Table 4 Prognostic indicators detected by Cox's univariate and multivariate analyses in patients with ER-positive primary breast cancer

Variable	Univariate			Multivariate		
	HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Disease-free survival						
SDF-1						
"High expression"	1		0.0047	1		0.015
"Low expression"	1.87	(1.22–2.67)		1.70	(1.12–2.52)	
Clinical stage						
1 or 2	1		<0.0001	1		<0.0001
3 or 4	2.87	(1.96–4.19)		2.53	(1.70–3.75)	
Nuclear grade[a]						
1, 2	1		<0.0001	1		0.0041
3	2.28	(1.59–3.30)		1.77	(1.20–2.62)	
Overall survival						
SDF-1						
"High expression"	1		0.036	1		0.046
"Low expression"	1.89	(1.05–3.21)		1.86	(1.01–3.26)	
Clinical stage						
1 or 2	1		0.0009	1		0.0040
3 or 4	2.57	(1.50–4.39)		2.28	(1.32–3.94)	
Nuclear grade[a]						
1, 2	1		0.0008	1		0.019
3	2.42	(1.45–4.19)		1.92	(1.12–3.37)	

Abbreviation: *95%CI* 95% confidence interval

[a] There was no relapse and death among the cases with ER-positive and nuclear grade 1, so we combined the category of "nuclear grade 1" with "2" and performed subset analyses of ER-positive patients

correlated with the amount of SDF-1 mRNA. Tumors with cytoplasmic-dominant immunoreactivity had a higher level of SDF-1 mRNA than those with negative and membrane-dominant immunoreactivity. Therefore, we evaluated SDF-1 protein expression immunohistochemically in the present cohort of 223 invasive breast cancers based on criteria emphasizing its intracellular distribution. In previous studies, SDF-1 immunoreactivity was detected on the cell membrane of gastric and ovarian tumor cells [32, 44], and in the cytoplasm of colorectal tumor cells [31]. No previous report has described both membranous and cytoplasmic immunoreactivity in a single type of cancer. Further studies are needed to validate the reproducibility of the present criteria for judgment of immunohistochemical data.

We showed that "high SDF-1 expression" was significantly correlated with nuclear expression of CXCR4 in all 223 breast cancers. This finding was concordant with previously reported results indicating that SDF-1 stimulation induced rapid nuclear internalization of CXCR4 [45, 46], and confirmed that the CXCR4/SDF-1 axis plays an important role in the progression of breast cancer.

In summary, the present study revealed that breast cancers showing "high SDF-1 expression" have a higher frequency of ER positivity, HER2 negativity, lower nuclear grade, and better patient outcome, not only overall, but also in patients with ER-positive tumors. Examination of SDF-1 expression in ER-positive invasive breast cancers might be useful for identification of patients with a potentially better clinical outcome, and could help avoid the prescription of unnecessary chemotherapy for them.

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The Breast Journal

LETTER TO THE EDITOR

Early Reduction in Standardized Uptake Value After One Cycle of Neoadjuvant Chemotherapy Measured by Sequential FDG PET/CT is an Independent Predictor of Pathological Response of Primary Breast Cancer

To the Editor:

^{18}F-fluorodeoxyglucose (FDG) levels on positron emission tomography (PET) reflect glucose metabolism in cancer cells (1). Currently, FDG PET combined with computed tomography (FDG PET/CT) has become employed as a non-invasive method for imaging glucose metabolism in tumors (2,3). The aim of the present study was to evaluate whether early metabolic changes after one cycle of neoadjuvant chemotherapy in FDG uptake evaluated by maximal standardized uptake value (SUV_{max}) could predict a pathological response of primary breast cancers.

Thirty-two tumors in 30 patients having primary invasive breast cancer were investigated. All patients had received a standard neoadjuvant chemotherapy regimen comprising four cycles of epirubicine (90 mg/m^2) and cyclophosphamide (600 mg/m^2) on a triweekly basis and sequential use of 12 cycles of weekly paclitaxel (80 mg/m^2) (25 patients) or four cycles of triweekly docetaxel (60 mg/m^2) (five patients). The procedure of FDG PET/CT has been described (4). Sequential FDG PET/CT (Biograph LSO Emotion; 3D model, Siemens, Germany) was performed at the baseline (baseline PET/CT), after one cycle of chemotherapy (PET/CT2), after four cycles of chemotherapy (PET/CT3), and prior to surgery (PET/CT4). Tumors showing a 40% reduction or more in SUV_{max} on PET/CT2, when compared with the baseline PET/CT, were defined as metabolic responders and those showing a change of less than 40% in SUV_{max} were considered to be metabolic nonresponders (Fig. 1).

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Baseline characteristics of patients and tumors, determined by conventional modalities, are shown in Table 1. Baseline SUV_{max} was significantly higher in metabolic responders [10.2 ± 6.4 SD] than nonresponders (6.7 ± 3.1 SD) ($p = 0.05$). The percentage of tumors with nuclear grade 3 was significantly higher among the metabolic responders (71%) than among the nonresponders (16%) ($p = 0.03$). There were no significant differences between metabolic responders and nonresponders with regard to patient age, T-stage, nodal status, hormone receptor status, or HER2 status.

