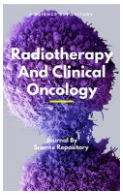

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Research Article

The Correlation between Lymphoma Cell Size and PET–CT Metabolic Activity in Follicular Lymphomas

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ABSTRACT

The purpose of this study was to correlate the average lymphoma cell size and metabolic activity in follicular lymphoma (FL) by comparing to the pathological grading, in a cohort of FL subjects.

Methods: 64 patients with FL were retrospectively studied. 44 cases were grade 1-2 (FL1-2) and 20 were grade 3 (FL3). The average tumor cell size was measured based on flow cytometric analysis of the relative forward scatters (FSC) of tumor cells relative to that of internal T-cells in the lymphoma tissue. The metabolic activity was measured using maximal standardized uptake value (SUV) of the involved lymph node by ^{18}F-FDG PET–CT scan. The SUV was normalized to a glucose level of 100 mg/dl using the formula: SUV100 = SUV x {[100 mg/dl]/[Glc]}^{g} where g = [-0.5] and [Glc] was the glucose level recorded at the time of PET scan. The lymphoma cell size (FSC) and metabolic activity (SUV100) was cross-tabbed and correlated using cut off values determined by discriminant analysis.

Results: Average lymphoma cell size of FL3 was significantly ($p < 0.0005$) greater than that of FL1-2. There was significant correlation between lymphoma cell size (FSC) and metabolic activity (SUV100) ($p = 0.021$), suggesting the possibility of combining these two objective measurements to grade FL.

Conclusions: The significant difference of average lymphoma cell size between FL3 and FL1-2 validate the utility of flow cytometry in grading FL. The significant correlation between cell size and metabolic activity suggests that metabolic activity can further serve as an independent criterion in grading.

Introduction

Follicular Lymphoma (FL) is a common lymphoma with variable clinical aggressiveness, depending on histological grade. It accounts for approximately 20 to 30% of all non-Hodgkin lymphomas [1, 2]. The prognosis of FL is very heterogeneous, and the therapeutic options are increasingly risk-adapted. Histologically, FL has been graded into grade 1-2 and grade 3 by visual counting and estimation of the absolute number of Centro blasts in neoplastic follicles [3]. However, this grading method is subjective and inconsistent. Flow cytometry appears more objective and consistent in evaluation of lymphoma cell size variation of FL. A significant variation in metabolic activity has also been observed in FL studied by [18F] fluoro-2-deoxy-D-glucose (^{18}F-FDG) positron emission tomography (PET) [4, 5]. The purpose of this study was to correlate the average lymphoma cell size measured by flow cytometry and metabolic activity based on ^{18}F-FDG PET–CT scan in FL, compared to the pathological grading, in a cohort of FL subjects.

Materials and Methods

Over a period of four years, 64 consecutive patients with FL proven pathologically were enrolled in this study. Patients were ineligible if they

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had other concomitant malignancy, or if they had received prior radiation therapy or chemotherapy. This study has been reviewed and approved by the Human Investigation Committee for enrollment. These patients included 25 males and 39 females with median age of 61 years. FLs are classified using the World Health Organization (WHO) criteria and graded from grades 1–3 based on the number of centroblasts per high power field (hpf), as grade 1 (0–5 centroblasts per hpf), grade 2 (6–15 centroblasts per hpf), and grade 3 (>15 centroblasts per hpf). 44 cases were grade 1-2 (FL1-2, defined as indolent) and 20 were grade 3 (FL3, defined as aggressive) based on current WHO classification [3].

