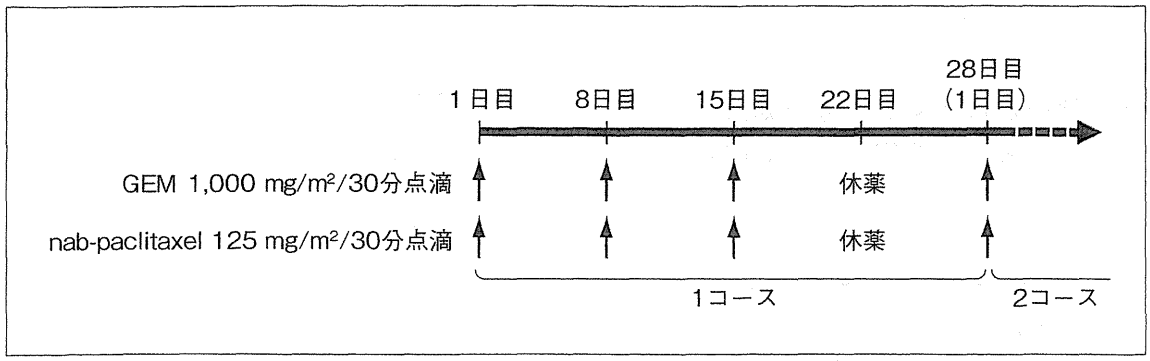

図5. Nab-paclitaxel + GEM療法

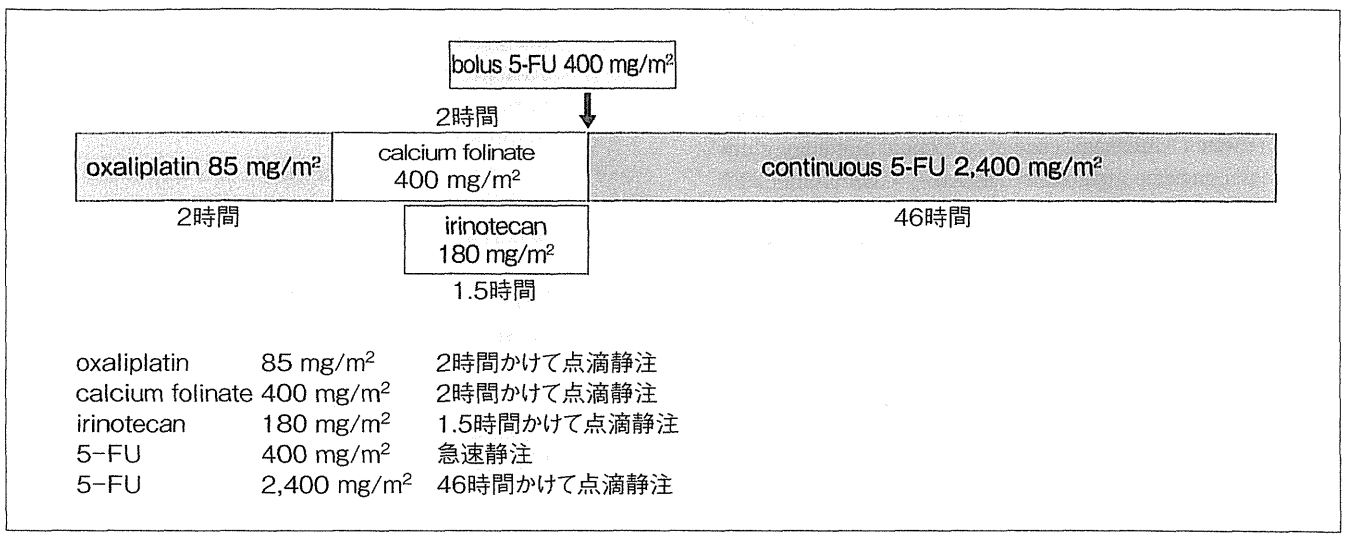

図6. FOLFIRINOX療法(1コース：2週間)

で，日常臨床の現場では全身状態などの状況に応じてこれらのレジメンを使い分けていくのが適切と思われる．後二者は2013年6月現在国内で保険診療での使用はできないため，早期の臨床導入が望まれる．

Nab-paclitaxel + GEM，FOLFIRINOXの投与スケジュールを図5，6に示す．GEMは比較的副作用が少ない抗癌薬であるが，まれに間質性肺炎を起こすため(1％)，呼吸困難や空咳などの呼吸器症状の出現時や熱発時は注意を要する．S-1に関しては，排便コントロールが不良となりがちな膵癌患者においては下痢の予防・管理が重要である．また，腎障害がある場合は5-FUの異化代謝酵素阻害薬gimeracilの腎排泄が低下し，副作用が増強する可能性があるため，適正使用の目安に従いクレアチニンクリアランスに応じた減量が必要となる．Erlotinib + GEMでは特に間質性肺炎(8.5％)への注意が必要で，治療開始前の十分な安全性情報の説明と治療確認シートの交付，治療中の頻回の画像検査など慎重な対応(適正使用ガイドを参照)が求められている．挫瘡様皮疹に対しては，ステロイド剤外用やminocycline hydrochloride内服の対応がとられている．Nab-paclitaxel + GEMやFOLFIRINOXでは前述の副作用を念頭において対応する必要があるが，いずれも限られた患者数での情報であるため，臨床導入後も副作用情報の共有が重要である．

おわりに

胆道癌，膵癌ともに以前はGEM単剤，S-1単剤といった，比較的副作用も軽く，シンプルな治療レジメンが主体であったが，近年になり治療開発がすすむにつれ併用療法が主体となりつつある．このため治療レジメンが複雑化し，副作用管理の重要性が増している．分子標的治療薬の開発にも関心が集まっており，今後有効性が示された

分子標的薬が登場すればバイオマーカーによる個別化医療もすすんでいくことが予想される．そのため，癌薬物療法に関する十分な知識を有しトレーニングを積んだ専門家による対応が重要となってくる．

◆ ◆ ◆ 文 献 ◆ ◆ ◆

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胆と膵 Vol. 34 (8) p. 601～606, 2013

膵・胆道癌薬物療法：臨床試験を読む！—最新の動向と実地診療へのインパクト—

局所進行または転移性膵癌に対するGEM＋S-1併用療法，S-1単剤療法，またはGEM単剤療法のランダム化第Ⅲ相試験：GEST試験

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森実　千種[1)]・池田　公史[2)]・奥坂　拓志[1)]

要約：Gemcitabine（GEM）の登場以降，GEM単剤療法が切除不能進行膵癌に対する標準治療であった。一方，S-1は膵癌に対するkey drugとして本邦で単剤あるいはGEMとの併用で独自に開発が進められてきた。そこで進行膵癌を対象にGEM単剤療法に対するS-1単剤療法の非劣性，GEM＋S-1併用療法（GS療法）の優越性を検証するためのランダム化第Ⅲ相試験（GEST試験）が日本と台湾で実施された。結果，生存期間中央値はGEM群8.8ヵ月，S-1群9.7ヵ月，GS群10.1ヵ月でGEM単剤療法に対するS-1単剤療法の非劣性が示された（ハザード比：0.96，97.5%信頼区間：0.78～1.18，$p<0.001$）。しかし，GS療法の優越性は示されなかった（ハザード比：0.88，97.5%信頼区間：0.71～1.08，$p=0.15$）。本試験の結果により，S-1単剤療法は局所進行および転移性膵癌に対する一次治療の選択肢の一つとなることが示された。

Key words：切除不能進行膵癌，GEM単剤療法，S-1単剤療法，GEM＋S-1併用療法（GS療法）

はじめに

5-fuluorouracil（5-FU）とgemcitabine（GEM）を比較した第Ⅲ相試験の結果，主要評価項目の症状緩和効果（GEM群：25%，5-FU群：5%）のみならず全生存期間（overall survival：OS）に関してもGEMの優越性が示された［生存期間中央値（median survival time：MST）：GEM群5.65ヵ月 vs. 5-FU群4.41ヵ月，$p=0.0025$］[1)]。以降，GEM単剤療法が切除不能進行膵癌に対する標準治療とされてきた。

その後さらなる治療成績の向上を目指して，GEMと他の抗癌剤との併用療法が数多く試みられてきたが，第Ⅲ相試験で有意なOSの延長を示したのはGEM＋erlotinib併用療法のみであった（MST：GEM＋erlotinib群6.24 vs. GEM群5.91，ハザード比：0.82，$p=0.038$）[2)]。しかしGEM＋erlotinib併用療法に関しても，OSの改善が大きくなかったことから副作用やコストを考慮した臨床的意義に関してはコンセンサスが得られておらず，GEM単剤療法に置き換わる位置づけにはならなかった。