The average degree of decrease in SUV_{max} at PET/CT2 in comparison with the baseline SUV_{max} was 57.9% (±11.7 SD) in metabolic responders and 10.3% (±15.7 SD) in metabolic nonresponders ($p < 0.0001$). Clinical response after completion of chemotherapy was measured using ultrasound or CT combined with FDG PET, and evaluated based on RECIST. On the basis of clinical response, five (71%) and two (29%) of the seven tumors with a metabolic response exhibited partial response (PR) and complete response (CR), respectively, while 18 (72%) of the 25 nonresponding tumors had PR. No nonresponding tumors showed CR. Metabolic responders showed a significantly excellent clinical response rate (100%) in terms of PR or CR in comparison with that (72%) for non-responding tumors ($p = 0.001$) (Table 2a).

Histological criteria for assessment of therapeutic response were based on General Rules for Clinical and Pathological Recording of Breast Cancer 2008 (5). Among a total of 32 tumors, six (19%) and two (6%) were found to have grade 3, or pathological complete response (pCR), and to have grade 2b, or near pCR, where only a few residual invasive cancer cells were seen, respectively. Among the seven tumors with a metabolic response, three (43%) and two (29%) showed pCR and near pCR, respectively. Among the 25 tumors without metabolic response,

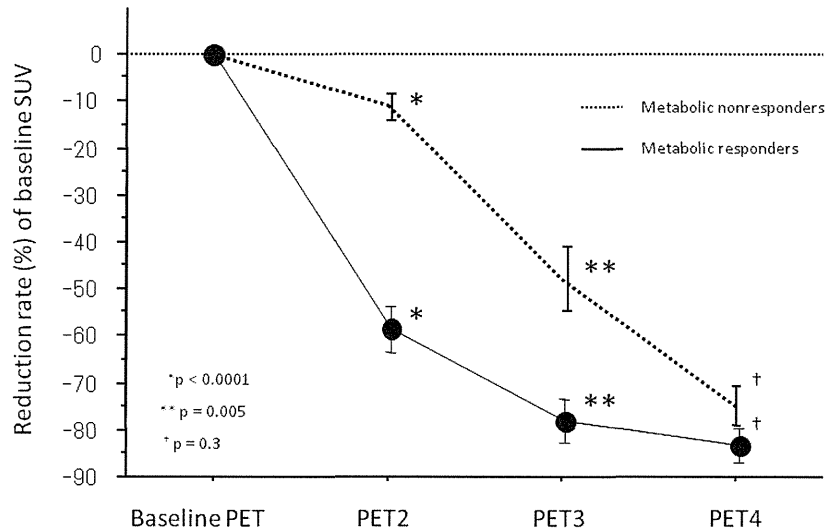

Figure 1. Reduction in standarized uptake value (SUV) of primary breast cancer after adjuvant chemotherapy, detected by FDG PET/CT. Mean reduction rate of SUV during neoadjuvant chemotherapy was significantly different at PET/CT2 ($p < 0.0001$) and at PET/CT3 ($p = 0.005$) but not at PET/CT4 ($p = 0.3$) between tumors showing,and not showing a metabolic response. Thirty-two tumors were divided into metabolic responders and metabolic nonresponders based on a cut-off value of 40% reduction in SUV at PET/CT2 compared with baseline PET/CT.

Table 1. Patients and tumors characteristics between metabolic responders and nonresponders

Variables	Total *n* = 32 (%)	Responder *n* = 7 (%)	Nonresponder *n* = 25 (%)	p-value
Age, years (average ± SD)	54.9 ± 10.1	58.9 ± 14.5	53.5 ± 8.0	0.2
Baseline SUV (average ± SD)	7.5 ± 4.2	10.2 ± 6.4	6.7 ± 3.1	0.05
T-stage				
1	5 (16)	1 (14)	4 (16)	0.3
2	21 (66)	5 (72)	16 (64)	
3	3 (9)	0 (0)	3 (12)	
4	3 (9)	1 (14)	2 (8)	
Nodal status				
Negative	14 (4)	3 (43)	11 (44)	0.9
Positive	18 (5)	4 (57)	14 (56)	
Nuclear grade				
1 and 2	23 (72)	2 (29)	22 (88)	0.03
3	9 (28)	5 (71)	3 (12)	
Hormone receptor status				
ER and/or PR positive	25 (78)	4 (57)	21 (84)	0.5
ER and PR negative	7 (22)	3 (43)	4 (16)	
HER2 status				
0, 1+, 2+	25 (78)	3 (43)	22 (88)	0.06
3+	7 (22)	4 (57)	3 (12)	

SD, standard derivation; SUV, standardized uptake value; ER, estrogen receptor; PR, progesterone receptor.

Table 2. Clinical and pathological response to neoadjuvant chemotherapy between metabolic responders and nonresponders

(a) Post-chemotherapeutic clinical response

	PD	SD	PR	CR	Total
Metabolic responder	0	0	5	2	7
Metabolic nonresponder	2	5	18	0	25
Total	2	5	23	2	32
					p = 0.001

(b) Post-chemotherapeutic pathological response

	pPD	pPR	Near pCR	pCR	Total
Metabolic responder	0	2	2	3	7
Metabolic nonresponder	1	21	0	3	25
Total	1	23	2	6	32
					p = 0.01

PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response; pCR, pathological CR.

three (12%) showed pCR, but none showed near pCR. Tumors showing a metabolic response showed a significantly higher percentage of pCR/near pCR than tumors showing no metabolic response ($p = 0.01$) (Table 2b).

