Patient Age and Biological Aggressiveness of Endometrial Carcinoma

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Abstact. *Background: Advanced age is associated with a significantly worse prognosis of endometrial carcinoma patients. The aim of this study was to test whether age is a poor-risk factor in endometrial carcinoma because tumors arising in older patients are biologically different from those diagnosed in patients of an earlier age. Materials and Methods: Formalin-fixed, paraffin-embedded samples from 136 previously untreated patients with endometrial carcinoma were studied by means of immunohistochemistry. The expression of molecular markers associated with hormone responsiveness (estrogen and progesterone receptors), proliferation (Ki67, C-ERB-B2, p53), invasiveness (E-cadherin) and apoptosis (BCL2 and p53) was analyzed. The obtained expression levels, together with all available clinical and pathological features were tested for correlations with the patients age and survival. Results: Advanced patient age showed a direct correlation with tumor stage (r=0.29, p=0.0008) and mutant p53 expression (r=0.25, p=0.004), and an inverse correlation with E-cadherin expression (r=–0.28, p=0.001). Patient age above the 25th percentile (57 years) of the age distribution was significantly associated with a worse prognosis (p=0.018). Conclusion: It appears that with advancing age, endometrial carcinoma exhibits a more aggressive tumor phenotype, characterized by mutant p53 expression and down-regulation of E-cadherin expression, and that this, in its turn, results in tumors being diagnosed at a more advanced stage in older patients.*

Advanced age is considered to be a poor-risk factor in endometrial carcinoma, although it is still debated whether this is due to a genuine biological effect, or to other, general high risk factors associated with older age. Farley *et al*. (1), in a multivariate analysis, found that age was a significant independent predictor of outcome in endometrioid carcinoma, and that the decreased survival associated with age was not related to surgical stage or grade. They speculated that the decreased survival they had observed could involve molecular differences in the endometrial carcinomas arising in older, as opposed to younger, women. Similarly, in a multivariate analysis, Jolly *et al*. (2) also found that advanced age, together with higher stage and grade, was associated with significantly lower cause-specific and overall survival of patients with endometrial carcinoma, independently of all other tested variables. In contrast with this, Fleming *et al*. (3), in a very recent report, found that, after adjusting for tumor grade and coronary disease, age ≥70 years was no longer significantly associated with survival in their cohort of patients with stage I and II endometrial carcinoma.

In view of this controversy, we designed the present study in order to test the hypothesis that advanced patient age might indeed be associated with biological differences in endometrial carcinoma, and if so, these should be reflected in significant differences in the expression of distinct molecular markers.

Materials and Methods

Formalin-fixed, paraffin-embedded samples from 136 previously untreated patients with endometrial carcinoma diagnosed at the Marqués de Valdecilla University Hospital, Santander, Spain, between 1st January 2006 and 31st December 2009 were studied. The age range was 42-92 years, with a mean value of 66 years. The histological varieties were as follows: pure endometrioid, 95; mixed, predominantly endometrioid, 19; papillary serous, 7; clear cell, 5; papillary, 2; solid, undifferentiated, 2; other, 6.

Out of the 136 patients, 124 were subsequently operated upon at our center. Of them, disease in 83 was deemed as stage I, in 15 stage II, in 23 stage III and in 3 stage IV. The remaining 12 patients were deemed to have inoperable disease at the time of diagnosis, either for medical reasons, or due to advanced stage, and were subject to primary radiotherapeutic or combined radiotherapeutic and medical treatment.

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By means of immunohistochemistry, we analyzed the expression of molecular markers associated with hormone responsiveness (estrogen and progesterone receptors), proliferation (Ki67, C-ERB-B2, p53), invasiveness (E-cadherin) and apoptosis (BCL2 and p53). The immunohistochemical reactions were carried out under identical conditions, after heat-induced epitope retrieval in citrate buffer, using a pressure boiler and the Dako EnVision system in an automatic Dako Autostainer (both from Dako, Glostrup, Denmark). The antibodies employed and their relative concentrations are summarized in Table I. We used the scoring system described in previous reports (4-6), which includes both the number of reactive tumor cells (fewer than 10%, 10-50%, >50%) and the intensity of staining (none, less than the positive control, as high as the positive control). This results in a semiquantitative score ranging from 0 to 6, which allows for statistical correlation analyses to be carried out as described below.

Tumor stage and grade were also included as variables in the study. The older, 1988 FIGO staging system was employed because it uses three different grades of myometrial invasion, which we felt might be more revealing from a biological point of view in terms of the degree of local aggressivenes. The final surgical stage was converted for statistical purposes into a continuous variable as follows: IA, B, C=1, 2, 3; extension to the cervix, 4; extension to the small pelvis, 5; lymph node extension, 6.

