Triple negative endometrial cancer: Incidence and prognosis in a monoinstitutional series of 220 patients

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Abstract. Endometrial cancer (EC) represents the most frequently occuring gynecological tumor worldwide. The aim of the present study was to estimate the prognostic value of triple negative phenotype (TNP) in EC, and any associations with to pathological and clinical characteristics. The present study includes 220 cases of patients with EC who underwent to surgery at the Guglielmo da Saliceto Hospital of Piacenza (Italy) and the expressions of estrogen receptor (ER), progesterone receptor (PR) and oncoprotein c-erbB-2 (HER2) expression were examined. Pearson's Chi-square and Fisher's exact test were used to evaluate the association of TNP cases with variables associated with a worse prognosis. Progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier curves. A total of 26 patients (12%) had a TNP, and these cases had a higher percentage of high-risk histology, an advanced stage of disease at the time of diagnosis, with shorter PFS and OS when compared to non-TNP. The present study confirmed that TNP represents prognostic significance in EC.

Introduction

Endometrial cancer (EC) represents the most frequent gynecologic tumor in developed countries (1). The majority of patients presents with early stage, well-differentiated, limited myometrial invasive tumors and favorable prognosis, but a subset of women recurs and do not survive from the disease (2). EC is hormone-dependent and, like breast cancer, can express markers such as estrogen receptors (ERs), progesterone receptors (PRs) and oncoprotein c-erbB-2 (HER2) (3,4). Breast cancer can be characterized into several subgroups based on immunohistochemistry: Luminal A (ER^+ and/or PR^+, $HER2^-$, low Ki67); luminal B (ER and/or PR^+, $HER2^-$, high Ki67); non-luminal (ER^-, PR^-, and $HER2^+$); and triple negatives (ER^-, PR^-, and $HER2^-$) (5). This classification finds clinical application in terms of prognosis and treatment personalization. Since $HER2^+$ and triple negative (TN) subtypes show a poor prognosis, patients are treated with more aggressive treatments (chemotherapy and immunotherapy), both in adjuvant and in metastatic disease (6-8). Particularly, triple negative phenotype (TNP) is often associated with unfavorable pathological features such as high nuclear grade, high proliferation rate, increased risk to show distant metastasis at the diagnosis or to recur after surgery. The absence of ER, PR and HER2 influence the poor response to treatments currently available causing poor prognosis of this subgroup of patients (9). Furthermore, no therapeutic targets have yet been established in this subgroup of breast cancer (10). Ongoing studies are focalizing on target therapy. For example, PARP inhibitors have demonstrated a potential benefit in association with chemotherapy in patients affected by TN breast cancer (11). EC molecular subtypes based on ER, PR and HER2 status have proven to differ in terms of prognosis and clinicopathological data: ER and PR expression leads to a more favorable prognosis, while overexpression of p53, HER2 and epidermal growth factor receptor (EGFR) all predict a poor prognosis (3,4,12-14). Nevertheless, currently the choice of treatment in EC is not conditioned by molecular features of the tumor.

The aim of our study is to estimate the prognostic value of TNP in EC related to pathological and clinical characteristics.

Materials and methods

This retrospective study includes two hundred and twenty patients diagnosed with EC at the Guglielmo da Saliceto hospital of Piacenza (Northern Italy) between January 1, 2000 and December 31, 2010. All patients underwent

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Abbreviations: DFS, disease-free survival; EC, endometrial cancer; ER, estrogen receptor; EGFR, epidermal growth factor receptor; ESMO, European Society of Medical Oncology; FIGO, Internazional Federation of Gynecology and Obstetrics; FISH, fluorescence *in situ* hybridization; OS, overall survival; PFS, progression-free survival; PR, progesterone receptor; TNEC, triple negative endometrial cancer; TNP, triple negative phenotype

