Pankreascancer

Måling av tumorstørrelse i pankreascancer: ulike målemetoder fører til ulike resultater

My Linh Tran

Prosjektoppgave
20 studiepoeng

Det Medisinske fakultet
Universitetet i Oslo

31.03.2022

Innholdsfortegnelse

Abstract

In the 8th edition of the TNM classification for pancreatic ductal adenocarcinoma (PDAC), stages T1 to T3 are defined by tumour size, size measurement being deemed objective and accurate. This study investigated whether various, currently used approaches to tumour measurement result in different tumour sizes and differences in T-stage assignment. In a series of 315 resected PDAC, tumour sizes were measured as follows: macroscopically in a single or in two perpendicular planes, and with or without microscopic corroboration. Comparison of the resulting tumour sizes showed that both macroscopic measurement in two planes and microscopic corroboration gave significantly different results (p<0.001). Compared to the most simple approach - macroscopic measurement in one plane - the comprehensive approach - macroscopic measurement in two planes with microscopic corroboration – resulted in a larger tumour size in 263 (83%) of cases (mean absolute size difference: 10 mm, mean relative size change: 36%). T-stage assignment differed in 142 (45%) of cases between the simple and comprehensive approach, and affected 87%, 38% and 48% of the cases deemed to be stage T1, T2 and T3, respectively. In conclusion, tumour size and T-stage are highly approach-dependent. Consensus on an accurate method is required to ensure comparability of these basic data.

Innledning

Patologi er tradisjonelt ansett som gullstandard for diagnostikk og cancer staging. Cancer staging er viktig både med tanke på vurdering av behandling, prognose, kliniske studier og deres sammenlignbarhet, kommunikasjon på tvers av spesialiteter og institusjoner. Tatt i betraktning at tumor-staging spiller en sentral rolle i ovennevnte er det desto viktigere at man stager tumores likt uavhengig av patolog og institusjon. Ifølge retningslinjene skal man basere T-stagingen av pankreascancer på den største tumordiameteren, helst målt i tre dimensjoner. Dette kan høres elementært og simpelt ut, men i praksis er det ikke like lett fordi tumoren vanligvis ikke er synlig ved ekstern inspeksjon av operasjonspreparatet og da nasjonale- og internasjonale retningslinjene ikke beskriver hvordan man skal måle tumorstørrelsen i tre dimensjoner. Dermed er det i praksis fortsatt stor variasjon mellom institusjoner og patologer i hvordan man dissekerer og måler tumorstørrelser. Siden nøyaktig

måling av tumorstørrelse er avgjørende for riktig T-stadium har denne studien som hensikt å sammenligne resultatene som fremkommer av de ulike målemetodene, altså to-dimensjonell (i ett plan) versus tre-dimensjonell (i to plan vinkelrett på hverandre) måling og makroskopisk måling med eller uten mikroskopisk korrigering. Videre ser studien på hvordan resultatene av ulike målemetoder påvirker tumorstørrelse og T-stadium. *Vår hypotese er at de ulike målemetodene vil medføre større endringer i «største diameter» og at dette vil medføre endringer i T-stadium.*

Studentens rolle i oppgaven

Min veileder dr. Caroline Verbeke har dissekert, vurdert, staget pankreastumores og registrert dette i OUS sine datasystemer. Jeg har i første skriveperiode arbeidet med å hente ut alle relevante data for hver enkelt pasient (type operasjonspreparat, lokalisasjon av tumor, tumorstørrelse målt makroskopisk og mikroskopisk i to og tre dimensjoner, T-stadium, N-stadium, vaskulær status, lymfatisk status, perinevral status, reseksjonsstatus).

Siden det kom ut ny versjon av UICC TNM staging system for pankreascancer i 2017, gikk jeg gjennom tumorstørrelsene for casus registrert mellom 2015-2018 og omregistrerte T-stadium i henhold til den nyeste 8.utgaven av TNM-staging systemet (1).

Deretter gjorde jeg diverse analyser av dataene i excel og SPSS. Etter å ha fremstilt data gjorde jeg i samarbeid med veilederen min en tolkning av resultatene, da særlig med henblikk på hvilken betydning dette har for prosedyrer for disseksjon og undersøkelse av operasjonspreparater med pankreascancer. Til slutt fremstilte jeg resultatene i tabeller/figurer som har blitt brukt i et manuskript som er sendt inn til et peer-reviewed, open-access tidsskrift (Cancers).

Bakgrunn

Patofysiologi

PC står for 85-90% av alle neoplastiske tumores i pankreas. Av alle PC er ca. 70% lokalisert i caput, 5-10% i corpus og 10-15% i cauda. PC danner solide, faste svulster

som er dårlig avgrenset og infiltrerer omkringliggende ekstrapankreatiske vevsstrukturer (2). PC utvikles via non-invasive prekusorlesjoner, der den vanligste typen er **pankreatisk intraepitelial neoplasi** men kan også utvikles fra **intraduktalt papillær mucinøs neoplasi** eller **mucinøs cystisk neoplasi** (3).

Epidemiologi

PC er den vanligste typen av cancer i pankreas og er den cancertypen jeg har valgt å fokusere oppgaven min på. PC er fremdeles en sjelden krefttype idag, men man har sett en økende trend i insidens de siste tiårene. Denne økningen kan i stor grad tilskrives bedring i både diagnostikk og påvisning av sykdommen, men noe må også tilskrives økt og mer nøyaktig registrering i nasjonale kreftregistre. Dessuten er alder en risikofaktor for PC og siden gjennomsnittlig levealder har økt de siste tiårene er det også naturlig at insidens av PC øker i takt med dette. I tillegg har forekomsten av fedme og overvekt, som er en kjent risikofaktor for PC, økt de siste tiårene og kan dermed være med på å forklare den økende insidensen av PC (4). I 2020 var insidensen av PC estimert til å være rundt 140,116 (18.7 per 100 000) i Europa, mens mortaliteten lå på rundt 132,134 (17,6 per 100 000). Her ser vi at tallene for insidens og mortalitet nesten går parallelt og gjenspeiler den dårlige prognose PC har. I Norge var det 701 tilfeller av PC i 2020 ifølge Kreftregisteret (5). For PC har man sett en stabil økning i mortalitet mens det for andre cancer typer sakte, men sikkert faller. PC har nå blitt rangert som den 4. vanligste årsaken til cancer-relatert mortalitet i Europa og rangert til nr. 3 i USA (6). PC er også assosiert med en 30% økning i **helsejusterte leveår** det siste tiåret (6).

5-årsoverlevelsen for PC er fortsatt lav der sykdomsinsidens og mortalitet nesten sammenfaller.

Pasientgrupper, behandling og prognose

Resektabel	Borderline	Lokalavansert	Metastatisk
10 - 15%	10 - 20%	20 - 30%	50 - 60%

Kirurgi + Adj kjemoterapi	Neoadj kjemoterapi + Kirurgi + Adj kjemoterapi	Palliativ kjemoterapi	Palliativ kjemoterapi
20 -24 mnd 5 års = 20%	20 -24 mnd 5 års = 20%	9 -12 mnd 5 års = 0%	5 -9 mnd 5 års = 0%

Risikofaktorer

PC er en kompleks og multifaktoriell cancersykdom, og både arvelige og miljøfaktorer spiller en viktig rolle. Kjente livsstils- og miljøfaktorer forklarer rundt 40% av sykdommen (6). Arvelige risikofaktorer, dvs. Bestemte kimbanemutasjoner og såkalt familiær PC (hittil uten kjent underliggende gendefekt) er viktige risikofaktorer med tanke på å identifisere høy-risikogrupper, men er årsak til under 10% av alle tilfeller av PC (4).

Alder er en viktig risikofaktoren for å utvikle PC. Andre faktorer som øker risikoen mindre enn 5 ganger er familiehistorie (førstegradsslektning med PC), røyking, fedme (BMI > 30kg/m^2), stort alkoholforbruk (> 4 enheter/dag), diabetes over tid (> 5år), BRCA1 bærer, Lynch syndrom og familiær adenomatøs polypose. Faktorer som øker risikoen mellom 5-10 ganger er familiehistorie (to førstegradsslektninger med PC), BRCA2 bærer, kronisk pankreatitt og cystisk fibrose. (7) Det er en rekke genetiske tilstander som gir mer enn 10 ganger økt risiko for PC hvor Peutz-Jeghers syndrom, hereditær pankreatitt (autosomal dominant PRSS1-mutasjon) og familiær atypisk multippel føflekk melanom er de fremste representanter (7).

Symptomer og klinisk bilde

Symptomene ved PC oppstår relativt sent i sykdomsforløpet og de fleste av symptomene er diffuse, slik at pasienter ikke søker legehjelp eller at symptomene feiltolkes. Dette medfører derfor ofte til forsinket diagnostikk. Vekttap, nedsatt appetitt, tretthet, ubehag i abdomen, kvalme/oppkast er blant noen av de diffuse symptomene på PC. Noen pasienter med PC utvikler nyoppstått diabetes/glukoseintoleranse seks måneder eller mer før kreftdiagnosen, og nye studier viser at det er en kausal sammenheng mellom PC og redusert glukosetoleranse. Men mesteparten av pasienter med nyoppstått diabetes har likevel ingen underliggende PC, slik at glukoseintoleranse alene ikke betraktes som et alarmerende tegn. Senere i forløpet kan pasientene oppleve smerter i abdomen som kan være postprandiale og medføre redusert matinntak. Ved avansert sykdom med peritonealcarcinose vil pasientene kunne oppleve ascites.

Tumores i pankreashodet gir ofte symptomer tidligere på grunn av obstruksjon av ductus choledocus som kan medføre stille ikterus og/eller pankreatitt anfall. Tumores lokalisert i pankreaskroppen/halen er ofte asymptomatiske og rekker ofte å metastasere eller utvikle omfattende lokal vekst i nærliggende organer før de blir diagnostisert (2, 3).

Overvåkning

Det finnes ingen spesifikke, kostnads-effektive og non-invasive screening-undersøkelser for PC, og siden PC har lav insidens, er screening på befolkningsnivå ikke er aktuelt. Screening for PC i høyrisikogrupper anbefales bare som ledd i et forskningsprogram (7).

Diagnostikk

Ved mistanke om PC er CT abdomen førstevalget i utredningen. Dersom CT viser forandringer som gir mistanke om tumor og det er kurative hensikter, så suppleres det med flerfase CT pankreas protokoll. Basert på CT-undersøkelsene gjør radiologene en stadievurdering med TNM-klassifikasjon og kartlegging av karanatomien for vurdering av resektabilitet. Endoskopisk ultralyd-veiledet biopsi er idag førstevalget dersom det er behov for vevsdiagnostikk av pankreaslesjoner (2).

Andre modaliteter som MR og eventuelt PET-CT gir ytterligere informasjon om svulstens metabolisme, metastaser og fungerer som et supplement ved vanskelig diagnostikk (2).

Per idag er det kun én tumormarkør, karbohydrat-antigen 19-9 (CA 19-9) som er i klinisk bruk. Denne markøren brukes for å overvåke effekten av kjemoterapi og fungerer som en markør for recidiv (2).

Idag er det rundt 15-20% av PC pasienter som diagnostiseres på et tidlig tidspunkt hvor kurativ kirurgi er aktuelt, mens 80% allerede har lokal-invasiv sykdom og/eller fjernmetastase (8).

TNM staging

TNM staging systemet kategoriserer pasientene i ulike grupper på basis av hvor avansert sykdommen er. For å kunne predikere sykdomsforløpet og velge det beste behandlingsalternativet er det viktig å ha en nøyaktig beskrivelse av sykdomsutbredelse i form av TNM-staging. TNM-staging tillater objektiv kommunikasjon på tvers av spesialiteter og institusjoner. Her står «T» for beskrivelse av primær tumor, «N» for regional lymfeknutestatus og «M» for metastase. Dataene som brukes i denne studien har blitt innhentet mellom 2015-2020 og underveis har det kommet en ny (8.) utgave av TNM-stagingsystemet

I den 7. utgaven ble stadiene kategorisert på følgende måte: T1: tumor begrenset til pankreas og < 2cm, T2: tumor begrenset til pankreas >2cm, T3: tumor ekspanderer utenfor pankreas men uten å involvere arterier, T4: tumor involverer a. mesenterica superior og truncus coeliacus (ikke resektabel primær tumor), N0: ingen lymfeknute metastase, N1: regional lymfeknutemetastase (8).