一方，本邦では経口フッ化ピリミジン薬であるS-1の開発が膵癌に対して独自に進められてきた。進行膵癌に対するS-1単剤療法の第Ⅱ相試験では37.5%の奏効割合と9.2ヵ月のMSTが示され[3)]，GEM＋S-1併用療法（GS療法）の二つの第Ⅱ相試験ではそれぞれ44.4%と48.5%の奏効割合と10.1ヵ月と12.5ヵ月のMSTが示された[4,5)]。これらの有望な試験結果を受けて，GEM単剤療法に対するS-1単剤療法のOSにおける非劣性とGEM単剤療法に対するGS療法の優越性

Randomized Phase III Study of Gemcitabine Plus S-1, S-1 Alone, or Gemcitabine Alone in Patients with Locally Advanced and Metastatic Pancreatic Cancer in Japan and Taiwan：GEST Study

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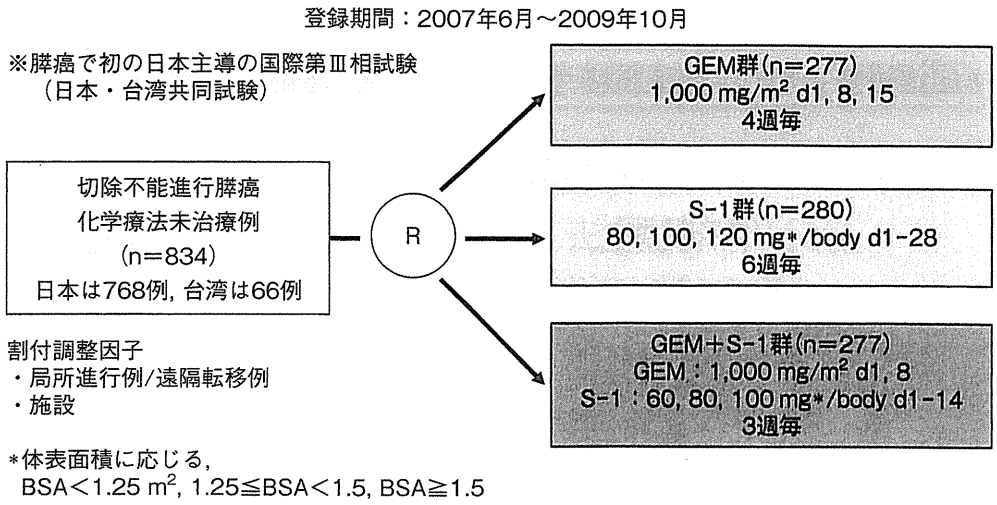

図 1　試験デザイン

を検証することを目的にランダム化第Ⅲ相試験（Gemcitabine and S-1 Trial：GEST）が日本と台湾で実施された。

Ⅰ．対象および方法

1．試験デザイン（図 1）

本研究はオープンラベル多施設共同ランダム化第Ⅲ相試験である。主目的は，局所進行膵癌および転移性膵癌を対象に OS に関する GEM 単剤療法に対する S-1 単剤療法の非劣性，および GEM 単剤療法に対する GS 療法の優越性を検証することであり，病期（局所進行/遠隔転移）と施設が割付調整因子に用いられた。

2．対象（表 1）

病理組織学的検査にて腺癌もしくは腺扁平上皮癌であることが確認された局所進行または転移性膵癌で，膵癌に対する手術以外の前治療歴なし，全身状態良好（ECOG Performance Status 0 または 1），年齢 20～79 歳などを主な適格基準とした。

3．評価項目および統計方法（表 2）

主要評価項目は OS，副次的評価項目は無増悪生存期間（progression-free survival：PFS），奏効割合，有害事象・副作用発現割合，QOL（quality of life）に定められた。

統計学的事項に関しては非劣性と優越性の二つの仮説を検証することから試験全体の有意水準を片側 0.025 に抑えるために，それぞれの比較における有意水準を片側 0.0125 とした。MST を GEM 群 7.5ヵ月，S-1 群 8ヵ月，GS 群 10.5ヵ月と想定し，非劣性の許容限界をハザード比 1.33 に設定した場合，1群あたり 250 例とすると，どちらの仮説検証についても 90%以上の検出力を確保できることから 3 群合わせて総症例数 750 例を目標症例数とした。

表 1　主な適格基準

1．腺癌又は腺扁平上皮癌であることが病理組織学的検査にて確認されている膵癌を有する
2．切除が不能な進行膵癌（局所進行膵癌，再発膵癌を含む）である
3．膵癌に対する切除以外の前治療が実施されていない
4．登録時の年齢が 20 歳以上 80 歳未満
5．ECOG Performance Status が 0 または 1
6．主要臓器の機能が十分に保持されている
7．肺線維症または間質性肺炎，水様性の下痢がない

Ⅱ．結　　果

1．患者背景（表 3）

2007 年 6 月から 2009 年 10 月までの間に 834 人の切除不能進行膵癌患者（日本から 768 人，台湾から 66 人）が登録された。それらの中から同意取得の逸脱があった 2 人を除いた 832 人が FAS（full analysis set）集団とされた。各群間の患者背景に特記すべき差は認められず，比較可能性は担保されていた。

2．有効性（図 2，3，表 4）

MST は GEM 群 8.8ヵ月，S-1 群 9.7ヵ月，GS 群 10.1ヵ月であった。GEM 単剤療法に対する S-1 単剤療法の非劣性に関しては，ハザード比の 97.5%信頼区間（confidence interval：CI）が事前に設定した非劣性マージンの 1.33 を超えておらず，GEM 単剤療法に対する S-1 単剤療法の非劣性が示された（ハザード比：0.96，97.5% CI：0.78～1.18，$p<0.001$）。一方で GEM 単剤療法に対する GS 療法の優越性は示されなかった（ハザー

表 2　評価項目および統計方法

主要評価項目：全生存期間（OS）
副次的評価項目：無増悪生存期間（PFS），奏効割合 有害事象・副作用発現割合，QOL（EQ-5D※に基づく）
計画したサンプルサイズ：各群 250 例で合計 750 例（680 イベント）
優越性の検定（GEM vs. GS）：OS における 7.5ヵ月（GEM 群）から 10.5ヵ月（GS 群）への延長（HR：0.71）
非劣性の検定（GEM vs. S-1）：OS における S-1 群の設定 8.0ヵ月 （非劣性マージン HR≦1.33）
検出力：90%（おのおのの比較における有意水準：片側 0.0125）

※EQ-5D：Euro-QOL 5D　健康水準の変化を基数的に評価するための QOL 質問票

表 3　患者背景

	GEM 群 n＝277	S-1 群 n＝280	GS 群 n＝275
年齢中央値（歳）	65	64	65
女性/男性（%）	39/61	39/61	43/57
ECOG PS 0/1（%）	65/35	64/36	63/37
局所進行/転移性（%）	24/76	24/76	25/75
膵切除歴（%）	8	6	10
減黄例（%）	27	23	24

ド比：0.88，97.5% CI：0.71～1.08，p＝0.015）（図 2）。

PFS に関しては中央値が GEM 群 4.1ヵ月，S-1 群 3.8ヵ月，GS 群 5.7ヵ月で，GEM 単剤療法に対すると S-1 単剤療法の非劣性（ハザード比：1.09，97.5% CI：0.90～1.33，p＝0.02）と，GEM 単剤療法に対する GS 療法の優越性が示された（ハザード比：0.66，97.5% CI：0.54～0.81，p＜0.001）（図 3）。

奏効割合は GEM 群 13%，S-1 群 21%，GS 群 29% であり，S-1 単剤療法（p＝0.02）および GS 療法（p＜0.001）は GEM 単剤療法と比較して有意に高かった（表 4）。

QOL については quality-adjusted life-years（QALYs）に関する解析が行われ，死亡例を 0 として扱った場合，GEM 群，S-1 群，GS 群の QALYs 中央値はそれぞれ 0.401，0.420，0.525 であった。QALYs は GEM 群と S-1 群の間に差は認められず（p＝0.56），GS 群は GEM 群よりも有意に良好であった（p＜0.001）。

3．安全性（表 5）

GEM 単剤療法では S-1 単剤療法に比べて骨髄抑制が強く発現し，肝機能障害の発現頻度も多かった。また grade 3 以上の間質性肺炎が 2% 認められた。S-1 単剤療法では主に下痢や食欲不振といった消化器毒性が認められた。GS 療法では GEM と S-1 の両者の毒性プロファイルが認められ，GEM 単剤療法に比べてさらに骨髄抑制が強い傾向があった。しかしながらいずれの群も認容可能なものであると判断された。

