary outcomes were the intraoperative number of circulating tumour cells and cluster mobilization. Further, their potential impact on 3-year distant metastasis disease-free survival and overall survival was assessed.

Results: A total of 101 patients with periampullary tumours were randomized (51 in the superior mesenteric artery group and 50 in the no-touch group) and 63 patients with pancreatic ductal adenocarcinoma (34 in the superior mesenteric artery group and 29 in the no-touch group) were analysed. Circulating tumour cells and cluster mobilization were similar in both the no-touch group and the superior mesenteric artery group at all time points. There were no significant differences between surgical groups with regard to the median metastasis disease-free survival (12.4 (interquartile range 6.1–not reached) months in the superior mesenteric artery group and 18.1 (interquartile range 12.1–not reached) months in the no-touch group; $P = 0.730$). Patients with intraoperative cluster mobilization from the beginning to the end of surgery developed significantly more distant metastases within the first year after surgery ($P = 0.023$). Two intraoperative factors (the superior mesenteric artery approach ($P = 0.025$) and vein resection ($P < 0.001$)) were predictive factors for cluster mobilization.

Conclusion: Patients undergoing pancreatoduodenectomy using either the no-touch approach or the superior mesenteric artery approach had similar circulating tumour cells and cluster mobilization and similar overall survival and metastasis disease-free survival. A high intraoperative cluster dissemination during pancreatoduodenectomy was a predictive factor for early metastases in patients with pancreatic ductal adenocarcinoma.

Registration number: NCT03340844 (http://www.clinicaltrials.gov)—CETUPANC trial.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy with poor overall survival (OS) due to the clinically silent progression of the disease and appearance of metastases at the time of diagnosis or early after surgery[1,2].

It has been suggested that patients die, on average, 2 years after the pancreatic tumour cells acquire the ability to metastasize[3]. This cancer cell capacity is enhanced by the heterogeneous pancreatic cancer microenvironment, which consists of fibrotic stromal cells with different subtypes of extracellular matrix and infiltrating inflammatory cells[4–7].

However, even in cases with early diagnosis, many patients develop metastases shortly after complete surgical resection[8,9]. This could be explained by the presence of non-detected micrometastases at the time of diagnosis. On the other hand, it has been suggested that cancers may be disseminated through the bloodstream by the physiological stress associated with surgical trauma and manipulation of the tumour[10,11]. Some techniques used to remove pancreatic head cancer, such as the superior mesenteric artery (SMA) approach, require more mobilization and manipulation of the tumour before dissection of the venous drainage. This can potentially promote tumour cell dissemination through the portal venous system[12,13]. In contrast, the no-touch (NT) approach, which disconnects the tumour vasculature before manipulation, could avoid intraoperative tumour spread[14–16].

Liquid biopsy using different modalities, such as circulating tumour cells (CTCs), cell-free circulating tumour DNA, and circulating tumour extracellular vesicles, represents an interesting novel tool for monitoring of disease[17,18]. Using this technique, a breakthrough has recently been reached in predicting the survival of patients with PDAC by detecting CTCs and clusters in preoperative liquid biopsy analysis[19,20]. Likewise, intraoperative tumour dissemination assessment can be performed; Hirota *et al.*[15] and Gall *et al.*[16] evaluated the detection of CEA mRNA and CTCs respectively in the portal vein blood after tumour resection. These pilot studies were conducted on a limited number of patients; however, both studies showed increased mRNA and CTC mobilization in conventional pancreatoduodenectomy (PD) when compared with the NT approach. However, no association was observed between tumour marker mobilization and the development of postoperative metastases. Currently, there is limited evidence on the impact of the surgical approach on intraoperative neoplastic cell dissemination and subsequent metastatic spread in patients with PDAC.

Consequently, the CETUPANC trial was designed to compare two surgical approaches for PD (the SMA approach *versus* the NT approach) with regard to the number of intraoperative CTCs and cluster mobilization (determined using intraoperative liquid biopsy) and their effect on distant metastatic development in patients with PDAC.

Methods

Trial design

This randomized clinical trial (RCT) was a multicentre, individually randomized, patient-blinded trial conducted in two parallel groups at ten university hospitals with specialized hepatopancreatobiliary surgery units (Virgen del Rocío University Hospital of Seville (the main centre), Badajoz University Hospital, University Hospital of Salamanca, University Hospital October 12 in Madrid, Terrassa Mutual University Hospital, Hospital Clinico of Barcelona, Valencia Clinical Hospital, Miguel Servet University Hospital of Zaragoza, Princess University Hospital of Madrid, and Hospital Clinico of Madrid). These hospitals and units were selected on the basis of quality criteria published by the Pancreatoduodenectomy Multicentric Spanish Group, requiring a minimum of 31 PD in each centre per year[21]. All surgeons had previous experience with the surgical techniques investigated in this study (at least 15 procedures performed for each approach).

Approval from the Ethical Committee of Hospitals was obtained in accordance with the Declaration of Helsinki (date: 21 December 2016; identification number: 1510-M1-17). The study was registered in ClinicalTrials.gov (NCT03340844) and followed the CONSORT guidelines[22]. The original and translated study protocol and additional amendments are included in the *Supplementary material*.

Patients

The inclusion criteria were patients over 18 years of age with radiologically resectable PDAC of the head of the pancreas, with less than 180° contact with the portal vein[23] who signed the informed consent form. Histological confirmation was carried out after surgery. Preoperative staging consisted of triphasic CT and PET-CT when necessary. Patients were selected for upfront surgery based on the National Comprehensive Cancer Network criteria[23] independent of the level of carbohydrate antigen 19-9 (CA 19-9) present.

The exclusion criteria were histology other than PDAC, high-risk patients with severe disease (ASA grade IV[24]), neoadjuvant chemotherapy, liver metastases or peritoneal carcinomatosis detected during surgery, unresectable tumour due to arterial infiltration, and macroscopic residual tumour.

