의학석사 학위논문

종합적 *KRAS* 변이 부담, 솔질세포검사, p53 면역세포검사를 이용한 췌장암환자의 휘플씨 수술검체의 방사상 절제연 평가의 유효성

Effectiveness of radial resection margin assessment of pancreatic ductal adenocarcinoma of Whipple operation specimens by combining *KRAS* mutational burden, brushing cytology, and p53 immunocytochemistry

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신 재 훈

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이 논문을 의학석사 학위 논문으로 제출함

2022 년　　2 월

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국문요약

연구 배경

췌장선암 환자의 췌십이지장절제 검체의 방사상 절제연 평가는 국소적 재발 예측과 수술 후 보조요법 결정에 중요하다. 최근 선행 항암화학요법이 국소 진행형 췌장암을 경계성 절제가능한 혹은 절제가능한 췌장암으로 만들어 주어서, 점점 더 적용되는 추세이다. 그러나 선행 항암화학요법을 받은 환자들에서 병리 절제연이 예후에 미치는 영향은 잘 알려져 있지 않다. 본 연구는 선행 항암화학요법을 받은 환자들에서의 병리 절제연이 예후에 미치는 영향을 평가하고, 좀 더 나은 방사상 절제연 평가를 위한 방법을 개발하고자 한다.

연구 방법

본 연구는 전향적 연구로서, 췌십이지장절제술을 받은 126 명의 췌장암 환자의 수술 검체의 방사상 절제연에서 병리 절제연을 평가하였고, 면봉표본채취법으로 절제연의 세포를 수집하여 췌장암에서 높은 빈도로 관찰되는 *KRAS* 유전자의 돌연변이를 민감도가 높은 droplet digital PCR (ddPCR)을 이용하여 *KRAS* 변이부담(mutational burden)을 측정하고 이를 이용한 분자 절제연을 평가하였다. 또한, 세포도말, 군집절편 (cell block), p53 면역세포검사를 종합적으로 평가한 세포 절제연 (cytologic resection margin)을 평가하였고, 병리, 분자, 세포 절제연이 무재발 생존율에 미치는 영향을 비교, 분석하였다.

결과

총 126 명의 환자가 포함기준을 만족하였다. 55 명의 환자는 선행 항암화학요법을 받았고, 71 명의 환자는 수술 전 어떠한 치료도 받지 않았다. 중앙추적기간은 10 개월이었다. 병리 절제연 양성은 15 증례 (11.9%)에서 관찰되었다. 90 증례 (71.4%)에서는 적어도 한 개 이상의 *KRAS* 돌연변이가 관찰되었고, 분자 절제연 양성은 32 증례 (25.4%)에서 관찰되었다. 세포 절제연 양성은 36 증례 (28.6%)에서 관찰되었다.

선행항암화학요법을 받은 그룹에서 병리 절제연의 상태에 따른 무재발 생존기간의 통계학적 차이는 관찰되지 않았다. 반면, 분자 절제연 양성 환자군의 무재발 생존기간 (중앙값, 6 개월)은 분자절제연 음성 환자군 (17 개월)보다 통계학적으로 유의하게 짧았다. 또한, 세포 절제연 양성 환자군 (7 개월)도 세포 절제연 음성 환자군 (17 개월)보다 통계적으로 유의하게 짧은 무재발 생존율을 보였다.

결론

병리학적 방사상 절제연 평가는 선행 항암화학요법을 받은 환자들에서는 무재발 생존율과 관련성을 보이지 않았다. 반면 분자, 세포학적 방사상 절제연 평가는 선행 항암화학요법을 받은 췌장선암 환자들의 무재발 생존율에 대한 유용한 정보를 제공한다.

차 례

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with poor prognosis[1]. Surgical resection with adjuvant chemotherapy has been the standard treatment for patients with localized PDAC[6]. However, at the time of diagnosis, at most 20% of patients are considered candidates for surgical resection because most patients have advanced and unresectable disease[7]. Worse prognostic factors for recurrence and survival after resection of PDAC included PDACs with large size, metastasis of lymph node[9], poor differentiation (high tumor grade)[10], involvement of resection margins[11,12], lymphovascular invasion[13], perineural invasion[14], peripancreatic fat invasion[15], and involvement of large vessels, such as superior mesenteric or portal veins[16].

Of which, resection margin status is a very important prognostic factor. When PDAC is located on pancreatic head, Whipple operation or pylorus-preserving pancreaticoduodenectomy (PPPD) is performed. Whipple or PPPD specimens have several margins, such as pancreatic neck, bile duct, duodenal, jejunal, and radial or uncinate margins. Radial or uncinate margin is the most important margin because the radial margin is the most common site of local recurrence after surgery[17]. In addition, involvement of the radial margin is closely related with decreased survival[17]. The radial margin is composed of the superior mesenteric vein (SMV) groove and the superior mesenteric artery (SMA) margin[18]. The true resection margin is the SMA margin, and the SMV groove is false margin which is just the concavity where the SMV comes in

contact with retroperitoneal surface of the pancreas[19]. These two margins are the most common sites to be R1 resection [18]. R1 resection is when cancer cells directly involve or are located within 1mm from the radial margin[20]. In addition to anatomical difficulty to approach[21], another reason of frequent involvement of the radial margin is that the radial margin is hard to evaluate during operation because the margin area is wide. Meanwhile, pancreatic neck and bile duct margin can be checked during surgical resection with frozen section evaluation [22]. At present, pathologic evaluation of the radial margin is done by submission of a few representative sections where the tumor is the closest to the radial margin during gross examination[23]. However, there is a possibility of underestimating the radial margin status. Although entire submission of the whole radial margin can be a solution to overcome the problem of underestimation[22], it is time consuming and labor-intensive.

In recent years, neoadjuvant chemotherapy for locally advanced PDAC has been increasingly applied due to its ability to lower disease stage and transform locally advanced PDAC into borderline resectable or resectable PDAC[8]. However, the impact of pathologic margin status for patients who got neoadjuvant treatment remains uncertain. Several studies have reported that margin status had prognostic impact on survival outcomes in neoadjuvant setting[24-26]. However, other studies showed that margin status did not hold the prognostic significance in neoadjuvant setting[27-29]. These controversies made us validate prognostic impact of pathologic margin status

in neoadjuvant treatment setting, and investigate better method to evaluate radial margin status.

The *KRAS* gene encodes the protein KRAS, which acts as a molecular switch for various cellular processes[30]. PDAC is the most frequently *KRAS* mutating cancers with almost 100% *KRAS* mutation frequency[31]. In the majority of PDAC cases, an activating point mutation occurs on codons 11, 12, 13, 61 or 146[30]. The *KRAS* mutation is unlikely to be found in noncancerous peripancreatic soft tissue including the radial margin. In the previous study by Kim et al.[32], *KRAS* mutational status was assessed in the radial margin as complementary method to pathologic evaluation. In addition to *KRAS* mutation, inactivating mutations in tumor suppressor genes such as *TP53*, *CDKN2A*/p16, and *SMAD4* cooperate with *KRAS* mutation to cause aggressive PDAC tumor growth[33].

The aim of this study is to evaluate and compare the impact of pathologic margin status on survival outcome in neoadjuvant setting and treatment naïve setting. Also, in an effort to investigate better method to evaluate radial margin, we evaluated and compared pathologic margin status, *KRAS* mutational status, cytomorphologic feature, and p53 immunocytochemical staining pattern in the radial margin.

MATERIALS AND METHODS

Study population

We included 154 surgically resected PDAC patients, regardless of neoadjuvant treatment status, who gave consent to our study from November, 2019 to February, 2021 at Asan Medical Center. Approval from the institutional review board (approval number: 2019-1683) was obtained.

Pathologic margin evaluation

The radial margin was inked black and cut parallel to the plane of the radial margin in about 5 to 10 mm in thickness[34]. One or two representative sections, where the tumor was the closest to the radial margin grossly, was submitted as a perpendicular margin. Two pathologists (JH and SM) independently evaluated the radial margin status, and agreement was reached in all cases. The positive pathologic margin was defined when tumor cells directly infiltrated or were present within 1mm from the radial margin.

Sample collection

After identification of radial margin through external examination, we collected sample only in true radial margin, which was SMA margin, not SMV groove. The Whatman FTA swab (GE Healthcare Life Sciences, Buckinghamshire, United Kingdom) was used to rub the radial margin (**Figure 1**). Then, the swabbed cells were collected in a 5 ml tube for *KRAS* mutation analysis and a 15ml tube for cytologic evaluation, respectively.

