

Cellular Proteolysis and Oncology

Haemostatic abnormalities and thrombotic disorders in malignant lymphoma

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Summary

We examined haemostatic abnormalities and thrombotic disorders in 217 patients with malignant lymphoma. Plasma levels of fibrinogen and D-dimer were significantly higher in patients with malignant lymphoma than in healthy subjects. The incidence of severe complications, such as disseminated intravascular coagulation (DIC) and interstitial pneumonia (IP), differed with each clinical stage or histological type, but they occurred frequently in stage IV or natural killer (NK) cell lymphoma. Plasma levels of fibrinogen degradation products (FDP) and D-dimer, leukocyte tissue factor (TF) mRNA and plasma TF antigen were significantly higher in stage IV than in stage I, II or III. Plasma levels of FDP, D-dimer, and leukocyte TF mRNA in NK cell lymphoma

were markedly higher than in other types of lymphoma. Immunohistochemical staining of NK cell lymphoma revealed that granulocyte macrophage colony-stimulating factor was positive in tumour cells, whereas von Willebrand factor and TF were positive in vascular endothelial cells of surrounding tissue. Our results suggested that patients with stage IV disease and NK cell lymphoma were in abnormal thrombotic and haemostatic state, and may frequently develop DIC and IP. One of the mechanisms of DIC and IP may involve elevated cytokine production by lymphoma cells, which can stimulate the expression of TF in blood cells or surrounding tissue.

Keywords

Malignant lymphoma, disseminated intravascular coagulation, interstitial pneumonia, thrombotic diseases, leukocyte tissue factor

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Introduction

Thrombotic disorders, including deep vein thrombosis (DVT), pulmonary embolism (PE), and disseminated intravascular coagulation (DIC) are the most frequent complications and the second cause of death in patients with malignant disease (1), such as solid tumours (2, 3), acute promyelocytic leukaemia (APL) (4) and malignant lymphoma (5). In addition, these patients are more likely to develop a thrombotic disorder during chemotherapy (3, 6). One mechanism of hypercoagulability in patients with malignant diseases is the action of tissue factor (TF), which may be tumour cell-derived (7–9) or may originate from the tumour-associated environment (10). TF is a transmembrane glycoprotein

that plays an essential role in initiation of the TF coagulation pathway. TF serves as the receptor that binds to its essential cofactors, factor VII and activated FVII, leading to thrombin generation and fibrin formation (11). In malignant disease, it was reported that TF plays an important role in coagulation abnormalities, and also in abnormal angiogenesis (12–14), cell migration (15), progression (16) and metastasis (17–19). Previous studies showed a significant increases in plasma TF antigen (17, 20) and leukocyte TF mRNA expression (21) in malignant diseases. It was suggested that tumour cells express TF, and that elevated cytokines (tumour necrosis factor [TNF] [22] or interleukin-1 [IL-1] [23]) may stimulate endothelial cells or leukocytes to express TF. Furthermore, in cases complicated with infection, in-

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creased lipopolysaccharide (LPS) expression (24) or consequent inflammatory cytokines may upregulate TF expression.

In hematopoietic malignancy, a recent study showed that the incidence of malignant lymphoma was higher than that of acute myeloblastic leukaemia (AML) (25). It is widely recognized that patients with malignant lymphoma are in a hypercoagulable state, and frequently have associated thrombotic disorders during the clinical course (26). In particular, DIC is one of the most lethal complications and its prognosis remains poor. Recently, a close association between coagulation and inflammation has been suggested (27). Interstitial pneumonia (IP) is a non-bacter-

ial infectious lung condition of an unknown aetiology, and considered to occur often during chemotherapy of hematopoietic tumours. Pathological findings suggest that it is associated with hypercoagulation and inflammation. Malignant lymphoma has been implicated as the underlying disease in DIC and IP.

In this report, we evaluated haemostatic abnormalities in 217 cases of malignant lymphoma to investigate the pathogenetic role of TF in haemostasis and inflammation.

