TBILISI STATE MEDICAL UNIVERSITY

By the right of manuscript

Irina Dvalishvili

THE PROGNOSIS OF UTERINE ENDOMETRIOID CARCINOMA BY IMMUNOHISTOCHEMICAL EXPRESSION OF CATHEPSIN D AND CADHERIN E

14.00.14 – Oncology

A U T O R E F E R E N C E

of Dissertation Presented to Acquire the Scientific Degree of Candidate of Medical Sciences

TBILISI

2006

This study has been performed at National Cancer Center of Georgia and Department of Pathological Anatomy of Tbilisi State Medical University

Scientific Supervisor – Professor L. Charkviani
M.D., Ph.D., Sc.D.

Scientific Consultant – Professor G. Burkadze
M.D., Ph.D., Sc.D.

Official Opponents – Professor Vl. Kuchava
M.D., Ph.D., Sc.D.

B. Tkeshelashvili
M.D., Ph.D., Sc.D.

The Defense of the Dissertation will be held _____ 2006, at ___ Scientific Counsil meeting m.14.27 №8 at Tbilisi State Medical University (0177, Tbilisi, 33 Vazha-Pshavela Avenue)

Dissertation is available at the library of Tbilisi State Medical University (0160, Tbilisi, 29 Vazha-Pshavela Avenue)

Autoreferences were sent on ____ _____ 2006

Secretary of Scientific Qualifying Council,
M.D., Ph.D., Sc.D. Professor G. Lobzhanidze

GENERAL CHARACTERIZATION OF STUDY

Actuality of the problem. Endometrial carcinoma is the third most common gynecological cancer worldwide, following uterine cervix and ovarian cancers (Morrows and Curtin JP, 1998). The estimated total number of new cases of endometrial cancer is 142 000 per year or 3.7% of cancers in women (Parkin DM et al, 1999).

The prognosis of endometrial cancer is based on a variety of factors. The prognostic factors are usually related to the pathological changes detected during surgery (DiSaia and Creasman WT, 1997). Clinical stage is found to be the most important prognostic factor (Ascher Smet al, 2001). The majority of endometrial cancers are endometrioid adenocarcinomas (Creasman WT et al, 2004). Histological grade seems also to be an important prognostic factor. In the patients with well differentiated adenocarcinomas endometrium or superficial myometrium is involved, and extrauterine spead is uncommon. Poorly differentiated tumors tend to be more aggressive and deeply invade myometrium (Saez F. et al, 2000), metastatic spead is not uncommon (Kilgore LC et al, 1995).

The risk of metastasis increases with the depth of myometrial invasion (DelMaschio A. et al, 1993), and the latter is associated with tumor grade (Fleischer AC et al, 1987, Dubinsky TJ et al, 1999). The cytological changes of peritoneum are correlated to other prognostic factors such as depth of myometrial invasion and lymph node metastases (Farghaly SA, 2004).

To date, there are new prognostic factors, which are related to the advances of molecular biology. However, their importance is still disputable. These prognostic factors are as follow: evaluation of proliferation fraction (S phase) of tumor cells, high expression of genes (HER-2/neu, TP53).

In past years the role of cadherins, especially E cadherin, has been intensively studied. The expression and/or function of E-cadherin is lost in relation to tumor progression (Takeichi, 1993; Birchmeier and Behrens, 1994). Therefore, E-cadherin can be considered as an invasion supressor gene (van der Linden PJ. 1996).

In normal endometrium the expression of E cadherin and beta-catenin is higher during proliferative phase and decreases in secretory phase (Fujimoto J. et al, 1998). It has been suggested that the expression of E-cadherin and beta-catenin is significantly lower in uterine endometrioid adenocarcinoma than in normal endometrium. Furthermore, their expression is lower in poorly differentiated cancers in comparison to well differentiated cancers (Shih HC et al, 2004). However, Fujimoto J. et al. (1996) have showed that the expression of mRNA of E cadherin, alpha- and beta-catenins is significantly lower in proliferative phase. van der Linden PJ et al. (1995) have reported that the expression of cadherin does not change according to the

phases of menstrual cycle. Sakuragi N. et al. (1994) suggest that the expression of cadherin E is associated with tumor differentiation, depth of myometrial invasion and presence of metastates in paraaortic lymph nodes.

Tumor invasion is greatly influenced by remodeling of extracellular matrix. Endoproteases degrade the components of extracellular matrix directly or activate another proteases (van der Stappen JW et al, 1996). It has been reported that cathepsin D is differentially expressed in different phases of normal menstrual cycle. Camier B. et al. (1996) have showed that the expression of cathepsin D is significantly higher in secretory phase in comparison to the proliferative phase, also in endometrial adenocarcinoma in comparison to normal endometrium and correlated to the depth of myometrial invasion (Maudelonde T. et al, 1990).

