self-renewal potential, so that the latent AML1/ETO fusion transcript may have been correlated with daughter leukemic cells without self-renewal potential [27].

The significance of MRD in CBF-AML has not yet been precisely evaluated because of the persistence of AML1/ETO and CBFβ/MYH11 in long survivors [13,32,33]. However, a lower frequency of gene fusion, especially undetectable MRD, is reportedly associated with long relapse-free survival (RFS) [13,34-36]. Therefore, our results suggest that ASCT with a graft that is PCR-negative for CBF-AML could be indicated not only for patients with adverse factors but also those with persistent MRD detectable by RQ-PCR after postremission therapy.

In conclusion, we have analyzed a series of consecutive CBF-AML patients, and found that those with ASCT had excellent EFS. Even if MRD was detectable in BM, it was possible to harvest a PCR-negative graft. Our ASCT strategy was based on graft MRD, and this was thought to have contributed to the excellent EFS and overcome other adverse factors. A large trial of ASCT with a PCR-negative graft is warranted for CBF-AML, especially in patients with adverse factors or with remnant MRD in BM after postremission therapy.

CONFLICT OF INTEREST

The authors report no potential conflicts of interest.

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bjh research paper

Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma

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Summary

Autotaxin (ATX) is a tumour cell motility-stimulating factor originally isolated from melanoma cell supernatants. ATX is identical to lysophospholipase D, which produces a bioactive lipid mediator, lysophosphatidic acid (LPA), from lysophosphatidylcholine. ATX is overexpressed in various malignancies, including Hodgkin lymphoma, and ATX may stimulate tumour progression via LPA production. The present study measured the serum ATX antigen levels in patients with haematological malignancies using a recently developed automated enzyme immunoassay. The serum ATX antigen levels in patients with B-cell neoplasms, especially follicular lymphoma (FL), were higher than those in healthy subjects. Serum ATX antigen levels in FL patients were associated with tumour burden and changed in parallel with the patients' clinical courses. The serum ATX antigen levels were little affected by inflammation, unlike the soluble interleukin-2 receptor and β2-microglobulin levels. As expected, the plasma LPA levels in FL patients were correlated with the serum ATX antigen levels. Given that leukaemic tumour cells from FL patients expressed ATX, the shedding of ATX from lymphoma cells probably leads to the elevation of serum ATX antigen levels. Our results suggest that the serum ATX antigen level may be a promising and novel marker for FL.

Keywords: autotaxin, lysophospholipase D, lysophosphatidic acid, lysophospholipid, follicular lymphoma.

Lysophosphatidic acid (LPA: monoacyl-*sn*-glycero-3-phosphate) is a lipid mediator with a variety of biological actions, such as cell proliferation and survival, cell migration and invasion, platelet activation and aggregation and smooth muscle cell contraction (Moolenaar *et al*, 2004; Birgbauer & Chun, 2006). In addition, mounting evidence points to a role for LPA in cancer progression (Mills & Moolenaar, 2003). Recent studies have shown that LPA is related to the

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initiation or progression of ovarian (Xu *et al*, 1995), prostate (Xie *et al*, 2002), and other cancers (Xu *et al*, 1995; Shida *et al*, 2003). LPA acts via specific G protein-coupled receptors (GPCRs) on the cell surface and activates a variety of signalling pathways (Valentine *et al*, 2008). Five LPA-specific GPCRs are as follows: LPA_{1-3}, which belong to the endothelial differentiation gene (EDG) family (Hecht *et al*, 1996; An *et al*, 1998; Bandoh *et al*, 1999), LPA_4/p2y9/GPR23 (Noguchi *et al*, 2003), and LPA_5/GPR92 (Lee *et al*, 2006). The aberrant expression of LPA receptors has been detected in various cancers (Mills & Moolenaar, 2003), while an association between LPA signalling and tumour progression in mouse models has been reported (Boucharaba *et al*, 2004; Yang *et al*, 2005).

Lysophosphatidic acid is present in human serum, plasma, saliva, follicular fluid and malignant effusions at a physiologically significant level (Moolenaar *et al*, 2004; Nakamura *et al*, 2007a). Several pathways contribute to LPA production, and it is now clear that extracellular LPA is mainly produced through the action of lysophospholipase D (lysoPLD), which converts lysophosphatidylcholine (LPC) to LPA (Meyer zu Heringdorf & Jakobs, 2007). Recently, lysoPLD was purified from human plasma (Tokumura *et al*, 2002) and fetal bovine (Umezu-Goto *et al*, 2002) and was found to be identical to autotaxin (ATX).

Autotaxin is a 125-kDa glycoprotein and a potent cell motility-stimulating factor originally isolated from the conditioned medium of A2058 human melanoma cells (Stracke *et al*, 1992). ATX belongs to the ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) family and is known as ENPP2. ATX is widely expressed, with the highest mRNA levels detected in the brain, placenta, ovary, and small intestine (Lee *et al*, 1996). ATX is also overexpressed in several human malignancies, such as glioblastoma multiforme (Kishi *et al*, 2006), prostate cancer (Zhao *et al*, 2007), and Hodgkin lymphoma (HL) (Baumforth *et al*, 2005). Based on the cloning of lysoPLD and the resultant finding that ATX is identical to plasma/serum lysoPLD, ATX has been confirmed to regulate cell motility through the production of LPA and LPA's effects on LPA receptors. Indeed, recent studies have shown that ATX stimulates the cell motility of various cancer cells *in vitro* through LPA formation and its interaction with LPA_1 (Hama *et al*, 2004; Kishi *et al*, 2006) although a key role for ATX in vascular development has also been reported (van Meeteren *et al*, 2006; Tanaka *et al*, 2006).

Several studies have shown that the addition of exogenous LPA stimulates cell proliferation and protects tumour cells from apoptosis in haematological malignancies. However, little is known about the association between the mechanism of LPA production via ATX and haematological malignancies. LPA acts as a survival factor in B-cell neoplasms. LPA stimulates the proliferation and immunoglobulin formation of B lymphoblasts (Rosskopf *et al*, 1998). LPA also protects B-cell lines and primary chronic lymphocytic leukaemia (CLL) cells from apoptosis (Hu *et al*, 2005; Satoh *et al*, 2007). Furthermore, the induction of ATX by the Epstein Barr virus (EBV) has been suggested to promote the growth and survival of HL cells; the up-regulation of ATX increased the generation of LPA and led to the enhanced growth and survival of HL cells (Baumforth *et al*, 2005).

Recently, we, for the first time, developed an automated enzyme immunoassay for measuring serum ATX antigen levels (Nakamura *et al*, 2008a). Using this new assay system, we previously reported the clinical significance of serum ATX measurements. In the present study, we measured the serum ATX antigen levels in patients with haematological malignancies and evaluated the usefulness of this parameter for clinical laboratory testing.

Materials and methods

Patients

We enrolled 161 patients with haematological malignancies who were treated at the Department of Haematology and Oncology, the University of Tokyo Hospital (Tokyo, Japan) and the Division of Transfusion, Tokyo Metropolitan Fuchu Hospital (Tokyo, Japan) between 2005 and 2007. The serum samples used in this study were residual samples from after the completion of the requested clinical laboratory tests. Plasma samples for the measurement of LPA and LPC were available from six patients with follicular lymphoma (FL). Informed consent was obtained from the patients for the usage of the samples. This study was approved by the Institutional Research Ethics Committee of the Faculty of Medicine, the University of Tokyo, and that of Tokyo Metropolitan Fuchu Hospital.

Table I shows the clinical diagnoses (according to the World Health Organization classification) (Jaffe *et al*, 2001) of the patients enrolled in the study. The patients with lymphoma were staged according to Ann Arbor classification (Carbone *et al*, 1971) by means of a physical examination, a computed tomography examination of the neck, chest, abdomen and pelvis, a bone marrow aspiration and biopsy, a haemogram and differential cell count, and routine biochemistry tests. Performance status was assessed using the Eastern Cooperative Oncology Group (ECOG) scale. In the patients with FL, the prognostic factors and the tumour burden were assessed according to the Follicular Lymphoma International Prognostic Index (FLIPI) (Solal-Céligny *et al*, 2004), and the Groupe d'Etude des Lymphomas Folliculaires (GELF) criteria (Brice *et al*, 1997) respectively.

As a control group, blood was collected from the antecubital vein of 120 healthy adult volunteers who had given their informed consent. To obtain the serum samples, whole blood specimens were directly collected into glass tubes and left to stand for 15 min at room temperature to allow blood clots to form. Then, the serum was separated by centrifugation at 1500 × *g* for 5 min.

Table I. Serum ATX antigen levels in patients with haematological malignancies.