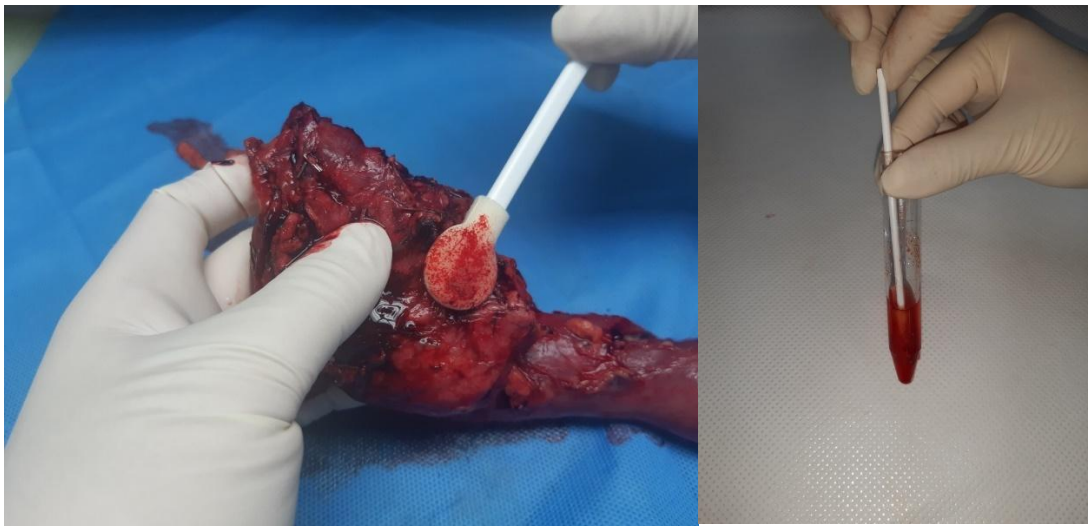

Figure 1. Swabbing and collection of cells on the radial margin. The radial margin from Whipple or PPPD specimen is rubbed with a swab. The swabbed cells are collected in a 5ml tube with 70% ethanol.

Evaluation of *KRAS* mutation

The swabbed cells from the radial margin were collected with 70% ethanol and washed 3 times with 1X PBS and harvested by centrifugation at 13,000 revolutions/min for five minutes. Genomic DNA was extracted using the QIAamp DNA Micro kit (Qiagen, Hilden, Germany) by the company's protocol after overnight incubation with proteinase K. With the Nanodrop2000 (Thermo Scientific, Wilmington, Del), DNA quality was evaluated by measuring the 260/280 absorbance ratio. Droplet digital PCR (ddPCR) was used to detect seven hotspot *KRAS* mutations at codons 12 and 13 (G12A, G12C, G12D, G12R, G12S, G12V, and G13D). 20X *KRAS* probes fluorescently labeled with FAM (mutant; Bio-Rad, Hercules, California) or HEX (wild-type; Bio-Rad), and 2X

ddPCR Supermix buffer were loaded into the Droplet Generator (Bio-Rad) with DG oil to create DNA droplet, which is 5ng DNA. The generated DNA droplets were incubated at 95°C for 10 minutes, and the *KRAS* target regions were amplified at 94°C for 30 seconds and 55°C for 1 minute for 40 cycles. The temperature ramp increment was 2.5°C/s in all PCR steps by using the C-1000 thermal cycler (Bio-Rad). The fluorescence intensity of the probes in droplets was assessed to determine whether wild-type or mutant *KRAS* with the QX100 Droplet Reader (Bio-Rad). The ddPCR data was processed by the QuantaSoft v.1.6.6 (Bio-Rad). To minimize the rate of false positivity of wild-type or mutant *KRAS*, we used 3 no-template controls for seven hotspot *KRAS* mutations. Independently, the *KRAS* mutation rates of three wild-type *KRAS* controls (normal pancreas tissue) were calculated. The mutation threshold was defined as the "mean + 3 SDs"[32,35]. Finally, each mutation rate of 7 hotspot *KRAS* mutations were summed.

Total cytology score: smear, cell block and p53 immunocytochemistry

The swabbed cells from the radial margin were washed and collected with preservative solution (YD diagnostics, Yongin, Korea). After centrifugation at 2,000 revolutions/min for 5 minutes, the collected cells were applied directly onto the slides and stained based on the fixation method by Papanicolaou (Pap) method. The swabbed cells on Pap smear were cytomorphologically scored as follows: negative, 0; atypical, 1; positive, 2 (**Figure 2**). After making a smear slide, the remaining cells were fixed with 95% alcohol for 5 minutes and

centrifuged at 2,000 revolutions/min for 5 minutes again. The sediments were processed into a paraffin block and stained by Hematoxylin and Eosin (H&E). The cell block was scored as follows: negative, 0; atypical, 1; positive, 2 (**Figure 3**). P53 immunocytochemistry was applied to the paraffin section, and scored as follows: normal, 0; abnormal, 2 (**Figure 4**). Lastly, total cytology score was calculated by the sum of the above three components, which ranged from zero to six. Two pathologists (JH and SM) independently evaluated total cytology score, and the Cohen's kappa coefficient (κ) was 0.861.

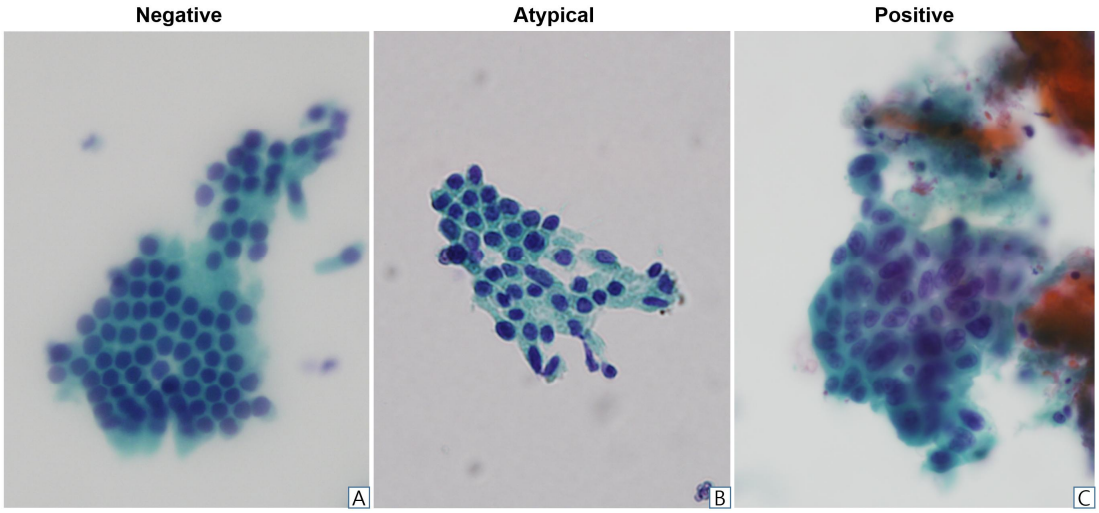

Figure 2. Representative images of cytology on Pap smear of radial margin. (A) Negative is scored zero point when atypical or malignant cells are not present at all. (B) Atypical is scored one point when nucleus is hyperchromatic, and shows mild atypia short of positive. Nuclear size variation is less than 4:1, and not many atypical cell clusters are observed. (C) Positive is scored two points when nucleus is hyperchromatic and shows overt pleomorphism. Nuclear size variation is greater than 4:1.

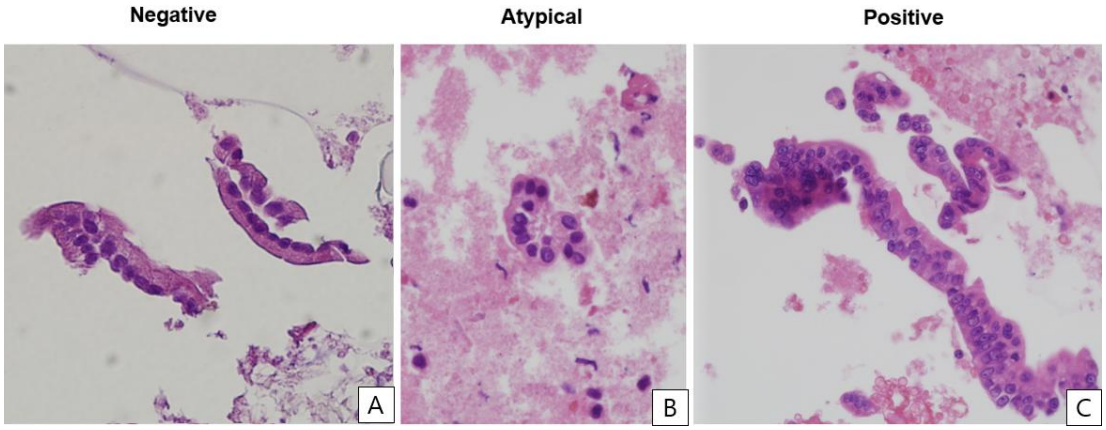

Figure 3. Representative images of cell block on H&E of radial margin. Each criterion is the same as Figure 2.

