Quantitative versus qualitative in the analysis of cervical squamous cell carcinoma

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Abstract

Our work aimed to reveal and explore connections between the cellular morphometric features and the differentiation degree of cervical squamous cell carcinoma. Six cases were studied, for which biopsy was performed before the radiation treatment. The microscopic exam diagnosed two cases of well-differentiated squamous cell carcinoma and four cases of moderately differentiated squamous cell carcinoma. The morphometric technique was developed in the Zeiss KS400 software environment, by implementing a script, which allowed measuring the cytoplasmic and nuclear areas and calculating the nucleo-cytoplasmic ratios. For each studied case, characterized by a number of 500-measured cells (randomly selected), the paper presents the mean, minimum and maximum values of the two types of areas, as well as the histograms of the nucleo-cytoplasmic ratios. These numerical results showed that the quantitative approach could play an important role in enlarging the perspectives on the evaluation of the differentiation degree in invasive squamous cell carcinoma. Special attention was paid to the nucleo-cytoplasmic ratios, since we supposed they are able to provide enough information for quantifying several levels in the differentiation of the cells. Although we have obtained encouraging results, our current work cannot be regarded as fully confirming this assumption, because of the small number of cases. By involving larger groups of patients in our further researches, we expect to get a solid confirmation, and, thus, to support the refinements of the radiotherapy schemes.

Keywords: cervical carcinoma, nucleo-cytoplasmic ratio, computer-aided morphometry, radiotherapy.

Introduction

Cervical cancer is the second most common cancer among women worldwide, with almost half a million new cases each year [1]. Therefore, the increasing of the treatment efficiency and the introduction of certain prognostic criteria formulated in quantitative terms represent major problems for specialists.

For the cases diagnosed in initial stages, current therapy modalities are effective, but for the advanced cases, there still are controversies on the best schemes of treatment. However, the majority of the specialists consider that the elective attitude is radiotherapy, followed by surgery [2-4].

Regarding the prognostic, the traditional histopathological diagnosis benefit from the contribution of the data obtained through morphometric investigations. This technique is sustained in the last decade through the development of certain software specialized instruments, which grounds imaging analysis assisted by computer, with applicability in numerous types of pathology [5].

For the uterine cervix carcinoma, the reported morphometric researches mainly refer to evaluations at cellular level, based on either biopsy fragments [6-9] or cervical smears [10-16].

Our concerns focused on computerized morphometric studies of the effects of radiotherapy upon neoplastic lesions.

In the literature we did not find articles approaching the morphometric characterization of the cervical carcinoma, pre- and postradiotherapy, that is why our prime interest was the evaluation of the tumoral volume percentage in those two situations [17].

The purpose of the current paper is to develop an analysis at cellular level, based on the nucleo-cytoplasmic ratio, in cervical squamous cell carcinomas. The knowledge of this ratio pre-radiation is important because values close to 1 show that the tumor is more undifferentiated and, implicitly, more sensitive to radiotherapy [2, 3].

Thus, we aim to investigate the existence of a correlation between the cellular morphometric features (such as cytoplasmic area, nuclear area, nucleo-cytoplasmic ratio) and the differentiation degree of carcinoma (including its type).

Material and methods

There were investigated six cases of uterine cervical carcinoma, for which biopsy was performed before the radiation treatment. The specimens were routinely processed for the histopathological examination.

Computed-aided morphometric analysis was performed in Zeiss KS400 software environment available at the Histology Department, University of Medicine and Pharmacy "Gr. T. Popa" Iassy.

The material was represented by digital images of the relevant microscopic fields selected from each case. The digital images were acquired from the specimens with a video camera and stored on a PC compatible machine.

We have designed and implemented a Zeiss KS400 script, named CELL, which extracts the morphometric features. The programming technique used in developing the CELL code relies on our previous experience in the digital processing of histopathological images [17, 18].

From the morphometric point of view, **each case** was considered as characterized through a number of 500 cells, interactively selected from several images by the help of the Zeiss KS400 graphic editor.

On **each image**, the contours of the cells and nuclei were drawn during two successive stages, so as to permit the construction of two binary mask-images, one for cells, and the other for nuclei.

The boolean operation XOR applied to these two binary images leads to the binary mask-image for the cytoplasm regions.