Diagnosis	No. of patients (M/F)	Age, years (mean ± SD)	Serum ATX antigen, mg/l (mean ± SD)
AML	26 (14/12)	53·2 ± 16·2	0·864 ± 0·293
MDS	5 (2/3)	48·2 ± 17·9	0·929 ± 0·301
CML-BC	3 (2/1)	60·0 ± 14·7	1·019 ± 0·266
B-cell neoplasms			
Precursor B-ALL	7 (3/4)	52·3 ± 16·9	1·088 ± 0·345
CLL	14 (10/4)	64·0 ± 12·8	1·037 ± 0·355
FL	25 (15/10)	63·0 ± 11·2	1·471 ± 0·693
DLBCL	28 (18/10)	64·5 ± 10·6	0·936 ± 0·387
MLBCL	1 (1/0)	31·0	0·704
MCL	8 (7/1)	64·8 ± 11·7	1·107 ± 0·320
LPL	3 (3/0)	75·3 ± 6·8	1·750 ± 0·989
Plasma cell myeloma	8 (5/3)	67·4 ± 11·9	0·816 ± 0·175
HCL	1 (0/1)	69·0	1·291
Burkitt lymphoma	1 (1/0)	59·0	1·382
T-cell and NK-cell neoplasms			
T-ALL	4 (3/1)	38·0 ± 12·1	0·795 ± 0·179
T-LGL	1 (1/0)	67·0	0·768
ATL	3 (2/1)	49·0 ± 8·2	1·047 ± 0·047
Extra-nodal NK/T-cell lymphoma, nasal type	2 (0/2)	60·0 ± 5·7	0·769 ± 0·127
AITL	3 (3/0)	66·0 ± 8·2	2·021 ± 0·667
ALCL	2 (2/0)	38·0 ± 24·0	1·133 ± 0·533
Primary cutaneous-ALCL	2 (1/1)	61·0 ± 15·6	0·992 ± 0·205
MF	1 (1/0)	57·0	0·814
Sezary syndrome	1 (0/1)	58·0	1·713
PTCL, unspecified	1 (0/1)	60·0	1·240
Hodgkin lymphoma	11 (5/6)	37·8 ± 18·0	0·952 ± 0·273
Healthy subjects	120 (74/46)	40·4 ± 10·3	0·731 ± 0·176

AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; CML-BC, chronic myeloiod leukaemia blastic crisis; Precursor B-ALL, precursor B lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MLBCL, mediastinal large B-cell lymphoma; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; HCL, hairy cell leukaemia; T-ALL, precursor T lymphoblastic leukaemia; T-LGL, T-cell large granular lymphocytic leukaemia; ATL, adult T-cell leukaemia; NK, natural killer cell; AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; Primary cutaneous-ALCL, primary cutaneous anaplastic large cell lymphoma; MF, mycosis fungoides; PTCL, unspecified, peripheral T-cell lymphoma, unspecified.

Measurement of serum ATX antigen

Anti-human ATX monoclonal antibodies were produced by immunization with recombinant human ATX expressed in a baculovirus system. An automated immunoassay for the quantitative determination of ATX was then established, and human serum samples were assayed using an automated immunoassay analyzer AIA-system (TOSOH Corp., Tokyo, Japan), as previously described (Nakamura *et al*, 2008a).

Serum ATX antigen levels were previously found to be significantly higher among females than among males in healthy subjects (Nakamura *et al*, 2008a). Therefore, to compare the serum ATX antigen levels in both sexes, we defined the ATX ratio as follows: the individual's serum ATX antigen level divided by the mean of the serum ATX antigen levels in healthy subjects of the same sex (males, 0·656 mg/l; females, 0·852 mg/l).

Measurement of plasma LPA

Blood samples were treated with ethylene-diamine-tetra-acetic acid and citrate-theophylline-adenosine-dipyridamole (BD Biosciences, Tokyo, Japan). The samples were centrifuged at 2500 × *g* for 30 min at 4°C and the plasmas obtained were stored at −80°C until LPA measurement (Nakamura *et al*, 2007a). The plasma LPA level was determined using a colorimetric assay with an enzymatic cycling method, as previously described (Kishimoto *et al*, 2003).

Measurement of plasma LPC

The plasma LPC level was determined using a specific enzymatic assay, as previously described (Kishimoto *et al*, 2002).

Flow cytometry

Flow cytometry was performed by following the Guidelines for Performing Surface Antigen Analysis on Haematopoietic Malignant Cells (Japanese Committee for Clinical Laboratory Standards (JCCLS) H2-P V1.0), proposed by the Subcommittee on Flow Cytometry, Area Committee on Haematology (Japanese Committee for Clinical Laboratory Standards; JCCLS Area Committee on Haematology Subcommittee on Flow Cytometry, 2003). Blood samples were adjusted to obtain white blood cell counts of 5 to 10×10^9/l. For indirect immunofluorescence staining, the cells were incubated with anti-ATX monoclonal antibody (3D1), which was generated as previously described (Tanaka *et al*, 2004). Then, the samples were washed with phosphate-buffered saline and incubated with fluorescein isothiocyanate (FITC)-labelled anti-rat IgG (BD Biosciences, San Jose, CA, USA), followed by washing with phosphate-buffered saline.

The cells were further stained with specific antibody solution to identify the blood cell types and leukaemic tumour cells. The following antibodies were employed to distinguish the cell types: phycoerythrin (PE)-conjugated mouse anti-human CD20 (BD Biosciences) for B-lymphocytes; and PE-conjugated mouse anti-human CD10 (BD Biosciences) for FL. Subsequent two-colour flow cytometry was performed using a FACSCalibur flow cytometer (BD Biosciences), and the data were analysed using the CELLQUEST software (BD Biosciences).

Statistical analysis

The Steel–Dwass test, a non-parametric multiple comparison procedure, was performed to compare medians among groups (Dwass, 1960; Steel, 1960). Comparisons between two groups were performed using the non-parametric Wilcoxon rank-sum test. Correlations between serum ATX antigen levels and clinical laboratory data were obtained using linear regression analysis. All data are expressed as the mean ± standard deviation (SD) unless indicated otherwise. *P* values less than 0·05 were considered statistically significant. All analyses were performed using JMP6 (SAS Institute, Cary, NC, USA).

Results

Serum ATX antigen levels in patients with haematological malignancies

Serum ATX antigen levels were measured in 161 patients with various haematological malignancies and in 120 healthy subjects. The patient characteristics and the values of the serum ATX antigen levels are summarized in Table I. Patients who had been treated just before the serum ATX measurement was obtained were excluded. As shown in Table I, the mean value of the serum ATX antigen levels in 120 healthy subjects (74 males and 46 females) was 0·731 ± 0·176 mg/l. Elevated ATX antigen levels were found in most patients with B-cell neoplasms, especially in those with FL, when compared with the levels in healthy subjects.

We then compared the values among the groups that contained over 10 patients using the Steel–Dwass test (Fig 1). The serum ATX antigen levels in the patients with FL ($P < 0·001$), CLL ($P < 0·001$), diffuse large B-cell lymphoma (DLBCL) ($P = 0·03$), and HL ($P = 0·01$) were significantly higher than those in the healthy subjects. On the other hand, no significant difference was found between the serum ATX antigen levels in the patients with acute myeloid leukaemia (AML) and the healthy subjects ($P = 0·08$). The serum ATX antigen levels in the patients with FL were significantly higher than those in the patients with AML ($P = 0·01$) or DLBCL ($P = 0·01$).

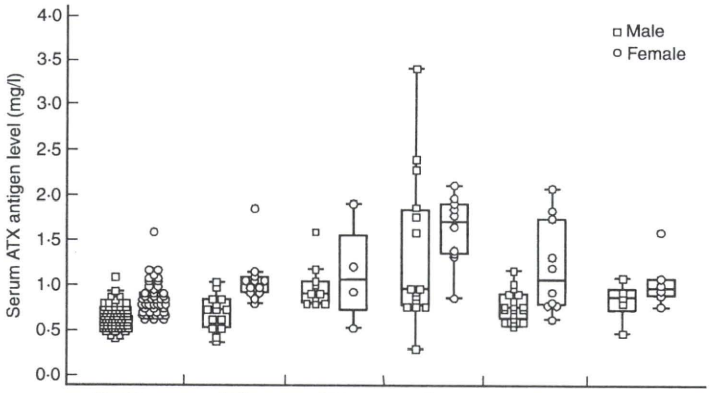
In the healthy subjects, the serum ATX antigen levels were significantly higher ($P < 0·001$) among females (0·852 ± 0·184 mg/l) than among males (0·656 ± 0·121 mg/l), as evaluated using the Wilcoxon rank-sum test. Therefore, we compared the serum ATX antigen levels among the groups according to sex. In males, the serum ATX antigen levels in the patients with FL ($P < 0·001$), CLL ($P < 0·001$), and DLBCL ($P = 0·02$) were significantly higher than those in the healthy subjects. In females, the serum ATX antigen levels in the patients with FL ($P < 0·001$) and AML ($P = 0·03$) were significantly higher than those in the healthy subjects. In addition, the serum ATX antigen levels in the patients with FL were significantly ($P = 0·03$) higher than those in the patients with AML.