Flow cytometry analysis

The routine lymphoma immunophenotyping procedure was performed as below. Fresh tissue was teased apart with scalpel and forceps, and then washed with a 0.1% bovine serum albumin and phosphate-buffered saline mixture. Following two more washes, the suspension was filtered through a 74-μm nylon mesh. Cells were incubated with a panel of antibodies in the dark at room temperature for 30 min and then washed twice. Samples were run on a Beckman Coulter *FC500* and analyzed using *CXP* software. For this study, list mode data of Kappa-FITC /Lambda-PE/CD20-ECD/CD10-PC5/CD5-PC were retrospectively reanalyzed. All these antibodies were obtained from Beckman Coulter (Miami, FL). The lymphoma cells were gated based on the expression of light chain (kappa and lambda) and B-cell markers (CD20 and CD10). T-cells were identified by T-cell markers (CD5). The average size of lymphoma cells was determined by measuring mean channels of forward scatter (FSC) which was standardized as relative FSC channels by subtracting the mean FSC of the internal reactive T-cells from the mean FSC of lymphoma cells. A positive value indicated larger tumor cells in comparison with T-cells while a negative value implied smaller tumor cell.

PET–CT scan and analysis

Patients received an average of 555 MBq of ^{18}F-FDG with the PET–CT scan started at 1.5-hour post-injection under standard 4-6 hour fasting condition with blood glucose recorded. The pre-scan fasting blood glucose was 93.14 ± 23.31 mg/dl. The PET data were acquired with a 3-min scan time per bed position with a total PET acquisition time of 18-24 minutes. A complete patient study typically involved six to eight overlapping bed positions to cover the entire body which included the neck, thorax, abdomen, and pelvis. The images were reconstructed using non-time-of-flight iterative reconstruction algorithms. An ordered subsets expectation maximization (OSEM) algorithm with 30 chronologically ordered subsets and 2 iterations were used. Attenuation was corrected using the CT transmission data. A 7.0-mm post-reconstruction smoothing filter was used. The image matrix size was 256 × 256 mm. An initial scout scan was obtained first to define the imaging field for the CT component of image acquisition, which used the following imaging parameters: 140 kVp, 120–200 mA, 0.8 s per CT rotation, pitch 1.75:1, and detector configuration of 16 × 1.25 mm, 3-mm slice thickness with oral contrast only. Sixty-four pre-treatment PET–CT scans within 2 months of biopsy were analyzed and the maximum SUV, defined as tumor activity divided by dose injected per body mass, was measured by searching the maximum value within a volume of interest over the biopsy region. All the SUVs were corrected by glucose level to the standard level of 100 mg/dl using the formula published previously [6, 7]:

$SUV100 = SUV \times (100/[Glc])^{g}$ where $g = -0.5$

The difference of average lymphoma cell sizes between FL1-2 (indolent) and FL3 (aggressive) was compared with Student t test. Then the difference of SUV100 between FL with small and large sizes were also compared with Student t test. Finally, the correlation between lymphoma cell size (relative FSC) and metabolic activity (SUV100) was cross-tabbed using the cell size and SUV100 cut off values determined by discriminant analysis and tested by Chi-Square test. A two-tail p-value less than 0.05 was considered significant in all tests.

Results

Table 1 and 2 revealed age, gender, blood glucose at the time of injection, tumor cell size, original SUV, and SUV100 for patients with grade 3 FL and grade 1-2 FL respectively. Average lymphoma cell size of FL3 (relative FSC of 92, range of -32 to 243) was significantly ($p < 0.0005$) greater than that of FL1-2 (relative FSC of -15, range of -83 to 153). Discriminant analysis suggested that the best cell size cut-off value was -18 for classifying the histologically determined grade 3 and grade 1-2 FL (Figure 1). There was significant difference between SUV100 when using this cut-off by flow cytometry to grade FL into two dichotomous group ($p = 0.018$) and the best SUV100 cut-off value was determined to be 11.5 by discriminant analysis (Figure 2). Cross-tabbing the entire group using the cell size (FSC) and SUV100 cut off values obtained from discriminant analysis, there was significant correlation between lymphoma cell size (relative FSC) and metabolic activity (SUV100) ($p = 0.021$ by Chi-Square test), suggesting the possibility of combining these two-objective measurements to risk stratify FL (Figure 3).

Using the special reference lines of Y=11.5 for SUV100 and X=(-18) for relative FSC, there was only one patient with aggressive risk of lymphoma fell wrongly into this quadrant among a total of 20 patients. Nineteen out of 20 patients (95%) were classified in agreement with pathology.

