癌でイエロー色細胞が多いかについてであるが，文献的な報告はない．あくまで推測であるが，発現するサイトケラチンの差異と，それによる染色液の色素拡散性の違いが原因と考えられる．色素拡散性は，色素分子の大きさと細胞質の構築の疎密度の両者が大きく関連している．サイトケラチンは上皮性細胞の細胞骨格を成すフィラメントであるが，分子量や生化学的分析によって約20種類の蛋白に分類される．肺癌と咽喉頭癌でサイトケラチンの蛋白発現が一部異なることが知られており[21,22]，それにより細胞質構造の疎密度に差が生じ，結果的に染色性が異なるのではないかと考えられる．

喀痰細胞診で発見される咽喉頭癌は無症状のT1の喉頭癌が多いといわれている[6]．当施設での発見例も喉頭癌が多く，Tis～T1の早期症例が大半であった．また今回，病期での検討はできなかったが，過去の喉頭癌の報告では病期やT因子が進行すれば異型細胞の出現数は増加するが[20,23]，T因子と癌細胞の形態には差はみられない[8]といわれている．

喉頭癌は早期発見が重要な予後良好因子である[23]．肺癌検診の集検喀痰細胞診においては，喉頭癌の発見も念頭におき，前述した喉頭癌に特徴的な細胞所見に留意することで喉頭癌の発見に寄与できる可能性があると考えられた．

V. 結　　語

集検喀痰細胞診において，喉頭癌と早期中心型肺癌では異なる細胞所見を呈していた．これらの細胞像に着目することで，より詳細な情報を臨床側に提供でき，喉頭癌の早期発見に寄与できる可能性が示唆された．

本論文の要旨は第47回日本臨床細胞学会秋期大会（2008年11月，東京）にて発表した．

謝辞　稿を終えるにあたり，御教示いただきました福島県立医科大学公衆衛生学講座後藤あや先生に厚く御礼申し上げます．

Abstract

Objective : Upper respiratory cancer may occasionally be detected in sputum cytology. We compared cytological features of laryngeal cancer and early bronchial lung cancer detected in cytology screening.

Study Design : 8 cases of laryngeal cancer and 14 early bronchial lung cancer cases detected in cytology mass screening between 1994 and 2006 were investigated. Severely atypical and squamous cancer cells extracted from specimens were compared statistically in terms of : staining properties ; size, shape, and brightness of cytoplasm ; properties of cell borders ; nuclear number and shape ; and nuclear chromatin.

Results : Both groups showed mildly increased chromatin. Approximately 90% of cells were small- to medium-sized orange- and yellow-staining with mild nuclear atypia. Laryngeal cancer specimens had numerous orange-staining cells with no cellular brightness, while early bronchial lung cancer specimens exhibited a significantly high numbers of yellow-staining cells and cellular brightness. Receiver operating characteristic (ROC) curve analysis identified ≧84% of non bright orange-staining cells with 100% sensitivity and 100% specificity for laryngeal cancer.

Conclusions : Sputum cytology findings differed between laryngeal cancer and early bronchial lung cancer-findings suggesting that focusing on cytology features may help to detect laryngeal cancer early.

文　　献

1) Neel, H.B. 3rd., Sanderson, D.R., Fontana, R.S., Taylor, W.F., Woolner, L.B. Sputum cytologic diagnosis of upper respiratory tract cancer. Second report. Ann Otol Rhinol Laryngol 1981 ; 90 : 312-315.
2) Fontana, R. S., Sanderson, D. R., Taylor, W. F., Woolner, L. B., Miller, W. E., Muhm, J. R., et al. Early lung cancer detection : results of the initial (prevalence) radiologic and cytologic screening in the Mayo Clinic study. Am Rev Respir Dis 1984 ; 130 : 561-565.
3) Frost, J. K., Ball, W. C. Jr., Levin, M. L., Tockman, M. S., Baker, R. R., Carter, D., et al. Early lung cancer detection : results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. Am Rev Respir Dis 1984 ; 130 : 549-554.
4) Flehinger, B. J., Melamed, M. R., Zaman, M. B., Heelan, R. T., Perchick, W. B., Martini, N. Early lung cancer detection : results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloan-Kettering study. Am Rev Respir Dis 1984 ; 130 : 555-560.
5) 斎藤裕夫，小野　勇，海老原　敏，吉積　隆，池田茂人，小野良祐．喀痰細胞診から発見に至った頭頸部癌（肺癌検診と頭頸部癌）．癌の臨床 1985 ; 31 : 1665-1668.
6) 佐藤雅美，斎藤泰紀，永元則義，遠藤千顕，薄田勝男，高橋里美・ほか．肺癌集検喀痰細胞診によって発見された肺癌以外の悪性腫瘍症例の検討．気管支学 1992 ; 14 : 328-333.
7) 森谷浩史，柳沼康之，渋谷広子，高橋一弘，黒沢美枝子，佐藤美賀子・ほか．喀痰細胞診陽性症例の精検方法に関する一考察．CT・口腔耳鼻科的診察の有用性．日臨床細胞誌 1993 ; 32 : 853-859.
8) 南雲サチ子，宝来　威，松田　実．喉頭癌および下咽頭癌の喀痰細胞診．日臨細胞誌 1987 ; 26 : 427-432.
9) Sato, M., Saito, Y., Usuda, K., Takahashi, S., Sagawa, M., Fujimura, S. Occult lung cancer beyond bronchoscopic visibility in sputum-cytology positive patients. Lung Cancer 1998 ; 20 : 17-24.
10) 日本肺癌学会．肺癌取扱い規約第6版．東京：金原出版株式会社 ; 2003. 90-91.

11) 細胞検査士会．細胞診標本作製マニュアル（呼吸器）．2003.
12) 近　京子，中嶋隆太郎，小野寺美枝，白鳥まゆみ，佐藤博俊，佐藤信二・ほか．早期肺扁平上皮癌のスクリーニングと判定における細胞質染色性の光輝性の意義と染色法の検討．日臨細胞誌 1999；38：15-22.
13) 佐藤美賀子，二瓶憲俊，千葉聖子，室井祥江，塚原　孝，高橋一弘・ほか．肺扁平上皮内癌の細胞像　癌未確定例由来の異型細胞との比較．日臨細胞誌 2005；44：56-62.
14) 三竿貴彦，佐藤　功，小林琢哉，伊達　学．喀痰細胞診D判定で発見され確定診断までに3年を経過した早期喉頭癌の1例．気管支学 2000；22：276-279.
15) 佐川元保，斎藤泰紀，遠藤千顕，高橋里美，薄田勝男，菅間敬治・ほか．Tx肺癌例との鑑別が困難であった上気道癌　喀痰細胞診陽性で部位診断困難例に対する精査方法に関する考察．肺癌 1991；31：259-263.
16) 佐藤丈晴，二瓶憲俊，神尾淳子，高橋一弘，石田　卓，森村　豊・ほか．肺の初期浸潤癌と細胞診　集検喀痰細胞診で発見された上皮内癌と初期浸潤癌の細胞像について．日臨細胞誌 2007；46（Suppl. 2）：376.
17) 神尾淳子，二瓶憲俊，安達則子，高橋一弘，冨田　健，石田　卓・ほか．集検喀痰細胞診で発見された上気道癌の細胞像について．日臨細胞誌 2005；44（Suppl. 1）：165.
18) 佐藤丈晴，神尾淳子，冨田　健，石田　卓，森村　豊，森谷浩史・ほか．集検喀痰細胞診で発見された上皮内癌の細胞像について．日臨細胞誌 2004；43（Suppl. 2）：454.
19) 斎藤泰紀，今井　督，薄田勝男，菅間敬治，佐川元保，永元則義・ほか．気管支原発早期扁平上皮癌の細胞診断学的検討．日臨細胞誌 1985；24：686-698.
20) Matsuda, M., Nagumo, S., Horai, T., Yoshino, K. Cytologic diagnosis of laryngeal and hypopharyngeal squamous cell carcinoma in sputum. Acta Cytologica 1988；32：655-657.
21) Terry, R. M., Grey, C., Bird, C. C. Aberrant expression of low molecular weight cytokeratins in primary and secondary squamous carcinoma of the head and neck. J Laryngol Otol 1986；100：1283-1287.
22) Chin, N. W., Zhang, R., Nostro, D. C., Lanks, K. W. A novel monoclonal antibody to cytokeratin 18 with reactivity toward lung squamous cell carcinomas and adenocarcinomas of various sites. Am J Pathol 1992；140：1061-1069.
23) Sasaki, C. T., Jassin, B. Cancer of the pharynx and larynx. Am J Med 2001；111（Suppl 8A）：118S-123S.

コメント COMMENT

細胞診用検体の採取と評価

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細胞診は生検を補完する重要な診断法である．生検がピンポイントで組織を採取するのに対し，ブラシによる擦過ではより広範囲から細胞を採取できる．

擦過ブラシの使い方

気管支内視鏡で用いる擦過ブラシはシース（外套）を有するものを使用することが多い．そのようなブラシで擦過動作を行う場合，助手がブラシをシースから出し固定した状態で術者が右手でシースを動かす方法と，術者はブラシのシースを固定し助手がブラシをシースから出し入れする方法がある．前者はブラシにかかる抵抗感で検体が採取されていることを術者がわかる例があり，より確実に擦過ができるが，気管支鏡が動いて検体採取場所がずれることがある．後者では術者と助手の息が合わないと出血や気胸のリスクが高くなりうる．どちらを行うかは症例により選択する．

当科で行った検討では，末梢悪性病変に対し生検とブラシ擦過細胞診の両方を施行した場合にどちらか一方のみで診断される例がみられた．したがって生検に細胞診を併用すると診断率が向上する[1]（表3）．生検と擦過細胞診のどちらを先に行うかは明確な基準がない．かつては細胞診標本の背景の血液が少なくなるように擦過細胞診を先に行うよう勧められていたが，超音波プローブとガイドシース（EBUS-GS）を用いた末梢病変の診断においては，鉗子生検とブラシによる細胞診採取を行う順番は診断率に影響を与えない[2]．

表3　擦過細胞診と生検の両方を行った症例での診断の可否

(a) 細径の気管支鏡（P240，Olympus，外径5.2 mm）での診断．悪性病変12例中，擦過細胞診と生検のいずれかのみで診断がついた症例が6例あった．

		擦過細胞診 陽性	擦過細胞診 陰性	計
生検	陽性	5	0	5
	陰性	6	1	7
計		11	1	12

(b) 極細径の気管支鏡（XP240，Olympus，外径2.8 mm）での診断．悪性病変30例中，擦過細胞診と生検のいずれかのみで診断がついた症例が8例あった．