Because of the difference in serum ATX antigen levels between females and males, we also compared the ATX ratios among the groups. Similar results were obtained when the ATX ratios were compared among the groups (data not shown), as was the case with the ATX antigen levels.

These results indicate that serum ATX antigen levels in patients with B-cell neoplasms, especially those with FL, are specifically higher than those in healthy subjects.

Correlations between serum ATX antigen levels and clinical parameters in patients with FL

Next, we analysed the correlations between the serum ATX antigen levels and clinical parameters, including prognostic factors, in 25 patients with FL. Because the patients with FL showed various disease statuses, the patients were divided into two groups according to each individual's clinical parameters; the groups were then compared using the



No. of patients (M/F)	120 (74/46)	26 (14/12)	14 (10/4)	25 (15/10)	28 (18/10)	11 (5/6)
P (versus control) All		0·08	<0·001	<0·001	0·03	0·01
P (versus control) Male		0·95	<0·001	<0·001	0·02	0·30
P (versus controll) Female		0·03	0·83	<0·001	0·36	0·31

Fig 1. Serum ATX antigen levels in patients with haematological malignancies compared with healthy subjects. Serum ATX antigen levels were measured using an automated enzyme immunoassay. We compared the serum ATX antigen levels among healthy subjects and disease groups that contained over 10 patients. Samples from males and females are shown as open squares and open circles, respectively. The central boxes represent the values from the lower to upper quartiles (25 to 75 percentiles), and the middle lines represent the medians. The vertical lines extend from the minimum to the maximum value, excluding the outside values, which are displayed as separate points. An outside value is defined as a value that is smaller than the lower quartile minus 1·5 times the interquartile range, or larger than the upper quartile plus 1·5 times the interquartile range. To compare the levels among the groups, the Steel–Dwass test, a non-parametric multiple comparison procedure, was performed. *P* values less than 0·05 indicate significant differences.

Wilcoxon rank-sum test. Thirteen patients were analysed at the time of diagnosis, before any treatment had been administered. Twelve patients had been previously diagnosed and treated for FL, but had either progressive disease or disease relapse at the time of the serum ATX antigen measurement. The performance status was grade 0 or 1 in all the patients. Table II gives the clinical characteristics and the individual serum ATX antigen levels.

The serum ATX antigen levels were significantly higher among patients with a lactate dehydrogenase (LDH) level greater than the upper normal limit ($P = 0{\cdot}02$), a β2-microglobulin level greater than 3 mg/l ($P = 0{\cdot}01$), a tumour diameter greater than 7 cm ($P = 0{\cdot}03$), or the presence of a high tumour burden ($P = 0{\cdot}008$), compared with their counterparts. The levels were also significantly higher among patients with disease relapse or disease progression, compared with those in patients at the time of diagnosis ($P = 0{\cdot}02$). No significant relationships were found when sex, age groups, clinical stage, the presence of B symptoms, the presence of bone marrow involvement, histological subgroups, or the FLIPI scores were evaluated. The serum ATX antigen levels in patients with stage IV disease were higher than those in patients with stages I to III, although the difference was not significant ($P = 0{\cdot}12$; $1{\cdot}643 \pm 0{\cdot}763$ and $1{\cdot}213 \pm 0{\cdot}500$ mg/l respectively). Considering these results, we regarded the serum ATX antigen levels to be associated with tumour burden in patients with FL.

Correlations of serum ATX antigen levels with soluble interleukin-2 receptor, β2-microglobulin, and LDH in patients with FL

Next, we analysed the relationship between the serum ATX antigen levels and the biomarkers for lymphoma, such as soluble interleukin-2 receptor (sIL-2R), β2-microglobulin, and LDH, in the patients with FL. As shown in Fig 2, the serum ATX antigen levels were significantly and positively correlated with sIL-2R ($r = 0{\cdot}594$, $P < 0{\cdot}001$, $n = 115$), β2-microglobulin ($r = 0{\cdot}465$, $P < 0{\cdot}001$, $n = 58$), and LDH ($r = 0{\cdot}495$, $P < 0{\cdot}001$, $n = 154$), as determined using linear regression analysis. Because the serum levels of sIL-2R and β2-microglobulin are known to be elevated in patients with inflammatory or infectious conditions (Bethea & Forman, 1990; Rubin & Nelson, 1990), we analysed the relationship of serum ATX antigen, sIL-2R, and β2-microglobulin levels to inflammation markers, such as C-reactive protein (CRP), to evaluate the specificity of this marker as a laboratory test. The serum sIL-2R levels and the β2-microglobulin levels were significantly correlated with CRP ($r = 0{\cdot}566$, $P < 0{\cdot}001$, $n = 91$; $r = 0{\cdot}516$, $P < 0{\cdot}001$, $n = 55$, respectively). In contrast, no significant correlation was found between the serum ATX antigen levels and CRP (Fig 2D). These results suggest that the serum ATX antigen levels are little affected by inflammation and are a more specific biomarker for lymphoma than sIL-2R and β2-microglobulin.

Table II. Correlations between serum ATX antigen levels and clinical parameters in the patients with FL.

Characteristic	No. of patients	Serum ATX antigen, mg/l (mean ± SD)	P
Sex			
Male	15	1·369 ± 0·836	0·17
Female	10	1·624 ± 0·385	
Age			
<60 years	10	1·495 ± 0·521	0·68
≥60 years	15	1·455 ± 0·804	
Disease status			
At diagnosis	13	1·149 ± 0·542	0·02*
At relapse or in progression	12	1·819 ± 0·687	
Ann Arbor stage			
I–III	10	1·213 ± 0·500	0·12
IV	15	1·643 ± 0·763	
B symptoms			
Absence	22	1·443 ± 0·717	0·45
Presence	3	1·679 ± 0·531	
Serum LDH			
Less than or equal to ULN	10	1·105 ± 0·615	0·02*
Greater than ULN	15	1·715 ± 0·648	
Serum β2-microglobulin†			
Less than or equal to 3 mg/l	9	1·054 ± 0·516	0·01*
Greater than 3 mg/l	4	2·005 ± 0·278	
Bone marrow involvement			
Absence	14	1·311 ± 0·514	0·19
Presence	11	1·674 ± 0·852	
Histological findings†			
Grade 1, 2	19	1·492 ± 0·722	0·64
Grade 3	5	1·534 ± 0·634	
Tumour diameter			
Less than or equal to 7 cm	13	1·253 ± 0·801	0·03*
Greater than 7 cm	12	1·706 ± 0·479	
High tumour burden (GELF criteria)			
Absence	7	0·928 ± 0·201	0·008*
Presence	18	1·682 ± 0·703	
FLIPI score			
Low/intermediate risk	12	1·245 ± 0·513	0·14
High risk	13	1·680 ± 0·787	

*Statistically significant as determined using the Wilcoxon rank-sum test.
†Data were missing for serum β2-microglobulin (12 patients) and histological findings (one patient).
LDH, lactate dehydrogenase; ULN, upper limit of normal; GELF, Groupe d'Etude des Lymphomas Folliculaires; FLIPI, Follicular Lymphoma International Prognostic Index.

Relationship between serum ATX antigen levels, clinical laboratory data and clinical course in patients with FL

We further analysed the relations of serum ATX antigen levels to laboratory data and clinical course in patients with FL (Fig 3). The serum ATX antigen levels were measured at various times during the clinical courses of four patients with FL.

Patient 1 was a 51-year-old man who had been newly diagnosed as having FL. He was treated with R-CHOP therapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone) and obtained a partial response. Because of progressive disease, rituximab was administered once a week eight times as salvage therapy. In this patient, the serum ATX antigen levels and sIL-2R levels changed in parallel with the clinical course. Patient 2 was a 65-year-old man with refractory FL who had been treated repeatedly until the time of the serum ATX antigen measurement. Although this patient's serum ATX antigen levels and sIL-2R levels decreased after R-2CDA-MIT therapy (rituximab, cladribine, and mitoxantrone), the levels increased in parallel with tumour progression. The levels once again decreased after modified R-ESHAP therapy (rituximab, etoposide, carboplatin, cytarabine and methylprednisolone). Patients 3 and 4 were females with refractory FL. The serum ATX antigen levels and sIL-2R levels in these patients decreased after R-2CDA-MIT therapy. These results suggest that the serum ATX antigen levels change in parallel with the clinical course in patients with FL.