I den nye utgaven ble T1-T3 kategoriene definert på basis av tumorstørrelse, siden vurdering av ekstrapankreatisk vekst (som skapte bekymring rundt nøyaktigheten og variasjon blant patologer og radiologer) er vanskelig og studier viste at tumorstørrelse alene korrelerer bedre med overlevelse. Dessuten fikk T1-stadium flere undergrupper. Det ble ikke gjort endringer i T4 kategorien. Den tidligere beskrivelsen av T4 inkluderte også «ikke resektabel» som et kriteriet og har nå blitt fjernet da dette punktet kan variere avhengig av institusjonen man befinner seg på.
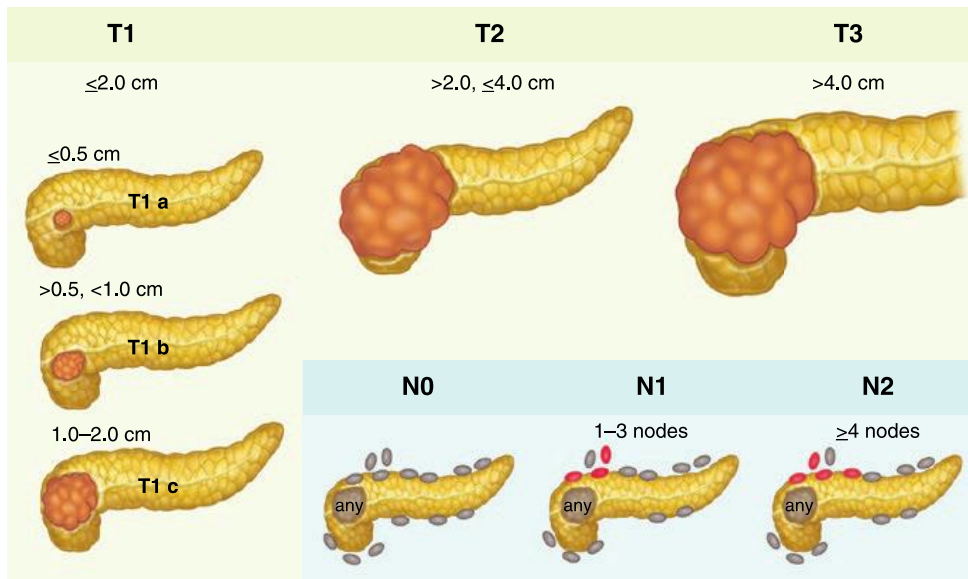
(1).

Inndeling av PC etter resektabilitet

Ikke-metastatisk PC inndeles i primært resektabel, borderline resektabel og lokalavansert tumor i henhold til National Comprehensive Cancer Network. Det er tumorens nærhet til og graden av evt kontakt med eller infiltrasjon i de store blodkarrene (A. mesenterica superior, A. hepatica communis, Truncus coeliacus, V. mesenterica superior, V. portae, V. cava inferior) som bestemmer om en tumor betraktes som primært resektabel, borderline resektabel eller lokalavansert (9).

Resektabilitets status	Arterieside	Venøs side
Resektabel	Ingen tumorkontakt med arterier [(truncus coeliacus (TC), a.mesenterica superior (AMS) eller a.hepatica communis (AHC)].	Ingen tumorkontakt med v. mesenterica superior (VMS) eller v.portae (VP) eller ≤ 180⁰ kontakt uten uregelmessighet i venekontur.
Borderline resektabel	**Caput/processus uncinatus:** – Solid tumorkontakt med AHC uten utbredelse til TC eller a.hepatica bifurkaturen forenlig med trygg reseksjon og rekonstruksjon. – Solid tumorkontakt med AMS ≤ 180⁰. – Solid tumorkontakt med variant arterieanatomi (f.eks aksessorisk høyre leverarterie, avvikende forløp av høyre leverarterie, avvikende forløp av AHC og utspringet av eventuell aksessorisk høyre leverarterie) og utbredelsen av tumorkontakt bør vurderes fordi det kan influere på kirurgisk planlegging. **Corpus/cauda pankreatis:** – Solid tumorkontakt med TC ≤ 180⁰. – Solid tumorkontakt med TC > 180⁰ uten at aorta er involvert og med intakt og ikke involvert a.gastroduodenale som kan muliggjøre en eventuell Appleby operasjon (enkelte plasserer dette kriteriet i ikke-resektabel kategori).	– Solid tumorkontakt med VMS eller VP på > 180⁰, kontakt ≤ 180⁰ med uregelmessig kontur av venen eller trombose av venen, men med vene både proksimalt og distalt for det involverte sted som tillater sikker og komplett reseksjon og venerekonstruksjon. – Solid tumorkontakt med v.cava inferior (VCI).
Lokalavansert	**Caput/processus uncinatus:** – Solid tumorkontakt med AMS > 180⁰. – Solid tumorkontakt med TC > 180⁰. **Corpus / cauda pankreatis:** – Solid tumorkontakt > 180⁰ med AMS eller TC. – Solid tumorkontakt med TC og aortaaffeksjon.	– Ikke rekonstruerbar VMS/VP som skyldes tumoraffeksjon eller okklusjon (kan skyldes tumor eller bare trombe).

(9).

Histopatologisk undersøkelse

PC graderes histologisk som høyt, moderat eller lavt differensiert basert på histopatologisk undersøkelse. Men histologisk undersøkelse kan også brukes i TNM-stagingen for å verifisere/bekrefte makroskopiske funn.

Behandling

Det finnes flere ulike behandlingsalternativer for PC og hvilken type behandling pasientene får avhenger av sykdomsutbredelse, funksjonsnivået til pasienten og målet med behandlingen. For PC er kirurgisk reseksjon eneste kurative behandling og etterfølgende behandling med adjuvant kjemoterapi har vist å være effektivt. For

pasienter med godt funksjonsnivå og borderline resektabel/lokalavansert sykdom er behandlingen gjerne neoadjuvant kjemoterapi etterfulgt av kirurgi. Pasienter med metastatisk sykdom og godt funksjonsnivå tilbys palliativ kjemoterapi og evt. kirurgisk bypass, mens de med redusert funksjonsnivå tilbys støttebehandling (7).

Man skiller mellom 3 prosedyrer for pankreas reseksjon: pankreatoduodenektomi, distal pankreatektomi og total panklreatektomi. Typen reseksjon som blir utført avhenger av tumorens lokalisasjon (7).

Følsomheten for kjemoterapi og stråling er begrenset hos de fleste PC pasienter. Tidligere var monoterapi med gemcitabine standard systemisk behandling, men dagens førstevalg er en kombinasjonsbehandling: gemcitabine med nab-paclitaxel eller FOLFIRINOX (2, 7).

Metode

Som nevnt tidligere er T-staging basert på den største tumordiameter, men i praksis måles den største diameteren på ulike måter. De fleste patologer begrenser tumorens størrelsesvurdering til en todimensjonell målemetode, hvor de etter gjennomført preparatdisseksjon identifiserer den preparatskiven der tumor ser størst ut. I denne skiven måles den største tumordiameteren, og i tillegg måles en sekundær tumordiameter som ligger vinkelrett i forhold til den største diameteren. Få patologer vil ta en størrelsesvurdering basert på tre dimensjoner. Måling i en tredje dimensjon gjøres ved at man måler lengden til tumor på tvers av skivene, slik det fremkommer i figurene under. Den største av tumorstørrelsene målt i tre dimensjoner vil da bestemme T-stadium.

De to metodene nevnt ovenfor kan enten vurderes kun makroskopisk eller kontrolleres og ved behov korrigeres med mikroskopisk vurdering. Måling i tre dimensjoner kombinert med mikroskopisk kontroll/korrigering betraktes å være den mest nøyaktige måten å måle tumorstørrelse på, senere referert til som «den mest presise metoden».
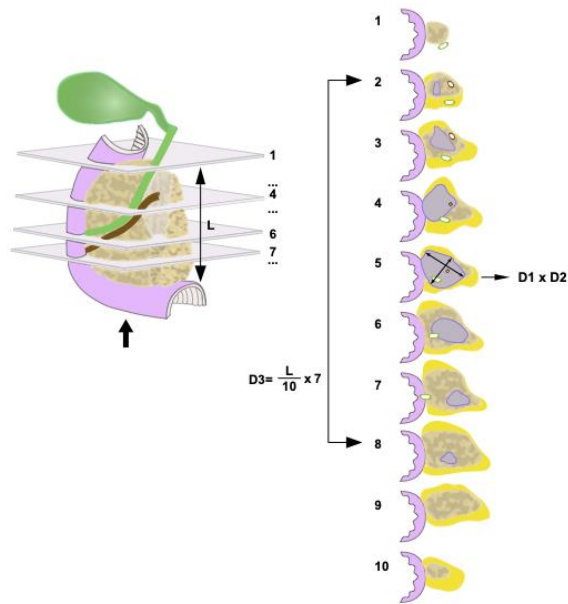
Figur 1: disseksjon og størrelsesvurdering av tumorstørrelse i pankreatoduodenektomi-preparater.
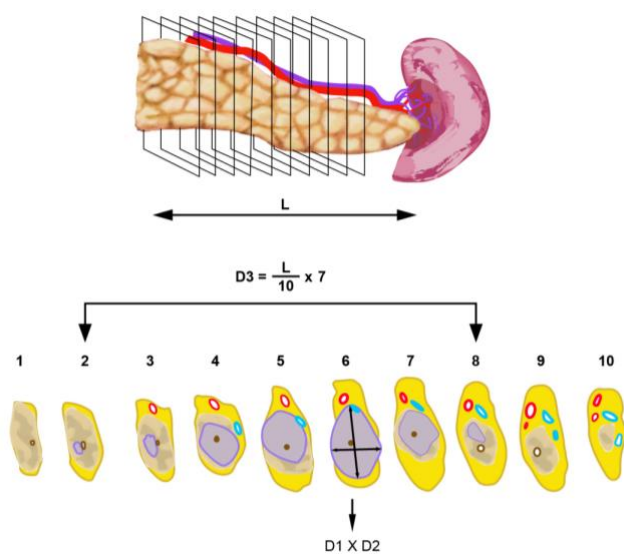
Figur 2: disseksjon og størrelsesvurdering av tumorstørrelse i pankreatektomi-preparater.

Makroskopisk undersøkelse

Preparater fra pankreatoduodenektomi (PDE) ble beskåret i et aksialt plan. Med en tykkelse på skivene mellom 2-3 mm, resulterte et PDE-preparat gjennomsnittlig i 10-15 skiver. Disse skivene ble lagt ved siden av hverandre i sekvensiell rekkefølge og deretter fotografert. Deretter ble skiven med størst tumormasse identifisert og den største tumorstørrelsen ble målt i denne skiven, følgelig den nest største diameter

13

målt som en vinkelrett linje på den største diameteren. Deretter kalkuleres den tredje dimensjonen på tvers av skivene ved å bruke følgende formel:

$$D3 = \left(\frac{Totallengde\ av\ pankreas\ i\ kraniokaudalretning}{Totalt\ antall\ skiver} \right) * antall\ skiver\ med\ tumor$$

I tilfeller hvor tumor kun var synlig på den ene siden, av henholdsvis første eller siste skive, ble halve tykkelsen av skiven brukt til å beregne tumorstørrelsen. For eksempel, størrelsen til en tumor som går over 8 sekvensielle preparatskiver, med en tykkelse på 3 mm, og som kun er synlig på den ene siden av skive nr. 8, vil beregnes som følgende: 3*7mm + ½*3mm = 22.5mm

Preparater fra distal pankreatektomi ble beskåret i et saggital plan, altså vinkelrett på den longitudinelle aksen av corpus og cauda pancreatis. Tumorstørrelse ble så målt på lik linje som for pancreatoduodenektomipreparatene.

Mikroskopisk undersøkelse

For mikroskopisk bekreftelse eller korreksjon av den makroskopisk målte todimensjonale tumorstørrelsen, ble skiven med størst tumormasse komplett innstøpt. Den største tumordimensjonen i denne skiven ble vurdert ved at tumorens periferi ble markert med tusj på histologiglasset, slik at den største tumorstørrelsen kunne direkte måles på histologiglasset. Måling av tumorstørrelsen på tvers av skivene ble kontrollert mikroskopisk og kalkulert som nevnt ovenfor.

T-stadium

De ulike målemetodene vil i mange tilfeller medføre ulike størrelser som maksimal tumorstørrelse. Dermed ble det registrert T-stadium basert på største tumordiameter målt med de ulike målemetodene.

