ANTICANCER RESEARCH *43*: 683-687 (2023)
doi:10.21873/anticanres.16206

Ki67 and E-cadherin Are Independent Predictors of Long-term Survival in Endometrial Carcinoma

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Abstract. *Background/Aim: In previous studies, we identified estrogen receptor, progesterone receptor, Ki67, p53, c-erb-B2, and E-cadherin to be individually associated with the prognosis of endometrial carcinoma. In the present study, we aimed to identify which of the aforementioned are associated with survival after long-term follow-up. Patients and Methods: A total of 106 patients were followed until their demise, or for a median of 120 months in the case of survival (range=84-240 months). At the end of the study, 38 patients had died, and 68 were alive. The association of the studied variables with survival was analyzed by means of a Weibull regression model. Results: A final, restricted model adjusted for age, stage, and histological variety showed both Ki67 and E-cadherin to be independent predictors of a shorter and a longer survival, respectively. Conclusion: Immunohistochemistry for Ki67 and E-cadherin is a cheap and relatively easy-to-interpret laboratory procedure for predicting survival of patients with endometrial carcinoma in clinical practice.*

Endometrial cancer is generally, and wrongly, considered to be a "benign" sort of cancer. This is mainly due to its most prominent clinical feature, *i.e.*, its propensity to announce its presence by way of vaginal bleeding, which in its turn leads to most endometrial cancers being diagnosed at a very early, more curable, stage. However, stage by stage, endometrial cancer is as deadly as any other cancer. Furthermore, even within every single stage of the disease, different biological features define different levels of aggressiveness and hence a different prognosis. Several of these biological traits and their relationship with each other and with tumor aggressiveness have been identified in the past, among others, by our own research group (1-3). However, at present, there is still no consensus about which of them, if any, are significantly, and independently, associated with a better or worse outcome of the disease.

With this in mind, we have submitted our series of patients belonging primarily to our previous studies to a long-term follow up, until the event of death, or for a median period of ten years in case of survival. The pertinent clinical and analytical data were then processed in an attempt to identify which biological features may constitute an improvement in the definition of the prognosis of individual patients.

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Key Words: Endometrial carcinoma, Ki67, E-cadherin.

Patients and Methods

In all, 106 patients were followed until their demise, or for a median of 120 months in the case of survival (range=84-240 months). At the end of the study, 38 patients had died, and 68 were alive. Our whole, larger series of endometrial carcinoma patients was purged of those surviving patients with a follow-up period of less than 72 months, because this was the period in which the bulk (33/38, 86.8%) of death events occurred, with five outliers scattered among the rest of the follow-up period. The series was also purged of those cases not submitted to a complete surgical staging at our center, with an unclear histology, or with two or more missing immunohistochemical data.

Histologically, 89 [84%] were endometrioid carcinomas, followed by the papillary serous [9], mucinous [3], clear cell [2], solid [2] and mixed clear cell/solid variety [1].

Immunohistochemistry. The expression of estrogen and progesterone receptors (ER and PR), Ki67, c-erb-B2, p53, Bcl-2, and E-cadherin were studied by means of immunohistochemistry.
The immunohistochemical technique employed and antibodies used have been described extensively elsewhere (1-3).

Briefly, 5 μM sections were obtained from the corresponding paraffin blocks, and subsequently processed in an automatic Dako autostainer, using the dedicated Dako EnVision system (Dako, Glostrup, Denmark). The antibodies used were also purchased from Dako in prediluted,

Table I. *Full multivariate model. Crude hazard ratios (columns 3 and 4) and hazard ratios (columns 5 and 6), obtained by means of Weibull regression.*