Correlations between serum ATX antigen levels and plasma LPA levels

As described above, ATX exerts a lysoPLD activity, which converts LPC to LPA (Tokumura *et al*, 2002; Umezu-Goto *et al*, 2002). To examine whether the serum ATX antigen levels play a role in determining the plasma LPA levels, we measured the plasma LPA levels in patients with FL; note that it is LPA, not ATX that actually plays a pathophysiological role. The plasma LPA levels were significantly and positively correlated with the serum ATX antigen levels ($r = 0·905$, $P = 0·01$, $n = 6$) (Fig 4A). On the other hand, no significant correlation was found between the plasma LPA levels and the plasma LPC levels ($r = -0·402$, $P = 0·43$, $n = 6$) (Fig 4B). These results suggest that the plasma LPA levels depend on the levels of ATX (the enzyme that produces LPA) but not LPC (the substrate).

Expression of ATX in leukaemic tumour cells from patients with FL

Autotaxin is synthesized as a secreted protein and is released into the extracellular space (Jansen *et al*, 2005; Koike *et al*, 2006). To examine the source of the elevated serum ATX antigen level, we determined the surface expression of ATX in peripheral blood cells from healthy subjects and patients with FL. The leukaemic tumour cells from the patients with FL were found to express ATX (Fig 5B). On the other hand, normal peripheral blood cells, including B-cells, from healthy subjects failed to express ATX (Fig 5A). Although the results were highly reproducible, i.e., similar results were obtained in three patients with FL and in five healthy subjects, the ATX fluorescence observed by flow cytometry may reflect the cell

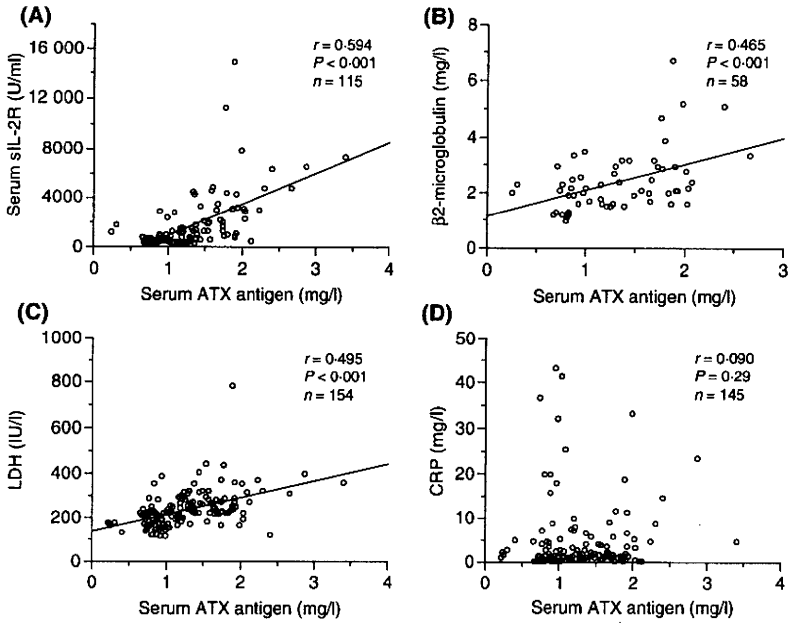


Fig 2. Correlations of serum ATX antigen levels with sIL-2R (A), β2-microglobulin (B), LDH (C), and CRP (D) in patients with FL. The correlations of serum ATX antigen levels with clinical laboratory data were examined using linear regression analysis in 25 patients with FL.

targeting of secreted ATX by cell surface molecules such as integrins, as reported recently (Kanda *et al*, 2008).

Discussion

This study demonstrated that the serum ATX antigen levels in patients with B-cell neoplasms, especially those with FL, were higher than those in healthy subjects. The serum ATX antigen levels in the patients with FL were found to be associated with the tumour burden and to change in parallel with the clinical course. In addition, the serum ATX antigen levels were little affected by inflammation, in contrast to other biomarkers for lymphoma, such as sIL-2R and β2-microglobulin. Our study is the first to report the usefulness of serum ATX measurements not only in B-cell neoplasms (especially FL), but also in haematological malignancies. As expected, as ATX is a key enzyme for converting LPC to LPA and as plasma LPA levels are correlated with the serum ATX activity in patients with chronic liver disease (Watanabe *et al*, 2007a), the serum ATX antigen levels and the plasma LPA levels were correlated in patients with FL. In the plasma (van Meeteren *et al*, 2006) and serum (Tanaka *et al*, 2006) from ATX-deficient heterozygous mice, both the ATX activity and LPA levels have been shown to be about half of those from wild-type mice, while transgenic overexpression of lipid phosphate phosphatase-1 reportedly failed to affect plasma LPA levels in mice (Yue *et al*, 2004). Although the plasma LPA level can be controlled by balance between LPA production (through the action of ATX activity) and degradation (through the action of lipid phosphate phosphatases), the former may be more important *in vivo*.

We have attempted to apply the measurement of serum ATX to clinical laboratory testing (Nakamura *et al*, 2007a,b,c; Nakamura *et al*, 2008a,b; Watanabe *et al*, 2007a). We previously reported the levels of serum ATX activity and serum ATX antigen in patients with various diseases and conditions: levels were elevated in patients with chronic liver disease (Watanabe *et al*, 2007a; Nakamura *et al*, 2008a), decreased in postoperative prostate cancer patients (Nakamura *et al*, 2007c), and the application to hypoalbuminemia differentiation (Nakamura *et al*, 2008a). Reportedly, the serum ATX activity level was significantly higher in normal pregnant females than in non-pregnant healthy females (Tokumura *et al*, 2000, 2002). From the results of this study, B-cell neoplasms, especially FL, should be added to the list of pathophysiological conditions in which serum ATX levels are altered.

Autotaxin was originally identified as a tumour cell motility factor (Stracke *et al*, 1992), and mounting evidence points to a link between ATX and cancer, such as tumour progression, metastasis, and angiogenesis (Mills & Moolenaar, 2003; Moolenaar *et al*, 2004; Birgbauer & Chun, 2006). However, elevated ATX activity in cancer patients has rarely been reported. Many studies have suggested a causal link between LPA and ovarian cancer (Mills & Moolenaar, 2003), and plasma LPA levels were found to be elevated in patients with ovarian cancer (Xu *et al*, 1998). Because ATX is a key enzyme producing LPA, the association between ATX and ovarian cancer once received attention. However, whether plasma LPA levels are elevated in patients with ovarian cancers is controversial (Baker *et al*, 2002), and a recent study suggests that no difference in serum ATX activity exists between ovarian cancer

