ADULT TYPE GRANULOSA CELL TUMOR – MORPHOLOGICAL FEATURES

IOANA BUDA[1], RALUCA BALAN[2], EDUARD CRAUCIUC[2], OVIDIU TOMA[3], CORNELIA AMALINEI [2]

Keywords: adult type granulosa cell tumor, AGCT, immunohistochemistry

Abstract: Adult granulosa cell tumors (AGCT) account for approximately 1-2% of all ovarian tumors and 95% of all GCT. They occur more often in postmenopausal women, with a peak incidence between 50 and 55 years. Nine cases of AGCT were diagnosed in the Clinical Hospital of Obstetrics and Gynecology Iasi, in a 10 years period. The age of the patients ranged between 35 and 67 years, 4 of them (44.44%) being postmenopausal. The macroscopical appearance showed that all were unilateral tumors – 6 of them (66.66%) solid and 3 cystic (33.33%). After paraffin-embedding and usual stainings, we observed the granulosa cells as small, cuboidal to polygonal cells, arranged in anastomosing cords, and gland-like structures filled with an acidophilic material recalling immature follicles (Call-Exner bodies). Two tumors presented microfollicular and macrofollicular patterns. Three tumors (33.33%) presented also a thecoma component formed by sheets of cuboidal to polygonal cells (specifically called granulosa-theca cell tumors). In two of them both granulosa and theca cells presented aspects of luteinization (luteinized granulosa-theca cell tumors). Only one tumor (11.11%) was associated with simple hyperplasia of the endometrium (suggesting an active endocrine tumor). One tumor was associated with an ovarian endometrioid adenocarcinoma. Although considered as benign tumors, only 5-25% of them being malignant, with an indolent course and only local recurrences, in the reported cases the tumors were mainly malignant (66.66%), two of them being highly aggressive, as vascular invasion was noted, and one of them was also advanced, with histologically confirmed implants of abdominal peritoneal surfaces.

INTRODUCTION

Sex cord-stromal ovarian tumors are composed of granulosa cells, theca cells, and their luteinized derivatives: Sertoli cells, Leydig cells, and fibroblasts of gonadal stromal origin, singly or in various combinations and in varying degrees of differentiation. Sex cord-stromal tumors account for approximately 8-10% of all ovarian tumors, with granulosa cell tumors accounting for about half of the cases. Granulosa cell tumors (GCTs) account for about 4-5% of all ovarian tumors. They are usually unilateral, variable in size, and are considered neoplasms of a low grade of malignancy. GCTs that occur typically in middle-aged and older women are referred to as adult type, differing in several important features from those arising in young adults and children, referred as juvenile type (Cronje, 1998; Gershenson, 2005; Mom, 2007).
Adult granulosa cell tumors (AGCTs) account for approximately 1-5% of all ovarian tumors and 95% of GCTs, occur more often in postmenopausal than premenopausal women, with a peak incidence between 50 and 55 years of age. Clinically, they are the most common estrogenic ovarian tumors, aspect confirmed by specimens of endometrium that may diagnose hyperplasia, carcinoma (in less than 5% of cases). The endometrial changes associated with AGCTs are represented by endometrial manifestations: *metropathia hemorrhagica*, amenorrhea or postmenopausal bleeding, and breasts manifestations: sweeling and tenderness. Elevated levels of estrogens are demonstrated also by increased maturation of squamous epithelial cells observed in cervico-vaginal cytologic smears (Miller, 1997; Ko, 1999; Uygun, 2003). Rarely, induction of a secretory endometrium, associated with progesterone production or virilisation (hirsutism), as an androgenic effect may be encountered. Androgen production seems to be highly associated with the morphologic formation of thin-walled cysts.

MATERIAL AND METHODS

Nine cases of AGCT were diagnosed in the Clinical Hospital of Obstetrics and Gynecology Iasi, in a 10 years period. The age of the patients ranged between 35 and 67 years, 4 of them (44.44%) being postmenopausal. Routine histologic processing, by formalin fixation, paraffin-embedding and usual stainings was performed. Immunohistochemistry, using antibodies against cytokeratin (MNF 116 and 20), epithelial membrane antigen (EMA), vimentin, epidermal growth factor receptor (EGFR), Ki-67, HER-2, and MUC-1 was additionally performed.

RESULTS AND DISCUSSIONS

The macroscopical appearance showed that all tumors were unilateral. Six tumors (66.66%) were predominantly solid and 3 tumors (33.33%) were predominantly cystic. Their consistency was mainly firm. Sectioning surfaces revealed a gray-white or yellow color, depending on the luteinized areas, with common hemorrhagic areas.

