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Prognosis of Lymphoma From a Fine-needle Aspirate

Heikki Joensuu, Kalle Alanen and Pekka J. Klemi

The prognostic value of S-phase fraction (SPF) determined by flow cytometry from a fine-needle aspirate was investigated in a prospective series of 52 non-Hodgkin lymphomas. The aspirates were drawn either at diagnosis (n = 16) or at lymphoma recurrence (n = 36). Patients with lymphoma with a large SPF (> 10%, n = 24) had only a 21% 3-year survival rate corrected for intercurrent deaths as calculated from the date of aspiration, whereas a smaller SPF was associated with a 71% 3-year survival rate (n = 28, P = 0.0009). SPF size also correlated with Working Formulation grading (P = 0.002). In a multivariate analysis the relative risk of death from lymphomas with a large SPF was 4.01 (1.60–10.1), whereas histological grading, age, and sex had no additional independent prognostic value. SPF determined from a fine needle aspirate had unexpectedly good prognostic value, and the result suggests that the method is of clinical importance.
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INTRODUCTION

HISTOLOGICAL EXAMINATION of excised lymphoma tissue is essential not only for making the distinction between Hodgkin's disease and non-Hodgkin lymphoma, but also for further subtyping of lymphoma to augment selection of treatment and to estimate the biological aggressiveness of lymphoma. Aggressiveness of lymphoma can also be assessed by several techniques other than histology, e.g. by measuring the relative number of cells in the DNA synthesis phase of the cell cycle (the S-phase fraction, SPF) by DNA flow cytometry or image cytomery. The SPF can be determined rapidly by flow cytometry, and both Hodgkin's and non-Hodgkin's lymphomas with a large SPF have been shown to have inferior outcome as compared with lymphomas with a low SPF [1–13]. Unlike the SPF, DNA ploidy appears to have relatively little prognostic value in lymphomas [3–5, 7–19]. A few authors report that the SPF may be superior to histology in predicting survival in lymphoma [5, 6, 9, 20]. These studies have been performed from surgically

excised tissue, and often retrospectively from formalin-fixed, paraffin-embedded tissue.

Although the diagnosis of malignant lymphoma can often be made by fine-needle aspiration biopsy morphology alone, it may be particularly difficult to distinguish low grade lymphoma from benign reactive lymphoid cells in a fine-needle aspirate [21], and a histological biopsy is needed for reliable subtyping of lymphoma. However, fine-needle aspiration biopsy is an almost atraumatic procedure, easily repeatable, cost-effective, and the cytological diagnosis can be reported rapidly within a few hours from aspiration. In the present prospective study we determined the SPF of lymphoma from a fine needle aspirate in order to establish whether the size of the SPF correlates with the histological subtype of lymphoma, and whether fine-needle aspiration together with flow cytometric nuclear DNA content determination can be used to assess prognosis of lymphoma.

PATIENTS AND METHODS

Patients

Fine needle aspirates of 85 patients with histologically diagnosed lymphoma were analysed for nuclear DNA content in the University Central Hospital of Turku in 1986–1989. The presence of lymphoma in the aspirated tumour was confirmed in 43 cases by a subsequent surgical removal and histological examination of the same tumour from which the aspirate was drawn. The rest of the cases, where the tumour from which aspiration was performed was not excised, were accepted in the analysis if the cytological diagnosis from the same tumour was malignant lymphoma, and if histological diagnosis of lymphoma was available from some other site of the body than the aspiration site. These criteria lead to the exclusion of 11 cases from the series, where the cytological diagnosis of the tumour was either benign, an equivocal finding for malignancy, or an insufficient sample. DNA ploidy determination was not successful in 6 (8%) of the remaining 74 cases (< 5 000 nuclei in the aspirate, $n = 4$; poor quality of the DNA histogram with a coefficient of variation of the diploid peak > 8.0, $n = 2$), and in further 4 cases calculation of the SPF had not been done (the cytometrist had failed for an unknown reason to report the SPF from a technically acceptable DNA histogram, $n = 2$; presence of excessive cell debris or overlapping DNA stemlines prohibiting calculation of the SPF, $n = 2$). 52 of the remaining 64 patients had non-Hodgkin lymphoma, and form the basis of the study. Sixteen aspirations were done at the time of the diagnosis, and the rest ($n = 36$) at the time of lymphoma recurrence.