Discussion

The definition of three grades (grades 1–3) of follicular lymphoma is based on an increasing number of centroblasts per high power field as recognized in the WHO classification of follicular lymphoma [3]. Accurate grading of FL is limited by sampling errors in selection of the sites of lymph nodes, and in the tissue biopsy specimen within the lymph node and microscopic fields. Moreover, issues with adequacy of the sample and potential inter-observer variations are additional limitations in histological assessment. Accurate grading has profound clinical implications as follicular lymphoma grades 1 and 2 are usually characterized by an indolent clinical course while follicular lymphoma grade 3 is a more aggressive disease which requires systemic treatment [3, 8-10]. Thus, it is clinically desirable to classify follicular lymphoma using more objective parameters.

PET using ^{18}F-FDG has been recognized as an important clinical tool, particularly in oncology and is now routinely used in the detection,

staging and treatment response evaluation of various tumors [11–15]. PET scan has also emerged as a major imaging modality for the staging and follow-up of patients with non-Hodgkin's lymphoma (NHL) [16, 17]. Studies have also shown that follicular lymphoma reveals a varying degree of FDG avidity [4, 5]. This metabolic spectrum of follicular lymphoma may offer an opportunity to extract clinically useful information. The standardized uptake value (SUV) is a semi-quantitative measure of the glucose metabolism based on the degree of FDG uptake, which is derived from the tumor activity divided by dose per body mass in the attenuation-corrected PET images [18-20]. It may improve the definition of abnormal areas by reducing subjective visual assessment. It is currently common practice to use ^{18}F-FDG PET scan as an imaging tool in the evaluation of lymphoma, supplemented by semi-quantitative information extracted in the form of SUV, to aid in therapeutic decision-making and prognostication [18]. To assure the uniformity due to glucose variations, all the SUVs in this study were corrected to a uniform glucose level of 100 mg/dl (SUV100) before entering into statistical analysis [6, 7].

Increased cell size was shown in the current study to be associated with more aggressive grade in FL (Figure 1). It had been shown previously that increased tumor metabolism by SUV100 was also associated with aggressive grade of lymphoma [6, 7]. However, cell size and metabolic activity may not be always predictive of each other. Thus, the current study cross tabbed these two independent parameters for correlation (Figure 2). A more accurate risk stratification may be possible using a combination of cell size and metabolic activity (Figure 3). If both relative FSC and SUV100 are low, the patient has a high chance of being in the low or indolent risk of lymphoma and observation may be justified. However, ongoing clinical validation is needed to evaluate the combined independent prognostic values of cell size measurement and metabolic activity for FL.

Table 1: Tumor cell size determined by flow cytometry and SUV of high grade (grade 3) follicular lymphoma

Patient No.	Age	Gender	Glc	Tumor cell size	SUV	SUV100
1	57	female	88	-56	6.8	6.4
2	68	female	83	178	4.5	4.1
3	76	female	130	12	4.9	5.6
4	68	female	224	91	5.8	8.7
5	78	female	88	131	6.3	5.9
6	77	female	89	38	7.2	6.8
7	60	female	76	-15	8.0	7.0
8	79	male	122	126	8.0	8.8
9	58	female	86	186	8.6	8.0
10	76	female	77	163	9.5	8.3
11	49	female	71	152	13.7	11.5
12	63	female	97	165	14.3	14.1
13	53	female	98	26	14.8	14.7
14	43	male	82	29	17.0	15.4
15	47	female	88	-10	17.2	16.1
16	63	female	94	108	18.8	18.2
17	58	female	92	20	21.0	20.1
18	60	male	95	-32	23.6	23.0
19	71	male	87	137	25.8	24.1
20	52	female	90	243	27.0	25.6

Glc: glucose level at the time of PET-CT scan exam SUV: standardized uptake value

