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Morphometric Characterization of Small Cell Lymphocytic Lymphoma

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ABSTRACT
The morphometry in histopathology is used to characterize cell populations belonging to different tissues and to identify differences in their parameters with prognostic implications. To achieve morphometric examination were selected 6 of 24 cases identified as small cell lymphocytic lymphoma. For each case analysis was done on five fields, for each field measuring the parameters of 20 cells. The studied parameters were for cytoplasm: cytoplasmic area, maximum and minimum cytoplasmic diameter, cytoplasmic perimeter; for nucleus were measured: nuclear area, minimum and maximum nuclear diameter, nuclear perimeter, nuclear contour index, nuclear ellipticity index, nuclear irregularity index. Also the nucleo-cytoplasmic ratio was calculated in all studied cases. Small cell lymphocytic lymphoma is characterized in morphometric terms having a small cytoplasmic area (average 29.206) and also a small nuclear area (mean 28.939) having a nucleo-cytoplasmic ratio appearance suggestive for adult lymphocyte. A nuclear contour index small value (3.946), ellipticity index value also small (3.521) and small nuclear irregularity index (3.965). Standard deviations, in any of the studied morphometric categories, is around or below 1 suggesting monomorphic cell appearance. These morphometric and microscopic features characterized mainly by a small population of adult lymphocytes, monomorphic, with rounded hipercromic nuclei, dense chromatin, support the framing into indolent lymphoma group in terms of clinical outcome.

Keywords: lymphoma, morphometry, histopathology

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Introduction

The morphometry in histopathology field is used to characterize cell populations belonging to different tissues and to identify differences in their parameters with prognostic implications. The morphometry may be used in the development of automated diagnostic programs.

Non-Hodgkin lymphomas are a heterogeneous group so morphological and immunological differentiation into subclasses is important for patient outcomes. Differentiating lymphomas starts from the microscopic appearance continue with special stains and immunohistochemical analysis and in the end morphometric evaluation.

Small lymphocytic lymphoma represents 14.8% of all lymphoid neoplasms and approximately 20% of

B cell malignant lymphomas. The overall incidence is 5.17 per 100,000 people/year. The condition is more common in Caucasians than in African Americans and is very rare in Asians. It is more common in men than in women (WHO classification ratio male: female is about 2: 1). Small lymphocytic lymphoma is a malignancy of adults, the incidence peak in the sixth and seventh decade of life. It is unusual in patients younger than 40 years. After age 40, presents a linear increase in the incidence [1,2,3].

Material and methods

To achieve morphometric examination were selected 6 of 24 cases identified as small cell lymphocytic lymphoma. Cases selected for morphometry were reviewed and representative regions were selected for study in each case. For each case analysis was done on five fields, for each field measuring the parameters of 20 cells, so morphometric measurements were made on 100 cells for each case. Were selected for measurement cell with clear nuclear outlines. The studied parameters were for cytoplasm: cytoplasmic area, maximum and minimum cytoplasmic diameter, cytoplasmic perimeter; for nucleus were measured: nuclear area, minimum and maximum nuclear diameter, nuclear perimeter, nuclear contour index, nuclear ellipticity index, nuclear irregularity index. Also the nucleo-cytoplasmic ratio was calculated in all studied cases. Nuclear indices were calculated from the measured nuclear area and perimeter. Elevated nuclear contour index shows the deviation from a circle, the other two indices, the ellipticity and irregularity, provides detailed data on the shape of the nucleus. Increasing irregularity index values above 3.54 reflect the existence of the more irregular nuclei as the index value is greater.

Getting morphometric data by measuring cellular parameters mentioned above was made using a Nikon E600 microscope equipped with Nikon DN100 camera for viewing microscopic slides chosen for evaluation, LuciaNet software running on a computer with Pentium processor and Windows operating system to obtain values parameters. Data obtained from the measurements were processed by Microsoft Excel calculating the average and standard deviation for each case.

Results and discussion:

In literature, small lymphocytic lymphoma is described as having lymph node architecture totally or partially replaced by lymphoma cells. Neoplasms usually affects lymph follicles and sinuses extending beyond the capsule fat. The growth is diffuse, dim, with imprecisely defined nodular areas, known as proliferation centers, pseudofollicular centers or pseudofolliculs identified in tumor cells diffusely distributed areas [1,4].

Microscopic pattern identified in the 6 cases was varied, the most common type of microscopic appearance was the destroyed follicular lymphoid tissue structure, with marked proliferation of small adult lymphocytes rounded nuclei, monomorphic, with nuclear hyperchromasia and dense chromatin, tahychromasia, reduced cytoplasm (Figures 1-3).

