

MORPHOLOGIC EVALUATION OF RADIOTHERAPY IN CERVICAL CARCINOMA – A COMPUTERIZED MORPHOMETRIC APPROACH

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Summary. Our work analyses the effects of radiotherapy in cervical carcinoma by computerized morphometry techniques that evaluate the morphologic changes in the specimens taken from pre and post-irradiation biopsies of 8 cases. These cases were diagnosed as squamous cell carcinoma, undifferentiated (1 case), moderately (5 cases) and well differentiated (2 cases). For each case, 40 relevant microscopic fields were chosen, reflecting the morphologic aspects before (20 fields) and after irradiation (20 fields). The digitised images of these fields were used for measuring the tumoral and stromal areas by a program we implemented in the Zeiss KS400 environment. The program provides a mean tumoral/stromal area for each case, computed by averaging the results of the 20 measurements performed on the pre and post-irradiation images. In terms of a stereological interpretation, each mean tumoral area expresses the percentage of the tumoral volume and can be used as an index for quantifying the efficiency of the radiotherapy. Such an index allows developing comparative discussions on cases that belong either to the same diagnosis entity, or to different diagnosis entities. These discussions yield the idea that the morphologic classification of a case is not sufficient to accurately predict the efficiency of irradiation, and a systematic approach to the morphometric features, before and after irradiation, may contribute to the refinement of the prediction.

Key words: computerized morphometry, cervical carcinoma, radiotherapy.

INTRODUCTION

Despite falling incidence, the cervical carcinoma represents the tenth leading cause of cancer death (American Cancer Society, 2002), the mortality rate remaining near 3.0 deaths per 100 000 women, for the period 1998–2002.

In 2002, the prediction of the American Cancer Society, for the whole year, in the United States, was approximately 13 000 new cases and 4100 cervical cancer-related deaths.

These facts motivate the great interest shown by current researches that aim to reduce the mortality rate to 2.0 deaths per 100 000 women as a target for 2010 (U.S. Department of Health and Human Services, 2000).

There exist efficient methods for the early diagnosis of the cervical carcinoma by cytologic (Papanicolaou test) and clinical (pelvic examination) screening. The screening programs yielded satisfactory results in western countries.

In Romania, the early diagnosis of cervical carcinoma is still not organized adequately, in the current practice, most cases being discovered in advance stages and requiring a combined therapy, with modest results, in general. Within the framework of this therapy, a significant role is played by pre-surgery irradiation.

This paper aims to evaluate the effects of radiotherapy on the neoplastic lesions (in the sense of reducing their size), by morphometry techniques measuring the percentage of the tumoral volume in specimens taken from pre and post-irradiation biopsies.

Nowadays, these techniques are fully assisted by the computer and the appropriate processing of the digitised images ensures high precision in extracting the geometric features on which the measurement relies. The digital image processing requires specialized software environments, much more complex and more reliable than the early computer programs for morphometry based on different types of grids.

On the best of our knowledge, there are no papers reporting quantitative approaches to the evaluation of radiotherapy effects. However, the literature includes a number of studies related to our work, proving the importance of the morphometry researches developed for exploring cervical carcinomas (Tosi *et al.*, 1992; Yacoub *et al.*, 1994; Poulin *et al.*, 1999; Cenci *et al.*, 2000a, b; Weyn *et al.*, 2000; Swartz *et al.*, 2003).

MATERIAL AND METHODS

The studied lot comprised 8 cases of cervical carcinomas, investigated at the Caritas Hospital, București. Each case was biopsied, before and after irradiation. The collected fragments were processed for pathological exam. The pre-irradiation biopsies were used to diagnose the cases and to recommend the radiotherapy protocols. The post-irradiation biopsies allowed the evaluation of the radiotherapy results.

The pre and post-irradiation biopsies were studied from the computerized morphometry point of view.

For each of the above cases, we have chosen 20 relevant microscopic fields from both the pre-irradiation and the post-irradiation biopsies.

The digitized images of these fields represented the material used for the computer-assisted analysis we ran under the Zeiss KS400 environment (operational at the Histology Department of the “Gr. T. Popa” University of Medicine and Pharmacy, Iași).

To perform the measurements, we designed and implemented a specialized procedure (macro) called COL, which computes the tumoral and stromal areas existing in the analyzed microscopic fields.

