

The Configuration of Biomolecular Markers in Cancer of the Uterine Cervix. Personalized Therapy. Monitoring and Prognosis

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Rezumat

Configurația markerilor biomoleculari în cancerul de col uterin. Atitudine terapeutică personalizată. Monitorizare și prognostic

Introducere: Există forme agresive de cancer de col uterin, rezistente la radio/chimioterapie, care ar putea fi depistate cu ajutorul markerilor biomoleculari. În ultima perioadă, pentru stadiile II-III B, se utilizează pentru distrugerea tumorilor și hipertermia generată de diverse surse de energie. Scopul studiului este de a propune adăugarea la protocolul terapeutic a unei tehnici rapide și simple de hemostază în neoplasmul de col sângerând cu anemie acută și stabilirea unor criterii biomoleculare ca indicatori ai agresivității și ai responsivității la tratament.

Metodă: Am folosit hipertermia prin radiofrecvență (RFA) pentru tumorile de col sângerânde și însoțite de anemie acută secundară. Corelat cu experiența personală asupra 16 pacienți, am constatat că markerii Ki67, p53 și Bcl-2 pot fi asimilați unor forme agresive de cancer cu rezistivitate la radio/chimioterapie.

Rezultate: Rezultatele au demonstrat rolul hemostatic al metodei, RFA obținând hemostaza rapid (20 min). Secundar, am obținut

diminuarea volumului tumoral la nivel local și potențarea responsivității la radiochimioterapie.

Concluzii: Odată cu stabilirea diagnosticului de neoplasm agresiv și rezistent la radiochimioterapie, markerii biomoleculari ar putea influența și atitudinea terapeutică personalizată (radiofrecvență, chimioterapie neoadjuvantă), ceea ce, pe termen lung, poate echivala cu prelungirea vieții pacientelor și ameliorarea condițiilor lor de viață.

Cuvinte cheie: markeri biomoleculari Ki67, p53 și Bcl-2, tumoră col uterin, hipertermie

Abstract

Introduction: The paper deals with the diagnosis of some aggressive forms of uterine cervix cancers, resistant to radio/chemotherapy, using biomolecular markers. For this study, the destruction of tumours in stages II-III B is carried out by hyperthermia induced by different sources of energy. The aimed targets are to embed a quick and simple technique of haemostasis used in bleeding uterine cervix tumours associated with acute anaemia in the treatment protocols and to identify biomolecular criteria revealing tumour aggressiveness and treatment response. *Method:* The proposed method consists in radiofrequency ablation (RFA) applied to uterine cervix bleeding tumours with acute secondary anaemia. Studying 16 patients displaying aggressive cancer forms resistant to radio/chemotherapy treated by the abovementioned method, we assessed that the commonly present markers: Ki67, p53 and Bcl-2, may be a substantial indication of such cases. Aggressiveness and

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treatment resistance was defined based on clinical and para-clinical investigations.

Results: RFA haemostasis achieved in approximately 20 min proved the efficiency of this method. A secondary important effect was local tumour volume decrease, resulting in the improvement of radio-chemotherapy responsiveness.

Conclusions: Once an aggressive and radio-chemotherapy resistant cancer is diagnosed, the quantitative, qualitative and associative presence of the biomolecular markers mentioned hereinbefore, could influence the personalised treatment attitude (radiofrequency, neoadjuvant chemotherapy), which on the long term, may increase patient survival and life quality improvement.

Key words: biomolecular markers Ki67, p53 and Bcl-2, tumour of the uterine cervix, hyperthermia

Introduction

Annually, worldwide, there are around 500,000 new cases of cancer of the uterine cervix. It occupies the fourth place in the hierarchy of female cancer, continuing to be a major problem for the public healthcare compartment (1). According to the Minister of Health, cancer of the uterine cervix represents in Romania 15% of malignant tumours, with the first place among genital cancers in women (67%), and is the second cause of death through cancer.

