Purpose: Usually the diagnosis of lymphoma is made by histologic examination (most often with fine needle aspiration or lymph node biopsy). It is hoped that an addition of DNA cytometry would provide diagnostic and prognostic information based on objective measurements of proliferation and ploidy. However, detailed review of 9 recent articles on the subject reveals inconclusive results.

Principles: The amount of DNA within a nucleus can be determined by using fluorescent dyes which bind stoichiometrically to nucleic acids. The fluorescence intensity measured by flow cytometer is proportional to the DNA content. A histogram can be constructed by plotting number of nuclei at varying fluorescence intensity as a population of cells is analyzed. Proportions of cells in various phases of cell cycle can be determined from such a histogram. For example, the majority of cells are in the G0 (resting state) / G1 (pre-synthetic phase) in Figure (from Dressler and Bartow, 1989). The second smaller peak represents cells in G2 (post-synthetic) + M (mitotic) phase. The cells in G2 phase cannot be distinguished from the cells in M phase by this technique. The area between the two peaks represent cells in S (synthetic) phase. The cells in various phases overlap and there is no clear border between the phases. A proliferative index is a measure of proportion of cells in S phase or cells in S and G2+M phases. Methods used to calculate proliferative index in studies reviewed here vary from use of manual technique to use of computer programs based on mathematical models (Table). In studies which have looked at S phase fraction in adult solid tumors, a high S phase value generally correlates with more aggressive behavior (Dressler and Bartow, 1989).

Ploidy or DNA content is another parameter measured using DNA cytometry. Normal tissue and diploid tumors have normal amounts DNA and aneuploid tumors have abnormally high or low amounts of DNA. In normal cells and in diploid tumors, the cells in G0/G1 phase have 2N amounts of DNA and cells in G2 + M phase have 4N amounts of DNA. The cells in S phase have intermediate amounts of DNA (2N to 4N). In aneuploid tumors, the position of G0/G1 peak would be shifted from that of normal tissue. The DNA index or the relative amount of DNA in
the aneuploid tumor relative to normal tissue can be calculated by dividing the
fluorescence channel number of the aneuploid population by that of normal
population. If the G0/G1 peak is shifted to the right, the tumor is hyperploid and
DNA index is >1. On the other hand, if the G0/G1 peak is shifted to the left, the tumor
is hypoploid and DNA index is <1. Aneuploidy has been found to correlate with
increasing tumor grade and stage and with worse prognosis in adult solid tumors in
general (Dressler and Bartow, 1989).

Ploidy: Most of the studies summarized in the Table concludes that there is no
significant association between ploidy and clinical outcome of NHL (Cowan et al.,
However, some studies support favorable outcome with diploid tumors or
unfavorable outcome with aneuploid tumors (Joensuu et al., 1990; Lehtinen et al.,
1989; Christensson et al., 1988; and McLaughlin et al., 1988). On the other hand,
Wooldridge et al. (1988) concludes that DNA aneuploidy has significant correlation
with longer duration of complete remission and survival. The source of this
discrepancy is not apparent. Since all the patients in this study were untreated at the
time of analysis, better response to therapy may explain better outcome of aneuploid
tumors. Relatively small number of samples analyzed (48) and restriction of samples
to diffuse cell and mixed cell non-Hodgkin's lymphoma (NHL) may also have
contributed to this discrepancy.

Proliferative Index: Association of low proliferative index with favorable outcome
or association of high proliferative index with unfavorable outcome is supported by
some of the studies (Joensuu et al., 1990; Jalkanen et al., 1990; Wooldridge et al., 1988;
and McLaughlin et al., 1988). Other studies show no correlation between proliferative
index and clinical outcome or show such correlation only with a subgroup of cases
(Cowan et al., 1989; Lehtinen et al., 1989; O'Brien et al., 1989; Christensson et al. 1988,
and Egerter et al., 1988). Lehtinen et al. (1989) concludes that high S phase fraction
correlates with shorter survival in histologically favorable group, but not in
histologically unfavorable group. In contrast, Christensson et al. (1988) finds that low
S-phase value correlates with favorable outcome in high grade but not in low grade
NHL.

McLaughlin et al. (1988) have shown that, in patients who were followed
beyond 12 to 24 months, higher proliferative activity ((S+G2M)>18%) seems be
associated with more durable remission. Although this correlation is not statistically
significant, such observation is contrary to a premise that higher proliferative index correlates with worse outcome.

While a number of studies seem to support the relationship between low proliferative index with favorable outcome or vice versa, it would be very difficult to apply published information to actual clinical cases since cut offs for proliferation index vary widely between different studies (High S-phase fraction defined to be >3.1% in Lehtinen et al, 1989 to low proliferative index (percentage of cells in S and G2M) defined to be <22% in O'brien et al., 1989).

Conclusion: Would adding DNA cytometry to the diagnostic work-up of NHL provide useful information? The review of recent publications reveals inconclusive results in regards to correlation between aneuploidy and prognosis and between high proliferative index and prognosis. Adding DNA cytometry to a diagnostic work-up of NHL is unlikely to be valuable at this point since consensus in current literature is lacking. Also, since histological examination can give some idea about the proliferative capacity of tumor (i.e. mitotic index) information gained by DNA cytometry is not unique. It is not clear whether the lack of consensus is due to true lack of correlation or due to numerous differences in methodologies (i.e. dye, instrument, calculation, sample type; see Table).
Figure: DNA histogram
## TABLE: DNA cytometry of Non-Hodgkin's lymphoma.