The development of the COL macro exploited the general principles formulated by Căruntu (2003), which were particularized for the current work.

The COL macro was programmed in the KLIC language available under Zeiss KS400.

In the following we briefly present the sequence of operations performed by COL, for each case, separately considered before and after radiotherapy.

By $case(k, t)$ we generically refer to the k -th case ($k = 1, \dots, 8$) before radiotherapy ($t = 0$) and after radiotherapy ($t = 1$), and by $field(i, k, t)$ to the i -th microscopic field ($i = 1, \dots, 20$) chosen for $case(k, t)$.

Given $case(k, t)$, for each $field(i, k, t)$, COL loads the corresponding digitized image and calls the graphical editor in order to interactively select the tumoral zones, by drawing their contours in the graphical plane (e.g. Figure 1, corresponding to $field(7, 3, 1)$).

The closed curves defined by these contours are copied, in white color, on a black binary image (e.g. Figure 2, corresponding to $field(7, 3, 1)$) and then, they are filled (as holes) also with white colour.

The resulting white zones are used as binary masks for measuring the tumoral areas, which, by summation, yield the *total tumoral area* of $field(i, k, t)$, abbreviated as $TTA(field(i, k, t))$.

The *total stromal area* of $field(i, k, t)$, abbreviated as $TSA(field(i, k, t))$ is calculated as the difference between the whole area of $field(i, k, t)$ and $TTA(field(i, k, t))$.

The values of $TTA(field(i, k, t))$ and $TSA(field(i, k, t))$ are stored in a database.

After finishing the measurements for $field(1, k, t), \dots, field(20, k, t)$, COL computes the *mean values* of the *tumoral areas* and *stromal areas* characterizing $case(k, t)$, namely:

$$MTA(case(k, t)) = \frac{\sum_{i=1}^{20} TTA(field(i, k, t))}{20}, \text{ and}$$

$$MSA(case(k, t)) = \frac{\sum_{i=1}^{20} TSA(field(i, k, t))}{20}$$

Finally, COL expresses these two areas in a percentage form defined as:

$$MTA\%(case(k, t)) = \frac{MTA(case(k, t))}{MTA(case(k, t)) + MSA(case(k, t))}, \text{ and}$$

$$MSA\%(case(k,t)) = \frac{MSA(case(k,t))}{MTA(case(k,t)) + MSA(case(k,t))}$$

and it also calculates their ratio (tumoral to stromal):

$$MTA2MSA(case(k,t)) = \frac{MTA(case(k,t))}{MSA(case(k,t))}.$$

RESULTS

The eight cases were pathologically diagnosed as:

- squamous cell carcinoma, with small cells (undifferentiated): one case ($k=1$).
- squamous cell carcinoma, with large cells, without keratinization (moderately differentiated): five cases ($k=2,3,4,5,6$).
- squamous cell carcinoma, with large cells, with keratinization (well differentiated): two cases ($k=7,8$).

These diagnoses together with the results of the measurements are presented in Table 1.

DISCUSSIONS

In accordance with the therapeutic protocol of the cervical cancer, irradiation has the role to reduce the existing tumoral mass (Pointou, 1991; Le Bourgeois, 1992; Perez *et al.*, 1992). Our computerized morphometry results prove the opportunity to refine, by quantitative information, the microscopic analysis (of qualitative nature) of typical changes occurring in cervical carcinoma, as a consequence of radiotherapy.

The effectiveness of this therapy can be quantified, in the sense of using the percentage of the tumoral area $MTA\%(case(k,t))$ at times $t=0$ (before irradiation) and $t=1$ (after irradiation), as an index for the illness evolution, corresponding to each studied case.

In terms of a stereological interpretation, the proposed index $MTA\%(case(k,t))$ has a 3-D meaning that reflects the percentage of the tumoral volume. Such a numerical measure gives the possibility to develop comparisons between cases belonging either to the same diagnosis entity, or to different diagnosis entities.

To focus the discussions on the usage of the proposed index, our comparative study organizes the eight cases into four groups, relying on the morphometric features before the irradiation.