Materials and methods

Subjects

Established global coagulation markers (activated partial thromboplastin time [APTT]), prothrombin time [PT], fibrinogen [Fbg], fibrinogen degradation products [FDP], D-dimer) were measured in 217 patients (age, 63 ± 14 years, mean \pm SD, male: female = 131: 86) with malignant lymphoma, who were treated or underwent chemotherapy at the Mie University School of Medicine between April 1, 1998 and March 31, 2003. The measurements were performed prior to chemotherapy or radiotherapy in newly diagnosed patients or patients in relapse following 6 months of a therapy-free period. The patients were diagnosed according to the Revised European and American lymphoma (REAL) classification (28). Clinical staging was classified according to the Ann Arbor classification system.

Thirty healthy volunteers (age, 27 ± 5 years, male: female = 31: 6) were recruited as controls. The clinical stage, histopathological type, protocol of therapy, incidence of IP/ARDS and DIC of participating patients are shown in table 1. We retrospectively analysed the clinical data and coagulation markers. During the last year of the study, we continuously and prospectively examined plasma TF antigen and leukocyte TF mRNA. Leukocyte RNA was extracted from ethylenediaminetetraacetic acid (EDTA)-peripheral whole blood within 3 hours post-venepuncture and plasma samples were stored at -80°C until measurement.

Informed consent was obtained from the patients in advance and the study was approved by the Mie University Review Board for human studies.

Histopathological examination and immunohistochemical staining

Histopathological and immunohistochemical examinations were performed on natural killer (NK) cell lymphoma tissue and nasal CD8-positive T cell lymphoma tissue. Sections of tissues measuring approximately 5×5 mm were fixed immediately, embedded in optimal cutting temperature (OCT) compound in 15×15 mm cryomolds and rapidly frozen in liquid nitrogen and stored at -80°C until use. For histopathological examination, samples were stained with hematoxylin and eosin.

For immunohistochemical staining of TF, granulocyte macrophage colony-stimulating factor (GM-CSF) or von Willebrand factor (vWF) antigen, samples were subjected to the avidin-biotin method as described previously (16). Briefly, immunohistochemical staining was performed on freshly prepared cryostat sections placed on silane-coated slides. Sections were fixed in 4% paraformaldehyde (pH 7.0) at 4°C for 10 min. After two washes in ethanol, they were air dried for 90 min at -20°C . Next,

Table 1: Incidence of severe complications associated with malignant lymphoma according to clinical stage/histological type and anticancer protocol.

		IP/ARDS	DIC
Patients	n=217	9 (4.1%)	7 (3.2%)
Age (mean \pm SD)	63 \pm 14		
Sex (Male:Female)	M131:F86		
Clinical Stage			
Stage I	37	1 (2.7%)	1 (2.7%)
Stage II	42	3 (7.1%)	
Stage III	33	1 (3.0%)	
Stage IV	64	4 (6.2%)	6 (9.4%)
unknown	31		
Histological Type			
DLBCL	86	5 (5.8%)	1 (1.2%)
FL	26		
MALT	19		
HD	12	1 (8.3%)	
MCL	10		
MZBCL	8		
ATL	4		
NK	7		3 (42.9%)
Burkitt	7	2 (28.6%)	2 (28.6%)
Others	38	1 (2.6%)	1 (2.6%)
Chemotherapy			
CHOP/R-CHOP	84	4	1
DeVIC	8		2
ABVD	4	1	
Radiation	13		
VAD	2	1	
LSG	3		
VPI6, PDN	1	1	
No therapy	12	1	1
Others	21		1
Multiple protocol	69	1	2

Table 2: Haemostatic abnormalities encountered in patients with malignant lymphoma.