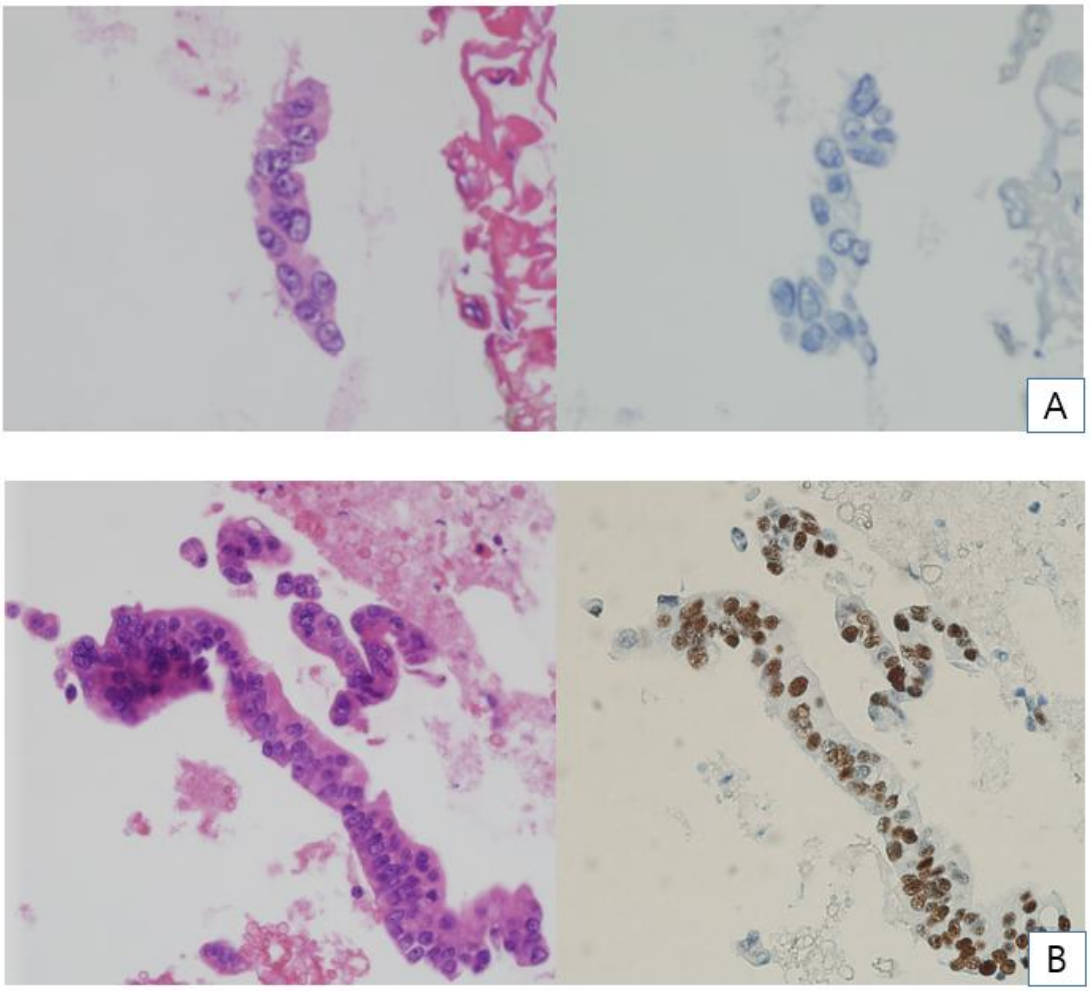

Figure 4. Representative images of p53 immunocytochemistry of cell block. (A) Total loss or (B) diffusely strong p53 expression patterns are regarded as abnormal p53 expression. Abnormal pattern is scored two points and normal pattern is scored zero point.

Determining cutoff point for *KRAS* mutation and total cytology score

To set the cutoff for discriminating the recurrence free survival (RFS) the best, the survival data of patients were divided and compared according to the summed *KRAS* mutation rate and total cytology score. Classification and regression tree (CART) model was applied[36].

Statistical analysis

The radial margin status was compared with other clinicopathologic factors, such as patient age, sex, tumor size, lymphovascular invasion (LVI), perineural invasion (PNI), differentiation, T category, lymph node metastasis. SPSS Statistics 27.0 (IBM, Armonk, NY) was used. Associations between categorical variables were analyzed by the Pearson χ^2 test. RFS rate was calculated by the Kaplan-Meier method, and statistical significance was evaluated by the log-rank test and the Cox proportional hazards regression model. P value less than 0.05 was considered statistically significant.

RESULTS

Clinicopathologic characteristics of patients

Out of 154 surgically resected PDAC patients, 126 patients underwent Whipple or PPPD; 24 patients underwent distal pancreatectomy, and 4 patients underwent total or subtotal pancreatectomy (Figure 5). Out of 126 patients who got Whipple or PPPD operation, 55 patients underwent neoadjuvant chemotherapy, and 71 patients did not get any treatment before surgery. The clinicopathologic characteristics of the patients in this study are summarized in Table 1. The mean age of the patients was 65.5 ± 9.6 years. Among 126 patients, 55 were female and 71 were male. The mean tumor size was 2.7 ± 1.2 cm. LVI was present in 69 cases (54.8%), and PNI was present in 97 cases (77.0%). Most cases were designated as moderately differentiated (94 cases, 74.6%) followed by well differentiated (18 cases, 14.3%) and poorly differentiated (14 cases, 11.1%). Most cases were T2 (77 cases, 61.1%) followed by T1 (38 cases, 30.2%) and T3 (11 cases, 8.7%) stage according to the eighth edition of the American Joint Committee on Cancer TNM staging system[37]. Regarding conventional pathologic radial margin status, 15 (11.9%) cases were R1 and 111 (88.1%) cases were R0.

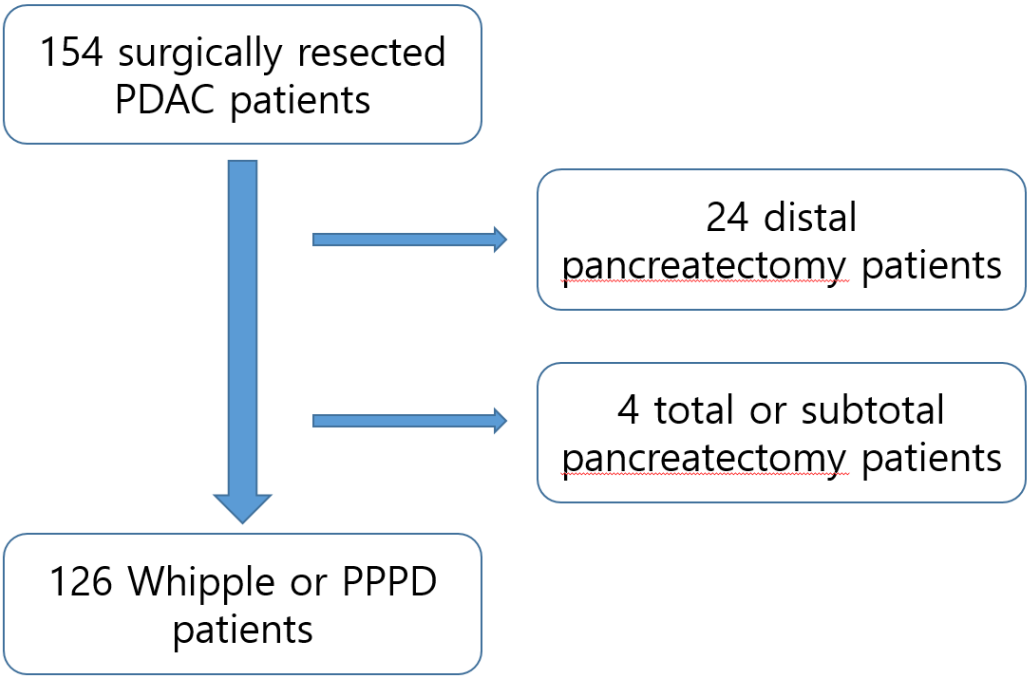

Figure 5. Study population included in our study. One hundred twenty-six patients who underwent Whipple or PPPD were included.