Resultater

Oversiktstabell

Tumor størrelse	Hele pankreas	Caput	Corpus og cauda
2D macro			
Gjennomsnitt (SD), mm	30 (10)	29,9 (8)	31 (15)
Median (IQR), mm	30	30 (27 – 40)	27 (21 – 36)
Range, mm	9 – 110	11 – 51	9 – 110
2D micro			
Gjennomsnitt (SD), mm	31 (15)	32 (7)	31 (15)
Median (Q1, Q3), mm	29	32 (27 – 36)	29 (21 – 36)
Range, mm	6 – 110	14 – 61	6 – 110
3D macro			
Gjennomsnitt (SD), mm	35 (14)	32 (10)	45 (19)
Median (Q1, Q3), mm	32	30	43
Range, mm	12 – 100	12 – 61	13 – 100
3D micro			
Gjennomsnitt (SD), mm	38 (14)	35 (10)	45 (19)
Median (Q1, Q3), mm	36	35	43
Range, mm	13 – 100	15 – 75	13 – 100

Endring i T-stadium	Ingen endring	Skift til høyere T-stadium	Skift til lavere T-stadium
2D macro – 2D micro			
Caput	186 (80)	30 (13)	15 (7)
Corpus/cauda	72 (85,7)	3 (3,6)	9 (10,7)
2D macro – 3D macro			
Caput	161 (70)	36 (15)	34 (15)
Corpus/cauda	39 (46)	39 (46)	6 (8)
2D macro – gullstandard			
Caput	129 (56)	80 (35)	22 (9)
Corpus/cauda	44 (52)	37 (44)	3 (4)

Analyser

På basis av dataene som ble uthentet ble det utført ulike analyser, først og fremst sammenligninger mellom tumorstørrelsene som resulterte fra de ulike målemetodene. Den viktigste sammenligningen ble gjort mellom «den enkleste metoden» metoden og «den mest presise» metoden. «Den enkleste» metoden ble definert som metoden hvor man kun måler tumorstørrelse basert på makroskopisk vurdering av tumor i én enkelt skive. Med «den mest presise» metoden blir den største tumorstørrelsen identifisert ved å kombinere makroskopisk undersøkelse med mikroskopisk kontroll både i én enkelt skive og på tvers av skivene, slik at tumorstørrelsen måles i tre dimensjoner.

Funn

Ut fra analysene som ble gjort av våre data kom det frem 4 hovedfunn:

(1) måling av tumorstørrelse i tre dimensjoner resulterer i en signifikant større maksimal tumordiameter enn når tumorstørrelse måles kun i to dimensjoner, både ved makroskopisk og mikroskopisk måling,

(2) størrelsesforskjellen mellom tredimensjonell og todimensjonell måling var større ved mikroskopisk kontroll enn ved kun makroskopisk måling,

(3) størrelsesdifferansen mellom tredimensjonell og todimensjonell måling er større for tumores i corpus/cauda enn i caput

(4) ved tredimensjonelle måling er den største tumordiameteren for PC i corpus/cauda signifikant større enn for PC i caput.

De mest overraskende resultatene var den signifikante endringen i T-stadium avhengig av målemetodene som ble brukt. Ved sammenligning av «den mest presise» metoden opp mot «den enkleste» metoden så man at det ble en endring i T-stadium i 142 (45%) av alle casus. Hvorav det i 117 (37%) av alle casus ble gjort en underestimering av tumorstørrelsen, slik at T-stadium måtte oppjusteres. I et mindre antall casus, 25 (8%), ble tumorstørrelsen overestimert slik at T-stadium måtte nedjusteres. I 80 (35%) av alle casus i caput ble størrelsen underestimert slik at T-stadium måtte oppjusteres. Samme observasjoner gjaldt for tumores i corpus/cauda hvor man i hele 37 (44%) casus så at størrelsen ble underestimert og dermed måtte oppjustere T-stadium. Dessuten ble størrelsen overestimert i 22 (10%) av alle casus i caput og i 3 (4%) av alle casus i corpus/cauda.

Diskusjon

Denne studien viser at måten tumorstørrelse blir målt på har en betydelig innflytelse på den største tumorstørrelsen som blir registrert og dermed også på T-stadium. Resultatene fra denne studien viser at måling av tumorstørrelse i to ulike plan med mikroskopisk korrigering resulterer i en annen, ofte større tumorstørrelse enn ved kun makroskopisk måling i ett enkelt plan. Størrelsesforskjellene medførte en oppjustering i T-stadium i 38% av alle kasusene og en nedjustering i 8% av alle kasusene i denne studien.

Ulik vekst av tumor avhengig av lokalisasjon

Oversikten over gjennomsnittlig tumorstørrelse i henholdsvis caput og corpus viser noe variasjon i tumorstørrelse. Først av alt ser man at størrelsesforskjellen mellom lengden målt på tvers av skivene og største diameteren målt i en skive er størst i corpus/cauda sammenlignet med caput. Dette illustrerer at PC i corpus/cauda ofte følger og vokser etter formen til organet og ikke ekspansivt ut av organet, følgelig får tumores der en mer avlang form. I tillegg viser oversikten til at tumores i caput gjerne er mer runde i formen. I praksis betyr dette at dersom tumorstørrelse ikke blir målt i to ulike plan, så vil den største tumorstørrelsen bli undervurdert i en signifikant andel av kasusene, særlig for PC i corpus/cauda.

Mikroskopisk undersøkelse

Internasjonale retningslinjer anbefaler mikroskopisk korrigering av tumorstørrelsene som er målt makroskopisk. Årsaken til denne anbefalingen er at PC vokser diffust, slik at tumorveksten er vanskelig å avgjøre makroskopisk. Mikroskopisk korrigering gjøres vanligvis bare for den største tumorstørrelsen målt i én skive. Denne studien viste at mikroskopisk korrigering av tumorstørrelse i caput ga en signifikant størrelsesforskjell, mens størrelsesforskjellen i corpus/cauda ikke var signifikante. En tenkelig årsak til at sistnevnte forskjell ikke var signifikant er at tumores i corpus/cauda ofte har ekspandert og fyller nærmest hele tverrsnittet av pankreas. Dermed vil ikke mikroskopisk korrigering avdekke store forskjeller i tumorstørrelse målt i dette planet.

Implikasjoner

Funnene i denne studien har flere implikasjoner. Først av alt ser man at tumorstørrelse og T-stadium i mange tilfeller endres avhengig av hvilken målemetode som tas i bruk. Dermed vil ikke tumorstørrelse og T-stadium være sammenlignbart mellom ulike institusjoner som bruker forskjellige målemetoder. I tillegg vil manglende tredimensjonell tumorvurdering resultere i unøyaktig vurdering av størrelse og T-stadium i en signifikant andel av alle casus, dette kan da medføre en risiko for bias i multisenter kliniske studier. Dessuten vil data i kreftregistre ikke være sammenlignbart ved bruk av ulike målemetoder som medfører ulik størrelsesvurdering. For at data fra ulike institusjoner skal være sammenlignbart er det viktig at det oppnås en konsensus om retningslinjer for makroskopisk undersøkelse og vurdering av tumorstørrelse. En viktig faktor ved valg av målemetode er at metoden skal være standardisert og enkel å gjennomføre i praksis. På bakgrunn av dette kunne det tenkes at en universell målemetode kunne være kun makroskopisk vurdering i én skive. En slik målemetode ville sørget for sammenlignbare og reproduserbare data, men ville medført mindre nøyaktige og presise målinger av tumorstørrelse og dermed mindre nøyaktig T-stadium. Dessuten er forskjellen mellom «den enkleste» og «den mest presise» metoden ikke konstant, men varierer mellom under- og overestimering slik at det ikke blir mulig å beregne/predikere en «tenkt» tredimensjonell størrelse.

For det andre så eksisterer det idag ingen praktisk veiledning for hvordan man skal vurdere tumorstørrelse i tre dimensjoner. Makroskopisk måling av tumor på tvers av skivene avdekker ofte en større tumorstørrelse enn måling kun i én skive. Videre vil mikroskopisk kontroll av de ulike tumordimensjonene gi en større presisjon i vurdering av tumorstørrelsen.

Begrensninger

Denne studien har flere begrensninger. Først og fremst er analysene kun utført på pasienter fra én og samme institusjon som kan medføre bias. Men siden både kliniske, kirurgiske og patologiske metoder er basert på internasjonale retningslinjer, er bias antakeligvis begrenset. En videre begrensning er at denne studien kun brukte én disseksjonsmetode der pankreatoduodenektomi preparater ble dissekert i et aksialt plan, mens enkelte institusjoner dissekerer preparatene langs ductus choledochus og ductus pancreaticus. Det viktigste for måling av tumorstørrelse er at størrelsen måles i to ulike plan som ligger vinkelrett på hverandre, noe som er tilfelle ved begge metodene beskrevet over. Dermed har disseksjonsretning trolig liten innvirkning på måling av tumorstørrelse.

For det andre er studien basert på en relativt liten kohort (n = 315). Likevel ser man i resultatdelen at gjennomsnitt og mediantumorstørrelse for denne studiekohorten for to-dimensjonal måling er relativt sammenlignbart med data fra andre studier med større kohorter. I tillegg ble visse pasientgrupper ekskludert, f.eks. pasienter som gjennomgikk neoadjuvant behandling. Dette er særlig relevant da det er en økende populasjonsgruppe. Årsaken til eksklusjonen skyldes at det foreligger vanskeligheter med å måle nøyaktig tumorstørrelse etter cellegiftbehandling, fordi den ofte etterlater en mer fragmentert tumormasse.

En siste begrensning er at man i denne studien ikke har sett på korrelasjonen mellom tumorstørrelse, T-stadium og overlevelse. Grunnen til dette er at overlevelse avhenger av mange andre faktorer enn bare tumorstørrelse og T-stadium: f.eks lymfeknutestatus (N-stadium) og adjuvant behandling har en betydelig innflytelse på overlevelse.

Styrkene med denne studien er at studiekohorten er hentet fra en relativt kort og ny tidsperiode (2015-2020). Dessuten har det blitt brukt detaljerte, standardiserte patologiske undersøkelsesmetoder. En siste styrke med denne studien er at det ble foretatt fotografering av alle casus etter makroskopisk beskjæring, som muliggjør retrospektiv kontroll av makroskopiske funn.

Konklusjon

Til tross for at histopatologisk undersøkelse er en hjørnesteinen i diagnostikk av PC er det overraskende at noe så elementært som å vurdere tumorstørrelse ikke er standardisert og likt mellom ulike institusjoner. Resultatene fra studien viser at ulike målemetoder resulterer i signifikante forskjeller i største tumordiameter og dermed i signifikante endringer i pT-stadium. Dersom målemetodene varierer mellom ulike institusjoner og patologer vil tumorstørrelse og pT-stadium ikke være reproduserbart og det blir dermed vanskelig og meningsløst å sammenligne stadium-relaterte utfall. Etter å ha arbeidet med denne oppgaven har jeg først og fremst innsett hvor mye arbeid det ligger bak en artikkel, både når det kommer til forarbeid, analyser og selve skriveprosessen. Jeg har også innsett at resultater i ulike studier kan vinkles/fremstilles på spesifikke måter for å få frem et bestemt budskap. Slik at jeg har lært at det kan være viktig å lese vitenskapelige artikler med et mer kritisk øye. Dessuten har jeg fått erfare at det er vanskelig å skrive på en objektiv og nøytral måte, slik man skal i vitenskapelige artikler.

Kilder

1.	Brierley J, Gospodarwicz MK, Wittekind C. TNM classification of malignant tumours. Eighth edition. ed. Chichester, West Sussex, UK,Hoboken, NJ: John Wiley & Sons, Inc.; 2017.
2.	Birkeland KI, Gullestad L, Aabakken L, Toverud KC. Indremedisin : 1. Drammen: Vett & Viten; 2017.
3.	Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet. 2011;378(9791):607-20.
4.	Maisonneuve P. Epidemiology and burden of pancreatic cancer. Presse Med. 2019;48(3 Pt 2):e113-e23.
5.	Kreftregisteret (2021). Årsrapport 2020: resultater og forbedringstiltak fra kvalitetsregister for pankreaskreft. [cited 2022 17. Feb]. Available from: https://www.kreftregisteret.no/globalassets/publikasjoner-og-rapporter/arsrapporter/publisert-2021/arsrapport-kvalitetsregister-for-pankreaskreft-2020.pdf.
6.	Michl P, Löhr M, Neoptolemos JP, Capurso G, Rebours V, Malats N, et al. UEG position paper on pancreatic cancer. Bringing pancreatic cancer to the 21st century: Prevent, detect, and treat the disease earlier and better. United European Gastroenterol J. 2021;9(7):860-71.
7.	Loveday BPT, Lipton L, Thomson BN. Pancreatic cancer: An update on diagnosis and management. Aust J Gen Pract. 2019;48(12):826-31.
8.	Soreide K, Stattner S. Textbook of Pancreatic Cancer: Principles and Practice of Surgical Oncology. Cham: Cham: Springer International Publishing AG; 2021.
9.	Helsedirektoratet. Nasjonalt handlingsprogram med retningslinjer for diagnostikk, behandling og oppfølging av pasienter med pancreaskreft Oslo: Helsedirektoratet; 2017 [updated 05. juli 2021; cited 2022 17. Feb]. Available from: https://www.helsedirektoratet.no/retningslinjer/pancreaskreft-bukspyttkjertelkreft-handlingsprogram.