		擦過細胞診 陽性	擦過細胞診 陰性	計
生検	陽性	17	2	19
	陰性	6	5	11
計		23	7	30

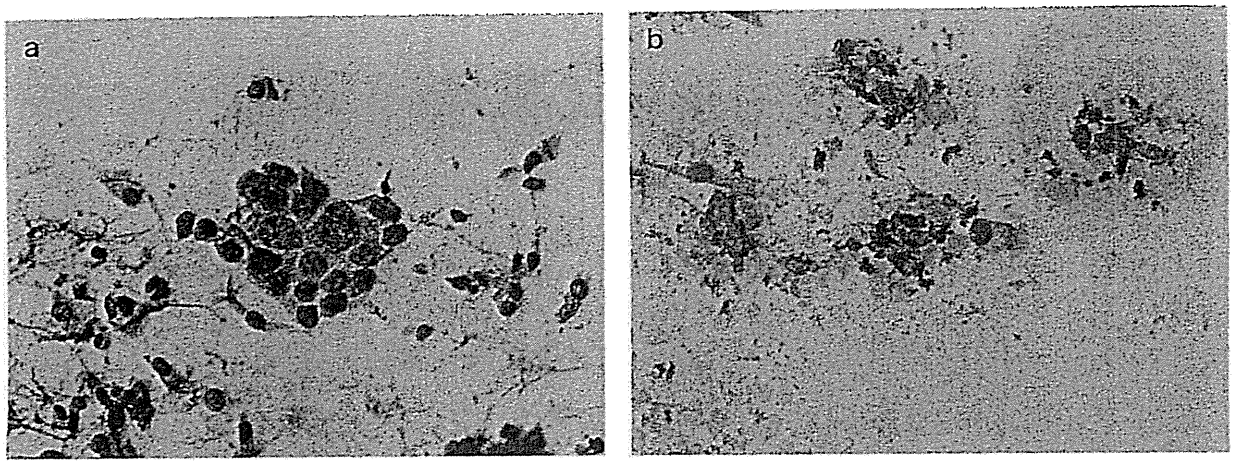


図 15　標本に対する乾燥の影響

(a) 適切に作成された標本．細胞の観察が容易である．高分化腺癌と診断された．
(b) 乾燥した標本．細胞が膨化し形態に変化が起こり，染色色調も異常である．このような標本では良悪性の診断はできない．

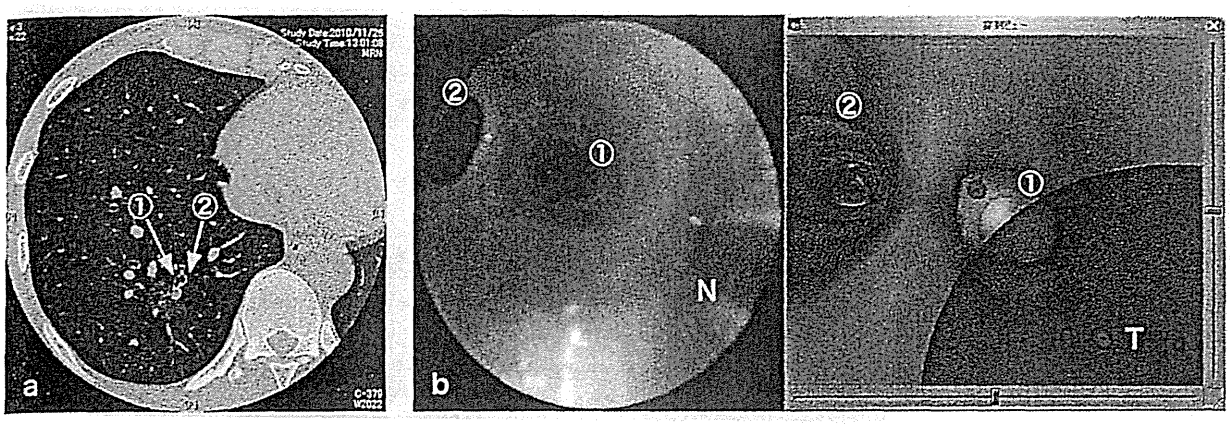
検体作成のコツ

適正な細胞診断上，最も重要な点の 1 つに検体の作成手技がある．パパニコロウ染色が通常用いられるが，そのためにスライドガラス上に検体を塗抹する場合の留意点が 2 つある．1 つはエタノール固定を行うまでの乾燥を極力避けるために，ごく短時間での塗抹操作を行うことである．乾燥検体では細胞の染色性が低下し，膨化変性が起こるため診断が非常に難しくなる（図 15）．なお細胞乾燥は気管支内でブラシをシースから長時間出した状態でも起こりうる．2 つ目は適度に薄く均等に細胞を塗抹することである．検体を薄く広げるために，スライドガラスを相互にすり合わせることがあるが，強い圧を加えると小細胞癌などは細胞挫滅が起こりうるので愛護的に行う．また塗抹を行ったあとのブラシには多数の細胞が残存している．それらは生食の入った試験管中でブラシを洗って回収でき[3]，回収液を用いて EGFR 遺伝子変異などの検索を行うことも可能である[4]．いずれにしても貴重な検体を無駄にすべきではない．

スライドガラス作成は検査技師が行うのが望ましいが，実際の現場では医師や看護師が作成することが多いであろう．初心者は熟練者の検体処理の見学や練習をしたのちに本番に臨むべきである．術者自らも標本の検鏡をすることもおすすめしたい．細胞像を知るのみならず採取細胞量の多寡，細胞の変性の程度がわかるので，検体採取・標本作成のテクニック向上に役立つ．

ブラシ擦過ができない時の穿刺細胞診

病変が気管支と交通のない場所に位置する場合，あるいはリンパ節から経気管支的に検体を採取する場合には針吸引が有用である．たとえば図 16 に示すように病変が気管支周囲にあり，ブラシや鉗子では気管支外の病変から検体を採取できないような症例に用いる．ナビゲーションシステムを利用すると穿刺精度がより向上する．針吸引された細胞はブラシ採取の場合よりも乾燥しやすいため，迅速な処理が必要である．なお吸引時には継続的に十分な陰圧が加わるように助手が注意する．血液が



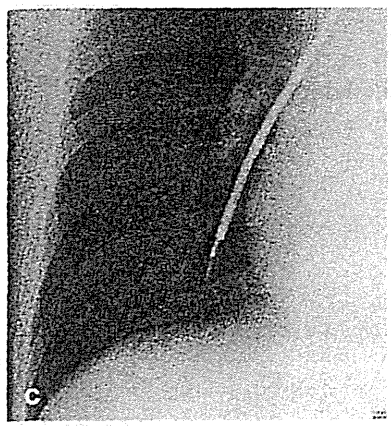


図 16　気管支壁外病変に対する穿刺細胞診

(a) CT で右 $B^{10}{}_{bi\alpha}$ (①) と $B^{10}{}_{bi\beta}$ (②) の近くに 18 mm 大の ground-glass opacity (GGO) 病変がある．病変は気管支の周囲にある．

(b) 左は仮想気管支鏡内腔像 (CT 画像の DICOM データより Bf-NAVI®，オリンパスメディカルシステムズ社で作成) で，右 $B^{10}{}_{bi\alpha}$ (①) と $B^{10}{}_{bi\beta}$ (②) と気管支壁から透見した病変 (T) の位置関係がわかる．右は実際に穿刺をしている写真で針 (N) が気管支壁を貫いて病変部に到達している．吸引物の細胞診結果は高分化腺癌で，採取された腫瘍細胞の EGFR 遺伝子変異陽性も確認された．この症例では気管支内腔に病変を認めず，内腔からの鉗子生検，ブラシ擦過では診断が不能であった．

(c) 穿刺時の透視写真．GGO は透視では確認できないため仮想内視鏡によるナビゲーションが有用であった．

多量に吸引される場合，陰圧を直ちに解除し落ち着いて針を抜去し止血をはかる．

文献

1) Tachihara M, Ishida T, Kanazawa K, et al. A virtual bronchoscopic navigation system under X-ray fluoroscopy for transbronchial diagnosis of small peripheral pulmonary lesions. Lung Cancer. 2007; 57 :322-7.

2) 立原素子，石田　卓，二階堂雄文，他．ガイドシース併用気管支腔内超音波断層法 (EBUS-GS) を用いた気管支鏡検査 ―生検と擦過，どちらを先行させるべきか―．気管支学．2010; 32: 395-401.

3) Kawaraya M, Gemba K, Ueoka H, et al. Evaluation of various cytological examinations by bronchoscopy in the diagnosis of peripheral lung cancer. Br J Cancer. 2003; 89: 1885-8.

4) 峯村浩之，金沢賢也，関根聡子，他．経気管支擦過細胞診検体にて EGFR 遺伝子変異陽性を検出した原発性肺癌における予後の検討．肺癌．2011; 51: 436.

INTERNAL MEDICINE

□ CASE REPORT □

Four Cases of Trousseau's Syndrome Associated with Lung Adenocarcinoma

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Abstract

Cancer patients are at high risk of venous thromboembolism (VTE), and the combination of these two conditions is well known as Trousseau's syndrome. Here we present four cases of Trousseau's syndrome associated with advanced lung adenocarcinoma. In addition to fibrinogen degradation products (FDP) and D-dimer, the levels of mucin-producing markers, such as KL-6, were elevated. There is a possibility that mucin production may be associated with cancer-related VTE.

Key words: lung cancer, venous thromboembolism, Trousseau's syndrome, mucin, KL-6

(Intern Med 51: 1099-1102, 2012)
(DOI: 10.2169/internalmedicine.51.6453)

Introduction

The relationship between venous thromboembolic features and malignancy was first described by Armand Trousseau in 1865 (1). Recently cancer has become recognized as a prothrombotic state. Trousseau's syndrome has been applied to various clinical conditions, ranging from brain infarction to any kind of coagulopathy occurring in cancer (2). Here we define Trousseau's syndrome as cancer-related thromboembolism, and present four cases of this syndrome associated with lung adenocarcinoma.

Case Reports

Case 1

A 31-year-old non-smoker man presented with cough and was referred to our hospital. Chest X-ray demonstrated a mass in the right lower lung field and chest computed tomography (CT) showed a right lower lobe mass with right pleural effusion (Fig. 1A, B). Bronchoscopy was performed, and lung adenocarcinoma was diagnosed histologically. Chemotherapy was scheduled, but before it could be administered, right hemiplegia and articulatory disorder suddenly developed. Brain magnetic resonance imaging (MRI) showed infarction in the left parietal lobe. Heparin was administered and blood flow was resumed. In laboratory findings, the elevation of FDP, D-dimer, tissue factor (TF) and sialylated carbohydrate antigen KL-6 were seen (Table 1). Immunostaining was positive for Periodic acid-Schiff (PAS), suggesting a mucin-producing tumor (Fig. 2A, B). We diagnosed Trousseau's syndrome accompanying lung cancer, and performed heparin injections with chemotherapy. He received cisplatin (60 mg/m^2) and oral S-1 (120 mg/day) as first-line chemotherapy. His best response to chemotherapy was a partial response, which continued for four cycles. Although he received up to third-line chemotherapy, he died of progressive tumor 7 months after the diagnosis of lung cancer. Heparin injection was continued during chemotherapy and thromboembolism did not occur again.