Table 1. Clinicopathologic characteristics

Characteristics	Variable	No. (%)
Age, years	≤60	35 (27.8)
	>60	91 (72.2)
Sex	Male	71 (56.3)
	Female	55 (43.7)
Size, cm	≤2	38 (30.2)
	>2	88 (69.8)
Lymphovascular invasion	Absent	57 (45.2)
	Present	69 (54.8)
Perineural invasion	Absent	29 (23.0)
	Present	97 (77.0)
Differentiation	Well	18 (14.3)
	Moderate	94 (74.6)

	Poor	14 (11.1)
Lymph node metastasis	Absent	65 (51.6)
	Present	61 (48.4)
T category	T1	38 (30.2)
	T2	77 (61.1)
	T3	11 (8.7)
	T4	0 (0)
Pathologic radial margin	Negative	111 (88.1)
	Positive	15 (11.9)

KRAS mutational status in the radial margin

The representative *KRAS* mutation results by ddPCR are illustrated in **Figure 6**. The frequency of seven types of *KRAS* mutations were as follows: 32.5% (41 cases) for G12D, 31.0% (39 cases) for G12V, 17.5% (22 cases) for G12S, 11.9% (15 cases) for G12R, 10.3% (13 cases) for G13D, 9.5% (12 cases) for G12C, and 1.6% (2 cases) for G12A. The G12D and G12V mutations were the two most common *KRAS* mutations. One case harbored four types of *KRAS* mutations, and eight cases harbored three types of *KRAS* mutations. Thirty-five cases showed two types of *KRAS* mutations; 46 cases showed single type of *KRAS* mutation, and 36 cases showed no *KRAS* mutation. The *KRAS* mutation rates (mean ± standard deviation [SD]) from highest to lowest were as follows: G12V (1.26 [2.10]), G12C (0.91 [2.39]), G12R (0.87 [2.16]), G12D (0.72 [1.40]), G12A (0.16 [0.15]), G12S (0.13 [0.22]), and G13D (0.09 [0.10]). To determine overall *KRAS* mutational status which represent so called "molecular margin", we summed the seven types of *KRAS* mutation rates. The

mean value of the summed *KRAS* mutation rate of all cases was 0.73 ± 2.34 and ranged from 0 to 19.15%. To set the cutoff, patients' RFS times were plotted against the summed *KRAS* mutation rates. By CART model, the cutoff point of 0.365% was determined. When the summed *KRAS* mutation rate was greater or equal to 0.365%, we defined this as positive molecular margin. When the summed *KRAS* mutation rate was less than 0.365%, we defined this as negative molecular margin. Thirty-two (25.4%) cases were molecular margin positive, and 94 (74.6%) cases were molecular margin negative.

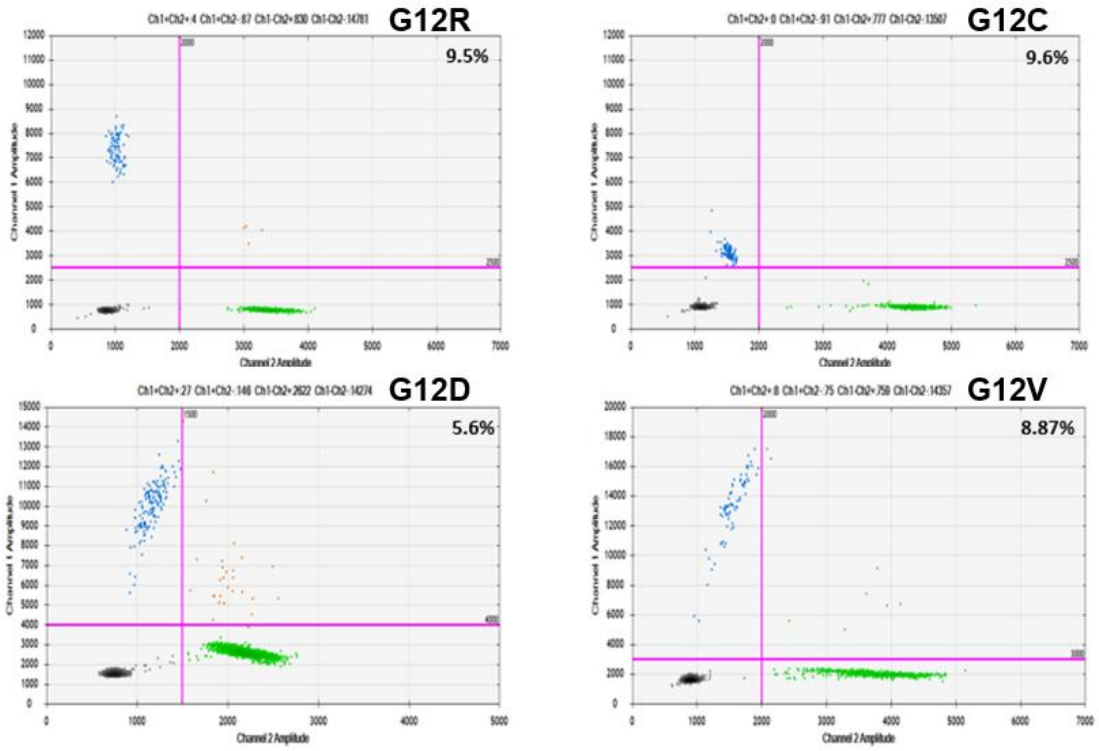

Figure 6. Representative images of *KRAS* mutations by ddPCR. Green dots on X axis represent wild-type *KRAS* probes fluorescently labeled with HEX. Blue dots on Y axis show mutant *KRAS* probes fluorescently labeled with FAM. Orange dots represent *KRAS* probes simultaneously labeled with HEX and FAM.

The sum of the fluorescent intensity of blue and orange dots correlates with the *KRAS* mutation rate.

Smear, cell block and p53 immunocytochemistry in the radial margin

On smear, 96 (76.2%) cases were negative; 20 (15.9%) cases were atypical, and 10 (7.9%) cases were positive. On cell block, 111 (88.1%) cases were negative; 8 (6.3%) cases were atypical, and 7 (5.6%) cases were positive. On p53 immunocytochemical staining, 120 (95.2%) cases showed normal pattern, and 6 (4.8%) cases showed abnormal pattern which was total loss or diffusely strong expression. The distribution of total cytology score, the sum of the above three components, was as follows: 71.4% (90 cases) for score zero, 13.5% (17 cases) for score one, 9.5% (12 cases) for score two, 0.8% (1 case) for score three, 2.4% (3 cases) for score four, and 2.4% (3 cases) for score six. By CART model, the cutoff of score 0.5 was calculated. Because total cytology score can only exist as whole number, we determined the cutoff point as score one. When the total cytology score was greater or equal to one, we called this as positive cytologic margin. When the total cytology score was zero, we called this as negative cytologic margin. Thirty-six (28.6%) cases were cytologic margin positive, and 90 (71.4%) cases were cytologic margin negative.

Association with pathologic resection margin and other clinicopathologic factors

Associations between pathologic margin and other clinicopathologic factors

are summarized in Table 2. Positive pathologic margin was associated with poor differentiation (P = 0.02).

Table 2. Association with pathologic resection margin and other clinicopathologic factors

		Pathologic Margin, No. (%)		
Characteristics		Negative	Positive	P
Age, years	≤60	31 (88.6)	4 (11.4)	1.00
	>60	80 (87.9)	11 (12.1)	
Sex	Male	63 (88.7%)	8 (11.3%)	0.80
	Female	48 (87.3)	7 (12.7)	
Size, cm	≤2	34 (89.5)	4 (10.5)	1.00
	>2	77 (87.5)	11 (12.5)	
LVI	Absent	50 (87.7)	7 (12.3)	0.91
	Present	61 (88.4)	8 (11.6)	
PNI	Absent	28 (96.6)	1 (3.4)	0.19
	Present	83 (85.6)	14 (14.4)	
Differentiation	Well	16 (88.9)	2 (11.1)	0.02*
	Moderate	86 (91.5)	8 (8.5)	
	Poor	9 (64.3)	5 (35.7)	
Lymph node metastasis	Absent	58 (89.2)	7 (10.8)	0.69
	Present	53 (86.9)	8 (13.1)	

* indicates $P < 0.05$.