Tumour size and T-stage in pancreatic cancer resection specimens depend on the pathology examination approach

My Linh Tran [1], Maia Blomhoff Holm [1,2] and Caroline Sophie Verbeke [1,2,*]

[1] Department of Pathology, Faculty of Medicine, University of Oslo, Norway; m.l.tran@studmed.uio.no (M.L.T.); m.b.holm@medisin.uio.no (M.B.H.)
[2] Department of Pathology, Oslo University Hospital, Norway
* Correspondence: c.s.verbeke@medisin.uio.no; Tel.: +47 405 578 36

Simple Summary: Tumour size is considered a key oncological feature, as it reflects the local tumour burden. In pancreatic cancer, tumour size also constitutes the defining criterion for staging of the primary tumour (T-stage), which provides essential information for patient treatment, risk stratification in clinical trials, and cancer registries. While measurement of tumour size in pancreatic cancer resection specimens is considered accurate and reproducible, this has not been formally proven. This study prospectively investigated whether and in how far various approaches to tumour size measurement result in different tumour sizes and, consequently, different T-stages. The study findings show that tumour size and T-stage are different in a significant proportion of cases, depending on whether (i) the tumour was measured in one or two planes and (ii) the macroscopic tumour size was corroborated microscopically. Hence, divergence in pathology practice may limit comparability of tumour size and T-stage between institutions

Abstract: In the 8th edition of the TNM classification for pancreatic ductal adenocarcinoma (PDAC), stages T1 to T3 are defined by tumour size, size measurement being deemed objective and accurate. This study investigated whether various, currently used approaches to tumour measurement result in different tumour sizes and differences in T-stage assignment. In a series of 315 resected PDAC, tumour sizes were measured as follows: macroscopically in a single or in two perpendicular planes, and with or without microscopic corroboration. Comparison of the resulting tumour sizes showed that both macroscopic measurement in two planes and microscopic corroboration gave significantly different results (p<0.001). Compared to the most simple approach - macroscopic measurement in one plane - the comprehensive approach - macroscopic measurement in two planes with microscopic corroboration – resulted in a larger tumour size in 263 (83%) of cases (mean absolute size difference: 10 mm, mean relative size change: 36%). T-stage assignment differed in 142 (45%) of cases between the simple and comprehensive approach, and affected 87%, 38% and 48% of the cases deemed to be stage T1, T2 and T3, respectively. In conclusion, tumour size and T-stage are highly approach-dependent. Consensus on an accurate method is required to ensure comparability of these basic data.

Keywords: pancreatic cancer, pathology, tumour size, T-stage

1. Introduction

For most solid cancer entities, including pancreatic cancer, the size of the tumour is considered of key oncological importance, as it reflects the primary tumour burden. Moreover, according to the 8th edition of the Tumour, Node, Metastasis (TNM) classification (8th edition) published by the Union for International Cancer Control (UICC) and the American Joint Commission on Cancer (AJCC) [1,2], tumour size is the defining criterion for the T-categories T1-T3. While the definition of categories T1 and T2 re-

mained unchanged (T1: ≤20 mm, T2: >20 mm to ≤40 mm) compared to the previous edition [3,4], invasion outside the pancreas as the defining criterion for T3 was replaced by tumour size exceeding 40 mm. Hence, the 8th edition of the AJCC/UICC TNM classification introduced criteria for T1 to T3 that are exclusively based on tumour size, the underlying assumption being that measurement of the largest tumour size is objective and straightforward, and therefore accurate and reproducible.

Because tumour staging is key to both individual patient management, clinical research and cancer registries, tumour size is a core data item in national and international data sets for pancreatic cancer [5-8]. However, in spite of the clinical importance attached to tumour size, (inter-)national recommendations for the pathology reporting of surgical resection specimens with ductal adenocarcinoma of the pancreas provide only limited guidance as to how tumour size should be measured. Most guidelines recommend microscopic corroboration of the largest tumour size that was measured macroscopically [5-8]. The reason for this recommendation is that pancreatic cancer grows in a highly dispersed fashion, such that the exact tumour boundary may not be visible by naked-eye inspection [9]. Furthermore, atrophy of flanking pancreatic parenchyma and fibrosis often blur the macroscopic delineation of the tumour. In recognition of the fact that most cancers do not have a perfect spherical shape, it is important that the *largest* tumour size is recorded. This requires measurement in multiple planes, and in practice specimen dissection techniques allow for measurement in two planes: the plane in which the specimen is sliced and the plane perpendicular to that.

In practice, most pathologists record the tumour size during specimen grossing by measuring the tumour - usually in two dimensions - on the specimen slice in which the tumour appears at its largest expanse. Some will subsequently check the macroscopic size measurement during microscopic examination. Measurement of a third dimension, in the plane across specimen slices, may be done by some pathologists for cancers in the pancreatic body or tail, for example in the context of a clinical study, but this approach is usually omitted for cancers located in the head of the pancreas [10].

Given the importance of accurate measurement of the largest tumour size for correct T-staging, this study aimed at comparing the results of the above approaches, that is, two-dimensional versus three-dimensional measurement and macroscopic measurement with or without microscopic corroboration. Furthermore, the study aimed at investigating if and in how far different approaches to tumour size measurement affected assignment to T-stage.

2. Materials and Methods

2.1. Study cohort

This retrospective observational study is based on a cohort of pancreatic ductal adenocarcinomas that were identified from a prospective database at the Department of Pathology at Oslo University Hospital. Included were all ductal adenocarcinomas of the pancreas that were surgically resected at Oslo University Hospital, Norway, between January 1, 2015 and December 30, 2020. Excluded were tumours that had been treated neoadjuvantly, pancreatic ductal adenocarcinoma that had developed from intraductal papillary mucinous neoplasia, and any other tumour entities in the pancreas, including mixed tumour entities, such as mixed neuroendocrine-non-neuroendocrine neoplasms.

2.2. Data collection

For each case in the study cohort, the following data were recorded: type of surgical resection, tumour site (head or body/tail of pancreas), T- and N-stage (according to the 8th edition of the AJCC/UICC TNM classification), presence or absence of lymphatic, vascular and perineural invasion, and resection margin status. Regarding the tumour

dimensions, two sets of sizes were extracted from the database: (1) the maximum tumour size measured macroscopically in one specimen slice and the maximum tumour size measured across specimen slices, and (2) the same tumour dimensions corroborated by microscopic measurement.
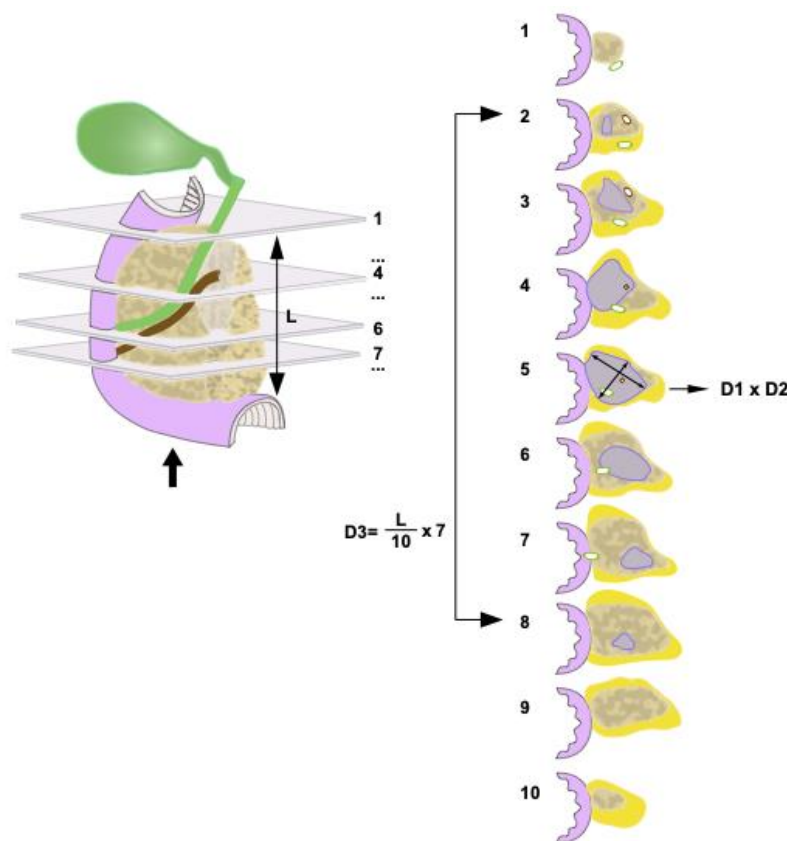
Figure 1. Tumour size measurement in pancreatoduodenectomy specimens. Following specimen slicing in the axial plane, the maximum tumour dimension (D1) is measured in the specimen slice where the tumour is largest. A second tumour dimension can be measured in the same slice perpendicular to the first measurement (D2); this size is not considered in this study, as it is by definition not the largest. The dimension measured in the plane perpendicular to the axial plane (D3) is calculated based on the craniocaudal length of the pancreatic head (L), the total number of axial specimen slices and the number of tumour-bearing slices. In case the tumour was visible on only one side of either the first or the last specimen slice, half of the slice thickness was considered in the calculation. To ensure correct identification of the cranial and caudal ends of the tumour (macroscopically assumed in specimen slices 2 and 8), both adjacent slices (i.e., slices 1 and 9) were also embedded.

2.3. Pathology examination

Grossing of all specimens was undertaken in line with national guidelines [11] and according to a rigorous departmental standard operating procedure, as described previously [12,13]. Briefly, pancreatoduodenectomy specimens were dissected by axial slicing, yielding for each case between 10-15 slices. During macroscopic examination, the largest tumour size were routinely measured in the plane of specimen slicing and the plane perpendicular to that, as follows (Figure 1). Once the specimen slices were laid out in sequential order, the specimen slice in which the tumour was at its largest expanse was identified. The largest tumour dimension was measured in that slice (hereafter referred to as "2D-macro"). The second tumour dimension, which was measured in the same specimen slice, perpendicularly to the largest dimension, was not considered in the current study, as it did not represent the largest tumour size in that plane. Furthermore, the number of the axial specimen slices that were involved by tumour were recorded. The

tumour dimension in the craniocaudal plane was then calculated based on the length of pancreatic head in craniocaudal direction (measured before axial slicing), the total number of specimen slices, and the number of tumour-bearing slices, according to the following formula:

$$\left(\frac{\text{craniocaudal length of pancreas}}{\text{total number of axial slices}}\right) \times \text{number of axial slices with tumour.}$$

This (third) tumour size is in the following referred to as "3D-macro".

With this approach, the accuracy of the measurement in the craniocaudal direction was determined by the thickness of the specimen slices. In some cases, tumour tissue was visible only on one side, but not on both sides of the specimen slice(s) containing the edge of the tumour. To increase accuracy on such occasions, only half of the slice thickness for that particular slice (or for both slices) was used in the size calculation. Given an average slice thickness of 3 mm, the accuracy of size measurement across specimen slices was 1.5 mm.

Distal pancreatectomy specimens were dissected by serial slicing in the sagittal plane, that is, perpendicular to the longitudinal axis of the pancreatic body and tail. A similar procedure was followed to evaluate the maximum tumour size in three dimensions: by measuring the maximum tumour size in the slice with the largest tumour expanse (2D-macro) and by calculating the tumour size across sagittal specimen slices (3D-macro), as illustrated in Figure 2.

Total pancreatectomy specimens were divided by the pathologist into a "pancreatoduodenectomy" and a "distal pancreatectomy" part, in which the tumour dimensions were measured as described above.

To allow review of the macroscopic findings, each case was photodocumented by an overview image of all specimen slices as well as close-up images of individual slices.
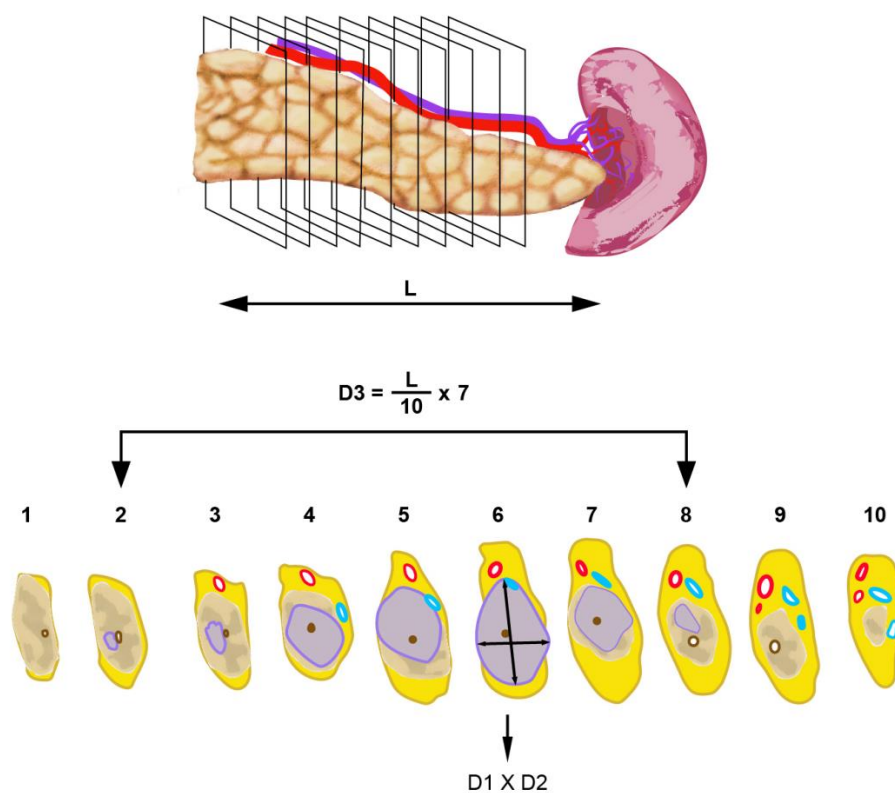
Figure 2. Tumour size measurement in distal pancreatectomy specimens. Following specimen slicing in the sagittal plane, the maximum tumour dimensions (D1 and D3) are measured in a similar fashion as described in Figure 1.