Univariate analysis showed that metabolic response [$p = 0.005$, hazard ratio (HR) = 18.3, 95% confidential interval (CI) 2.4–140.4], HER2 overexpression ($p = 0.005$, HR = 18.3, 95% CI 2.4–140.4), and hormone-receptor negativity ($p = 0.04$, HR = 7.0, 95% CI 1.1–44.1) were predictive of pCR/near-pCR to neoadjuvant chemotherapy. Nuclear grade was

marginally associated with pCR/near-pCR (p = 0.1, HR = 4.2, 95% CI 0.6–27.4). No significant association between other factors and pCR/near-pCR was found. Multivariate analysis employing a logistic regression model showed that metabolic response remained an independent variable for predicting pCR/near-pCR (p = 0.05, HR = 11.9, 95% CI 1.1–104.9), but HER2 overexpression and hormone-receptor negativity were not (p = 0.1 and 0.4 respectively).

This study indicated that assessment of FDG PET/CT after one cycle of chemotherapy has independent value for early prediction of a pathologic response of primary breast cancer to standard neoadjuvant chemotherapy regimen followed by taxane.

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● 臨床生物学的特性

乳癌の circulating endothelial cell

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要旨

血管新生は悪性腫瘍の特異的性質であり，抗腫瘍薬の標的としても重要視され，さまざまな分子標的薬が開発されている．乳癌に対しても血管新生阻害薬の効果が注目されている．しかし，血管新生の状況や血管新生阻害薬の治療効果を反映するバイオマーカーは少ない．その中で末梢循環内皮細胞（circulating endothelial cell：CEC）は血管新生や血管新生阻害薬の効果予測のサロゲートマーカーとしての有用性が示唆されている．CEC についての総論と共に，乳癌治療における CEC の現況について述べる．

はじめに

固形腫瘍においては，腫瘍の増大に伴い微小環境は低酸素や低栄養の状態となり，それに対応しがん血管新生が行われる．すなわち，がん血管新生は悪性腫瘍の特異的な現象である[1)2)]．がん血管新生に関係する分子は，抗悪性腫瘍薬の標的として重要視され，現在，さまざまな分子標的薬が開発されている．乳癌の領域でも，転移性乳癌に対し行われた3つの前向き比較第Ⅲ相試験において，従来型の殺細胞薬（cytotoxic drug）に対するベバシズマブの無増悪生存期間に関する上乗せ効果が示された[3~5)]

キーワード：CEC，CEP，血管新生，乳癌，バイオマーカー

末梢循環内皮細胞（CEC）の定義と測定方法

CEC は血液中に出現した血管内皮細胞である．何らかの原因で生じた血管傷害により，血管壁の内皮細胞が剥がれ落ちて出現すると考えられている．正常でも少数は出現しており[6)7)]，ある種の悪性腫瘍[6~9)]，敗血症[10)]，機械刺激[11)]，冠動脈疾患[12)13)] などにより増加するとの報告もある．

一方，CEC と並んで評される血液中の細胞群には，末梢循環内皮前駆細胞（CEP）がある．血管新生は，局所において既存の血管内皮細胞が誘導されて管腔形成するだけではなく，骨髄の血球血管芽細胞や血管芽細胞から分化した血管内皮前駆細胞が血液中に誘導され，局所の血管新生の場に遊走・定着し血管内皮細胞となる．この血液中の血管内皮前駆細胞が CEP であると考えられており，腫瘍の血管新生のサロゲートマーカーとして CEC と共に多くの報告[14)] がされているが，役割の詳細については明らかにはされていない．

CEC・CEP の測定方法の原理は，細胞の表面抗原に特異的な抗体を用いて識別し，数の測定を行う．フローサイトメトリーなどの技術の発展により，幾つかの表面抗原を組み合わせることで，特定の細胞群の抽出がより簡便となった．近年では主に蛍光染色・特異抗体標識磁気ビーズ・フローサイトメトリーが用いられる．多くは CD31／CD146（血管内皮に出現）陽性，CD45（リンパ球，骨髄系細胞に出現）陰性，核内 DNA 陽性の細胞を CEC とするが，研究により組み合わせは異なっており，統一された見解には至っていない．また，アポトーシス細胞と生細胞，活性化細胞と休止細胞の亜分類を行い，より詳細な解析を行うなどの検討もされている．表1に表面マーカーを示す．

CEC の測定で商品化されているものには，CellSearch system®（Veridex 社）がある．この方法は，まず全血から抗 CD146 抗体標識磁気ビーズを用い CD146 陽性細胞を磁気的に分離し，さらに蛍光染色により核内 DNA 陽性，CD105 陽性，CD45 陰性細胞を抽出し，それらを CEC としている．しかし，コストが1回あたり 72,000 円と高価である．

