

# Quantitative analysis of non-Hodgkin's lymphoma

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**SUMMARY** A preliminary attempt has been made to characterise a small series of non-Hodgkin's lymphomas (NHL) by morphometric means using the Quantimet 720 and Kontron MOP/AMO<sub>3</sub> image analysis systems. In most cases it was found that the distribution of nuclear area and correlation between mean nuclear area and frequency per unit field, corresponded closely with tumour classification determined by light microscopy. These results suggest that it may be possible to devise an objective and reproducible grading system for NHL using quantitative morphometric techniques.

The multiplicity of classification systems for non-Hodgkin's lymphomas (NHL) manifests the difficulty experienced by most pathologists in defining the histological pattern of lymphoid tumours accurately and reproducibly. Although attempts have been made to refine lymphoma classification by study of the ultrastructural,<sup>1</sup> immunohistological<sup>2</sup> and surface membrane properties of tumour cells<sup>3</sup> these have failed so far to provide sufficient discrimination to supplant more conventional morphological assessments by light microscopy. An alternative approach, as yet little explored, concerns the use of image analysers for quantitative morphometric analysis of lymphoma cell populations. This possibility has recently been examined by Crocker and Curran<sup>4</sup> using a Zeiss Microvideomat to measure the mean nuclear diameter of cells in imprints from neoplastic and reactive lymph nodes. Although some discrimination was obtained between these conditions the television-based analyser employed provided poor definition of closely adjacent cells precluding its use for tissue sections.

We report here the preliminary results of an investigation in which we have used both a television-based image analyser (Quantimet 720) and a semiautomatic electronic planimeter (Kontron MOP/AMO<sub>3</sub>) to undertake the morphometric analysis of tissue sections from a small series of NHL. The results suggest that with these instruments it is possible to define a malignant lymphoma by its nuclear size distribution and frequency with only minor modifications to the routine preparation of histological sections.

## Material and methods

### CASES STUDIED

Lymph nodes from 10 patients with NHL were fixed in 10% neutral buffered formol saline and processed for embedding in hydroxyethylmethacrylate resin. Sections (1  $\mu$ m) were cut on a Leitz microtome using a steel knife and stained by Slidders' iron haematoxylin method.<sup>5</sup> The histological diagnosis in each case was established independently by each author using the British National Lymphoma Investigation classification system.<sup>6</sup> Four tumours were analysed using both the Quantimet 720 and Kontron MOP/AMO<sub>3</sub> system and 6 with the Kontron MOP/AMO<sub>3</sub> system alone. Preliminary studies showed that direct measurement of cell boundaries and cytoplasmic features was precluded by poor definition of these structures; nuclei, on the other hand, were readily delineated and could be used to characterise the tumour cell population.

### QUANTIMET 720 SYSTEM

With the Quantimet 720 system (Cambridge Scientific Instruments) the image generated by the light microscope is scanned by a television camera and features in the monochrome video-image separated according to their grey-scale level. A setting was chosen which detects the maximum nuclear detail without overflow into cytoplasmic features. The video-image was edited using a "light pen" to separate the fused images of adjacent nuclei and restore undetected nuclear detail. The total field and individual features were measured automatically and small non-nuclear particles excluded by setting a suitable particle size threshold.

#### KONTRON MOP/AMO<sub>3</sub> SYSTEM

With the Kontron MOP/AMO<sub>3</sub> system (Reichert-Jung) the digitising tablet was used to receive the projected image of the histological section. The boundaries of the microscope fields and cell nuclei were defined by the operator using an electromagnetic pen linked to the tablet through a co-ordinate-producing unit and microprocessor. Displacement of the pen around the features of the image was recorded electronically and interpreted as a measurement of area by a preset programme in the microprocessor.

#### MORPHOMETRIC ANALYSIS

For each tumour sample the area of the nuclear profile was measured in 200 to 400 cells and the number of nuclei determined in random microscope fields of uniform size. The mean value for nuclear area was plotted against the mean frequency of nuclei per unit field ( $\mu\text{m}^2 \times 10^4$ ) and 95% confidence limits calculated to describe the probable limits of the population.

Frequency histograms were constructed using the Johnson-Saltykov subtraction-correction procedure<sup>7</sup> which corrects for nuclear sections of submaximal size.

#### Results

##### HISTOLOGICAL CLASSIFICATION

Details of the cases studied are shown in the Table. Histologically, nine of the ten tumours had a diffuse growth pattern. Two were classified as well differen-

tiated lymphocytic, three poorly differentiated lymphocytic, two predominantly large undifferentiated cell and two mixed small lymphoid and large undifferentiated cell in type. The remaining tumour displayed a nodular growth pattern and was composed of small follicle centre cells.