Key words: endometrial cancer, triple negative endometrial cancer, gynecologic cancer

total abdominal hysterectomy with or without bilateral salpingo-oophorectomy. Only patients with tissue available for staging and histological review were included; fifty eight cases of EC were excluded because biopsy was not followed by hysterectomy. Postoperatively, some only three patients received adjuvant treatment, one triple negative endometrial cancer (TNEC) and two non-TNECs. Follow-up information was available until December 2016; the follow-up period ranged from a minimum of 55 to a maximum of 170 months. A database with demographic, clinical and pathologic information for each patient was created. The study was approved by the Institutional Review Board of Hospital of Piacenza, and conducted according to the Declaration of Helsinki. Pathologist performed specimens review of all endometrial cancers, redefining the histologic type, grade, depth of myometrial invasion, and immunohistochemical expression of ER, PR, HER2 and Ki67. The same tissue from formalin-fixed, paraffin-embedded donor blocks were precisely arrayed into a new recipient paraffin block (35x20 mm). Paraffin-embedded tissue was cut at 3 μm thickness and placed on positively charged slides. Slides were placed in a 60˚C oven for 1 h, cooled, then deparaffinized. Immunohistochemical techniques can be used to demonstrate the presence of antigens in tissue. PR clone 16 and ER clone 6F11 antibodies were specifically optimized for use in a dedicated automation system (Leica Biosystems Ltd., Newcastle, UK), in combination with Bond Polymer Refine Detection, a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. This detection system avoids the use of streptavidin and biotin, and therefore eliminates nonspecific staining as a result of endogenous biotin. The system is based on consecutive application of: i) Specimen, incubated with hydrogen peroxide to quench endogenous peroxidase activity; ii) ready-to-use primary PR (or ER) antibody; iii) post-primary antibody solution, which enhances the penetration of subsequent polymer reagents; iv) poly-HRP anti-mouse/rabbit IgG reagent, localizing the primary antibody; and v) the chromogenic substrate 3,3'-diaminobenzidine (DAB), allowing the revelation of the antibody complex via a brown precipitate. A blue counterstain of cell nuclei is provided by Hematoxylin coloration. For ER and PR, immunohistochemical results were evaluated both as a percentage of nuclear staining and as intensity of staining. The results were recorded as 3+ for strong or weak nuclear staining in >50% of cells, 2+ for strong or weak nuclear staining in 10 to 50% of cells, and 1+ for strong or weak nuclear staining in <10%. Cases with no evidence of nuclear staining, or only rare scattered positive cells, were recorded as negative (0). HER2 expression was performed using Pathway HER2 CB11 clone) on the Leica automated system (Leica Biosystems Ltd.) according to the manufacturer's recommended protocol. Positive tissue controls are used to indicate correctly prepared tissues and proper staining techniques. One positive tissue control should be included for each set of test conditions in each staining run. The score was recorded as 3+ for complete strong membrane staining in >10% of tumor cells, 2+ for complete moderate membrane staining in >10%, 1+ for incomplete staining or complete staining in <10% of tumor cells, and 0 for no staining or staining without a membranous pattern. Biomarker expression for ER, PR and HER2 was designated as positive for 3+ and 2+, and negative for 1+ and 0. In most cases, we used fluorescence *in situ* hybridization (FISH) for the determination of HER2 gene amplification status when the test results were borderline (2+). HER2 receptors receive signals that stimulate the growth of cancer cells. Tumors staining negative for ER, PR, and HER2 were designated as TNP and those with one or more positive stains were designated as non-TNPs. All 220 cases of EC underwent comprehensive surgery and were therefore were stratified as low, intermediate and high risk according to the European Society of Medical Oncology (ESMO) guidelines, which are based on both pathological and surgical staging. Risk groups were related to the expression of ER, PR and HER2.

Statistical analysis. Pearson's Chi-square (χ^2) and Fisher's exact test were used to evaluate the association of TNP cases with several variables associated with a worse prognosis. Progression-free survival (PFS) and overall survival (OS) were analyzed with Kaplan-Meier curves. This function uses the Kaplan-Meier procedure to estimate the survival function. All patients were set to a standard starting time (t_0), and cases were censored as they quit follow-up. The log-rank test was used to compare the groups (TNEC vs. non-TNEC). All tests were two-tailed, and the P<0.0001 was considered to indicate a statistically significant difference.