表1　末梢循環内皮細胞（CEC），末梢循環内皮前駆細胞（CEP）の主なマーカー

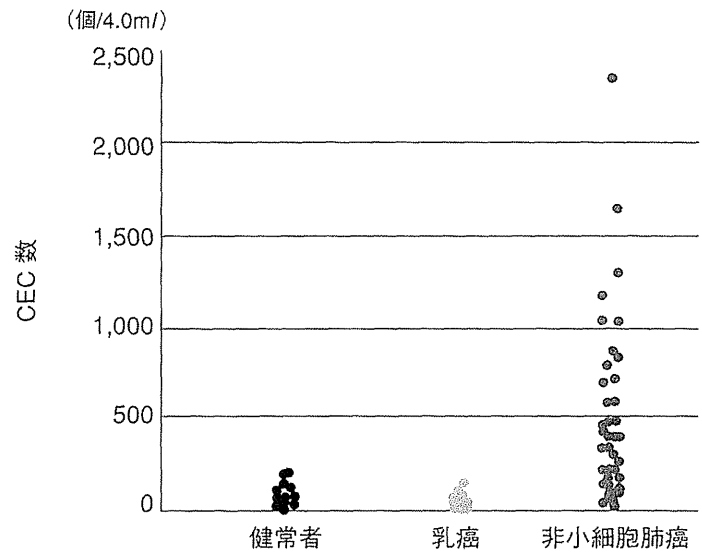
マーカー	別名	CEC	CEP	血小板	リンパ球	成熟骨髄細胞	血球前駆細胞
DNA	−	+	+		+	+	+
RNA	−	+	+	+	+	+	+
CD45	PTPRC				+	+	+
CD133	prominin		+				+
CD34	Stem cell marker		+				+
CD105	endoglin	+ (activated)	+				+
CD146	MCAM	+	+	+ (activated)			
CD144	VE-cadherin	+	+				+
CD31	PECAM-1	+	+	+	+	+	+
CD13	−	+	+				+
CD117	C-KIT		+				
VEGFR1	Flt-1	+					
VEGFR2	KDR	+	+				
vWF	−	+		+			

vWF：von Willebrand factor，MCAM：melanoma cell adhesion molecule，
PECAM-1：platelet/endothelial cell adhesion molecule-1，KDR：kinase insert domain receptor，
PTPRC：protein tyrosine phosphatase, receptor type, C,

悪性腫瘍と末梢循環内皮細胞（CEC）

多くの報告では健常者に比べ悪性腫瘍患者で CEC が上昇するとしている[6~9]．だが，がん種，進行期によってばらつきがある．Rowand ら[6]は 255 人の健常者の血液を用い，CEC を CD146 陽性，ジアミジノフェニルインドール（DAPI）陽性，CD105-phycoerythrin（PE）陽性，CD45 陰性の細胞としてフローサイトで検出し，報告している．結果，健常者 255 人中，極端に高値を示した 6 人を除く 249 人の CEC の平均値は 21 ± 18 / 4 m*l*，中央値は 15（0 ～ 97）/ 4 m*l* であった．一方，さまざまな転移性がん 206 人についても検討を行ったところ，全がんの平均値は 111 ± 255 / 4 m*l*，中央値は 34（0 ～ 1,939）/ 4 m*l* と健常者に比較し高値であった．しかし，がん種により CEC の平均値，中央値は異なり，肺癌 35 例は 146 ± 270 / 4 m*l*，75（11 ～ 1,546）/ 4 m*l*，乳癌 50 例は 78 ± 96 / 4 m*l*，38（1 ～ 471）/ 4 m*l*，大腸癌 49 例は 86 ± 204 / 4 m*l*，29（0 ～ 1,375）/ 4 m*l* であった．

図1　健常者，乳癌，肺癌での末梢循環内皮細胞（CEC）数の比較



	CEC 数（個/4.0ml）
健常者（n=50）	35.9 ± 38.4
乳癌（n=37）	23.5 ± 28.4
非小細胞肺癌（n=56）	459 ± 710

我々がCellSearch system®を用いて検討したところ，健常者50例の平均値は 35.9 ± 38.4 / 4 m*l*，ⅢB，Ⅳ期の非小細胞肺癌 56 例では 459 ± 710 / 4 m*l* であり，腫瘍サイズ，進行期などの臨床背景には関係性は認めなかった[7]．一方，早期乳癌 37 例の検討では，CEC の平均は 23.5 ± 28.4 / 4 m*l* であり，健常者に比し，明らかな上昇は認めなかった（図1）．現在も多がん種で検討を進めているが，がん種によるばらつきを認めている．

血管新生阻害薬の予測因子

血管内皮増殖因子（VEGF）を抗原とするマウス抗ヒト VEGF モノクローナル抗体のヒト組換え型抗体であるベバシズマブは早期より開発が行われ，大腸癌[15)16)]，非小細胞肺癌[6)]，乳癌[3)17)18)]，腎癌[19)]，卵巣癌などの多がん種にわたり大規模な第Ⅲ相試験が行われてきた．転移・