Table 1
Diagnoses of the considered cases accompanied by the computerized morphometry results

Case <i>k</i>	Squamous cell carcinoma	Time $t = 0$ (before irradiation)		Time $t = 1$ (after irradiation)	
		$MTA(case(k,0))$	$MSA(case(k,0))$	$MTA(case(k,1))$	$MSA(case(k,1))$
		$MTA\%(case(k,0))$	$MSA\%(case(k,0))$	$MTA\%(case(k,1))$	$MSA\%(case(k,1))$
		$MTA2MSA(case(k,0))$		$MTA2MSA(case(k,1))$	
1.	small cells, undifferentiated	929881.31 μm^2	173198.68 μm^2	304323.73 μm^2	724426.26 μm^2
		84.30%	15.70%	29.58%	70.42%
		5.36		0.42	
2.	large cells, moderately differentiated	827829.87 μm^2	286635.12 μm^2	344727.71 μm^2	695272.28 μm^2
		74.28%	25.72%	33.14%	66.86%
		2.88		0.49	
3.	large cells, moderately differentiated	679259.02 μm^2	371790.97 μm^2	409989.20 μm^2	702046.79 μm^2
		64.62%	35.38%	36.87%	63.13%
		1.82		0.58	
4.	large cells, moderately differentiated	949790.10 μm^2	155427.89 μm^2	488003.95 μm^2	555822.04 μm^2
		85.94%	14.06%	46.75%	53.25%
		6.11		0.87	
5.	large cells, moderately differentiated	762224.78 μm^2	296040.21 μm^2	130274.01 μm^2	933545.98 μm^2
		72.02%	27.98%	12.24%	87.76%
		2.57		0.13	
6.	large cells, moderately differentiated	769174.93 μm^2	280741.06 μm^2	59694.27 μm^2	981101.72 μm^2
		73.26%	26.74%	5.73	94.27%
		2.73		0.06	
7.	large cells, well differentiated	669585.96 μm^2	371910.03 μm^2	426070.29 μm^2	597801.70 μm^2
		64.32%	35.68%	41.61%	58.39%
		1.80		0.71	
8.	large cells, well differentiated	570357.57 μm^2	539924.42 μm^2	85051.42 μm^2	892208.57 μm^2
		51.37%	48.63%	7.97%	92.03
		1.05		0.08	

Towards this end, we consider an integer approximation $n = 1, 2, 3, \dots$ for each value $MTA2MSA(case(k,0))$, $k = 1, \dots, 8$, and we use the writing $MTA2MSA(case(k,0)) \approx n$ when the condition $MTA2MSA(case(k,0)) \in [n - 0.5, n + 0.5)$ is fulfilled.

The four groups are:

- group 1, defined by $n = 1$, including case $k = 8$.
- group 2, defined by $n = 2$, including cases $k = 3, 7$.
- group 3, defined by $n = 3$, including cases $k = 2, 5, 6$.
- group 4, defined by $n > 3$, including cases $k = 1, 4$, and the progress of the discussions will be guided in terms of the two types of comparisons, mentioned

above. For a complete understanding of the details, maximum attention should be paid to the numerical values displayed by Table 1.

GROUP 1

Case 8 (squamous cell carcinoma, with large cells, with keratinization, well differentiated) had a good evolution after irradiation. The radiotherapy drastically reduced (6.45 times) the percentage of the tumoral volume, from 51.37% to 7.97%. This result was unexpected, since, in literature, good answers to radiotherapy are mentioned especially for squamous cell carcinoma with small cells, undifferentiated.

GROUP 2

Case 3 (squamous cell carcinoma, with large cells, without keratinization, moderately differentiated) and case 7 (squamous cell carcinoma, with large cells, with keratinization, well differentiated) had modest evolutions after irradiation. Although the two cases belong to different diagnosis entities, the percentage of the tumoral volume decreased almost similarly, namely 1.75 times for case 3 (from 64.62% to 36.87%) and 1.54 times for case 7 (from 64.32% to 41.61%).

GROUP 3

Cases 2, 5 and 6 belong to the same diagnosis entity, squamous cell carcinoma, with large cells, without keratinization, moderately differentiated. Nevertheless, their responses to radiotherapy were quite different. Case 6 had an excellent evolution, the percentage of tumoral volume decreasing 12.78 times (from 73.26% to 5.73%).

Case 5 presented a good evolution, the percentage of tumoral volume decreasing 5.88 times (from 72.02% to 21.24%). The evolution of case 2 was modest, with a tumoral volume reduction of 2.24 times (from 74.28% to 33.14%), close to the result of case 3 (belonging to group 2).