Marker	Categories	Hazard ratio (95%CI)	*p*-Value	Hazard ratio (95%CI)	*p*-Value
Age	Continuous	1.09 (1.05, 1.12)	<0.001	1.10 (1.06, 1.14)	<0.001
Grade	1	1 (ref.)	-	1 (ref.)	-
	2	0.94 (0.22, 3.93)	0.93	1.51 (0.35, 6.57)	0.58
	3	2.76 (0.84, 9.06)	0.09	3.38 (0.97, 11.7)	0.06
Stage	1	1 (ref.)	-	1 (ref.)	-
	2	3.65 (1.56, 8.54)	0.003	2.69 (1.11, 6.52)	0.03
	3	5.84 (2.69, 12.7)	<0.001	5.96 (2.62, 13.5)	<0.001
	4	33.7 (7.13, 159.2)	<0.001	14.0 (2.91, 67.7)	0.001
Ki67 categorical	≤10	1 (ref.)	-	1 (ref.)	-
	11-19	1.51 (0.25, 9.01)	0.65	1.01 (0.15, 6.84)	0.99
	20+	2.63 (0.78, 8.85)	0.12	2.52 (0.63, 10.0)	0.19
Ki67	Continuous	1.008 (0.996, 1.020)	0.18	1.005 (0.992, 1.020)	0.45
RE	1 *vs.* 0	0.78 (0.35, 1.75)	0.54	1.10 (0.47, 2.55)	0.83
PR	1 *vs.* 0	0.53 (0.24, 1.16)	0.11	1.21 (0.48, 3.06)	0.69
C-ERB-B2	1 *vs.* 0	1.66 (0.76, 3.62)	0.20	1.04 (0.42, 2.62)	0.93
Bcl-2	1 *vs.* 0	0.69 (0.30, 1.59)	0.39	0.69 (0.25, 1.86)	0.46
E-cadherin	1 *vs.* 0	0.35 (0.16, 0.79)	0.01	0.47 (0.19, 1.17)	0.10
P53	1 *vs.* 0	1.39 (0.58, 3.31)	0.46	0.51 (0.19, 1.35)	0.17

ready-to-use form, with the only exception of the c-erb-B2 antibody, which was diluted 1:50 in our laboratory prior to use.

All specimens were diagnosed by the same pathologist (IGR), thus ensuring uniformity of interpretation. A final score was obtained from two parameters: number of stained cells (1: 0-10%; 2: 11-50%; 3: >50%), and intensity of staining (absent: 0; weaker than the positive control: 1; similar to the positive control: 2; stronger than the positive control: 3). The addition of both partial scores gave a final score which ranged from 0 to 6.

We used as cutoff levels for positivity the same ones obtained from our previous studies on the biological relevance of the different markers tested (1-3), with the only exception of p53. Thus, only cases in the very upper range (scores 5 & 6) were considered positive in the case of ER, PR, c-erb-B2, and E-cadherin, whereas Bcl-2 was considered positive from score 2 upwards. This is very practical for clinical use, since a strongly positive immunohistochemical reaction in most tumor cells and any degree of positivity in any number of tumor cells are relatively easy to interpret. Ki67 was treated as a continuous variable, expressed as the percentage of positive cells. The case of p53, as mentioned, is particular. In our previous studies (1-3), we had applied a cutoff upwards of 10% staining nuclei, often used in practice for the identification of the mutant protein. However, recent evidence (4) points towards the fact that, in the particular case of endometrial carcinoma, as addressed here, only very strong nuclear staining in virtually all tumor cells represents mutant p53 protein. We have thus shifted our cutoff correspondingly to the upper tertile of the distribution (scores 5 & 6), at variance with our previous reports.

Ethics. The study, involving data and specimens from actual patients treated at the institution, was approved by the Ethics Committee (code 2021.405) of Hospital Universitario Valdecilla, Santander, Spain.

Statistics. The statistical analysis was carried out after blinding all personal data pertaining to the individual patients studied. Differences in survival were calculated by means of the Kaplan–Meier method and visualized by the corresponding curves.

Table II. *Restricted multivariate model adjusted for age and histological variety.*

Marker	Categories	Hazard ratio (95% CI)	*p*-Value
Ki67 categorical	≤10	1 (ref.)	-
	11-19	1.76 (0.42, 7.30)	0.44
	20+	4.81 (1.74, 13.3)	0.003
Ki67	Continuous	1.017 (1.007, 1.027)	0.001
RE	1 *vs.* 0	0.84 (0.41, 1.72)	0.64
PR	1 *vs.* 0	0.55 (0.27, 1.14)	0.11
C-ERB-B2	1 *vs.* 0	1.45 (0.75, 2.81)	0.27
Bcl-2	1 *vs.* 0	0.70 (0.33, 1.45)	0.33
E-cadherin	1 *vs.* 0	0.41 (0.20, 0.85)	0.02
P53	1 *vs.* 0	0.95 (0.47, 1.91)	0.88

Hazard ratios were calculated by means of a Weibull regression model (5). Successive restricted models, eliminating confounding variables yielded a final restricted model best adapted to practical use in the clinical setting. Values were considered significant when $p<0.05$.