The expression of cathepsin D is primarily limited to the glandular epithelium, stromal cells are weakly positive only. Atypical endometrial hyperplasias show higher expression of cathepsin D in comparison to the normal endometrium, and endometrial carcinoma is negative (Sato S. 1993., Mylonas I. et al, 2003., Ioachim E. et al., 2003).

in 1989 Rochefort H et al., (1989) supposed that high expression of cathepsin D is correlated to the aggressiveness of endometrial adenocarcinoma. Cathepsin D seems to be an independent prognostic factor for endometrial cancer, its low expression is associated with worse clinical outcome (Falcon O. et al, 1999). The positivity for cathepsin D is related to the size of primary tumor. The patients with vascular invasion and positive pelvic lymph nodes tend to be positive for cathepsin D. The prognosis is better in cathepsin D-negative cancers (Inoue Y. et al, 1999).

Purpose of study was to evaluate the prognostic significance of immunohistochemical expression of cathepsin D and E cadherin in uterine endometrioid adenocarcinomas.

Tasks of study: - Clinical study of patients with grade 1, 2 and 3 endometrioid adenocarcinomas.

- Histological study of patients with grade 1, 2 and 3 endometrioid adenocarcinomas.
- Immunohistochemical study of cathepsin D and E cadherin in patients with grade 1, 2 and 3 endometrioid adenocarcinomas.
- Evaluation of clinicalpathological prognostic factors in patients with grade 1, 2 and 3 endometrioid adenocarcinomas.

The scientific innovation of study: - Most of clinicopathological factors we have examined seem to be correlated to the histological grade of uterine endometrioid carcinoma.

- The analysis of clinicopathological prognostic factors in grade 1, 2 and 3 endometrioid adenocarcinomas such as obesity, family history of endometrial cancer, vaginal bleeding, cervical involvement, positive peritoneal cytology, parametrium invasion, myometrial

invasion, adnexal metastasis, vessel permeation at the time of diagnosis, recurrence within two years, pre-surgery value of CA125.

- The loss of E-cadherin expression (negativity) is associated with the higher histological grade of uterine endometrioid adenocarcinoma, depth of myometrial invasion, lymph node positivity, coexistence of obesity and vaginal bleeding. It seems that local invasion and metastatic spread of tumor should be preceded by the loss of E-cadherin expression in tumor cells, which progressively occurs in carcinogenesis. The expression of cathepsin D is associated with the higher histological grade of endometrioid adenocarcinoma, depth of myometrial invasion, lymph node positivity, coexistence of obesity and vaginal bleeding. It seems that local invasion and metastatic spread of tumor should be preceded by the expression of cathepsin D in stromal cells, which can be assessed in grade 1 and 2 endometrioid adenocarcinomas.

- The expression of cathepsin D is negatively correlated to the expression of E cadherin. In grade 1 endometrioid carcinomas E cadherin is negative, weakly and moderately positive; in grade 2 endometrioid carcinomas E cadherin is negative and weakly positive; in grade 3 endometrioid carcinomas E cadherin is negative. In grade 1 endometrioid carcinomas cathepsin D is negative, weakly and moderately positive; in grade 2 endometrioid carcinomas cathepsin D is moderately and strongly positive; in grade 3 endometrioid carcinomas cathepsin D is strongly positive. Therefore, negativity for E cadherin and positivity for cathepsin D can be used as poor prognostic factors and more aggressive chemotherapy regimen should be used.

The practical value of the study: - Besides histological examination of uterine endometrioid adenocarcinoma, immunohistochemistry should be used to determine the expression of cathepsin D and E cadherin.

- In patients with grade 1 and 3 endometrioid carcinomas the choice of treatment strategy should be based on the histological grade of tumor (in grade 1 adenocarcinoma less aggressive chemotherapy or radiotherapy is recommended, and grade 3 adenocarcinoma more aggressive chemotherapy is preferable), and in patients with grade 2 endometrioid carcinomas treatment should be based on the clinical stage.

- The positivity for cathepsin D and negativity for E cadherin indicate higher probability of invasion and metastatic spread of tumor, therefore, more aggressive chemotherapy regimen is recommended.

The basic theses to be reported at the defense: - The results of clinical study of patients with grade 1, 2 and 3 endometrioid adenocarcinomas; - The results of histological study of patients with grade 1, 2 and 3 endometrioid adenocarcinomas; - The results of immunohistochemical study of cathepsin D and E cadherin in patients with grade 1, 2 and 3

endometrioid adenocarcinomas; - Clinicalpathological prognostic factors in patients with grade 1, 2 and 3 endometrioid adenocarcinomas.

Approbation of study. The materials of the study have been reported and discussed at the ???

Publications: 3 scientific articles have been published on the theme of dissertation.

Structure and Volume of Study. The study consists of the following parts: Introduction, Review of Literature, Materials and Methods, Results of Study, Discussion of Results, Conclusions, Practical Recommendations, References (161 sources). The study includes 141 printed pages, 20 tables, 17 pictures and 21 graphs.

MATERIALS AND METHODS

We have studied 104 postmenopausal women registered in National Cancer Center of Georgia from 2003 to 2005 with diagnosis of endometrioid adenocarcinoma. Surgical treatment included radical hysterectomy. The clinical stage of tumor was determined according to the FIGO classification: T1 (FIGO I) - tumor confined to corpus uteri; T2 (FIGO II) - tumor invades cervix but does not extend beyond uterus; T3 (FIGO III) - local or regional spread within pelvis; T4 (FIGO IVA) - tumor extends beyond pelvis to invades bladder mucosa or bowel mucosa.