	All patients	Patients complicated DIC or IP	Healthy subjects
n=	217	15	37
APTT (sec)	31.3±7.5	35.9±37.8*	30.6±1.4
PT (sec)	11.9±1.7	12.0±1.3	11.4±0.6
Fibrinogen (mg/ml)	338±133 [¶]	388±464 [¶]	221±23
FDP (mg/dl)	12.9±32.5	18.5±30.7*	3.9±1.7
D-dimer (µg/ml)	3.3±4.6 [¶]	5.4±8.9 [§]	0.3±0.2
n=	70	6	37
Leukocyte TF mRNA ratio	1563±2883 [¶]	3509±4514*	109±144
Plasma TF antigen (pg/ml)	328±243 [§]	271±290*	180±115

Data are mean ± SD. *P<0.05, [§]P<0.01, [¶]P<0.001, compared with healthy subjects. (Data of IP and DIC were measured at the time of accident).

the slides were incubated in horse serum for 20 min at room temperature to block non-specific binding. The slides were then incubated with monoclonal antibody to TF (#4508CJ; American Diagnostica, Greenwich, CT), granulocyte/macrophage colony-stimulating factor (GM-CSF, Genzyme, Cambridge, MA), or vWF (DAKO, Carpinteria, CA) (29) at a dilution of 1:100. After incubation and appropriate washing with phosphate-buffered saline (PBS), the slides were incubated with the secondary antibody, biotinylated horse anti-mouse IgG₁ at room temperature for 30 min and then rinsed in Tris-buffered saline (TBS). The slides were incubated with alkaline phosphatase-conjugated streptavidin at room temperature for 30 min. To reduce the endogenous alkaline phosphatase activity, the slides were reacted with levamisole dissolved in 0.2 M Tris HCl, pH 8.25. After incubation with a mixture of hexazotized new fuchsin and naphthol AS-BI phosphoric acid at room temperature for 20 min, TF, GM-CSF or vWF antigens were detected as red colour. The slides

Table 3: Haemostatic abnormalities according to clinical stage.

	Stage I	Stage II	Stage III	Stage IV
n=	37	42	33	64
APTT (sec)	30.9±6.6	30.6±7.3	29.5±4.6	32.6±10.0*
PT (sec)	12.1±2.7	11.6±1.1	11.7±1.4	12.0±1.5
Fibrinogen (mg/ml)	357±102	345±147	319±127	363±159
FDP (mg/dl)	3.7±2.3	4.6±3.1	5.9±6.0	15.8±40.7 [¶] †
D-dimer (µg/ml)	1.1±0.8	2.1±2.6	2.9±3.4	4.8±6.2 [§] †
n=	17	12	6	35
Leukocyte TF mRNA ratio	742±854	473±172	693±311	2454±3827 [¶] ‡***
Plasma TF antigen (pg/ml)	234±93	212±123	199±57	392±223 [§] ***

Data are mean ± SD. [¶]p<0.05, compared with stage I; [§]p<0.01, compared with stage I; [†]p<0.05, compared with stage II; [‡]p<0.01, compared with stage II; *p<0.05, compared with stage III; ***<0.01, compared with stage III

were then washed in distilled water and counterstained with hematoxylin.

Real-time quantitative PCR of TF mRNA

We measured leukocyte TF and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and standardized TF gene expression by GAPDH. For detection of TF and GAPDH gene expression at the mRNA level, RNA was extracted from EDTA peripheral whole blood using the Qiagen RNeasy blood mini kit (Qiagen Inc, Chatsworth, CA) according to the instructions provided by the manufacturer. The cDNA was synthesized using the Qiagen First-strand cDNA synthesis kit based on the procedure outlined by the supplier (30).

The fluorogenic probe and the primer designed for the amplification of cDNA were derived from the TF and GAPDH transcripts. Real-time quantitative polymerase chain reaction (PCR) was performed based on fluorescent TaqMan methodology (PE Applied Biosystems) and a threshold cycle (Ct) value for each sample was calculated by determining the point at which the fluorescence exceeded a threshold limit. The relationship between logarithm of starting template cDNA and Ct values appears to be linear, shown as a standard curve ($R^2 = 0.996-0.999$). The Ct value of each clinical sample was plotted on the standard curve, and the copy number was computed by Sequence Detector version 1.6 (PE Applied Biosystems), a software package (Ver. 1.0, Applied Biosystems, Foster City, CA) for data analysis. Data described in the text represent the mean Ct values of triplicate PCR amplifications. A plasmid containing the full-length cDNA of TF, PCR2.1-TM, was used as a positive control.