27 (52%) of the 52 patients were male, and the median age was 66.5 years (range, 17–88 years). 14 patients had low grade, 22 intermediate grade, 15 high grade, and 1 unclassified non-Hodgkin lymphoma by the Working Formulation scheme. Patients still alive have been followed-up after SPF determination for the median of 23 months (range, from 1 to 50 months). 24 patients have died, 22 from lymphoma, and 2 from an intercurrent cause.

Treatment following aspiration was individual. Lymphomas of low grade malignancy were usually treated with chlorambucil possibly combined with prednisone ($n = 6$), COP (cyclophosphamide, vincristine, and prednisone, $n = 6$), or radiotherapy ($n = 8$), but five other chemotherapy regimens had also been used. Patients with intermediate or high grade lymphomas had received 12 different types of chemotherapy protocols (the most commonly used was CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; $n = 9$) in addition to radiotherapy ($n = 22$).

Aspiration technique

The aspirations and cytological investigations were made mainly by two pathologists. A syringe holder and a needle (0.6 mm outer diameter) were used in aspiration [22]. The tumours were punctured 2–3 times. A part of each aspirate was ejected onto a glass slide, smeared, air-dried, and later stained with May-Grünwald-Giemsa (MGG) stain. The rest of the aspirate was used for analysis of the nuclear DNA content, and it was ejected into a test-tube containing citric acid buffered saline ($n = 19$), 50% ethanol ($n = 7$), or citric acid buffered saline and 99% ethanol 1:1 ($n = 38, 23$). When no preservative was used the aspirate was analysed for the nuclear DNA content within a few hours, and when the ethanol containing preservatives were used, within a few days after aspiration.

DNA flow cytometry

DNA was stained with propidium iodide, and flow cytometry was done with a FacStar flow cytometer (Becton-Dickinson Immunocytometry Systems) as described in detail elsewhere [11, 23]. For each histogram 20 000 particles were analysed. Chicken red blood cells were used as an internal control. The mean coefficient of variation (CV) of the diploid peaks was 3.3% (SD, 1.2%, range, 1.7–7.5%). Histograms with 1 symmetrical G0/G1 peak were considered to be DNA diploid. Histograms with 2 G0/G1 peaks present were classified as DNA aneuploid, and if the aneuploid G0/G1 peak was present at 4N, as tetraploid. If only 1 G0/G1 peak was present, but it was clearly asymmetrical (with a "shoulder"), the histogram was classified as near-diploid. DNA index (DI) was calculated from the ratio of the modal channel numbers of the diploid and aneuploid peaks.

SPF was analysed using the rectangular method. The height of the rectangle was measured as the mean of about a 10 to 15 channel long segment at the lowest part of the curve over the S-region, which was usually in the middle or near the G2 peak. No correction for the background debris was made. In aneuploid or tetraploid cases with a large DNA index (> 1.3), SPF was calculated for the aneuploid/tetraploid stemline only. In aneuploid cases with a smaller DNA index, SPF was calculated for both of the G1 peaks combined as for near diploid histograms [24]. DNA ploidy and SPF analyses were done blindly without any knowledge of the clinical, histological, or cytological data.