The binary mask-images for cytoplasm regions and nuclei are further used to measure the cytoplasmic areas (*CitArea* vector) and the nuclear areas (*NcArea* vector), as well as to compute the nucleo-cytoplasmic ratio (*NcRatio* vector, $NcRatio(i) = \frac{NcArea(i)}{CitArea(i)}$), where i counts the cells selected on the processed image.

The results of the measurements performed on all the images pertaining to a case are stored in a database, and the final size of the vectors *CitArea*, *NcArea*, *NcRatio* is 500 (i.e. the total numbers of cells per case).

After the complete exploration of the six considered cases, six different databases are constructed.

The information from the databases is accessible for numerical computations and graphical plots.

Results and discussions

The six cases (below identified from case 1 to case 6) were histopathological diagnosed as follows: (i) moderately differentiated nonkeratinizing squamous cell carcinoma (cases 1-4) and (ii) well-differentiated keratinizing squamous cell carcinoma (cases 5, 6).

The operation principles of the CELL script are illustrated by the help of Figures 1-3. Figure 1 reproduces an image pertaining to case 6, on which the cell and nuclear contours are drawn in green and blue, respectively.

Figures 2 and 3 reproduce the binary mask-images for cytoplasm regions and nuclei. The CELL script uses these two binary images for performing the measurements according to the method detailed in the above section.

The results of our measurements for all the six cases are summarized in Table 1.

Each row of the table describes a case, giving the mean, minimum and maximum values of the vectors *CitArea*, *NcArea*, *NcRatio*.

Table 1 - ***Summary of the measurement results***

Case number/ differentiation degree	*CitArea* (μm^2)			*NcArea* (μm^2)			*NcRatio*		
	Mean value	Minimum value	Maximum value	Mean value	Minimum value	Maximum value	Mean value	Minimum value	Maximum value
Case 1 / moderately differentiated	260.36	138.85	651.18	130.77	50.30	259.99	0.53	0.19	0.95
Case 2 / moderately differentiated	91.73	56.34	229.40	31.72	12.87	134.82	0.34	0.17	0.78
Case 3 / moderately differentiated	64.07	42.45	243.56	43.18	22.57	146.63	0.67	0.39	0.92
Case 4 / moderately differentiated	155.44	86.12	293.39	70.51	24.95	260.79	0.45	0.21	1.02
Case 5 / well-differentiated	140.68	62.78	520.78	75.37	26.56	326.8	0.52	0.22	0.96
Case 6 / well-differentiated	74.66	33.65	310.35	56.53	19.26	167.96	0.81	0.4	1.3

The effective analysis of the cases (including relevant comparisons) requires a systematic exploitation of the whole numerical information stored in the databases. Therefore we have constructed the histograms corresponding to *CitArea*, *NcArea*, *NcRatio* for all the six cases.

In order to keep the length of the current text within reasonable limits, we reproduce only the *NcRatio* histograms (Figures 4–9), since, in our opinion, this type of values is the most significant for the correlation with the differentiation degree.

The six histograms are uniformly scaled ($[0, 1.4] \times [0, 30.0]$), to facilitate direct visual comparisons between the cases.

Nevertheless the following information referring to the nuclear and cytoplasmic areas (i.e. implicitly to the *CitArea, NcArea* histograms), which is complementary to Table 1, ensures the completeness of our morphometric approach:

- **Case 1**: the distribution of the cytoplasmic areas is concentrated between 150 and 300 μm^2 (about 60% of cells); the distribution of the nuclear areas is concentrated between 75 and 150 μm^2 (about 70% of cells);

- **Case 2**: the distribution of the cytoplasmic areas is concentrated between 50 and 100 μm^2 (about 75% of cells); the distribution of the nuclear areas is concentrated between 20 and 40 μm^2 (about 75% of cells);

- **Case 3**: the distribution of the cytoplasmic areas is concentrated between 50 and 75 μm^2 (about 70% of cells); the distribution of the nuclear areas is concentrated between 30 and 50 μm^2 (about 70% of cells);

- **Case 4**: the distribution of the cytoplasmic areas is concentrated between 100 and 200 μm^2 (about 70% of cells); the distribution of the nuclear areas is concentrated between 25 and 100 μm^2 (over 80% of cells);

- **Case 5**: the distribution of the cytoplasmic areas is concentrated between 50 and 200 μm^2 (about 75% of cells); the distribution of the nuclear areas is concentrated between 25 and 100 μm^2 (over 80% of cells);

- **Case 6**: the distribution of the cytoplasmic areas is concentrated between 25 and 100 μm^2 (about 75% of cells); the distribution of the nuclear areas is concentrated between 25 and 75 μm^2 (about 75% of cells).