Results

Two hundred and twenty patients were included in our study. All patients were Caucasian. The median age at the diagnosis was 67 years (range, 36-89). One hundred and ninety-nine showed endometrioid histotype (90.5%) and 21 were high risk histological type, 11 with papillar serous type (5%), 9 clear cell (4%), and 1 carcinosarcoma (0.5%). Eighty-five (38.6%) patients were placed in the G1 grading class, 87 (39.5%) were G2 and 48 (21.9%) G3. Ninety-five cases (43.2%) showed deep myometrial invasion (>50%) and 105 (56.8%) low invasion <50%. Sixty-four (29%) patients showed low Ki67 (≤30%) and 156 (71%) Ki67 >30%. Regarding stage at the diagnosis, 173 cases (78.6%) were staged in according to the revised 2009 FIGO (International Federation of Gynecology and Obstetrics) staging system for EC, resulting in 18 (8.2%) stage II, 8 (3.7%) III, and 21 (9.5%) IV.

Twenty-six (12%) patients showed a TNP. The clinicopathological characteristics of the two groups of patients included in the study are summarized in Table I. TN cases had a higher percentage of grade 3 (42.3 vs. 19%), high risk histology (34.6 vs. 6.2%), advanced stage (38.5 vs. 9.8%) and high grade disease (42.3 vs. 28.8%) compared to the non-TN subgroup. In this pattern of patient the deep myometrial invasion, lymph node metastasis and cervical involvement were similar between two groups. Relapses were significantly higher in the TN patients group (39.1 vs. 12.3%). Outcome was also more favorable for non-TN cases (Table II). Kaplan-Meier plots showed significantly shorter PFS and OS in TN patients compared to non-TN cases (log-rank test, P<0.0001; Fig. 1). We could only calculate the median disease-free survival (DFS) for TNECs (34 months), as other estimates did not reach 0.5. The 5-year OS rate was 34.8% in TNPs compared to 64.7% in control group.

Table I. Clinicopathological features of triple negative endometrial cancer (TNEC) and non-triple negative endometrial cancer (NON-TNEC).

	TNEC + non-TNEC (n=220)		TNEC (n=26)		Non-TNEC (n=194)		
Variable	n	%	n	%	n	%	P-value
Median age at diagnosis	67		66		71		
Patients <65	87	39.50	5	19.20	82	42.30	0.0004
Patients ≥65	133	60.50	21	80.80	112	57.70	
Stage							
I	173	78.60	15	57.70	158	81.40	<0.0001
II	18	8.20	1	3.80	17	8.80	
III	8	3.60	3	11.50	5	2.60	
IV	21	9.50	7	26.90	14	7.20	
Histology							
Endometriod	199	90.50	17	65.40	182	93.80	<0.0001
Clear cell	9	4.10	4	15.40	5	2.60	
Serous	11	5.00	5	19.20	6	3.10	
Carcinosarcoma	1	0.50	0	0.00	1	0.50	
Grading							
G1	85	38.60	4	15.40	81	41.80	<0.0001
G2	87	39.50	11	42.30	76	39.20	
G3	48	21.80	11	42.30	37	19.10	
Myometrial invasion							
<50%	125	56.80	14	53.80	111	57.20	0.6727
>50%	95	43.20	12	46.20	83	42.80	
Early	191	86.80	16	61.50	175	90.20	<0.0001
Advanced	29	13.20	10	38.50	19	9.80	
Risk group							
Low to intermediate[a]	153	69.50	15	57.70	138	71.10	0.057
High[b]	67	30.50	11	42.30	56	28.90	

[a]Endometrioid G1, G2; [b]endometriod G3, clear cell, serous, carcinosarcoma. TNEC, triple negative endometrial cancer; NON-TNEC, non-triple negative endometrial cancer; G, grading. Risk group was assessed according to the 2016 ESMO-ESGO-ESTRO guidelines (25).