Association with molecular resection margin and other clinicopathologic factors

Associations between molecular margin and other clinicopathologic factors are summarized in Table 3. Positive molecular margin was associated with positive cytologic margin ($P < 0.01$). Positive molecular margin showed tendency for

positive pathologic margin, but did not show statistical significance ($P = 0.06$).

Table 3. Association with molecular resection margin and other clinicopathologic factors

		Molecular Margin, No. (%)		
Characteristics		Negative	Positive	*P*
Age, years	≤60	29 (82.9)	6 (17.1)	0.19
	>60	65 (71.4)	26 (28.6)	
Sex	Male	54 (76.1)	17 (23.9)	0.67
	Female	40 (72.7)	15 (27.3)	
Size, cm	≤2	29 (76.3)	9 (23.7)	0.77
	>2	65 (73.9)	23 (26.1)	
LVI	Absent	45 (78.9)	12 (21.1)	0.31
	Present	49 (71.0)	20 (29.0)	
PNI	Absent	23 (79.3)	6 (20.7)	0.51
	Present	71 (73.2)	26 (26.8)	
Differentiation	Well	13 (72.2)	5 (27.8)	0.84
	Moderate	71 (75.5)	23 (24.5)	
	Poor	10 (71.4)	4 (28.6)	
Lymph node metastasis	Absent	51 (78.5)	14 (21.5)	0.30
	Present	43 (70.5)	18 (29.5)	
Pathologic margin	Negative	86 (77.5)	25 (22.5)	0.06
	Positive	8 (53.3)	7 (46.7)	
Cytologic margin	Negative	77 (85.6)	13 (14.4)	<0.01*
	Positive	17 (47.2)	19 (52.8)	

* indicates $P < 0.05$.

Association with cytologic resection margin and other clinicopathologic factors

Associations between cytologic margin and other clinicopathologic factors are summarized in Table 4. Positive cytologic margin was associated with positive pathologic margin ($P < 0.01$) and molecular margin ($P < 0.01$).

Table 4. Association with cytologic resection margin and other clinicopathologic factors

		Cytologic Margin, No. (%)		
Characteristics		Negative	Positive	*P*
Age, years	≤60	24 (68.6)	11 (31.4)	0.66
	>60	66 (72.5)	25 (27.5)	
Sex	Male	52 (73.2)	19 (26.8)	0.61
	Female	38 (69.1)	17 (30.9)	
Size, cm	≤2	26 (68.4)	12 (31.6)	0.62
	>2	64 (72.7)	24 (27.3)	
LVI	Absent	41 (71.9)	16 (28.1)	0.91
	Present	49 (71.0)	20 (29.0)	
PNI	Absent	22 (75.9)	7 (24.1)	0.55
	Present	68 (70.1)	29 (29.9)	
Differentiation	Well	13 (72.2)	5 (27.8)	0.17
	Moderate	70 (74.5)	24 (25.5)	
	Poor	7 (50.0)	7 (50.0)	
Lymph node metastasis	Absent	50 (76.9)	15 (23.1)	0.16
	Present	40 (65.6)	21 (34.4)	
Pathologic margin	Negative	85 (76.6)	26 (23.4)	<0.01*
	Positive	5 (33.3)	10 (66.7)	

* indicates $P < 0.05$.

RFS based on pathologic, molecular, and cytologic radial margin status

The median RFS time in patients with negative pathologic margin was 15 months, and the median RFS time in patients with positive pathologic margin was 6 months (Figure 7A, hazard ratio (HR) = 2.40, 95% confidence interval (CI) = 1.28–4.49; $P = 0.006$). The median RFS time in patients with negative molecular margin was 16 months, and the median RFS time in patients with

positive molecular margin was 6 months (**Figure 7B**, HR = 2.96, 95% CI = 1.79-4.92; $P < 0.001$). The median RFS time in patients with negative cytologic margin was 16 months, and the median RFS time in patients with positive cytologic margin was 6 months (**Figure 7C**, HR = 2.62, 95% CI = 1.55-4.41; $P < 0.001$). The all three radial margin status were statistically associated with shorter RFS. However, molecular and cytologic radial margin status were slightly better predictive for RFS than pathologic radial margin. Median follow up period was 10 months (range 1 to 21 months).

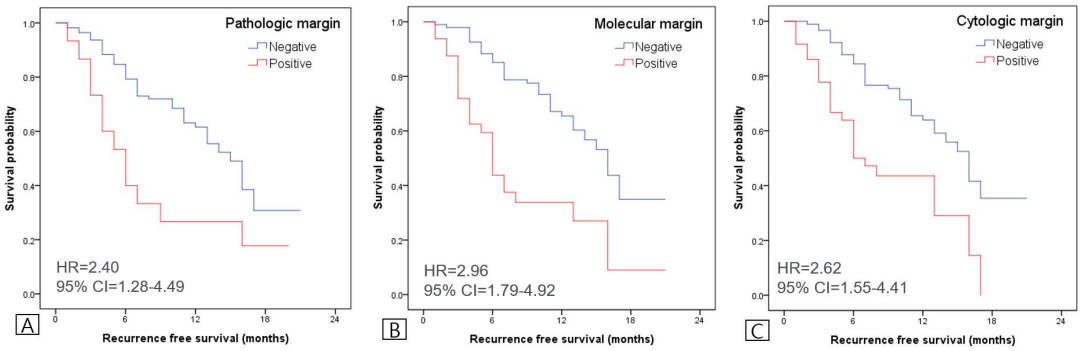

Figure 7. Kaplan-Meier RFS analyses based on pathologic, molecular, and cytologic radial margin status. (A) Patients with positive pathologic margin (median, 6 months) had significantly shorter RFS than those with negative pathologic margin (15 months). (B) Patients with positive molecular margin (6 months) had significantly shorter RFS than those with negative molecular margin (16 months). (C) Patients with positive cytologic margin (6 months) had significantly shorter RFS than those with negative cytologic margin (16 months).

Univariate and multivariate analyses for RFS

In addition to pathologic, molecular, and cytologic radial margin status, tumor size greater than 2 cm (P = 0.02), LVI (P = 0.03), PNI (P = 0.03), tumor differentiation (P < 0.01), and lymph node metastasis (P < 0.01) were associated with shorter RFS in univariate analysis (Table 5). However, age (P = 0.82) and sex (P = 0.24) did not show statistical significance. By multivariate analysis, tumor differentiation (P < 0.01), lymph node metastasis (P = 0.03), molecular margin (P < 0.01), and cytologic margin (P = 0.05) remained independent worse prognostic factors (Table 5). Pathologic radial margin showed tendency for shorter RFS, but did not show statistical significance (P = 0.13).

Table 5. Univariate and multivariate analyses for RFS

Characteristics		Univariate Analysis		Multivariate Analysis	
		Median Survival, mo	P	HR (95% CI)	P
Age, years	≤60	13	0.82	-	-
	>60	14			
Sex	Male	13	0.24	-	-
	Female	16			
Size, cm	≤2	17	0.02*		0.11
	>2	13			
LVI	Absent	16	0.03*		0.49
	Present	12			
PNI	Absent	17	0.03*		0.36
	Present	13			
Differentiation	Well	17	<0.01*	1	<0.01*
	Moderate	14		2.35 (1.06-5.19)	

	Poor	4		5.64 (2.08-15.34)	
Lymph node metastasis	Absent	16	<0.01*	1	0.03*
	Present	11		1.74 (1.06-2.84)	
Pathologic margin	Negative	15	<0.01*		0.13
	Positive	6			
Molecular margin	Negative	16	<0.01*	1	<0.01*
	Positive	6		2.90 (1.60-5.26)	
Cytologic margin	Negative	16	<0.01*	1	0.05*
	Positive	6		1.83 (0.99-3.37)	

* indicates $P < 0.05$.

RFS based on pathologic, molecular, and cytologic radial margin status in neoadjuvant chemotherapy subgroup

The median RFS time in patients with negative pathologic margin was 16 months, and the median RFS time in patients with positive pathologic margin was 9 months (Figure 8A, HR = 1.53, 95% CI = 0.53-4.43; P = 0.434). Pathologic radial margin positivity showed no statistical significance. However, molecular radial margin showed statistical significance (Figure 8B, median survival: negative, 17 months; positive, 6 months; HR = 3.66, 95% CI = 1.54-8.73; P = 0.003). In addition, cytologic radial margin showed statistical significance (Figure 8C, negative, 17 months; positive, 7 months; HR = 2.58, 95% CI = 1.15-5.79; P = 0.022).