<table>
<thead>
<tr>
<th>Type(s) of Lymphomas</th>
<th># of cases</th>
<th>Dye</th>
<th>Instrument</th>
<th>Analysis of proliferative index</th>
<th>Sample Type</th>
<th>Ploidy</th>
<th>Proliferative Index</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>High &amp; intermediate grade NHL</td>
<td>225</td>
<td>DAPI</td>
<td>EPIC S V flow cytometer</td>
<td>Manual Technique</td>
<td>Paraffin</td>
<td>No significant difference in response rate, relapse-free survival or over all survival. Not an independent prognostic factor for response, relapse-free survival or over all survival.</td>
<td>PI (percentage of cells in S and G2M) correlate with histological subtype. Not an independent prognostic factor for response, relapse-free survival or over all survival.</td>
<td>Cowan et al. 1989 (United Kingdom)</td>
</tr>
<tr>
<td>NHL</td>
<td>37</td>
<td>Propidium Iodide</td>
<td>FACStar</td>
<td>Calculated using rectangular method.</td>
<td>Paraffin</td>
<td>Diploto tumors were associated with more favorable survival.</td>
<td>Low S-phase fraction (6%) correlated strongly with survival and post-relapse survival.</td>
<td>Joensuu et al. 1990 (Finland)</td>
</tr>
<tr>
<td>NHL</td>
<td>104</td>
<td>Propidium Iodide</td>
<td>FACStar</td>
<td>Calculated using rectangular method.</td>
<td>Paraffin</td>
<td>No correlation with survival.</td>
<td>Large S-phase fraction (&gt;12%) correlated with unfavorable histologic type (Working Formulation) and poor survival.</td>
<td>Jalkanen et al. 1990 (Finland)</td>
</tr>
<tr>
<td>High grade NHL</td>
<td>110</td>
<td>DAPI (Quirke et al. 1986; Hedley et al., 1983)</td>
<td>EPIC S V flow cytometer</td>
<td>N/A</td>
<td>Paraffin</td>
<td>No association found between ploidy and remission induction.</td>
<td>Low PI (percentage of cells in S and G2M; &lt;22%) correlate with longer survival (statistically not significant). No association with remission induction.</td>
<td>O'Brien et al. 1989 (United Kingdom)</td>
</tr>
<tr>
<td>Histologically favorable &amp; unfavorable NHL</td>
<td>82 + 117</td>
<td>Ethidium Bromide</td>
<td>EPICS C flow cytometer</td>
<td>Computer programs based on mathematical models. (Baisch et al., 1975)</td>
<td>Paraffin</td>
<td>Among unfavorable NHLs, aneuploidy correlated with worse prognosis (P&lt;0.01). Overall, the DNA aneuploidy &amp; diploid cases had similar prognosis.</td>
<td>No prognostic difference with regard to S-phase fraction in unfavorable group. High S-phase fraction (&gt;3.1%) correlated with shorter survival in favorable NHL (P&lt;0.01).</td>
<td>Lehtinen et al. 1989 (Finland)</td>
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<tr>
<td>Grade</td>
<td>Dyes</td>
<td>Method</td>
<td>DNA analysis</td>
<td>Outcome Notes</td>
<td>Ref.</td>
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<tr>
<td>Low</td>
<td>Ethidium Bromide</td>
<td>Rapid flow cytometer</td>
<td>Fresh biopsy material</td>
<td>In low grade NHL, aneuploidy associated with poor response &amp; shorter clinical remission ($P &lt; 0.05$). Ploidy does not correlate with survival.</td>
<td>Christensson et al. 1988 (Sweden)</td>
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<td>Low $S$-phase value (&lt;5.6%) in high grade histology correlate with complete remission ($P &lt; 0.05$) and longer survival ($P &lt; 0.02$). In the low grade NHL group the $S$-phase value did not correlate to response. $S$-phase correlated to survival for patients with the lymphocytic (p &lt; 0.05) and follicle center cell derived (P &lt; 0.01) but not in blastic NHL.</td>
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<td>Paraffin DNA aneuploidy associated with a significantly longer duration of complete remission ($P &lt; 0.01$). Aneuploidy correlated with longer survival ($P &lt; 0.01$).</td>
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<td>Tumors with low PI (&gt; 80% of cells in 60/61 phase) were associated with more initial complete remission ($P &lt; 0.02$). Low PI value correlated with longer survival ($P &lt; 0.01$).</td>
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<tr>
<td>High</td>
<td>Propidium Iodide</td>
<td>EPICS C flow cytometer</td>
<td>Paraffin</td>
<td>Significantly better outcome was noted for previously untreated patients with diploid DNA content.</td>
<td>Wooldridge et al. 1988 (Nebraska, U.S.A.)</td>
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<td>Significantly better outcome was noted for previously untreated patients with low proliferative activity during 12 mo. follow-up. Of patients followed beyond 12-24 mo., those with high proliferative activity ($S + 62M &gt; 10%$) appeared to have the most durable remission (statistically not significant). In relapsing patients, low ($S + 62M$) correlated with longer survival.</td>
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<td>Acridine Orange</td>
<td>ICP-22 mercury-arc cytometer</td>
<td>Fresh biopsy material</td>
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<td>McLaughlin et al. 1988 (Texas, U.S.A.)</td>
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<tr>
<td>lymphoma</td>
<td>29 controls</td>
<td>Propidium iodide</td>
<td>EPICS C flow cytometer</td>
<td>EPICS C program disk software</td>
<td>Paraffin</td>
<td>Patients with and without DNA aneuploid tumors did not have significantly different tumor grade, response to therapy, or clinical outcome.</td>
<td>No significant differences were found in PI (S+62+M) or S-phase fraction of cases with and without response to initial treatment, and good and bad clinical outcomes.</td>
<td>Egerter et al. 1988 (Los Angeles, U.S.A.)</td>
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REFERENCES