The classical methods to establish an early diagnosis of neoplasm of the uterine cervix are based on screening methods which use the means of clinical and paraclinical exploration. For instance, the use of genital clinical exam and of the Babeş-Papanicolau/Bethesda test, at the same time as abdominal/transvaginal ultrasound have led to early detection and cure, or to a long-time survival in those patients. The goal of this paper is to extend the method of investigation to other tumour markers which, according to recent studies, and also from our personal experience, have demonstrated the possibility of identification of some aggressive cancer forms resistant to radio/chemotherapy. The method proposed is improved by previously established imagery diagnostic criteria (ultrasound, CT scan/MRI or PET-CT), in order to obtain a correct staging of the tumour development. Apart from their prognostic value, these biomarkers could influence the treatment attitude in several situations, which on the long term may equal the decrease in hospital admission costs, patient increased survival and improvement in the quality of life.

Once the diagnosis of neoplasm resistant to radio/chemotherapy is established, the alternative of radiofrequency ablation may be possible (with or without neoadjuvant chemotherapy) in bleeding tumours of the uterine cervix with secondary anaemia (2,3) and in advanced stages (II-III, IV-hepatic metastases) (4,5). The main indication of radiofrequency ablation (RFA) is for hepatic metastases. The new element proposed by us is to extend the method to cancers of

the uterine cervix that are chemo/radio resistant and invasive. In turn, this method will be able to diminish the costs of the treatment used for the correction of the anaemia and for decrease in tumour volume (2,3). The method used at present for haemostasis is performed under intra-vaginal ultrasound control, being followed by radio/chemotherapy and the Wertheim procedure (when possible).

Material and Method

It has been noticed that the response of certain forms of cancers of the uterine cervix is weaker in radio/chemotherapy (6), and finding a method to early diagnose it may lead to an improvement in prognosis and in the clinical outcome. The most important prognostic factors are: stage, tumour volume, invasion of the parameters, vascular invasion, lymph node metastases and distant metastases. A new approach in the identification of aggressive tumours of the cervix, and with resistance to radiotherapy, is represented by the study of tumour markers. These do not replace biopsy with a pathology examination of the specimen, but are useful in tumour staging, treatment monitoring and detection of the disease recurrence, and also useful as prediction factors.

During the time when we performed RFA in tumours of the uterine cervix with vaginal bleeding accompanied by secondary acute anaemia (61 patients, Hb=7-11g%) and which did not respond to conservative treatment (haemostasis, mesh wrap and locally applied haemostatic agents, administration of iv haemostatic products) (2) we have noticed that some patients had a worse clinical outcome under radiofrequency ablation (increased time - more than 20 minutes - and a big quantity of absorbed energy, more than 40-50kj) necessary for haemostasis, and also a bad response to radio-chemotherapy. From the pathologist's point of view, 35 patients presented with infiltrative forms (stages IIB-IIIB) of squamous carcinoma (40-56kj), 5 cases were adeno-carcinomas (50-55kj) and 2 cases were sarcomas (52-55,5kj). We mention that only in 12 patients out of the 61 (19.6%), Wertheim procedure was performed after RFA followed by radio-chemotherapy due to the advanced stage of the disease, or due to the partial response to the standard treatment (2).

It was for this fact that we decided to search for the agents that determine tumour aggressiveness and treatment resistance. The personal experience acquired during the development of application of RFA to perform haemostasis to uterine cervix cancer, correlated with the literature data (6,7), show that markers: ki67, p53 and Bcl-2 can be associated with aggressive cancer forms with resistance to radiochemotherapy. By establishing the diagnosis of neoplasm resistant to radio/chemotherapy, those biomarkers may influence also some personalised treatment attitudes (radiofrequency ablation, neoadjuvant chemotherapy, radio/chemotherapy, etc.).

The paper aims to identify the configuration of markers: Ki67, p53 and Bcl-2 in tumours of the uterine cervix in stages II-III by correlating their presence with the association mode, their quantity in percentages, with the clinical and paraclinical examination (biochemistry and imagery), HPV presence, but also with the treatment response (RFA, radio-chemotherapy)

and, last but not least, with the presence of the markers on the pathology specimens with residual tumours post Wertheim. Starting from the contribution of different authors, a set of markers were studied:

- Ki67 - is a nuclear protein which shows cell proliferation and is present during all the active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from the cells which are at rest (G0). (8,9)
- p53 (the guardian of the genome) - the protein controls cell proliferation and apoptosis (programmed cell death). This functions as a suppressor and stops the development of the cancer cells. When DNA is damaged, gene p53 is activated and protein p53 interacts with other proteins called CDK inhibitors/ cyclin (cyclin dependent kinases), including p16, p27 and p21. This action determines the detention of the cell cycle at the R point in G1, in order to allow the DNA to repair itself. If the repair of the DNA succeeded, p21 signals CDK/cyclin in order for the cell cycle to continue. When DNA repair is not possible, the signals from proteins p53 and other regulating proteins such as bax, bcl-2 and c-myc, determine the induction of apoptosis and eliminates the cells with erroneous genetic information. Therefore, p53 is considered to be a factor of control in cellular division. Gene p53 mutation and its presence in excess determine the loss of control in cell proliferation and accumulation of oncogenic mutations frequently encountered in invasive cancers. It seems that HPV (strains 16,18), through the viral proteins E6 and E7, interferes in the control of the cell cycle. E6 induces the degrading and inhibition of p53, resulting in tumour progression. (10,11)
- Bcl-2 - is considered to be a cell membrane protein which regulates apoptosis. A high level of bcl-2 proteins protects the cells from early death. The decrease in the rate of cell destruction is thus obtained. An inadequate expression of bcl-2 may prolong the life of the deteriorated cells with an increase in the risk of malignant transformation and a stop in p-53-mediated apoptosis. The modification of this gene is considered to be the generator of radiotherapy resistant tumours of the uterine cervix (9,12,13).

Other markers used in the immunohistochemical diagnosis of cervix tumours are: p16 (a suppressor protein, implicated in cancer prevention, which holds an important role in the regulation of the cell cycle, by slowing the cell progression from G1 to S phase) and p21 which prevents cell proliferation. Some authors studied the influence of the presence of tumour markers p53 and bcl-2 on patients' survival in tumours of the uterine cervix, considering that bcl-2 may be used as a prognostic factor (7). Different researchers suggested that a series of markers, such as: p16, p21, p53, CK8, CK17, Ki67, Bcl-2, as well as some cyclins (D1, E, A, B1), may be associated with the increase of the CIN grade, the tumour stage, tumour aggressiveness, resistance to radiotherapy and patient life span decrease (11,14,15,16,17).

Radio/chemotherapy is considered a standard treatment for cancers in stages I-II. In advanced stages, efficient treatment of

the disease is a challenge. This is why initially in 2010, and afterwards in 2012, Franckena and others (4,5) started using hyperthermia in association with radiotherapy in the local treatment of the disease, the procedure taking place under MRI control. Likewise, hyperthermia procedures (among which RFA) may be accepted as a way of complementary treatment in advanced tumours of the uterine cervix and in inoperable forms, without increase in toxicity, and may represent an alternative to chemotherapy for the patients which cannot benefit from this treatment.

This method is only slightly aggressive, well tolerated by the patient, performed under safety conditions under MRI monitoring, and can be associated with brachytherapy (5).

The RFA procedure we performed at the level of the uterine cervix allows the decrease in tumour volume. This can be monitored intraoperatively through transvaginal ultrasound and postoperatively through CT scan/MRI. We cannot perform tumour ablation at the level of the parametria. The risks of the procedure are: lesion of the urinary bladder, of the rectum, of the ureter, the apparition of complex vaginal fistulae and ureteral stenosis or perforations in the abdominal cavity (2).

Results

In 62 patients included in the study group the results have demonstrated the haemostatic role of the method, RFA achieving quick haemostasis (20 min). Secondly, we obtained local decrease in tumour volume and the enhancement of the effects of radio/chemotherapy.

Immunohistochemistry tests were done in 16 patients with ages between 37 and 76 and with a mean of 56.3 years. These presented in the following disease stages: IA – 1 case (6.25%), IIA – 2 cases (12.50%), IIB – 3 cases (18.75%) and IIIB – 10 cases (62.50%). The pathology department identified 10 cases of non-keratinised squamous carcinoma (62.50%), 4 cases of keratinised squamous carcinoma (25%) and 2 cases of adenosquamous carcinoma (12.50%). Tumour dimension measured on the CT scan and correlated with the clinical examination has varied between 3/3-10/8 cm with a mean of 5.53/4.27 cm.