Measurement of coagulation markers and plasma TF antigen

Plasma PT and APTT were determined by the one-stage method of Quick using Thromborel S (Behringwerke, Marburg, Germany) and Pathromtin SL (Behringwerke), respectively. Plasma levels of fibrinogen were measured by a clotting method using Multiblin U (Behringwerke). Plasma levels of FDP and D-dimer were measured by a latex agglutination method using Lpia FDP (Dia-Iatron, Tokyo) and D-dimer (Kokusai-Shiyaku, Kobe, Japan), respectively. Plasma TF antigen was measured using the immunobind TF sandwich ELISA kit (American Diagnostica).

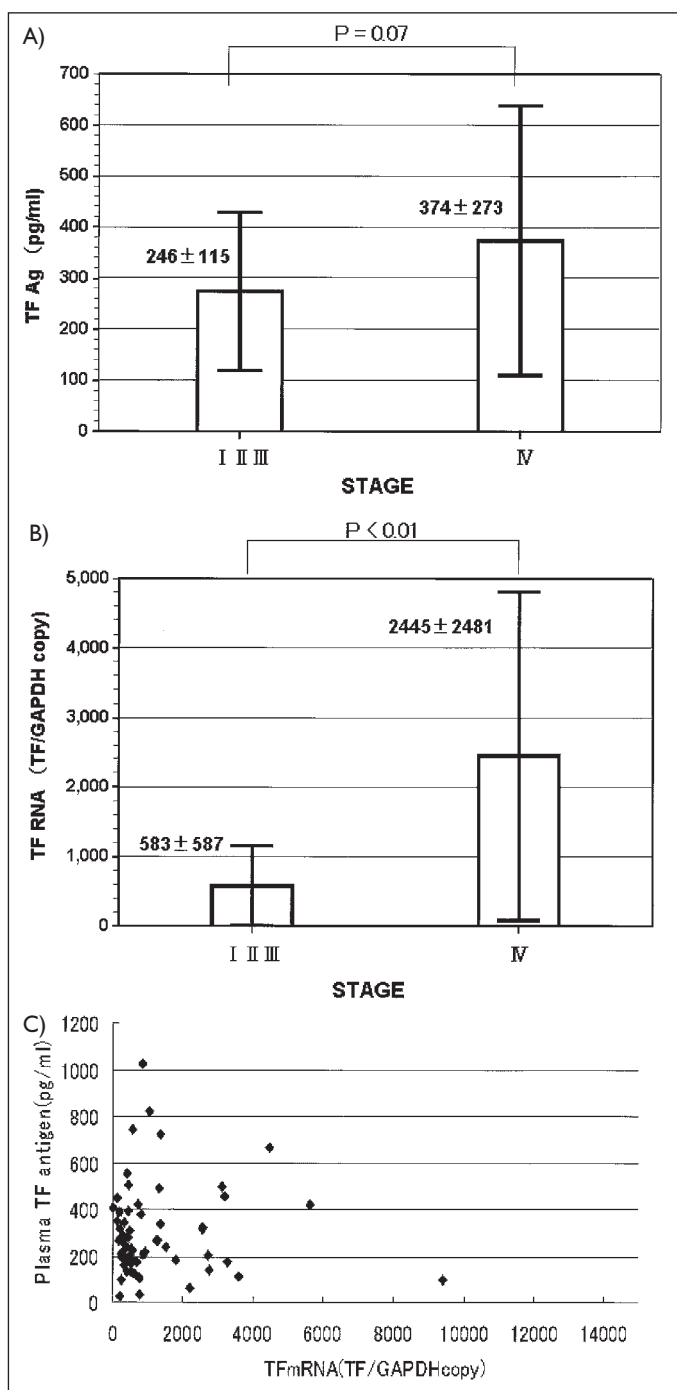


Figure 1: (A) Plasma TF antigen levels in DLBCL. TF antigen levels were significantly higher in patients with stage IV than in those of stage I, II, and III ($p = 0.07$). (B) Leukocyte TF mRNA levels in DLBCL. TF mRNA was significantly higher in patients with stage IV than in those of stage I, II, and III ($p < 0.001$). (C) There was no correlation between leukocyte TF mRNA levels in all patients and plasma TF antigen ($r = -0.049$, $p = \text{NS}$).

Diagnosis of DIC and IP

The diagnosis of DIC was based on the overt-DIC criteria established by the International Society of Thrombosis and Haemostasis (ISTH) (31). Diagnosis of IP was based on CT scan findings (ground-glass opacity, diffuse reticulonodular shadowing) and

decreased PaO_2 , normal range or rather decreased PaCO_2 , with clinical symptoms such as severe dyspnea or dry cough. Bacterial or fungal pneumonia were excluded by negative sputum culture. During the clinical course, one patient developed PE before diagnosis of malignant lymphoma and was treated with warfarin before chemotherapy, thus data of this patient were excluded from analysis.

Statistical analysis

Statistical analysis was carried out using Statmate for Windows. Data are expressed as mean \pm SD. Analyses of leukocyte TF mRNA and plasma TF antigen and coagulation markers were performed by Mann-Whitney's U test. Pearson correlations were performed to determine the association between leukocyte TF mRNA and plasma TF antigen. Data are expressed as mean \pm standard deviation. A p value of < 0.05 was considered significant.

Results