#### MORPHOMETRIC ANALYSIS

##### *Nuclear size and frequency per unit field*

The results of plotting mean nuclear area against mean number of nuclei per unit field measured with the Kontron MOP/AMO<sub>3</sub> system are shown in Fig. 1. A spectrum of nuclear size and distribution is seen ranging from small crowded nuclei to larger well separated nuclei; reduction in frequency and increase in size of nuclei correspond to a loss of histological differentiation. Eight of the 10 cases studied can be distinguished with more than 95% confidence limits. Disparity between the morphometric and histological assessments is apparent for one of the well differentiated lymphocytic tumours and one of the poorly differentiated lymphocytic tumours. Different values for mean nuclear area and number of nuclei per unit field, with larger confidence limits and less clear cut tumour separations, were obtained with the Quantimet 720 system than with the Kontron MOP/AMO<sub>3</sub> system (Table).

##### *Nuclear size distribution*

The distribution of nuclear sizes for each tumour after correction by the Johnson-Saltykov procedure are shown in Fig. 2; the nuclear section group sizes

##### *Details of non-Hodgkin's lymphomas studied*

Case	Sex	Age at diagnosis (yr)	Survival (months)	Histological diagnosis	Mean number nuclei per 10 000 $\mu\text{m}^2$		Mean nuclear area ( $\mu\text{m}^2$ )	
					Kontron MOP/AMO <sub>3</sub>	Quantimet 720	Kontron MOP/AMO <sub>3</sub>	Quantimet 720
1	M	49	> 48	Diffuse, well-differentiated lymphocytic lymphoma	257.5	—	9.5	—
2	F	70	11	Diffuse, well-differentiated lymphocytic lymphoma	142.8	141.5	21.1	24.8
3	F	73	> 36	Nodular, small follicle centre cell lymphoma	141.2	—	13.2	—
4	M	70	1	Diffuse, mixed small lymphoid and undifferentiated large cell lymphoma	160.6	—	16.4	—
5	M	55	35	Diffuse, mixed small lymphoid and undifferentiated large cell lymphoma	123.9	—	15.7	—
6	F	64	23	Diffuse, poorly-differentiated lymphocytic lymphoma	150.5	—	16.3	—
7	M	15	> 48	Diffuse, poorly-differentiated lymphocytic lymphoma	133.8	146.4	30.7	32.4
8	F	54	> 60	Diffuse, poorly-differentiated lymphocytic lymphoma	110.5	132.2	24.8	28.6
9	F	71	22	Diffuse, undifferentiated large cell lymphoma	88.1	—	27.2	—
10	M	15	8	Diffuse, undifferentiated large cell lymphoma	39.2	44.8	39.1	36.6

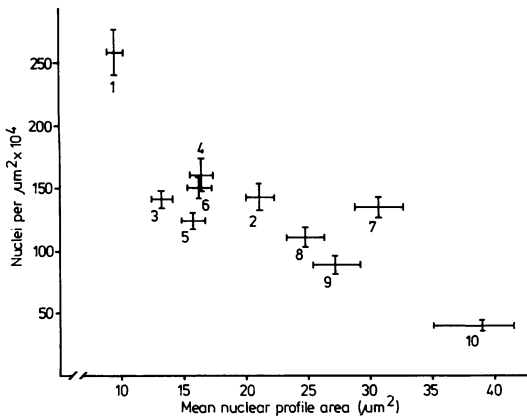


Fig. 1 Relation of mean nuclear profile area and number of nuclei per unit field in 10 cases of non-Hodgkin's lymphoma as measured by Kontron MOP/AMO<sub>3</sub> system. 95% confidence limits for nuclear area calculated from log-values. Histological diagnoses are listed in the Table.

in the undifferentiated and two of the poorly differentiated lymphocytic tumours is readily apparent but there is a wide scatter of nuclear sizes in the remaining poorly differentiated lymphocytic lymphoma which contains proportions of nuclei of size groups 8 and 9 comparable to the two mixed tumours and in excess of those found in the nodular small follicle centre cell lymphoma.