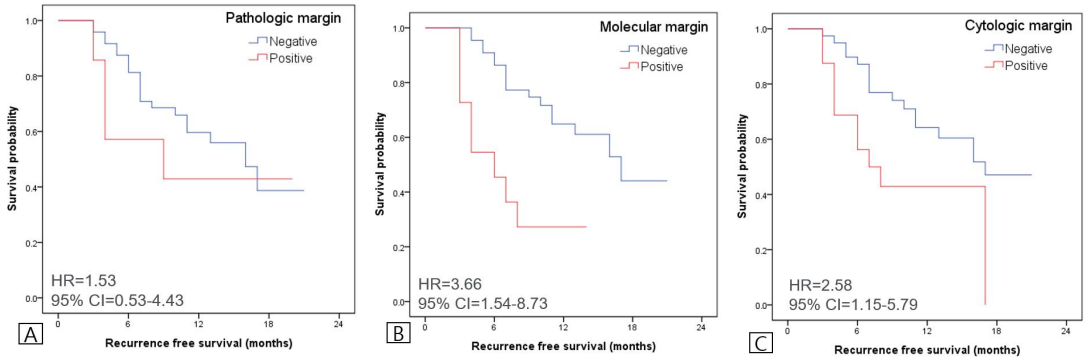

Figure 8. Kaplan-Meier RFS based on pathologic, molecular, and cytologic radial margin status in neoadjuvant chemotherapy subgroup. (A) Patients with positive pathologic margin (median, 9 months) did not show statistically significant difference from those with negative pathologic margin (16 months). (B) Patients with positive molecular margin (6 months) had significantly shorter RFS than those with negative molecular margin (17 months). (C) Patients with positive cytologic margin (7 months) had significantly shorter RFS than those with negative cytologic margin (17 months).

RFS based on pathologic, molecular, and cytologic radial margin status in treatment naïve subgroup

The median RFS time in patients with negative pathologic margin was 14 months, and the median RFS time in patients with positive pathologic margin was 5 months (**Figure 9A**, HR = 3.58, 95% CI = 1.63-7.85; P = 0.001). The median RFS time in patients with negative molecular margin was 15 months, and the median RFS time in patients with positive molecular margin was 6 months (**Figure 9B**, HR = 2.44, 95% CI = 1.30-4.58; P = 0.006). The median RFS time in patients with negative cytologic margin was 15 months, and the

median RFS time in patients with positive cytologic margin was 6 months (**Figure 9C**, HR = 2.64, 95% CI = 1.32-5.27; P = 0.006). The all three radial margin positive status were statistically associated with poor RFS.

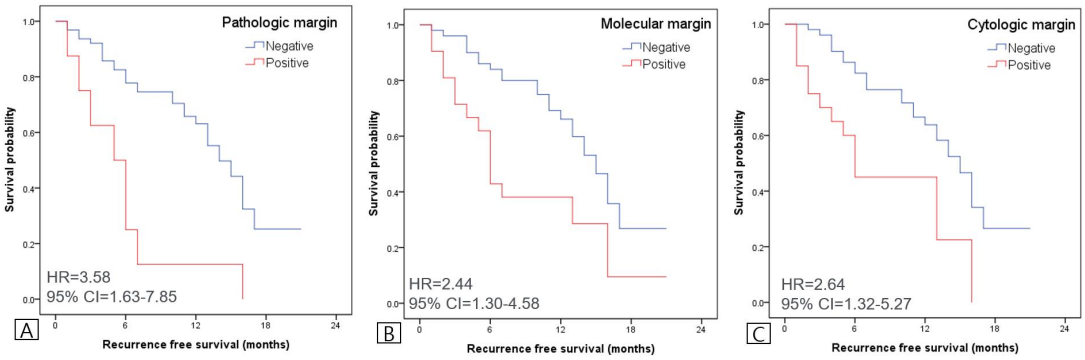

Figure 9. Kaplan-Meier RFS based on pathologic, molecular, and cytologic radial margin status in treatment naïve subgroup. (A) Patients with positive pathologic margin (median, 5 months) showed significant difference from those with negative pathologic margin (14 months). (B) Patients with positive molecular margin (6 months) had significantly shorter RFS than those with negative molecular margin (15 months). (C) Patients with positive cytologic margin (6 months) had significantly shorter RFS than those with negative cytologic margin (15 months).

DISCUSSION

Margin status has been known as an independent prognostic factor on RFS in PDAC patients undergoing curative surgical resection[23]. However, the impact of margin status for patients who got neoadjuvant treatment remains uncertain. Several studies have reported that margin status had prognostic

impact on survival outcomes in neoadjuvant setting[24-26]. However, in our study, we observed that margin positivity offered incomplete information about RFS in neoadjuvant treatment setting. These results are consistent with other studies which showed that margin status did not hold the prognostic significance in neoadjuvant setting[27-29].

We applied so called "1-mm rule" according to the previous studies[23,46,47], which concluded that resection margin distance of 1mm or less was associated with worse survival. Locoregional recurrence is closely associated with R1 resection[23]. In addition, tumor spreading by lymphovascular or perineural invasion might contribute to locoregional recurrence. By univariate analysis in our study, pathologic radial margin (P < 0.01) and PNI (P = 0.03) were associated short RFS. LVI (P = 0.03) might contribute to distant metastasis as well as local recurrence.

When we detect *KRAS* mutations, we used ddPCR method, ddPCR is one of the most sensitive techniques, which can detect one mutant out of 10,000 cells[48]. In PDACs, ddPCR has been applied as a tool to evaluate circulating tumor DNA (ctDNA), which could be used in noninvasive serial monitoring of clinical response or detecting early signs of relapse after surgical resection [49-51]. In addition, PCR method has been tried to assess margin status of PDAC as evaluation of *KRAS* mutations[32,52-54]. There were four previous studies of *KRAS* mutation evaluation in PDAC resection margin[32,52-54]. Kim *et al.*[52] assessed *KRAS* mutation in 70 histologically free pancreatic transection and radial margins by quantitative real time PCR.

Kim et al.[52] reported a significant difference in overall survival between patients with *KRAS* mutation positive margins and negative margins (median, 15 and 55 months; $P = 0.0008$). Turrini *et al.*[53] evaluated *KRAS* mutation in 22 paraffin embedded tissue blocks of R0 venous resection margin (portal vein bed) by quantitative real time PCR. They observed a significant difference of median overall survival (positive, 16 months; negative, 25 months; $P = 0.04$), which showed that *KRAS* mutation detection had strong impact on survival[53]. Ohigashi *et al.*[54] evaluated *KRAS* mutation by PCR in additional peri-SMA tissue and compared with conventional histologic evaluation method. The author concluded the *KRAS* mutation assay was more sensitive than histologic examination. Lastly, Kim *et al.*[32] evaluated *KRAS* mutation by ddPCR from 81 patients with a swab rubbing radial margin. Kim *et al.*[32] reported that patients with positive molecular margin, in which *KRAS* mutation rate of 4.19% or greater, had significantly worse RFS (median, 7 months) than those with negative molecular margin (12 months). In addition, they demonstrated patients with either pathologic or molecular margin positive had significantly worse RFS (median, 9 months) than those with both pathologic and molecular margin negative (18 months) when pathologic and molecular margins were combined [32]. While the previous studies only included cases without having neoadjuvant treatment, we included all cases regardless of neoadjuvant treatment status. We observed that molecular margin status correlated better with RFS than pathologic margin status especially in neoadjuvant chemotherapy group. In our study, 90 out of 126 cases (71.4%) showed at least

one *KRAS* mutation. This high *KRAS* mutation detected rate might explain the reason of frequent recurrence after surgical resection of PDAC patients. Mutant *KRAS* was believed from chronic pancreatitis[55,56]. However, it was finally revealed that *KRAS* mutation detected in the previous studies of chronic pancreatitis was associated with presence of PanIN[57]. With pathologic review of radial resection margin of the surgically resected PDACs, no PanIN lesions were observed in the radial margin.