Clinically, we evaluated the presence of the following prognostic factors: family history of endometrial cancer, obesity, vaginal bleeding, recurrence within two years (disease-free interval), vessel permeation, myometrial invasion (<1, 2/3, >2/3), cervical involvement, positive lymph nodes, positive peritoneal cytology, parametrium invasion, adnexal metastasis, pre-surgery value of CA125 (>35 U/ml, ≤35 U/ml).

Surgical specimens were fixed in 10% neutral buffered formalin solution and embedded in paraffin (Bio-Optica Milano). 4 μm sections were stained by hematoxylin and eosin, von Gieson, and histological type of cancer, metastatic lesion of lymph nodes and depth of myometrial invasion were evaluated.

Histological grade of cancer was assessed by FIGO grading system: FIGO 1 - composed primarily of well formed glands; <5% nonsquamous solid component; FIGO 2 - 6-50% nonsquamous solid component; FIGO 3 - more than 50% nonsquamous solid component; lacks well formed glands.

According to the histological study by hematoxylin-eosin the patients were distributed into three groups: group 1 – 35 patients with grade 1 endometrioid carcinoma, group 2 - 44 patients with grade 2 endometrioid carcinoma, group 3 - grade 3 endometrioid carcinoma.

All samples were analyzed by immunohistochemistry for cathepsin D and E cadherin (Dako, Denmark). We have assessed the number of cathepsin D-positive and E cadherin-positive cells and degree of positivity (low, moderate, high) in 10 fields of view based on 500 cells (with low, moderate and high expression), X400: 1 X the percentage of cells with low expression + 2 X the percentage of cells with moderate expression + 3 X the percentage of cells with high expression. Evaluation criteria: 0-50 – negative, 51-100 – low positivity, 101-200 – moderate positivity, 201-300 – high positivity. Statistical significance of differences was evaluated by t-test and correlation analysis.

THE RESULTS AND DISCUSSION

The results of histological study

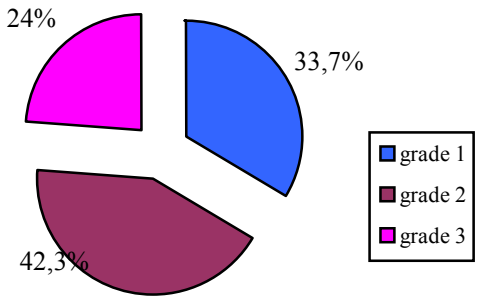
Histological study of 104 surgical specimens by hematoxylin and eosin allowed to make a diagnosis of endometrioid carcinomas which was based on the following morphologic characteristics: there are endometrial type glands with different differentiation or various grade atypia, papillary structures, lining epithelial cells are elongated, pleomorphic, some of them contain nucleoli, interglandular stroma is scant.

Histological grades of endometrioid carcinomas were assessed by FIGO grading system. Surgical specimens of 35 patients were primarily composed of well formed glands, with <5% nonsquamous solid component (grade 1). These patients were included in group 1 (33,7%).

In surgical specimens of 44 patients besides well differentiated glandular structures solid areas were also found, and nonsquamous solid component was recorded as 6-50% (grade 2). These patients were included in group 2 (42.3%).

25 Surgical specimens lacked glandular structures, and nonsquamous solid component was found in more than 50% of tumor (grade 3). These patients were included in group 3 (24%) (graph 1).

Diag. 1. The groups of patients according to the histological grade of endometrioid adenocarcinoma



The results of clinical study

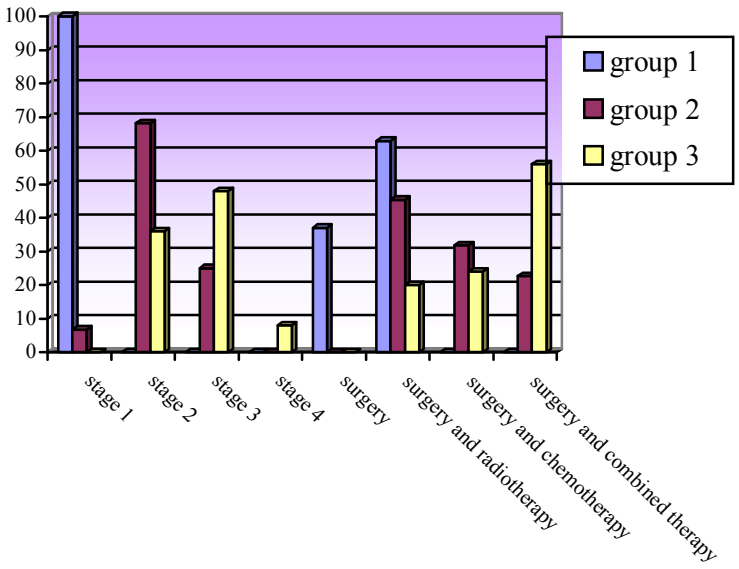
All G1 tumors were at stage 1. 13 patients (37.1%) with G1 tumors have been treated by surgery alone, and 22 patients (62.9%) have been treated by surgery and radiotherapy.