Case 2

A 64-year-old man presented with back pain, which was diagnosed as metastasis to the lumbar vertebra. Chest X-ray showed a tumor in right upper lung field. The tumor was diagnosed as stage IV adenocarcinoma. Laboratory data demonstrated elevation of FDP, D-dimer, CEA, and KL-6 (Table 1). Enhanced CT revealed deep vein thrombosis (DVT) of the left popliteal vein. He received only first-line chemo-

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Received for publication August 25, 2011; Accepted for publication January 24, 2012
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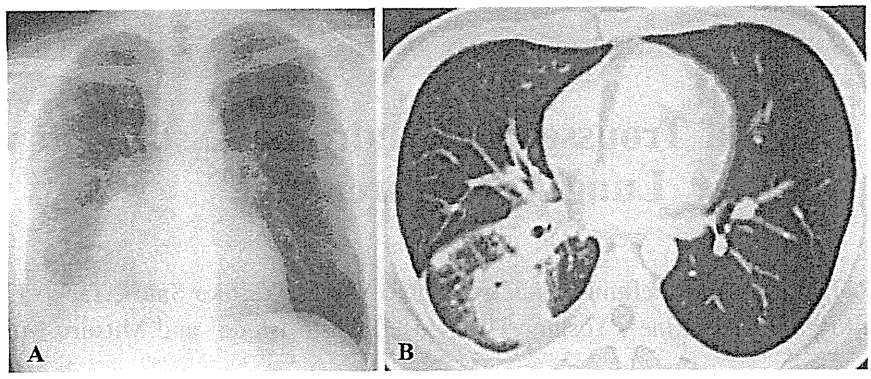

Figure 1. Chest X-ray of Case 1 showed radiolucent fall in the right lower lung field (A). CT demonstrated a mass 6 cm in diameter in the right lower lobe with right pleural effusion (B).

Table 1. Characteristics of 4 Cases of Lung Adenocaricnoma with Trousseau's Syndrome

	Case 1	Case 2	Case 3	Case 4
Age	30	64	70	56
Sex	M	M	M	M
histology	Ad	Ad	Ad	Ad
stage	IV	IV	IV	IV
Leukocyte (/μL)	9,400	10,100	10,600	7,300
Platelet counts ($\times10^4$/μL)	21.5	25.8	28.3	20.7
TF (pg/mL)	712	446	344	260
CEA (<5ng/mL)	0.6	180	208	580
CA19-9 (<37U/mL)	3.1	9.6	2,626	4.1
KL-6 (<450U/mL)	1,036	1,757	758	4,857
FDP (<1.0μg/mL)	92	33.6	47	9.7
D-dimer (<5μg/mL)	55.4	22.1	22.3	7.3
Complication	brain infarction	DVT	DVT	DVT
Therapy of Trousseau's syndrome	UFH	UFH	UFH	UFH
Mucin-producing histologic type	+	-	+	-

Ad, adenocaricnoma; TF, Tissue factor; CEA, carcinoembryonic antigen; DVT, deep vein thrombosis; UFH, unfractionated heparin.

therapy in combination with heparin, and he died from progressive tumor in 5 months.

Case 3

A 70-year-old man presented with dyspnea and hemoptysis. Investigation led to a diagnosis of mucin-producing stage IV lung adenocarcinoma. Laboratory data showed elevation of FDP, D-dimer, CEA, CA19-9, and KL-6. Occlusion of the right pulmonary artery was found on enhanced CT (Fig. 3A). He also had pain of the left foot, and DVT was found on enhanced CT (Fig. 3B). He received chemotherapy in combination with heparin. After decreasing the level of FDP and D-dimer to within the normal range, administration of heparin was ended. He received up to second-line chemotherapy and the effect of chemotherapy was a good partial response. He is alive for 2 years after the diagnosis of lung cancer.

Case 4

A 56-year-old man presented with cough and dyspnea. He was diagnosed as having stage IV lung adenocarcinoma. Laboratory data showed elevated FDP, D-dimer, CEA, and KL-6. DVT of the left popliteal vein was found on enhanced CT. He received chemotherapy with heparin, but the tumor progressed. We changed his treatment to erlotinib (Tarceva®) as second-line chemotherapy and the tumor decreased markedly. When FDP and D-dimer were decreased to within the normal range, we stopped the administration of heparin. He remains alive 3 years after the diagnosis of lung cancer.

The patient characteristics and laboratory data of the present four cases are listed in Table 1. These data were examined when patients were hospitalized before administration of heparin. Plasma TF level was measured with the IMUBINED Tissue Factor ELISA Kit (American Diagnostica Inc., Greenwich, CT, USA). All four were stage IV adenocarcinoma and the elevation of FDP and D-dimer was seen in all of them. Two of the patients (Cases 1 and 3) appeared to have mucin-secreting adenocarcinomas histopathologically.

Meanwhile, the serum levels of KL-6 (reference range, ≦ 500 U/mL), a type of mucin, were elevated in all four patients (mean ± SD, 2,102.0±1,884.3 U/mL) despite there being no evidence of interstitial lung disease. These levels were higher than those in the other 11 patients with advanced adenocarcinoma without Trousseau's syndrome (499.1±200.4 U/mL) (Fig. 4A). In contrast, plasma TF levels in the above-mentioned patients with adenocarcinoma (440.5±196.3 pg/mL) were not particularly high among patients (564.5±331.5 pg/mL) (Fig. 4B).

Discussion

Cancer has become recognized as a major risk factor for VTE, increasing the risk about 6-10 fold, and the association is well known as Trousseau's syndrome (3-5). Patients with cancer account for 0.6-13.6% of cases of VTE (6-8). Multiple interacting mechanisms are thought to explain the increased incidence of thrombosis in patients with malignancies. The main causes are prothrombotic agents such as TF, mucin, cysteine proteinase (CP) and plasminogen activator

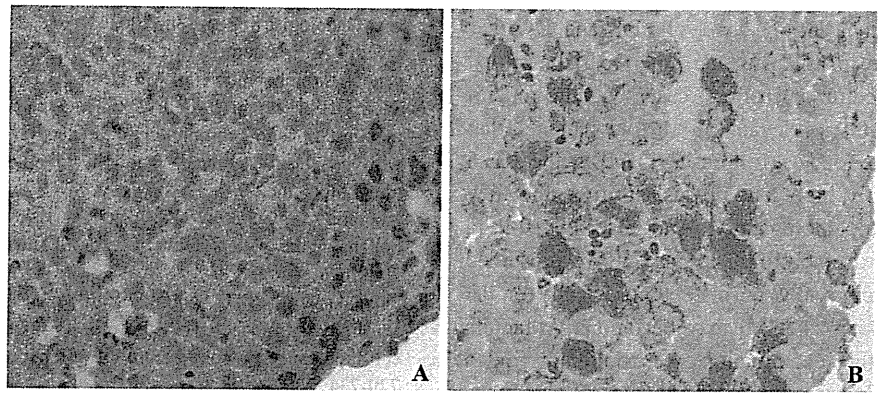

Figure 2. Histological examination demonstrated adenocarcinoma (A: Case 1, Hematoxylin and Eosin staining, ×20). Periodic acid-Schiff stain (PAS) was positive, and it suggested mucin-producing tumor (B: Case 1, PAS stain, ×20).

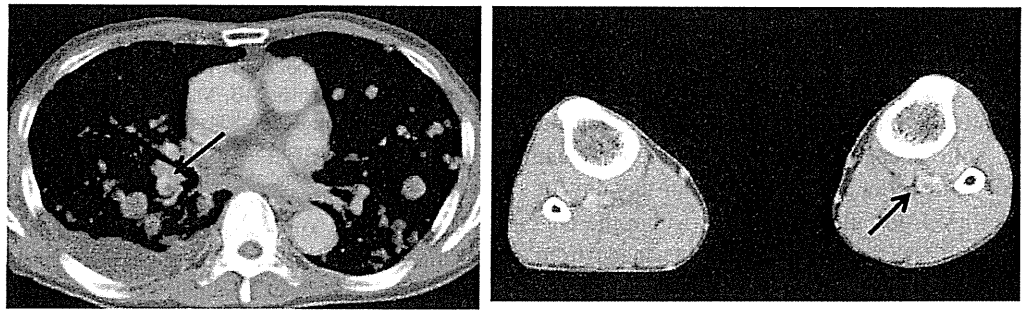

Figure 3. Enhanced CT of Case 3 revealed thrombus of the right pulmonary artery (A) and left popliteal vein (B) (arrow).

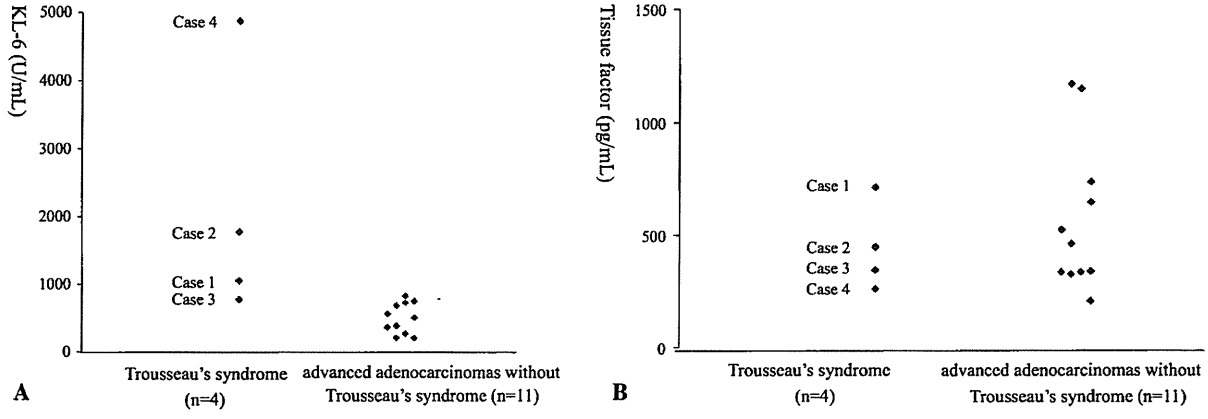

Figure 4. KL-6 level (A) and plasma level of tissue factor (B) in the present cases and advanced adenocarcinomas without Trousseau's syndrome.

inhibitor -1 (PAI-1) which are produced by tumor cells (2, 9-11). TF is a transmembrane glycoprotein which contributes to a variety of pathologic processes, such as thrombosis, metastasis, and angiogenesis (2, 9-13). Mucins produced by cancer are large glycosylated molecules which act as ligands for the selectins. Mucins are thought to be a trigger of coagulation (14) and cause induction of disseminated thrombosis (2, 15). Meanwhile, the clinical risk factors and candidate laboratory biomarkers predictive of cancer-associated VTE have recently been assessed (7, 8, 16, 17). These factors were reported as tumor type, stage of disease, hospitalization, elevation of platelet, leukocyte counts and D-dimer. In addition, it is reported that VTE rates are higher in patients with adenocarcinoma than in those with squamous cell carcinoma in lung cancer (18, 19). In the present cases, they all were adenocarci-

noma at advanced stage, and the level of FDP and D-dimer were high. They were in line with the reported risk factors. Moreover, what was common in the 4 cases was the mucin-producing tumor. KL-6 was elevated in all four patients, that suggested mucin secretion. As for Cases 1 and 3, the KL-6 level was not markedly high, but it was clarified that they produced mucin histopathologically. And also CA19-9, a type of mucin, was extremely high in case 3. We presumed that the mucin-producing subtype histopathologically or the elevation of marker such as KL-6 may be predictive factors of coagulopathy. In this examination, we could not find a correlation between Trousseau's syndrome and TF.