Results

In our initial, crude model (Table I), traditional prognostic clinical variables, such as stage, tumor grade, and age were so overwhelmingly strong, that they overshadowed all other variables tested. However, the interest of this study lies

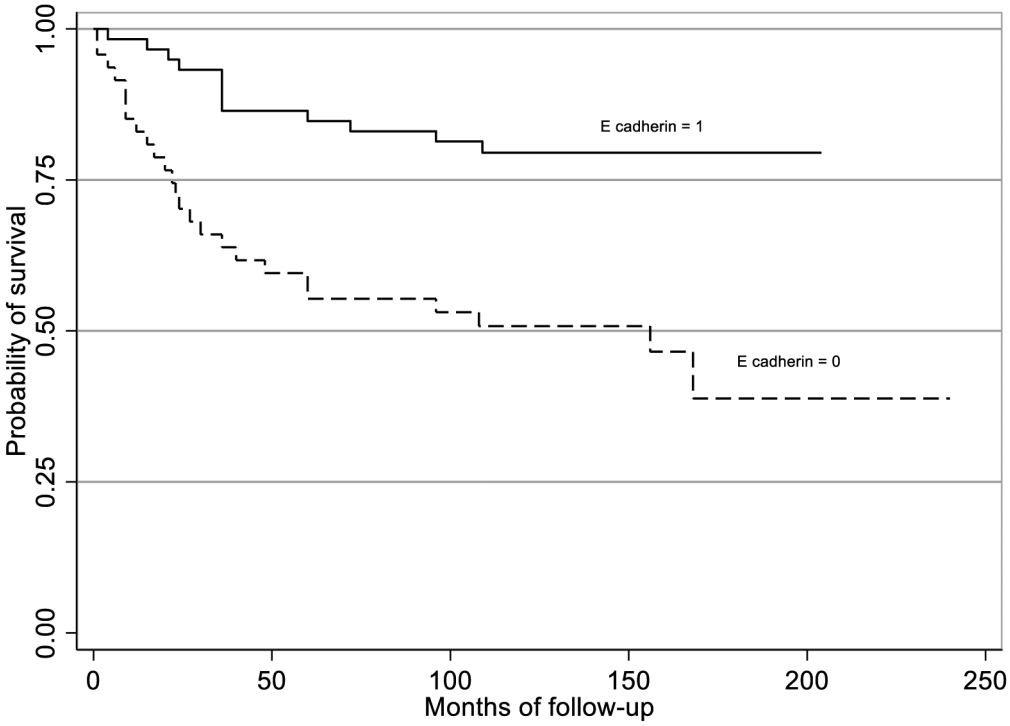

Figure 1. *Survival of endometrial carcinoma patients according to the tumor presence (1) or absence (0) of E-cadherin expression.*

precisely in ascertaining the influence of molecular marker expression independently of clinical parameters, and independently of patient age, since endometrial carcinoma is a cancer typical of old or very old patients, many of whom tend to die over a relatively short period of time from causes entirely different from the treated cancer. By adjusting for these variables in our model, the real significance of the molecular markers tested emerges.

Our final restricted model (Table II) shows that E-cadherin is a predictor of better survival, whereas Ki67 is a predictor of worse survival, both independently of age, stage, and histology (Table I, Figure 1 and Figure 2), which have been eliminated by adjustment for them. PR expression, identified in the initial, crude model (Table I) as potentially interesting, did not attain statistical significance in the final model as an independent variable, most probably due to its interrelationship with most clinical and molecular parameters, as evidenced in our previous publications on the subject (1-3).

Survival in relationship with Ki67 and E-cadherin expression is visualized in the corresponding Kaplan–Meier curves (Figure 1 and Figure 2). The Ki67 variable, which is continuous, has been dichotomized at the 20% level, which is an often-used cutoff in the clinical setting, relatively easy to interpret in practice, and has been identified as the most discriminating one by means of the "CRITLEVEL" statistical procedure (6).