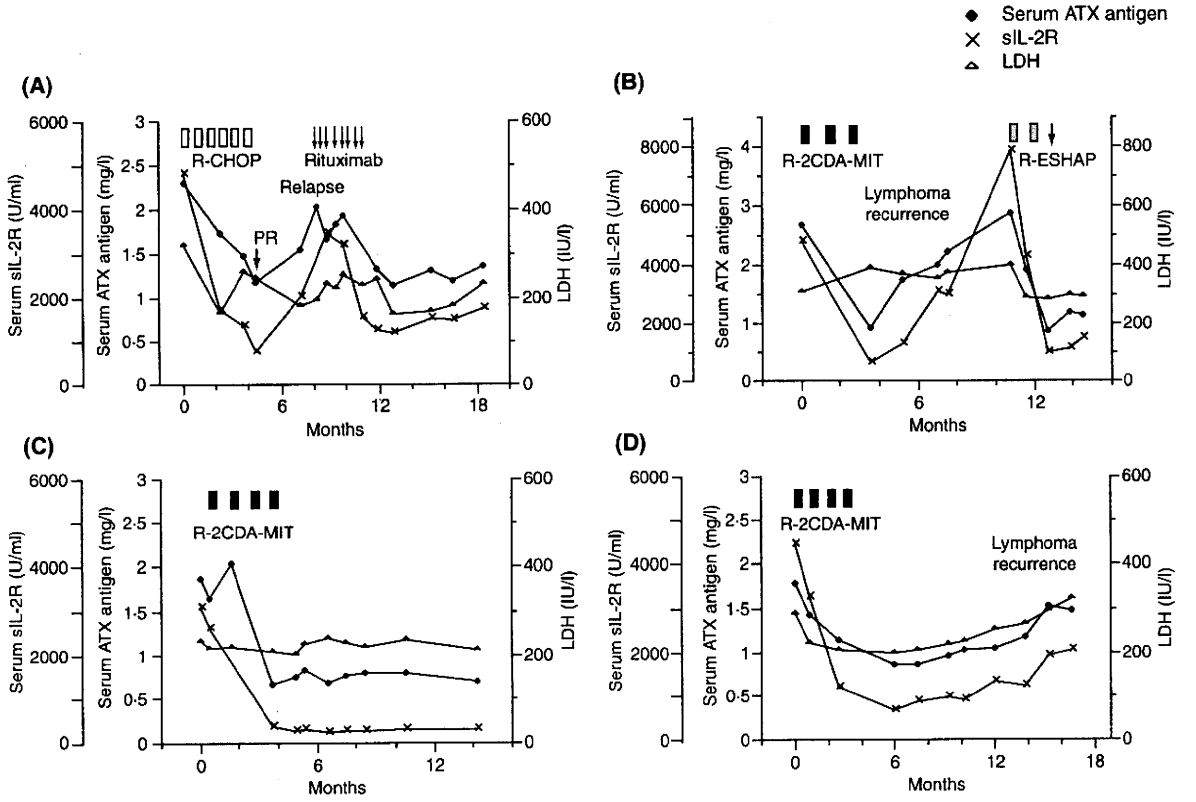


Fig 3. Relationship between serum ATX antigen levels, clinical laboratory data and clinical course in patients with FL. Closed circles, crosses, and open triangles represent serum ATX antigen, sIL-2R, and LDH levels, respectively. (A) Clinical course of Patient 1, a 51-year-old man newly diagnosed with FL. (B) Clinical course of Patient 2, a 65-year-old man with refractory FL. (C) Clinical course of Patient 3, a 59-year-old woman with refractory FL. (D) Clinical course of Patient 4, a 73-year-old woman with refractory FL. R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone; R-2CDA-MIT, rituximab, cladribine and mitoxantrone; R-ESHAP, rituximab, etoposide, carboplatin, cytarabine and methylprednisolone.

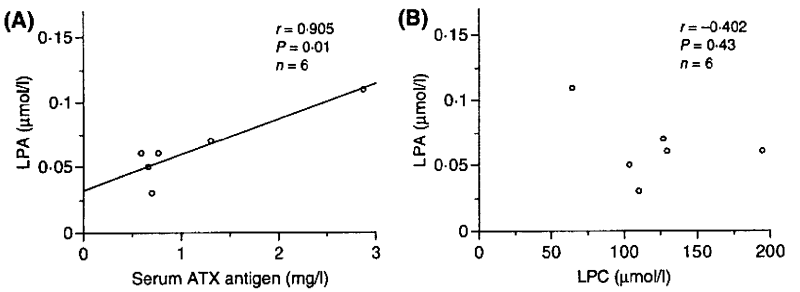


Fig 4. Correlations of plasma LPA levels with serum ATX antigen levels (A) and plasma LPC levels (B) in patients with FL. The plasma LPA levels were measured using a colorimetric assay, and the plasma LPC levels were measured using a specific enzymatic assay. The correlations of the plasma LPA levels with the serum ATX antigen levels and the plasma LPC levels were examined using linear regression analysis in six patients with FL.

patients and healthy females (Tokumura *et al*, 2007). LPA is also an autocrine mediator in prostate cancer cells (Xie *et al*, 2002), and ATX is up-regulated in stromal cells from prostate cancer patients (Zhao *et al*, 2007). Although LPA and ATX are thought to be associated with prostate cancer, no significant difference in serum ATX activity has been found between prostate cancer patients and healthy subjects (Nakamura *et al*, 2007c). Accordingly, the present study is the first report to show an elevation in serum ATX antigen levels in patients with malignancies.

In this study, the serum ATX antigen levels in patients with FL were found to be significantly higher than those in healthy

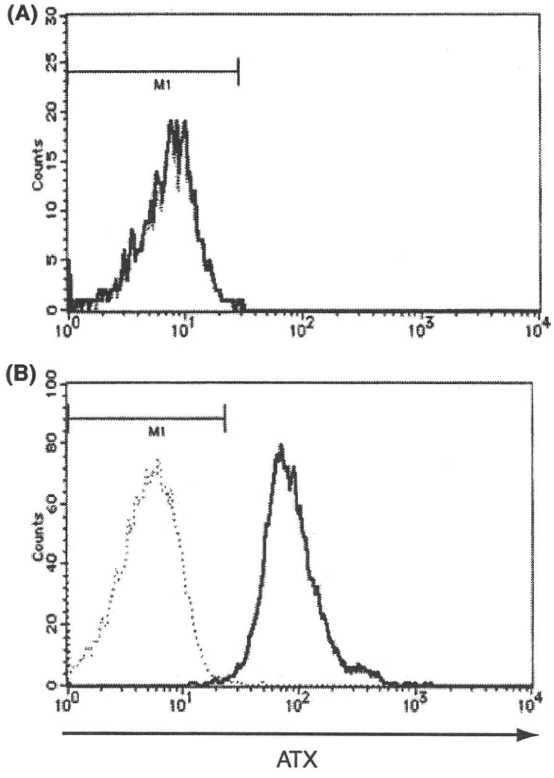


Fig 5. Analysis of the surface expression of ATX in leukaemic tumour cells from patients with FL. We examined the surface expression of ATX in peripheral blood cells using flow cytometry. (A) Normal B-lymphocytes from healthy subjects. (B) Leukaemic tumour cells from patients with FL. The solid line histogram represents staining with anti-ATX antibody, while the dotted line represents the control.

subjects. Furthermore, leukaemic tumour cells from patients with FL were shown to express ATX. These results suggest that the shedding of ATX from lymphoma cells leads to an elevation of serum ATX antigen levels. As described above, the serum ATX activity and ATX antigen level are elevated in patients with chronic liver disease (Watanabe *et al*, 2007a; Nakamura *et al*, 2008a). A recent study using hepatectomized rats suggested that elevated ATX activity in rats with liver injury was caused by a decrease in ATX clearance (Watanabe *et al*, 2007b). Accordingly, the mechanism of elevated ATX antigen levels in FL is opposite to that in chronic liver disease. Although the mechanism by which the plasma/serum ATX level is regulated remains to be solved, both ATX production and clearance should probably be considered.

As yet, little is known about the association between ATX and haematological malignancies. One recent study has shown that the induction of ATX by EBV promoted the growth and survival of HL cells and that ATX expression in lymphoid tissues was mainly restricted to EBV-positive Hodgkin and Reed-Sternberg cells and CD30-positive anaplastic large cell lymphoma (Baumforth *et al*, 2005). In the present study, however, no major difference in the serum ATX antigen levels was found between HL patients and healthy subjects. Although the levels in the total patients (males plus females) with HL were significantly higher than those in the healthy subjects, the difference was marginal. In HL lesions, the major cell constituents are normal or reactive lymphocytes, not tumour cells like Hodgkin and Reed-Sternberg cells; this difference may explain why no difference in ATX antigen levels was observed between HL patients and healthy subjects.

LPA has also been suggested to act as a survival factor in B-cell neoplasms (Rosskopf *et al*, 1998; Hu *et al*, 2005; Satoh *et al*, 2007). As expected from the results of studies on chronic liver disease (Watanabe *et al*, 2007a; Nakamura *et al*, 2008a), the plasma LPA levels in patients with FL are elevated, in parallel with the serum ATX antigen levels. These results indicate that the autocrine or paracrine production of LPA contributes to tumour progression, although direct evidence of this mechanism has not been reported. Considering the specificity of ATX expression in lymphoma cells, ATX could be used as a pharmacological target; the blockage of LPA production via ATX inhibition might be a useful anticancer therapy.

In conclusion, the serum ATX antigen level is a promising marker for FL. Considering the specificity of ATX and the action of LPA as a tumour growth factor for B-cell neoplasms, therapeutical applications may be an important goal of future studies.

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Conventional allogeneic hematopoietic stem cell transplantation for lymphoma may overcome the poor prognosis associated with a positive FDG-PET scan before transplantation

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A positive scan in pretransplantation fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has been shown to be associated with a poor prognosis in patients with lymphoma undergoing high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). For those with a positive FDG-PET scan, treatment that includes allogeneic stem cell transplantation (allo-SCT) may be an alternative. However, it is uncertain whether allo-SCT can overcome a poor prognosis. Therefore, we conducted a retrospective analysis of 14 patients with lymphoma who had undergone FDG-PET scan within one month before allo-SCT at our institution. Eleven patients were FDG-PET-positive and three were negative. With a median follow-up of 17 months (range: 6–44) after allo-SCT, the cumulative incidence of progression was 29.3% in FDG-PET-positive patients and 0% in the FDG-PET-negative patients. Four of the 11 patients who had post-transplantation FDG-PET showed FDG-avid lesions on the first post-transplantation scan. In two of the four, regression of the lesions was observed during the scheduled reduction of immunosuppressant without donor lymphocyte infusion and remained without progression at the last follow-up (34 and 8 months). Durable responses after allo-SCT, at least with conventional conditioning regimens, can be expected in patients with FDG-PET-positive lesions before transplantation. Thus, conventional allo-SCT could be an attractive modality compared to ASCT for patients with positive FDG-PET after the completion of conventional salvage chemotherapy, and particularly for patients with T and NK-cell lymphomas. Am. J. Hematol. 83:477–481, 2008. © 2008 Wiley-Liss, Inc.