4．サブグループ解析（図 4）

ベースライン時の患者背景因子別に OS のサブグループ解析を行った。GEM 群と S-1 群の間に有意な交互作用を認める因子はみられなかった。一方，GS 群に関しては局所進行例および PS 1 の患者において GEM 群よりも OS が良好な傾向が認められた。

Ⅲ．考　察

本研究は切除不能進行膵癌患者を対象とした一次化学療法に関するランダム化第Ⅲ相試験であり，GEM 単剤療法に対する S-1 単剤療法の非劣性と，GS 療法の優越性が検証された。その結果，GEM 単剤療法に対する S-1 単剤療法の非劣性は証明され，GS 療法の優越性は示されなかった。

主要評価項目である OS に関して，S-1 単剤療法を受けた患者群は GEM 単剤療法を受けた患者群と同様の生存曲線を示し，副次評価項目の PFS に関しても非劣性が証明された。したがって，本研究の対象になった PS 0-1 の切除不能進行膵癌患者に対して，S-1 単剤療法は GEM 単剤療法と同等の効果を有する薬剤であることが明らかになった。二つの薬剤の毒性プロファイルは若干異なっており，好中球減少などの骨髄抑制は GEM 単剤療法に多く，下痢などの消化器毒性は S-1 単剤療法に多い傾向があったが，いずれの薬剤においても重篤な有害事象は稀であり，忍容性は良好であった。また，QOL 解析においても S-1 単剤療法と GEM 単剤療法は同等であることが示された。以上より，S-1 単剤療法は GEM 単剤療法と同等の効果と安全性を有する薬剤であることが本研究で示され，切除不能進行膵癌に対する標準治療の一つになることが証明された。切除不能進行膵癌に対して長らく標準治療とされてきた GEM 単剤療法に対して，単剤で非劣性を示したのは S-1 単剤療法が初めてである。GEM 単剤療法と S-1 単剤療法の使い分けに関しては，患者背景因子を用いた OS のサブグループ解析にて GEM 群と S-1

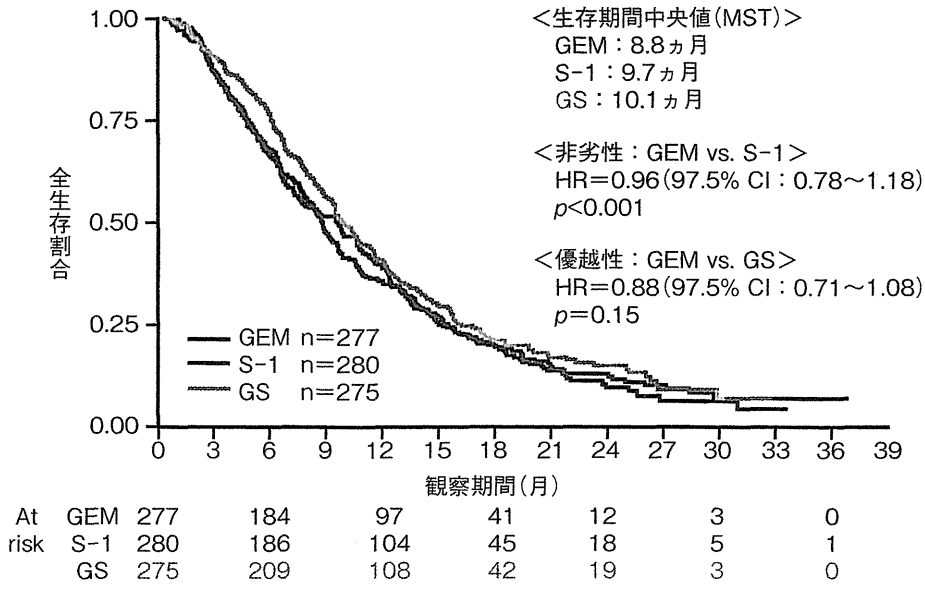

図 2 全生存期間

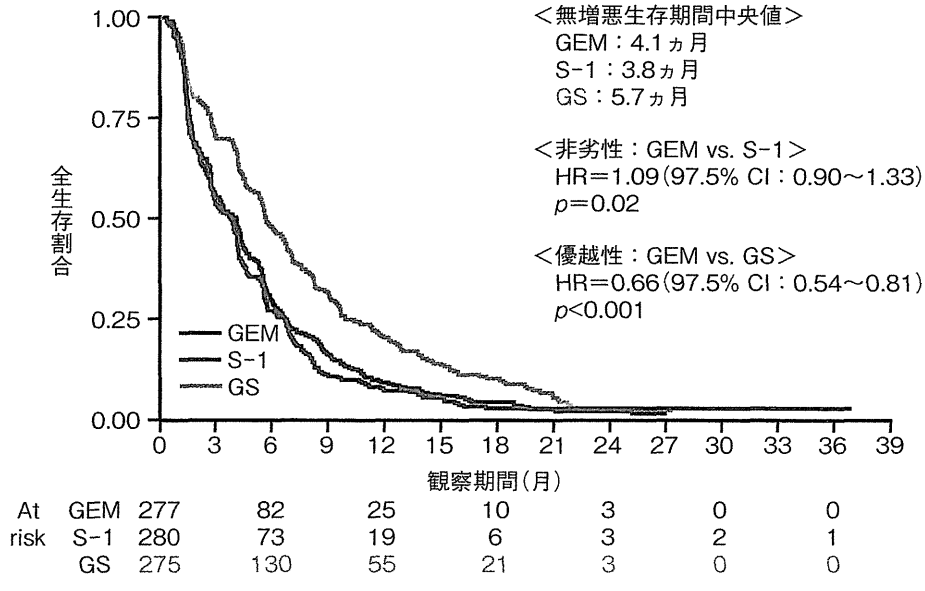

図 3 無増悪生存期間

表 4 奏効割合

	GEM 群	S-1 群	GS 群
症例数	241	248	242
CR＋PR	32	52	71
奏効割合	**13%**	**21%**	**29%**

GEM 群 vs. S-1 群：$p=0.02$
GEM 群 vs. GS 群：$p<0.001$

群の間に有意な交互作用を示す因子はみられなかったことや，GEM や S-1 の効果を治療前に予測できるバイオマーカーなどが見つかっていないことから，それぞれの薬剤の利点や毒性プロファイルを患者に説明した上で，患者の状況に合わせて両者を使い分けていくことが現実的な方法と考えられる。例えば治療開始前より下痢などの消化器症状がある患者には GEM を，骨髄機能が低下している患者には S-1 を，といったような使い分けの治療戦略が考えられる。

一方，GS 療法に関しては，GEM 単剤療法よりも奏効割合が有意に高く，PFS や QOL も明らかに優れていたが，OS に関してはやや良い傾向にあったものの優越性は示されなかった。PFS に関しては有意に良好であったにも関わらず，OS で優越性を示すことができなかった理由の一つには，後治療の影響が考えられる。二次治療移行率は GEM 群が 69%，S-1 群が 71%，

表 5 主な有害事象

有害事象	GEM 群（n＝273）		S-1 群（n＝272）		GS 群（n＝267）	
	Any（%）	Gr. ≧3（%）	Any（%）	Gr. ≧3（%）	Any（%）	Gr. ≧3（%）
貧血	80	14	68	10	85	17
白血球減少	76	**19**	43	4	88	**38**
好中球減少	68	**41**	34	9	83	**62**
血小板減少	78	**11**	46	2	81	**17**
ALT 増加	58	**15**	42	6	60	11
AST 増加	60	**15**	49	8	61	12
ビリルビン増加	26	10	53	14	39	9
クレアチニン増加	18	1	19	1	16	0.4
疲労	45	4	53	7	66	5
脱毛	11	—	3	—	18	—
皮疹	28	1	19	1	41	4
食欲不振	58	7	66	11	65	9
下痢	21	1	39	**6**	38	**5**
口内炎	14	0	25	1	34	2
悪心	43	2	54	2	55	**5**
嘔吐	27	1	32	2	34	**5**
発熱性好中球減少	0.4	0.4	0.4	0.4	2	**2**
間質性肺炎	3	**2**	0.4	0	2	1