For immunohistochemistry markers we established several grades of malignancy according to the percentage of their presence at tumour level:

- For Ki67 we established the following grades of malignancy: negative (0%), low (<50%), intermediary (50-75%), high (>75%). Ki67 was found in a low proportion in 3 cases (all post-treatment), intermediary in 5 cases (31.25%), high in 11 cases (68.75%). We mention that post-radiotherapy, 3 cases presented with low malignancy residual tumours (<50%), and pre-radiotherapy those cases had presented with high and intermediary malignancy. Out of the 11 cases of high malignancy, 9 are in stage IIIB (81.81%), 1 in stage IIA (9.09%) and 1 in stage IIB (9.09%), and the tumour dimension was between 5/4 cm and 10/8 cm (in 7 of the 11 cases). (Table 1)
- For p53 we established the following grades of malignancy:

nancy: negative (0%), isolated (<25%), moderate (25-50%), highly positive (>50%). Thus, there were: 1 isolated case (6.25%), moderate 7 cases (43.75%) and highly positive 8 cases (50%). Out of the three cases post radiotherapy, initially, 2 had been highly positive and 1 had been moderated, and post-treatment they arrived at the moderate grade and respectively at the isolated grade. From the 8 cases of highly positive malignancy, 6 are IIIB (75%), 1 in stage IIA (12.5%) and 1 in stage IIB (12.5%). (Table 1)

- For Bcl-2 we established the following grades of malignancy: negative (0%), isolated (<25%), moderate (25-50%), highly positive (>50%). Thus, there were 4 negative cases (25%), 1 isolated case (6.25%), 6 moderate cases (37.5%) and 5 highly positive cases (31.25%). Out of the highly positive, a number of 3 cases were in stage IIIIB and 2 cases in stage IIB. The 3 cases with a tumour remnant after radiotherapy were: 1 case presented with 0% malignancy, 1 case of isolated malignancy (18%) and one case of moderate malignancy (30%), and they all had moderate and highly positive grading before treatment. (Table 1).

From the point of view of the association between tumour markers we had the following results: ki67 + p53 in 4 cases (25%), all in stage IIIIB, 12 cases with the pattern ki67 + p53 + bcl2 (75%) 1 case being in stage IIA (8.33%), 1 case IA (8.33%), 4 cases IIB (33.33%) and 6 cases IIIIB (50%). P16 was found in 7 cases (37.5%), out of which 5 were highly positive and 2 moderate. Out of the 5 with a high malignancy grade, 2 were stage IIB and 3 stage IIIIB. In 5 cases p16 was associated with the 3 markers (ki67 + p53 + bcl2) and in 2 cases with ki67 + p53. P21 was expressed in 2 patients and there was a case of moderate value (12.5%) and one case of highly positive malignancy (12.5%), the highly positive one being associated with ki67 + p53 + bcl2 + p16 + p21 (IIB), and the moderate one with ki67 + p53 + p16 + p21 (IIIIB), p16 and also p21 having had the highly positive character before treatment. HPV with oncogenic strands (16,18) was performed only in 6 patients, 4 being negative and 2 positive (HPV16). Tumour grading was G2 in 15 cases (93.75%) and G3 in a single case (6.25%). Case G3 was in stage IIIIB, with markers Ki67 + p53

+ p16 + p21. (Table 2)

From the perspective of the lab tests, the anaemic syndrome was present in 13 of the 16 patients (81.25%), the patients having a HB of between 6-13g% (N=12-18g%), and the 10 cases in stage IIIIB had a HB=6-11.5g%, with a mean of 10.02g%. The case of 6g% had the following markers' pattern: ki67+p53+p16+p21 (stage IIIIB and G3). From the 10 cases, 9 presented with high malignancy for ki67 and 6 for p53, and the tumour dimension was of more than 5/4 cm. CA 125 tumour marker, currently used in establishing the diagnosis of genital tumour was elevated in only 6 patients (37.5%) (3 in stages IIIIB) and was normalised after the treatment applied.