Concerning therapy for Trousseau's syndrome, treatment of the cancer itself is a priority. In addition, thrombolytic therapy and thromboprophylaxis are necessary. Compared with vitamin K antagonist (VKA), heparin is reported to provide a statistically significant reduction in VTE (20). This is because heparin has several antithrombotic mechanisms that VKA does not, such as inhibition of the binding of mucin to selectin and release of tissue factor pathway inhibitor from endothelial binding sites (2). We used heparin in combination with chemotherapy for the present four cases, and continued it as long as possible with the aim for FDP and D-dimer levels to fallen adequately within the normal range. In Case 1, the anticoagulant was changed from heparin to Warfarin® once, but FDP, and D-dimer levels began to rise again. This episode supports the favorable effect of heparin compared with VKA in this syndrome.

In conclusion, we reported four cases of lung adenocarcinoma with Trousseau's syndrome. In addition to the elevated levels of FDP, D-dimer in advanced adenocarcinoma, we presume that there is a possibility that mucin production may be predictive factor of cancer-related VTE.

The authors state that they have no Conflict of Interest (COI).

References

1. Trousseau A. Plegmasia alba dolens. Clin med Hotel-Dieu Paris **3**: 654-712, 1865.
2. Varki A. Trousseau's syndrome: multiple definitions and multiple mechanisms. Blood **110**: 1723-1729, 2007.
3. Bick RL. Cancer-associated thrombosis. N Engl J Med **349**: 109-111, 2003.
4. Kakkar AK, Levine M, Pinedo HM, Wolff R, Wong J. Venous thrombosis in cancer patients: insights from the FRONTLINE survey. Oncologist **8**: 381-388, 2003.
5. Piccioli A, Prandoni P, Ewenstein BM, Goldhaber SZ. Cancer and venous thromboembolism. Am Heart J **132**: 850-855, 1996.
6. Sack GH Jr, Levin J, Bell WR. Trousseau's syndrome and other manifestations of chronic disseminated coagulopathy in patients with neoplasms: clinical, pathophysiologic, and therapeutic features. Medicine **56**: 1-37, 1977.
7. Khorana AA, Connolly GC. Assessing risk of venous thromboembolism in the patient with cancer. J Clin Oncol **27**: 4839-4847, 2009.
8. Tagalakis V, Levi D, Agulnik JS, Cohen V, Kasymjanova G, Small D. High risk of deep vein thrombosis in patients with non-small cell lung cancer: a cohort study of 493 patients. J Thorac Oncol **2**: 729-734, 2007.
9. Rak J, Yu JL, Luyendyk J, Mackman N. Oncogenes, Trousseau syndrome, and cancer-related changes in the coagulome of mice and humans. Cancer Res **66**: 10643-10646, 2006.
10. Kuderer NM, Ortel TL, Francis CW. Impact of venous thromboembolism and anticoagulationon cancer and cancer survival. J Clin Oncol **27**: 4902-4911, 2009.
11. Sato T, Tsujino I, Ikeda D, Ieko M, Nishimura M. Trousseau's syndrome associated with tissue factor produced by pulmonary adenocarcinoma. Thorax **61**: 1009-1010, 2006.
12. Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. J Clin Oncol **27**: 4834-4838, 2009.
13. Khorana AA, Ahrendt SA, Ryan CK, et al. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. Clin Cancer Res **13**: 2870-2875, 2007.
14. Pineo GF, Brain MC, Gallus AS, Hirsh J, Hatton MW, Regoeczi E. Tumors, mucus production, and hypercoagulability. Ann NY Acad Sci **230**: 262-270, 1974.
15. Kim YJ, Borsig L, Han HL, Varki NM, Varki A. Distinct selectin ligands on colon carcinoma mucins can mediate pathological interactions among platelets, leukocytes, and endothelium. Am J Pathol **155**: 461-472, 1999.
16. Lyman GH, Khorana AA, Falanga A, et al. American Society of Clinical Oncology Guideline: recommendations for venous thromboembolism prophylaxis and treatment in patients with cancer. J Clin Oncol **25**: 5490-5505, 2007.
17. Mandala M, Falanga A, Roila F. Venous thromboembolism in cancer patients: ESMO Clinical Practice Guidelines for the management. Ann Oncol **21**[Suppl 5]: v274-v276, 2010.
18. Blom JW, Osanto S, Rosendaal FR. The risk of a venous thrombotic event in lung cancer patients : higher risk for adenocarcinoma than squamous cell carcinoma. J Thromb Haemost **2**: 1760-1765, 2004.
19. Chew HK, Davies AM, Wun T, Harvey D, Zhou H, White RH. The incidence of venous thromboembolism among patients with primary lung cancer. J Thromb Haemost **6**: 601-608, 2008.
20. Akl EA, Barba M, Rohilla S, et al. Low-molecular-weight heparins are superior to vitamin K antagonists for the long term treatment of venous thromboembolism in patients with cancer: a cochrane systematic review. J Exp Clin Cancer Res **27**: 21, 2008.

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Statement of Interest: None declared.

REFERENCES

1 Raghu G, Collard HR, Egan JJ, *et al.* An Official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; 183: 788–824.
2 du Bois RM. Strategies for treating idiopathic pulmonary fibrosis. *Nat Rev Drug Discov* 2010; 9: 129–140.
3 Papiris SA, Manali ED, Kolilekas L, *et al.* Clinical review: idiopathic pulmonary fibrosis acute exacerbations – unravelling Ariadne's thread. *Crit Care* 2010; 14: 246.
4 National Heart, Lung, and Blood Institute. NIH News. Commonly used three-drug regimen for idiopathic pulmonary fibrosis found harmful. October 21, 2011. www.nih.gov/news/health/oct2011/nhlbi-21.htm Date last accessed: January 22, 2012.
5 Azuma A, Nukiwa T, Tsuboi E, *et al.* Double-blind, placebo-controlled trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2005; 171: 1040–1047.
6 Taniguchi H, Ebina M, Kondoh Y, *et al.* Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2010; 35: 821–829.
7 Noble PW, Albera C, Bradford WZ, *et al.* Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* 2011; 377: 1760–1769.
8 Collard HR, King TE Jr, Bartelson BB, *et al.* Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2003; 168: 538–542.
9 du Bois RM, Weycker D, Albera C, *et al.* Forced vital capacity in patients with idiopathic pulmonary fibrosis: test properties and minimal clinically important difference. *Am J Respir Crit Care Med* 2011; 184: 1382–1389.
10 Guyatt GH, Osoba D, Wu AW, *et al.* Methods to explain the clinical significance of health status measures. *Mayo Clin Proc* 2002; 77: 371–383.
11 Man-Son-Hing M, Laupacis A, O'Rourke K, *et al.* Determination of the clinical importance of study results. *J Gen Intern Med* 2002; 17: 469–476.

DOI: 10.1183/09031936.00114311

Primary pulmonary extranodal natural killer/T-cell lymphoma: nasal type with multiple nodules

To the Editors:

Primary pulmonary lymphoma (PPL) is an uncommon disease representing only 3–4% of extranodal non-Hodgkin's lymphoma (NHL), and <1% of NHL [1]. The true incidence of PPL other than NHL is unknown, while primary pulmonary T-cell lymphoma is extremely rare. Natural killer (NK)/T-cell lymphoma usually shows an extranodal presentation. Nasal NK/T-cell lymphomas occur in the nose and the upper aerodigestive tract. Extranasal NK/T-cell lymphomas represent the counterpart of nasal NK/T-cell lymphomas and can involve every other part of the body. Primary sites of involvement are mainly the skin, soft tissue, gastrointestinal tract and testis [2]. Although some cases of primary pulmonary T-cell lymphoma have been reported, there are only two reports written in English to date [3, 4]. Here, we describe a case of primary pulmonary NK/T-cell lymphoma with multiple nodules in both lung fields.

The patient was a 50-yr-old Japanese male with an abnormal shadow on chest radiography in a health examination (fig. 1a). He was an office worker with no history of dust inhalation or asbestos exposure and was a current smoker (50 pack-yrs). He had a history of hypertension and gallstones. He had a high fever of over 39°C for 10 days before the health examination, and had appetite loss and general fatigue. A chest computed tomography (CT) on admission revealed multiple nodules, measuring a maximum of 40 mm in size, were in both lung fields (fig. 1b–d). Administration of broad-spectrum antibiotics did not resolve his symptoms. Bronchoscopic examination and CT-guided needle biopsy did not give a definite diagnosis. Laboratory data showed elevation of lactate dehydrogenase, transaminases, hepatobiliary enzymes and an inflammatory reaction without cytopenia. Two sets of blood cultures were both negative and no tumour markers were elevated. Anti-neutrophil cytoplasmic antibodies were negative and soluble interleukin (IL)-2 receptor was elevated to 5,060 $U \cdot mL^{-1}$. Although we first planned a surgical biopsy to make a definitive diagnosis, it was not performed due to the rapid deterioration in his performance status. The final needle aspiration showed lymphocytes of various morphologies invading the blood vessels.