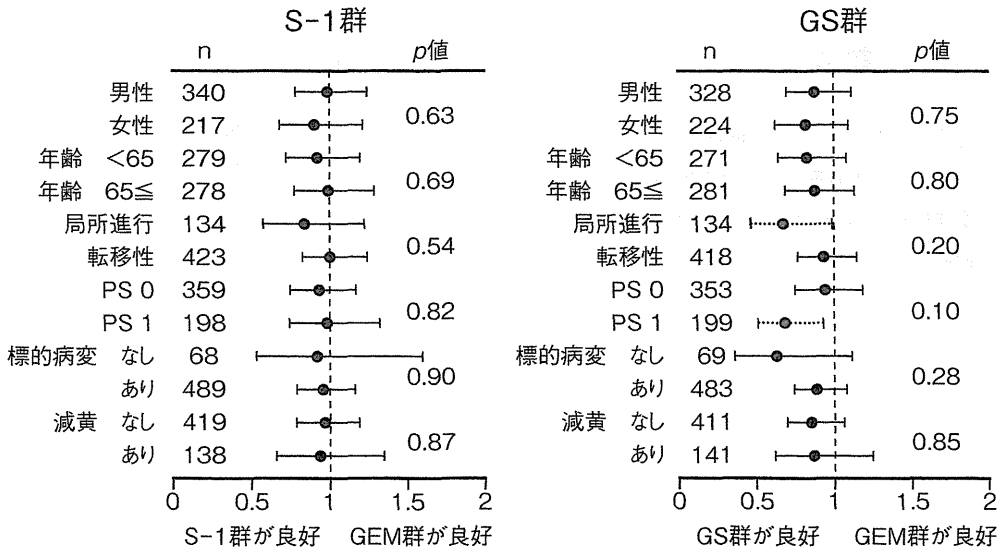

図 4 サブグループ解析（全生存期間）

GS 群が 67％で群間に差は認められなかった。一方，二次治療の内容に関して，GEM 群では 51％で S-1 を，S-1 群では 59％で GEM を，GS 群では 58％で一次治療において不応となった GEM や S-1 を使用した後治療が行われていた。本研究における GEM 群の MST は 8.8ヵ月とこれまでに報告された GEM 単剤療法の成績と比較して長く，その理由として GEM 群の約半数で S-1 を使用した二次治療を受けていたことが考えられる。GEM 単剤療法に抵抗性となった膵癌に対する S-1 単剤療法の第Ⅱ相試験では 15％の奏効割合と 58％の病勢制御割合が報告されており[6]，有望な後治療が控えていなかった GS 群とは対照的に，GEM 群では後治療に S-1 が控えていたことが，PFS で開いた差が OS で縮まった理由として考えられる。GS 療法は PFS で優越性を示したものの，OS では有意差を示せなかったこと，また単剤療法と比較して毒性がやや強かった事実を踏まえると，本研究より GEM と S-1 の使用に関しては同時（concurrent）に投与する必要はなく，

連続的（sequential）に投与すれば良いことが示唆される。ただし，GS療法に関しては，既述したようないくつかの利点を有することや，サブグループ解析では局所進行例やPS 1例に良好な傾向がみられたことから，GS療法の利点が活かせる対象や治療戦略を見出す研究の余地は残されていると思われる。

近年，海外では5-FUを含む多剤併用レジメンのFOLFIRINOX療法がGEM単剤療法に対して明瞭な優越性を示し，切除不能進行膵癌に対する新たな一次治療の一つとして普及しつつある。しかしFOLFIRINOX療法では5-FUを持続静注するために中心静脈リザーバーの留置が必要となる。本研究によりS-1が膵癌に対するkey drugであることが示されたことから，今後は5-FUの持続静注を経口薬のS-1に置き換えた5-FU based regimenの開発が進むことが期待される。また，本邦での状況と異なり，S-1はまだ世界的に普及した薬剤ではないが，本研究や術後化学療法におけるGEM単剤療法に対するS-1単剤療法の優越性を示したJASPAC01試験の結果を受けて，今後膵癌の治療開発においてS-1への関心が世界的にも高まることが予測される。

おわりに

本研究により，切除不能進行膵癌においてGEM単剤療法に対するS-1単剤療法の非劣性が証明され，S-1単剤療法は局所進行膵癌および転移性膵癌に対する一次治療の選択肢の一つになることが示された。今後S-1を含んだ治療開発が進み，膵癌の治療成績がさらに向上することを期待したい。

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Association between variations in the fat mass and obesity-associated gene and pancreatic cancer risk: a case–control study in Japan

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Abstract

Background: It is clear that genetic variations in the fat mass and obesity-associated (FTO) gene affect body mass index and the risk of obesity. Given the mounting evidence showing a positive association between obesity and pancreatic cancer, this study aimed to investigate the relation between variants in the FTO gene, obesity and pancreatic cancer risk.

Methods: We conducted a hospital-based case–control study in Japan to investigate whether genetic variations in the FTO gene were associated with pancreatic cancer risk. We genotyped rs9939609 in the FTO gene of 360 cases and 400 control subjects. An unconditional logistic model was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for the association between rs9939609 and pancreatic cancer risk.

Results: The minor allele frequency of rs9939609 was 0.18 among control subjects. BMI was not associated with pancreatic cancer risk. Compared with individuals with the common homozygous TT genotype, those with the heterozygous TA genotype and the minor homozygous AA genotype had a 48% (OR=1.48; 95%CI: 1.07–2.04), and 66% increased risk (OR=1.66; 95%CI: 0.70–3.90), respectively, of pancreatic cancer after adjustment for sex, age, body mass index, cigarette smoking and history of diabetes. The per-allele OR was 1.41 (95%CI: 1.07–1.85). There were no significant interactions between TA/AA genotypes and body mass index.

Conclusions: Our findings indicate that rs9939609 in the FTO gene is associated with pancreatic cancer risk in Japanese subjects, possibly through a mechanism that is independent of obesity. Further investigation and replication of our results is required in other independent samples.

Keywords: The fat mass and obesity-associated gene, Pancreatic cancer, rs9939609, Case–control study

Background

In 2010, approximately 28,000 Japanese subjects died from pancreatic cancer, making it the fifth leading cause of cancer deaths in Japan [1]. Despite extensive research efforts, the etiology of pancreatic cancer remains poorly understood. Cigarette smoking and long-standing type II diabetes are two well-established risk factors, based on consistent findings from epidemiologic studies [2,3]. In addition, being overweight and obese have been implicated in the development of pancreatic cancer [4], with statistically significant, positive associations observed in large cohort studies conducted in Western countries [5-7], and corroborated in at least four meta-analyses [8-11] and three pooled analyses [12-14]. The positive association between body mass index (BMI) and pancreatic cancer, however, has not been clearly observed in Asian populations. To date, four cohort studies have examined the association between BMI and pancreatic cancer in Asians, but the results have been inconsistent and inconclusive [15-18].

Recently, genome-wide association (GWA) studies have identified at least 30 loci that affect BMI and the

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risk of obesity [19]. Among these loci, the fat mass and obesity-associated (FTO) gene, which was first identified in a GWA study of diabetes in 2007 [20], has the strongest influence on BMI and obesity. Rs9939609, located in the first intron of the FTO gene, was found to be associated with both BMI and type II diabetes in subsequent GWA studies in diverse populations [21-23]. The association of rs9939609 with various traits, including hip circumference, energy intake and total mortality has also been studied [24-26]. In addition, rs9939609 genotypes have been linked with the risk of prostate, breast and endometrial cancers [27-29]. The association between genetic variations in the FTO gene and the risk of pancreatic cancer, however, is not clear. Of the three studies that examined this association, only one case–control study, conducted at the MD Anderson Cancer Center in the United States, reported that the minor A allele of FTO, rs9939609, was associated with an increased risk of pancreatic cancer among overweight subjects [30]. Another two studies examined rs8050136 of the FTO gene, with one study reporting a positive association [31], and the other no association [32].

Given the mounting evidence showing a positive association between obesity and pancreatic cancer, we hypothesized that variants in the FTO gene may be associated with pancreatic cancer risk through effects on obesity or other mechanisms. In a search of the literature for obesity-related genetic variants, we found that FTO rs9939609 was the most widely studied single nucleotide polymorphism (SNP), and has been found to exert strong effects on BMI, as well as diabetes. Furthermore, it showed strong linkage disequilibrium with other SNPs in the FTO gene, such as rs8050135 and rs17817449 [22]. We therefore investigated the association between FTO rs9939609 and pancreatic cancer risk in a case–control study in Japan.