The statistical analysis was performed using the GraphPad Prism (GraphPad Software, San Diego, CA, USA) biostatistical package. The correlation between patients' age and the tested variables was studied by means of the Spearman´s rank correlation test for nonparametric variables. The relationship between all variables and the survival was studied by means of the Kaplan-Meier curves and the log-rank test. The cut-off for younger and older age was calculated using the CRITLEVEL procedure (7). Results were considered significant when *p*-value was <0.05.

Table I. *Antibodies and relative dilutions used in the present study.*

Antigen	Clone	Manufacturer	Dilution
Estrogen receptor	SP1	Dako	Prediluited
Progesterone receptor	PgR 636	Dako	Prediluited
c-ERB-B2	CB11	Dako	1:50
p53	DO7	Dako	Prediluited
BCL2	124	Dako	Prediluited
E-Cadherin	NCH-38	Dako	Prediluited
Ki67	MiB-1	Dako	Prediluited

Results

Of all the tested variables, growing patient age showed a direct correlation with tumor extension (r=0.29, p=0.0008) and mutant p53 expression (r=0.25, p=0.004) and an inverse correlation with the E-cadherin expression (r=–0.28, p=0.001). At the closure of the study, 27 patients had died of their disease. Patient age above the 25th percentile of the distribution (57 years, 32/136 patients) was associated with a significantly worse outcome (Figure 1). This was not due to the accumulation of cases with an unfavorable histological type in the older-age cohort, since a separate analysis for patients harboring carcinomas of the more favorable, and more frequent, endometrioid variety, showed the same results. In fact, all patients 57 years - old or younger of this subanalysis were alive at the closure of this study (Figure 2). All results are summarized in Table II.

Since it might be hypothesized that the correlations obtained between patient age, p53 and E-cadherin expression might be a by-product of the strong direct correlation found between age and tumor stage, we carried out a separate analysis of the correlation between tumor extension and the remaining variables. Interestingly, tumor extension correlated significantly in a direct fashion with tumor grade (r=0.27, p=0.02), as would be expected, and inversely with BCL2 expression (r=–0.20, p=0.024) and the progesterone receptor expression (r=–0.19, p=0.032), but did not correlate with either p53 (r=0.10, p=0.24) or E-cadherin (r=–0.11, p=0.22) expression.

Furthermore, since it has been reported that less favorable histological variants of endometrial carcinoma, *i.e.* papillary serous and clear cell carcinomas, are more frequent in older patients, we carried out separate subanalyses for carcinomas with pure endometrioid (n=95) and non-endometrioid or mixed histology (n=41). Indeed, non endometrioid histology had a strong, statistically significant association with mutant p53 (r=0.40, p=0.01) and diminished E-cadherin expression (r=0.48, p=0.002). The same trend was observed for pure endometrioid carcinomas, albeit not reaching statistical significance (r=0.18, p=0.07 and r=–0.17, p=0.10, respectively). However, the distribution of non-endometrioid histology among patients was not different according to the age in our series (r=–0.04, p=0.60), so that the biological differences of the tumors harbored by younger *vs.* older patients cannot be attributed solely to a predominance of a particular histological type in either cohort.

Discussion

To our knowledge, this is the first time that molecular features of endometrial carcinoma have been systematically tested in relationship to the age of the patients, in an attempt to identify a biological pattern characteristic of younger and older patients, and to settle the question of whether the worse prognosis observed in older endometrial carcinoma patients is attributable to older age *per se*, or to distinct biological features of the tumors arising in that age group. We have found that older age is significantly associated with a biologically more aggressive phenotype, with tumors of a more advanced stage, significantly more frequently expressing mutant p53 and significantly less frequently expressing E-cadherin. Expression of mutant p53 by endometrial carcinoma has been repeatedly shown to be

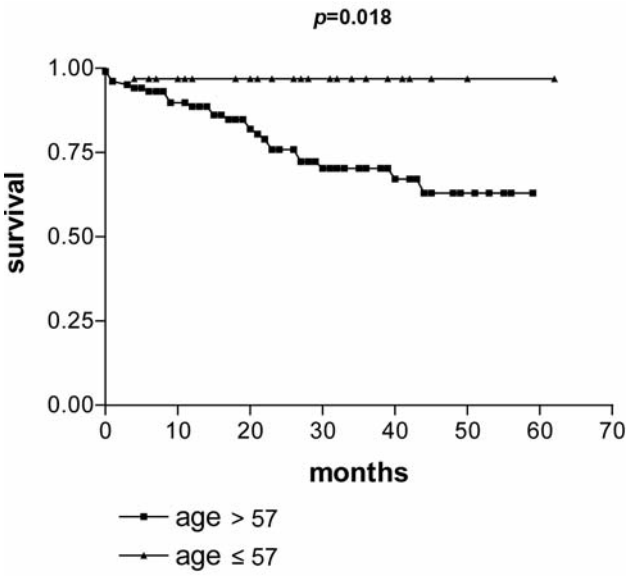

Figure 1. *Influence of patients' age on survival in endometrial carcinoma patients (n=136). The age cut-off was set at the 25th percentile of the age distribution. Analysis was performed by means of Kaplan-Meier curves.*