Trial procedures

To evaluate the potential role of tumour manipulation in tumour cell mobilization, patients were allocated in a 1 : 1 ratio to either the SMA group or the NT group by random assignment and stratified by participating centres to balance the groups. To ensure standardization of the techniques and steps in the determinations, a consensus meeting of surgeons was held before the start of the study.

In the SMA group, the pancreatic head was exposed early by lowering the angle of the colon and a Kocher manoeuvre until exposure of the left renal vein. The SMA was then located and dissected at its origin from the aorta, surrounding it with a vessel loop. Next, at the inframesocolic level, the superior mesenteric vein and SMA were located, dissected, and surrounded with loops. Once the SMA had been identified and marked, at the proximal level and at the level of the mesenteric root, most of the connective, lymphatic, and nervous tissue that forms the lateral portion of the retroportal process was very carefully cut. Following the axis formed by the SMA, gently pulling it through the previously placed loop, it was separated from the pancreatic tissue and the portal vein, identifying and sectioning both pancreatoduodenal arteries. After these manoeuvres, the hepatic pedicle was dissected and the stomach, jejunum, and mesojejunal area were sectioned. Then the jejunum was uncrossed behind the mesenteric vessels and the pancreas was sectioned at the level of the neck. Next, the posterolateral aspect of the portal vein was dissected, sectioning its tributary branches to access the retroperitoneal tissue lateral to the axis of the SMA, the area that had already been practically divided and of which usually at this point of the intervention only a small part remains. It was sectioned in a

cephalic direction, always on the right lateral edge of the artery, until finally completing the excision of the piece.

In the NT group, dissection of the greater omentum was carried out, separating it from the thin peritoneal sheath of the transverse mesocolon, advancing in a cephalic direction until the gastrocolic trunk was identified, the gateway to the superior mesenteric vein below the neck of the pancreas. The gastrocolic trunk was sectioned and the dissection of the superior mesenteric vein was continued below the pancreas. After this manoeuvre, the greater omentum was removed and the area of the hepatoduodenal ligament and gallbladder was dissected, allowing identification and dissection of the left and right hepatic arteries, with continuation towards the gastroduodenal artery for subsequent sectioning. Subsequently, lymphadenectomy was continued on the common hepatic artery without reaching the exit of the coeliac trunk. Once the bile duct was sectioned, the portal vein was dissected, removing all lympho-fatty tissue circumferentially. This leads to the entrance of the portal vein into the pancreas from its upper part. The stomach was then prepared and cut using a mechanical stapler. After these steps, Treitz's angle was opened, sectioning the jejunum at 10–15 cm from it after sectioning its mesentery. It was then time to complete the dissection of the anterior side of the portal mesenteric axis below the neck of the pancreas and, after this, the pancreas was sectioned at the level of the neck. Once all these steps were completed, the small vessels that run from the mesenteric-portal axis to the head of the pancreas were delicately dissected and sectioned, achieving the disconnection of venous drainage from the head of the pancreas to the portal vein. After the pancreatic head was completely disconnected from the portal vein, the wide Kocher manoeuvre was performed and the sectioned jejunum was uncrossed behind the mesenteric vessels. Finally, the excision of the surgical specimen was completed by sectioning the retroperitoneal edge on the right lateral wall of the SMA. Standardization of the technique was especially agreed upon in patients who underwent the NT technique requiring venous resection. In these cases, complete and wide dissection of the superior mesenteric vein and portal vein axis (SMV-PV) in the upper and lower parts of the pancreas was performed and taped. Once the pancreas was sectioned at the level of the pancreatic neck, by lifting the vascular tap, the thin pancreatic veins that drain to the SMV-PV were identified, ligated, and divided until the tumour invaded the axis. Then, the involved SMV-PV was clamped, after which wedge or segmental resection of the vein was performed, followed by the completion of the Kocher manoeuvre and removal of the specimen. Subsequently, the SMV-PV was reconstructed.

Adherence of surgeons to the protocol was assessed every 6 months by means of a video call. A review of patient report forms was carried out by the quality department of the clinical assay department of the main centre. In addition, a follow-up meeting was held halfway through recruitment.

Randomization and blinding

A randomization sequence was created at the Clinical Trial Unit of the main centre by the trial monitoring team using the open source software OxMaR (Oxford Minimization and Randomization)[25]. Patients were allocated in a 1 : 1 ratio to either the SMA group or the NT group by random assignment in four blocks and stratified by participating centres to balance the groups. The sequence was hidden from researchers and surgeons until the moment of the surgery. The main centre was advised on the date of surgery and direct telephone communication was established in the operating room. Randomization was done intraoperatively once the surgeon confirmed the absence of metastasis or carcinomatosis. The patients were blinded after assignment to the intervention. Data quality control was performed by external outcome assessors who were not blinded to the group assignment.

Intraoperative sampling for determining circulating tumour cells and clusters

A total of four intraoperative portal vein samples (S0 to S3) were obtained for each patient in both groups for CTC and cluster measurements using intraoperative liquid biopsy. A total of 7 ml of whole blood was obtained for each sample through direct portal vein puncture with a hypodermic needle (25G × 1 inch). The puncture hole was covered with moist gauze after the blood was drawn to reduce potential bleeding.

S0 was obtained before tumour manipulation after minimal bile duct dissection in both of the groups. S1 was obtained in the NT group after ligation of the pancreatic vessels that drained into the portal vein to avoid tumour manipulation. In the SMA group, S1 was obtained after extensive SMA dissection before retropancreatic portal vein dissection. Finally, S2 was obtained after resection and S3 just before abdominal closure in both of the groups.