Methods

Study subjects

Our study is an ongoing hospital-based case–control study focusing on the role of genetic polymorphisms and gene-environment interaction in pancreatic cancer. For the present analysis, eligible cases were patients aged older than 20 years, who were newly diagnosed with pancreatic cancer in five hospitals located in central, north and Tokyo metropolitan areas from April 1, 2010 through May 15, 2012. The diagnosis of pancreatic cancer was based on imaging modalities or pathologic reports. The response rate among cases was 85% (441/516) as of July 1, 2012. Almost all of the cases were approached within a week after the diagnosis of pancreatic cancer, and very few cases died before they were invited to participate in our study. During the same period, we recruited control subjects with no diagnosis of cancer from inpatients and outpatients from the participating hospitals where the cases were enrolled, as well as relatives of inpatients, and individuals undergoing a medical checkup in one of the participating hospitals. Control subjects were eligible if they were more than 20 years old and had no prior cancer diagnoses. Recruitment of controls was accomplished by approaching eligible participants in the hospitals who satisfied the study requirements, and the response rate was 98% (525/534). Control subjects had a variety of diseases, such as anemia, gastric ulcer, and irritable bowel syndrome. Control subjects were matched with case patients according to sex and age (within 10-year categories). As a result, data from 360 case patients and 400 control subjects were included in the present analysis.

All subjects provided written, informed consent. This study was approved by the ethical board of Aichi Medical University (Nagakute, Japan), the Institutional Review Board (IRB) of Cancer Institute Hospital (Tokyo, Japan), the IRB of Kanagawa Cancer Center Hospital (Kanagawa, Japan), the IRB of Tokyo Metropolitan Komagome Hospital (Tokyo, Japan), and the IRB of Sapporo Medical University (Sapporo, Japan).

Data collection

Study subjects were asked to fill out a self-administered questionnaire including information on demographic characteristics, medical history, and lifestyle factors, such as cigarette smoking, alcohol consumption and dietary intake. For body weight, data on usual weight over the year prior to study entry as well as weight at age 20 were reported by the study participants. For current or former smokers, we collected detailed data on smoking exposure, including smoking status (never, former, or current smokers), average number of cigarettes smoked per day, age at starting and quitting, and duration of smoking. For subjects with type II diabetes, we recorded the age at diagnosis. In addition to the questionnaire survey, all consenting participants provided a 7-mL venous blood sample. Genomic DNA was extracted from peripheral lymphocytes at SRL Hachioji Laboratory and then stored at -30°C at the Department of Public Health, Aichi Medical University.

Genotyping assays

Genotyping was performed using the Taqman SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) at the laboratory of Aichi Cancer Center Research Institute, Nagoya, Japan. Laboratory staff were blinded to case or control status. Four quality control samples were included in each assay, and the successful genotyping rate was 100%.

Statistical analysis

Case–control differences in selected demographic characteristics and risk factors were evaluated using t tests (for continuous variables) and Chi-square tests (for categorical variables). A chi-square test was used to test genotype frequencies in control subjects for Hardy-Weinberg equilibrium (HWE) by comparing observed genotype frequencies with those expected under HWE. A co-dominant genomic model was assumed for SNP effects. Unconditional logistic regression methods were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between rs9939609 genotypes and pancreatic cancer risk. Homozygous carriers of the common FTO rs9939609 T allele served as the reference group. All analyses were adjusted for age (continuous), sex (male or female), BMI (<20, 20–22.4, 22.5-24.9, ≥25.0), history of diabetes (yes or no), and cigarette smoking (current, former, never smokers). ORs were also estimated for the variant allele on the basis of a log-additive model. The interaction of genotype-BMI and genotype-history of diabetes with respect to pancreatic cancer risk was assessed using the likelihood ratio test. Because recent-onset diabetes may result from pancreatic cancer, we performed an analysis excluding cases who had onset of diabetes within 2 years prior to the diagnosis of pancreatic cancer.

All P-values were two-sided, with $P<0.05$ indicating statistical significance. All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

The distribution of genotypes among control subjects did not deviate from the Hardy-Weinberg equilibrium (P=0.94). The minor allele frequency (MAF) was 0.18 among control subjects. Table 1 summarizes the characteristics of cases and controls. Both groups had a similar distribution of sex and 10-year age groups. The mean age was 65.1±8.1 years for cases, and 58.5±9.1 years for controls. Cases were more likely to be current smokers and have a history of diabetes compared with controls. Current smokers had an approximately 2.9-fold increased risk of pancreatic cancer compared with nonsmokers, after adjustment for age, sex, BMI, and history of diabetes (OR=2.86; 95%CI: 1.79-4.57). Individuals who had a BMI of 30 or more had a 1.21-fold increased risk, but the association was not statistically significant. Similar results were obtained in an additional analysis in which BMI at age 20 was used (data not shown). Risk of pancreatic cancer was significantly increased among subjects reporting a history of diabetes (OR=2.94; 95%CI: 1.90-4.57). The significant, positive association remained after excluding pancreatic cancer cases with recent-onset diabetes (OR=1.92; 95%CI: 1.20–3.08). Among control subjects, the mean BMI was 22.7±3.1 for the TT genotype, 23.2±3.3 for the TA genotype, and 21.1±2.9 for the AA genotype.

Table 1 Association between variations in the fat mass and obesity-associated gene and pancreatic cancer risk: a case–control study in Japan

Characteristics	Case patients (N=360)	Control subjects (N=400)	OR (95% CI)
Age group			Matching factor
<50	12 (3.3)	19 (4.8)	
50-59	44 (12.2)	79 (19.8)	
60-69	141 (39.2)	170 (42.5)	
70-79	138 (38.3)	115 (28.8)	
≥80	25 (6.9)	17 (4.3)	
Sex			Matching factor
Female	215 (59.7)	226 (56.5)	
Male	145 (40.3)	174 (43.5)	
Body mass index (kg/m^2)			
<25	278 (77.2)	312 (78.0)	1.00
25.0-29.9	64 (17.8)	75 (18.7)	0.96 (0.65-1.43)
≥30	16 (4.4)	12 (3.0)	1.21 (0.53-2.77)
Unknown	2 (0.6)	1(0.3)	-
Smoking status			
Non-smokers	145 (40.2)	202 (50.5)	1.00
Former smokers	119 (33.1)	140 (35.0)	1.23 (0.82-1.85)
Current Smokers	96 (26.7)	58 (14.5)	2.86 (1.79-4.57)
History of diabetes			
No	269 (74.7)	362 (90.5)	1.00
Yes	87 (24.2)	35 (8.7)	2.94 (1.90-4.57)
Unknown	4 (1.1)	3 (0.8)	-

OR: odds ratio; CI: confidence interval.
OR was adjusted for sex, age, smoking status and history of diabetes.

Table 2 shows the association between variants in the FTO gene (rs9939609) and pancreatic cancer risk. Compared with individuals with the TT genotype, the multivariate adjusted OR for developing pancreatic cancer was 1.48 (95%CI: 1.07–2.04) among those with the TA

Table 2 Association between the FTO rs9939609 and pancreatic cancer risk

FTO rs9939609	Cases	Control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	213	271	1.00	1.00
TA	133	116	1.49 (1.09-2.03)	1.48 (1.07-2.04)
AA	14	13	1.49 (0.67-3.29)	1.66 (0.70-3.90)

OR: odds ratio ; CI: confidence interval.
Multivariable adjusted OR: adjusted for age, sex, body mass index, cigarette smoking and history of diabetes.

genotype, and 1.66 (95%CI: 0.70–3.90) among those with the AA genotype. Under the dominant model, the OR was 1.49 (95%CI: 1.09–2.05) among carriers of the TA/AA genotype. Under the log-additive model, each additional copy of minor allele A was associated with a 1.4-fold increased risk of pancreatic cancer (OR=1.41, 95% CI: 1.07–1.85).

We found no significant interaction between FTO rs9939609 and BMI (Table 3). Individuals with both a TA/AA genotype and a history of diabetes had a 3.7-fold increased risk of pancreatic cancer compared with those with a TT genotype and no history of diabetes (Table 4), but a test for the interaction was not statistically significant.

Table 4 Joint associations of the FTO rs 9939609 and history of diabetes with respect to pancreatic cancer risk

Genotype	History of diabetes	Cases/control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	No	163/243	1.00	1.00
TA/AA	No	106/119	1.38 (0.99-1.93)	1.41 (1.00-1.98)
TT	Yes	34/26	1.76 (1.01-3.07)	1.70 (0.96-3.00)
TA/AA	Yes	24/8	4.03 (1.75-9.24)	3.70 (1.59-8.63)
				P for interaction=0.28

Cases were excluded if the onset of diabetes was within 2 years prior to the diagnosis of pancreatic cancer.
Multivariable OR: adjusted for age, sex, body mass index, and cigarette smoking.