Introduction

Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) has become a standard imaging test in the management of lymphoma in both initial staging [1,2] and response assessment [3–6]. Response criteria that incorporate FDG-PET can better predict the prognosis than the original International Workshop Criteria [7,8], and revised response criteria for lymphoma using FDG-PET have been published [9]. In addition, the results of FDG-PET before and/or after hematopoietic stem cell transplantation for lymphoma have been reported to have prognostic significance. Several groups have reported that the probability of durable responses with high-dose chemotherapy followed by ASCT for lymphoma is lower in patients with positive FDG-PET before ASCT than in patients with negative FDG-PET [6,10–15]. For those with positive FDG-PET before ASCT, allogeneic hematopoietic stem cell transplantation (allo-SCT) can be an attractive alternative treatment, since the use of uncontaminated graft and a potential graft-versus-lymphoma (GVL) effect are expected to reduce the probability of relapse. To date, however, there has been no report on whether such an approach could overcome the poor prognosis associated with positive pretransplantation FDG-PET. Moreover, the significance of pretransplantation FDG-PET in predicting the clinical outcome of allo-SCT has not yet been reported. Thus, to assess whether patients with positive pretransplantation FDG-PET could achieve a durable response after allo-SCT, we conducted a retrospective analysis of such patients.

Results

Patient population

We identified 14 patients who had undergone FDG-PET scan before allo-SCT for lymphoma. The patient characteristics are shown in Table I. The median follow-up for surviving patients was 17.0 months after allo-SCT (range: 6–44). There were no transplant-related deaths before day 100. Acute GVHD was observed in nine patients (grade I, 2; grade II, 7; grade III–IV, 0). The median time from transplantation to the onset of acute GVHD was 30 days (range: 13–60). Chronic GVHD occurred in eight patients (5 extensive, 3 limited). We did not find any relationship between GVHD occurrence and tumor regression.

Pretransplantation FDG-PET and the outcome of allo-SCT

Eleven patients (79%) had FDG-PET-positive lesions before allo-SCT. All of these lesions had been detected by CT scan. In three patients (21%), there were no FDG-PET-positive lesions, although FDG-avidity had been confirmed before chemotherapy was started in each patient. Of the 14 patients, 12 were alive at the last follow-up. Of the 11 patients with positive pretransplantation FDG-PET, nine were alive without progression of lymphoma. Two patients (patients 7 and 8) suffered progression on days 61 and

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TABLE I. Patient Characteristics

Patient	Sex	Age at diagnosis	Donor source	Conditioning	Diagnosis	Disease status	SUVmax	Diameter max (mm)	Status at the last follow up	Follow up (months)
01	M	37	uBMT	CY/TBI	LBL	PR1	7.3	52	Alive: remission	42.9
02	M	56	rPBSCT	FLU/CY	FL grade 2	PR2	5.7	37	Alive: remission	34.4
03	F	47	uBMT	CY/TBI	FL grade 1	PR2	4.9	16	Alive: remission	31.3
04	M	53	rPBSCT	CY/TBI	MCL	CR1	Negative	–	Alive: remission	30.1
05	F	42	rPBSCT	CY/TBI	Nasal NK/T	PR2	3.6	23	Alive: remission	26.2
06	F	28	uBMT	CY/TBI	t-CTCL	Primary R.	7.1	23	Alive: remission	14.2
07	M	45	rPBSCT	CY/TBI	ALCL	Primary R.	6.0	30	Dead: progression	6.3
08	F	25	CBT	FLU/MEL/TBI	HL	Relapse 2	11.5	40	Alive: progression	7.2
09	F	46	uBMT	CY/TBI	ATLL	CR1	Negative	–	Alive: remission	8.1
10	F	32	rPBSCT	CY/TBI	t-CTCL	Primary R.	6.9	28	Alive: remission	7.5
11	F	48	uBMT	CY/TBI	FL grade 3	Primary R.	10.2	38	Alive: remission	6.2
12	M	47	rPBSCT	CY/TBI	ATLL	PR1	7.8	31	Alive: remission	19.9
13	F	36	uBMT	BU/MEL	ANKL	CR1	Negative	–	Dead: GVHD	6.6
14	M	42	rBMT	CY/TBI	FL grade 1	PR2	6.3	59	Alive: regression	10.4

uBMT, unrelated bone marrow transplantation; rPBSCT, related peripheral blood stem cell transplantation; CBT, cord blood transplantation; rBMT, related bone marrow transplantation; CY/TBI, Cyclophosphamide 60 mg/kg × 2 days + Total Body Irradiation 2 Gy × 2 × 3 days; FLU/CY, Fludarabine 25 mg/m^2 × 5 days + Cyclophosphamide 60 mg/kg × 2 days; FLU/MEL/TBI, Fludarabine 30 mg/m^2 × 5 days + Melphalan 140 mg/m^2 × 1 day + Total Body Irradiation 2 Gy × 2 × 1 day; BU/MEL, Busulfan 4 mg/kg × 4 days + Melphalan 140 mg/m^2 × 1 day; LBL, lymphoblastic lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; Nasal NK/T, Extranodal NK/T-cell lymphoma, nasal type; ATLL, adult T-cell leukemia/lymphoma; t-CTCL, tumor-stage Cutaneous T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ANKL, aggressive NK cell leukemia; disease status, status at transplantation; CR, complete response; PR, partial response, Primary R., primary refractory; SUVmax, maximum standard uptake value in pretransplantation FDG-PET scan; diameter max, maximum diameter (long axis) of the largest lymphoma lesion in pretransplantation CT scan; GVHD, graft-versus-host disease.

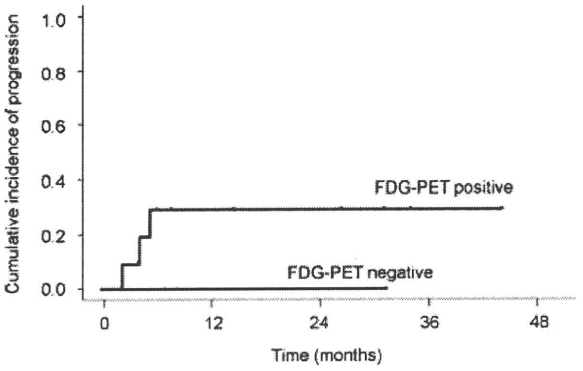

Figure 1. Cumulative incidence of progression in 11 patients with a positive pre-transplantation FDG-PET scan compared to that in 3 patients with a negative pre-transplantation FDG-PET scan.

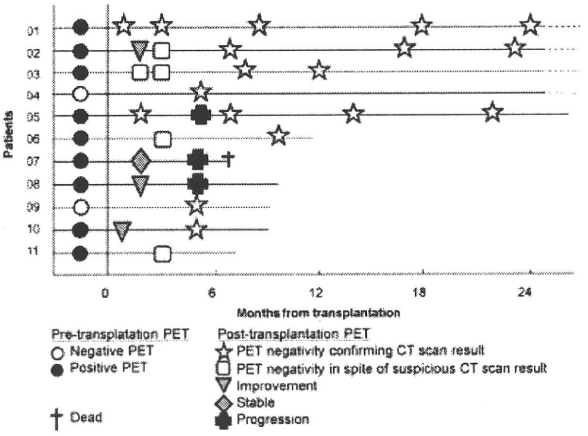

Figure 2. Patient timelines. Results of pretransplantation and post-transplantation FDG-PET scans were performed in 11 patients. Four patients showed FDG-PET-positive lesions on the first post-transplantation PET scan (patient 2, 7, 8, 10). All of them were positive for pretransplantation FDG-PET. Improvement of FDG-PET uptake was observed in patients 2 and 10 during the scheduled reduction of immunosuppressant and remained without progression at the last follow-up (34 and 8 months).

111, respectively and patient 7 died of lymphoma progression on day 190. Among the three patients who had a negative scan in pre-transplantation FDG-PET, one died of severe GVHD with no evidence of disease progression on day 198 (patient 13), while the two other patients remained without progression at the last follow-up. The cumulative incidence of progression at a median follow-up of 17.0 months from allo-SCT was 29.3% in patients with positive pretransplantation FDG-PET and 0% in patients with negative pretransplantation FDG-PET (see Fig. 1). OS at the median follow-up was 90.9% in patients with a positive scan in pretransplantation FDG-PET.

Follow-up FDG-PET

A total of 30 post-transplantation FDG-PET scans were performed in 11 patients (patients 1–11, the median number of follow-up scans per patient: 2, range 1–5) to evaluate the response and to monitor disease progression (see Fig. 2). The median time to the first FDG-PET scan after transplantation was 72 days (range: 28–142). In seven patients (patients 1, 3, 4, 5, 6, 9, 11), the first post-transplantation FDG-PET was negative, including three patients who had residual disease in CT scans (patients 3, 6, 11). Among these seven patients, five had positive pretransplantation FDG-PET scans (patients 1, 3, 5, 6, 11). All of these seven patients but one (patient 5) remained free from progression. Patient 5 achieved a CR defined by CT scan after allo-SCT on day 57 and was negative in the first post-transplantation FDG-PET scan on day 72. However, the second FDG-PET scan and CT scan on day 140 revealed disease recurrence. Cyclosporine was rapidly reduced within one month from the recurrence and this patient remained in remission (26-month follow-up). Including this patient, none of the patients received DLI for residual disease or recurrent disease.