Circulating tumour cell isolation, detection, and enumeration protocol

The first blood sample was discarded to exclude epithelial cells that were dislodged via vein puncture. Samples were collected in K2-EDTA Vacutainer tubes. To achieve sample stability, the samples were transported by a company experienced in transporting biological samples. Biospecimens were stored at 4° C during transportation and processed within 24 h of collection.

All CTC and cluster determinations were performed at the main centre. Blood samples were enriched in peripheral mononuclear blood cells using gradient centrifugation with Histopaque®-1119 and CTCs were isolated using the IsoFlux™ platform. The IsoFlux™ Epithelial-to-Mesenchymal Transition CTC Enrichment Kit (EMT Enrichment Kit, Fluxion, CA, USA; Catalogue No. 910-0106) was used to perform CTC enrichment and the enriched CTCs were fixed and stained with fluorescent reagents (IsoFlux™ Circulating Tumor Cell Enumeration Kit, Fluxion, CA, USA; Catalogue No. 910-0093). The fluorescent reagents included anti-CK-fluorescein isothiocyanate (FITC), anti-CD45-indocarbocyanine (Cy3), and Hoechst 33342. CTC detection and enumeration were performed using fluorescence microscopy. A cluster has been defined as the aggregation of two or more CTCs[26].

Finally, the Hough transform algorithm was used to count CTCs and clusters (89% sensitivity and 91% accuracy)[20,27].

Follow-up

Follow-up was initiated on the day of surgery and continued throughout the ensuing 3 years. Follow-up visits included CT and CA 19-9 testing every 6 months. A patient could undergo CT or MRI outside of standard surveillance because of symptoms or when required according to clinical criteria. Patients with non-standard CT were not excluded from the study. Review of the radiological scans was not centralized.

Primary outcomes

Primary outcomes were the intraoperative number of CTCs (cells/ml) and cluster (clusters/ml) mobilization during PD. Their association with the appearance of distant metastases was also assessed.

Secondary outcomes

Metastasis disease-free survival (MDFS) was defined as the time from surgery to the appearance of metastases during the 3 years of follow-up. Distant metastases included liver or lung metastases. Local/lymph node recurrence and OS were also assessed.

The pathological study was carried out according to a specific protocol based on the approach of Verbeke *et al.*[28]. R0/R1 rates, tumour grade differentiation (G1–G3), and TNM classification of malignant tumours were defined according to the guideline criteria[29–31]. Surgical complications were classified according to the Clavien–Dindo classification[32] and the International Study Group of Pancreatic Surgery[33–35].

Statistical analysis

In the context of the CETUPANC trial, an additional specific sample size was calculated for PDAC evaluation according to previous studies[15,16]. Smaller differences in the mobilization of tumour cell markers were considered. Accepting an α risk of 0.05 and a β risk of 0.01 in a two-sided test, at least 27 subjects were necessary in each group to find a statistically significant proportion difference of tumour cell mobilization expected to be 0.13 in the NT group and 0.50 in the SMA group. During follow-up, a dropout rate of 15% was anticipated.

The cohort characteristics were compared between the surgical approaches using a *t* test, a Wilcoxon rank sum test, or a chi-squared test. The analyses were performed in accordance with the modified intention-to-treat principle, where patients stayed in their allocated group, but were excluded from analyses after randomization when PD was not performed or the final pathology was not PDAC.

The intraoperative samples were evaluated not only independently (S0, S1, S2, and S3) but also as change (Δ) compared with baseline: S1–S0 (Δ mobilization-to-baseline), S2–S0 (Δ resection-to-baseline), and S3–S0 (Δ end-to-baseline). A more positive difference in Δ values indicated higher CTCs and/or cluster dissemination at each sampling time.

Patient follow-up was tested using an independent Cox proportional hazards regression model for MDFS as the outcome. The model was implemented using a backward elimination process, leaving only the significant variables in the model. Significant variables that fulfilled the proportional hazard assumption were univariately substudied using Kaplan–Meier (K-M) curves with the log rank test, categorical variable strata, or descriptive statistical metrics for continuous variables. Only the K–M curves with the lowest *P* values were studied together with the surgical approaches in conjunction with the curve strata. Finally, an additional regression logistic model was used to study the impact of categorical variables on candidate K–M curve strata. All tests, except those noted, were two-sided, considering a significance level of $\alpha = 0.05$, and were analysed using R version 4.0.5.

Results

Of the 881 patients with periampullary tumours evaluated by the tumour boards, 101 were randomized, 93 patients who met the preoperative and intraoperative inclusion criteria were allocated to a surgical approach, and 63 patients with PDAC were included for the final analysis. Finally, 34 patients were included in the SMA group and 29 patients were included in the NT group (*Fig. 1*). Recruitment was performed for 31 months from 17 January 2018 to 24 July 2020. *Table 1* shows the demographic data for both groups.

Primary outcomes

The distributions of the number of CTCs and clusters in each intraoperative sample according to the NT and SMA approach are depicted in *Fig. 2*. The determination carried out after manipulation of the tumour until portal venous disconnection (S1) showed no differences between the surgical groups with regard to either the CTCs or the cluster values. The main peaks of CTCs and cluster mobilization were observed after complete resection (S2) (*Fig. 2*). At this sample point, the NT group showed a higher mobilization of clusters (median of 27 (interquartile range (i.q.r.) 9–53) *versus* 12 (i.q.r. 6–29); $P = 0.042$). CTCs also showed a higher mobilization at this point in the NT group, although the difference was not statistically significant (median of 306 (i.q.r. 185–604) *versus* 217 (i.q.r. 129–409)). Finally, before abdominal closure (S3), there were no differences in CTCs and cluster measurements between the NT approach and the SMA approach.

Secondary outcomes

There were no significant differences between surgical groups with regard to R0/R1 rate and surgical complications (*Table 1*). No complications were associated with the portal vein puncture. Bleeding stopped spontaneously in all patients with no other haemostatic approach except moist gauze application.