The association with DIC and IP was examined in all patients. Throughout the clinical course, seven patients (3.2%) were diagnosed with overt-DIC (age, 53 ± 17 years; male: female = 3:4) and 10 patients (4.1%) were diagnosed with IP (59 ± 13 years; 5:5). The association with DIC was frequently observed in stage IV or NK cell lymphoma. DIC occurred in only one case with stage I disease. Association with IP was observed in diffuse large B cell lymphoma (DLBCL), Hodgkin's disease (HD) and Burkitt's lymphoma. Although several therapeutic regimens were used, there was no clear correlation between the type of anti-cancer therapy and the incidence of IP (Table 1).

Coagulation markers appeared to worsen in the test group compared with the healthy subjects. APTT and PT were slightly longer in patients with malignant lymphoma than in healthy subjects. Plasma levels of FDP were markedly elevated in some patients with malignant lymphoma but there was no significant difference in FDP between patients and healthy subjects. Plasma levels of fibrinogen and D-dimer were significantly higher in patients with malignant lymphoma compared with healthy subjects ($p < 0.001$). The mean expression levels of leukocyte TF mRNA ($p < 0.001$) and plasma TF antigen ($p < 0.01$) were significantly higher in patients with malignant lymphoma than in healthy subjects. Furthermore, they were relatively high at the point of development of DIC or IP (Table 2).

There were no significant differences in APTT, PT and fibrinogen among the four clinical stages. However, plasma levels of FDP and D-dimer were significantly higher in stage IV than in stage I or II. Leukocyte TF mRNA and plasma TF antigen were significantly higher in patients with stage IV than in those with stage I, II or III ($p < 0.05$) (Table 3). In patients with DLBCL, the leukocyte TF mRNA levels and plasma TF antigen were significantly higher in stage IV than in stages I, II and III (TF mRNA: $p < 0.001$, TF antigen: $p = 0.07$, Fig. 1A, B). Leukocyte TF mRNA did not correlate with plasma TF antigen ($r = -0.049$, $p = \text{NS}$, Fig. 1C).

There were no significant differences in APTT, PT and fibrinogen levels among the nine histological groups, probably due to the small number of subjects in each group. On the other hand, FDP levels were markedly elevated in patients with Burkitt's and

Table 4: Haemostatic abnormalities according to histological type.

