Study of nuclear diameters in non-Hodgkin's lymphomas

J CROCKER, EL JONES, RC CURRAN

From the Department of Pathology, The Medical School, University of Birmingham, Edgbaston, Birmingham B15 2TJ

SUMMARY The mean maximum nuclear diameter ($D_{\max}$) in 21 cases of non-Hodgkin's lymphoma (NHL) has been determined, using the Reichert-Jung (Kontron) MOP-AMO₃ user-controlled image analyser. Nuclear diameters of high-grade malignancy NHL were found to be considerably greater than those of low-grade malignancy lymphomas, although there was some overlap of their ranges. These findings confirm objectively subjective estimates of nuclear size in NHL. The relative usefulness of the user-controlled (interactive) image analyser for the measurement of nuclei in tissue sections is compared with that of a fully automatic machine.

The assessment of nuclear morphology and size are of importance in the histological diagnosis of malignant lymphomas, especially non-Hodgkin's lymphomas (NHL).¹ Accuracy in the typing of NHL is of considerable value in the prognosis and management of these diseases and any procedure which improves the accuracy is useful. In the past assessment of nuclear size in lymphomas have generally been subjective but Crocker and Curran,² with the aid of a fully automatic image analyser, measured mean nuclear diameters in reactive lymph nodes, palatine tonsils, NHL and Hodgkin's disease (HD). This approach has now been applied on a wider scale to NHL using a user-controlled (interactive) image analyser, the Reichert-Jung (Kontron) MOP-AMO₃.

Material and methods

LYMPH NODES

Twenty-one lymph nodes were examined, from the same number of patients. These were histologically diagnosed and typed according to the Kiel classification¹ prior to measurement of nuclear diameters. All measurements were performed "blind", the specimens being coded by numbers. There were six centrocytic, nine centrocytic-centroblastic, two centroblastic, and four immunoblastic lymphomas (Table).

FIXATION AND STAINING

The lymph nodes were obtained directly after

removal and cut into slices (approximately 2 mm thick) with a degreased razor blade. Slices from the specimens were fixed for 24 h in 10% formol-saline solution. The slices were then processed to paraffin wax, sectioned at 4 μ m, stained by Harris's haematoxylin and eosin (HE), dehydrated, cleared and mounted in balsam.

NUCLEAR DIAMETER MEASUREMENT

The use of the MOP-AMO₃ has been described previously.³ The instrument was used with a camera lucida drawing tube and microscope above a graphic tablet. Measurements were made by drawing around the optical image of each nucleus. Five hundred nuclei were measured in each specimen. For accuracy in outlining each nucleus, the sections were examined with a 100 $\times$ oil-immersion lens, and consecutive fields were studied. Only lymphoid cell nuclei were measured—that is, endothelial cells, fibroblasts and histiocytes were excluded. A simple eyepiece grid was used to ensure that nuclei were not measured more than once.

Fields were selected at random, the first field examined being the first to fall under the objective lens when the slide was placed on the microscope stage. Adjacent fields were then examined, moving from left to right.

When the section was from a follicle centre cell lymphoma (especially the centrocytic-centroblastic type) or from a case of follicular hyperplasia, care was taken to include nuclei from the follicles and interfollicular areas in the measurement process, in approximately equal proportions. As with diffuse

Type of NHL and corrected mean D_{max}

Type of NHL	First measurement		Repeat measurement	
	Corrected mean D_{max} (μm)	Standard error of mean	Corrected mean D_{max} (μm)	Standard error of mean
Centrocytic	11.6	0.10	11.8	0.11
"	13.6	0.12	13.2	0.10
"	13.0	0.13	13.2	0.13
"	14.2	0.10	14.2	0.12
"	12.0	0.08	11.8	0.09
"	13.2	0.11	13.6	0.12
Centrocytic-centroblastic	12.0	0.11	12.2	0.11
"	14.7	0.16	14.5	0.14
"	12.2	0.10	12.2	0.12
"	13.2	0.08	13.0	0.10
"	14.1	0.14	13.8	0.13
"	12.0	0.08	12.4	0.08
"	14.4	0.13	14.1	0.14
"	14.1	0.14	13.9	0.13
"	16.1	0.12	15.8	0.13
Centroblastic	15.5	0.13	15.7	0.13
"	16.3	0.17	16.0	0.15
Immunoblastic	25.2	0.12	25.0	0.13
"	18.2	0.16	17.9	0.14
"	20.1	0.20	20.3	0.19
"	22.3	0.13	22.5	0.13

lymphomas, the follicular and interfollicular fields were selected randomly.