After paraffin-embedding and usual stainings, microscopy revealed the granulosa cells as small, cuboidal to polygonal cells, arranged in anastomosing cords. The diagnosis was supported by characteristic cytologic features on high power magnification: pale, relatively uniform nuclei, with variable proportions of nuclear grooves (figure 1).

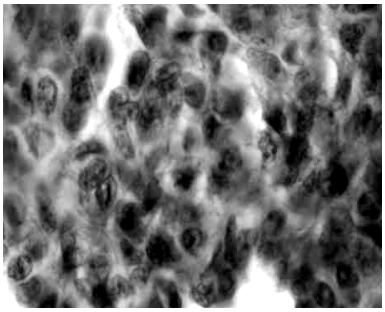

Fig. 1. AGCT- granulosa cells with scanty cytoplasm, pale, ovalar, angular, and few grooved nuclei.

Microscopically, GCTs are composed of granulosa cells, theca cells, and fibroblasts in varying amounts and combinations. The term granulosa-theca cell tumor had been applied to all tumors in which both cell types were identified, regardless of the amounts present. Young and Scully proposed a system that required a tumor to be composed of at least 25% of the second cell type before the tumor could be designated as a true granulosa-theca cell tumor (Young, 1984). Otherwise, the tumor would be designated as a granulosa cell tumor or a theca cell tumor, based on the predominant cell type. This has led to some confusion in the literature because some theca cell tumors, which are essentially benign neoplasms, have been given the dual designation of granulosa-theca cell tumors, suggesting a malignant potential among this benign group of tumors. AGCTs had multiple histopathological aspects, including well-differentiated and less differentiated types. The well-differentiated group was composed of microfollicular, macrofollicular, trabecular, and insular patterns, as already described in literature (Young, 1994; Lane, 1999; Robertson, 2002).

Two tumors (22.22% of cases) presented microfollicular and macrofollicular patterns. Microfollicular pattern was the most common histological presentation and contained characteristic Call-Exner bodies, consisting of gland-like structures of granulosa cells surrounding eosinophilic fluid and basement membrane material, recalling immature follicles (simulating the Call-Exner bodies of the developing ovarian follicles). The microfollicles were separated by well-differentiated interconnected, haphazardly arranged granulosa cells that contained scanty cytoplasm and pale, angular or often ovalar, grooved nuclei (Long, 2000; Rubin, 2000; Uygun, 2003).

The macrofollicular pattern was characterized by cysts lined by a single layer of well-differentiated granulosa cells, combined or not with theca cells. The distinction between macrofollicular pattern and follicular cysts was made by using cytological criteria.

Trabecular (figure 1) and insular (figure 4) patterns contained cells arranged in nests and bands, with an intervening fibrothecomatous stroma.

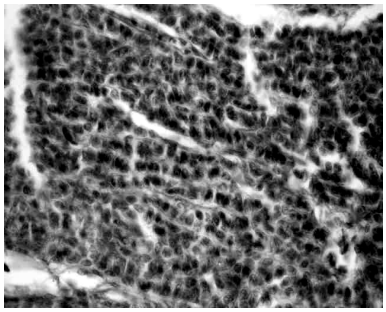

Fig. 2. AGCT- trabecular pattern.

In the solid tubular areas, uniformly cellular structures, with hollow tubules or gland-like aspect were noted (figure 3). In tubular patterns, the differential diagnosis with a mixed granulosa cell and Sertoli cell tumor, or gynandroblastoma was necessary.

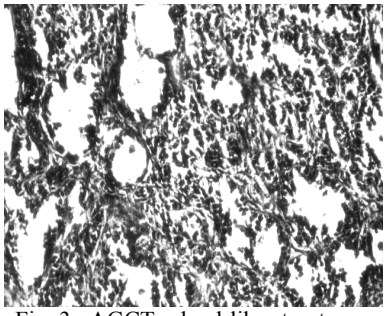

Fig. 3. AGCT- gland-like structures.

In insular areas, islands of neoplastic cells, separated by a conspicuous stromal component, composed of fibroblasts and theca cells (Evans, 1980; Segal, 1995), with variable eosinophilic or vacuolated lipid-rich cytoplasm were noted (figure 4).

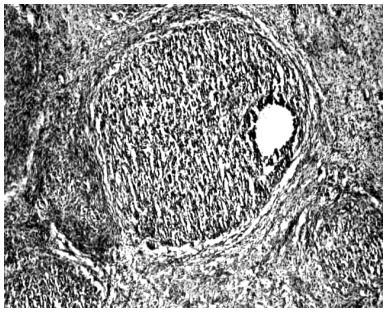

Fig. 4. AGCT- islands (one containing a microfollicle) separated by fibrothecomatous stroma.