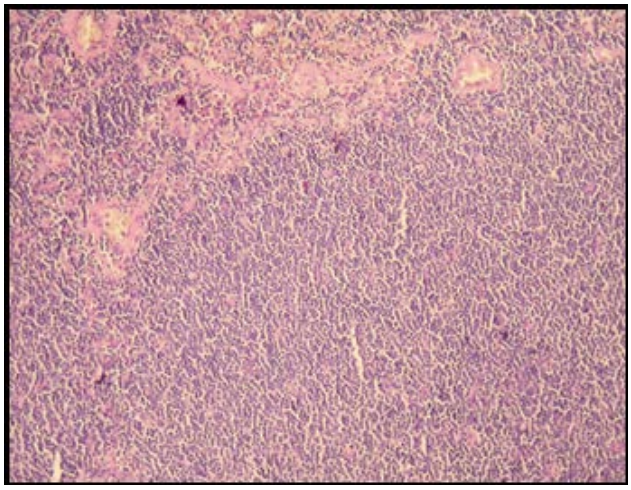

Figure 1 - Microscopic appearance of small cell lymphocytic lymphoma (HE x 10)

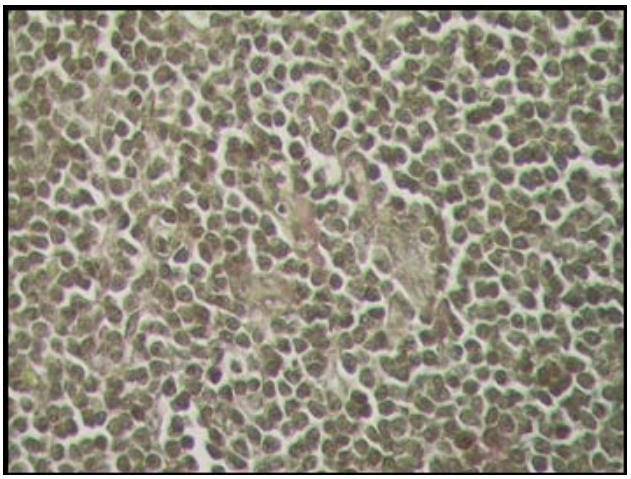

Figure 2 - Microscopic appearance of small cell lymphocytic lymphoma (VG x 40)

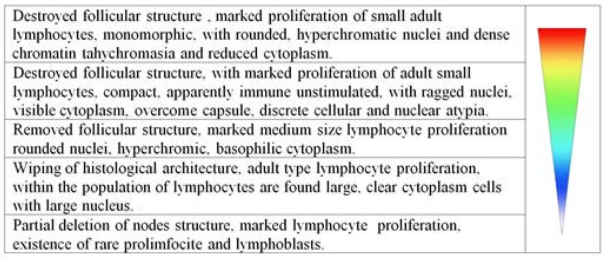

Figure 3 - Microscopic aspects of small cell lymphocytic lymphoma

This is highlighted by morphometric measurements, nuclear irregularity index with maximum value of 4.07 and mean 3.96 ± 0.02 emphasizing regular appearance of nuclei.

Cytoplasmic area in small cell lymphocytic lymphoma cases varied within a fairly narrow range of values between 27 and 31.67µ and also average cytoplasmic areas in each case vary over a narrow range of values between 28.458µ and 29.468µ (Figure 4). Standard deviation for cytoplasmic area of each case shows a uniform population of cells. Standard deviation values were in the range 1.53 - 1.83 (Table I). According to international morphometry studies carried out in recent years, values of between 27µ and 32µ cell area correspond to small cell lymphomas [5]. Cytoplasmic areas obtained by morphometric measurements made on cases of this study fall within this range confirming the microscopic appearance on histopathological examinations.

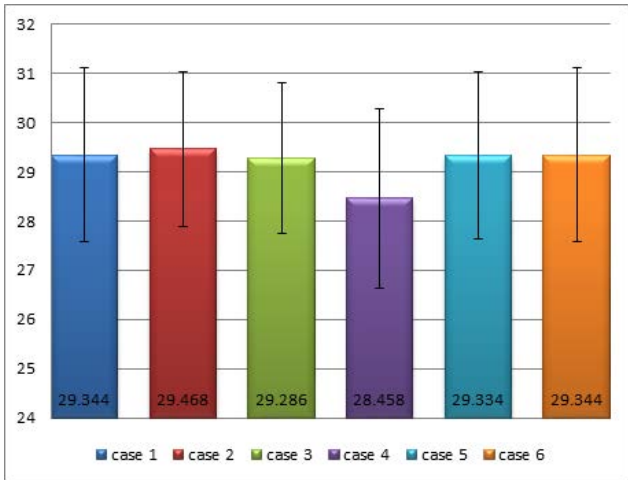

Figure 4 - Mean cytoplasmic area in small cell lymphocytic lymphoma cases

Morphometric measurements of nuclear areas highlights the trend, framing in a narrow range that has characterized also cytoplasmic areas values. Thus, nuclear area has values in the range of 25.69 - 32.20µ, and the average value of nuclear areas are between 27.93µ and 30.09µ (Figure 5).