Discussion

To the best of our knowledge, this is a study on the relationship of molecular markers with survival of patients with endometrial cancer on the longest follow-up ever published. This eliminates the confounding effect exerted by markers with a promising significant short-term influence on survival, which disappears eventually, common to many similar studies.

Of all the molecular markers under scrutiny, only two retained their independent long-term prognostic value after multivariate analysis: Ki67, representing proliferation of the tumor cells, and E-cadherin, representing adhesiveness to their neighboring cells. This is completely understandable from the biological point of view. Excessive proliferation is the first step in the oncogenic transformation of normal cells, and proliferation is a paramount prognostic factor for almost any cancer, endometrial carcinoma included. Atram *et al*., in a very recent study, corroborated this for endometrial carcinoma (7). However, they also found p53 to have independent prognostic value, which we did not. This may have to do with the confounding effect mentioned above, due to a shorter period of follow-up. It may be also attributable to the different cutoff level of positivity contemplated in our study, or to the stricter statistical analysis used by us (Weibull regression), compared to the traditional Cox proportional hazards model employed by Atram *et al*. Furthermore, in one of our previous studies

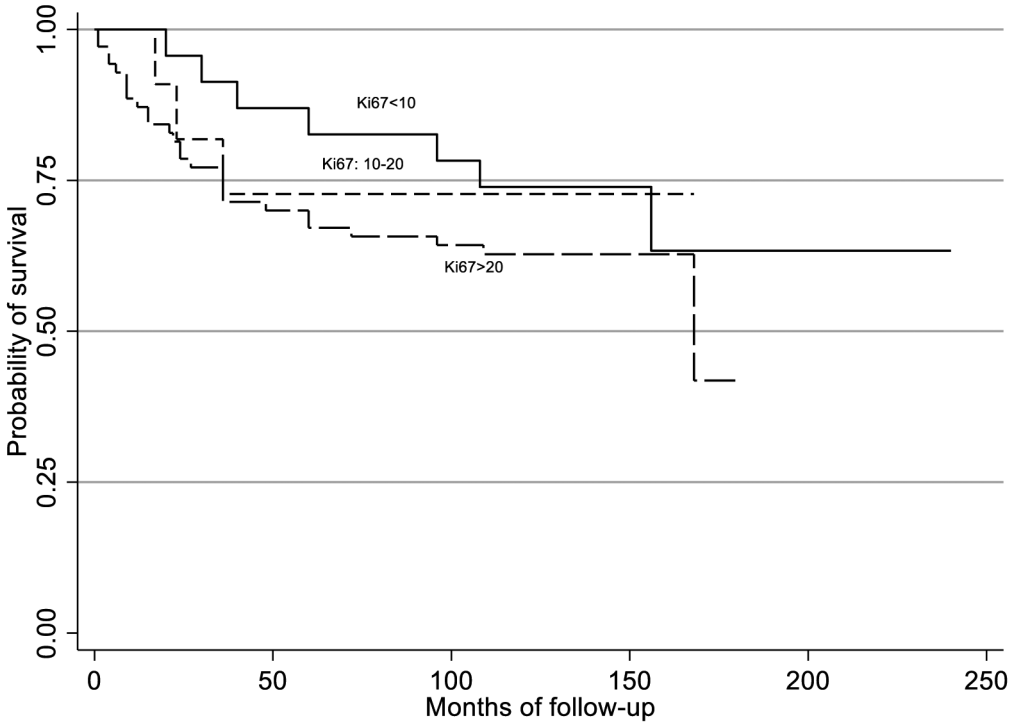

Figure 2. *Survival of endometrial carcinoma patients according to the percentage of tumor cells expressing Ki67.*

(1), we showed that p53 is significantly co-expressed with E-cadherin, and this alone may have downgraded the significance of p53 in our multivariate model, the prognostic weight of E-cadherin finally predominating. In any case, both Ki67 and p53 reflect proliferation, albeit from a different perspective (proliferation rate and proliferation control, respectively), so that there is no conceptual discrepancy between our findings and those of Atram *et al*.

Flindris *et al*., finally, have recently reported a very interesting highly significant association between NRIP1 (nuclear receptor interacting protein 1) and Ki67 expression in endometrial cancer (8). In their study, NRIP1 expression was also strongly correlated with myometrial invasion, so that Ki67 expression in endometrial cancer may not only reflect a higher proliferation rate of the tumor cells, but also, indirectly, higher tumor invasiveness.