At the end of the measurement sequence, the data were printed as a histogram by the microprocessor unit, together with a median and mean value of the D_{max} . The microprocessor was programmed to exclude nuclei with a D_{max} of $< 5 \mu\text{m}$; and it printed 40 size classes with a class interval of $0.5 \mu\text{m}$. Correction for section thickness effect was performed with the use of Weibel curves as described by Steer.⁴ As the MOP-AMO₃ was programmed so that features less than $5 \mu\text{m}$ in diameter were not included in the measurement procedure, the spherical truncation effect was reduced; nonetheless a correction factor of $1.1 \times$ the measured data was calculated, assuming a minimum measurable nuclear diameter of $5 \mu\text{m}$ and a maximum nuclear diameter in the range of $20 \mu\text{m}$. The entire procedure was repeated for each specimen in order to check the reproducibility of the method.

In addition, the corrected data were mainly plotted on probability graph paper as a cumulative percentage of the number of nuclei for each D_{max} class against, on the x-axis, the D_{max} , according to the method described by Berry.⁵ This was performed to demonstrate different populations of cells by virtue of their nuclear D_{max} ranges.

Results

The mean D_{max} for each of the lymph nodes studied is shown in Fig. 1. All 21 specimens proved suitable

Fig. 1 The mean D_{max} for each specimen, with Kiel subtype.

for nuclear sizing and cell overlap did not cause problems. Low-grade malignancy NHL cases range from $11.6 \mu\text{m}$ to $16.1 \mu\text{m}$ in mean D_{max} while high-grade malignancy have a mean D_{max} ranging from $15.5 \mu\text{m}$ to $25.2 \mu\text{m}$.

The Table shows the results of repeating the procedure for each specimen; there is excellent reproducibility.

Centrocytic lymphomas tend to have a lower mean D_{max} than centrocytic-centroblastic NHL, which in turn have a lower mean D_{max} than centroblastic types. Immunoblastic lymphomas have the highest mean D_{max} , with a wide range of values.

Typical histograms of nuclear D_{max} distribution for a low-grade and a high-grade lymphoma are shown in Fig. 2.

The cumulative probability plots for each specimen (Fig. 3) show various positions along the x-axis, but all plots have in common a non-linearity: for

Fig. 2 Two examples of the histogram plotted from the print-out from the MOP-AMO₃. The upper histogram shows the $D_{\max}$ distribution for a centrocytic-centroblastic (low-grade) lymphoma; the lower for an immunoblastic lymphoma. The broken vertical line represents the value for the mean $D_{\max}$.

each specimen it is possible to project two lines along the data. This implies that two populations of cells exist (as assessed by $D_{\max}$) in all 21 specimens. High-grade lymphomas tend to have probability plots which lie in a higher $D_{\max}$ range than those for low-grade NHL. In addition, although the low-grade NHL specimens have a small population of large nuclei, high-grade NHL specimens have a greater content of even larger nuclei.

Discussion

The appearance and size of nuclei in NHL are of diagnostic importance. However as in other areas of histopathology these features are usually assessed subjectively. Cytophotometric measurement of nuclear DNA content has been used diagnostically in mycosis fungoides,⁶ as has the degree of nuclear convolution.⁷ In the latter study a semiautomatic (interactive) image analyser was used with electron

micrographs of isolated cells from mycosis fungoides and Sézary's syndrome.

Crocker and Curran² used a fully automatic image-analyser, the Zeiss Microvideomat, in a study of nuclear diameters in NHL, HD, reactive lymph nodes and palatine tonsils. The data resulting from this investigation showed differing ranges of nuclear diameters in these tissues and were considered to be of potential diagnostic value, particularly in the differentiation of reactive lymph nodes from those involved by NHL. However, a limitation of the technique was the necessity for fresh tissue from which to produce cell imprints, since the Microvideomat proved unable to measure adjacent or overlapping nuclei in conventional tissue sections. A method was therefore sought that would allow us to measure cell nuclei in sections of lymphomas, and the answer was provided by the user-controlled (interactive) image analyser distributed by Reichert-Jung (Kontron), the MOP-AMO₃. With this machine the user draws around features of interest (for example, the image formed by a camera lucida drawing tube) with a sensitive pen. The information is fed into a microprocessor which can produce various data such as maximum diameter, shape factor, perimeter or area. Alternatively the machine can be used as a rapid and convenient means of counting cells.³ The limitations of certain fully automatic image-analysers are therefore overcome, as the user can draw around selected features. Hence adjacent or overlapping nuclei in tissue sections can be sized with ease and considerable accuracy.