In contrast, the other four showed FDG-PET-positive lesions at the first post-transplantation PET scan (days 38, 77, 64, 30 in patients 2, 7, 8, and 10, respectively). In all of these patients, pre-transplantation FDG-PET was positive, and the same sites remained FDG-positive at the first post-transplantation FDG-PET scan. Two (patients 2, 8) of the four patients received RIC. Regression of lesions detected by CT scans that was associated with an improvement of FDG-PET findings in terms of reduced levels of SUVmax was observed in patients 2 and 10 during the scheduled reduction of cyclosporine, and these patients remained free from progression at the last follow-up (34 months and 8 months, respectively). In patients 7 and 8, the first FDG-PET showed stable disease (day 64) and improvement (day 77), respectively, compared to the pretransplantation FDG-PET. However, follow-up CT scans showed disease progression soon after the first FDG-PET scans in both patients. In patient 7, the second FDG-PET on day 112 showed disease progression and the patient died of septic shock on day 190. In patient 8, rapid tapering of cyclosporine provided transient regression of the disease. However, the second FDG-PET on day 111 revealed disease progression and the patient was alive with disease at the last follow-up (day 216).

Outcome of patients with T and NK-cell lymphomas

The present study included seven patients (patients 5, 6, 7, 9, 10, 12, 13) with T and NK-cell lymphomas. Of these seven, four had chemorefractory disease and five had positive pretransplantation FDG-PET scans. Five patients were alive in CR at the last follow-up (median follow-up: 8 months). In patients 5 and 10, tumor regression was observed in the follow-up FDG-PET scan findings in terms of reduced levels of SUVmax along with decrease in the transverse diameter of tumors in the CT scan findings by only the rapid or scheduled reduction of cyclosporine, respectively, which suggested a potential GVL effect. They remained in remission (26- and 8-month follow-up, respectively).

Discussion

Functional imaging with FDG-PET is now considered to play an important role in staging and response evaluation of lymphoma. Moreover, FDG-PET has also been studied with regard to its ability to predict the outcome of a specific therapy for lymphoma such as ASCT [6,10–15]. To the best of our knowledge, however, only one previous study has addressed the utility of FDG-PET after allogeneic transplantation [16] and there has been no report on the outcome of allo-SCT for lymphoma in patients with positive pretransplantation FDG-PET lesions.

In the present study, the cumulative incidence of progression was 29.3% at 17.0 months from allo-SCT in patients with positive pretransplantation FDG-PET. This supports the idea that allo-SCT could be an attractive modality for the treatment of lymphoma with positive pretransplantation

FDG-PET, since a dismal outcome has been reported in patients undergoing ASCT for aggressive lymphomas with positive pretransplantation FDG-PET lesions [6,10–15]. Although the duration of follow-up in the present study was shorter than in studies on ASCT, several studies have shown that progression after allo-SCT for lymphoma was usually seen within 1 year [17–19]. Therefore, we consider that the duration of follow-up in the current study was long enough for us to discuss the prognosis after allo-SCT for lymphoma.

In this study, six of the seven patients with negative post-transplantation FDG-PET remained progression-free, which supports the previous report by Hart et al., who analyzed patients received allo-SCT with RIC for lymphoma [16]. In that study, the authors proposed that post-transplantation FDG-PET may be useful for guiding DLI after allo-SCT with RIC. In contrast, in the current study, two patients with FDG-avid lesions at the first post-transplantation FDG-PET showed regression along with previously scheduled tapering of cyclosporine, without undergoing DLI. Post-transplantation FDG-positivity may reflect inflammatory responses to a conditioning regimen or even an immune reaction corresponding to the GVL effect. Thus, the clinical significance of FDG-avid lesions that remain after allo-SCT for lymphoma may require further evaluation. In this regard, the optimal timing for post-transplantation FDG-PET should be determined.

The present study has several limitations. First, histological subtypes included in the present study were more heterogeneous than in the previous studies on FDG-PET before ASCT [6,10–15], although most patients in our study had aggressive histological subtypes. During the period of this retrospective analysis, no patient underwent allo-SCT for DLBCL, which reflects the preferred use of ASCT for this subtype. On the other hand, a half of our patients had T and NK-cell lymphomas. Our approach reflected the dismal outcome with conventional chemotherapy or ASCT and the promising data with allo-SCT for these subtypes such as adult T-cell leukemia/lymphoma, advanced stage NK-cell lymphoma, and advanced stage cutaneous T-cell lymphoma [20–22]. The histological subtypes included in the present study, including T and NK-cell lymphomas, have been shown to be FDG-avid [23,24], and FDG-avidity had been confirmed in each patient at the initial staging. Thus, therapy-monitoring with FDG-PET may be supported for these subtypes. Nevertheless, the prognostic significance of pretransplantation FDG-PET should be confirmed in major subtypes such as DLBCL and HL, preferably in future prospective studies. Second, most of the patients received a conventional conditioning regimen and only two received RIC. Thus, the conclusion of the present study should be restricted to cases of allo-SCT who received conventional pretransplantation conditioning, and it remains to be determined whether patients with positive pretransplantation FDG-PET can achieve a durable response after allo-SCT with RIC. Third, this retrospective study may have an inherent patient-selection bias. Although we did not find any patient in whom allo-SCT was canceled based solely on the results of FDG-PET, it could be possible that the result somehow affected the decision as to whether allo-SCT should be recommended. For example, patients with very extensive FDG-PET uptake before allo-SCT may not have been referred to allo-SCT. Although this study did not address the relationship between the intensity of FDG-uptake and the prognosis, this point might be worth further evaluation. Fourth, there might be a false-positive lesion in FDG-PET which reflects an inflammatory process after radiation or infection. We did not routinely perform biopsy for residual lesions before allo-SCT, and we defined PET-positive lesions as in the study by Spaeson et al. [15], both of which were consistent with the recent consensus report on FDG-PET in response assessment [25].

In conclusion, this study suggested that patients with positive pretransplantation FDG-PET could achieve a durable response after allo-SCT as long as a conventional conditioning regimen is used. Therefore, conventional allo-SCT could be an attractive modality compared to ASCT for patients with positive FDG-PET after the completion of conventional salvage chemotherapy, and particularly for patients with T and NK-cell lymphomas. Prospective studies of allo-SCT with conventional pretransplantation conditioning regimens for such patients could be justifiable. However, the role of pretransplantation FDG-PET may be different in allo-SCT with RIC and this point requires further evaluation.

Methods

Patient population

We reviewed a database of adult patients who underwent allo-SCT for Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) at the University of Tokyo Hospital, Japan, between November 2003 and December 2006. We identified 14 patients who had undergone FDG-PET scan within one month before transplantation. Allo-SCT was performed for chemoresistant disease in five patients, whereas nine patients had chemosensitive disease. Some patients were transplanted in the first complete response (CR) or chemosensitive relapse. These patients had a subtype for which conventional chemotherapy or ASCT was unlikely to confer a long-term survival according to the literature that had been published at the time [20–22]. Conditioning regimens were high-dose cyclophosphamide with total body irradiation (CY/TBI; CY 120 mg/kg, TBI 12 Gy) in 11 patients, busulfan and melphalan (BU/MEL; BU 16 mg/kg, MEL 140 mg/m^2) in one patient (patient 13), and reduced-intensity conditioning (RIC) regimens in two patients (FLU/CY; fludarabine 125 mg/m^2, CY 120 mg/kg in patient 2, FLU/MEL/TBI; fludarabine 150 mg/m^2, melphalan 140 mg/m^2, TBI 4 Gy in patient 8). RIC regimens were selected because of advanced age or comorbidity. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and a short course of methotrexate in all patients. T-cell depletion of the graft was not performed. None of the patients had allo-SCT after failure with ASCT in this analysis. We did not use the results of FDG-PET as the sole criterion to exclude allo-SCT or to introduce donor lymphocyte infusion (DLI) after allo-SCT.