	DLBCL	FL	MALT	HD	MCL	MZBCL	ATL	NK	Burkitt
n=	86	26	19	12	10	8	4	7	7
APTT (sec)	30.9±8.0	29.6±5.1	31.9±8.1	33.0±10.0	27.5±4.1	27.0±4.2	29.8±4.8	31.3±3.7	41.7±8.9
PT (sec)	12.0±2.1	11.4±0.9	12.2±2.2	12.3±1.7	11.5±0.7	11.0±0.5	12.0±1.2	11.9±1.3	12.0±1.3
Fibrinogen (mg/ml)	336±103	290±71	293±101	448±230	358±131	359±86	300±52	386±35	505±239
FDP (mg/dl)	5.2±3.7	2.5±2.1	0.6±0.2	4.6±1.1	11.3±10.2	1.2±0.8	3.1±1.4	29.7±39.1	12.5±7.9
D-dimer (µg/ml)	3.4±3.9	2.1±2.1	0.4±0.2	2.4±2.1	2.8±2.2	0.8±0.6	2.0±2.5	9.6±9.9	0.7±0.2
n=	27	9	5	3	4	4	3	4	4
TF mRNA ratio	2036±3069	522±378	903±1047	843±615	324±366	1789±2559	812±565	4890±5806	430±294
TF antigen (pg/ml)	291±211	330±222	224±87	254±27	393±17	234±162	453±319	339±285	293±197

Data are mean ± SD

NK cell lymphoma, and D-dimer levels tended to be higher in NK cell lymphoma albeit statistically insignificant. There were no significant differences in APTT, PT, fibrinogen, FDP and D-dimer among the nine types of lymphoma. Leukocyte TF mRNA was markedly increased in some cases of NK cell lymphoma compared with other groups, but there were no significant differences in plasma TF antigen among the nine groups, probably due to the small number of patients per group (Table 4).

On histopathological examination, characteristic pathological changes such as diffuse necrosis, granulocytic change and angiocentricity (tumour cell invasion around vessels) were observed in NK cell lymphoma. Immunohistochemical studies of NK cell lymphoma showed strong staining for TF and vWF in vascular endothelial cells surrounding lymphoma tissue, rather than in tumour cells. In contrast, GM-CSF was detected in tumour cells themselves. In contrast, immunohistochemical staining of CD8-positive T cell lymphoma showed negative TF and GM-CSF staining in both tumour cells and vascular endothelial cells. vWF was positive in vascular endothelial cells of tumour but the staining was weak compared to that in NK cell lymphoma (Fig. 2).

Discussion

Haemostatic abnormalities and thrombotic disorders are frequently observed during the clinical course of malignant tumours. A hypercoagulable state may contribute to tumour progression and angiogenesis (32, 33). It is widely understood that hematopoietic tumours, such as AML and malignant lymphoma, are the most frequent diseases underlying DIC. The incidence of DIC in malignant lymphoma is not as high, but the absolute numbers of patients have increased recently. DIC is a potentially lethal complication, and it is therefore important to evaluate haemostatic abnormalities in malignant lymphoma. In this study, global coagulation markers were worse in all patients with malignant lymphoma compared with healthy subjects. In particular, plasma levels of fibrinogen and D-dimer were significantly different (Table 2). Previous studies indicated that plasma levels of fibrinogen and D-dimer were significantly higher in patients with metastatic gastric tumours than normal volunteers (34).

Elevated plasma levels of fibrinogen suggest complications of infection or inflammation, while elevated plasma levels of D-dimer suggest a hypercoagulable and fibrinolytic state in malignant lymphoma. Interestingly, Palumbo and colleagues (35) suggested that fibrinogen is an important determinant of the metastatic potential of circulating tumour cells and plasma levels of D-dimer have been reported to be markedly increased in solid tumours (36, 37) and malignant lymphoma (38) and correlated strongly with future thrombosis (39). During the course of our study, seven patients out of 217 (3.2%) developed DIC. Our results support the notion that malignant lymphoma predisposes to DIC.