At this point, we suspected pulmonary lymphoma, such as lymphomatoid granulomatosis. The Otolaryngologists investigation of the upper respiratory tracts showed no specific findings. Lung nodules increased and grew larger, and hypoxia progressed. His clinical condition deteriorated so rapidly that we had to start treatment without a definitive diagnosis. He was treated with methylprednisolone (mPSL) pulse therapy (1,000 mg per day for 3 days) twice followed by cyclophosphamide pulse therapy (500 mg per day) as a salvage therapy. Progressive hypoxia was temporarily improved; however, his respiratory condition immediately worsened. Direct haemoperfusion using a polymyxin B immobilised fibre column was also performed;

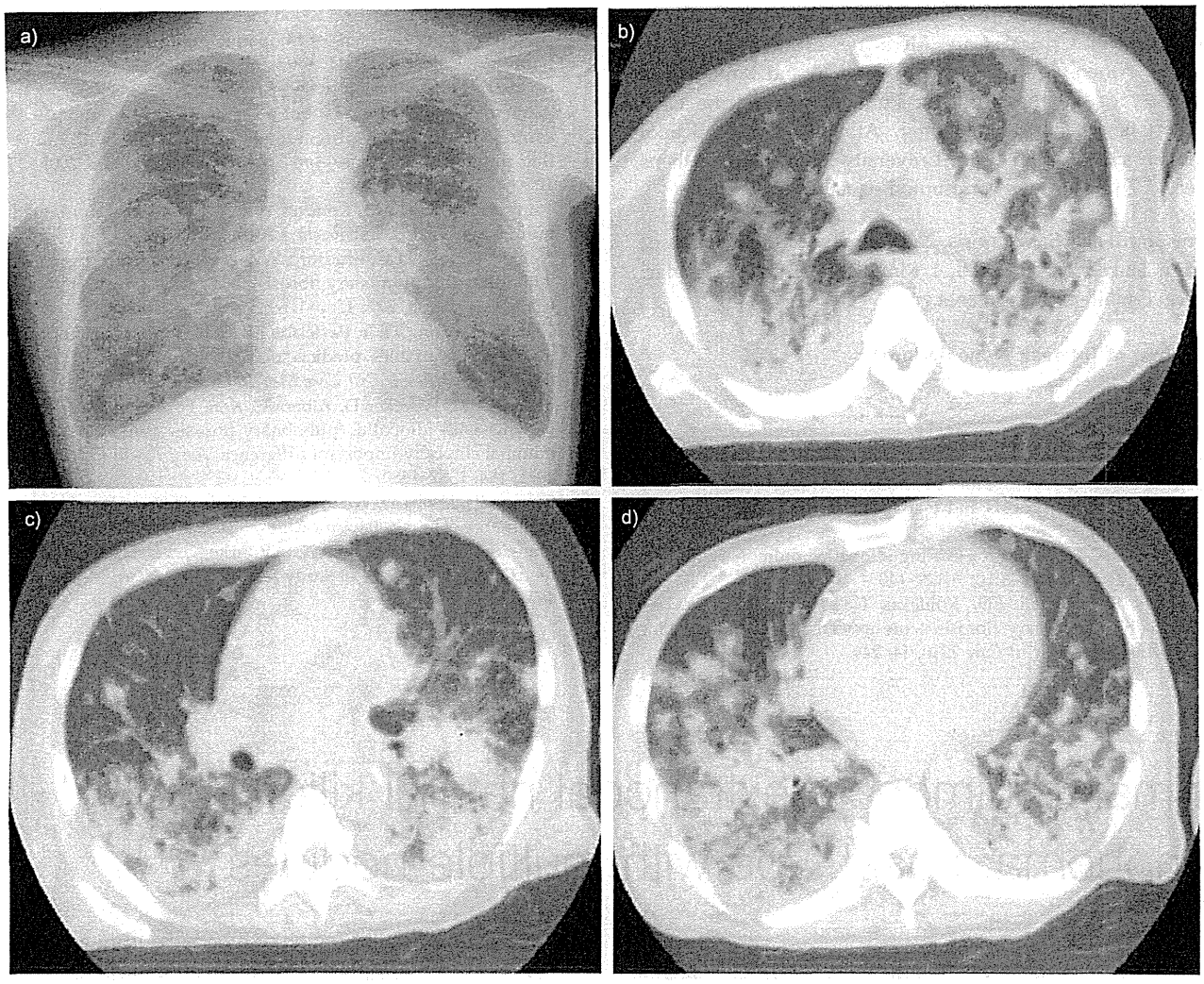

FIGURE 1. a) Chest radiograph, and b) upper, c) middle and d) lower computed tomography images on admission showing bilateral nodules in both lung fields.

however, it improved the condition only temporarily. At 10 days after the second mPSL pulse therapy, when continuous haemodiafiltration was started for progressive renal insufficiency, he developed marked hepatosplenomegaly. His clinical condition continued to deteriorate in spite of the intensive treatment. His respiratory condition worsened progressively and he died of multiple organ failure on the 29th day after admission.

The *post mortem* examination showed multiple whitish nodular lesions in both lungs. These nodular lesions were also seen on the cut surface of the heart. Slight lymphadenopathy was seen in the mediastinum, paratracheal, bifurcation and bilateral hilar regions. The spleen was swollen and fragile, and the liver was markedly enlarged.

The microscopic examination showed that medium to large sized atypical lymphoid cells proliferated in both lungs, the myocardium, the spleen, the portal areas, both adrenal glands, the interstitium of both kidneys, the marrow of the examined bones (sternum, vertebrae and rib), the lymph nodes in the mediastinum, and the paratracheal, bifurcation and bilateral hilar regions, but not in the entire gastrointestinal tract. The cells stained positively for cytoplasmic CD3, CD56 and Epstein–Barr virus (EBV)-encoded small RNA (fig. 2b–d), but negatively for CD20 and CD79 (B-cell markers). As CD3 and CD56 are T- and NK-cell markers, respectively, the immunocytochemistry showed that these atypical lymphoid cells were of T- and NK-cell lineages. Based on the neoplastic morphology and the immunohistochemical examination of the atypical lymphocytes, the pathological diagnosis was extranodal NK/T-cell lymphoma (nasal type).

According to the World Health Organization 2008 classification, NK-cell tumours are classified into two types: 1) extranodal NK/T-cell lymphoma (nasal type); and 2) aggressive NK-cell leukaemia. NK/T-cell lymphomas are uncommon but are more prevalent in Asia, Mexico, and Central and South America [5]. In the present case, the abdominal CT, which was taken 10 days before admission, showed no involvement of other organs except the lungs, and the physical examination on admission showed no hepatosplenomegaly. In addition, the *post mortem* examination did not indicate the involvement of the upper respiratory tract and the entire gastrointestinal tract. It is

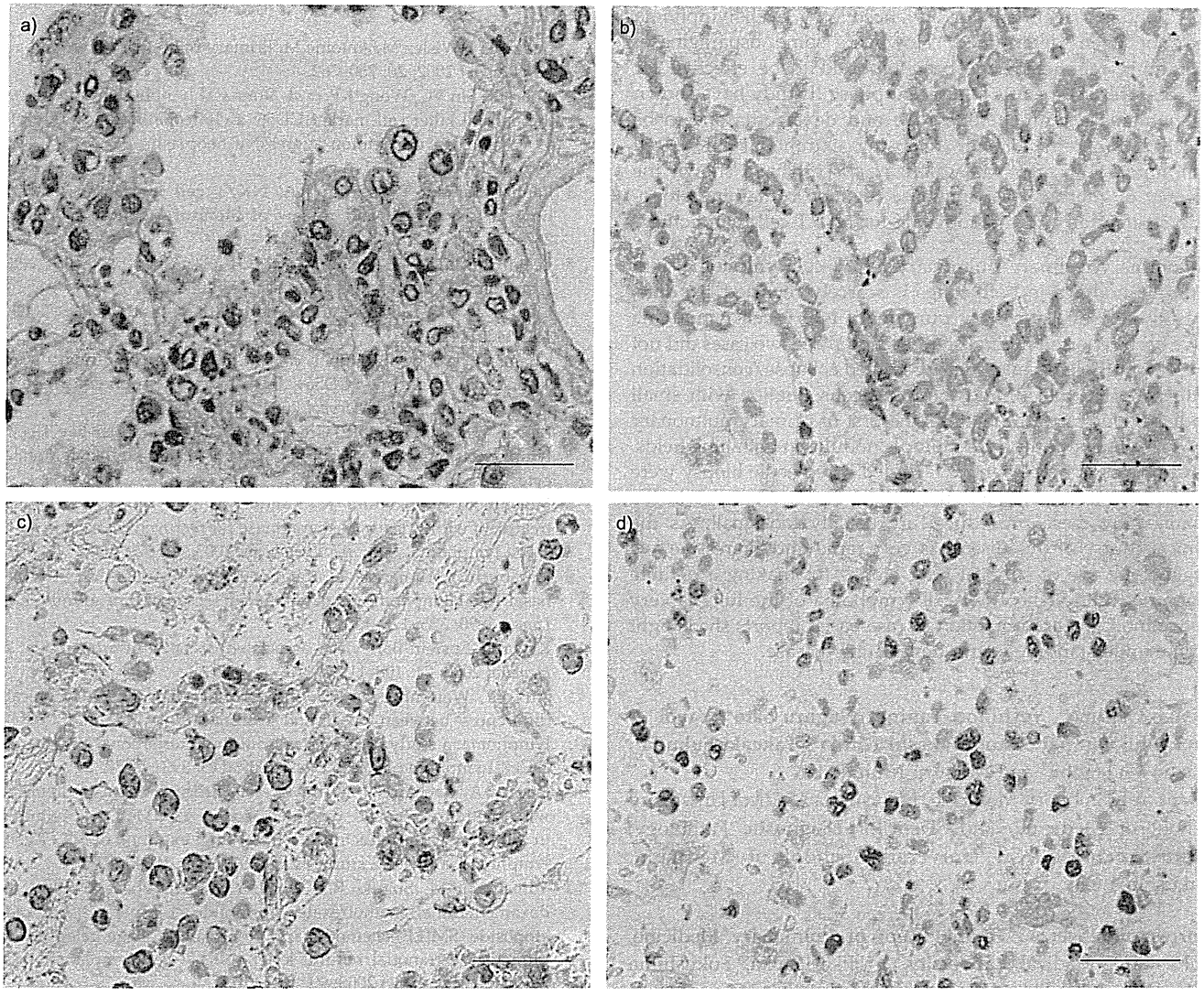

FIGURE 2. *Post mortem* lung histology showing proliferation of medium to large sized atypical lymphoid cells infiltrating alveolar septa. a) Haematoxylin and eosin staining. Positive staining for b) CD3, c) CD56 and d) Epstein–Barr virus-encoded small RNA was seen by immunohistochemical analysis. Scale bar = 50 μm.

difficult to locate the definite primary site in the present case; however, it is reasonable to suggest that the lung is the primary site, based on the clinical and radiological findings obtained before and at admission. Although lung involvement is sometimes seen in the end stage of extranodal NK/T-cell lymphoma [6–8], there have been cases of the lesions found only in the lungs even in the early stage [3, 4].