The median OS was 19 (i.q.r. 10–not reached) months. There were no significant differences between groups (18 (i.q.r. 9–not reached) months in the SMA group *versus* 23 (i.q.r. 11–not reached) months in the NT group; $P = 0.440$).

A total of 21 patients (33%) presented with local recurrence during follow-up, without differences between surgical groups (median not reached (i.q.r. 9–not reached) months in the SMA group *versus* median not reached (i.q.r. 12–not reached) months in the NT group; $P = 0.900$).

Related to the metastasis analysis, 33 (56%) patients had a systemic recurrence during the 3 years of follow-up. *Figure 3* shows the MDFS comparison between groups. The median MDFS was 12 (i.q.r. 6.1–not reached) months and 18 (i.q.r. 12.1–not reached) months in the SMA group and in the NT group respectively ($P = 0.730$). Regarding the development of early metastases during the first year, 53% of patients in the SMA group presented with distant metastases *versus* 38% of patients in the NT group.

Multivariate analysis

To evaluate the potential factors determining MDFS, both groups were combined in the multivariate analysis. The logistic regression showed that MDFS was associated with two preoperative factors (CA 19.9 (HR 1.00 (95% c.i. 1.0001 to 1.0003); $P < 0.001$) and vascular invasion presence (HR 2.98 (95% c.i. 1.34 to 6.65); $P = 0.007$)) and one intraoperative factor (intraoperative cluster Δ end-to-baseline (HR 1.01 per cluster/ml (95% c.i. 1.00 to 1.03); $P = 0.031$)). The full model is included in the *Supplementary material*.

Due to the finding in the previous additional analysis, related to the association of cluster mobilization with distant metastasis, a univariate K–M analysis (using the cluster Δ end-to-baseline quartiles to detect the best cut-off for predicting MDFS) was performed. The additional analysis showed that the cluster Δ end-to-baseline cut-off that best separated the cohort for MDFS was 14 clusters/ml in the third quartile (*Fig. 4*). *Table 2* shows the cohort characteristics for cluster end-to-baseline groups

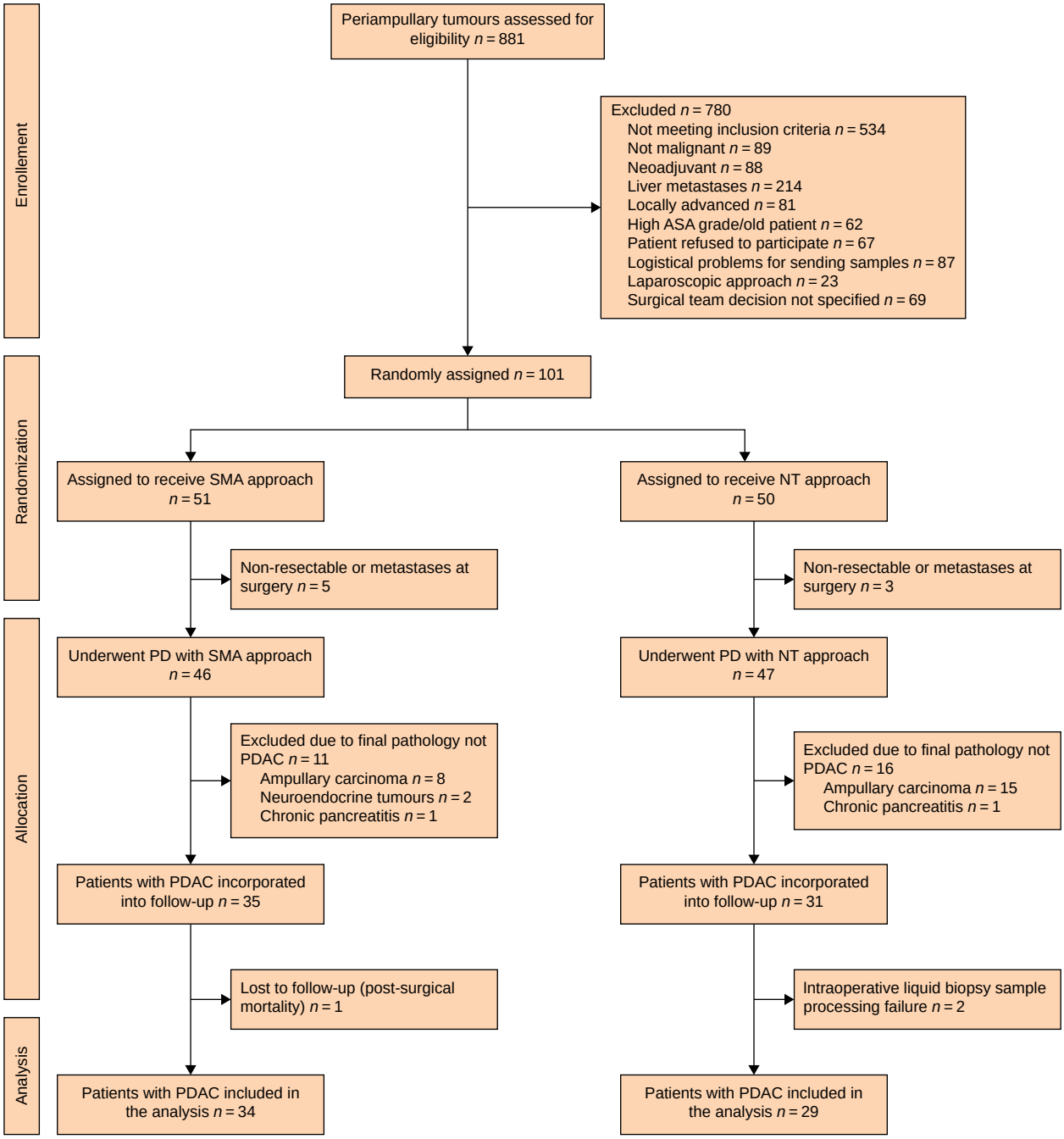


Fig. 1 CONSORT flow diagram

The diagram shows the flow of participants in the two arms of the clinical trial. SMA, superior mesenteric artery; NT, no-touch; PD, pancreatoduodenectomy; PDAC, pancreatic ductal adenocarcinoma.

according to this cut-off. Patients with higher cluster dissemination during surgery (Δ end-to-baseline greater than 14 clusters/ml) had significantly higher metastases within the first year ($P = 0.023$). This association disappeared at 2 and 3 years ($P = 0.052$ and $P = 0.064$ respectively) (*Fig. 4*).