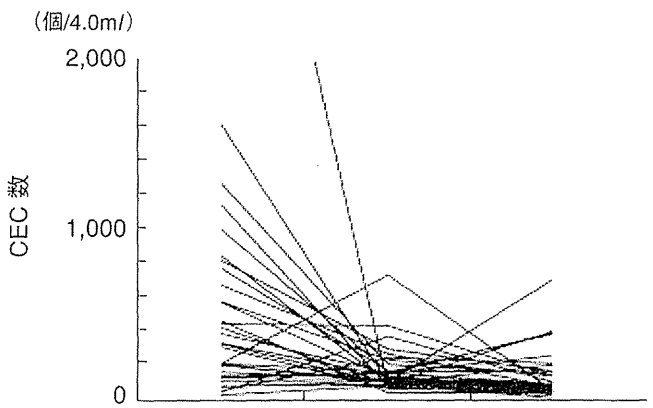
再発大腸癌，非小細胞肺癌では生存期間を，乳癌では無増悪生存期間を延長している．また，卵巣癌では術後補助療法の標準治療法であるパクリタキセル/カルボプラチン併用に比べ，ベバシズマブ併用かつ維持療法をした群で有意に無病生存期間を延ばしたという報告が2010年度の米国臨床腫瘍学会（ASCO）で行われる予定である．このように，臨床においてはベバシズマブの有用性は実証されている．

また，その他にVEGF受容体（VEGFR），血小板由来増殖因子受容体（PDGFR），*fms*様チロシンキナーゼ3（FLT3），KITをターゲットにする多標的阻害薬であるsunitinibやRaf，VEGFR-2, 3，FLT3，c-Kitの阻害薬であるsorafenibの有用性も報告されている．sunitinibは腎癌[20]で無増悪生存期間を，消化管間質性腫瘍（GIST）[21]で生存期間を延長させている．sorafenibは腎癌[22]，肝臓癌[23]で生存期間を延ばしている．

血管新生阻害薬の効果予測因子としては，過去にさまざまな検討がされている．腫瘍内の微小血管密度（MVD）[24]は組織のがんの血管に特異的な抗体で血管内皮を染色し鏡験にて観察する方法であるが，組織採取が侵襲的であること，生検による採取部位が全体像を反映するか否かの問題などがある．血清中のVEGF，インターロイキン8（IL8）の報告がある．Motzerら[25]は転移性腎癌に対するsunitinibの第Ⅱ相試験で，VEGFや胎盤成長因子（PLGF）を治療前後に測定した．結果，VEGF，PLGFは上昇，VEGFR2は低下した．しかし，小規模な検討であり，十分な検証もされておらず，確立した効果予測因子に成りえていない．その他，ダイナミック造影MRI（DCE-MRI）は腫瘍内の血流や透過性を画像で解析する方法であり，臨床試験でも行われている[26]．しかし，施設が限られることなど簡便さに欠けることから，十分な検証がされていない．

1978年にHladovecら[27]が血液中に上皮由来の細胞が含まれていることを報告してから，CECについてのさまざまな検討がされてきた．*In vivo*では悪性リンパ腫細胞株をマウスに移植したところ，腫瘍体積の増加に伴い，CECが上昇したとの報告[28]がある．またBeaudryらは，マウスにVEGFR2チロシンキナーゼ阻害薬であるvandetanibを投与した場合，肺癌細胞株を移植していないマウスで

図2 非小細胞肺癌におけるパクリタキセル/カルボプラチン投与後の末梢循環内皮細胞（CEC）の推移（文献[7]より引用）



	治療前	day 8	day 22
mean ± SD	595 ± 832	176 ± 141*	173 ± 189*

*：P< 0.05

は CEC の変化はなかったが，肺癌細胞株を移植したマウスでは投与前に比較し投与後には CEC が上昇したと報告[29]している．この結果から，筆者らは，がんにおける CEC の上昇は腫瘍の内皮細胞由来であると結論づけている．一方我々は，進行非小細胞肺癌 34 例に対し，パクリタキセル/カルボプラチン併用療法を行った症例の治療前後で，CellSearch system®を用い CEC を測定した[7]．治療前の CEC は腫瘍体積との相関はなく，薬剤投与による変化としては治療前平均（595 ± 832 / 4 ml）に比較し，治療後 8 日目（176 ± 141 / 4 ml），22 日目（173 ± 189 / 4 ml）には低下した．また，400 / 4 ml 以上の高値群は低値群に比較し，無増悪生存期間が有意に良好であった（P = 0.019）（図 2）．しかし，ゲムシタビン/シスプラチン併用療法を行った進行非小細胞肺癌に対し同様の検討を行ったところ，治療前平均（306 ± 463 / 4 ml）に比較し，治療後 8 日目（450 ± 726 / 4 ml），22 日目（509 ± 833 / 4 ml）には上昇した．また，400 / 4 ml 以上の高値群は低値群に比較し，無増悪生存期間が不良であった．パクリタキセルやドセタキセルは微小管阻害薬であるが，抗血管形成の作用につい

ての報告[30)31)]があり，腫瘍血管への影響が大きいなどの理由が考えられるが，いまだ実証はされていない．以上のように *in vivo* や臨床での検討には一定の見解がない．しかし，いずれも小規模な検討であり，*in vivo* も含め，より大規模な検討が必要であろう．