EBV is frequently seen in tumour cells of NK/T-cell lymphoma. The first report showing the aetiological role of EBV in the development of NK/T-cell lymphoma was published in 1990 [9]. A strong relationship between EBV infection and NK/T-cell lymphoma has been suggested. However, NK/T- and T-cell peripheral lymphomas are responsible for the majority of lymphoma-associated haemophagocytic syndrome (HPS), and it has been reported that about half of lymphoma-associated HPS is associated with EBV [10]. The patient did not fulfil the diagnostic criteria of the 2004 haemophagocytic lymphohistiocytosis guidelines on admission, but developed fever, splenomegaly, cytopenia, elevated levels of ferritin and soluble IL-2 receptor in the end stage. Although haemophagocytosis in bone marrow was not proven, we can say that the patient developed HPS during disease progression.

NK/T-cell lymphoma usually presents a highly aggressive clinical course and the prognosis is generally poor. The International Peripheral T-cell Lymphoma Project demonstrated that extranasal NK/T-cell lymphoma (nasal type) has worse clinical features and survival rate, even in cases with apparently localised disease, than nasal NK/T-cell lymphoma in extranodal NK/T-cell lymphoma [11]. The present case is considered to be at high risk at the time of admission according to the international prognostic index, from which the median overall survival for patients with stage III/IV extranasal disease is 0.28 yrs. The overall clinical course of this case was ~2 months, which is consistent with the previous report [11].

The standard treatment for advanced NK/T-cell lymphoma has not been determined. However, some asparaginase-containing regimens have been reported to be effective for this disease. The results of a recent phase II study have shown L-asparaginase with methotrexate and dexamethasone to be effective for refractory or relapsing extranodal NK/T-cell lymphoma [12], and YAMAGUCHI *et al.* [13] have shown the efficacy of another L-asparaginase regimen containing etoposide, ifosfamide and methotrexate for NK/T-cell lymphoma.

In summary, primary pulmonary NK/T-cell lymphoma with multiple nodules in both lungs is scarcely reported. To date, only two cases of primary pulmonary NK/T-cell lymphoma have been reported in English [3, 4], and these two cases did not present multiple nodules. One presented collapse/consolidation with pleural effusion, and the other consolidation with small nodules. Our case is, therefore, the first of primary pulmonary NK/T-cell lymphoma with multiple nodules in both lung fields. Transbronchial biopsy and CT-guided core needle biopsy were not helpful for making a definite diagnosis because specimens obtained by these procedures are usually too small. As the clinical course of this disease is very rapid and aggressive, we suggest the use of surgical lung biopsy for making a definite diagnosis. Although NK/T-cell lymphoma of the lung is very uncommon, the accumulation of case-based reports sheds light on the understanding of this devastating disease.

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Statement of Interest: None declared.

REFERENCES

1 Cadranel J, Wislez M, Antoine M. Primary pulmonary lymphoma. *Eur Respir J* 2002; 20: 750–762.
2 Chan JK, Sin VC, Wong KF, *et al.* Nonnasal lymphoma expressing the natural killer cell marker CD56: a clinicopathologic study of 49 cases of an uncommon aggressive neoplasm. *Blood* 1997; 89: 4501–4513.
3 Laohaburanakit P, Hardin KA. NK/T cell lymphoma of the lung: a case report and review of literature. *Thorax* 2006; 61: 267–270.
4 Lee BH, Kim SY, Kim MY, *et al.* CT of nasal-type T/NK cell lymphoma in the lung. *J Thorac Imaging* 2006; 21: 37–39.
5 Liang X, Graham DK. Natural killer cell neoplasms. *Cancer* 2008; 112: 1425–1436.
6 Chang SE, Yoon GS, Huh J, *et al.* Comparison of primary and secondary cutaneous CD56+ NK/T cell lymphomas. *Appl Immunohistochem Mol Morphol* 2002; 10: 163–170.
7 Macon WR, Williams ME, Greer JP, *et al.* Natural killer-like T-cell lymphomas: aggressive lymphomas of T-large granular lymphocytes. *Blood* 1996; 87: 1474–1483.
8 Matsumoto Y, Nomura K, Kanda-Akano Y, *et al.* Successful treatment with *Erwinia* L-asparaginase for recurrent natural killer/T cell lymphoma. *Leukemia Lymphoma* 2003; 44: 879–882.
9 Harabuchi Y, Yamanaka N, Kataura A, *et al.* Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. *Lancet* 1990; 335: 128–130.
10 Kawa K. Epstein-Barr virus–associated diseases in humans. *Int J Hematol* 2000; 71: 108–117.
11 Au WY, Weisenburger DD, Intragumtornchai T, *et al.* Clinical differences between nasal and extranasal natural killer/T-cell lymphoma: a study of 136 cases from the International Peripheral T-Cell Lymphoma Project. *Blood* 2009; 113: 3931–3937.
12 Jaccard A, Gachard N, Marin B, *et al.* Efficacy of L-asparaginase with methotrexate and dexamethasone (AspaMetDex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study. *Blood* 2011; 117: 1834–1839.
13 Yamaguchi M, Suzuki R, Kwong YL, *et al.* Phase I study of dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide (SMILE) chemotherapy for advanced-stage, relapsed or refractory extranodal natural killer (NK)/T-cell lymphoma and leukemia. *Cancer Sci* 2008; 99: 1016–1020.

DOI: 10.1183/09031936.00123911

Original Article

Journal of Oncology Pharmacy Practice

J Oncol Pharm Practice
0(0) 1–10

DOI: 10.1177/1078155212459667
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Exposure of family members to antineoplastic drugs via excreta of treated cancer patients

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Abstract

Purposes: (a) To measure the urinary excretion of antineoplastic drugs of three patients during 48 h after the administration of cyclophosphamide (two patients) and 5-fluorouracil (one patient). (b) To evaluate environmental contamination with antineoplastic drugs via excreta of patients in the home setting. (c) To evaluate exposure of family members to antineoplastic drugs by measuring the drugs in their urine during the 48 h after completion of the chemotherapy by the patients.

Methods: Two patients were administered cyclophosphamide by i.v. bolus injection. One patient was administered 5-fluorouracil by i.v. bolus injection and thereafter immediately administered the same drug by continuous infusion for 46 h. Urine samples from the patients administered cyclophosphamide and their family members, and wipe samples from their home environment, were analysed for the unchanged form of cyclophosphamide. For 5-fluorouracil, the urine samples from the patient and the family member were analysed for the 5-fluorouracil metabolite α-fluoro-β-alanine. Wipe samples were analysed for 5-fluorouracil. Drugs were detected and quantified with gas chromatography in tandem with mass spectroscopy-mass spectroscopy or by high-performance liquid chromatography with ultraviolet-light detection.

Results: A total of 35 and 16 urine samples were collected from the three patients and their family members, respectively. The drugs were detected in all samples.

Cyclophosphamide was detected at levels of 0.03–7.34 ng/cm^2 in 8 of the 12 wipe samples obtained from the homes of the patients administered cyclophosphamide. For the patient administered 5-fluorouracil, drug levels in his home environment were below the limit of detection.

Conclusion: We demonstrated contamination of the home setting and exposure of family members to cyclophosphamide via the excreta of outpatient receiving chemotherapy. Exposure of the family member of the patient administered 5-fluorouracil was also demonstrated. These findings indicate the importance of strict precautions by the members of treated cancer patients as well as healthcare workers, to reduce the risk of exposure to antineoplastic drugs.

Keywords

Drug exposure, antineoplastic agents, drug contamination, cyclophosphamide, 5-fluorouracil

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Introduction

With the development of antineoplastic drugs, the treatment of malignant tumours has been advancing rapidly. Antineoplastic drugs primarily exert cytotoxic effects on tumour cells, but often also affect normal cells. In other words, most antineoplastic drugs are harmful to all cells in cancer patients, causing both beneficial and toxic effects. These drugs not only exert therapeutic effects in cancer patients but also have the potential to harm healthcare workers who prepare and administer antineoplastic drugs, such as pharmacists and nurses, as a result of occupational exposure to these drugs. To date, numerous studies have demonstrated the occurrence of adverse effects due to occupational exposure to antineoplastic drugs, including acute allergic reactions, carcinogenicity, genotoxicity, fetal abortion and congenital anomalies.[1–13]

Antineoplastic drugs are classified as hazardous drugs that exhibit cytotoxicity. Various guidelines have been issued to ensure their safe use (National Institute for Occupational Safety and Health;[14] Occupational Safety and Health Administration;[15] American Society of Health System Pharmacists;[16] Oncology Nursing Society;[17] Health, & Safety Executive[18]). The guidelines recommend adopting preventive measures for 48 h after drug administration, because the unchanged form of an administered cytotoxic antineoplastic drug (or its active metabolites) may be part of the substances excreted by the patient. The guidelines also stress that drug-specific durations are desirable, as the duration of excretion of hazardous drugs varies. These guidelines also recommend that one should wear protective clothing/equipment (gloves, gown, goggles and a face shield if any scattering of bodily fluids is likely) while handling urine, faeces, blood or vomitus. Furthermore, these guidelines state that caution should be exercised during flushing the toilet (performed twice) after appropriately placing the toilet seat cover following disposal of the excreta of the patients administered cytotoxic drugs within the previous 48 h. However, no studies have provided evidence supporting the efficacy of flushing the toilet twice

The aforementioned guidelines[14–18] provide an outline for procedures, which healthcare professionals, janitorial staff and the family members of patients should conform to, in the hospital and at home.

The administration of chemotherapy in the hospital setting is different from that of an outpatient setting. Administration methods for outpatient chemotherapy (such as FOLFOX and FOLFIRI) include bolus i.v. administration of an antineoplastic and also continuous infusion of 5-fluorouracil (5-FU) for approximately 46 h. In the latter case, the antineoplastic drug is continuously administered over a long period of time including while the patient is at home. This further increases the risk of exposure of the patient's family members and associates to the antineoplastic drug via the patient's excreta. Following outpatient chemotherapy administration, cancer patients spend most of their time at home or in the workplace. The exposure of the associates of a cancer patient to an antineoplastic drug via the patient's excreta can negatively affect their health, similarly as in the case of occupational exposure to these hazardous drugs. Thus, it is necessary to consider that the people at risk of exposure to antineoplastic drugs not only include healthcare workers but also the family members and associates of the patient. As far as we know, no papers have published the antineoplastic drug contamination of the homes of cancer patients via their excreta, and at present, it remains unclear whether the family members of these patients are exposed to these drugs.

The present study was conducted with the following three objectives: (a) to measure the urinary excretion of antineoplastic drugs of three treated patients during 48 h after the administration of cyclophosphamide (CPM; two patients) and 5-fluorouracil (5-FU; one patient); (b) to evaluate environmental contamination with antineoplastic drugs via excreta of patients in the home setting and (c) to evaluate exposure of family members to antineoplastic drugs by measuring the drugs in their urine during the 48 h after completion of the chemotherapy by the patients.