Finally, to determine the factors associated with intraoperative cluster mobilization, a logistic regression was performed using cluster Δ end-to-baseline as the dependent variable, considering cluster Δ end-to-baseline greater than 14 clusters as the reference category. The main factors associated with cluster mobilization greater than 14 clusters/ml were preoperative CA 19-9 (OR 1.0005 per CA 19-9 U/ml (95% c.i. 1.0004 to 1.0006); $P = 0.032$) and two intraoperative factors: the SMA approach (OR 4.4457 (95% c.i. 4.3653 to 4.5206); $P = 0.025$) and portal/superior mesenteric vein resection (OR 10.2467 (95% c.i. 6.5224 to 13.9410); $P < 0.001$). The full model is included in the *Supplementary material*.

Discussion

The present trial investigated intraoperative CTCs and cluster mobilization during PD in patients with PDAC, comparing two surgical approaches; both the NT approach and the SMA approach had similar tumour cells and cluster mobilization by the end of the surgery and no differences between surgical

Table 1 Cohort description (comparison of baseline, intraoperative, and surgical parameters according to surgical approach)

Variable	Surgical approach	
	No-touch (*n* = 29)	Superior mesenteric artery (*n* = 34)
Baseline parameters		
Male	18 (62)	14 (41.1)
Female	11 (38)	20 (58.8)
Age (years), median (i.q.r.)	65 (54–73)	64 (57–74)
Diabetes mellitus	7 (24)	6 (18)
Arterial hypertension	9 (31)	18 (53)
Dyslipidaemia	16 (55)	9 (26)
Preoperative biliary stent	17 (59)	26 (76)
CA 19-9 (U/ml), median (i.q.r.)	149 (52–357)	143 (38–551)
Baseline intraoperative liquid biopsy		
CTCs in S0 (cells/ml), median (i.q.r.)	199 (118–428)	228 (70–383)
Clusters in S0 (cells/ml), median (i.q.r.)	13 (4–34)	9 (2–19)
Surgical parameters		
Surgery time (min), median (i.q.r.)	295 (270–360)	300 (270–357)
Vein resection	9 (31)	8 (23)
Blood loss (ml), median (i.q.r.)	275 (112–600)	243.8 (175–550)
Blood transfusion	6 (21)	6 (18)
Number of harvested lymph nodes, median (i.q.r.)	16 (12–24)	17 (12–24)
Histological characteristics		
Tumour size (mm), median (i.q.r)	2.5 (2.0–3.5)	2.9 (2–3.5)
Tumour stage		
I (IA/IB)	6 (21) (1 (3)/5 (17))	10 (29) (2 (6)/8 (23))
II (IIA/IIB)	19 (65) (3 (10)/16 (55))	13 (38) (3 (9)/10 (29))
III	4 (14)	11 (32)
Tumour grade		
G1	11 (38)	6 (18)
G2	15 (52)	19 (56)
G3	3 (10)	9 (25)
Vascular invasion	16 (55)	22 (65)
Lymphatic invasion	14 (48)	16 (47)
Neural invasion	19 (65)	30 (88)
R0/R1 resection	18 (62)/11 (38)	23 (68)/11 (32)
Postoperative parameters		
Clavien–Dindo grade III/IV complications[32]	7 (24)	5 (15)
Biliary fistula	2 (7)	3 (9)
Pancreatic fistula	8 (27)	3 (9)
Delayed gastric emptying	2 (7)	8 (23)
Haemorrhage	3 (10)	3 (9)
Readmission	5 (17)	3 (9)
Time until chemotherapy (days), median (i.q.r)	94 (84–103)	98 (92–108)
Adjuvant chemotherapy		
No treatment	5 (17)	9 (26)
Gemcitabine	10 (34)	10 (29)
Capecitabine	5 (17)	3 (9)
Folfirinox	9 (31)	10 (29)
Oxaliplatin + irinotecan + 5-fluorouracil	0 (0)	1 (3)
Abraxane	0 (0)	1 (3)
Postoperative CA 19-9 (U/ml), median (i.q.r.)		
3 months	27 (11–294)	34 (12–540)
6 months	30 (10–93)	64 (10–354)
12 months	28 (8–159)	27 (10–591)
18 months	25 (9–353)	42 (7–5671)
24 months	18 (8–242)	12 (2–944)
36 months	10 (6–25)	9 (2–214)

Values are *n* (%) unless otherwise indicated. i.q.r., interquartile range; CA 19-9, carbohydrate antigen 19-9; CTCs, circulating tumour cells; S0, intraoperative portal vein baseline sample.

techniques were observed with regard to MDFS and OS. The study showed that a high intraoperative cluster dissemination during PD was a predictive factor for early metastasis within the first year in patients with PDAC of the pancreatic head.

To determine whether tumour manipulation could influence tumour cell dissemination, only two prospective pilot studies have analysed the mobilization of tumour cell markers during PD, comparing conventional PD with the NT approach[15,16]. Whereas Hirota *et al.*[15] evaluated the detection of CEA mRNA, Gall *et al.*[16] determined the CTC levels in the portal vein after tumour resection.