**Discussion**

Our results show that it is feasible to undertake morphometric analysis of tissue sections from lymphoid tumours either with an electronic planimeter like the Kontron MOP/AMO<sub>3</sub> or a television-based image analyser such as the Quantimet 720. In eight of the 10 cases studied it was possible to separate tumours by nuclear size distribution and frequency and the sequencing order corresponded closely with the histological grading determined by light microscopy. However, in two instances the results were at variance and it is of interest that the

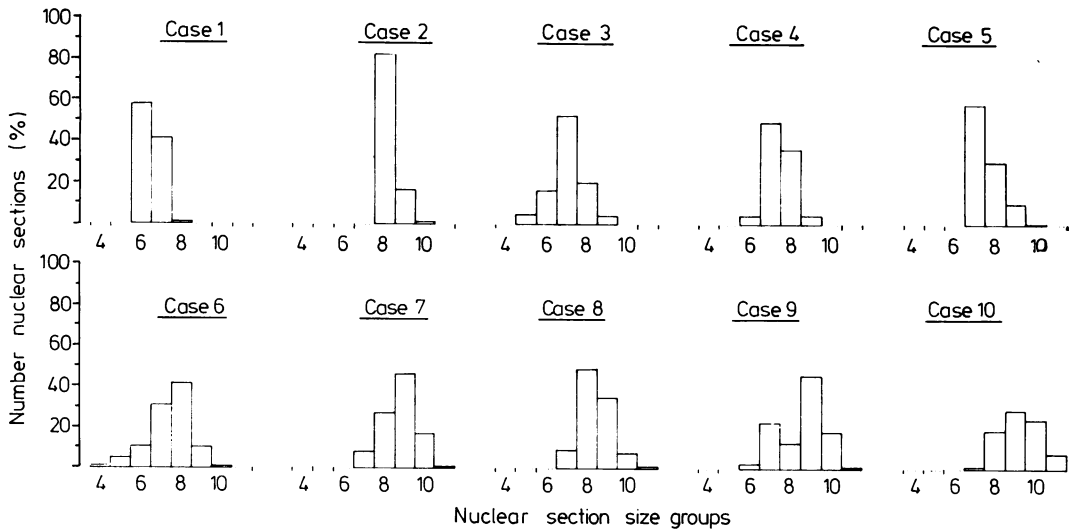


Fig. 2 Nuclear size distribution measured by Kontron MOP/AMO<sub>3</sub> in 10 cases of non-Hodgkin's lymphoma. Histological diagnoses are listed in the Table. Nuclear section group sizes are expressed according to Saltykov's first working table<sup>7</sup> which is based on a linear-logarithmic scale of nuclear diameter.

are expressed according to Saltykov's first working table<sup>7</sup> which is based on a linear-logarithmic scale of nuclear diameter. Small nuclei predominate in one of the well differentiated lymphomas but in the other there is again disparity between the morphometric and histological assessments with morphometric analysis revealing larger nuclear sizes than estimated by light microscopy. The high proportion of large nuclei (nuclear profile size groups 9, 10, 11)

patient with the apparently well differentiated lymphocytic tumour, where morphometric analysis revealed a larger mean nuclear size than estimated by light microscopy, succumbed within a year to an aggressive widespread lymphoma.

In contrast to our findings, Crocker and Curran<sup>4</sup> were unable to relate the mean diameter of neoplastic lymphoid cells to specific subtypes of NHL. However, their studies were conducted with imprints

of unfixed lymph nodes whereas we employed fixed and processed tissues and the discrepancy between our findings may result from differences in the method of sample preparation.

The preparation of thin plastic sections and a high-contrast nuclear stain are relatively minor modifications to the procedures normally employed for routine histological analysis of lymphomas. However, these thin sections only partially solved the problem of defining boundary features in the image detected by the television-based image analyser (Quantimet 720). Indeed we found that considerable electronic image-editing was necessary to separate fused nuclear images, ensure the inclusion of undetected nuclear features and delete all non-nuclear material. This may explain the discrepancy in the results obtained with the two systems. Where the analysis system relies on the operator's manual definition of the feature under study, as in the Kontron MOP/AMO<sub>3</sub> system, morphometric data is more readily and reliably obtained. This type of instrument also has the virtue of being considerably less expensive.

The limited information available from this preliminary study precludes the accurate definition of morphometric subtypes of NHL by nuclear size distribution or frequency per unit field. However, the results are sufficiently encouraging to suggest that with a larger series of cases arbitrary gradings could be devised for classification purposes. It is suggested that the morphometric approach to the study of lymphomas offers the prospect of a more reproducible and objective method of grading these tumours than is currently possible by conventional

histological techniques.

We are indebted to Cambridge Scientific Instruments (Quantimet 720) and Reichert-Jung (Kontron MOP/AMO<sub>3</sub>) for making their image analysis systems available to us. We are also grateful to Mr D Loney, Department of Pathology, and to Mr R Edwards, Department of Statistics, University of Leeds for technical advice and comments on the statistical analysis of data. CCB was supported by a grant from the Yorkshire Cancer Research Campaign.

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