3 cases (6.8%) of G2 tumors were at stage 1, 30 tumors (68.2%) were at stage II, and 11 tumors (25%) were at stage III. Among them, 20 patients (45.5%) with G2 tumors have been treated by surgery and radiotherapy, 14 patients (31.8%) have been treated by surgery and chemotherapy, and 10 patients (22.7%) have been treated with surgery and combination of multiple therapies.

9 G3 tumors (36%) were at stage II, 12 tumors (48%) were at stage III, and 2 tumors (8%) were at stage IV. 6 patients (24%) with G3 tumors have been treated by surgery and radiotherapy, 5 patients (20%) have been treated by surgery and chemotherapy, and 14 patients (56%) have been treated with surgery and combination of multiple therapies (graph 2).

The patients of group 1 did not report family history of endometrial cancer, 18 patients (51,4%) were obese. 8 patients (22,9%) had vaginal bleeding, none of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation and recurrence, all patients were lymph node negative, ascites cell analysis was negative, depth of myometrial invasion was less than 1/3 of myometrium, and CA125 pre-surgery values were less than 35 U/ml.

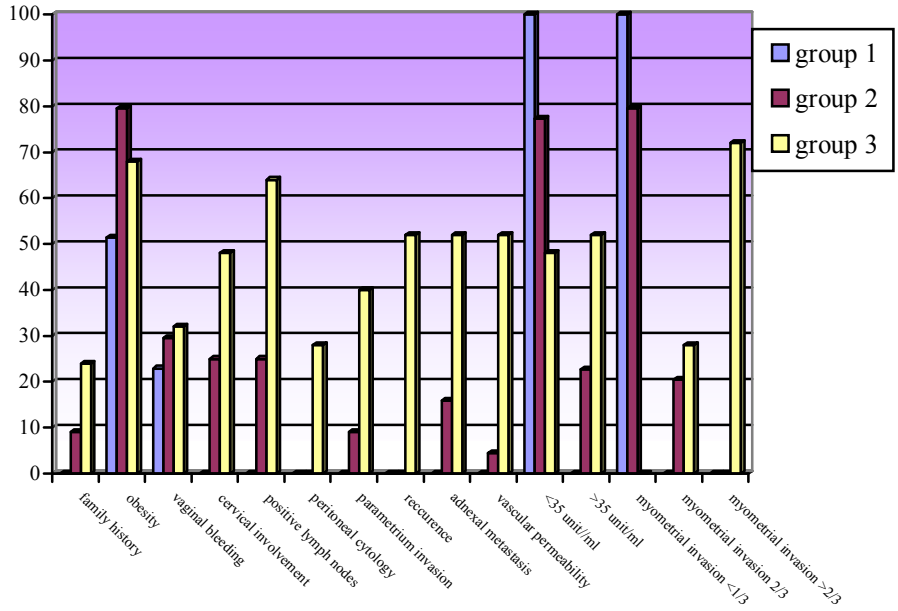
Diag. 2. Comparison of treatment choices and clinical stages among groups



Among the patients of group 2 family history was reported in 4 cases (9,1%), 35 patients (79,5%) were obese, 13 patients (29,5%) had vaginal bleeding, 11 patients (25%) showed cervical involvement, 4 patients (9,1%) showed parametrium invasion, 7 patients (15,9%) showed adnexal metastasis, 2 patients (4,5%) showed vessel permeation, none of them showed recurrence, positive lymph nodes were detected in 11 patients (25%), ascites cell analysis was negative, depth of myometrial invasion was <1/3 of myometrium in 35 patients (79,5%), and 2/3 in 9 patients (9,1%). CA125 pre-surgery values were less than 35 U/ml in 34 patients(77,3%), and >35 U/ml in 10 patients (22,7%).

Among the patients of group 3 family history was reported in 6 cases (24%), 17 patients (68%) were obese, 8 patients (32%) had vaginal bleeding, 12 patients (48%) showed cervical involvement, 10 patients (40%) showed parametrium invasion, 13 patients (52%) showed adnexal metastasis, 9 patients showed vessel permeation (36%), 13 patients showed recurrence (52%), positive lymph nodes were detected in 16 patients (64%), ascites cell analysis was positive in 7 patients (28%), depth of myometrial invasion was 2/3 of myometrium in 7 patients (28%), and >2/3 in 18 patients (72%), CA125 pre-surgery values were <35 U/ml in 12 patients (48%), and >35 U/ml in 13 patients (52%) (graph 3).

Graph 3. Comparison of clinicopathological prognostic factors among groups

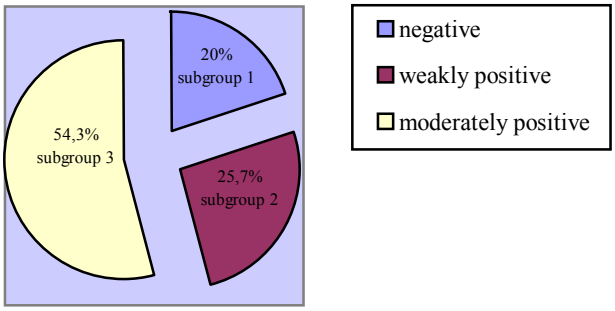


The results of immunohistochemical study

The results of immunohistochemical study of E cadherin. In group 1, on the basis of the grade of positivity for E-cadherin, three subgroups were identified: subgroup 1 – negative (7 patients, 20%), subgroup 2 – low positivity (9 patients, 25.7%), subgroup 3 – moderate positivity (19 patents, 54.3%). E-cadherin positivity assessed in subgroup 1: 1X7 + 2X8 + 3X9 = 50 (negative), subgroup 2: 1X18 + 2X17 + 3X13 = 91 (low positivity); subgroup 3: 1X24 + 2X38 + 3X27 = 181 (moderate positivity) (graph 4).