The key point of the NT approach in PD is to perform the resection after complete disconnection of tumour vein drainage into the portal vein without tumour mobilization. Theoretically, this approach could avoid tumour cell dissemination. In the present study, this technique was compared with the standard SMA approach for PD in which a Kocher manoeuvre and posterior SMA combined with mesenteric root dissection were performed before the ligation and sectioning of the tumour venous drainage into the portal vein. Theoretically this could increase tumour cell dissemination.

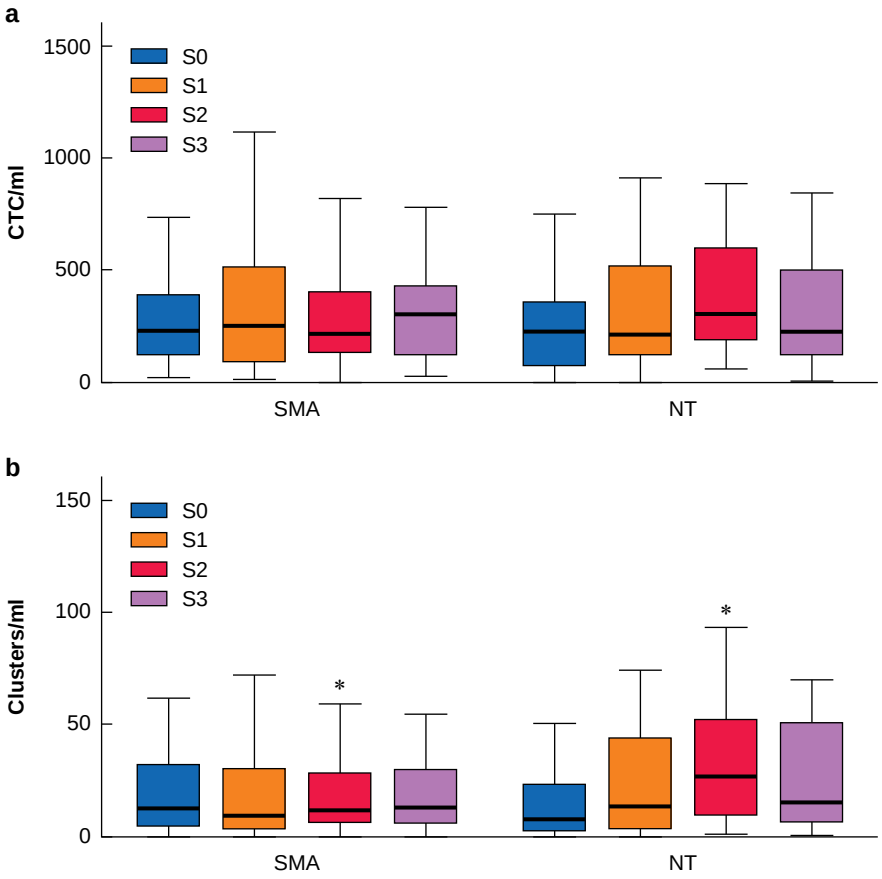


Fig. 2 Circulating tumour cells and clusters over time

a Circulating tumour cells. **b** Clusters. Box plots depicting the distribution of circulating tumour cells and clusters in each intraoperative portal vein sample from S0 to S3 by surgical technique. *Statistically significant difference between the two groups ($P = 0.042$). SMA, superior mesenteric artery; NT, no-touch; S0, sample obtained before tumour manipulation after minimal bile duct dissection in both of the groups; S1, sample obtained after ligation of the pancreatic vessels that drained into the portal vein to avoid tumour manipulation in the no-touch group and sample obtained after extensive superior mesenteric artery dissection before retropancreatic portal vein dissection in the superior mesenteric artery group; S2, sample obtained after resection in both of the groups; S3, sample obtained just before abdominal closure in both of the groups.

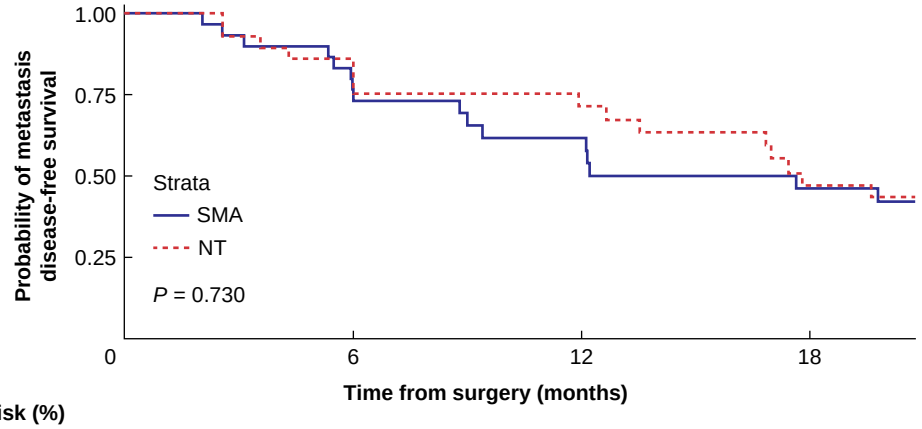
For the evaluation of intraoperative tumour cell mobilization during PD, not only free CTCs, as in the Gall *et al.*[16] study, but also CTC clusters were determined in the portal vein in the present study. Whereas the previous studies analysed tumour markers in two intraoperative portal vein samples (at the beginning of resection and after resection), the present study determined CTCs and clusters at four strategic points (at the beginning of the intervention, after portal vein disconnection from the tumour, after tumour resection, and before abdominal closure). The findings of the present study are partially consistent with those of Gall *et al.*[16] and Hirota *et al.*[15] with a main peak of both CTCs and clusters observed after specimen resection. However, whereas Gall *et al.*[16] and Hirota *et al.*[15] showed fewer CTCs and less mRNA mobilization in patients with the NT approach after resection, in the present study, both CTCs and clusters were higher in the NT group in this sample. This was an unexpected finding. Unfortunately, there is a lack of evidence in this setting because most studies that recommend the use of the NT approach are retrospective with several biases[36,37].