Fluorine-18 FDG-PET

FDG-PET was performed as previously described [24]. FDG-PET scans were performed with a whole-body PET camera, ADVANCE (GE Healthcare). Emission data were acquired 60 min after the intravenous administration of 296 MBq of F-18 FDG. PET images were reconstructed using Ge-68 for attenuation correction with the ordered-subsets expectation maximization. Two experienced radiologists scrutinized all scans. Interpretation of the PET images followed the approach in a previous study [15] and was consistent with the recommendations in the recent publication from the International Harmonization Project [25]. Briefly, focal or diffuse FDG uptake above background in a location that is incompatible with normal anatomy or physiology was interpreted as abnormal and was considered to be indicative of a lymphoma lesion. In areas with abnormal FDG accumulation, the standard uptake value (SUV) was calculated according to the standard formula. We did not use SUV as a specific cutoff value for positivity but rather considered the result of FDG-PET to be positive if FDG-PET detected a lymphoma lesion in at least one site that was also detected by physical examination, CT, or BM examination on the basis of the definition in a previous report [23]. In CT scan, a nodule with a long-axis length of more than 1.5 cm at any site was considered to be a lymphoma lesion. In patients with no FDG-PET scan after allo-SCT, disease status was determined by the original CT criteria [7].

Statistical analysis

Time to progression was measured from the day of stem cell infusion (day 0) until the time of disease relapse or progression, or disease-related death, with censoring at the last follow up. The cumulative incidences of progression were evaluated using Gray's method, while considering death before progression as a competing risk [26]. Overall

survival (OS) was measured from day 0 until the date of death, with censoring at the time of the last follow up. OS was calculated according to the Kaplan–Meier method.

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False-positive *Aspergillus* galactomannan antigenaemia after haematopoietic stem cell transplantation

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***Objectives*: Although *Aspergillus* galactomannan (GM) antigen detection is widely applied in the diagnosis of invasive aspergillosis (IA), false-positive reactions with fungus-derived antibiotics, other fungal genera or the passage of dietary GM through injured mucosa are a matter of concern. The aim of this study was to investigate the cumulative incidence and risk factors for false-positive GM antigenaemia.**

***Patients and methods*: The records of 157 adult allogeneic haematopoietic stem cell transplantation (HSCT) recipients were retrospectively analysed. Episodes of positive GM antigenaemia, defined as two consecutive GM results with an optical density index above 0.6, were classified into true, false and inconclusive GM antigenaemia by reviewing the clinical course.**

***Results*: Twenty-five patients developed proven or probable IA with a 1 year cumulative incidence of 12.9%, whereas 50 experienced positive GM antigenaemia with an incidence of 32.2%. Among the total 58 positive episodes of the 50 patients, 29 were considered false-positive. The positive predictive value (PPV) was lower during the first 100 days than beyond 100 days after HSCT (37.5% versus 58.8%). Gastrointestinal chronic graft-versus-host disease (GVHD) was identified as the only independent significant factor for the increased incidence of false-positive GM antigenaemia (PPV 0% versus 66.7%, $P = 0.02$).**

***Conclusions*: GM antigen results must be considered cautiously in conjunction with other diagnostic procedures including computed tomography scans, especially during the first 100 days after HSCT and in patients with gastrointestinal chronic GVHD.**

Keywords: fungal infections, invasive aspergillosis, chronic GVHD, gastrointestinal tract, mucosal damage

Introduction

Invasive aspergillosis (IA) remains one of the leading infectious causes of death after allogeneic haematopoietic stem cell transplantation (HSCT), despite new antifungal agents that have become available in recent years.[1] The high mortality rate of IA was mainly attributed to the difficulty of diagnosis at the early stage of the disease, because histopathological examinations require invasive procedures and fungal cultures have low specificity and sensitivity in detecting IA.

Monitoring of the circulating *Aspergillus* galactomannan (GM) antigen by the sandwich enzyme-linked immunosorbent assay (ELISA) is a feasible non-invasive biological method for early diagnosis of IA.[2] The GM ELISA test has sensitivity of 67% to 100% and specificity of 81% to 99% in neutropenic patients and allogeneic transplant recipients,[3–6] and was introduced as microbiological evidence in the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) criteria for opportunistic invasive fungal infection.[7] However, a concern is the false-positive reactions,

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which may lead to inappropriate invasive investigation or over-treatment with antifungal agents. Previous studies have reported various risk factors for the false-positive results, including early childhood,[3] the development of chronic graft-versus-host disease (GVHD),[8] the passage of GM of food origin[9,10] and certain exoantigens from other fungal genera[11] or fungus-derived antibiotics.[12,13] However, little is known about the exact mechanism of false-positive reactions with these factors.

To clarify the cause of false-positive results, we retrospectively analysed the incidence and risk factors for false-positive GM antigenaemia in allogeneic HSCT recipients.

Patients and methods

Study population

GM ELISA became available at the University of Tokyo Hospital as a routine diagnostic test in February 2000. During a 5 year period (February 2000 to May 2005), 163 consecutive adult patients (>16 years old) underwent allogeneic HSCT at the University of Tokyo Hospital. The medical records of 157 patients who had at least two GM ELISA tests after HSCT were available for a retrospective analysis of positive GM antigenaemia. The median follow-up was 519 days (range, 15–2090 days) after HSCT. The patient characteristics are shown in Table 1. Acute leukaemia in first remission, chronic myelogenous leukaemia in first chronic phase, myelodysplastic syndrome with refractory anaemia or refractory anaemia with ringed sideroblasts, and aplastic anaemia were defined as low-risk diseases, whereas others were considered high-risk diseases. Donors other than human leucocyte antigen (HLA)-matched sibling donors were defined as alternative donors.

Transplantation procedure

The conventional preparative regimen for leukaemia/lymphoma was mainly performed with either cyclophosphamide/total body irradiation (TBI)-based regimens or busulfan/cyclophosphamide-based regimens. In cyclophosphamide/TBI-based regimens, the dose of cyclophosphamide was decreased and etoposide was added instead in patients with impaired cardiac function. Fludarabine-based regimens were used as reduced-intensity regimens for elderly or clinically infirm patients.[14] Cyclosporin A or tacrolimus was administered combined with short-term methotrexate for prophylaxis against GVHD. Alemtuzumab was added for patients who received a graft from an HLA-mismatched donor.[15] Methyl-prednisolone or prednisolone at 1 or 2 mg/kg was added for patients who developed grade II–IV acute GVHD, whereas prednisolone at 0.5 mg/kg or more was added for patients who developed extensive chronic GVHD. Prophylaxis against bacterial, herpes simplex virus and *Pneumocystis jirovecii* infections consisted of tosufloxacin, aciclovir and sulfamethoxazole/trimethoprim.

Antigen detection

GM assay was performed at least every other week after HSCT until discharge from the hospital in the majority of patients. In the outpatient setting, the monitoring of GM was continued at each visit in patients who were receiving immunosuppressive therapy, at the discretion of attending physicians. Circulating *Aspergillus* GM was detected using a sandwich immunocapture ELISA (Platelia Aspergillus, Bio-Rad, Marnes-la-Coquette, France) using a rat anti-GM monoclonal antibody.[2] The technique was performed as recommended by the manufacturer. The optical absorbance of specimens and controls was determined with a spectrophotometer set at 450 and 620 nm wavelengths. The optical density (OD) index for each sample was calculated by dividing the optical absorbance of the clinical sample by that of the threshold control. Two consecutive serum samples with an OD index of 0.6 or more were considered positive.[16]

Table 1. Patients' characteristics

Characteristic	Total patients
Sex (male/female)	105/52
Age, median (range)	41 (16–66)
Underlying disease	
acute leukaemia	70
CML	26
MDS	22
SAA	8
other	31
Graft source	
PBSC	69
BM	88
Donor type	
matched sibling	58
mismatched related	15
unrelated	84
Preparative regimen	
Cy (Etp)/TBI-based regimens	105
Bu/Cy-based regimens	15
ATG-based regimens for SAA	5
Flu-based RIC	32
GVHD prophylaxis	
CsA+MTX	115
tacrolimus+MTX	18
alemtuzumab+CsA+MTX	24
Acute GVHD	
grade 0–I	87
grade II–IV	69
Chronic GVHD	
extensive	57
limited	30
none	47

CML, chronic myelogenous leukaemia; MDS, myelodysplastic syndrome; SAA, severe aplastic anaemia; PBSC, peripheral blood stem cell; BM, bone marrow; Cy, cyclophosphamide; Etp, etoposide; TBI, total body irradiation; Bu, busulfan; ATG, antithymocyte globulin; Flu, fludarabine; RIC, reduced intensity conditioning; GVHD, graft-versus-host disease; CsA, cyclosporin A; MTX, methotrexate.

Antifungal prophylaxis and treatment for IA

As antifungal prophylaxis, fluconazole at 200 mg was principally given daily from day −14 until the end of immunosuppressive therapy. For patients with a history of IA, intravenous micafungin at 150–300 mg or oral itraconazole at 200 mg was administered instead. All patients were isolated in high-efficiency particulate air (HEPA)-filtered rooms from the start of the conditioning regimen to engraftment. Febrile neutropenia was treated with broad-spectrum antibiotics in accordance with