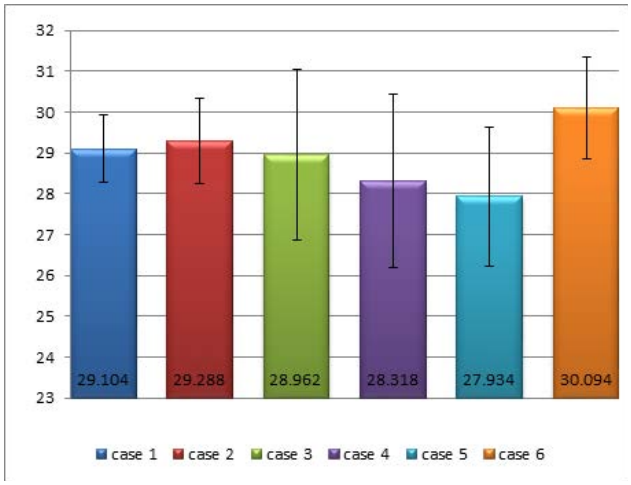

Figure 5 - The average nuclear area in small cell lymphocytic lymphoma cases

The standard deviation for the average nuclear areas meet the general trend of the measured parameter being within the range of 0.82 to 2.13 with an average of 1.6 (Table I). International

studies previously conducted nuclear areas for small cell lymphoma were within the range 16.7 - 21.5μ, averaging 19μ, whereas in this study nuclear area has an average 28.93μ hovering above the upper limit obtained previously, showing a cell population with relatively large nuclei compared to cytoplasm [6].

Table I - Averages morphometric parameters measured in small cell lymphocytic lymphoma cases

	Overall average	Overall SD	The minimum	The maximum
cytoplasmic area	29.206	1.585	27	31.67
nuclear area	28.939	1.608	25.69	32.2
nuclear perimeter	21.205	0.405	20.42	21.84
Nuclear shape index	3.946	0.124	3.73	4.27
ellipticity index	3.521	0.127	3.29	3.86
nuclear irregularity index	3.965	0.022	3.91	4.07
nucleo-cytoplasmic ratio	0.992	0.043	0.928	1.071

Nucleo-cytoplasmic ratio is relatively constant, with an average of 0.992 and a standard deviation of 0.004 showing monomorphic, cohesive microscopic appearance of lymphocyte in small cell lymphocytic lymphoma confirming the microscopic aspects described.

Morphometric nuclear contour index considers the degree of indentation of the nucleus being a parameter dependent on the perimeter and nuclear area. The higher nuclear contour index, the obvious nuclear indentations (have greater depth and lower angle). Although we expected that small cell lymphocytic lymphoma have small nuclear contour index because microscopic described as comprising lymphocyte cells of adult type, without cleavage and visible indentations, its nuclear contour index has medium high values compared with the data obtained for other types of lymphoma (3.946) [7].

Ellipticity index is a parameter measured by morphometry who appreciate nucleus form depending on its axes, considering it to an ellipse, offers data closer to reality on nucleus shape and size, being in fact an improved version of the nuclear contour index, and can differentiate an irregular nucleus in the true sense, from a regular but elliptical nucleus. The higher nuclear ellipticity index values, the nucleus is more elongated [8]. Lymphocytic lymphoma cell has a narrow ellipticity index (3.521), suggesting round nuclei.

Nuclear irregularity index is calculated by adding a constant (3.54), what is the value of the ellipticity of a circle having a radius equal to unit, to substraction of nuclear contour index and the ellipticity index. In international literature nuclear irregularity indices values below 3.79 designate regular cell outline and values over 4.29 designate cleaved cells with irregular contour. Nuclear index irregularity is also an embodiment of the nuclear shape index much more accurate to characterize the shape of the nucleus [6,8]. Small cell lymphocytic lymphoma has nuclear irregularity index values close to the threshold value of 3.79 (3.965). According to the international literature small lymphocytic lymphoma has nuclear irregularity index values comprised within the interval corresponding to the cases with regular shape, round or oval lymphocytes, corresponding to a higher nuclear contour index and ellipticity index [5,8].

Conclusions

Small cell lymphocytic lymphoma is characterized in morphometric terms having a small cytoplasmic area (average 29.206) and also a small nuclear area (mean 28.939) having a nucleo-cytoplasmic ratio appearance suggestive for adult lymphocyte. A nuclear contour index small value, ellipticity index value also small and small nuclear irregularity index. Standard deviations, in any of the

studied morphometric categories, is around or below 1 suggesting monomorphic cell appearance. These morphometric and microscopic features characterized mainly by a small population of adult lymphocytes, monomorphic, with rounded hipercromic nuclei, dense chromatin, support the framing into indolent lymphoma group in terms of clinical outcome.

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