Regarding the potential role of intraoperative tumour cell dissemination in the appearance of distant metastases, broadly speaking, surgical resection has long been linked to increased metastasis via tumour cell spread during surgery[38,39]. However, to date, no studies have demonstrated the potential role of the intraoperative dissemination of tumour cells in the development of metastases in pancreatic cancer. The study by Gall *et al.*[16] failed to demonstrate any correlation between isolated CTCs and OS or disease-free survival.

The present RCT studied the mobilization of not only CTCs but also the CTC clusters and included tumour cell dissemination by the end of surgery. These have been highly interesting when analysing MDFS. In fact, multivariate analysis showed that the metastatic phenomenon was associated with preoperative CA 19-9 levels, the presence of vascular invasion, and increased intraoperative cluster mobilization from the beginning to the end of surgery. CTC and cluster determination after mobilization and resection failed to show any correlation with metastasis.

Whereas both CA 19-9 levels and vascular invasion are well-known predictive factors at baseline[40,41], cluster Δ end-to-baseline measurement is a new dynamic predictive marker in the field of oncology, leading to the new concept of intraoperative liquid biopsy. According to the results of the present study, the highest impact of cluster dissemination during surgery on metastasis development occurs during the first year, decreasing gradually through the first 3 years. In patients with high cluster mobilization, the OS prognosis decreased dramatically.

An additional logistic regression analysis was performed to determine the intraoperative factors related to cluster mobilization. Interestingly, using the SMA technique with the Kocher manoeuvre and tumour mobilization prior to tumour venous drainage ligation, the risk of cluster mobilization greater than 14 clusters/ml



No. at risk (%)

SMA	34 (100)	23 (68)	16 (47)	12 (35)
NT	29 (100)	24 (83)	18 (62)	12 (41)

Fig. 3 Metastasis disease-free survival analysis

An at-risk table is shown beneath the graph. SMA, superior mesenteric artery; NT, no-touch.

Table 2 Cohort characteristics for cluster end-to-baseline groups

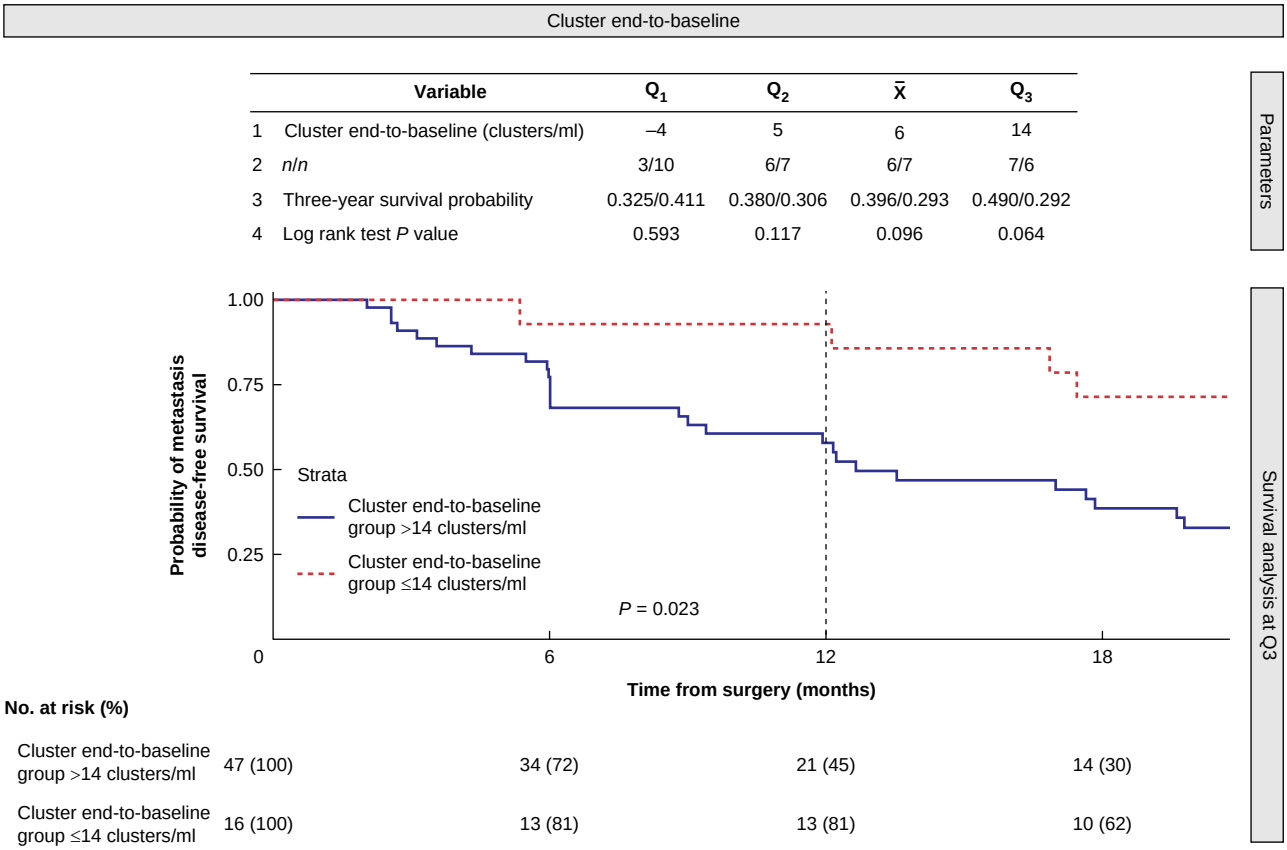
Variable	Cluster end-to-baseline group (clusters/ml)		P
	>14 (*n* = 47)	≤14 (*n* = 16)	
Baseline parameters			
Male	23 (49)	9 (56)	0.773
Female	24 (51)	7 (44)	
Age (years), median (i.q.r.)	63 (57–73)	66 (59–75)	0.472
Diabetes mellitus	9 (19)	4 (25)	0.723
Arterial hypertension	19 (40)	8 (50)	0.566
Dyslipidaemia	17 (36)	8 (50)	0.383
Preoperative biliary stent	34 (72)	9 (56)	0.351
CA 19-9 (U/ml), median (i.q.r.)	149 (63–366)	93 (29–950)	0.069
Baseline intraoperative liquid biopsy			
CTCs in S0 (cells/ml), median (i.q.r.)	227 (115–350)	310 (126–443)	0.731
Clusters in S0 (cells/ml), median (i.q.r.)	10 (4–29)	9 (5–21)	0.433
Surgical parameters			
Surgery time (min), median (i.q.r.)	300 (270–360)	285 (265–302)	0.118
Vein resection	17 (36)	0 (0)	0.003
Blood transfusion	10 (21)	2 (12)	0.714
Blood loss (ml), median (i.q.r.)	300 (200–600)	350 (200–650)	0.572
Number of harvested lymph nodes, median (i.q.r.)	17 (12–24)	16 (12–24)	0.821
Histological characteristics			
Tumour size (mm), median (i.q.r.)	3.0 (2.0–3.5)	3.1 (2.5–3.5)	0.364
Tumour stage			0.261
I (IA/IB)	13 (28) (3 (6)/10 (21))	3 (19) (0 (0)/3 (19))	
II (IIA/IIB)	21 (45) (4 (8)/17 (36))	11 (69) (2 (12)/9 (56))	
III	13 (28)	2 (12)	
Tumour grade			0.583
G1	12 (25)	5 (31)	
G2	27 (57)	7 (44)	
G3	8 (17)	4 (25)	
Vascular invasion	29 (62)	9 (56)	0.771
Lymphatic invasion	22 (47)	8 (50)	1.000
Neural invasion	38 (81)	11 (69)	0.319
R0/R1 resection	31 (66)/16 (34)	10 (62)/6 (38)	1.000
Postoperative parameters			
Clavien–Dindo grade III/IV complications[32]	8 (17)	4 (25)	0.481
Readmission	6 (13)	2 (12)	1.000