乳癌と末梢循環内皮細胞（CEC）

乳癌においても CEC の報告は幾つかある．Furstenberger ら[9)]は，アンスラサイクリン系薬剤単独もしくはタキサン系薬剤を追加する術前化学療法を施行した乳癌患者の CEC を測定した．測定方法は CD45 陰性，CD146，CD31，CD34 陽性細胞を CEC とし，フローサイトメトリーで計測し，1 μl 中の CEC で検討をしている．結果，治療前では健常者に比して有意に高く（1.3 CEC / μl vs. 5.7 CEC / μl, P＜0.01），化学療法後に低下したと報告している．ヒト上皮増殖因子受容体 2（HER2）陰性もしくはトラスツズマブ抵抗性の HER2 陽性転移性乳癌に対して行われたベバシズマブとタキサン系薬剤の併用療法の有効性をみた MO19391 試験では，付随研究として 67 例で治療前後の CEC を CellSearch system® を用いて測定した[32)]．結果は 45 歳以上（P＝0.01）で CEC が高く，乳酸脱水素酸素（LDH）の上昇（P＜0.01）とも関係したが，転移場所や個数とは関係しなかった．治療前，3 コース目投与前の CEC の中央値はそれぞれ，17（1 ～ 769）CEC / 4 ml，26（2 ～ 335）CEC / 4 ml で，治療後に有意に上昇した（p＝0.013）．また，カットオフ値を 20 CEC / 4 ml にすると，治療後に上昇した場合には無病生存期間が有意に延長した（P＝0.003）．

Calleri ら[33)]はシクロホスファミドとカペシタビン内服とベバシズマブ点滴静注を併用した 46 人について，six-color フローサイトメトリーを用い，DNA 陽性，CD45 陰性，CD31 陽性，CD146 陽性細胞を CEC として治療前後に測定を行った．結果，治療前の CEC（viable）が 2.2 / μl 以上の場合，無病生存期間が有意に長く（P＝0.021），また，増悪時には治療前（P＝0.0002）や治療開始 2 ヵ月目（P＝0.039）に比較して有意に低下すると報告している．

Goon ら[34)]によると，160 例の乳癌患者より採取した血液を用い，CD45 陰性，CD146 陽性，CD34 陽性細胞を CEC として，フローサ

イトメトリーで計測を行い，臨床背景との比較を行った．結果，Nottingham Prognostic Index（NPI）で予後不良群は，予後良好もしくは中等度群に比較し CEC が高かった：8（4～8）cells / m*l*，8（4～12）cells / m*l* vs. 14（8～22）cells / m*l*，P<0.001．また，多変量解析では血管浸潤が多いほど（P<0.05），腫瘍サイズが大きいほど（P<0.001）CEC が有意に高かった．だが，手術前後での変化は認めなかった．

以上のように，乳癌における CEC の検討は広く行われているが，進行期，腫瘍体積，転移部位，個数との関係や治療による変化についても一定の見解はない．また，既存の報告，我々の検討（図1）でも乳癌における CEC の基礎値は肺癌などと比較して低く，カットオフ値を置くうえでも難しい問題がある．

新しい血管形成関連細胞

CEC，CEP に加え，新しい血液中の細胞集団の報告がされ始めている．Bogos ら[35]はリンパ毛細管の増生に関与すると考えられる CD34 陽性，VEGFR3 陽性細胞である lymphatic / vascular endothelial progenitor cell（LVEPC）が非小細胞肺癌において増加しており，また，リンパ管侵襲や予後に関与していると報告している．Grunewald らは，腫瘍から分泌される VEGF により骨髄から動員された CD45 陽性，VEGFR1 陽性，VEGFR2 陰性の recruited blood circulating cells（RBCCs）について報告[36]している．そのほか，tumour-associated stroma cells（TASCs）[37]や TIE2-expressing monocytes（TEMs）[38]や tumour-associated dendric cells（TADCs）[39]などもあり，腫瘍の血管新生に関与すると考えられる．

おわりに

CEC は血管新生や血管新生阻害薬の効果予測因子として有望であるが，いまだ実用段階に至っていない．今後，乳癌治療においても血管新生阻害薬の役割は大きいと予想される．大規模な前向き臨床試験の中で，治療の選択に役立つマーカーの探索が急務であると考える．

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Circulating Endothelial Cell in the Treatment of Breast Cancer

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ORIGINAL ARTICLE

Paclitaxel-induced peripheral neuropathy in patients receiving adjuvant chemotherapy for breast cancer

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Abstract
Background The long-term outcomes and risk factors of paclitaxel-induced peripheral neuropathy (PIPN) have not yet been fully elucidated.
Methods We identified 219 breast cancer patients who received paclitaxel as adjuvant chemotherapy between 2002 and 2009. We retrospectively analyzed the incidence, time to onset, duration, and risk factors for PIPN by chart review.
Results Of the 219 patients, 212 developed PIPN (97%) during a median follow-up time of 57 months (range 5.3–95.5). Median time to PIPN onset was 21 days (range 11–101) for the entire patient population: 35 days (range 14–77) for weekly administration and 21 days (range 11–101) for tri-weekly administration. PIPN caused termination of paclitaxel treatment in 7 patients (4%). Median duration of PIPN was 727 days (range 14–2621 days). PIPN persisted in 64 and 41% of patients at 1 and 3 years after initiating paclitaxel, respectively. Age ≥60 years and severity of PIPN were significantly associated with PIPN duration.
Conclusions PIPN persists longer in older patients and in those who experience severe neuropathy. Further studies to identify the risk factors for PIPN are warranted.