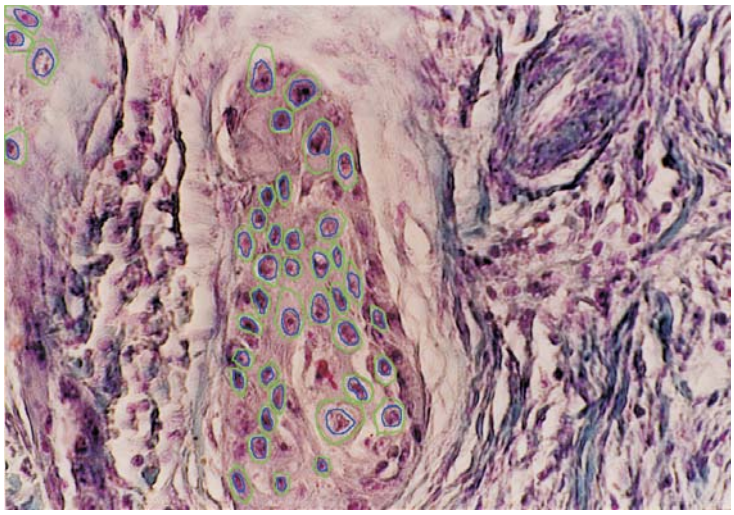

Figure 1 – *Color image pertaining to case 6; example used for illustrating the operation principles of the CELL script (HE, ×400)*

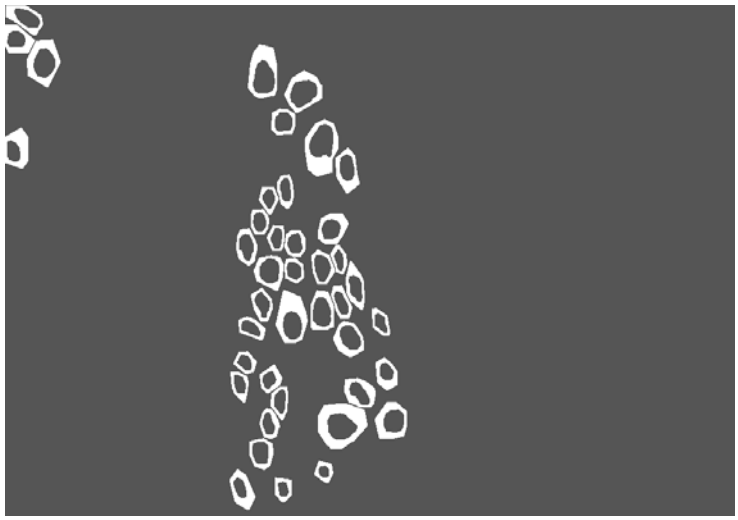

Figure 2 – *Binary mask-image for the cytoplasm regions, provided by the CELL script; example constructed starting from the color image in Figure 1*

Figure 3 – *Binary mask-image for the nuclei, provided by the CELL script; example constructed starting from the color image in Figure 1*