154

155 *Tissue sampling* was extensive in each case and included complete embedding of (i)
156 the specimen slice deemed to contain the largest tumour cross-section and (ii) the slices
157 that contained the craniocaudal or mediolateral ends of the tumour. In order to detect
158 possible tumour extension in the craniocaudal or mediolateral direction that was invisi-
159 ble to naked-eye inspection, the slice (or multiple slices in cases with an unclear tumour
160 boundary) flanking the presumed ends of the tumour were also embedded (Figures 1
161 and 2). Embedding of the specimen slices was done either by using whole mount blocks
162 or by dividing the specimen slices by rectilinear cuts into tissue samples of standard
163 block size such that the entire tumour bed could be reconstructed during microscopic
164 examination.

165 For *microscopic measurement* of the tumour dimensions, the invasive front of the
166 tumour was annotated under the microscope in the tissue section(s) representing the
167 specimen slice that was identified as containing the tumour at its largest expanse. The
168 largest tumour dimension, referred to as 2D-micro, was measured in that tissue section.
169 For evaluation of the largest tumour dimension across specimen slices, the number of
170 specimen slices with microscopically detectable primary tumour growth was recorded
171 and used for the calculation of this tumour dimension (hereafter referred to as 3D-micro),
172 as outlined above.

173

174 *2.4. Statistical analysis*

175 Descriptive statistics regarding the clinicopathological features of the study cohort
176 are presented as numbers and percentages, both for the entire series and separately for
177 cancers in the pancreatic head and body/tail. The measured tumour dimensions are pre-
178 sented both as means with standard deviations and as medians with interquartile range.
179 Also presented is the overall range for each tumour dimension. Group-wise comparisons
180 between tumours in the pancreatic head and body/tail were performed with independent
181 sample t-tests, while paired comparisons between the different measurement approaches
182 were assessed with paired-samples t-tests. A two-sided p-value of <0.05 was considered
183 significant. Statistical Package for the Social Sciences, Version 26 (SPSS Inc., Hong Kong)
184 was used for all analyses.

185

186 **3. Results**

187 *3.1. Study cohort*

188 A total of 315 eligible cases were included in the study cohort, of which 231 tumours
189 were located in the head of the pancreas and 84 in the pancreatic body/tail. Pathology
190 data for the entire cohort are presented in Table 1.

191 For the purpose of the analysis, the four tumours that were removed by total pan-
192 createctomy were included in the cohort of pancreatic head cancers, because these tu-
193 mours were located mainly in the pancreatic head, with limited extension into the
194 so-called pancreatic neck, that is, the transition to the pancreatic body.

195

196

197

198

199

200

201

202

203

204

205 **Table 1.** Clinicopathological features.

	Entire series (n = 315) (%)	Head of pancreas (n = 231) (%)	Body/tail of pancreas (n = 84) (%)
Specimen type			
Pancreatoduodenectomy			
• classical	80 (25)	80 (35)	0 (0)
• pylorus-preserving	147 (47)	147 (64)	0 (0)
Distal pancreatectomy	84 (27)	0 (0)	84 (100)
Total pancreatectomy	4 (0.01)	4 (0.02)	0 (0)
T-stage*			
T1c	10 (3)	5 (2)	5 (6)
T2	190 (60)	155 (67)	35 (42)
T3	115 (37)	71 (31)	44 (52)
N-stage			
N0	32 (10)	18 (8)	14 (17)
N1	115 (37)	79 (34)	36 (43)
N2	168 (53)	134 (58)	34 (40)
Lymphatic invasion			
L0	22 (7)	11 (5)	11 (13)
L1	293 (93)	220 (95)	73 (87)
Vascular invasion			
V0	93 (30)	69 (30)	24 (29)
V1	199 (63)	161 (69)	38 (45)
V2	23 (7)	1 (1)	22 (26)
Perineural invasion			
Pn0	22 (7)	9 (4)	13 (15)
Pn1	293 (93)	222 (96)	71 (85)
Resection margin status			
R0	52 (17)	37 (16)	15 (18)
R1	263 (83)	194 (84)	69 (82)

206 * based on 2D-3D-micro measurement.

3.2. Tumour size

The results of tumour size assessment macroscopically in the plane of slicing (2D-macro) and across specimen slices (3D-macro) and following microscopic corroboration of both measurements (2D-micro, 3D-micro) are shown in Table 2. Results are stated collectively for the entire series and separately for cancers located in the pancreatic head or body/tail.

Comparison between both tumour locations revealed that the tumour size measured in the third dimension, that is, across specimen slices, was significantly larger for tumours in the body/tail than for cancers in the head region ($p < 0.001$), both following macroscopic measurement and microscopic corroboration. In contrast, there was no statistically significant difference between locations for the tumour size measured in the plane of slicing.

221 | **Table 2.** Tumour size obtained by various measurement approaches.

Tumour size (mm)	Entire series (n = 315)	Head of pancreas (n = 231)	Body/tail of pancreas (n = 84)
2D-macro			
Mean (SD)	30 (10)	30 (8)	31 (15)
Median (IQR)	30 (23 – 35)	30 (27 – 40)	27 (21 – 36)
Range	9 – 110	11 – 51	9 – 110
2D-micro			
Mean (SD)	32 (10)	32 (7)	31 (15)
Median (IQR)	31 (25 – 36)	32 (27 – 36)	29 (21 – 36)
Range	6 – 110	14 – 61	6 – 110
3D-macro			
Mean (SD)	35 (14)	32 (10)	45 (19)
Median (IQR)	32 (25 – 41)	30 (25 – 37)	43 (30 – 55)
Range, mm	12 – 100	12 – 61	13 – 100
3D-micro			
Mean (SD)	38 (14)	35 (10)	45 (19)
Median (IQR)	36 (28 – 45)	35 (28 – 41)	43 (32 – 59)
Range	13 – 100	15 – 75	13 – 100

222 | SD, standard deviation; IQR, interquartile range.

3.3. Differences in tumour size dependent on the measurement approach

Because tumour measurement is primarily done during macroscopic examination, it was first investigated if and in how far macroscopic measurement also in the plane *perpendicular* to the plane of serial slicing resulted in a different tumour size. Furthermore, the impact of microscopic corroboration on tumour size was analysed. The results of comparison of tumour sizes obtained by the different approaches are shown in Table 3.

3.3.1. Impact of measurement in the third dimension (2D-macro versus 3D-macro)

Macroscopic measurement in the plane perpendicular to the plane of slicing (i.e., across specimen slices; 3D-macro) resulted in tumour sizes that were significantly different from those measured in the plane of slicing (i.e., on a specimen slice; 2D-macro; $p = 0.003$ head, $p < 0.001$ body/tail). The difference in size was particularly large for cancers in the pancreatic body/tail, with a mean size difference of 17 mm. The relative change in size, that is, the difference in size between both measurements divided by the tumour size obtained by 2D macroscopic measurement, was 61%. In 69 (82%) of these cases, the 3D-macro size was *larger* than the 2D-macro size, the mean difference between both sizes being 19 mm (range: 1 - 73 mm), representing a mean relative change in size of 72% (range: 4% - 280%). Only in a single case, the tumour size measured in both planes was identical, whereas in 14 (17%) of body/tail cancers, the 3D-macro size was *smaller* than the one measured in 2D (mean relative change in size: -15%, range: -5% to -50%). As illustrated in Figure 3, the size of the tumour along the length of the pancreatic body/tail (3D-macro) can exceed considerably the cross-sectional size (2D-macro). The study series included one pancreatic cancer in the body/tail that stood out in this respect, because it was considerably wider than long (110 mm vs 55 mm). Interestingly, this outlier was diagnosed as an undifferentiated carcinoma with osteoclast-like giant cells, and it contained a large blood-filled cavity, which was the reason for the unusual shape and exceptionally large size of the tumour.

251

Table 3. Difference in tumour sizes obtained by various approaches.

Difference in tumour size	Head of pancreas			Body/tail of pancreas		
	Absolute (mm)	Relative* %	P-value	Absolute (mm)	Relative* %	P-value
2D-macro vs 2D-micro			< 0.001			0.73
Mean (SD)	4 (4)	17 (24)		3 (5)	11 (14)	
Range	-21 – 25	-50 – 227		-22 – 30	-52 – 60	
2D-macro vs 3D-macro			0.003			< 0.001
Mean (SD)	6 (6)	22 (23)		17 (16)	61 (59)	
Range	-27 – 24	-64 – 123		-55 – 73	-50 – 280	
3D-macro vs 3D-micro			< 0.001			0.83
Mean (SD)	5 (6)	19 (26)		7 (10)		
Range, mm	-22 – 28	-38 – 183		-60 – 29		
2D-macro vs 2D-3D-micro			< 0.001			< 0.001
Mean (SD)	7 (8)	28 (40)		16 (15)	58 (57)	
Range	-21 – 37	-50 – 308		-15 – 73	-36 – 290	

252 *relative difference in tumour size measured by approach A and B: $\frac{(\text{tumour size approach A}) - (\text{tumour size approach B})}{\text{tumour size approach A}} \times 100$.
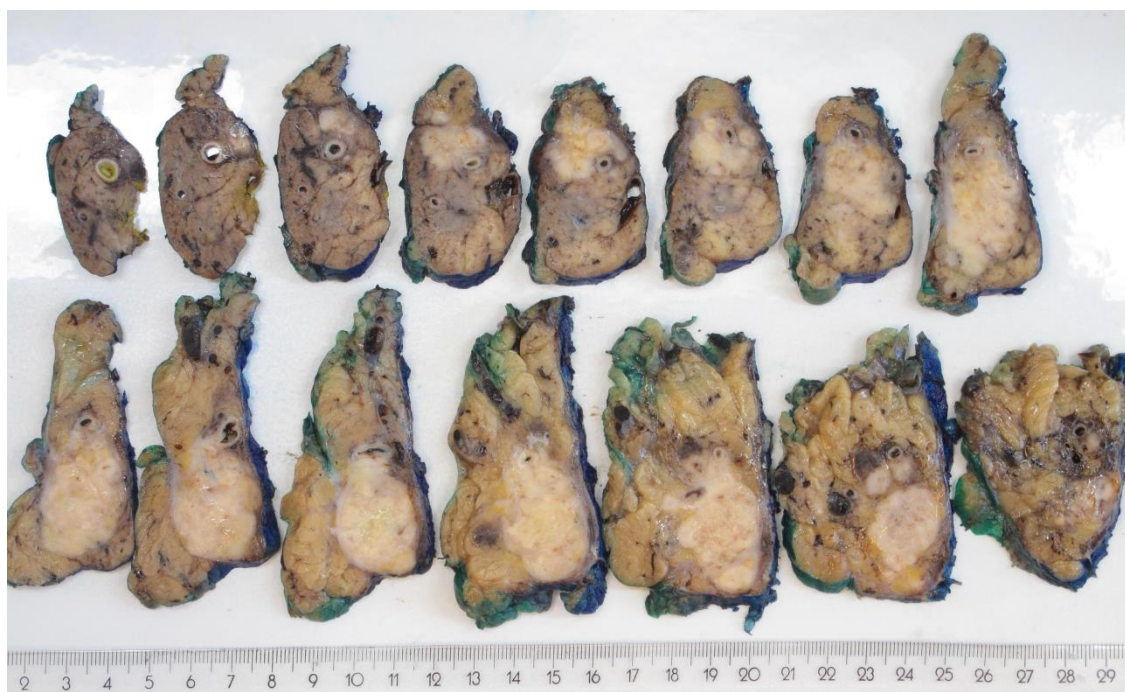
253 SD, standard deviation.

254

255

256 **Figure 3.** Oblong shape of pancreatic cancer in the body/tail. The 15 sagittal slices of a distal pancreatectomy specimen are laid out in
257 sequential order from upper-left to lower-right. Tumour tissue is visible from slice 3 (arrow) and extends through multiple subse-
258 quent slices. However, it is not clear which of the slices through the pancreatic tail contain tumour or just atrophic tissue. Micros-
259 copy revealed that only the two last slices were clear of tumour. The total length of the tumour (from slice 3 to 13, arrows; slice
260 thickness: 4 mm) is 44 mm, which is considerably larger than the maximum dimension in the sagittal plane (34 mm, measured in
261 slice 12) and corresponds with stage T3, not T2.