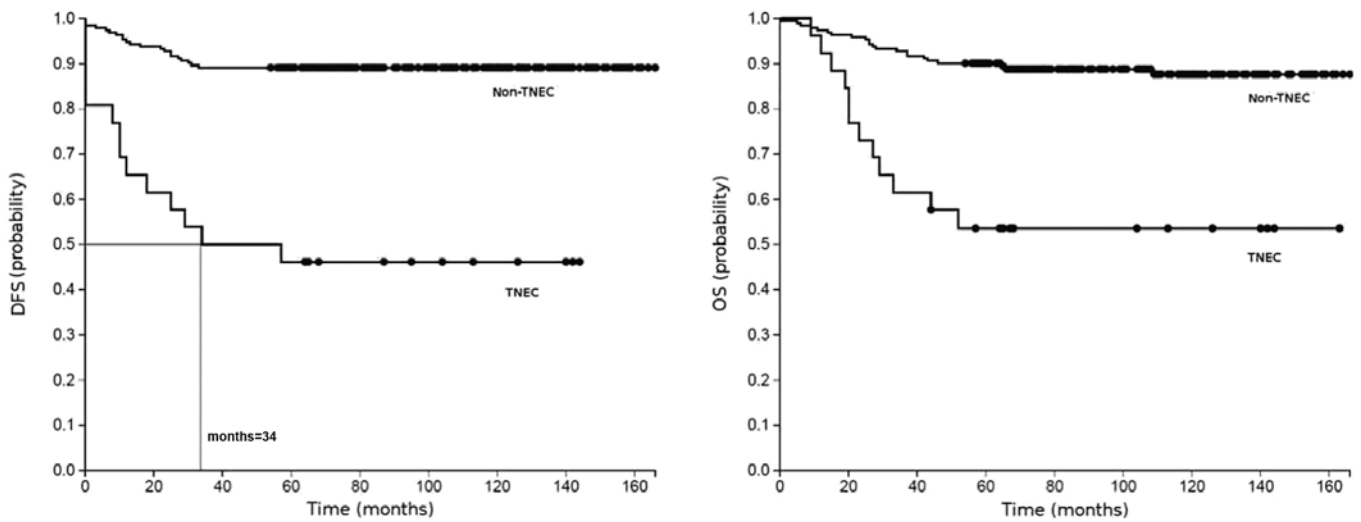

Figure 1. Kaplan-Meier plots for DFS and OS in TNECs and non-TNECs. Dots, censored cases (mortality from other cause or quit follow-up). DFS, disease-free survival; OS, overall survival; TNEC, triple negative endometrial cancer; non-TNEC, non-triple negative endometrial cancer.

Table II. Outcome of TNEC and non-TNEC.

	TNEC + non-TNEC (n=212)		TNEC (n=23)		Non-TNEC (n=189)		
Outcome	n	%	n	%	n	%	P-value
Alive	165	77.80	10	43.50	155	82.00	<0.0001
Deceased	47	22.20	13	56.50	34	18.00	
Alive without disease	158	74.50	9	39.10	149	78.80	<0.0001
Alive with disease	7	3.30	1	4.30	6	3.20	
Deceased (from disease)	34	16.00	12	52.20	22	11.60	
Deceased (other cause)	13	6.10	1	4.30	12	6.30	

TNEC, triple negative endometrial cancer; non-TNEC, non-triple negative endometrial cancer.