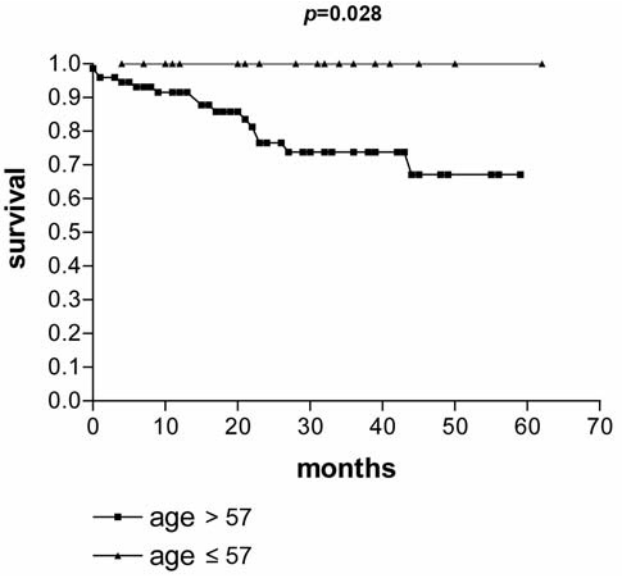

Figure 2. *Influence of patients' age on survival in endometrial carcinoma patients. Only patients with endometrioid histology (n=95) are presented. The age cut-off was set at the 25th percentile of the age distribution. Analysis was performed by means of Kaplan-Meier curves.*

associated with higher tumor aggressiveness and worse patient outcome (8). Down-regulation of E-cadherin expression, in its turn, has also been shown to be a negative prognostic factor (9). The tumors of our older age cohort did exhibit this combination of molecular features with a significantly higher frequency, which resulted in biologically more aggressive tumors at a more advanced stage. This was not attributable to the fact that more advanced-stage tumors are more likely to be found in older women, who tend to delay seeking medical care in the face of clinical symptoms. Indeed, as our subanalysis clearly shows, advanced stage was not significantly associated with the expression of either p53 or E-cadherin, thus the higher frequency of tumors showing this combination of molecular features in older patients cannot simply be a by-product of their being diagnosed at a more advanced stage. Rather, it may be speculated that it is probably the other way round, *i.e.* since endometrial carcinomas of elderly women tend to have a biologically more aggressive phenotype, they grow faster and metastasize earlier to the regional lymph nodes, and therefore tend to be diagnosed at a later stage. These findings have implications that go beyond the merely biological ones: life expectancy in developed countries is steadily rising. In fact, life expectancy for women, particularly in Spain where this investigation took place, is among the highest in the World. This means that the incidence of endometrial cancer, which typically increases with growing age, will significantly rise over the following years, and many of these tumors will be of a more aggressive variety. This, in its turn, means that these patients, according to present treatment protocols, will also be treated more aggressively, both from the surgical and the medical point of view. And this, finally, means that treatment-related morbidity and even mortality and, not to mention costs, will also increase, stretching the public healthcare system to the limit, often for a very little benefit in terms of survival, and above all, quality of life. All these issues have been addressed very recently by Sullivan *et al.* (10), who showed their concern regarding the inequalities that may arise from the immense burden posed on health care systems by the expenses cancer treatment generates in developed countries, which are not always justified by scientific evidence or appreciable benefits for the patients.

Table II. *Correlation of patients' age with clinical and biological variables of endometrial carcinoma. Spearman's rank correlation test.*

Variable	r	*p*-Value
Surgical stage	0.29	0.0008
Histological variety	–0.04	0.60
Estrogen receptor status	–0.07	0.45
Progesterone receptor status	–0.09	0.29
Tumor grade	0.13	0.12
Ki67	–0.03	0.69
c-ERB-B2	0.02	0.75
p53	0.25	0.004
BCL2	–0.05	0.58
E-Cadherin	–0.28	0.001

In conclusion, from our results it appears that, with advancing age, endometrial carcinoma results in a more aggressive tumor phenotype, characterized by mutant p53 expression and down-regulation of the expression of E-cadherin, and that this, in turn, causes the tumors to be at a more advanced stage at the moment of diagnosis in older patients. This will have a substantial impact on healthcare costs in developed countries, due to their demographic development.

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