Statistical analysis

Statistical analyses were done with the BMDP computer program (BMDP Statistical Software, Department of Biomathematics, University of California, Los Angeles, California). Comparisons of SPF distributions were done with the Kruskal–Wallis analysis of variance and the Mann–Whitney U-test. Cumulative survival was estimated with the product-limit method, and comparison of cumulative survival between groups was performed with the log-rank test. Survival corrected for intercurrent deaths was used in statistical calculations, and patients who died from other causes than lymphoma ($n = 2$) were withdrawn from the analysis at the date of death. The

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Table 1. DNA ploidy and S-phase fraction in 52 lymphomas

Lymphoma histology	n	S-phase fraction Mean (SD) %	Range %	DNA Ploidy* Di	ND	An	Te
Low grade	14	4.6 (2.8)	1.2–9.9	14	0	0	0
Intermediate grade	22	12.0 (8.4)	1.7–31.2	15	3	4	0
High grade	15	19.4 (13.9)	2.5–43.7	11	1	3	0
Unclassified	1	15.4		0	1	0	0

*Di, ND, An, and Te, DNA diploid, near diploid, aneuploid, and tetraploid, respectively.

relative importance of prognostic factors was analysed using Cox's proportional hazard model (BMDP 2L). All P-values are 2-tailed.

RESULTS

The SPFs measured correlated well with lymphoma histology (Table 1). There was no significant difference in the size of the SPF between intermediate grade and high grade malignant lymphomas (12.0% vs. 19.4%, respectively, $P = 0.13$), but the SPF was larger in high grade than in low grade lymphomas ($P = 0.001$), and in intermediate grade than in low grade lymphomas ($P = 0.006$).

The size of the SPF correlated well with prognosis (Table 2). The best cut-off value was 10%. Patients with a large SPF (> 10%, $n = 24$) had only a 21% 3-year survival rate corrected for intercurrent deaths as calculated from the date of aspiration, whereas a smaller SPF was associated with a 71% 3-year survival rate ($n = 28$, $P = 0.0009$, Fig. 1). Also the median value (9%) and the cut-off values for the highest (15%) and the lowest tertiles (5%) were strongly associated with survival.

The size of the SPF correlated well with the final outcome if the length of survival was calculated from the date of the diagnosis instead of the date of aspiration, even if the majority of the SPFs had been determined at lymphoma recurrence, and not at the time of the diagnosis. Using the best cut-off value of 10%, the 3-year survival rates of the patients with a low and a high SPF were 96% and 29%, respectively, and the 5-year survival rates 77% vs. 19%, respectively ($P < 0.0001$, Fig. 2).

Histological grading into low, intermediate, and high grade malignant lymphomas was also associated with survival if calculated from the date of aspiration ($P = 0.02$), whereas age at aspiration ($P = 0.08$) and sex ($P = 0.30$) were not. In order to find out if the size of the SPF had independent influence on

Table 2. Prognostic influence of S-phase fraction

Cut-off value	No. of patients ≤ cut-off n	No. of patients > cut-off n	3-year survival rate ≤ cut-off	3-year survival rate > cut-off	P
Best cut-off, ≤ 10% vs. > 10%	28	24	71%	21%	0.0009
Median, ≤ 9% vs. > 9%	26	26	73%	21%	0.002
Highest tertile, > 15%	34	18	61%	26%	0.02
Lowest tertile, ≤ 5%	15	37	78%	34%	0.02

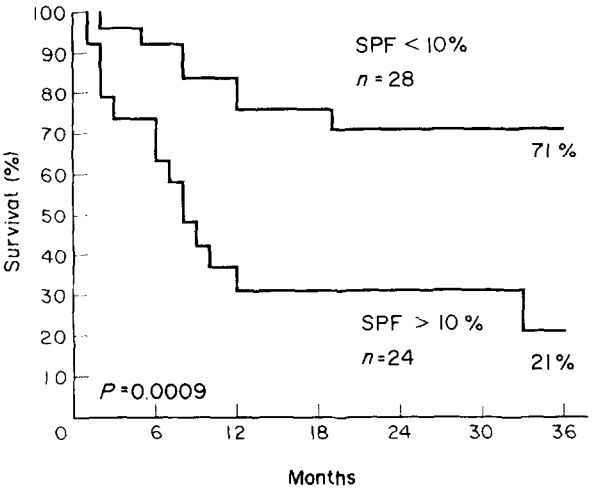

Fig. 1. Survival corrected for intercurrent deaths as calculated from the date of fine-needle aspiration by the size of the S-phase fraction among 52 patients with non-Hodgkin lymphoma.

survival it was entered into Cox's multivariate analysis (> 10% vs. ≤ 10%) together with histological grading (high vs. intermediate vs. low grade), age (> 60 vs. ≤ 60, the most effective cut-off), and gender. The size of the SPF turned out to be the only independent prognostic factor ($P = 0.002$). The relative risk of lymphomas with the SPF > 10% to cause death from lymphoma as compared with lymphomas with an SPF ≤ 10% was 4.01 (95% confidence interval from 1.60 to 10.1).