RFA pre-radio/chemotherapy was performed in 9 patients (56.25%), with the main goal of achieving haemostasis (secondarily, tumour decrease was also achieved as demonstrated intraoperatively through transvaginal ultrasound). There were 7 patients in stage IIIIB and 2 in stage IIB, and Hb=6-11.3g% with a mean of 9.84g%. The power generated in the majority of the patients was of 75W (a mean of 67.66W), the mean of the quantity of energy consumed was 44.83Kj, and the mean time during which haemostasis was achieved was of 22.11 minutes. The data show that for the patients included in the study a big quantity of energy was consumed (between 30 and 63kj) and it was necessary to use a longer time in order to achieve haemostasis. The results obtained from the previous experience in the use of RFA were on a mean of 46.7Kj and 18.24 minutes in squamous carcinoma. For instance the patient with a grading of G3 at a 75W power produced by the device, needed a haemostasis of 63 kj generated during 35 minutes. That patient after radio-chemotherapy and surgical treatment-total hysterectomy with bilateral adnexectomy- presented with a residual tumour at the level of the cervix and the uterus -as it was noticed on the surgical specimen- with a presence of ki67 and p53 markers which were half than before treatment. No presence was noticed for the bcl-2 marker.

All patients were treated through radiotherapy (the majority with external irradiation 25 series with 45Gy, plus brachytherapy 2 series with 15 Gy) and chemotherapy with Cisplatin (in some patients Paclitaxel, Docetaxel, Carboplatin were also added). After the treatment (radio-chemotherapy ±

Table 1. Presence of the markers in the tumour tissue - in percentages, on a half quantitative malignancy scale

	Negative (0%)	Low (<50%)	Intermediary (moderate) (50-75%)	High (>75%)		
Ki67	0	3 (16) post-radiotherapy (RT)	5 (16)	11 (16)		
				9 (11) std. IIIIB	1 (11) std. IIB	1 (11) std. IIA
P53	Negative 0%	Isolated (<25%)	Intermediary (moderate) (25-50%)	Highly positive (>50%)		
	0	1 (16), 1 (16) post-RT	7 (16), 2 (16) post-RT	8 (16)		
				6 (8) std. IIIIB	1 (8) std. IIB	1 (8) std. IIA
Bcl-2	Negative 0%	Isolated (<25%)	Intermediary (moderated) (25-50%)	Highly positive (>50%)		
	4 (16), 1 (16) post-RT	1 (16), 1 (16) post-RT	6 (16), 1 (16) post-RT	5 (16)		
				3 (5) std. IIIIB	2 (5) std. IIB	0 (5) std. IIA
p16	Negative 0%	Isolated (<25%)	Intermediary (moderated) (25-50%)	Highly positive (>50%)		
	0, 1 (16) post-RT	0	2 (16)	5 (16)		
				3 (5), 2 (16) post-RT std. IIIIB	2 (5) std. IIB	0 (5) std. IIA

Table 2. Correlation between tumour immunophenotype and stage (std)

Ki67 + p53	Ki67 + p53 + Bcl-2				Ki67 + p53 + Bcl-2 + p16			Ki67 + p53 + p16	Ki67 + p53 + p16 + p21
4 (16) std.IIIB	12 (16)				5 (16)				1 (16) std.IIIB
	6 (12) std.IIIB	4(12) std.IIB	1 (12) std.IIA	1 (12) std.IA	2 (5) std.IIIB	2 (5) std.IIB	1 (5) std.IIA	2 (16) std.IIIB	Hb=6g% G3 RFA (75W63Kj35)min

RFA and surgical treatment), 3 patients presented with a residual tumour on the specimen probes post-Wertheim procedure, and from those tissues markers ki67, p53 and bcl-2 were analysed. Important modifications of the values were identified (at least half from the original figures), reaching even values of 0 (bcl2). Out of the 16 patients, Wertheim procedure was performed post-radio/chemotherapy in only 12 cases (75%), while in 3 patients (12.5%) total hysterectomy with bilateral adnexectomy was performed and one patient (6.25%) refused the surgery, but accepted the radiofrequency ablation technique. Other associated procedures were: amputation of the uterine cervix, right hemicolectomy, internal digestive derivation and Hartmann procedure.