Discussion

The term 'triple negative' (TN) is used to define a specific subtype of breast cancer, which is characterized by absence of ER, PR, and HER2 expression (9). This subgroup of breast cancers is clinically more aggressive than other subgroups, causing poor prognosis with low response to therapies and short-term survival of patients (10,11). TNP is poorly investigated in other types of tumors, although some authors report that the loss of estrogen, progesterone, and HER2 receptors predict poor prognosis also in gynecological cancers. In EC, TNPs are observed in ~15-20% of patients and are related to unfavorable pathological features such as high risk histological type, deeper myometrial invasion, higher histological grade and clinical staging, and shorter survival (12-15). For example, Kothari *et al* reported that in a group of patients affected by EC the TNP was associated with advanced stage, high grade, and high risk histology, as well as poor survival, as compared to non-TNPs (16). A similar percentage of TNs observed in ovarian, endometrial, and breast cancers, may suggest a similar pathogenesis for these neoplasms (17). Our research, comparing the difference in clinicopathological parameters between TNEC and non-TNEC cases, confirms that the TNP has prognostic significance in EC. In our cohort, 12% of EC cases showed a TNP, which is consistent with previous studies. Very few patients underwent adjuvant chemotherapy and radiotherapy after surgical resection, and they were equally distributed between the two groups of TNECs and non-TNECs. Therefore, different treatments did not affect PFS and OS. Our data revealed that most TNECs showed high-grade features, such as advanced stage, high clinical grade, and high risk histology, as well as poor survival. This was also confirmed by other authors. TNEC cases show shorter progression-free and OS than non-TNEC cases, thus indicating that TNP may be an independent prognostic factor for progression-free and OS in EC. Although TN cases account for only a minority of patients among EC, this subgroup should be regarded as a clinically important subtype owing to its aggressive clinicopathological characteristics. Confirming the correlation between TNP and poor prognostic factors is important, as TNP in endometrial tumors could potentially predict a lack of response to specific therapies. This was widely described for TN breast cancers, which are not responsive to antiestrogens or trastuzumab (7,9,11). Studies continue to be conducted to find the best approaches to treat triple-negative tumors. Recent clinical trials are trying to investigate whether some targeted therapies are effective against triple-negative breast cancer (18). These treatments are aimed to different and novel targets. The specific role of some mutations in possible driver genes for these tumors is also under investigation. BRCA-1 mutations, which are present in a subgroup of TN breast cancer, deprive constitutively tumor cells of a DNA repair mechanism, increase platinum sensitivity, and seem to sensitize cells to the therapy with poly ADP-ribose polymerase (PARP-1) inhibitors (19). The mutation of PTEN gene leads to the loss of the suppressing effects of its encoded protein on the PI3-K/AKT pathway, and consequently to an increased activity of mTOR protein kinase, thus interfering with the cell cycle and apoptosis of tumor cell. Abnormal PTEN expression seems to improve the cellular response to therapies with mTOR inhibitors. PTEN gene mutations are also described in EC, as associated with positive prognostic factors (favorable histological type, lower histological grade, absence of myometrial invasion, and lower clinical staging). These mutations are also related to tumor response to chemotherapy (20,21). Clinical studies are being conducted on PARP-1 inhibitors and other drugs which can block mTOR and PI3K/AKT pathways. These studies are performed in all types of TN tumors, due to their similar pathogenesis and the equal role of DNA repair pathways (22). Many TN breast cancer cells overexpress epidermal growth factor receptors (EGFR), which receive signals that stimulate the growth of the cancer. Drugs targeting EGFR could block growth signals on the cancer cells also in EC (23). Other studies showed that a higher CD151 expression in TN breast cancers is associated with shorter survival time and poor prognosis, but data on the expression levels of these receptors are limited. A recent study examined the relation between tumor related macrophages and the TNEC. In this study, a higher percentage of tumor related macrophages was found in TN cancers (24). Due to their aggressive profiles and poor prognosis, TN cancers are important objects of research and may prove to benefit from individualized target therapies.

In conclusion, TNP EC represent a subset of EC with a worse prognosis with a shorter PFS and OS. Our results reported here are confirmed in other studies, the evaluation of ER, PR and HER-2 should be therefore integrated

into the prognostic factor of EC and this submit of TNP EC be considered a potential target for possible experimental treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RP and LC designed and supervised the trial. RP, CC, AMR, AU, FB, MP, CDN and LC were responsible for patients and data collection. RP and CC analyzed the data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval consent to participate

The present study was approved by the Local Ethics Committee of Piacenza General Hospital. Informed consent was previously obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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