There is considerable evidence for increased incidence of thrombotic disorders and DIC in patients with advanced cancer

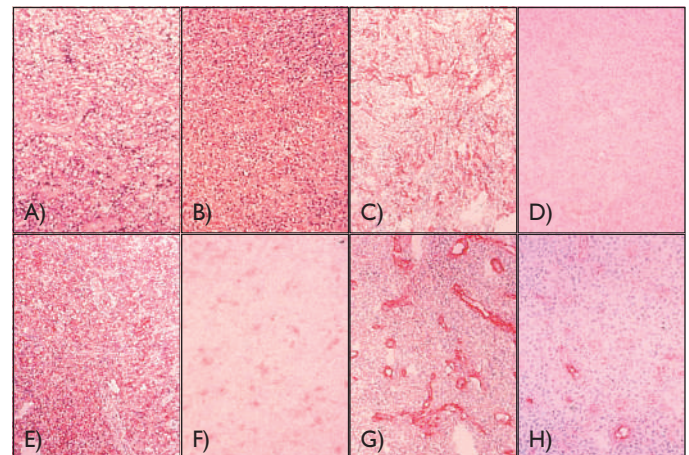


Figure 2: Histopathological and immunohistochemical staining of lymphoid tissues. (A, C, E, G) NK cell lymphoma, (B, D, F, H) CD8-positive T cell lymphoma. (A, B) Hematoxylin and eosin staining. Note the diffuse necrosis in (A). (C, D) TF antibody staining. Note the positive staining for TF in vascular endothelial cells of surrounding tissue of (C) but negative staining in (D). (E, F) GM-CSF staining. GM-CSF is positive in tumour cells of (E) but negative in (F). (G, H) vWF staining. vWF is strongly positive in vascular endothelial cells of (G) but weak in (H). Original magnification, ×100.

(40), particularly in progressive metastatic cases (36). In terms of histological classification, DIC frequently occurred in adenocarcinoma (36, 40). In our study of malignant lymphoma, the incidence of DIC was especially high in stage IV and NK cell or Burkitt's lymphoma. Global coagulation markers were impaired in those groups. The incidence of DIC in malignant lymphoma was considered distinct for each clinical stage or histological type of tumour. This finding suggests that coagulation abnormalities correlate not only with clinical stage or tumour volume, but also with histological type or species of malignancy.

DIC is frequent in patients with malignant tumours such as AML, solid cancer and malignant lymphoma. TF is an essential factor for DIC, and plasma TF antigens are markedly elevated in those conditions (20). However, it has been suggested that the main source of TF differs in each disease. We previously reported that TF antigen and activity in leukemic cell homogenate, especially in promyelocytic leukaemia (APL), were significantly higher than in mononuclear cells obtained from healthy subjects (41, 42). However, in adult lymphocytic leukaemia (ALL) and non-Hodgkin's lymphoma, TF antigen was not elevated compared with APL. That is, leukemic cells constitutively expressed TF. On the other hand, in solid cancer, TF antigen was positive on the tumour cell surface in lung, pancreatic and breast cancer as detected by immunohistochemical staining (8, 43). Our findings in immunohistochemical staining studies in malignant lymphoma showed that the surrounding tissue environment expressed TF whereas the tumour cells did not.

In the present study, our results showed that leukocyte TF mRNA and plasma TF antigen levels in malignant lymphoma were higher than those in healthy subjects, and that they were highest in stage IV ($p < 0.05$). Similar results were obtained in patients with DLBCL; plasma TF antigen levels were markedly high ($p = 0.07$) and leukocyte TF mRNA expression levels were significantly higher ($p < 0.001$) in stage IV than in stages I, II, and III. In patients with NK cell lymphoma or in those who developed DIC, leukocyte TF mRNA was markedly elevated, however, the difference was not significant probably due to the small number of patients in these groups. In DIC of malignant tumours, the source of elevated TF is unknown, but it is thought that tumour cells spontaneously express TF and that the activated tumour environment (endothelial cell or blood cell) secondarily expresses TF in response to inflammatory mediators (44, 45).