Only one previous study evaluated transaction resection margin of PDAC with cytologic method[58]. Tone and colleagues evaluated transaction resection margin of 34 PDAC cases with touch preparation and then the cytology result was compared with frozen section and final histology With a 100% of sensitivity and 92.9% of specificity 92.9%[58]. In our study, positive cytologic margin was also associated with positive pathologic margin. Out of 15 pathologic margin positive cases, 10 cases (66.7%) were cytologic margin positive, and out of 111 pathologic margin negative cases, 85 cases (76.6%) were cytologic margin negative. We differently approached and evaluated the radial margin by rubbing a swab, not touch preparation. The performance of cytologic margin status to predict RFS was almost equal to those of molecular margin status. Especially in neoadjuvant chemotherapy subgroup, cytologic margin status was correlated better with RFS than pathologic margin status. By multivariate analysis, cytologic margin status as well as molecular margin status remained independent prognostic factors. In contrast, pathologic margin status did not. The advantage of cytologic evaluation method is convenient,

cost-effective, and its result comes fast. We chose the smear method instead of liquid-based cytology method in order to reduce false negativity by minimalizing the loss of swabbed cells.

This study has several limitations. First, molecular test for *KRAS* mutation detection with ddPCR is still expensive compared to pathologic margin evaluation. Second, median follow-up period was relatively short, which was 10 months. Additional validation studies with longer follow-up period and larger sample size are required. Third, this study was subject to the selection bias because patients who gave consent to our study were only included.

In 111 pathologic margin negative cases, 25 cases (22.5%) and 26 cases (23.4%) were molecular and cytologic margin positive, respectively. These cases could be explained by sampling error of pathologic resection margin because submission of one or two representative sections with close to PDAC during gross examination. In addition, only 4 um thick sections of H&E slides were finally evaluated under microscope from 3 to 4mm-thick slab of formalin fixed and paraffin embedded tissues. Therefore, additional molecular and cytologic evaluation method of radial resection margin can overcome this limitation of evaluation of resection margin with conventional pathology method.

In conclusion, we evaluated and compared the radial margin status from Whipple or PPPD specimens based on conventional pathologic method, *KRAS* mutational status and cytomorphologic features (Table 6). Based on our study,

evaluation of molecular margin by *KRAS* mutation analysis, and assessment of cytologic margin by smear, cell block and p53 immunocytochemical staining may provide valuable recurrence and survival information especially in PDAC patients with neoadjuvant treatment.

Table 6. Comparison between the prognostic impact of each radial margin status on RFS

	Pathologic margin	Molecular margin	Cytologic margin
Neoadjuvant group	Not identified HR = 1.53 95% CI = 0.53-4.43 P = 0.434	Present HR = 3.66 95% CI = 1.54-8.73 P = 0.003*	Present HR = 2.58 95% CI = 1.15-5.79 P = 0.022*
Treatment naïve group	Present HR = 3.58 95% CI = 1.63-7.85 P = 0.001*	Present HR = 2.44 95% CI = 1.30-4.58 P = 0.006*	Present HR = 2.64 95% CI = 1.32-5.27 P = 0.006*

HR, hazard ratio; CI, confidence interval
* indicates $P < 0.05$.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
2. Lovecek M, Skalicky P, Klos D, et al. [Surgical therapy of pancreatic cancer - 5 years survival]. Rozhl Chir 2015; 94: 470-6.
3. Trikudanathan G, Lou E, Maitra A, Majumder S. Early detection of pancreatic cancer: current state and future opportunities. Curr Opin Gastroenterol 2021; 37: 532-8.
4. Hong S, Won YJ, Park YR, et al. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2017. Cancer Res Treat 2020; 52: 335-50.
5. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. Lancet 2011; 378: 607-20.
6. Janssen QP, Buettner S, Suker M, et al. Neoadjuvant FOLFIRINOX in Patients With Borderline Resectable Pancreatic Cancer: A Systematic Review and Patient-Level Meta-Analysis. J Natl Cancer Inst 2019; 111: 782-94.
7. Clancy TE. Surgery for Pancreatic Cancer. Hematol Oncol Clin North Am 2015; 29: 701-16.
8. Chen Z, Lv Y, Li H, Diao R, Zhou J, Yu T. Meta-analysis of FOLFIRINOX-based neoadjuvant therapy for locally advanced pancreatic cancer. Medicine (Baltimore) 2021; 100: e24068.
9. Allen PJ, Kuk D, Castillo CF, et al. Multi-institutional Validation Study

of the American Joint Commission on Cancer (8th Edition) Changes for T and N Staging in Patients With Pancreatic Adenocarcinoma. Ann Surg 2017; 265: 185-91.

10. Rochefort MM, Ankeny JS, Kadera BE, et al. Impact of tumor grade on pancreatic cancer prognosis: validation of a novel TNMG staging system. Ann Surg Oncol 2013; 20: 4322-9.

11. Paniccia A, Hosokawa P, Henderson W, et al. Characteristics of 10-Year Survivors of Pancreatic Ductal Adenocarcinoma. JAMA Surg 2015; 150: 701-10.

12. Gnerlich JL, Luka SR, Deshpande AD, et al. Microscopic margins and patterns of treatment failure in resected pancreatic adenocarcinoma. Arch Surg 2012; 147: 753-60.

13. Epstein JD, Kozak G, Fong ZV, et al. Microscopic lymphovascular invasion is an independent predictor of survival in resected pancreatic ductal adenocarcinoma. J Surg Oncol 2017; 116: 658-64.

14. Di Martino M, Ielpo B, de Nova JLM, et al. Lymph Node Ratio, Perineural Invasion and R1 Resection as Independent Prognostic Factors in Pancreatic Adenocarcinoma: A Retrospective Cohort Study. Surg Technol Int 2020; 36: 82-8.

15. Jamieson NB, Foulis AK, Oien KA, et al. Peripancreatic fat invasion is an independent predictor of poor outcome following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. J Gastrointest Surg 2011; 15: 512-24.

16. Tewari M. Significance of pathological positive superior mesenteric/portal venous invasion in pancreatic cancer. Hepatobiliary Pancreat Dis Int 2016; 15: 572-8.

17. Sabater L, Gomez-Mateo Mdel C, Lopez-Sebastian J, et al. Prognostic implications of the standardized study of resection margins in pancreatic cancers. Cir Esp 2014; 92: 532-8.

18. Peng Y, Xiu D, Jiang B, et al. [A clinical study about applying different R1 criteria to evaluate pancreatic head ductal adenocarcinoma specimens]. Zhonghua Wai Ke Za Zhi 2014; 52: 834-8.

19. Khalifa MA, Maksymov V, Rowsell C. Retroperitoneal margin of the pancreaticoduodenectomy specimen: anatomic mapping for the surgical pathologist. Virchows Arch 2009; 454: 125-31.

20. Daamen LA, van Goor I, Schouten TJ, et al. Microscopic resection margin status in pancreatic ductal adenocarcinoma - A nationwide analysis. Eur J Surg Oncol 2021; 47: 708-16.

21. Yamamoto J, Kudo H, Kyoden Y, et al. An anatomical review of various superior mesenteric artery-first approaches during pancreatoduodenectomy for pancreatic cancer. Surg Today 2021; 51: 872-9.

22. Maksymov V, Hogan M, Khalifa MA. An anatomical-based mapping analysis of the pancreaticoduodenectomy retroperitoneal margin highlights the urgent need for standardized assessment. HPB (Oxford) 2013; 15: 218-23.

23. Takahashi D, Kojima M, Sugimoto M, et al. Pathologic Evaluation of Surgical Margins in Pancreatic Cancer Specimens Using Color Coding With Tissue Marking Dyes. Pancreas 2018; 47: 830-6.

24. Maeda S, Moore AM, Yohanathan L, et al. Impact of resection margin status on survival in pancreatic cancer patients after neoadjuvant treatment and pancreatoduodenectomy. Surgery 2020; 167: 803-11.

25. Chatterjee D, Katz MH, Foo WC, et al. Prognostic Significance of New AJCC Tumor Stage in Patients With Pancreatic Ductal Adenocarcinoma Treated With Neoadjuvant Therapy. Am J Surg Pathol 2017; 41: 1097-104.

26. Liu L, Katz MH, Lee SM, et al. Superior Mesenteric Artery Margin of Posttherapy Pancreaticoduodenectomy and Prognosis in Patients With Pancreatic Ductal Adenocarcinoma. Am J Surg Pathol 2015; 39: 1395-403.

27. Schmocker RK, Delitto D, Wright MJ, et al. Impact of Margin Status on Survival in Patients with Pancreatic Ductal Adenocarcinoma Receiving Neoadjuvant Chemotherapy. J Am Coll Surg 2021; 232: 405-13.

28. He J, Blair AB, Groot VP, et al. Is a Pathological Complete Response Following Neoadjuvant Chemoradiation Associated With Prolonged Survival in Patients With Pancreatic Cancer? Ann Surg 2018; 268: 1-8.