Discussion

This was a hospital-based case–control study in Japan to investigate whether genetic variations in the FTO gene were associated with pancreatic cancer risk. The main findings of our study were: 1) individuals with the FTO rs9939609 TA genotype had a significant 1.5-fold increased risk of pancreatic cancer compared with those with the TT genotype; and 2) a combination of the FTO rs9939609 TA/AA genotype and a history of diabetes significantly increased the pancreatic cancer risk, with an OR of 3.70 (95%CI: 1.59–8.63).

We found that obesity, defined as a BMI of 30 or more, was associated with 1.2-fold increased risk of pancreatic cancer, but this association was not statistically significant. In contrast to evidence of a positive association between obesity and pancreatic cancer in Western countries, available data on the role of obesity in pancreatic cancer in Japanese are inconclusive. There have been no prospective studies that have observed a clear, dose–response relation between baseline BMI and pancreatic cancer risk in the Japanese population [15,16]. Given that less than 5% of the subjects were obese in this study, it might be difficult to observe significant associations. The small percentage of obese people may be the main reason for the inconclusive results on BMI and pancreatic cancer in Asians, including Japanese [15-18]. In addition, differences in body fat distribution, in genetic predisposition to obesity and in lifestyle factors between Caucasians and Asians may contribute to the inconsistent results on BMI and pancreatic cancer risk in Asian populations [33,34].

Table 3 Joint associations of the FTO rs9939609 and BMI with respect to pancreatic cancer risk

Genotype	BMI	Cases/control subjects	Age- and sex-adjusted OR	Multivariable-adjusted OR
TT	<25	166/220	1.00	1.00
TA/AA	<25	112/92	1.69 (1.20-2.40)	1.68 (1.18-2.41)
TT	≥25	45/51	1.29 (0.81-2.04)	1.20 (0.75-1.94)
TA/AA	≥25	35/36	1.35 (0.81-2.25)	1.21 (0.71-2.07)
				P for interaction=0.29

Multivariable OR: adjusted for age, sex, cigarette smoking and history of diabetes.

Because of the positive association between obesity and pancreatic cancer in Caucasians and the plausible mechanisms, several research groups have hypothesized that variants in obesity-related genes might be associated with pancreatic cancer risk. The association between rs9939609 in the FTO gene was reported in one previous hospital–based case–control study conducted at the MD Anderson Cancer Center, Texas, USA [30]. Of the 15 obesity- and diabetes-associated genotypes in the FTO gene, rs9939609 was found to be positively associated with pancreatic cancer risk in persons who were overweight, whereas no increased risk was observed in persons who had a BMI of less than 25 kg/m^2 [30]. In contrast, our study showed a significant, positive association between rs9939609 TA/AA genotype and pancreatic cancer risk in individuals with a BMI of less than 25 kg/m^2. We consider that the difference in minor allele frequency (MAF) may be the main reason, given the fact that the MAF was 18% in our study, much lower than the 38% in the MD Anderson Cancer Center case–control study. The possible differences in selection of cases and controls, patterns of linkage disequilibrium and effects of gene-gene interactions may also account for the inconsistent findings. In addition to rs9939609, rs8050136 in the FTO gene was found to be associated with pancreatic cancer risk in individuals of European ancestry [31]; however, no association was noted in another case–control study [32].

In our study, FTO rs9939609 genotypes were associated with pancreatic cancer risk. However, the mean BMI did not differ among rs9939609 genotypes for control subjects, and no significant interaction was observed between rs9939609 TA/AA genotypes and BMI with

respect to pancreatic cancer risk. It is possible that the positive association observed between rs9939609 genotypes and pancreatic cancer risk may be driven by a mechanism other than adiposity. Diabetes, a well-established risk factor for pancreatic cancer, is a possible candidate. There is evidence suggesting that Asian people are more susceptible to insulin resistance at a lesser degree of obesity than Caucasians [33,34]. Besides its close association with adiposity, FTO has been shown to be associated with susceptibility to type II diabetes [21,22]. We found that individuals with a TA/AA genotype and a history of diabetes were at a 3.7-fold increased risk of pancreatic cancer. However, a test for the interaction was not statistically significant. Another possibility is that FTO is just a proxy of as yet unidentified causal variants, and it is those variants that exert their effects on rs9939609 and influence pancreatic cancer risk. Given that the function of the FTO gene is largely unknown, further studies are needed to comprehensively evaluate multiple SNPs in the FTO gene and elucidate the mechanisms by which FTO rs9939609 influences pancreatic cancer risk.

Our study has several limitations. First, it is well-known that two significant issues, namely selection bias and recall bias, plague case–control studies. Our results might have been biased if hospital controls did not represent the same population from which the cases were derived. However, the allele frequencies observed among control subjects in our study were similar to those reported in the studies of Asian populations [22]. In particular, the MAF of FTO rs9939609 was 18% in our control subjects, which is very close to that reported from a sample of 100 Japanese included in the HapMap project. Moreover, the risk estimates for current smokers and individuals with a history of diabetes were comparable to those estimated from cohort or population-based case–control studies [2,3], providing indirect evidence that selection bias might not be a serious concern in our study. Second, as for recall bias, while the analysis of the association between pancreatic cancer and BMI based on self-reported weight and height might be affected by recall bias, the association with the obesity-related genotype was not. Third, although our study included a relatively large sample size compared with previous studies conducted in Japan, the sample size may not have been large enough to detect significant gene-environment interactions in subgroups. Finally, it is possible that the results could represent a chance association and therefore replication in other independent samples is required. Despite these limitations, there are several advantages of the hospital-based design adopted in our study, including rapid case ascertainment, a high response rate from both cases and controls, and high quality genotyping.

Conclusion

Our findings indicate that rs9939609 in the FTO gene is associated with pancreatic cancer risk in Japanese subjects, possibly through a mechanism that is independent of obesity. Because of the limited statistical power, our results need replication in other independent samples. The fast-increasing prevalence of overweight/obesity and type II diabetes in Asians provides a good opportunity to further address this association and its underlying mechanisms.

Competing interests

The authors declare no conflict of interest.

Authors' contribution

SK supervised the study, SK, YL, KY designed the study, YL drafted the manuscript and conducted the statistical analysis. JU and KM performed genotyping and SNP data analysis. HI, MU, NE, HN, MM participated in data collection. All authors read and approved the final manuscript.

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BRIEF ARTICLE

Ultrasound-guided *vs* endoscopic ultrasound-guided fine-needle aspiration for pancreatic cancer diagnosis

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Abstract

AIM: To clarify the effectiveness and safety of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) for the diagnosis of pancreatic cancer (PC).

METHODS: Patients who were diagnosed with unresectable, locally advanced or metastatic PC between February 2006 and September 2011 were selected for this retrospective study. FNA biopsy for pancreatic tumors had been performed percutaneously under extracorporeal ultrasound guidance until October 2009; then, beginning in November 2009, EUS-FNA has been performed. We reviewed the complete medical records of all patients who met the selection criteria for the following data: sex, age, location and size of the targeted tumor, histological and/or cytological findings, details of puncture procedures, time from day of puncture until day of definitive diagnosis, and details of severe adverse events.

RESULTS: Of the 121 patients who met the selection criteria, 46 had a percutaneous biopsy (Group A) and 75 had an EUS-FNA biopsy (Group B). Adequate cytological specimens were obtained in 42 Group A patients (91.3%) and all 75 Group B patients ($P = 0.0192$), and histological specimens were obtained in 41 Group A patients (89.1%) and 65 Group B patients (86.7%). Diagnosis of malignancy by cytology was positive in 33 Group A patients (78.6%) and 72 Group B patients (94.6%) ($P = 0.0079$). Malignancy by both cytology and pathology was found in 43 Group A (93.5%) and 73 Group B (97.3%) patients. The mean period from the puncture until the cytological diagnosis in Group B was 1.7 d, which was significantly shorter than that in Group A (4.1 d) ($P < 0.0001$). Severe adverse events were experienced in two Group A patients (4.3%) and in one Group B patient (1.3%).

CONCLUSION: EUS-FNA, as well as percutaneous needle aspiration, is an effective modality to obtain cytopathological confirmation in patients with advanced PC.