The antineoplastic drugs used in the current study have been extensively and frequently used in therapy. CPM has been used for adjuvant chemotherapy after breast cancer surgery. During chemotherapy, patients receive a bolus i.v. dose of CMP once every 3 to 4 weeks for several cycles at an outpatient facility. CPM is an alkylating agent, which is converted to its active metabolite, phosphoramide mustard, and has been classified as a genotoxic carcinogen in humans by the International Agency for Research on Cancer.[19] 5-FU is used to treat colon cancer. For FOLFOX and FOLFIRI therapies using 5-FU, patients are first administered a bolus i.v. dose of 5-FU at an outpatient facility, followed by continuous i.v. infusion for 46 h. This procedure is repeated every 2 weeks. 5-FU is administered by continuous i.v. infusion, making it necessary to manage long-term administration of the drug in a patient's home, which increases the risk of exposure of the family members and associates of the patient.

Patients and methods

Three cancer patients who were receiving chemotherapy at the Outpatient Chemotherapy Center of

University Hospital A in Japan were enrolled in this study. Each patient and their family members provided informed written consent to participate in the study. The antineoplastic drug administered in Patients 1 and 2 was CPM and in Patient 3 was 5-FU.

Case 1

Patient 1 was a 58-year-old female diagnosed with breast cancer. This was her third course of chemotherapy, and she was administered CPM 700 mg (500 mg/m^2) via an i.v. bolus on an outpatient basis. Her husband (Family Member 1), who was in his 60 s, underwent biological monitoring to measure CPM in his urine. Her husband was mandatorily retired and was often away from home pursuing his hobbies. Hence, his urine samples were collected only when he was at home during the 48-h post-administration period.

Case 2

Patient 2 was a 44-year-old female diagnosed with breast cancer. This was her fifth course of chemotherapy, and she was administered CPM 712 mg (500 mg/m^2) via an i.v. bolus on an outpatient basis. CPM was measured in the urine of her husband (Family Member 2), who was in his 40 s. The husband was away from home, at work, during the day and therefore, his urine samples were collected only during the time when he was at home, from approximately 19:00 in the evening until about 7:00 in the morning when he left for work (on two consecutive days).

Case 3

Patient 3 was a 78-year-old male diagnosed with rectal cancer who had undergone a colostomy. This was his eighth course of chemotherapy, and he was administered 5-FU 549 mg (400 mg/m^2) via an i.v. bolus on an outpatient basis. Immediately thereafter, a continuous i.v. infusion of 5-FU 3293 mg was initiated that lasted 46 h.

The patient's wife (Family Member 3) was in her 70 s and her urine samples were analysed for α-fluoro-β-alanine (FBAL). The wife remained at her husband's side throughout his outpatient treatment. At home, both the patient and his wife spent most of their time at a small table in their living room, including sleeping at the same site each night in Japanese-style beds. Their toilet was Japanese style, having no toilet seat lid. The collection of the wife's urine samples was stopped at 32 h due to personal circumstances.

The performance status was Grade 0 for all patients according to the Eastern Cooperative Oncology Group criteria.[20]

This survey was conducted with the approval of the Ethics Committee of Fukushima Medical University.

Urine sampling (biological monitoring) and wipe sampling (environmental monitoring)

After each patient completed outpatient chemotherapy in the hospital, the surveyor visited their homes and repeated the explanations of these tests and the urine sample collection method to the patients and their family members. A second visit was made to each patient's home 48 h after the completion of outpatient chemotherapy, during which time wipe sampling of the home environment was performed and the urine samples were collected.

Each patient and family member collected their own urine samples during the 48-h post-administration period. Each patient and family member was responsible for recording the frequency and time of voiding and measuring the urinary output at each urination. Ten millilitres of urine were collected (urine sample kit provided) and stored in a small freezer solely used for this purpose.

Wipe surveys were conducted in the homes of the three cancer patients to clarify the status of drug contamination during 48 h after administration. The surveyor visiting the patients' homes asked them how many times they had urinated and defecated after administration, and asked for information about vomiting, if occurred.

The surveyor identified possible areas of drug contamination based on the answers provided by each patient and conducted wipe surveys of these areas. The wipe samples were taken from 17 areas in the homes of the three patients. The areas from which wipe samples were taken included the toilet seat, toilet seat cover, flush handle, toilet door knob, floor around the toilet, toilet paper holder, toilet handrail and sink faucets. The wipe samples were collected in the following manner. After measuring the size of each target area, 17 mL of a 0.03 M NaOH solution were applied to each target area using a wipe kit to detect CPM and 5-FU. For target areas that were not flat, the solution was poured over the area and subsequently, the samples were collected using two pieces of nonwoven cloth, placed at a downhill location to soak up the solution.

All urine and wipe samples were immediately stored frozen at −20°C after sampling and during transport until sample preparation and analysis by Exposure Control Sweden AB (Bohus-Björkö, Sweden). The urine and wipe samples were collected using Cyto Urine kits and Cyto Wipe kits with Exposure Control Sweden AB. The methods to collect, store and exact

urine sampling and wipe sampling were performed according to standard procedures.[21–25]

Analyses of the samples

Analyses were performed to detect the unchanged form of CPM in the urine samples of the patients administered CPM and their family members, and in the wipe samples from their home environment. Analyses were performed to detect FBAL in the urine samples collected by the patient administered 5-FU and his family member. Wipe samples were analysed for 5-FU.

The wipe samples were prepared for analysis by adding a 0.03 M NaOH solution (total volume: 160 mL). After extraction, a portion of the extract was further purified according to the standard procedure.[21–25] The contamination per square centimetre was calculated by assuming 100% recovery and wipe efficiency. The limits of detection of CPM and 5-FU were 0.1 and 20 ng/mL NaOH, respectively.

The sample volume used for the determination of CPM and FBAL in urine was 5 mL. The limits of detection of CPM and FBAL were 0.01 and 5 ng/mL urine, respectively.[21–25]

CPM and FBAL were analysed with gas chromatography in tandem with mass spectroscopy-mass spectroscopy and 5-FU was analysed by high-performance liquid chromatography with ultraviolet-light detection.[21–25]

Results

There were 35 and 16 urine samples collected for the three patients and their family members, respectively. Antineoplastic drugs were detected in all samples. For Case 1, patient 1 urinated eight times (total volume: 1870 mL) and defecated twice during the 48-h post-administration period (Figure 1). The detected amount of CPM per urine sample ranged from 0.04 to 62.64 mg. The highest CPM excretion occurred at 3 h after drug administration. Thereafter, the amount excreted decreased gradually over time to 0.04 mg in the final urine sample. The total amount of CPM excreted in the urine during the 48-h post-administration period was 170.10 mg, representing 24.3% of the total administered dose.

Family Member 1 collected five urine samples (total volume: 1800 mL) during the 48-h post-administration period. CPM was detected in all samples. The detected amount of CPM per urine sample ranged from 24.0 to 35.0 ng, and the total detected amount was 152.0 ng.

For Case 2, patient 2 urinated 12 times (total volume: 1830 mL) and defecated twice during the 48-h post-administration period (Figure 2). The amount of CPM per urine sample ranged from 0.30 to 32.84 mg. The total amount of CPM during the 48-h post-administration period was 140.93 mg, representing 19.8% of the total administered dose.

Family Member 2 collected four urine samples (total volume: 900 mL) during the 48-h post-administration period. CPM was detected in all samples. Urine CPM levels ranged from 0.07 to 0.65 mg, and the total amount detected in the four samples was 1.81 mg.

For Case 3, patient 3 urinated 15 times (total volume: 1240 mL) during the 48-h post-administration period following outpatient 5-FU administration (including continuous infusion of 5-FU for 46 h) (Figure 3). This patient had a colostomy bag. The amount of FBAL per urine sample ranged from

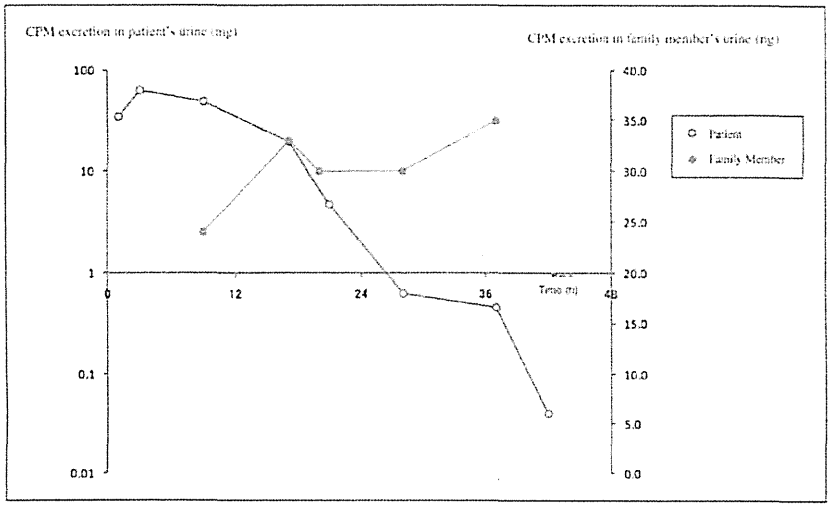

Figure 1. Time-course profiles of CPM excretion in urine samples of Patient 1 and Family Member 1. CPM: cyclophosphamide.

0.1 to 2.2 mg. The total amount of FBAL excreted in the urine was 16.9 mg, comprising 0.44% of the total administered dose.

Family Member 3 urinated eight times (total volume: 1120 mL) during the 32-h monitoring period. The amount of FBAL per urine sample ranged from 19.0 to 117.0 μg; the total amount in the urine was 421.0 μg.

The results from the wipe samples are presented in Table 1. For Case 1, wipe samples from four out of six positions were positive for CPM contamination. Of these areas, samples taken from the sink faucets and toilet seat exhibited the highest level of contamination of 3.02 and 0.57 ng/cm^2, respectively. The level of contamination on the floor around the toilet and toilet door knob was 0.03 and 0.09 ng/cm^2, respectively.

For Case 2, CPM contamination was detected on four out of the six positions. The levels of contamination were between 0.18 and 7.34 ng/cm^2. The toilet seat was the most contaminated position.

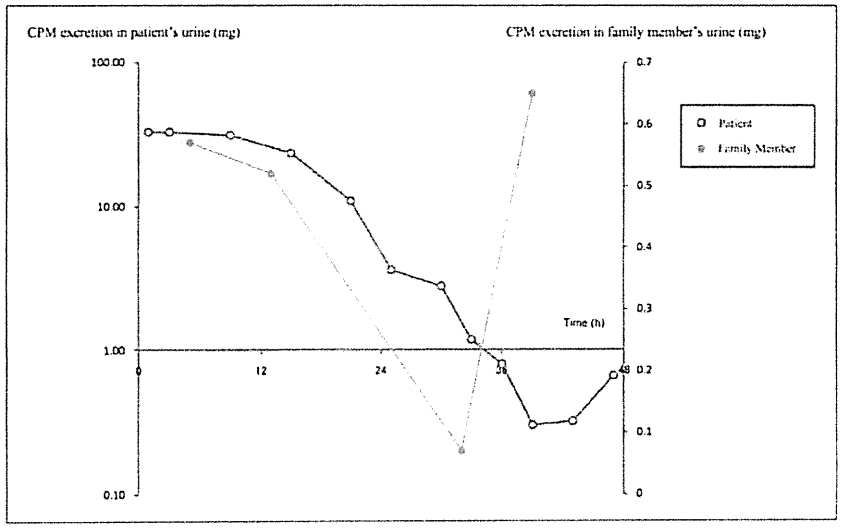

Figure 2. Time-course profiles of CPM excretion in urine samples of Patient 2 and Family Member 2. CPM: cyclophosphamide.