However, the evolution of case 2 seems to be more promising than that of case 3, because the percentage of the tumoral volume before irradiation was significantly higher for case 2 compared to case 3.

GROUP 4

Case 1 (squamous cell carcinoma, with small cells, undifferentiated) and case 4 (squamous carcinoma, with large cells, without keratinization, moderately differentiated) had modest evolutions after radiotherapy. The percentage of tumoral volume decreased 2.86 times for case 1 (from 84.30% to 29.38%) and 1.83% times for case 4 (from 85.94% to 46.75%).

However, these results can be considered better in comparison with cases 2, 6, 7 discussed above, if we take into account the much higher percentages of

tumoral volume before the irradiation of cases 1 and 4. On the other hand, the answer of case 1 compared to that of case 4 confirms the comments known from literature on the best evolution after radiotherapy of the squamous cell carcinomas, with small cells, undifferentiated. Our quantitative analysis reveals that some well and moderately differentiated types of squamous cell carcinomas can react to radiotherapy like the ones of undifferentiated type, although this fact is not clearly stated in literature. In other words, the morphologic classification of a case is not sufficient to accurately predict the efficiency of irradiation and a systematic approach to the morphometric features, before and after irradiation, may contribute to the refinement of the prediction.

CONCLUSIONS

The study we have developed in terms of morphometric criteria enriches the knowledge about the effects of radiotherapy on cervical carcinomas. Thus, the numerical information resulting from computer-assisted measurements compensates the subjectivity that can affect the pathological exam.

Moreover, by operating with mathematical-type descriptors of the microscopic observations, we were able to point out numerous details that usually remain hidden for a standard morphologic investigation.

Such details can be regarded as supplementary arguments for the diversity of cellular and tissue reactions, which is well known in cancer therapy. It is reasonable to expect that further research, using morphometric data collected from a larger lot of patients, might bring some rigorous explanations for this diversity, by a deeper insight into the role of other factors influencing the quality of the response to radiations.

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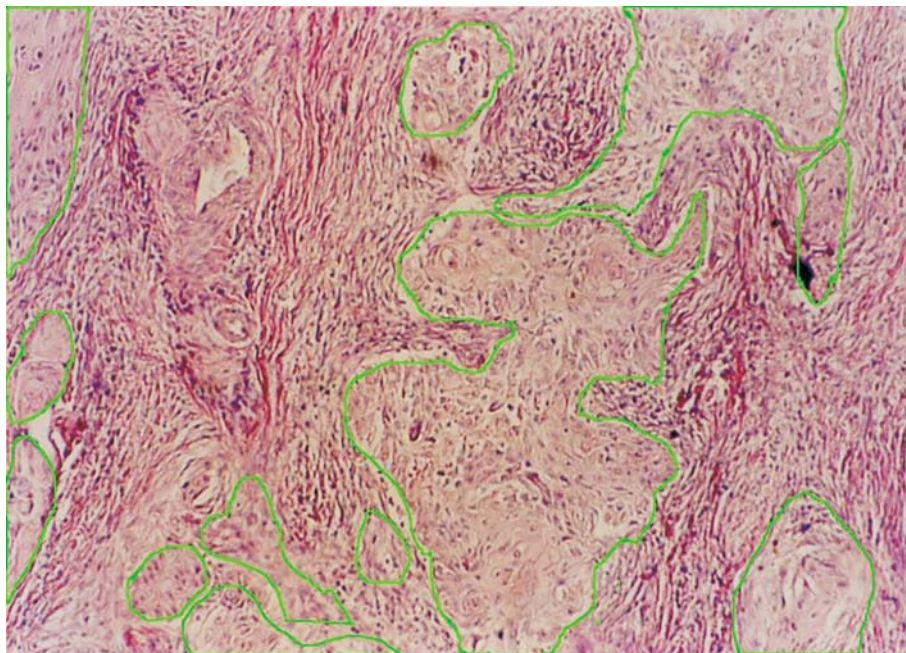


Figure 1 – Digitized image corresponding to with the contours of the tumoral areas
(original image, HE-stain, $\times 200$)



Figure 2 – Binary image resulted from the extraction of the contours in Figure 1