29. Bassi C, Marchegiani G, Dervenis C, et al. The 2016 update of the International Study Group (ISGPS) definition and grading of postoperative pancreatic fistula: 11 Years After. Surgery 2017; 161:

584-91.

30. Buscail L, Bournet B, Cordelier P. Role of oncogenic KRAS in the diagnosis, prognosis and treatment of pancreatic cancer. Nat Rev Gastroenterol Hepatol 2020; 17: 153-68.

31. Waters AM, Der CJ. KRAS: The Critical Driver and Therapeutic Target for Pancreatic Cancer. Cold Spring Harb Perspect Med 2018; 8.

32. Kim SJ, Kim MJ, Han JS, et al. Prediction of Recurrence With KRAS Mutational Burden Using Ultrasensitive Digital Polymerase Chain Reaction of Radial Resection Margin of Resected Pancreatic Ductal Adenocarcinoma. Pancreas 2019; 48: 400-11.

33. Grant TJ, Hua K, Singh A. Molecular Pathogenesis of Pancreatic Cancer. Prog Mol Biol Transl Sci 2016; 144: 241-75.

34. Adsay NV, Basturk O, Saka B, et al. Whipple made simple for surgical pathologists: orientation, dissection, and sampling of pancreaticoduodenectomy specimens for a more practical and accurate evaluation of pancreatic, distal common bile duct, and ampullary tumors. Am J Surg Pathol 2014; 38: 480-93.

35. Denis JA, Patroni A, Guillerm E, et al. Droplet digital PCR of circulating tumor cells from colorectal cancer patients can predict KRAS mutations before surgery. Mol Oncol 2016; 10: 1221-31.

36. Loh WY. Classification and regression trees. Wiley interdisciplinary reviews: data mining and knowledge discovery 2011; 1: 14-23.

37. Chun YS, Pawlik TM, Vauthey JN. 8th Edition of the AJCC Cancer

Staging Manual: Pancreas and Hepatobiliary Cancers. Ann Surg Oncol 2018; 25: 845-7.

38. Chang DK, Johns AL, Merrett ND, et al. Margin clearance and outcome in resected pancreatic cancer. J Clin Oncol 2009; 27: 2855-62.

39. Sugiura T, Uesaka K, Mihara K, et al. Margin status, recurrence pattern, and prognosis after resection of pancreatic cancer. Surgery 2013; 154: 1078-86.

40. Butturini G, Stocken DD, Wente MN, et al. Influence of resection margins and treatment on survival in patients with pancreatic cancer: meta-analysis of randomized controlled trials. Arch Surg 2008; 143: 75-83; discussion

41. Ethun CG, Kooby DA. The importance of surgical margins in pancreatic cancer. J Surg Oncol 2016; 113: 283-8.

42. Nitta T, Nakamura T, Mitsuhashi T, et al. The impact of margin status determined by the one-millimeter rule on tumor recurrence and survival following pancreaticoduodenectomy for pancreatic ductal adenocarcinoma. Surg Today 2017; 47: 490-7.

43. Menon KV, Gomez D, Smith AM, Anthoney A, Verbeke CS. Impact of margin status on survival following pancreatoduodenectomy for cancer: the Leeds Pathology Protocol (LEEPP). HPB (Oxford) 2009; 11: 18-24.

44. Brierley JD, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumours: John Wiley & Sons, 2017.

45. Campbell F, Foulis A, Verbeke C. Dataset for the histopathological

reporting of carcinomas of the pancreas, ampulla of Vater and common bile duct. The Royal College of Pathologists 2010.

46. Campbell F, Smith RA, Whelan P, et al. Classification of R1 resections for pancreatic cancer: the prognostic relevance of tumour involvement within 1 mm of a resection margin. Histopathology 2009; 55: 277-83.

47. van Roessel S, Kasumova GG, Tabatabaie O, et al. Pathological Margin Clearance and Survival After Pancreaticoduodenectomy in a US and European Pancreatic Center. Ann Surg Oncol 2018; 25: 1760-7.

48. Huggett JF, Cowen S, Foy CA. Considerations for digital PCR as an accurate molecular diagnostic tool. Clin Chem 2015; 61: 79-88.

49. Huerta M, Rosello S, Sabater L, et al. Circulating Tumor DNA Detection by Digital-Droplet PCR in Pancreatic Ductal Adenocarcinoma: A Systematic Review. Cancers (Basel) 2021; 13.

50. Alcaide M, Cheung M, Bushell K, et al. A Novel Multiplex Droplet Digital PCR Assay to Identify and Quantify KRAS Mutations in Clinical Specimens. J Mol Diagn 2019; 21: 214-27.

51. Ono Y, Sugitani A, Karasaki H, et al. An improved digital polymerase chain reaction protocol to capture low-copy KRAS mutations in plasma cell-free DNA by resolving 'subsampling' issues. Mol Oncol 2017; 11: 1448-58.

52. Kim J, Reber HA, Dry SM, et al. Unfavourable prognosis associated with K-ras gene mutation in pancreatic cancer surgical margins. Gut 2006; 55: 1598-605.

53. Turrini O, Gilabert M, Ewald J, Moutardier V, Delpero JR, Iovanna JL. Resectable pancreatic head adenocarcinoma: Is R0 resection an illusion? Genetic evaluation of venous resection margin affirmed unrecognized disease. J Visc Surg 2017; 154: 329-33.

54. Ohigashi H, Ishikawa O, Sasaki Y, et al. K-ras point mutation in the nerve plexuses around the superior mesenteric artery in resectable adenocarcinoma of the pancreatic head: distribution pattern and related factors. Arch Surg 2000; 135: 1450-5.

55. Luttges J, Diederichs A, Menke MA, Vogel I, Kremer B, Kloppel G. Ductal lesions in patients with chronic pancreatitis show K-ras mutations in a frequency similar to that in the normal pancreas and lack nuclear immunoreactivity for p53. Cancer 2000; 88: 2495-504.

56. Luttges J, Reinecke-Luthge A, Mollmann B, et al. Duct changes and K-ras mutations in the disease-free pancreas: analysis of type, age relation and spatial distribution. Virchows Arch 1999; 435: 461-8.

57. Lohr M, Kloppel G, Maisonneuve P, Lowenfels AB, Luttges J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. Neoplasia 2005; 7: 17-23.

58. Tone K, Kojima K, Hoshiai K, Kijima H, Kurose A. Utility of intraoperative cytology of resection margins in biliary tract and pancreas tumors. Diagnostic cytopathology 2015; 43: 366-73.

ABSTRACT

Background of Study

Radial margin evaluation of Whipple operation specimen of pancreatic ductal adenocarcinoma (PDAC) is important in predicting local recurrence and decision for adjuvant therapy. Neoadjuvant chemotherapy for locally advanced PDAC has been increasingly applied due to its ability to lower disease stage and transform locally advanced PDAC into borderline resectable or resectable PDAC. However, the impact of pathologic margin status for patients who got neoadjuvant treatment remains uncertain. We evaluated prognostic impact of pathologic margin status in neoadjuvant treatment setting, and investigated better method to evaluate radial margin status.

Materials and Methods

We prospectively collected a total of 126 Whipple or pyrolus preserving pancreaticoduodenectomy (PPPD) specimens of PDAC patients. We evaluated conventional pathologic margin status. We obtained swabbed cells from radial margin by brushing cytology, and assessed molecular radial margin by evaluating *KRAS* mutations which were observed with high frequency in PDAC by sensitive droplet digital polymerase chain reaction (ddPCR). In addition, we evaluated cytologic radial margin by combining smear, cell block and p53 immunocytochemical staining. RFS was measured based on pathologic, molecular, and cytologic radial margin status.

Results

One hundred twenty-six patients met inclusion criteria. Fifty-five patients underwent neoadjuvant chemotherapy, and 71 patients did not get any treatment before surgery. Median follow up period was 10 months. Fifteen (11.9%) cases were pathologic margin positive. Ninety (71.4%) cases showed at least one *KRAS* mutation, and 32 (25.4%) cases were classified as molecular margin positive. Cytologic margin positive cases were 36 (28.6%) cases. In neoadjuvant treatment subgroup, pathologic radial margin positivity showed no statistical difference of RFS. However, molecular radial margin showed statistical significance (median survival: negative, 17 months; positive, 6 months). In addition, cytologic radial margin showed statistical significance (median survival: negative, 17 months; positive, 7 months).

Conclusion

Pathologic radial margin status is not associated with RFS in neoadjuvant treatment setting. Molecular and cytologic radial margin evaluation provides prognostic information about RFS time for PDAC patients in neoadjuvant treatment setting.