DISCUSSION

The present results and others [25–27] indicate that SPF determination from a fine-needle aspirate drawn from lymphoma is feasible. According to Sneige *et al.*, the S + G2/M fraction size determined from a fine-needle aspirate resembles closely the one obtained from corresponding fresh surgical tissue [28].

We have previously made an attempt to study the prognostic significance of the SPF both in Hodgkin's [13] and non-Hodgkin lymphoma [11] from paraffin-embedded tissue taken at the time of the diagnosis. Although SPF turned out to have prognostic influence in both diseases in univariate and multivariate analyses, and correlated with the final outcome at least as

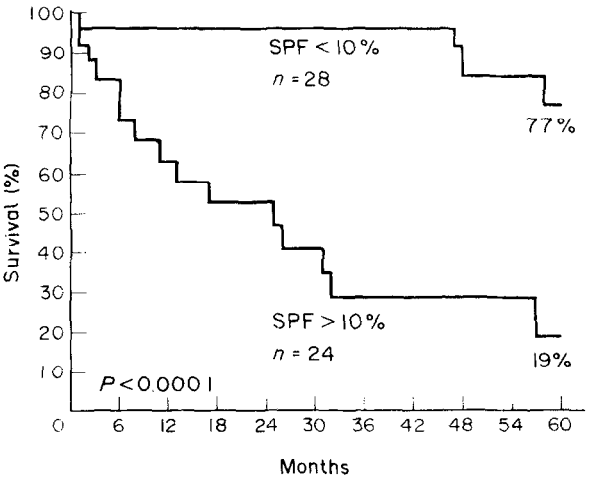

Fig. 2. Survival corrected for intercurrent deaths as calculated from the date of initial lymphoma diagnosis by the size of the S-phase fraction among 52 patients with non-Hodgkin lymphoma.

well as histological grading in non-Hodgkin lymphoma [11], the correlation between a large SPF and poor prognosis appears to be particularly strong in the present prospective series, where the size of the SPF determined several years ago was correlated with mortality caused by lymphoma. It is possible that the fine-needle aspiration biopsy technique is particularly suitable for SPF determination.

The size of the SPF correlated at least as well with prognosis if survival was calculated from the date of the first histological diagnosis of lymphoma as when it was calculated from the date of aspiration, which may have taken place years after the diagnosis. This suggests that in many lymphomas the rate of cell proliferation is probably similar at recurrence as it was at the time of the initial diagnosis of lymphoma. This finding is in line with another study [28] where the size of the SPF in a tissue biopsy specimen taken at the time of lymphoma diagnosis was compared with the SPF measured from a repeat biopsy sample taken several months or years after the first biopsy. The size of the SPF remained usually similar at lymphoma recurrence, although it was occasionally clearly increased at recurrence, especially in high grade malignant lymphomas.

Although to our knowledge this is the first study to demonstrate that the size the SPF determined from a fine-needle aspirate by flow cytometry is associated with prognosis in lymphoma, other data to support the use of fine-needle aspiration biopsy derived material for assessment of lymphoma prognosis are available. Vuckovic *et al.* [29] analysed the DNA content of 62 lymphomas from a fine-needle aspirate using microdensitometry and Feulgen staining, and found patients whose lymphoma contained less than 6% of cells with an increased DNA content to have mean survival of 81.3 months as compared with 18.5 months in those patients whose lymphoma contained 6% or more of such cells. Similarly, kinetic studies using tritiated thymidine uptake in lymphoma cells obtained through fine-needle aspiration biopsy have indicated a significant correlation between labelling index and survival [30]. However, this method is more laborious than DNA cytometry. Immunostaining of a fine-needle aspirate using the monoclonal antibody Ki-67 is also feasible, and deserves further study.