262

Statistically significant differences between 2D-macro and 3D-macro sizes were also observed in the series of cancers of the pancreatic head, although the change in size was overall smaller (mean size difference: 6 mm, median relative change in size: 22%) than in the series of tumours in the body/tail region. In 117 (51%) cases, 3D-macro sizes were larger than those obtained by 2D-macro measurement, the mean difference in size being 6 mm (mean relative change in size: 22%, range: 3%-123%). Tumour size was smaller in the 3D-plane in 92 (40%) cases, and in 22 (9%) cases, tumour size was identical in both planes.

3.3.2. Impact of microscopic corroboration

For cancers in the pancreatic head, microscopic corroboration of the macroscopic measurement either on a specimen slice (2D) or across axial slices (3D) resulted in significantly different sizes (p < 0.001; Table 3). The mean difference in size (and mean relative change in size) introduced by microscopic measurement was 4 mm (17%) or 5 mm (19%), respectively. Microscopic corroboration revealed mainly underestimation of the tumour size that was measured during macroscopic examination (Figure 4). Size *underestimation* by 2D- and 3D-macroscopic measurement was both common (in 136 (59%) and 119 (52%) of cases, respectively) and substantial (24% and 33% mean relative change in size, respectively), with up to 227% and 183% size change in individual cases, respectively. Size *overestimation* affected fewer cases (59 (25%) by 2D-macro, 35 (15%) by 3D-macro measurement) to a lesser degree (mean relative change in size: -13% and -14%, respectively).

For cancers in the pancreatic body/tail, microscopic corroboration of sizes in either plane did not reveal significantly different tumour sizes, although especially underestimation of size measured macroscopically across specimen slices was considerable (25% mean relative change in size, range 2-100%) and occurred in 37 (44%) of cases.
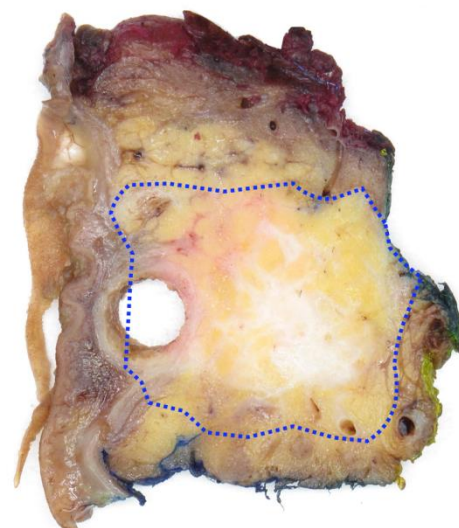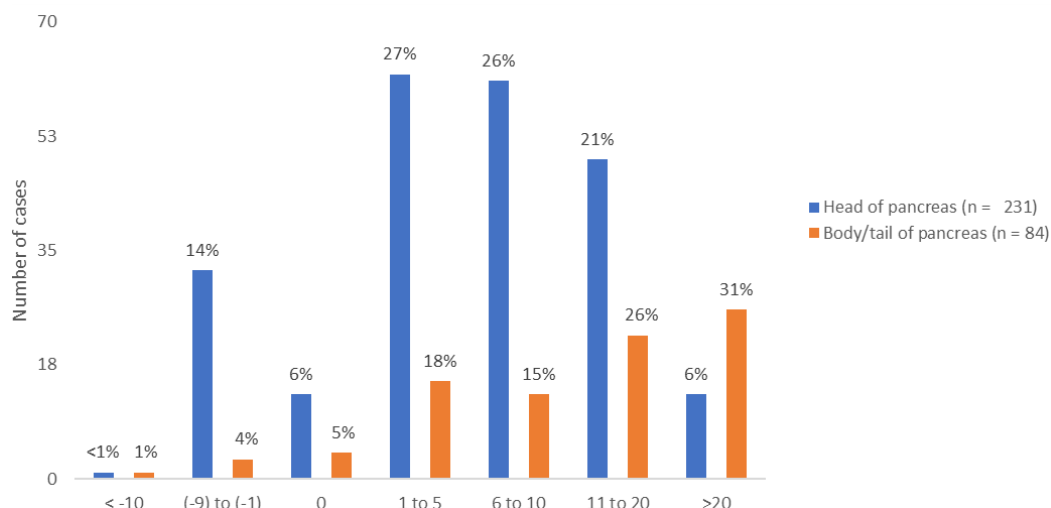

Figure 4. Microscopic corroboration of tumour size. This axial slice from a pancreatoduodenectomy specimen with a largely dilated common bile duct (metal stent removed) shows an ill-defined tumour. The true tumour border, as identified by microscopic examination of this tissue slice, is indicated on the macroscopic image (dotted line) and includes parts of pancreatic tissue that on naked-eye inspection appear clear of tumour. Consequently, the tumour size based on microscopic corroboration (2D-micro) exceeds the one assessed by naked-eye inspection only (2D-macro).

3.3.3. Impact of measurement in the third dimension combined with microscopic corroboration (2D-macro versus combined 2D-3D-micro)

In only 17 (5%) of all cases, identical maximum tumour sizes were obtained by both a simple procedure, which was based exclusively on macroscopic measurement on a specimen slice (2D-macro), and a comprehensive approach, which consisted of measuring also across specimen slices with microscopic corroboration of both macroscopic sizes (combined 2D-3D-micro). The difference in tumour size based on either approach was statistically significant (p < 0.001) irrespective of tumour site. In the vast majority of cancers - 185 (80%) in the pancreatic head and 76 (90%) in the body/tail - comprehensive measurement revealed a larger tumour size than the one obtained by 2D-macroscopic measurement only (Figure 5). Underestimation of the maximum tumour size based exclusively on 2D-macro measurement was not only frequent but also substantial: in 62

(27%) of cancers in the pancreatic head and 48 (57%) of tumours in the body/tail, under-estimation of size exceeded 10 mm. For pancreatic head cancers, the impact of the comprehensive approach (mean relative change in size: 28%) was larger than that of either the addition of microscopic corroboration of the 2D-macro measurement (mean relative change in size: 17%) or of macroscopic measurement across specimen slices (mean size difference: 6 mm, median relative change in size: 22%; Table 3). For cancers in the pancreatic body/tail, the comprehensive approach resulted in a substantially different tumour size (mean size difference: 16 mm, mean relative change in size: 58%). However, the impact was not significantly larger than that from measurement across specimen slices (mean relative change in size: 61%).

Figure 5. Difference in largest tumour size measured macroscopically in two dimensions (so-called simple approach) versus in three dimensions with microscopic corroboration (so-called comprehensive approach). The graph shows the number of pancreatic head and body/tail cancers (and percentages, indicated at the top of each bar) for the range of differences in size that were observed between both measurement approaches. Negative values indicate that the size obtained by the comprehensive approach was smaller than the one based on the simple approach. In the vast majority of cases, the tumour size measured by the comprehensive approach exceeded the size based on the simple approach.

3.4. Impact of the approach to tumour size measurement on T-stage assignment and distribution

3.4.1. T-stage assignment

For each cancer, T-stage was assigned based on the largest tumour size that was obtained by each of the various measurement approaches. Any difference in T-stage compared to the T-stage based on the simple approach, that is, macroscopic measurement on a specimen slice (2D-macro) was recorded separately for cancers in the pancreatic head and body/tail (Table 4, Supplementary Table S1).

When comparing measurement on a specimen slice without versus with microscopic corroboration (2D-macro versus 2D-micro), 57 (18%) cases in the entire study series changed T-category, with a shift affecting 19 of 38 (50%) of cancers categorised as T1 based on 2D-macro assessment, 21 of 231 (9%) of those deemed to be T2, and 17 of 46 (37%) cancers that were categorised as T3.

In 115 (37%) cases, T-stage assignment differed depending on whether tumour sizes were based on macroscopic measurement in two planes (3D-macro) or measurement in a single place (2D-macro). This change in T-stage affected 22 of 38 (58%) of the cancers that were categorised as T1 based on 2D-macro assessment, 72 of 231 (31%) of tumours deemed to be T2, and 20 of 46 (43%) cancers that were categorised as T3.

Table 4. Shift in T-stage assignment when comparing various approaches to tumour size measurement.

Shift in T-stage	None n (%)	Shift to higher T-stage n (%)	Shift to lower T-stage n (%)
2D-macro vs 2D micro			
Head	186 (80)	30 (13)	15 (7)
Body/tail	72 (86)	3 (3)	9 (11)
2D-macro vs 3D-macro			
Head	161 (70)	36 (15)	34 (15)
Body/tail	39 (46)	39 (46)	6 (8)
2-macro vs 2D-3D-micro			
Head	129 (56)	80 (35)	22 (9)
Body/tail	44 (51)	37 (45)	3 (4)

Comparison between the simple approach (2D-macro) and the comprehensive approach based on measurement in two planes and microscopic corroboration (2D-3D-micro) revealed an even larger shift in T-stage, namely in 142 of all cases (45%). This change in T-stage affected the vast majority of cancers deemed to be T1 based on the 2D-macro approach (33 of 38, 87%), and more than a third (87 of 231, 38%) and nearly half (22 of 46, 48%) of tumours in the categories T2 and T3, respectively. In the vast majority of cases with a change in T-stage, the T-stage shifted up (117 of 142, 82%), while in a smaller proportion (25 of 142, 18%), there was a down-shift. The shift was mainly by one T-stage, but in 8 cancers (6% of all cases with altered T-stage) the shift was across two T-stages (T1 to T3 in 6 cases, T3 to T1 in 2 cases). The single case in the entire series that was assigned to stage T1b based on 2D-macro measurement shifted to T1c, that is, it remained within the T1-category and was therefore not regarded as a change in T-stage. T-stage changed somewhat more frequently for cancers in the pancreatic body/tail than for those located in the pancreatic head (40 of 84 (48%) versus 102 of 213 (44%), respectively). Otherwise, the distribution of changes in the three T-categories was similar irrespective of tumour site.

3.4.2. T-stage distribution

The above-described shifts in T-stage resulted in a considerable change in T-stage distribution (Table 5). Tumour size measurement limited to the plane of slicing, without or with microscopic corroboration (2D-macro or 2D-micro), led to a higher proportion of T1-stage cancers (38 (12%) or 27 (9%), respectively) than when using the comprehensive approach (2D-3D-micro), according to which only 10 (3%) cancers were T1. While the proportion of cases assigned to stage T2 also decreased (from 213 (73%) or 245 (78%) to 191 (61%)), the percentage of cases in the T3-category more than doubled when using the comprehensive approach (from 46 (15%) or 43 (14%) to 114 (36%)). Similar changes in T-stage distribution were observed when analysing separately cancers in the pancreatic head or body/tail.

Macroscopic measurement in the plane across specimens slices (3D-macro) introduced changes in T-stage distribution mainly for cancers in the body/tail, where it resulted in a T-stage distribution that was very similar to the one based on the comprehensive approach (T1: 8%, T2: 38%, T3: 54%). In contrast, for cancers in the pancreatic head, T-stage distribution differed little from that based on 2D-macro measurement (T1: 13%, T2: 72%, T3: 15%).

Comparison with the T-stage distribution reported by other series shows that these are similar to the distribution based on the simple approach (2D-macro), but different from the one based on the comprehensive approach (2D-3D-micro; Supplementary Table S2).

Table 5. Distribution of T-stage based on various approaches to tumour size measurement.

Head of pancreas (n = 231)	2D-macro n (%)	2D-micro n (%)	2D-3D-micro n (%)
T1	26 (11)	10 (4)	5 (2)
T2	176 (76)	193 (84)	156 (68)
T3	29 (13)	28 (12)	70 (30)
Body/tail of pancreas (n = 84)			
T1	12 (14)	17 (20)	5 (6)
T2	55 (66)	52 (62)	35 (42)
T3	17 (20)	15 (18)	44 (52)

4. Discussion

Tumour size is a key oncological data item, because it reflects the primary tumour burden. As for multiple other cancer entities, tumour size is the defining criterion of T-stage (T1-T3) for PDAC. The clinical interest in and importance attributed to the TNM system is reflected by the considerable number of studies that soon after the release of the 8th edition of the UICC/AJCC classification system validated the new T- (and N-)staging criteria in European, Asian and US data sets [14-20]. While tumour size was the critical data item on which these studies were based, none actually described the method that was used to measure the size of the tumour. Several of the studies mentioned that size was measured during macroscopic examination [17,21,22], while others stated that - at least in part of the cases - the macroscopic size measurement was corroborated microscopically [14,15]. Microscopic corroboration is recommended by national and international pathology guidelines because of the generally poor macroscopic delineation of PDAC [5-8]. The latter results mainly from the highly dispersed growth of the cancer cells, which occurs especially in the tumour periphery and therefore precludes appreciation of the full width of the tumour by naked-eye inspection [9].

This study aimed at investigating whether and to which extent different ways of measuring the tumour resulted in different tumour sizes and in how far this divergence affected T-stage.