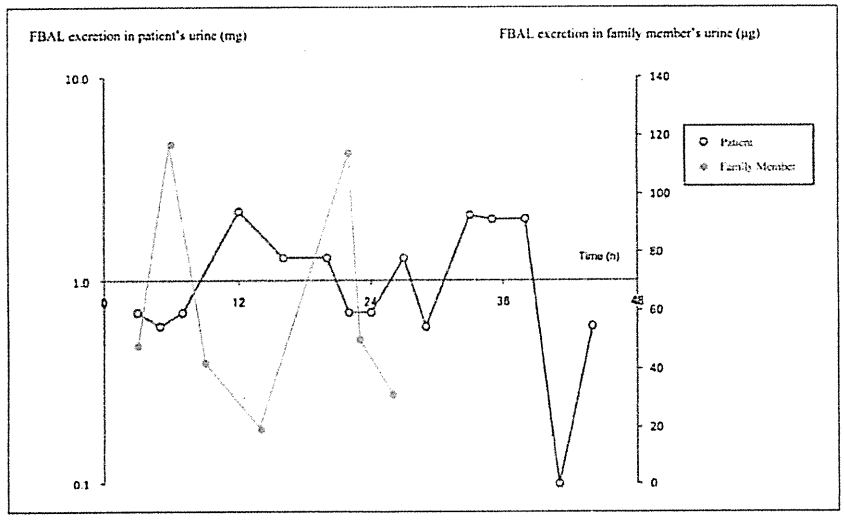

Figure 3. Time-course profiles of FBAL excretion in urine samples from Patient 3 and Family Member 3. FBAL: α-fluoro-β-alanine.

Table 1. Wipe test results: Surface levels of CPM and 5-FU.

	Surface description	Surface area (cm^2)	CPM (ng/mL NaOH)	CPM (ng)	CPM (ng/cm^2)
Case 1	Toilet seat	1960	7.03	1125	0.57
	Control panel	490	ND	–	–
	Flush handle	30	ND	–	–
	Toilet door knob	319	0.18	29	0.09
	Toilet floor	4000	0.63	101	0.03
	Sink faucets & surroundings	208	3.93	629	3.02
Case 2	Toilet seat	700	32.12	5139	7.34
	Toilet seat cover	1440	2.01	322	0.22
	Flush handle	100	ND	–	–
	Toilet door knob	260	0.30	48	0.18
	Toilet floor	1000	1.16	186	0.19
	Sink faucets & surroundings	100	ND	–	–
	Surface Description	Surface area (cm^2)	5-FU (ng/mL NaOH)	5-FU (ng)	5-FU (ng/cm^2)
Case 3	Toilet seat	3200	ND	–	–
	Toilet handrail	210	ND	–	–
	Toilet door knob	225	ND	–	–
	Portable pail	400	ND	–	–
	Paper holder	150	ND	–	–

CPM: cyclophosphamide; 5-FU: 5-fluorouracil; NaOH: sodium hydroxide; ND: not detected (CPM < 0.10 ng/mL NaOH, 5-FU <20 ng/mL NaOH).

The level of contamination on the toilet seat cover, floor around the toilet and toilet door knob was 0.22, 0.19 and 0.18 ng/cm^2, respectively.

For Case 3, wipe samples were taken from five positions and the level of 5-FU was below the limit of detection at each site.

None of the three patients vomited during the 48-h post-administration period.

Discussion

The present study assessed the amount of cytotoxic drug excreted in the urine of patients and their cohabiting family members during 48 h after the patients have received chemotherapy in an outpatient setting. The study also demonstrated environmental contamination with antineoplastic drugs at patients' home via excreta of the treated patients.

The percentage of the dose unchanged CPM in the urine of patients 1 and 2 during the 48-h post-administration period was approximately 24 and 19, respectively. According to previous studies, the percentage of CPM excreted in the urine during the 24-h period after CPM administration ranged from approximately 14 to 20 of the administrated dose.[26,27] Moreover, the present study demonstrated that CPM continued to be excreted at low levels in the urine of treated patients for at least 48 h after administration. Bagley et al.[28] injected radiolabelled CPM by i.v. and found that 62% of the administered dose was excreted in the urine within 2 days, whereas 1.8% and 1.2% were excreted in the faeces and expired air within 4 days after dosing, respectively.

Another important finding of the present study is that the administered cytotoxic drugs were detected in all urine samples collected by each cohabiting family member. Although the family members did not receive chemotherapy, CPM was detected in their urine samples collected during the post-administration period, indicating exposure to CPM. All five urine samples collected by Family Member 1 tested positive for CPM (range: 24.0 35.0 ng). However, urine samples were collected by Family Member 1 only when he was at home and thus, the number of samples was approximately half of the expected number. Therefore, it can be surmised that the actual level of exposure to the drug was higher than measured. Similarly, Family Member 2 collected four urine samples during the time that he was at home, and CPM was detected in all samples. The present study is the first to demonstrate CPM exposure of a family member of a patient at home.

The time-course profiles of CPM excretion in the urine of the Family Members 1 and 2 was also an interesting observation. Although the amount of CPM excreted by Patients 1 and 2 decreased over time following patient treatment, the urinary drug excretion remained fairly constant in Family Member 1, whereas it gradually decreased in Family Member 2, only to

rebound to a higher excretion later. These time-course profiles of CPM excretion by the family members can be ascribed to their continued exposure to the drug in their homes. Previous studies found that urinary CPM excretion by patients peaked at approximately 6–10 h after administration,[29,30] whereas in patients 1 and 2, the peaks occurred at approximately 3 h following administration. On the other hand, the time-course profiles of CPM excretion in the urine of the family members exhibited no clear peaks during the 48 h of monitoring.

In several studies, exposure to CPM was investigated using the urinary excretion of CPM as a biomarker. The mean amounts of CPM detected in the urine of workers due to occupational exposure were 5.2 μg/day in pharmacy personnels/nurses,[31] 1.36 μg/day in pharmacy technicians,[32] 0.79 μg/day in nurses/pharmacy technicians/cleaning women,[33] 0.47 μg/day in nurses,[34] 0.39 μg/day in hospital workers,[35] 0.18 μg/day in pharmacy technicians[36] and 0.05 μg/day in pharmacy technicians/nurses.[22] It can be assumed that the variation in these values reflects differences in the occupations and strict observance of precautions to prevent exposure to antineoplastic drugs. However, in our study, it is noteworthy that higher levels of CPM were detected in the urine samples of family members than in those of healthcare workers. Moreover, there is an important difference in the route of exposure to cytotoxic drugs between the previous reports and the present study. That is, in the case of healthcare workers, direct exposure via inhalation, skin contact, skin absorption and/or oral intake occurs during handling of the drugs. On the other hand, exposure of the family members at home occurred via contact with patient excreta containing the drug.

We estimated the areas in the patients' homes where CPM would likely be present. Our efforts at detecting the drug successfully confirmed the extent and level of CPM contamination in the home environment. Drug contamination was confirmed at four areas for both Patients 1 and 2. The areas with the highest levels of CPM contamination were the toilet seat, floor around the toilet, toilet door knob and sink faucets.

Yuki et al.[37] investigated CPM contamination in the homes of five female patients with breast cancer at 48 h after outpatient bolus i.v. administration of the drug. CPM was detected in 17 of 30 samples taken from the target areas. The toilet seat was contaminated by CPM in all the cases, and it also had the highest level of contamination ranging from 0.04 to 8.35 ng/cm^2. CPM contamination was also confirmed for the floor around the toilet (0.19–1.53 ng/cm^2), toilet door knob (0.79 ng/cm^2) and toilet seat lid (0.22 ng/cm^2). The sites and levels of CPM contamination in "patients' homes" and the "dose of unchanged CPM" in this study are similar to those reported by Yuki et al.[37]

In the US and Europe, reports have documented the detection of antineoplastic drugs in surface wipe examination of the handling sites of antineoplastic drugs in hospitals.[38–41] The present survey data cannot be compared with those from previously reported studies since no previous study has investigated antineoplastic drug contamination in the home environment of cancer patients receiving outpatient chemotherapy. In Japan, the values obtained from two surveys conducted to assess the extent of environmental CPM contamination in hospitals via the same wipe test method used in the present study were used as reference values. In the study conducted by Tanimura et al. involving six target areas in the chemotherapy preparation room, CPM contamination was demonstrated at levels of 0.01–0.09 ng/cm^2 in all six target areas in the first wipe test.[42] Sugiura and colleagues performed a survey of environmental CPM contamination at six hospitals in Japan and reported low contamination levels of ≤ 0.1 ng/cm^2 in the outpatient chemotherapy room, 0.01 ng/cm^2 on the table used for handling antineoplastic drugs and 0.04 ng/cm^2 on the floor under the drip in the fusion stand at half of the hospitals studied.[43] Compared with the results reported by these two studies, the CPM contamination levels detected in the present study were significantly greater, indicating a higher degree of environmental CPM contamination in the outpatient setting than in hospitals.

The high values of our findings for CPM contamination of the toilet seats, sink faucets, floor around the toilet and toilet door knobs at the patients home indicate that urine and faeces of the patients containing CPM contaminated the toilet environment due to splattering, and that this was further spread via the patients' hands, which had been contaminated during the process of cleaning themselves after urination/defecation. Variable amounts of hazardous drugs and their metabolites are excreted in the urine, stool, sweat and other bodily excreta of patients receiving the drugs.[17] Bed sheets of patients who were treated with CPM appeared to be contaminated by the drug.[44] Thus, we can surmise that they were repeatedly exposed to the drug.

Patient 3 was administered 5-FU via an i.v. bolus on an outpatient basis and then immediately started on a continuous i.v. infusion of 5-FU that lasted 46 h. The amount of FBAL per urine sample ranged from 0.1 to 2.0 mg. The total amount of FBAL excreted in the patient's urine during the 48-h post-administration period represented 0.44% of the total administered dose. FBAL was also detected in all urine samples collected by Family Member 3, demonstrating that she had been exposed to the 5-FU excreted by Patient 3. The concentration of FBAL excreted in the urine of Family Member 3 fluctuated greatly during the sampling period, and the total amount collected over a