Keywords Breast cancer · Paclitaxel · Peripheral neuropathy

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Introduction

Paclitaxel (PTX) is a key component of many therapeutic regimens in both early-stage and metastatic breast cancer [1–4]. PTX, a microtubule-stabilizing agent, binds to microtubules and abolishes their dynamic behavior, leading to inhibition of cell proliferation [5]. The agent is known to cause peripheral neurotoxicity (PN), which may result in discontinuation of treatment and poor quality of life.

The incidence of PTX-induced PN (PIPN) is known to depend on several factors, including dosages per cycle, treatment schedule, duration of infusion, cumulative dosage, and co-morbidity such as diabetes [6–11]. Although the clinical response of tumors to PTX is an important factor in selecting a chemotherapy regimen, it is also prudent to evaluate the risk of developing PN associated with each regimen, especially for patients already at high risk for neuropathy. The risk of sensory neuropathy is proportional to the dose of PTX administered. Grade 3 or 4 sensory neurotoxicity occurs in 20–35% of patients receiving 250 mg/m^2 every 3 weeks compared to 5–12% using doses ≤200 mg/m^2 every 3 weeks [12]. The weekly schedule is associated with higher neurotoxicity than the tri-weekly schedule. In a previous study, grade 3 neuropathy occurred significantly more often with the weekly regimen than with the tri-weekly regimen (24 vs. 12%) [13]. In another study, which compared weekly versus

tri-weekly PTX dosages, it was reported that grade 2, 3, or 4 neuropathy occurred more frequently with weekly than with tri-weekly PTX administration (27 vs. 20%, respectively) [14].

The time to onset of PIPN was previously determined in a phase III trial of patients with metastatic breast cancer treated with PTX (175 mg/m^2) every 3 weeks; the mean total dose at the onset of grade 2 neurotoxicity was 715 mg/m^2 [15]. However, there are limited data available describing the outcome of PIPN and risk factors of severe PN. We therefore conducted a retrospective study to determine the duration of PIPN and to identify potential factors predicting severe or persistent PN.

Patients and methods

Data collection

This study included breast cancer patients treated with PTX as adjuvant chemotherapy at the National Cancer Center Hospital between 2002 and 2009. All patients met the following criteria: female gender; age >18 years; recipients of lumpectomy or mastectomy; and presentation of more than one axillary lymph node metastasis, as determined pathologically. The following patients were excluded from this study: those previously treated with PTX, those who presented with severe neuropathy before initiating PTX treatment, and those who discontinued PTX treatment after only 1 cycle for any reason.

We performed chart reviews for all patients to obtain the following information: age; gender; stage; hormonal status; human epidermal growth factor receptor-2 (HER2) status; previous surgical procedures (lumpectomy or mastectomy); adjuvant chemotherapy; adjuvant radiotherapy; PTX administration schedule; date of the first documentation of PIPN; maximum grade of PIPN; date of disappearance of PIPN symptoms. This study was approved by the local institutional review board.

Treatment schedule

Chemotherapy consisted of anthracycline followed by PTX regimens as generally recommended for high-risk breast cancer patients, according to the St. Gallen risk criteria at our division [16, 17]. However, therapeutic options could vary based on the physician's discretion. Patients received either 80 mg/m^2 of PTX on days 1, 8, and 15 of each 21-day interval for 4 cycles, following anthracycline plus cyclophosphamide (AC) (weekly administration schedule), or 175 mg/m^2 of PTX on day 1 of each 21-day interval for 4 cycles, following AC (tri-weekly administration schedule).

Grading of PIPN

Patients were evaluated during and after chemotherapy by medical oncologists. We graded PIPN retrospectively according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0 [18]. Grade 1 PIPN had paresthesias including tingling, but not interfering with function, while grade 2 had sensory alterations or paresthesias interfering with function but not interfering with activities of daily living (ADL). Grade 3 had sensory alterations or paresthesias interfering with ADL. Patients were determined to have PIPN if their score for sensory neuropathy was grade 1 or higher. The severity of pain was not evaluated in this study because of insufficient data.

Statistical analysis

The time to onset of PIPN was defined as the time from the date of PTX administration to the date of the first documentation of PIPN. The duration of PIPN was defined as the time from the date of first documentation of PIPN to the date of disappearance of the PIPN symptoms described. The time to onset and duration of PIPN were estimated by the Kaplan–Meier method. We used multivariate Cox regression analysis to identify the variables associated with the time to onset and duration of PIPN. Furthermore, to identify the risk factors for PIPN above grade 2, we applied multivariate logistic regression analysis. A 2-sided $P < 0.05$ was considered statistically significant. All analyses were performed by SAS software, version 9.2 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

Of the 227 patients initially identified, 2 were excluded due to severe neuropathy induced by combination chemotherapy with AC before being treated with PTX. Several patients discontinued systemic therapy before completion of 1 cycle due to the following adverse events: severe liver dysfunction (grade 3) ($n = 3$), acute renal failure (grade 3) ($n = 1$), allergic reaction (grade 3) ($n = 1$), and interstitial pneumonitis (grade 3) ($n = 1$). Finally, a total of 219 patients were included; 212 patients (97%) developed PIPN which was characterized by numbness and tingling, while 7 had no PIPN symptoms. The maximum severity of PIPN reached in each of the 212 patients was as follows: grade 1, 159 patients (75%); grade 2, 45 patients (21%); and grade 3, 9 patients (4%). Two patients needed dose modifications due to PIPN above grade 2. No patients postponed or skipped the scheduled PTX due to PIPN.