The patients were followed up post-treatment for 9-72 months. Mean survival was of 32.65 months. Out of the patients registered, 2 died after 9, respectively 22 months. The deceased patients were in stages IIIB and presented a marker association of ki67, p53 and bcl-2 in highly positive values, one of the patients having an oncogenic HPV(HPV16), plus marker p16 present, being an adenosquamous carcinoma. Both patients received RFA for haemostasis and needed a high amount of energy and an increased time in order to achieve that.

Discussions

We consider that radiotherapists may accept the RFA method since it provides quick haemostasis (20 min) correcting the anaemia, it sterilises the tumour bulk due to the high work temperatures 66-105°C, which can also be monitored, and the decrease in tumour volume allows the association of RFA with brachytherapy as they have a favourable, cumulative effect. We mention that the procedure preserves the blood supply of the uterus and of the vagina by thus ensuring optimal conditions for radio-chemotherapy.

Adenosquamous carcinoma and the invasion of the lymph nodes are significant determinants in the decrease in survival, but chemotherapy is usually reserved for patients with metastases or recurrent disease. Recent studies have shown the decrease in tumour volume locally and under neoadjuvant chemotherapy (NAC)-mainly with cisplatin for 3 series every 21 days-after that, some cases become operable and sensitive to radiotherapy. But there is still the need to identify cell factors which could predict the response to NAC and which could optimize the treatment response. Likewise, p21 may be a prediction factor in the efficacy of NAC, the apoptosis increasing proportionally with it, and p53 is considered a prognosis factor in tumour progression. The expression of bcl-2 is tightly linked to Ki67, and bcl-2 also determines the response

to radiotherapy (resistivity) (18).

In clinical guidelines (ESMO 2012) neoadjuvant chemotherapy in advanced tumours of the uterine cervix is under question. Furthermore, the administration of this therapy followed by Wertheim procedure or radiotherapy might have an important role in the treatment of locally advanced cervical cancer (IB2-IIIB), 5-year patient survival increasing by 14% (19). The objectives of this treatment are represented by the destruction of clinical or subclinical metastases, the decrease in the invasion of the parameters, of the lymph node metastases, the decrease in the recurrence rate and the local decrease in tumour volume. The treatment infers a moderated hematologic toxicity (pancytopenia) (20,21).

Therefore in aggressive and treatment resistant cancers we propose the local decrease in tumour volume through the administration of personalised therapy: radiofrequency ablation and neoadjuvant chemotherapy (2-3 series of platinum salts). These can be followed by radical hysterectomy with lymphadenectomy (stages IB1, IIA) or radio/chemotherapy with cisplatin (stages IB2, IIB-IIIB).

Through this article, we suggest the study of the way in which those markers may become a prediction factor in treatment response and in the way in which one could supplement radio/chemotherapy with surgical methods, preferably of low aggressiveness, with the goal to increase survival and patient's quality of life. Some authors have noticed a significant increase in ki67 and p53 in squamous carcinoma in comparison with CIN III lesions (22).

The cases of high malignancy of ki67 were registered in 13 patients (81.25%), 9 of which in stage IIIB, with a mean of HB values of 10g%, 12 patients presenting with a pathology aspect of squamous carcinoma of the uterine cervix and a patient with adenosquamous carcinoma. We have noticed a significant association between the malignancy grade of ki67, tumour stage and the local tumour volume (the higher the ki67 malignancy the higher the stage of the disease and the tumour volume). We can infer that the bigger the ki67 values, the worst the patient's prognosis, as we have seen in the two deceased patients, or in the patients with a residual tumour on the specimens after the complex treatment. We consider that tumour marker ki67 can be a prognostic factor.

Protein p53 may also be a prognostic factor, as high values of this marker were found in advanced stages of the disease (IIIB), but also in patients with a tumour remnant or in the deceased ones, all of the above mentioned leading to a more severe prognostic. A positive association was noticed, strong and statistically significant between the intensity of p53 and the percentage of ki67, helping us establish a malignancy score: the higher the positivity of p53, the higher the

malignancy of ki67. The most frequent pattern was: Ki67+p53+Bcl-2 (12 cases out of 16 – 75%). The association remains significant for bcl-2 (6cases IIIB), more so if p16 was found as well. The cases with treatment resistance had: 2 patterns: Ki67+p53+bcl-2+p16, one case being an adenosquamous carcinoma in which the patient died after 22 months, and one case with ki67+p53+p16+p21. We mention that tumour marker CA-125 was increased only in 6 patients and became normal after the treatment and it was not used to demonstrate tumour aggressiveness and resistivity.