Using the MOP-AMO₃ we have examined 21 cases of NHL from the same number of patients. These were classified according to the Kiel convention.⁴ Apart from some overlap of mean $D_{\max}$ values between centrocytic-centroblastic and centroblastic NHL, the mean $D_{\max}$ for cases of high-grade NHL proved to be considerably greater than that for low-grade NHL. This confirmed the subjective view that the nuclei of high-grade lymphomas are larger than those of low-grade types. The $D_{\max}$ for immunoblastic lymphomas may be so large as to warrant the term "giant."

When we used the Zeiss Microvideomat to measure nuclei in cell imprints, two of 36 specimens proved uncountable. With the MOP-AMO₃ however, there were no such difficulties and all specimens could be measured with ease.

Section thickness is an important factor in the measurement of spherical or approximately spherical structures such as cell nuclei. Our sections were therefore cut at a standard thickness of 4 μm in order to reduce the effect of the nuclear cross-sections being non-equatorial. $D_{\max}$ values less than

Fig. 3 Cumulative percent lines plotted on probability paper. (a) shows high-grade NHL; the open dots represent immunoblastic NHL and the solid dots represent centroblastic NHL. (b) shows low-grade NHL; the open squares represent centrocytic-centroblastic NHL and the solid squares represent centrocytic NHL.

5 μm were rejected. Nonetheless a correction factor of $1.1 \times$ each measurement was necessary to overcome the spherical truncation effect. In addition all sizing procedures were repeated, with excellent reproducibility.

The findings from the probability-curves based on cumulative D_{max} data are interesting in that they demonstrate two populations of cells, by virtue of their D_{max} , for each specimen. In all specimens the majority of cells have a continuous range of nuclear D_{max} values, with a separate smaller range of larger-sized nuclei. Therefore, this technique demonstrates a small population of cells with larger nuclei, forming a separate population from the majority. This could be readily accounted for, for example, in centroblastic lymphomas by the presence of a small number of immunoblasts.

Similarly, in immunoblastic NHL there is often a small number of giant immunoblasts present. In the low-grade lymphomas the cellular populations often appear to divide, on cumulative probability curves, nearer to the middle of the overall range for D_{max} , indicating more equal numbers of small and large nuclei.

In conclusion therefore, we have found that the measurement of D_{max} in NHL has been greatly facilitated by the MOP-AMO₃, and our results suggest that the D_{max} is a useful additional feature to the diagnosis of NHL types. It would be interesting to measure other parameters, such as nuclear area and shape factor in NHL.

We are grateful to Mrs Mary Williams and Mr J Gregory for expert technical assistance and to Miss Angela Wright for preparing the manuscript. The camera lucida was provided by the Royal Society.

References

- 1 Lennert K. *Malignant lymphomas other than Hodgkin's disease*. New York: Springer-Verlag, 1978.
- 2 Crocker J, Curran RC. A study of nuclear diameters in lymph node imprints using the Zeiss Microvideomat. *J Clin Pathol* 1979;32:670-4.
- 3 Crocker J, Curran RC. A quantitative study of the immunoglobulin-containing cells in trephine samples of bone marrow. *J Clin Pathol* 1981;34:1080-2.
- 4 Steer MW. *Understanding cell structure*. Cambridge: Cambridge University Press, 1981.

- ⁵ Berry RE. Normal values in pathology. In: Sommers SC, ed. *Pathology Annual*. London: Butterworths, 1969;4:71–88.
- ⁶ Van Vloten WA, Van Duijn P, Schaberg A. Cytodiagnostic use of Feulgen-DNA measurements in cell imprints from the skin of patients with mycosis fungoides. *Br J Dermatol* 1974;91:365–71.
- ⁷ Van der Loo EM, Cornelisse CJ, Van Vloten WA, Van der Velde EA, Scheffer E, Meijer CJLM. Diagnostic morphometry of

isolated lymph node cells from patients with mycosis fungoides and Sézary's syndrome. *Virchows Archiv B* 1980;33:107–16.

Requests for reprints to: Dr J Crocker, Department of Pathology, The Medical School, Birmingham B15 2TJ, England.