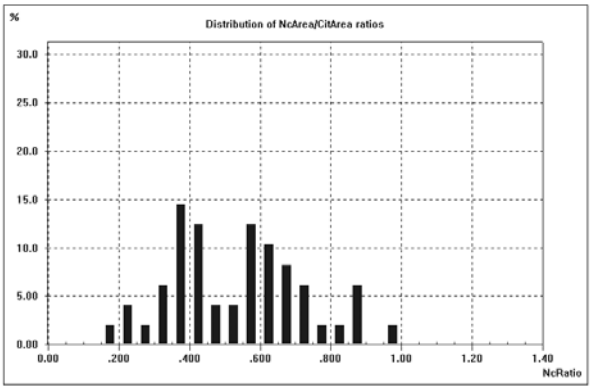

Figure 4 – ***NcRatio histogram for case 1***

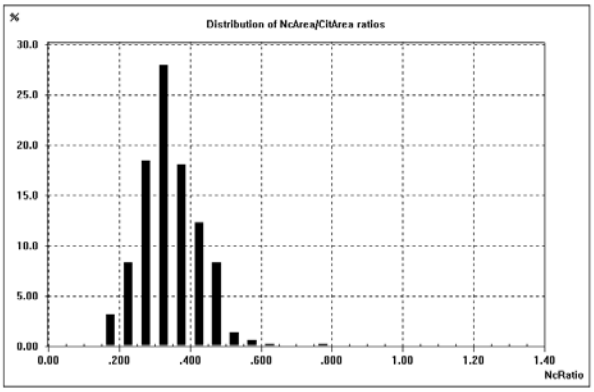

Figure 5 – ***NcRatio histogram for case 2***

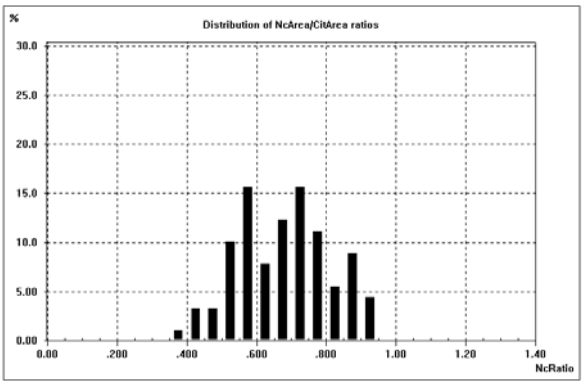

Figure 6 – ***NcRatio histogram for case 3***

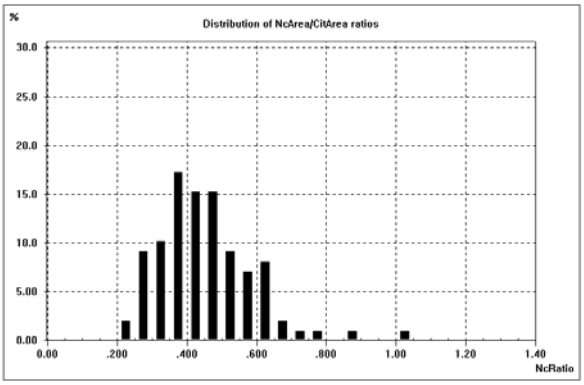

Figure 7 – ***NcRatio histogram for case 4***

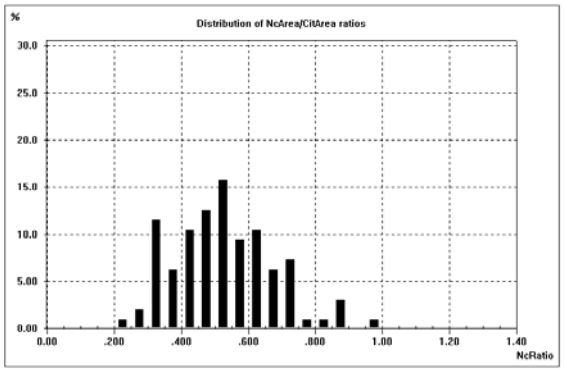

Figure 8 – ***NcRatio histogram for case 5***

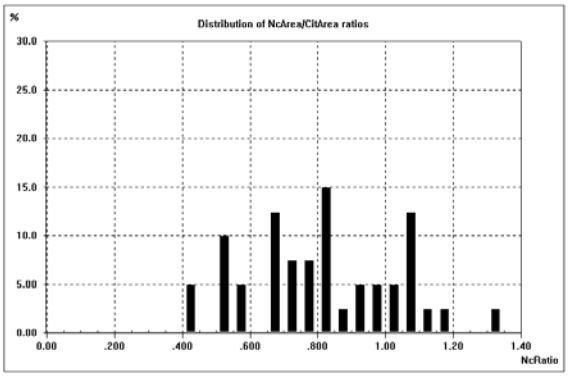

Figure 9 – ***NcRatio histogram for case 6***

Our results should be interpreted within the context of the recent morphometric studies in the field of uterine cervix pathology, focusing on different subjects, among which the following are close to our interest:

- the usage of the nuclear morphometry as a predictor of response to neoadjuvant chemotherapy plus radiotherapy [7], or as an intermediate endpoint biomarker for chemotherapy [19];
- the evaluation of karyometry and histometry in the prediction of survival, recurrence and response of early-stage invasive cervical carcinoma [8];
- the identification of certain quantitative features which can discriminate between normal and cancerous (consisting of adenocarcinoma and adenocarcinoma in situ) tissue samples [9].