Clinicopathological prognostic factors were studied in relation to E cadherin expression. In subgroup 1 family history was reported in none of patients, 4 patients (57.1%) were obese, 3 patients (42.9%) had vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was <1/3 of myometrium, and CA125 pre-surgery values were <35 U/ml in all patients.

Graph 4. Distribution of group 1 patients according to E cadherin expression



In subgroup 2 family history was reported in none of patients, 7 patients (77.8%) were obese, and 3 patients (33.3%) suffered from vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was <1/3 of myometrium, and CA125 pre-surgery values were <35 U/ml in all patients.

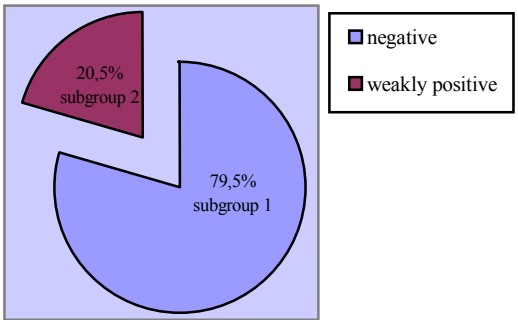
In subgroup 3 family history was reported in none of patients, 8 patients (42.1%) were obese, and 2 patients (10.5%) suffered from vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was <1/3 of myometrium, and CA125 pre-surgery values were <35 U/ml in all patients (table 1).

In group 2, on the basis of the grade of positivity for E-cadherin, two subgroups were identified: subgroup 1 – negative (35 patents, 79.5%), subgroup 2 – low positivity (9 patients, 20.5%). E-cadherin positivity assessed in subgroup 1: 1X10 + 2X11 + 3X9 = 48 (negative), subgroup 2: 1X16 + 2X22 + 3X13 = 99 (low positivity) (graph 5).

Table 1. Clinicopathological prognostic factors according to E cadherin expression in group 1

Prognostic factors		**Subgroup 1 (n=7)**	**Subgroup 2 (n=9)**	**Subgroup 3 (n=19)**
Family history		0	0	0
Obesity		4	7	8
Vaginal bleeding		3	3	2
Cervical involvement		0	0	0
Positive lymph nodes		0	0	0
Peritoneal cytology		negat.	negat.	negat.
Parametrium invasion		0	0	0
Recurrence		0	0	0
Adnexal metastasis		0	0	0
Vessel permeation		0	0	0
Pre-surgery value of CA125 (≤35 U/ml)		7	9	19
Pre-surgery value of CA125 (≥35 U/ml)		0	0	0
Myometrial invasion	<1/3	7	9	19
	2/3	0	0	0
	>2/3	0	0	0

Graph 5. Distribution of group 2 patients according to E cadherin expression



Clinicopathological prognostic factors were studied in relation to E cadherin expression. In subgroup 1 family history was reported in 2 cases (5.7%), 27 patients (71.1%) were obese, 7 patients (20%) suffered from vaginal bleeding, 8 patients (22.9%) showed cervical involvement, 3 patients (8.6%) showed parametrium invasion, 5 patients (14.3%) showed adnexal metastasis, 2 patients (5.7%) showed vessel permeation, none of them showed recurrence, positive lymph nodes were detected in 8 patients (22.9%), ascites cell analysis was negative, depth of myometrial invasion was <1/3 of myometrium in 8 patients (22.9%), and 2/3 in 27 patients (77.1%). CA125 pre-surgery values were <35 U/ml in 26 patients (74.3%), and >35 U/ml in 9 patients (25.7%).

Table 2. Clinicopathological prognostic factors according to E cadherin expression in group 2

Prognostic factors		**Subgroup 1 (n=35)**	**Subgroup 2 (n=9)**
Family history		2	2
Obesity		27	8
Vaginal bleeding		7	5
Cervical involvement		8	3
Positive lymph nodes		8	3
Peritoneal cytology		negat.	negat.
Parametrium invasion		3	1
Recurrence		0	0
Adnexal metastasis		5	2
Vessel permeation		2	0
Pre-surgery value of CA125 (≤35 U/ml)		22	12
Pre-surgery value of CA125 (≥35 U/ml)		13	1
Myometrial invasion	<1/3	26	9