The fine-needle biopsy method is quick and repeatable, and well accepted by the patients. If ethanol containing preservatives are used, analysis of the DNA content may be done several days or even months after aspiration, and the aspirate may be mailed. The fine-needle aspiration technique is also cost-effective, makes sampling of several tumours possible if discordant behaviour of lymphoma is suspected, and biological progression of lymphoma during the course of the disease may be detected by repeat aspirations [28].

In conclusion, the SPF as analysed from a fine-needle aspirate showed good correlation with mortality in lymphoma. Moreover, the prognostic value of the SPF determination was greater than that of histological grading in non-Hodgkin lymphoma in a multivariate analysis. Hence, the method appears to be of clinical value. In light of the close association with the size of the SPF and prognosis, new prospective studies where a tissue biopsy sample and a fine-needle aspirate are compared as starting material are warranted. In such studies the prognostic significance of the SPF may further increase after labelling procedures.

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A Randomised, Double-Blind Comparison of Granisetron with High-dose Metoclopramide, Dexamethasone and Diphenhydramine for Cisplatin-induced Emesis

An NCI Canada Clinical Trials Group Phase III Trial

David Warr, Andrew Wilan, Peter Venner, Joseph Pater, Leonard Kaizer, Francis Laberge, Jean Latreille, David Stewart, Gregory O'Connell, David Osoba, Kenneth Wilson, Alan Davis and Dianne Johnston

151 patients (149 evaluable) receiving their first course of chemotherapy containing cisplatin in a dose of at least 50 mg/m^2 were randomised to receive either a single dose of intravenous granisetron 80 μg/kg or intravenous metoclopramide 2 mg/kg every 2 h for five doses plus a single dose of dexamethasone 10 mg and diphenhydramine. After 24 h, there was no significant difference between groups with respect to nausesa or vomiting: in the granisetron group 46% of patients had no emesis, versus 44% of the standard group. Granisetron is an antiemetic agent with efficacy similar to that of high-dose metoclopramide plus dexamethasone.
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INTRODUCTION

CISPLATIN IS the most emetogenic cytotoxic agent [1]. The use of high-dose metoclopramide in combination with dexamethasone has decreased the frequency and severity of emesis [2–4] but a majority of patients will still vomit following therapy [5]. In addition, the adverse effects of restlessness and extrapyramidal symptoms due to dopamine receptor antagonism continue to be troublesome for some patients despite the use of diphenhydramine of lorazepam [6]. Thus there is a need for more effective, better tolerated antiemetics.

The hypothesis by Miner and Sanger that the antiemetic effect of metoclopramide might be due to an antagonistic effect at the 5-HT$_3$ receptor led to the discovery of a novel class of antiemetics that were remarkably effective in animals [7]. In patients with cancer, a recent double-blind study showed that a single dose of the selective 5-HT$_3$ antagonist granisetron was markedly superior to dexamethasone and prochlorperazine for moderately emetogenic chemotherapy [8]. There was prevention of emesis in 70% of patients with very little breakthrough beyond 12 h suggesting that additional administration of granisetron was not required. The only published comparative study for cisplatin-induced emesis thus far is a single-blind study by Chevallier *et al.* in which granisetron provided protection from cisplatin-induced emesis that was equivalent to an 8 h metoclopramide infusion plus dexamethasone [9].

The objective of this study was a double-blind comparison of the antiemetic activity of a single injection of granisetron with a standard therapy which, by consensus of the investigators, was an intermittent schedule of high-dose metoclopramide plus dexamethasone and diphenhydramine.

PATIENTS AND METHODS

Entry criteria

Patients were considered eligible if they were at least 18 years of age, spent less than 50% of the daytime in bed (Eastern Cooperative Oncology Group performance status < 3), had no