Histopathological examination of NK cell lymphoma revealed characteristic findings such as diffuse necrosis and angiocentricity. In terms of clinical findings in NK cell lymphoma, high-grade fever and high leukocyte count were observed despite small tumour volumes. Immunohistochemical staining of NK cell lymphoma revealed that expression of various cytokines (TNF and GM-CSF) in tumour cells, whereas TF was positive in vascular endothelial cells (Fig. 2). These results indicate that tumour-associated or -produced cytokine induced TF expression in the surrounding tissue and endothelial cells. Furthermore, it is possible that TF expression in vascular endothelial cells induced a hypercoagulable state that in turn induced local thrombosis and subsequent diffuse necrosis that was seen in histopathological examination of NK cell lymphoma. Histochemical staining of gastric cancer showed the presence of fibrinogen throughout the tumour stroma and fibrin and D-dimer staining at the host-tu-

mour interface whereas TF was present in cancer cells and tumour-associated macrophages (10). TF could induce a hypercoagulable state in malignant disease and has a close relationship with haemostatic abnormalities, but the mechanism of haemostatic abnormalities might differ in each disease.

Leukocyte TF mRNA did not always correlate with plasma TF antigen in each pathological state of malignant lymphoma. Furthermore, leukocyte TF mRNA was markedly increased in DIC high-risk groups. Leukocyte TF mRNA reflected activated leukocyte TF expression, and is possibly an important marker for predicting the development and/or prognosis of DIC in patients with malignant lymphoma. IP is one of the most lethal complications of malignant lymphoma. Histopathologically, intravascular or intraalveolar fibrin deposition is frequently seen in IP (46), and strong correlation between coagulation and inflammation has been postulated in IP. Previous studies also reported marked increase of procoagulant activity (PCA) in bronchoalveolar lavage fluid (BALF) of patients with ARDS compared with the control (47). One of the reasons for this finding was suggested to be high TF activity. In fact, alveolar macrophages and cuboidal epithelial cells were shown to express TF, and high levels of TF antigen were also found in BALF (48, 49). Other possible reasons include production by malignant cell and non-specific factors such as of acute phase reactants and necrosis (i.e., inflammation), abnormal protein metabolism (i.e., paraproteinaemia), and hemodynamic compromise (i.e., stasis). In addition, anticancer therapy may significantly increase the risk of thrombotic events by similar mechanisms, as well as by the release of procoagulants, damage of the endothelium (toxicity) with subsequent leakage of coagulation factors and inflammatory mediators, and depression of fibrinolytic activity. Our results showed no significant relationship between the incidence of IP and the type of anticancer therapy (patients who received several species of anticancer drugs were excluded because the influence on IP was not clear in such cases). However, previous studies reported that certain chemotherapeutic agents could induce a hypercoagulable state (50) or result in the development of IP (51–53). Considered together, further studies of the effects of various chemotherapeutic agents are needed. In our study, IP occurred in 10 patients out of 217 (4.1%) with malignant lymphoma. The incidence was high, particularly in NK and Burkitt's lymphoma. Global coagulation markers were impaired and leukocyte TF mRNA was higher in patients with IP. These findings suggest an enhanced inflammatory reaction in IP, and that TF plays an important role in fibrosis and abnormal coagulation. Activated leukocytes and increased cytokine levels are considered the main pathogenic cause of IP, which may be the same mechanism of DIC in malignant lymphoma.

TF may serve as an independent clinical molecular marker for the diagnosis of hypercoagulable and activated inflammatory states and prediction of future thrombotic disorders. We conclude that measurement of TF mRNA is useful for the management of lymphoma patients, and that in patients with elevated TF mRNA, especially those with stage IV or NK cell lymphoma, it is prudent to carefully monitor coagulation markers to help prevent DIC and IP.

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