Values are *n* (%) unless otherwise indicated. i.q.r., interquartile range; CA 19-9, carbohydrate antigen 19-9; CTCs, circulating tumour cells; S0, intraoperative portal vein baseline sample.

increased 4.5 times compared with the NT approach. Moreover, when the surgery required portal or superior mesenteric vein resection, the risk of higher cluster dissemination increased 10 times.

Despite this additional finding, surgical technique was not a factor determining the appearance of metastases and both NT and SMA approaches presented similar results in terms of MDFS and OS, similar to what was observed in a recent large randomized trial performed in patients with colon cancer[42].

Finally, regarding portal or superior mesenteric vein resection, in patients with resectable pancreatic cancer undergoing upfront



	Variable	Q_1	Q_2	$\bar{x}$	Q_3
1	Cluster end-to-baseline (clusters/ml)	–4	5	6	14
2	*n/n*	3/10	6/7	6/7	7/6
3	Three-year survival probability	0.325/0.411	0.380/0.306	0.396/0.293	0.490/0.292
4	Log rank test *P* value	0.593	0.117	0.096	0.064

Fig. 4 Spread of intraoperative tumour cell clusters as a factor associated with early metastasis

Descriptive statistics for cluster Δ end-to-baseline (S3–S0) to detect the best predictive cut-off for metastasis disease-free survival. The descriptive statistics include the first, second, and third quartiles (Q_1, Q_2, and Q_3) and the mean value ($\bar{x}$) for the cluster end-to-baseline (clusters/ml). The cohort was divided into two groups (over and under equal the cut-off value) and obtained the following metrics at 3-year follow-up: i) the number of patients at risk in each group (n/n); ii) the probability of metastasis disease-free survival (probability/probability), and iii) the log-rank test *P* value obtained between the two groups. Kaplan–Meier curves are presented for the descriptive statistical value with the most significant metastasis disease-free survival curve separation (Q3 with a cluster Δ end-to-baseline cut-off value of 14 clusters/ml). In addition, the 12-month log-rank test $P = 0.023$ is also presented.

surgery, the venous resection rate is approximately 20–27%[13,43]. This is similar to that observed in the present study (26.9%). Venous resection has been associated with a worse prognosis, which is attributed to a more advanced neoplastic process[44]. The association between venous resection and intraoperative cluster mobilization observed for the first time in the present study could also partially explain the evolution of these patients.

The main limitations of the present study are related to the small sample size and the high proportion of patients without PDAC. Therefore, the main finding regarding the association between intraoperative cluster spread and early metastasis should be validated in a large prospective study.

A future implication of the findings of the present study could be that patients with greater intraoperative tumour cell mobilization will require an individualized strategy due to the higher risk of early distant metastasis (closer follow-up, early adjuvant treatment, and patient information). Special studies could be performed about locally advanced PDAC, for which extended surgical resection has demonstrated increased survival[45]. Avoidance of surgical tumour manipulation when extended dissection and vascular resection are necessary is complicated[46].

Finally, although there is no evidence for a role of neoadjuvant therapy in resectable patients[47], some studies have demonstrated better results in clinical stage IB–III PDAC[48] and, in particular, in poorly differentiated resectable PDAC for which the risk of dissemination could be higher[49]. Thus, additional studies analysing the role of neoadjuvant therapy in the prevention of CTCs and cluster mobilization could be interesting.

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Disclosure

The authors declare no conflict of interest.

Supplementary material

Supplementary material is available at *BJS Open* online.

Data availability

Research data supporting this publication are not available from an open repository, although they can be shared by the author in selected cases.

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