Comparisons with the aforementioned research trends reveal the individuality of our work, which suggests using the nucleo-cytoplasmic ratio as a criterion for quantification of several levels in cell differentiation, in accordance with de following remarks:

- Four cases (three of moderately differentiated nonkeratinizing squamous cell carcinoma and one of well-differentiated keratinizing squamous cell carcinoma) exhibit mean values of the nucleo-cytoplasmic ratio between 0.45 and 0.67 – meaning that in a generic (average) cell, the nuclear area represents half to two thirds from the cytoplasmic area. On the other hand, in all these cases, the maximum value of the nucleo-cytoplasmic ratio is close to 1, i.e. there are real cells where the nuclear area is approximately equal to the cytoplasmic area.

▪ One case (well-differentiated keratinizing squamous cell carcinoma) exhibits 0.81 as the mean value of the nucleo-cytoplasmic ratio - meaning that in a generic (average) cell the nuclear area is almost equal to the cytoplasmic area. In this case the maximum value of the nucleo-cytoplasmic ratio is 1.4, i.e. there exist real cells where the nuclear area significantly exceeds the cytoplasmic area.

▪ One case (moderately differentiated nonkeratinizing squamous cell carcinoma) exhibits 0.34 as the mean value of the nucleo-cytoplasmic ratio - meaning that in a generic (average) cell the nuclear area represents less than half of the cytoplasmic area. In this case the maximum value of the nucleo-cytoplasmic ratio is 0.78, i.e. in the real cells the areas of the largest nuclei reach only three-quarters of the areas occupied by the cytoplasm.

Correlating the values of nucleo-cytoplasmic ratio obtained through computerized morphometry with the histopathologic diagnostic, there are two major observations:

▪ For well-differentiated keratinized squamous cell carcinoma, the value 0.81 of the mean nucleo-cytoplasmic ratio (that characterizes case 6) is totally unexpected. Such a value is surprising since cells with large nuclei and reduced cytoplasm are known as typical to undifferentiated carcinomas. At the same time, case 5 presents a mean nucleo-cytoplasmic ratio of 0.52, which agrees with the usual characterization of well-differentiated class.

▪ For the moderately differentiated nonkeratinizing squamous cell carcinoma, the widely spread values of the mean nucleo-cytoplasmic ratio 0.34 (case 2), 0.45 (case 4), 0.54 (case 1) and 0.67 (case 3) indicate that the differentiation degree within the same class of diagnosis may present large variations. Thus, on the basis of cellular morphometric evaluation, the cases of moderately differentiated nonkeratinizing squamous cell carcinoma could be graded according to the value of the nucleo-cytoplasmic ratio. This means a possible adaptation of radiation doses from one patient to another and, implicitly, a particularization of the radiotherapy.

The small number of cases studied by our work does not permit us to propose firm criteria for a refined classification of the differentiation degree, expressed in terms of intervals associated with the values of the mean nucleo-cytoplasmic ratio. However our results draw the attention on the great potential offered by the quantitative analysis in bringing supplementary information, extremely useful for diagnosis and therapy.

Conclusions

1. The cellular and nuclear morphometric features in invasive squamous cell carcinoma cover large ranges of numerical values that require adequate interpretation for ensuring a meaningful correlation with the qualitative aspects of the analysis.

2. Our paper shows that the quantitative approach can play an important role in enlarging the perspectives on the evaluation of the differentiation degree in invasive squamous cell carcinoma.

3. The cases presented and commented by us create a deeper understanding of the nucleo-cytoplasmic ratio as providing additional information, equally important for diagnosis and therapy. Although limited to a small number of cases, our morphometric study suggests that the nucleo-cytoplasmic ratio may be used in the analysis of the cervical squamous carcinoma as a synthetic index allowing the quantification of several levels in cell differentiation.

4. This work should be regarded as a preliminary investigation, with promising results, which provides a strong motivation for increasing the research efforts in the quantitative analysis of the invasive squamous cell carcinoma. Our results give us solid reasons to believe that reliable measurements on large group of cases would confirm our hypothesis on the quantification of several levels in cell differentiation by using the numerical values of the nucleo-cytoplasmic ratio.

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