Key words: Endoscopic ultrasound-guided fine needle aspiration; Percutaneous needle aspiration; Pancreatic cancer

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INTRODUCTION

Pancreatic cancer (PC) is currently the fifth leading cause of cancer-related mortality in Japan. Although complete surgical removal of the tumor is the only chance of cure, almost all PC patients are initially diagnosed as having advanced unresectable disease despite recent improvements in diagnostic techniques. In recent decades, techniques were developed to obtain proof of cancer from the primary tumor in PC patients. Pancreatic juice cytology *via* endoscopic retrograde pancreatography was initially developed to meet this challenge; however, in practical settings the positive rate for cancer cells has remained low, indicating the presence of false-negative results[1,2]. Ultrasonography-guided fine-needle aspiration (US-FNA) biopsy or computed tomography (CT)-guided FNA biopsy appears to provide a more definitive diagnosis of PC[3,4]. US-FNA is convenient but its usefulness is limited for masses in the pancreatic tail. In contrast, CT-guided FNA is the biopsy procedure of choice to assess pancreatic lesions. However, this technique is time-consuming and is limited by a substantial false-negative rate of approximately 20%[5]. In addition, there have been concerns about percutaneous cancer seeding[6,7]. Recently, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has been developed as a more feasible method to obtain definitive specimens for cytological and/or histological examinations for diagnosis of PC[8-12]. Three years ago, we began to perform EUS-FNA although until that time US-FNA was the standard technique at our institute.

In the current study, we retrospectively examined the diagnostic ability of EUS-FNA for PC compared with US-FNA.

MATERIALS AND METHODS

Patients

The inclusion criteria were: (1) the patient underwent US-FNA between February 2006 and October 2009 or EUS-FNA between November 2009 and September 2011 at the Cancer Institute Hospital, Tokyo, Japan for suspected PC; and (2) the patient was subsequently diagnosed as having clinical stage Ⅲ or Ⅳ PC. Unresectable PC, which was indicated by International Union Against Cancer clinical stage Ⅲ (locally advanced disease: T4N0-1 and M0) or Ⅳ (metastatic disease: T1-4N0-1 and M1), was diagnosed by CT.

The exclusion criteria were: (1) a contraindication for EUS (esophageal stenosis, duodenal stenosis, ileus, or perforation of the digestive tract); and (2) a contraindication for EUS-FNA and US-FNA (severe cardiovascular disease or respiratory disease, poor performance status, difficulty in visualization of the target, bleeding tendency, or impossibility of ensuring the puncture route).

Patients who met the selection criteria were identified from the database in our division, which was updated daily.

US- and EUS-FNA procedures

A short admission, usually for one or two nights, was mandatory according to the protocol for FNA biopsy of a suspected pancreatic tumor in our division. FNA biopsy for pancreatic tumors had been performed percutaneously under extracorporeal ultrasound guidance (US-FNA) until October 2009; then, beginning in November 2009, FNA biopsies have been performed under EUS guidance (EUS-FNA). In general, FNA examinations were performed and managed by Ishii H until October 2009 and by Matsuyama M since November 2009. Written informed consent was obtained from each patient before the examination.

US-FNA was performed using SSA-550A (Toshiba, Tokyo, Japan) as the ultrasound device and SONOPSY C1 21G (Hakko, Osaka, Japan) as the ultrasound-guided biopsy needle. After systemic premedication and percutaneous local anesthesia, FNA was performed 1-3 times repeatedly until adequate material was obtained. Pathological examination of the obtained materials and cytological examination of the needle-washing water were done. There was no on-site cytotechnologist during the performance of US-FNA.

EUS-FNA was performed using EU-ME1 and UCT240-AL5 (Olympus, Tokyo, Japan) as the EUS system and the Echo-Tip ULTRA 22G (Wilson-Cook, Bloomington, IN, United States) as the ultrasound-guided biopsy needle. After systemic premedication and pharyngeal local anesthesia, FNA was performed endoscopically *via* the stomach or duodenum. Aspiration puncture was repeated until an on-site cytology screener confirmed that adequate materials had been obtained.

After the examination, patients stayed in the hospital overnight and were discharged the following morning if no problems were revealed by physical examination, complete blood count tests and biochemistry tests that included serum amylase level. Three to 7 d later, the patients came to the outpatient clinic for an explanation of the results of the biopsy and examination for late adverse events, and were then able to start chemotherapy.

The final diagnosis was based on pathology results or clinical follow-up of > 6 mo.

Statistical analysis

We reviewed the complete medical records of all patients who met the selection criteria for the following data: sex, age, location and size of the targeted tumor, histological and/or cytological findings of the obtained specimens, details of puncture procedures, time from day of puncture until the day of definitive diagnosis, and details of severe adverse events, if any. The tumor status (location and size) was determined by dynamic CT before puncture. Frequency analysis was performed with Fisher's exact test for 2 × 2 tables, χ^2 test for 3 × 2 tables, and Mann-Whitney test. All analysis were performed using the statistical software SPSS 11.0J for Windows. Statistical significance was defined as a two-sided P value ≤ 0.05.

Table 1 Characteristics of patients and comparison of results of percutaneous biopsy with those of endoscopic ultrasound-guided fine-needle aspiration

	Percutaneous biopsy Group A	EUS-FNA Group B	*P* value
Patients	46	75	
Site of puncture			
Pancreas	46	74	> 0.9999
Head/body/tail	12/32/2	34/31/9	0.0114
Sex (male/female)	25/21	39/36	> 0.8525
Age, yr			> 0.8466
≥ 65	28	48	
< 65	18	27	
Tumor diameter, mm (range)	44.8 (18-111)	25.5 (7-70)	
≥ 40	30	25	0.0007
< 40	16	50	
Passes (range)	2.26 (1-4)	2.85 (2-5)	< 0.0001
Adequate specimens obtained[1] *n* (%)			
Cytology	42 (91.3)	75 (100)	0.0192
Histology	41 (89.1)	65 (86.7)	0.7812
Positivity for cancer *n* (%)			
Cytology	33 (78.6)	72 (94.6)	0.0079
Histology	33 (80.5)	51 (78.4)	> 0.9999
Total *n* (%)	43 (93.5)	73 (97.3)	0.3672
Complications *n* (%)	2 (4.3)	1 (1.3)	> 0.5567
	Fever[1]	Peritonitis[1]	
	Bleeding[1]		
Time from puncture to definitive diagnosis			
Cytology, d (range)	4.05 (0-8)	1.65 (0-5)	< 0.0001
Histology, d (range)	3.95 (2-7)	3.18 (2-10)	0.7066

[1]An on-site pathologist was available for endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) but not for ultrasonography-guided-FNA.

RESULTS

US-FNA was performed in 48 patients from February 2006 until October 2009. Two cases (renal cell carcinoma and malignant lymphoma) were excluded from the analysis of US-FNA because the patients did not have primary PC. EUS-FNA was attempted in 125 cases and was successfully performed in 123 cases from November 2009 until September 2011. Among these, 48 patients did not meet the selection criteria (lymph node metastasis, 34 cases; other pancreatic tumor, 10 cases; other abdominal tumor, three cases, and mediastinum tumor, one case). EUS-FNA could not be performed in two patients because of difficulty of visualization due to total gastrectomy in one case, and impossibility of ensuring the puncture route in the other. Thus, 46 patients who underwent US-FNA (Group A) and 75 who underwent EUS-FNA (Group B) were eligible for analysis.

Table 1 shows the characteristics of the study subjects. The distribution of the target tumor in the pancreas differed significantly between the two groups, with the tumor location more frequent in the pancreatic head/tail than in the pancreatic body in Group B. The maximum diameter of the target tumor ranged from 18 to 111 mm (median, 44.8 mm) in Group A and from 7 to 70 mm (median, 25.5 mm) in Group B. A significantly larger number of target tumors were < 40 mm in Group B than in Group A ($P = 0.0007$).

Table 1 shows a comparison of the results of percutaneous biopsy with those of EUS-FNA. Adequate cytological and histological specimens were obtained in 42 (91.3%) and 41 (89.1%) Group A patients ($n = 46$), respectively, and in 75 (100%) and 65 (86.7%) Group B patients ($n = 75$).

Results of cytology indicated the presence of cancer cells in 33 Group A patients (78.6%) and in 72 Group B patients (94.6%). Histological studies showed cancer tissue in 33 (80.5%) and 51 (78.4%) patients in Group A and Group B, respectively. In total, a cancer diagnosis was made in 43 Group A (93.5%) and 73 Group B (97.3%) patients by cytology and/or histology. These 116 patients were diagnosed with pancreatic adenocarcinoma by cytology/histology as well as by imaging and their subsequent clinical course. The final diagnosis of PC in the remaining five patients for whom there was no cytological or histological proof was confirmed by the clinical course until April 2012. The positive cytology/histology rate did not differ between the two groups.

Total puncture procedures per patient varied from one to five, with a median of 3. The frequency of multiple punctures, that is, > 2, was significantly higher in Group B than in Group A. Time from the day of puncture until the day of the final cytological diagnosis varied from 0 to 8 d (median, 4.1 d) in Group A and from 0 to 5 d (median, 1.7 d) in Group B. The period was significantly shorter in Group B than in Group A. The time from the day of puncture until the day of the final histological diagnosis varied from 2 to 7 d (median, 4.0 d) in Group A and 2 to 10 d (median, 3.2 d) in Group B, with no significant difference between the two groups.