The role played by RFA is also notable, which was applied to 9 patients (7 in stage IIIB and 2 in stage IIB), with a mean Hb of 9.8 g%, the goal being to achieve haemostasis (secondarily, local decrease in tumour volume and also tumour sterilisation due to the increased work temperatures were determined by ultrasound exam). These patients benefited from radio/chemotherapy in better conditions through the correction of the anaemia, but also due to the local hypoxia conditions through the vessels at the level of the uterus. In the patients in which RFA was performed along with the standard treatment, the mean survival was of 45.11 months, even though they were in advanced stages of the disease (7 in stage IIIB and 2 in stage IIB), anaemic and with the presence of the ki67 index of high malignancy (in 6 out of the 9 cases - 66.66%). In 4 patients of the 7 ones with the configuration Ki67+p53+Bcl-2 it was necessary to use high energy with a mean of 47.62kj (45-54,5kj) and an increased time in achieving haemostasis with a mean of 20.5 minutes (15-25 min). Other authors consider as well that in advanced cancers, the association of radio/chemotherapy with hyperthermia (RFA) has better effects than radiotherapy alone in which concerns treatment response without any supplementary increase in toxicity, the efficacy of the above being measured through Bax and Bcl-2 (23,24), but also through p53 and ki67 (25). The data from the literature show that 10% of the tumours with a diameter greater than 5 cm have a partial response in radiotherapy, even when chemotherapy is added, which would imply the need for a personalised treatment to use in those patients. Likewise, if the sensitivity of the tumours to radio/chemotherapy could be predicted, we could use a personalised treatment such as adapting the irradiation dosages, associating different chemotherapy agents, RFA, etc. (25)

In what concerns neoadjuvant chemotherapy, there are clinics which communicate favourable results after its use, mostly in the decrease in tumour volume, the patients being further exposed to either the surgical procedure, or to radio/chemotherapy, under better conditions, but also with good results in the lowering of metastase rates and decreased recurrence rates. It was noticed that some patients respond better to neoadjuvant chemotherapy than others, chemosensitivity being associated with the presence of p53, ki67 and bcl-2. Therefore an increased percentage of ki67 associated with the presence of p53 and bax suggest a better response to chemotherapy (26,27,28,29).

The cases studied so far have shown that the presence of the p16 marker, along with the ones studied, was associated with

an increased aggressiveness of the tumour cell and an increased treatment resistance, probably also caused by the infection with oncogenic typeHPV, as some other authors noticed as well (30,31). Research has demonstrated the importance of the studies of immunohistochemistry in the diagnosis, study of the mechanisms of tumour progression, and also in the evaluation of the prognosis in cancers of the uterine cervix (32).

Conclusions

Once the diagnosis of aggressive neoplasm with resistance to radio/chemotherapy is established, the configuration of these biomarkers could influence further treatment (radiofrequency ablation, neoadjuvant chemotherapy, radio/chemotherapy etc.). Personalised treatment is a trend set by other specialised institutions abroad, which on the long term may equal a significant decrease in the costs of hospital admission, increased patient survival and improvement in the quality of life.

Because of the small number of patients, we consider that future studies are necessary to confirm or deny the results found so far, in order for the patients to fully benefit from the treatment routine they are put through. Likewise, one could also add to the markers studied the following ones: EGFR (epidermal growth factor receptor), VEGF (vascular endothelial growth factor), COX-2 (Cyclo-oxygenases), HIF-1 α (hypoxia-inducible factor), but also the presence or absence of the intra/peritumour infiltrate formed by lymphocytes or plasma cells or of the desmoplastic reaction, which could all help determine radio-chemotherapy resistance and increase the treatment's efficacy.

Conflict of interest and source of funding

No conflicts of interests are declared.

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