SUV100: standardized uptake value normalized to a glucose level of 100 mg/dl

Table 2: Tumor cell size determined by flow cytometry and SUV of low grade (grade1-2) follicular lymphoma

Patient No.	Age	Gender	Glc	Tumor cell size	SUV	SUV100
21	71	female	79	-22	1.5	1.3
22	73	male	121	-27	1.7	1.9
23	52	female	99	-27	2.2	2.2
24	33	female	90	121	3.9	3.7
25	48	female	112	-78	4.0	4.2
26	67	male	68	-73	5.1	4.2
27	52	female	90	-26	5.1	4.8
28	48	male	98	-8	5.2	5.1
29	42	male	89	-43	5.3	5.0
30	57	male	92	153	5.6	5.4
31	55	female	110	-33	5.8	6.1
32	59	male	96	38	5.9	5.8

33	56	female	80	-43	6.0	5.4
34	79	female	92	-76	6.2	5.9
35	60	male	89	-42	7.1	6.7
36	46	male	77	-78	7.2	6.3
37	59	female	82	-35	8.0	7.2
38	63	female	76	-31	8.2	7.1
39	77	female	96	-81	9.0	8.8
40	51	female	91	-29	9.6	9.1
41	56	female	109	-9	10.0	10.4
42	57	male	86	-50	10.0	9.3
43	63	male	101	-83	11.4	11.5
44	58	male	101	38	11.6	11.7
45	81	male	105	-68	11.7	12.0
46	80	female	70	-72	12.0	10.0
47	85	female	86	-1	12.7	11.8
48	70	female	104	53	13.2	13.5
49	47	male	100	-45	13.4	13.4
50	45	male	74	-39	13.5	11.6
51	45	male	102	12	13.7	13.8
52	76	female	80	-45	14.0	12.5
53	80	female	99	-46	14.7	14.6
54	48	female	80	-4	14.7	13.1
55	72	male	99	34	15.4	15.3
56	73	male	114	-18	15.5	16.5
57	62	female	111	128	16.5	17.4
58	68	female	102	54	16.9	17.1
59	34	male	86	78	17.5	16.2
60	63	male	100	62	18.7	18.7
61	82	female	72	-65	22.5	19.1
62	78	male	88	-77	23.7	22.2
63	68	female	96	17	26.8	26.3
64	83	male	100	-14	27.4	27.4

Legends as in Table 1

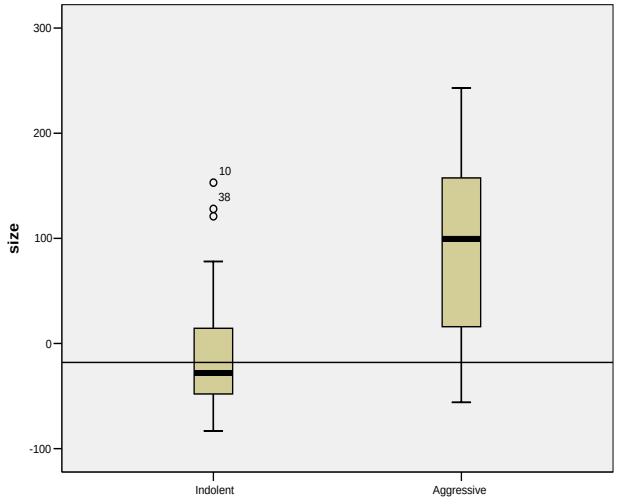
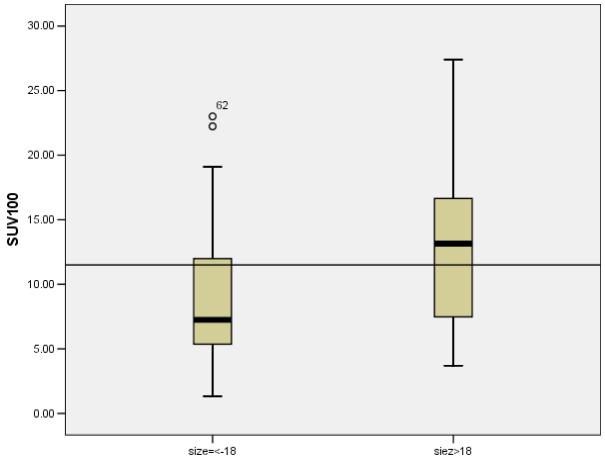
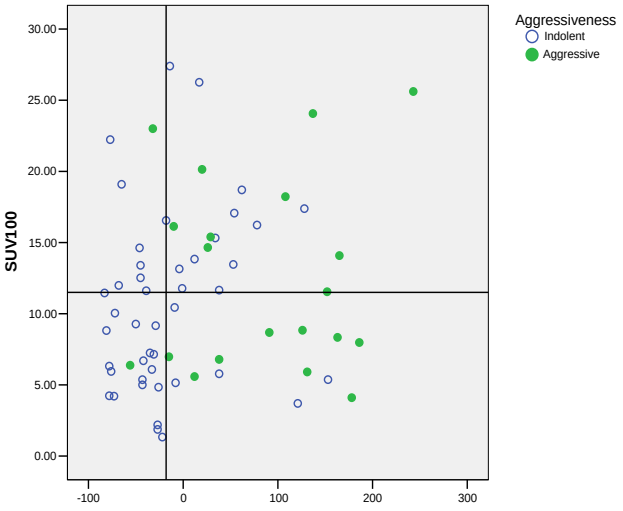