Baseline characteristics of the population are listed in Table 1. The median age of patients was 53 years (range 22–70). Eighteen patients had diabetes mellitus without neuropathy complications at baseline. Disease-free survival and overall survival were evaluated with a median follow-up time of 57.1 months (range 5.3–95.5). A total of 25 patients received weekly PTX: 23 following AC and 2 without AC. The remaining 194 patients received tri-weekly PTX: 182 following AC and 12 without AC. The mean dose intensity was 58 mg/week (range 16–80). Treatment cessation was deemed necessary in 9 patients (4%); reasons for cessation were PIPN (8 patients, 3 with grade 1, 1 with grade 2, and 5 with grade 3) and myelosuppression (1 patient).

Table 1 Patient characteristics

Variables	triPTX ($N = 188$)	wPTX ($N = 24$)	All ($N = 212$)
Age			
Median (range)	53 (22–70)	52 (32–68)	53 (22–70)
<60 (%)	141 (75.0)	17 (70.8)	158 (74.5)
≥60 (%)	47 (25.0)	7 (29.2)	54 (25.5)
Sex (%)			
Female	187 (99.5)	24 (100.0)	211 (99.5)
Male	1 (0.5)	0 (0.0)	1 (0.5)
Lymph (%)			
<4	118 (62.8)	12 (50.0)	130 (61.3)
≥4	70 (37.2)	12 (50.0)	82 (38.7)
Tumor size (%)			
<5 cm	153 (81.4)	18 (75.0)	171 (80.7)
≥5 cm	35 (18.6)	6 (25.0)	41 (19.3)
Surgery (%)			
Mastectomy	114 (60.3)	16 (66.7)	130 (61.3)
Lumpectomy	73 (39.2)	8 (33.3)	81 (38.2)
Excisional biopsy	1 (0.5)	0 (0.0)	1 (0.5)
Systemic therapy (%)			
Chemo	56 (29.8)	8 (33.3)	64 (30.2)
Chemo + endocrine	132 (70.2)	16 (66.7)	148 (69.8)
Radiation (%)			
No	69 (36.7)	8 (33.3)	77 (36.3)
Yes	119 (63.3)	16 (66.7)	135 (63.7)
Hormone (%)			
Negative	48 (25.5)	5 (20.8)	53 (25.0)
Positive	140 (74.5)	19 (79.2)	160 (75.0)
HER2 (%)			
Negative	156 (83.0)	16 (66.7)	172 (81.1)
Positive	32 (17.0)	8 (33.3)	40 (18.9)
Diabetes mellitus (%)			
No	171 (91.0)	23 (95.8)	194 (91.5)
Yes	17 (9.0)	1 (4.2)	18 (8.5)

triPTX tri-weekly paclitaxel, *wPTX* weekly paclitaxel, *chemo* chemotherapy

PIPN development time

The median time taken for the total patient group to develop PIPN was 21 days (range 11–101) (Fig. 1). With weekly administration of PTX, the median time taken to develop PIPN was also 21 days (range 11–101); the median time with tri-weekly administration was 35 days (range 14–77).

Cumulative dose

The mean cumulative dose at the onset of grade 1 or higher PIPN was 175 mg/m^2 for patients treated with PTX every 3 weeks and 320 mg/m^2 for weekly PTX patients.

Diabetes mellitus

Of 18 diabetic patients, all had PIPN and 3 had maximum grade 3 PIPN. Median time to PIPN onset was 21 days (range 20–21), and median duration of PIPN was 287 days (range 70–503). In patients without diabetes, median time to PIPN was 21 days (range 20–21), and median duration of PIPN was 231 days (range 190–271).

Risk factors correlated with PIPN

Multivariate analysis using a logistic regression model after stepwise selection revealed no significant correlations between time to PIPN onset and maximum PIPN severity (Table 2), while there were significant correlations between duration of PIPN and age (>60 years old) ($P = 0.027$) and between duration of PIPN and maximum PIPN severity ($P = 0.015$) (Table 3). Moreover, we could not identify

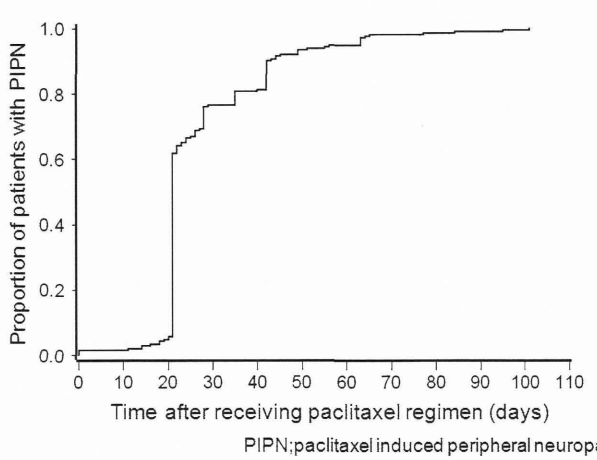

Fig. 1 Time taken for the total patient group to develop paclitaxel-induced peripheral neuropathy