Study of nuclear diameters in non-Hodgkin’s lymphomas

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SUMMARY The mean maximum nuclear diameter \( (D_{\text{max}}) \) in 21 cases of non-Hodgkin’s lymphoma (NHL) has been determined, using the Reichert-Jung (Kontron) MOP-AMO\(_3\) 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-AMO\(_3\).

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 classification\(^1\) 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 \(10\%\) formal saline solution. The slices were then processed to paraffin wax, sectioned at \(4\, \mu\text{m}\), stained by Harris’s haematoxylin and eosin (HE), dehydrated, cleared and mounted in balsam.

NUCLEAR DIAMETER MEASUREMENT

The use of the MOP-AMO\(_3\) 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
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 $D_{\text{max}}$. The microprocessor was programmed to exclude nuclei with a $D_{\text{max}}$ of < 5 $\mu$m; and it printed 40 size classes with a class interval of 0.5 $\mu$m. Correction for section thickness effect was performed with the use of Weibel curves as described by Steer. As the MOP-AMO$_3$ was programmed so that features less than 5 $\mu$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 $\mu$m and a maximum nuclear diameter in the range of 20 $\mu$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 $D_{\text{max}}$ class against, on the x-axis, the $D_{\text{max}}$, according to the method described by Berry. This was performed to demonstrate different populations of cells by virtue of their nuclear $D_{\text{max}}$ ranges.

**Results**

The mean $D_{\text{max}}$ for each of the lymph nodes studied is shown in Fig. 1. All 21 specimens proved suitable for nuclear sizing and cell overlap did not cause problems. Low-grade malignancy NHL cases range from 11.6 $\mu$m to 16.1 $\mu$m in mean $D_{\text{max}}$ while high-grade malignancy have a mean $D_{\text{max}}$ ranging from 15.5 $\mu$m to 25.2 $\mu$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 $D_{\text{max}}$ than centrocytic-centroblastic NHL, which in turn have a lower mean $D_{\text{max}}$ than centroblastic types. Immunoblastic lymphomas have the highest mean $D_{\text{max}}$ with a wide range of values.

**Fig. 1** The mean $D_{\text{max}}$ for each specimen, with Kiel subtype.
The data shows the mean 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.

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, as has the degree of nuclear convolution. 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 Curran 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. 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. 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
5 μm were rejected. Nonetheless a correction factor of 1-1 × 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-AMO, 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.

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References

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