Severe adverse events occurred in two Group A patients (4.3%) and in one Group B patient (1.3%). In Group A, one patient developed a high fever, which required hospitalization but resolved with only symptomatic treatment. The other Group A patient experienced upper gastrointestinal bleeding, which was confirmed by endoscopy to be related to the needle biopsy. This patient was treated by blood transfusion and antiulcer medication and was hospitalized for 1 wk without surgical intervention. The adverse event in Group B was an abdominal abscess that required surgical drainage. The patient experienced continuous abdominal pain one night after EUS-FNA, and dynamic CT demonstrated an abscess in front of the pancreatic body tumor, which was clearly related to the EUS-FNA puncture. Fortunately, she recovered after surgery and antibiotic therapy and could receive chemotherapy thereafter. There was no cancer seeding event up to 6 mo from the time of puncture in any patient in either group.

DISCUSSION

The aim of the current study was to investigate the results of two different approaches to obtain pancreatic biopsy specimens, which are a percutaneous approach and EUS-FNA, because this issue has seldom been ad-

dressed[12]. Our results confirmed the usefulness of EUS-FNA, especially with regard to cytology. The National Comprehensive Cancer Network Guidelines (2012) require that cytological or histological confirmation is needed for the diagnosis of unresectable pancreatic carcinoma[13]. In patients with stage IV PC, a biopsy of the metastatic lesion is preferred for proof of cancer. However, in those with stage III PC and some patients with stage IV PC in whom it is difficult to access metastatic sites for biopsy procedures, the primary tumor of the pancreas must be targeted to obtain proof of cancer. Pancreatic juice cytology was developed in the early 1980s and is still being performed; however, cancer cells cannot easily be observed by collection of pancreatic juice[1,2,14]. Percutaneous needle biopsy was developed with the expectation of a more definitive method to obtain proof of cancer from the primary pancreatic tumor[3,15,16]. Our institute then used percutaneous needle biopsy under extracorporeal US guidance as the standard for histological confirmation of the pancreatic primary tumor. Recently, EUS-FNA was introduced and was used mainly in high-volume cancer centers in Japan[17-22]. As a result of the risk of cancer seeding as well as other risks with percutaneous biopsy, we adopted EUS-FNA beginning in November 2009 in place of percutaneous biopsy. We expected that EUS-FNA would have advantages over a percutaneous procedure with regard to efficacy in confirmation of cancer and avoiding adverse reactions before administering chemotherapy to patients with PC.

Our results demonstrated that EUS-FNA is effective and feasible for obtaining proof of cancer in candidates for PC chemotherapy. In fact, EUS-FNA might have merits with regard to obtaining specimens from small tumors or tumors in the pancreatic tail, for which performance of percutaneous biopsy is difficult[2,23-27]. In this study, the location of the target tumor was most frequent at the body of the pancreas in Group A. In addition, the target tumors were larger in Group A than in Group B. These findings suggest that patients might have been excluded from Group A in which difficulty could be expected in making a puncture because the tumor was either small or difficult to delineate. In these cases, endoscopic retrograde cholangiopancreatography or liver biopsy might have been performed to obtain confirmation of malignancy, if possible.

Horwhat *et al*[12] have performed a randomized controlled trial of EUS-FNA and percutaneous biopsy of the pancreas (US- and CT-guided) in 2006. Although there was no statistically significant difference in accuracy between the two methods, the results showed that EUS-FNA had the advantage in the diagnosis of pancreatic malignancy. In our study, the diameters of the target tumors in the EUS-FNA group (Group B) were smaller than those in the US-FNA group (Group A) and the deviation of distribution around the puncture site was smaller in the EUS-FNA than the US-FNA group. Our results indicated high performance through the use of EUS-FNA and are not inconsistent with those of Horwhat *et al*[12]. In the present study, there was no analysis of accuracy in the two groups, because our institution is an oncology hospital and we rarely perform biopsies of benign cases.

The benefits of EUS-FNA might be maximized to make a pathological diagnosis in patients with an abdominal tumor of an uncertain type. The definite merit of our EUS-FNA procedure was thought to be rapid cytological results, but perhaps success in this regard was mainly due to the contribution of an on-site cytotechnologist and not to the EUS-FNA procedure itself. Iglesias-Garcia *et al*[28] have claimed that on-site cytological evaluation improves the diagnostic yield of EUS-guided FNA for the cytological diagnosis of solid pancreatic masses. Savoy *et al*[29] have pointed out that even trained endosonographers have variable and, in some cases, inferior abilities in interpreting on-site cytology in comparison with cytotechnologists. In the present study, we had adequate specimens for all cases in the EUS-FNA group. This is natural because we continued the examination until we obtained a sufficient quantity of specimens that were checked by the on-site cytotechnologist. On the contrary, there was no difference in the rate of adequate specimens obtained for histological examination between the EUS-FNA and US-FNA groups, because the collected tissue was checked by the examiner's naked eye in both groups. The presence of an on-site cytotechnologist to accompany EUS-FNA is considered to be necessary, at least, in high-volume centers.

In the present study, the positivity rate for malignancy was higher for EUS-FNA cytology than for histology. Supporting the current results, another study has shown that the positivity rate for malignancy in EUS-FNA cytology of the pancreas was higher than that in histology[30].

As previously reported, EUS-needle core biopsy is useful for histological and cytological diagnosis in terms of sample volume[31]. In addition, the combined results of EUS-FNA cytology and EUS-needle core biopsy have been reported to improve diagnosis[32-34]. However, to confirm the malignancy, EUS-FNA cytology is more useful than EUS-needle core biopsy[35]. This result is similar to the results of our study, indicating that cytology might be more useful than histology for the diagnosis of malignancy.

In the current study, there was no cancer seeding in any patient in either group. As previously reported, there were rare cases of seeding among patients who underwent US-guided FNA[36]. With regard to the puncture route, we suggest that there is less possibility of seeding in patients who undergo EUS-FNA than in patients who undergo US-FNA, although some recent studies have shown the possibility of seeding in patients who undergo EUS-FNA[37-39]. We did inform patients who were scheduled to undergo EUS-FNA about the possibility of this complication.

The limitations of our study included its retrospective nature. Furthermore, there were no cases of benign pancreatic conditions to enable an evaluation of US and EUS-FNA for accurate differentiation between malignant

and benign diseases.

In conclusion, EUS-FNA, as well as percutaneous needle aspiration, is an effective modality to obtain cytopathological confirmation in patients with advanced PC. EUS-FNA cytology was able to detect malignancy at a high rate. We believe that EUS-FNA has advantages for smaller tumors located deeply and for tumors in which the diagnosis is uncertain by various other imaging modalities.

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COMMENTS

Background

Ultrasonography-guided fine-needle aspiration (US-FNA) biopsy or computed tomography (CT)-guided FNA biopsy was used for histological/cytological diagnosis of pancreatic cancer (PC). US-FNA is limited to masses in the pancreatic tail. CT-guided FNA is time-consuming and limited by a substantial false-negative rate. There have been concerns about percutaneous cancer seeding and difficulty in puncturing for small tumors. Endoscopic ultrasound (EUS)-guided FNA has been developed as a more feasible method of obtaining definitive specimens for the diagnosis of PC. Studies on the results of the two different approaches to obtain pancreatic biopsy specimens, which are the percutaneous approach and EUS-FNA, have rarely been conducted.

Research frontiers

The benefits of EUS-FNA might be maximized to be able to make a pathological diagnosis in patients with an abdominal tumor of an uncertain type.

Innovations and breakthroughs

EUS-FNA is effective and feasible for obtaining proof of cancer in PC chemotherapy candidates. In fact, EUS-FNA might have advantages with regard to obtaining specimens from small tumors or tumors in the pancreatic tail, for which performance of percutaneous biopsy is difficult.

Applications

The results suggest that EUS-FNA is the best method of obtaining cytological samples for diagnosis of unresectable PC. This method can be used for other types of cancer.

Terminology

On-site cytotechnologist: An on-site cytotechnologist should attend the puncture examination to confirm quickly the existence of atypical cells. The information of the cytotechnologist is more appropriate than that of the endoscopist.

Peer review

This is a good descriptive study in which EUS-FNA is a feasible and safe technique to acquire pancreatic specimens. The results are interesting in that the advantages of EUS-FNA over the percutaneous procedure are time between examination and diagnosis, the possibility of puncture of small tumors, and tumors in the tail of the pancreas.

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