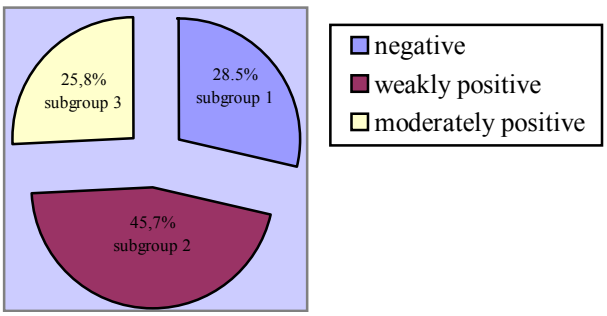
	2/3	9	0
	>2/3	0	0

In subgroup 2 family history was reported in 2 cases (22.2%), 8 patients (88.9%) were obese, 5 patients (55.6%) suffered from vaginal bleeding, 3 patients (33.3%) showed cervical involvement, 3 patients (11.1%) showed parametrium invasion, 2 patients (22.2%) showed adnexal metastasis, positive lymph nodes were detected in 3 patients (33.3%). None of them showed vessel permeation, recurrence, ascites cell analysis was negative, depth of myometrial invasion was <1/3 of myometrium in 8 patients (88.9%), and 2/3 in one patients (11.1%). CA125 pre-surgery values were <35 U/ml in 7 patients (77.8%), and >35 U/ml in 2 patients (22.2%) (table 2).

In all patients of the group 3 tumor cells were negative for E-cadherin (subgroup 1).

The results of immunohistochemical study of cathepsin D. In group 1 stromal cells were negative for cathepsin D. On the basis of the grade of positivity for cathepsin D in tumor cells, three subgroups were identified: subgroup 1 – negative (10 patients, 28.5%), subgroup 2 – low positivity (16 patents, 45.7%), subgroup 3 – moderate positivity (9 patents, 25.7%). Cathepsin D positivity assessed in subgroup 1: 1X5 + 2X7 + 3X8 = 43 (negative), subgroup 2: 1X21 + 2X19 + 3X12 = 98 (low positivity); subgroup 3: 1X27 + 2X41 + 3X25 = 184 (moderate positivity) (graph 6).

Graph 6. Distribution of group 1 patients
according to cathepsin D expression



Clinicopathological prognostic factors were studied in relation to cathepsin D expression. In subgroup 1 family history was reported in none of cases, 7 patients (70%) were obese, 2 patients (10.5%) suffered from vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was less than 1/3 of myometrium, and CA125 pre-surgery values were less than 35 U/ml in all patients.

In subgroup 2 family history was reported in none of cases, 7 patients (77.8%) were obese, 3 patients (33.3%) suffered from vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was <1/3 of myometrium, and CA125 pre-surgery values were <35 U/ml in all patients.

In subgroup 3 family history was reported in none of cases, 4 patients (57.1%) were obese, 3 patients (42.9%) suffered from vaginal bleeding. None of them showed cervical involvement, parametrium invasion, adnexal metastasis, vessel permeation, recurrence within two years after diagnosis, positive lymph nodes, and positive perinoteal cytology. Depth of myometrial invasion was <1/3 of myometrium, and CA125 pre-surgery values were <35 U/ml in all patients (table 3).

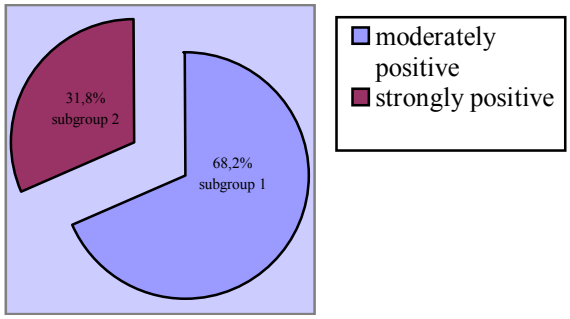
Table 3. Clinicopathological prognostic factors according to cathepsin D expression in group 1

Prognostic factors		**Subgroup 1 (n=10)**	**Subgroup 2 (n=16)**	**Subgroup 3 (n=9)**
Family history		0	0	0
Obesity		7	7	4
Vaginal bleeding		2	3	3
Cervical involvement		0	0	0
Positive lymph nodes		0	0	0
Peritoneal cytology		negat.	negat.	negat.
Parametrium invasion		0	0	0
Recurrence		0	0	0
Adnexal metastasis		0	0	0
Vessel permeation		0	0	0
Pre-surgery value of CA125 (≤35 U/ml)		10	9	7
Pre-surgery value of CA125 (≥35 U/ml)		0	0	0
Myometrial invasion	<1/3	10	19	7
	2/3	0	0	0
	>2/3	0	0	0

In group 2, on the basis of the grade of positivity for cathepsin D in tumor cells, two subgroups were identified: subgroup 1 – moderate positivity (30 patents, 68.2%), subgroup 2 – high positivity (14 patents, 31.8%). Cathepsin D positivity assessed in subgroup 1: 1X25 + 2X76 + 3X29 = 188 (moderate positivity), subgroup 2: 1X16 + 2X42 + 3X45 = 23 (high positivity). In

10 patients of subgroup 2 (71.4%) cathepsin D was expressed in stromal cells, with low expression in 6 cases (60%) and moderate expression in 4 cases (40%) (graph 7).