Figure 1: Average tumor cell size of indolent (grade 1-2) versus aggressive (grade 3) follicular lymphoma (FL) using flow cytometry analysis. There is significant difference in tumor cell size between indolent FL and aggressive FL ($p < 0.0005$). The best size cut-off value was -18 relative FSC determined by discriminant analysis.



lymphoma cell size: relative FSC=<-18, relative FSC>-18

Figure 2: The standardized uptake value corrected by glucose level to the standard level of 100 mg/dl (SUV100) versus follicular lymphoma (FL) with tumor cell size (relative FSC) smaller or greater than -18. Significant difference was demonstrated between SUV100 when using the cut-off value of -18 relative FSC by flow cytometry to grade FL into two dichotomous group ($p = 0.018$) and the best SUV100 cut-off value was determined to be 11.5 by discriminant analysis.



Lymphoma cell size: relative FSC

Figure 3: The classification of lymphoma indolent or aggressive behavior using cutoff values of 11.5 by SUV100 for metabolism and -18 by relative FSC for tumor cell size.

Conclusion

Clinically FL grade 3 is typically more aggressive than FL grade 1-2. The correlation of average lymphoma cell size and metabolic activity identified in this study in discriminating FL3 and FL1-2 validates the potential utility of flow cytometry and PET scan in grading FL. Using combined cell size and metabolism in refining biologic risk categories may be more reproducible, objective, and easy to use than manual counting of Centro blasts, with great potential for serving as an independent criterion in determining lymphoma aggressiveness in future.