Most commonly, pathologists assess tumour size during macroscopic examination of the surgical specimen. Measurement is usually done only in one plane, namely in the plane of specimen slicing. Following identification of the specimen slice in which the tumour is at its largest expanse, the tumour size is measured on this slice in *two* dimensions, that is, the dimension in which the tumour size is largest and the perpendicular dimension (which is of no further interest to this study, as it is de facto not the largest tumour size). In contrast to this simple procedure, a comprehensive approach includes in addition (i) measurement in a *third* dimension, namely in the plane that is perpendicular to the plane of specimen slicing (that is, across specimen slices), and (ii) microscopic corroboration of both sizes that were obtained by macroscopic measurement in both planes (Figures 1 and 2).

To compare the results of the various approaches to tumour size assessment, a set of four sizes were systematically measured in a prospective series of 315 consecutive, surgically resected, treatment-naïve pancreatic cancers. For each case, the following four sizes were recorded: the maximum tumour size measured (i) macroscopically on a

specimen slice (referred to as 2D-macro) and (ii) across specimen slices (3D-macro) as well as (iii, iv) the size in either plane obtained by microscopic corroboration (2D-micro, 3D-micro).

Overall, the study findings reveal that measurement in two planes and microscopic corroboration result in considerable size differences as compared to exclusive macroscopic measurement in a single plane.

Macroscopic measurement of tumour size both in the plane of sectioning or perpendicular to it, i.e., across specimen slices, resulted in significantly different tumour sizes compared to macroscopic measurement only in the plane of slicing (p = 0.003 head, p < 0.001 body/tail). The size discrepancy occurred irrespective of tumour site, but was particularly large for tumours in the pancreatic body/tail. In 82% of the latter cancers (69/84 cases), measurement limited to the plane of sectioning resulted in underestimation of tumour size by a mean of 19 mm or a 72% relative change in size. This marked difference in size between cross-sectional and longitudinal dimensions is explained by the fact that pancreatic cancers in the body/tail often have an oblong shape, as they grow extensively along the longitudinal axis of the organ without causing cross-sectional expansion to a similar extent (Figure 3). This macroscopic growth pattern is clearly different from that seen in other tumour entities, e.g., neuroendocrine tumour or solid pseudopapillary neoplasia, which usually have a more spherical shape.

As recommended by (inter-)national guidelines, it is common practice to corroborate by microscopic examination the tumour size that is measured macroscopically. This is usually done only for the measurement in the plane of sectioning, that is, the measurement taken on a specimen slice. The study shows that for cancers in the pancreatic head, tumour size was underestimated in 136 (59%) of cases by 24% if macroscopic measurement was not corroborated microscopically, and in a further 25%, size was overestimated by a mean of 13% (p < 0.001). For cancers in the body/tail, microscopic corroboration resulted in smaller size differences that were not statistically significant. The latter finding is not surprising, taking into consideration that the majority of these cancers already occupy the entire width of the pancreatic body/tail with - as outlined above - limited expansion of the latter.

Similar observations were made when comparing measurement of the third dimension (that is, across specimen slices) with or without microscopic corroboration. Again, significant size differences were observed for cancers in the pancreatic head (p < 0.001), with size underestimation in 52% (mean relative change in size 33%) if macroscopic measurement was not corroborated microscopically.   For cancers in the body/tail, change in size following microscopic corroboration did not reach statistical significance. However, in individual cases, large size discrepancies - both overestimations of up to 29 mm and underestimations of up to 60 mm - were recorded. The reason for these large differences is the fact that pancreatic tissue upstream from the tumour is often severely affected by atrophy and fibrosis, which renders macroscopic distinction from the cancer difficult. This may result in severe under- or overestimation of the longitudinal tumour size, especially for cancers located in the body of the pancreas, in which these macroscopically equivocal changes may extend throughout most of the pancreatic tail (Figure 3).

Finally, results from the most commonly used, simple approach of exclusive macroscopic measurement on a specimen slice were compared with those obtained by a comprehensive approach that combines macroscopic assessment in two planes *and* microscopic corroboration of both measurements. In only 5% of all cases, the largest tumour size was the same by either approach. Tumour sizes were significantly different irrespective of tumour site (p < 0.001). In 83% of all cases, the comprehensive approach revealed a larger tumour size, which in 35% exceeded by more than 10 mm the size obtained by the simple approach (Figure 5).

Because tumour size was found to differ significantly depending on the approach to measurement that was used, it was further investigated if and to which degree this af-

fected the *T-stage* in each case. While microscopic corroboration of tumour size measured in a specimen slice (2D-macro) affected the T-stage in a relatively limited number of cases (entire series: 18%, head: 20%, body/tail: 14%), macroscopic measurement in the plane across specimen slices resulted in a considerably larger T-shift, namely in 37% of all cases (head: 30%, body/tail: 54%). When comparing the simple approach, based on exclusive macroscopic 2D measurement, with the comprehensive approach of macroscopic measurement in two planes and microscopic corroboration, the T-stage changed in 45% of cancers (head: 44%, body/tail: 47%). In 82% of incorrectly staged cases, the shift was to a higher T-stage.

The shifts in T-stage that occurred depending on the approach to tumour measurement had a knock-on effect on T-stage distribution. When comparing the simple approach with the comprehensive approach, the latter reduced the proportion of T1 tumours to a dwindling 3% (versus 12% according to the simple approach), more than doubled the proportion of T3 tumours (36% versus 15%), and reduced the group of T2 tumours (61% versus 73%). These changes in T-stage distribution were nearly identical for cancers in the pancreatic head and body/tail. Measurement limited to the plane of slicing but with microscopic corroboration caused only minimal changes in T-stage distribution.

Taken together, the study demonstrates that various measurement approaches lead to significant differences in tumour size and, consequently, a different pT-assignment in a considerable number of cases. The currently most commonly used approach, which is based only on macroscopic measurement in the plane of specimen slicing, results in a different, incorrect T-stage assignment in 45% of pancreatic cancers as compared to the comprehensive approach.

To the best of our knowledge, this study is the first to show that different approaches to tumour measurement result in significantly different tumour sizes and, consequently, assignment to different T-stages.   The study is also the first to reveal that identification of the largest tumour size is dependent on two key aspects of the pathology examination method: measurement in two planes, i.e., on a specimen slice and across slices, and microscopic corroboration. While the latter is relevant irrespective of the tumour site, the former affects particularly (but not exclusively) the size assessment of tumours in the body/tail, resulting in a mean relative change in size of no less than 61%. Furthermore, the results show that because size discrepancies are both common *and* substantial, they lead to a shift in T-stage in 45% of tumours, if the comprehensive approach is used instead of the simple approach. As such, the assumption that measurement of tumour size is accurate and reproducible - and results in correct T-staging - only applies if the comprehensive method is used systematically.

The findings in this study have two important implications. First, given the current divergence in practice related to tumour size measurement, tumour size and T-stage assignment may not be comparable between institutions. While most guidelines acknowledge that microscopic corroboration is important [5,7,8], published studies show that this recommendation is not uniformly put into practice [15-17,19, 21,22]. Moreover, because the lack of three-dimensional tumour measurement results in inaccurate tumour size and T-stage in a significant proportion of cases - 95% and 45% in this series - , bias may be introduced in multicentre clinical trials and tumour registries. Furthermore, the correlation with patient outcome or other parameters that are tested for predictive or prognostic significance may be affected. In that respect, it is interesting to note that the T-stage distribution differs remarkably between the studies that validated the 8th edition of the AJCC/UICC TNM-classification system (T1: 12-20%, T2: 55-69%, T3-17-28%; Supplementary Table 2). These published data are similar to the ones observed in the current study for macroscopic size measurement only in one dimension. In contrast, the distribution is considerably different when based on the comprehensive approach to tumour size measurement, with a remarkably smaller T1-category (3%) and larger T3-category (36%), whilst, naturally, the T2-category is not dissimilar.

Since the ease of practice is an important factor to consider when deciding on which approach to follow, one could argue that universal adherence to the currently more commonly used approach, namely macroscopic measurement in the plane of specimen slicing without microscopic corroboration, would ensure comparability of data. However, while this would indeed improve reproducibility, it would not address the fact that - as outlined above - in a large proportion of cases both tumour size and T-stage are incorrect, with either under- or overestimation of a highly variable degree.

A second implication of this study is that (inter-)national data sets for PDAC should not only recommend tumour size measurement in three dimensions, but also provide practical guidance as to how this should be done, an important aspect that currently is lacking. Macroscopic measurement of the tumour dimension across specimen slices involves more detailed recording during macroscopic examination. Furthermore, microscopic corroboration of this tumour dimension requires additional tissue sampling of the slices that flank the first and last slice in which the cancer is seen on naked-eye inspection. However, this more comprehensive approach can be easily included in the standard operating protocol for specimen grossing (used by either pathologists or technical staff) and microscopic assessment.

The study has several limitations. First, the analysis is based on a single-institution cohort, which may have introduced bias. However, because clinical, surgical and pathology methods and decisions were standardised according to international guidelines [6,23], the bias is likely limited. Second, for pancreatoduodenectomy specimens, grossing was based on axial slicing, not bivalving as it is practised in some pathology departments. However, the different specimen dissection techniques are expected to have little if any impact when it comes to the measurement of tumour size, because the exact orientation of the dissection plane (axial versus along the main pancreatic and common bile ducts in the case of bivalving) is of little relevance as long as measurement is done in two perpendicular planes, which is the case for both techniques. More relevant is the slice thickness, as this determines the accuracy of both size measurement across specimen slices and the identification of the specimen slice with the largest tumour expanse. Slice thickness is not often mentioned in published studies, but when stated it lies around 3-5 mm [24]. In the current study, tumour size was measured with 1.5 mm increments across specimen slices. Dissection of distal pancreatectomy specimens by serial sagittal slicing is almost universally used and it is the preferred method for international multicentre trials [25].

A third limitation is the relatively small size of the cohort (n = 315). However, as discussed above, the mean and median tumour sizes observed in the current study for two-dimensional measurement are highly comparable with data from other studies [14-21], indicating that the impact of the size of the study series is likely small. The exclusion of specimens from neoadjuvantly treated patients represents a further limitation, especially as the proportion of the latter is rapidly increasing. However, the challenges met when measuring the size of residual cancer following neoadjuvant treatment are of an entirely different nature and have not been addressed as yet, making exclusion of this patient group unavoidable [26]. Last but not least, the study did not investigate the correlation between tumour size and/or T-stage and patient survival. The reason for this omission is the fact that survival is determined by multiple factors other than tumour size and T-stage, in particular N-status and, for instance, adjuvant treatment. Indeed, the intention of the TNM-system is in the first instance to allow unambiguous and precise communication of the tumour burden between multidisciplinary user groups, a goal to which the current study findings have contributed [27].

The strengths of the study lie in its prospective design, the fact that the study series is recent and covers a short period of time (2015-2020), and the use of a detailed, fully standardised pathology examination protocol. A further strength is the extensive annotation of the study series, including macroscopic photodocumentation of all cases, which enables review not only of the histology but also of the macroscopic findings.

593

5. Conclusions

594
595
596
597
598
599
600
601
602
603
604
605
606

While tumour size measurement is generally considered accurate and reproducible, the study results show that different measurement approaches result in significantly different maximum tumour sizes and, consequently, significantly different T-stage assignment. Especially measurement in the plane perpendicular to the plane of specimen slicing, but also microscopic corroboration of the macroscopic measurements, have a significant impact on the resulting tumour size and T-stage assignment. As long as pathology practice varies, tumour size and T-stage may be neither accurate nor reproducible. This puts at risk the meaningfulness of comparing stage-related outcomes between institutions, to the detriment of patient management, clinical research and cancer registries. Even if in the not-too-far future, artificial intelligence-assisted tumour size measurement may overcome current obstacles, study cohorts with correctly measured pancreatic cancers will be invaluable for establishing a robust ground truth [28].

626

627

References

628 1. Brierley, J.D.; Gospodarowicz, M.K.; Wittekind, D. *UICC: TNM classification of malignant tumours*, 8th ed.; Wiley-Blackwell:
629   Oxford, United Kingdom, 2017; pp. 93-95.
630 2. Kakar, S.; Pawlik, T.M.; Allen, P.J.; Vauthey, J.-N. In *AJCC cancer staging manual,* 8th ed.; Amin, M.B.; Edge, S.B.; Greene, F.L.;
631   Byrd, D.R.; Brookland, R.K.; Washington, M.K.; Gershenwald, J.E.; Compton, C.C.; Hess, K.R.; Sullivan, D.C.; Jessup, J.M.;
632   Brierley, J.D.; Gaspar, L.E.; Schilsky, R.L.; Balch, C.M.; Winchester, D.P.; Asare, E.A.; Madera, M.; Gress, D.M.; Meyer, L.R.,
633   Eds.; American Joint Committee on Cancer: Chicago, USA, 2017; pp. 337-347.
634 3. Robin, L.H.; Wittekind, C.; Gospodarowicz, M.K. *UICC: TNM classification of malignant tumours*, 7th ed.; Wiley-Blackwell:
635   Oxford, United Kingdom, 2009; pp. 132-135.
636 4. Edge, S.B.; Byrd, D.R.; Compton, C.C.; Fritz, A.G.; Greene, F.L.; Trotti, A. *AJCC cancer staging handbook,* 7th ed.; Springer: New
637   York, USA, 2010; pp.285-296.
638 5. College of American Pathologists (CAP) (2021). Protocol for the examination of specimens from patients with carcinoma of the
639   pancreas. Available online: https://documents.cap.org/protocols/Panc.Exo_4.2.0.2.REL_CAPCP.pdf (accessed on 17. Feb 2022)
640 6. Verbeke, C.; Brosens, L.; Campbell, F.; Del Chiaro, M.; Esposito, I.; Feakins, R.M.; Fukushima, N.; Gill, A.; Kakar, S.; Kench, J.;
641   Krasinskas, A.M.; van Laethem, J.L.; Schaeffer, D.F.; Washington, M.K. (2020). Carinoma of the exocrine pancreas Histo-
642   pathology reporting guide. International Collaboration on Cancer Reporting; Sydney, Australia. ISBN: 978-1-922324-03-0.
643 7. Title of Site. Available online: URL (accessed on Day Month Year).