Graph 7. Distribution of group 2 patients
according to cathepsin D expression



Clinicopathological prognostic factors were studied in relation to cathepsin D expression. In subgroup 1 family history was reported in 2 cases (6.7%), 25 patients (83.3%) were obese, 7 patients (20%) suffered from vaginal bleeding, 3 patients (10%) showed cervical involvement, 3 patients (10%) showed parametrium invasion, one patient (3.3%) showed adnexal metastasis, positive lymph nodes were detected in 3 patients (10%). None of them showed vessel permeation, recurrence, ascites cell analysis was negative, depth of myometrial invasion was <1/3 of myometrium in all patients. CA125 pre-surgery values were <5 U/ml in 23 patients (76.7%), and >35 U/ml in 7 patients (22.3%)

In subgroup 2 family history was reported in 2 cases (14.3%), 10 patients (7.1%) were obese, 6 patients (42,9%) suffered from vaginal bleeding, 8 patients (57.1%) showed cervical involvement, 3 patients (21.4%) showed parametrium invasion, 5 patients (35.7%) showed adnexal metastasis, positive lymph nodes were detected in 8 patients (67.1%). None of them showed vessel permeation, recurrence, ascites cell analysis was negative, depth of myometrial invasion was <1/3 of myometrium in 4 patients (28.6%), and 2/3 in 10 patients (71.4%). CA125 pre-surgery values were <35 U/ml in 11 patients (78.6%), and >35 U/ml in 2 patients (21.4%) (table 4).

In all patients of the group 3 tumor cells were strongly positive for cathepsin D (subgroup 1).

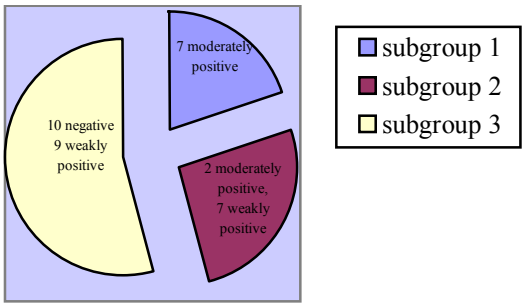
Table 4. Clinicopathological prognostic factors according
to cathepsin D expression in group 2

Prognostic factors	**Subgroup 1 (n=30)**	**Subgroup 2 (n=14)**
Family history	2	2

Obesity		25	10
Vaginal bleeding		7	6
Cervical involvement		3	8
Positive lymph nodes		3	8
Peritoneal cytology		negat.	negat.
Parametrium invasion		1	3
Recurrence		0	0
Adnexal metastasis		2	5
Vessel permeation		0	2
Pre-surgery value of CA125 (≤35 U/ml)		23	11
Pre-surgery value of CA125 (≥35 U/ml)		7	3
Myometrial invasion	<1/3	30	4
	2/3	0	10
	>2/3	0	0

Analysis of co-expression of E cadherin and cathepsin D. In group 1, on the basis of the grade of positivity for E-cadherin, three subgroups were identified: E cadherin was negative in 7 patients (subgroup 1), weakly positivite in 9 patients (subgroup 2), and moderately positive in 19 patents (subgroup 3). Among these cases, all patients of subgroup 1 showed moderate positivity for cathepsin D. In subgroup 2, 2 patients were moderately positive, and 7 patients were weakly positive for cathepsin D. In subgroup 3, 10 patients were negative, and 9 patients were weakly positive for cathepsin D (graph 8).

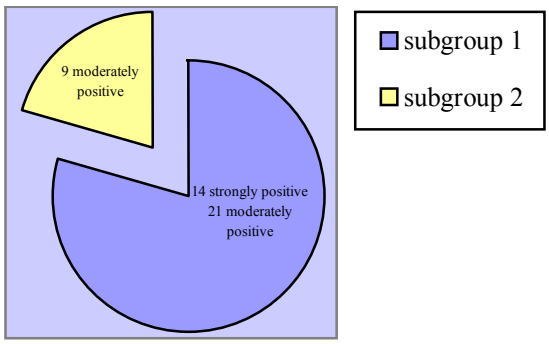
Graph 8. Comparison of co-expression of E cadherin and cathepsin D in group 1



In group 2, on the basis of the grade of positivity for E-cadherin, two subgroups were identified: E cadherin was negative in 35 patients (subgroup 1), and weakly positive in 9 patients (subgroup 2), and moderately positivite in 19 patents (subgroup 3). Among these cases, 14 patients of subgroup 1 showed strong positivity, and 21 patients were moderate posivitiy for cathepsin D. All patients of subgroup 2 were moderately positive for cathepsin D (graph 9).

In all 25 patients tumor cells were negative for E cadherin and strongly positive for cathepsin D. In this group the evaluation of cathepsin D expression was impossible due to the solid growth of tumor.

Graph 9. Comparison of co-expression of E cadherin and cathepsin D in group 2



CONCLUSIONS

1. Most of clinicopathological prognostic factors we have examined seem to be associated with histological grade of uterine endometrioid carcinoma.

2. The analysis of clinicopathological prognostic factors in G1 endometrioid adenocarcinoma cases has showed that about half of these patients are obese, vaginal bleeding is not common, no cervical involvement, parametrium invasion, adnexal metastasis and vessel permeation at the time of diagnosis, no recurrence within two years after diagnosis, pre-surgery value of CA125 is normal, and myometrial invasion is less than 1/3.