References

1. The Non-Hodgkin's Lymphoma Classification Project (1997) A clinical evaluation of the international lymphoma study group classification of non-Hodgkin's lymphoma. *Blood* 89: 3909-3918. [Crossref]
2. Lu P (2005) Staging and classification of lymphoma. *Semin Nucl Med* 35: 160-164. [Crossref]
3. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, et al. (1999) World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November 1997. *J Clin Oncol* 17: 3835-3849. [Crossref]
4. Wirth A, Foo M, Seymour JF, Macmanus MP, Hicks RJ (2008) Impact of 18F-fluorodeoxyglucose positron emission tomography on staging and management of early-stage follicular non-Hodgkin lymphoma. *Int J Radiat Oncol Biol Phys* 71: 213-219. [Crossref]
5. Karam M, Novak L, Cyriac J, Ali A, Nazeer T, et al. (2006) Role of fluorine-18 fluorodeoxyglucose positron emission tomography scan in the evaluation and follow-up of patients with low-grade lymphomas. *Cancer* 107: 175-183. [Crossref]
6. Tang B, Malysz J, Douglas-Nikitin V, Zekman R, Wong RH, et al. (2009) Correlating Metabolic Activity with Cellular Proliferation in Follicular lymphomas *Mol Imaging Biol* 11: 296-302. [Crossref]
7. Wong CY, Thie J, Parling-Lynch KJ, Zakalik D, Margolis JH, et al. (2005) Glucose-normalized standard uptake value from (18)F-FDG PET in classifying lymphomas. *J Nucl Med* 46: 1659-1663. [Crossref]
8. Wendum D, Sebban C, Gaulard P, Coiffier B, Tilly H, et al. (1997) Follicular large-cell lymphoma treated with intensive chemotherapy: an analysis of 89 cases included in the LNH87 trial and comparison with the outcome of diffuse large B-cell lymphoma. Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol* 15: 1654-1663. [Crossref]
9. Ganti AK, Weisenburger DD, Smith LM, Hans CP, Bociek RG, et al. (2006) Patients with grade 3 follicular lymphoma have prolonged relapse-free survival following anthracycline-based chemotherapy: the Nebraska Lymphoma Study Group Experience. *Ann Oncol* 17: 920-927. [Crossref]
10. Overman MJ, Feng L, Pro B, McLaughlin P, Hess M, et al. (2008) The addition of rituximab to CHOP chemotherapy improves overall and failure-free survival for follicular grade 3 lymphoma. *Ann Oncol* 19: 553-559. [Crossref]
11. Avril NE, Weber WA (2005) Monitoring response to treatment in patients utilizing PET. *Radiol Clin North Am* 43: 189-204. [Crossref]
12. Krak NC, Hoekstra OS, Lammertsma AA (2004) Measuring response to chemotherapy in locally advanced breast cancer: methodological considerations. *Eur J Nucl Med Mol Imaging* 31: 103-111. [Crossref]
13. Scheidhauer K, Walter C, Seemann MD (2004) FDG PET and other imaging modalities in the primary diagnosis of suspicious breast lesions. *Eur J Nucl Med Mol Imaging* 31: S70-S79. [Crossref]
14. Vansteenkiste J, Fischer BM, Dooms C, Mortensen J (2004) Positron emission tomography in prognostic and therapeutic assessment of lung cancer: systematic review. *Lancet Oncol* 5: 531-540. [Crossref]
15. Wahl RL, Zasadny K, Helvie M, Hutchins GD, Weber B, et al. (1993) Metabolic monitoring of breast cancer chemohormonotherapy using positron emission tomography: initial evaluation. *J Clin Oncol* 11: 2101-2111. [Crossref]
16. Kumar R, Maillard I, Schuster SJ, Alavi A (2004) Utility of fluorodeoxyglucose-PET imaging in the management of patients with Hodgkin's and non-Hodgkin's lymphomas. *Radiol Clin North Am* 42: 1083-1100. [Crossref]
17. Burton C, Ell P, Linch D (2004) The role of PET imaging in lymphoma. *Br J Haematol* 126: 772-784. [Crossref]
18. Wong CY, Thie J, Parling-Lynch KJ, Zakalik D, Wong RH, et al. Investigating the existence of quantum metabolic values in non-Hodgkin's lymphoma by 2-deoxy-2-[F-18] fluoro-D-glucose positron emission tomography. *Mol Imaging Biol* 9: 43-49. [Crossref]
19. Weber WA, Ziegler SI, Thodtmann R, Hanauske AR, Schwaiger M (1999) Reproducibility of metabolic measurements in malignant tumors using FDG PET. *J Nucl Med* 40: 1771-1777. [Crossref]

20. Young H, Baum R, Cremerius U, Herholz K, Hoekstra O, et al. (1999) Measurement of clinical and subclinical tumor response using [18F]-fluorodeoxyglucose and positron emission tomography: review and 1999 EORTC recommendations. European Organization for Research and Treatment of Cancer (EORTC) PET Study Group. *Eur J Cancer* 35: 1773-1782. [Crossref]