644 8. The Royal College of Pathologists (2019). Dataset for the histopathological reporting of carcinoma of the pancreas, ampulla of
645 Vater and common bileduct. 2019. Available online:
646 https://www.rcpath.org/uploads/assets/34910231-c106-4629-a2de9e9ae6f87ac1/G091-Dataset-for-histopathological-reporting-o
647 f-carcinomas-of-the-pancreas-ampulla-of-Vater-and-common-bile-duct.pdf (accessed on 17. Feb 2022).

648 9. Verbeke, C.S.; Knapp, J.; Gladhaug, I.P. Tumour growth is more dispersed in pancreatic head cancers than in rectal cancer:
649 implications for resection margin assessment. *Histopathol* **2011**, *59*, 1111-1121.

650 10. Van Hilst, J.; Korrel, M.; Lof, S.; Rooij, T.; Vissers, F.; Al-Sarireh, B.; Alseidi, A.; Bateman, A.C.; Björnsson, B.; Boggi, U.; Bratlie,
651 S.O.; Busch, O.; Butturini, G.; Casadei, R.; Dijk, F.; Dokmak, S.; Edwin, B.; van Eijck, C.; Esposito, A.; Fabre, J.-M.; Falconi, M.;
652 Ferrari, G.; Fuks, D.; Groot Koerkamp, B.; Hackert, T.; Keck, T.; Khatkov, I.; de Kleine, R.; Kokkola, A.; Kooby, D.A.; Lips, D.;
653 Luyer, M.; Marudanayagam, R.; Menon, K.; Molenaar, Q.; de Pastena, M.; Pietrabissa, A.; Rajak, R.; Rosso, E.; Sanchez Ve-
654 lazquez, P.; Saint Marc, O.; Shah, M.; Soonawalla, Z.; Tomazic, A.; Verbeke, C.; Verheij, J.; White, S.; Wilmink, H.W.; Zerbi, A.;
655 Dijkgraaf, M.G.; Besselink, M.G.; Abu Hilal, M.; European Consortium on Minimally Invasive Pancreatic Surgery (E-MIPS).
656 Minimally invasive versus open distal pancreatectomy for pancreatic ductal adenocarcinoma (DIPLOMA): study protocol for a
657 randomized controlled trial. *Trials* **2021**, *22*, 608-XX.

658 11. Nasjonalt handlingsprogram med retningslinjer for diagnostikk, behandling og oppfølging av pasienter med pancreaskreft.
659 Helsedirektoratet (2021). Available online:
660 https://www.helsedirektoratet.no/retningslinjer/pancreaskreft-bukspyttkjertelkreft-handlingsprogram (accessed on 17. Feb
661 2022).

662 12. Verbeke, C.S.; Gladhaug, I.P. Dissection of pancreatic resection specimens. *Surg Pathol Clin* **2016**, *9*, 523-538.

663 13. Campbell, F.; Verbeke, C.S. *Pathology of the pancreas: a practical approach*, 2nd ed.; Springer: Cham, Switzerland, 2021; pp. 33-59.

664 14. Van Roessel, S.; Kasumova, G.G.; Verheij, J.; Najarian, R.M.; Maggino, L.; de Pastena, M.; Malleo, G.; Marchegiani, G.; Salvia,
665 R.; Chau Ng, S.; de Geus, S.W.; Lof, S.; Giovinazzo, F.; van Dam, J.L.; Kent, T.S.; Busch, O.R.; van Eijck, C.H.; Groot Boerkamp,
666 B.; Abu Hillal, M.; Bassi, C.; Tseng, J.F.; Besselink, M.G. International validation of the eighth edition of the American Joint
667 Committee on Cancer (AJCC) TNM staging system in patients with resected pancreatic cancer. *JAMA Surg* **2018**, *154*, e183617.

668 15. Allen, P.J.; Kuk, D.; Castillo, C.F.; Basturk, O.; Wolfgang, C.L.; Cameron, J.L.; Lillemoe, K.D.; Ferrone, C.R.; Morales-Oyarvide,
669 V.; He, J.; Weiss, M.J.; Hruban, R.H.; Gönen, M.; Klimstra, D.S.; Mino-Kenudson, M. Multi-institutional validation study of the
670 American Joint Commission on Cancer (8th edition) changes for T and N staging in patients with pancreatic adenocarcinoma.
671 *Ann Surg* **2017**, *265*, 185-191.

672 16. Park, M.Y; Shin, S.H.; Song, K.B.; Hwang, D.; Lee, J.H.; Lee, Y.J.; Kim, S.C. Validation of the eighth edition of the American Joint
673 Committee on Cancer staging system and proposal of an improved staging system for pancreatic ductal adencoarcinoma. *Ann
674 Hepatobiliary Pancreat Surg* **2019**, *23*, 46-55.

675 17. Shin, D.W.; Lee, J.C.; Kim, J.; Woo, S.M.; Lee, W.J.; Han, S.S.; Park, S.-J.; Choi, K.S.; Cha, H.S.; Yoon, Y.-S.; Han, H.-S.; Hong,
676 E.K.; Hwang, J.-H. Validation of the American Joint Committee on Cancer 8th edition staging system for the pancreatic ductal
677 adenocarcinoma. *Eur J Surg Oncol* **2019**, *45*, 2159-2165.

678 18. Kamarajah, S.K.; Burns, W.R.; Frankel, T.L.; Cho, C.S.; Nathan, N. Validation of the American Joint Commission on Cancer
679 (AJCC) 8th edition staging system for patients with pancreatic adenocarcinoma: a Surveillance, Epidemiology and End Results
680 (SEER) analysis. *Ann Surg Oncol* **2017**, *24*, 2023-2030.

681 19. Kwon, W.; He, J.; Higuchi, R.; Son, D.; Lee, S.Y.; Kim, J.; Kim, H.; Kim, S.-W.; Wolfgang, C.L.; Cameron, J.L.; Yamamoto, M.;
682 Jang, J.-Y. Multinational validation of the American Joint Committee on Cancer 8th edition pancreatic cancer staging system in
683 a pancreas head cancer cohort. *J Hepatobiliary Pancreat Sci* **2018**, *25*, 481-427.

684 20. Liu, C.; Cheng, H.; Jin, K.; Guo, M.; Lu, Y.; Wang, Z.; Yang, C.; Long, J.; Ni, Q.; Yu, X.; Luo, G. Application of the eighth edition
685 of the American Joint Committee on Cancer staging for pancreatic adenocarcinoma. *Pancreas* **2018**, *47*, 742-747.

686 21. Saka, B.; Balci, S.; Basturk, O.; Bagci, P.; Postlewait, L.M.; Maithel, S.; Knight, J.; El-Rayes, B.; Kooby, D.; Sarmiento, J.; Muraki,
687 T.; Oliva, I.; Bandyopadhyay, S.; Akkas, G.; Goodman, M.; Reid, M.D.; Krasinskas, A.; Everett, R.; Adsay, V. Pancreatic ductal
688 adenocarcinoma is spread to the peripancreatic soft tissue in the majority of resected cases, rendering the AJCC T-stage pro-
689 tocol (7th edition) inapplicable and insignificant: a size-based staging system (pT1: ≤2, pT2: >2-≤4, pT3:>4 cm) is more valid and
690 clinically relevant. *Ann Surg Oncol* **2016**, *23*, 2010-2018.

691 22. Schlitter, A.M.; Jesinghaus, M.; Jäger, C.; Konukiewitz, B.; Muckenhuber, A.; Demir, I.E.; Bahra, M.; Denkert, C.; Friess, H.;
692 Kloeppel, G.; Ceyhan, G.O.; Weichert, W. pT but not pN stage of the 8th TNM classification significantly improves prognosti-
693 cation in pancreatic ductal adenocarcinom. *Eur J Cancer* **2017**, *84*, 121-129.

694 23. NCCN Guidelines version 2.2021. Pancreatic Adenocarcinoma. Available online:
695 https://www.nccn.org/professionals/physician_gls/pdf/ pancreatic_blocks.pdf (accessed on 17. Feb 2022).

696 24. Chandrasegaram, M.D.; Goldstein, D.; Simes, J.; Gebski, V.; Kench, J.G.; Gill, A.J.; Samra, J.S.; Merrett, N.D.; Richardson, A.J.;
697 Barbour, A.P. Meta-analysis of radical resection rates and margin assessment in pancreatic cancer. *Br J Surg* **2015**, *102*,
698 1459-1472.

699 25. Lof, S.; Rajak, R.; Vissers, F.; Korrel, M.; Bateman, A., Verheij, J.; Verbeke, C.; Cataldo, I.; Besselink, M.G.; Abu Hilal, M. DI-
700 PLOMA approach for standardised pathology assessment of distal pancreatectomy specimens. *J Vis Exp* **2020**, *156*.

701 26. Verbeke, C.; Häberle, L.; Lenggenhager, D.; Esposito, I. Pathology assessment of pancreatic cancer following neoadjuvant
702 treatment: Time to move on. *Pancreatol* **2018**, *18*, 467-476.

27. O´Sullivan, B.; Brierley, J.; Byrd, D.; Bosman, F.; Kehoe, S.; Kossary, C.; Pineros, M.; Van Eycken, E.; Weir, H.K.; Gospo-darowicz, M. The TNM classification of malignant tumours - towards common understanding and reasonable expectations. *Lancet Oncol* **2017**, *18*, 849-851.
28. Kriegsmann, M.; Kriegsmann, K.; Steinbuss, G.; Zgorzelski, C.; Kraft, A.; Gaida, M.M. Deep learning in pancreatic tissue: identification of anatomical structures, pancreatic intraepithelial neoplasia, and ductal adenocarcinoma. *Int J Mol Sci* **2021**, *22*, 5385.

756 **Supplementary material**

757

758 **Supplementary Table S1.** Change in T-stage assignment between approaches to tumour size measurement.

	2D-macro vs 2D micro		2D-macro vs 3D-macro		2D-macro vs 2D-3D-micro	
	Head	**Body/tail**	**Head**	**Body/tail**	**Head**	**Body/tail**
T1	**(n =26)***	**(n = 12)***			**(n =26)***	
No changes	8 (31)	11 (92)	12 (46)	4 (33)	1 (4)	4 (33)
T1 → T2	18 (69)	1 (8)	13 (50)	7 (58)	19 (73)	8 (67)
T1 → T3	0 (0)	0 (0)	1 (4)	1 (9)	6 (23)	0 (0)
T2	**(n = 176)***	**(n = 55)***				
No changes	162 (92)	48 (87)	137 (78)	22 (40)	119 (68)	25 (45)
T1 → T2	12 (7)	12 (7)	22 (12)	30 (55)	55 (31)	29 (53)
T1 → T3	2 (1)	5 (9)	1 (4)	3 (5)	2 (1)	1 (2)
T3	**(n = 29)***	**(n = 17)***				
No changes	16 (55)	13 (76)	12 (41)	14 (82)	9 (31)	15 (88)
T1 → T2	13 (45)	3 (18)	15 (52)	3 (18)	18 (62)	2 (12)
T1 → T3	0 (0)	1 (6)	2 (7)	0 (0)	2 (7)	0 (0)

759 *Based on 2D-macro assessment.

760
761

762 **Supplementary Table 2.** T-stage distribution in pancreatic cancer series.

Study	% T1	% T2	% T3
Current			
• 2D-macro	12	73	15
• 2D-3D-macro	3	61	36
Saka 2016 [21]			
• Institutional series	13	64	23
• SEER series	17	55	28
• "Ordinary cases"	20	56	24
Shin 2019 [17]	13.1	66.5	20.5
Kwon 2018 [19]	19.6	63.8	16.6
Park 2019 [16]	14.5	65.5	20.0
Schlitter 2017 [22]			
• Berlin series	12.0	69.3	18.7
• Munich series	13.5	65.3	21.2
Kamarajah 2017 [18]	17	58	25

763