3. The analysis of clinicopathological prognostic factors in G3 endometrioid adenocarcinoma cases has showed that family history was found in 24% of cases, 68% were obese, 32% of patients suffered from vaginal bleeding, 48% had cervical involvement, 40% parametrium invasion, 52% adnexal metastasis, 64% positive lymph nodes, 36% vessel permeation. In 52% of patients recurrences were found within two years after diagnosis. Positive peritoneal cytology was detected in 28% of cases. Myometrial invasion was 2/3 in 28% and >2/3 in 72% of patients. Furthermore, in 52% of cases reccurent tumors were developed within two years. CA125 pre-surgery values were <35 U/ml in 48%, and >35 U/ml in 52% of patients.

4. The analysis of clinicopathological prognostic factors in G2 endometrioid adenocarcinoma cases has showed that family history was found in 9.1% of cases, 79.5% were obese, 29.5% of patients suffered from vaginal bleeding, 25% had cervical involvement, 9.1% parametrium invasion, 15.9% adnexal metastasis, 25% positive lymph nodes, 4.5% vessel permeation. None of them showed recurrences within two years after diagnosis and positive peritoneal cytology. Myometrial invasion was <1/3 in 79.5% and 2/3 in 20.5% of patients. CA125 pre-surgery values were <35 U/ml in 77.3%, and >35 U/ml in 22.7% of patients. Therefore, G2 endometrioid adenocarcinoma can be considered as an intermediary form and should be managed according the clinical stage, not to the histological grade.

5. The loss of E-cadherin expression (negativity) is associated with the higher histological grade of uterine endometrioid adenocarcinoma, depth of myometrial invasion, lymph node positivity, coexistence of obesity and vaginal bleeding. It seems that local invasion and metastatic spread of tumor should be preceded by the loss of E-cadherin expression in tumor cells, which progressively occurs in carcinogenesis. Therefore, it can be used as a poor prognostic factors and more aggressive chemotherapy regimen should be used.

6. The expression of cathepsin D is associated with the higher histological grade of endometrioid adenocarcinoma, depth of myometrial invasion, lymph node positivity, coexistence of obesity and vaginal bleeding. It seems that local invasion and metastatic spread of tumor

should be preceded by the expression of cathepsin D in stromal cells which can be assessed in grade 1 and 2 endometrioid adenocarcinomas. Therefore, it can be used as a poor prognostic factors and more aggressive chemotherapy regimen should be used.

7. The expression of cathepsin D is negatively correlated to the expression of E cadherin. In grade 1 endometrioid carcinomas E cadherin is negative, weakly and moderately positive; in grade 2 endometrioid carcinomas E cadherin is negative and weakly positive; in grade 3 endometrioid carcinomas E cadherin is negative. In grade 1 endometrioid carcinomas cathepsin D is negative, weakly and moderately positive; in grade 2 endometrioid carcinomas cathepsin D is moderately and strongly positive; in grade 3 endometrioid carcinomas cathepsin D is strongly positive.

PRACTICAL RECOMMENDATIONS

1. Besides histological examination of uterine endometrioid adenocarcinoma, immunohistochemistry should be used to determine the expression of cathepsin D and E cadherin.

2. In patients with grade 1 and 3 endometrioid carcinomas the choice of treatment strategy should be based on the histological grade of tumor (in grade 1 adenocarcinoma less aggressive chemotherapy or radiotherapy is recommended, and grade 3 adenocarcinoma more aggressive chemotherapy is preferable), and in patients with grade 2 endometrioid carcinomas treatment should be based on the clinical stage.

3. The positivity for cathepsin D and negativity for E cadherin indicate higher probability of invasion and metastatic spread of tumor, therefore, more aggressive chemotherapy regimen is recommended.

List of Published Scientific Papers on the Theme of Dissertation:

1. Dvalishvili I, Charkviani L, Turashvili G, Burkadze G. The expression of cadherin E and clinical prognostic factors in uterine endometrioid adenocarcinoma. Georgian Med News. 2005 Nov;(128):17-21.
2. Dvalishvili I, Charkviani L, Charkviani T, Turashvili G, Burkadze G. Clinical prognostic factors and expression of cathepsin D in endometrioid adenocarcinoma. Georgian Med News. 2005 Sep;(126):27-31.
3. Dvalishvili I, Charkviani L, Charkviani T, Turashvili G, Burkadze G. Clinical Characteristics of Prognostic Factors in Uterine Endometrioid Adenocarcinoma of various grade.
4. Charkviani L, Charkviani T, Turashvili G, Burkadze G. The association of cathepsin D expression and clinical prognostic factors in end- dometrial adenocarcinoma. IGCS 15.2005. ESGO Istanbul.
5. Turashvili G, Burkadze G. Argyrophilic nucleolar organizer regions in the normal, hyperplastic and neoplastic endometrium. Annals of Oncology, 15:20, 2004 – ESMO, Vienna.
6. Turashvili G, Burkadze G. Detection of human papilomavirus in endocervical glandular lesions and relationship with p53 and MIB-1 expressions. EUROGIN Paris 2006.