Loss of E-cadherin expression, in its turn, reflects the ability of tumor cells to metastasize, which is what ultimately kills the host. Therefore, it is logical that E-cadherin expression might be a strong independent predictor of survival. Singh *et al*., in a study on advanced (stage IV) or recurrent endometrial carcinoma, found that both E-cadherin and p16 expression were independent predictors of survival (9), something corroborated now by our data for any stage of the disease.

However, endometrial carcinoma being an eminently hormone-dependent tumor, neither ER, nor PR expression showed a significant association with survival. PR expression with a corresponding *p*-value in the vicinity of significance (0.11), especially in such a strict multivariate model as the one employed by us, seems to have an influence on prognosis, and could be a factor eventually to be considered in practice in combination with the aforementioned two.

Conclusion

Immunohistochemistry for Ki67 and E-cadherin is a cheap and relatively easy to interpret laboratory procedure, especially at the cutoff levels established by us, which should allow for standardization or at least a high degree of interobserver agreement. This presents additional advantages for the use of these markers to better predict survival of patients with endometrial carcinoma in clinical practice.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

BMS and DE collected and processed all the data and participated in the final revision of the manuscript; JLL performed the statistical analysis and participated in the final revision of the manuscript; JS devised the study, wrote the first draft of the manuscript and its final version after revision by all Authors.

References

1 González-Rodilla I, Verna V, Muñoz AB, Estévez J, Boix M and Schneider J: Expression of the apoptosis-related genes Bcl-2 and p53 in clinical samples from endometrial carcinoma patients. Anticancer Res *31(12)*: 4191-4193, 2011. PMID: 22199279.

2 Gonzalez-Rodilla I, Boix M, Verna V, Muñoz AB, Estévez J, Jubete Y and Schneider J: Patient age and biological aggressiveness of endometrial carcinoma. Anticancer Res *32(5)*: 1817-1820, 2012. PMID: 22593467.

3 González-Rodilla I, Aller L, Llorca J, Muñoz AB, Verna V, Estévez J and Schneider J: The E-Cadherin expression vs. tumor cell proliferation paradox in endometrial cancer. Anticancer Res *33(11)*: 5091-5095, 2013. PMID: 24222154.

4 Köbel M, Ronnett BM, Singh N, Soslow RA, Gilks CB and McCluggage WG: Interpretation of P53 immunohistochemistry in endometrial carcinomas: Toward increased reproducibility. Int J Gynecol Pathol *38(Suppl 1)*: S123-S131, 2019. PMID: 29517499. DOI: 10.1097/PGP.0000000000000488

5 Zhang Z: Parametric regression model for survival data: Weibull regression model as an example. Ann Transl Med *4(24)*: 484, 2016. PMID: 28149846. DOI: 10.21037/atm.2016.08.45

6 Abel U, Berger J and Wiebelt H: CRITLEVEL: an exploratory procedure for the evaluation of quantitative prognostic factors. Methods Inf Med *23(3)*: 154-156, 1984. PMID: 6548543.

7 Atram MA, Shivkumar VB and Gangane NM: Immunohistochemical analysis of novel biomarkers cyclin D1, p53 And Ki67 in endometrial carcinoma: Clinicopathological significance and prognostic value. Gulf J Oncolog *1(38)*: 15-23, 2022. PMID: 35156640.

8 Flindris S, Katsoulas N, Goussia A, Lazaris AC, Navrozoglou I, Paschopoulos M and Thymara I: The expression of NRIP1 and LCOR in endometrioid endometrial cancer. In Vivo *35(5)*: 2631-2640, 2021. PMID: 34410950. DOI: 10.21873/invivo.12545

9 Singh M, Darcy KM, Brady WE, Clubwala R, Weber Z, Rittenbach JV, Akalin A, Whitney CW, Zaino R, Ramirez NC and Leslie KK: Cadherins, catenins and cell cycle regulators: impact on survival in a Gynecologic Oncology Group phase II endometrial cancer trial. Gynecol Oncol *123(2)*: 320-328, 2011. PMID: 21813170. DOI: 10.1016/j.ygyno.2011.07.005

Received November 28, 2022
Revised December 9, 2022
Accepted December 12, 2022