The less differentiated areas presented a watered-silk (moiré silk), gyriform, or diffuse (sarcomatoid) pattern (figure 5). The differential diagnosis with an undifferentiated ovarian carcinoma was based on monotonous nuclei pattern associated with some of the following

criteria: unilaterality, initial stage, absence of intracellular droplets or extracellular pools of mucin or psammoma bodies, and clinical course. The differential diagnosis with small cell carcinomas was based on the following criteria: older age, malignancy with protracted course, no hypercalcemia, usually estrogenic manifestations, fibrothecomatous stroma, and characteristic cytology with a variable mitotic rate (Cronje, 1998; Ko, 1999; Robinson, 1999).

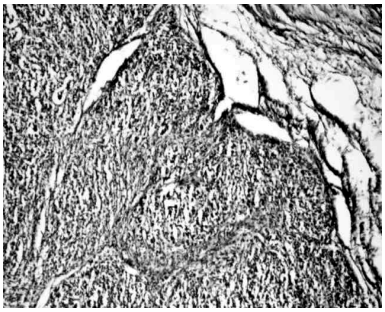

Fig. 5. AGCT- diffuse growth;
few Call-Exner bodies containing degenerated nuclei.

Three tumors (33.33%) presented also a thecoma component formed by sheets of cuboidal to polygonal cells (figure 6) (specifically called granulosa-theca cell tumors). The presence of theca cells probably reflected a response of the ovarian stroma to the growth of granulosa cells rather than the coexistence of a second neoplastic cell component, as the theca-like cells represent a nonspecific component of a large variety of ovarian tumors, both benign and malignant, both primary or metastatic. Theca cell component is responsible for androgens production, eventually converted by granulosa cells aromatase into estrogens, according to the two-cell theory of hormonal production by the normal graafian follicle (Lack, 1981; Page, 2000). Another evidence favoring the interpretation of thecal component as reactive is represented by the absence of theca cells in AGCTs extended beyond the ovary concomitantly with the lack of estrogenic manifestations. The differential diagnosis with steroid cell tumors was made in these cases. In two of them both granulosa and theca cells presented luteinization aspects, as neoplastic cells presented abundant dense or vacuolated cytoplasm (luteinized granulosa-theca cell tumors).

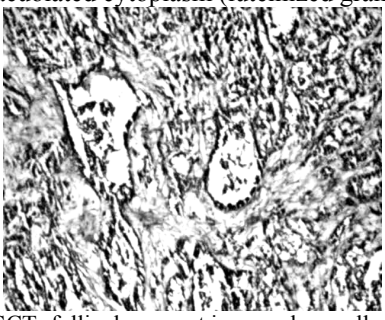

Fig. 6. AGCT- follicular aspect in granulosa cell component
with interspersed thecomatous, luteinized cells.

Only one tumor (11.11%) was associated with simple hyperplasia of the endometrium, suggesting an active endocrine tumor.

One tumor was associated with an endometrioid ovarian carcinoma, an extremely rare possibility. This association raises the issue of the simultaneous development of two different types of tumors or of tumor progression, with the development of a second type from a clonal derivative of the first tumor. No remnants of ovarian endometriosis were noted, to enforce the hypothesis of the development of an endometrioid ovarian carcinoma from a preexisting condition.
After the removal of the tumors, the manifestations of hyperestrinism regressed. No recurrences were noted, in the benign AGCTs (2 cases), but they are still under monitorisation, as the recurrence may be noted two or even three decades after the initial therapy.
Six of tumors (66.66%) presented areas of malignant transformation, 3 of them being associated with tumoral necrosis, 1 with calcifications, and 2 with vascular invasion. They were classified as stage IA (1 case), stage IIB (3 cases) and stage IIIC (2 case). Optimal treatments, total hysterectomy, associated with bilateral salpingo-oophorectomy, combined with chemotherapy/radiation (Lee, 1999; Spencer, 1999) were applied.
Immunohistochemistry revealed a negative reaction for cytokeratin 20, EMA (figure 9), HER-2 and MUC-1, a weak positivity of cytokeratin MNF 116, and a moderate to strong positivity for vimentin (figure 10), EGFR, and Ki-67, corresponding to the reported immunohistochemical characteristics of granulosa cell tumors, and to the increased proliferative activity in our group of study. Other useful markers in granulosa tumors may be CD 99, inhibin, desmoplakin, calretinin and Smooth Muscle Actin (SMA), as stromal tumor markers (Sehouli, 2004; Villella, 2007).

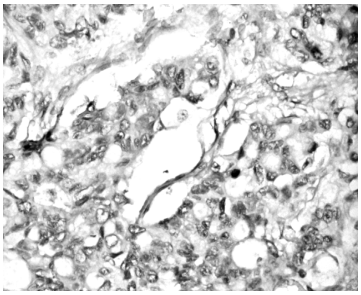

Fig. 9. EMA immunonegativity in AGCT (x 10).

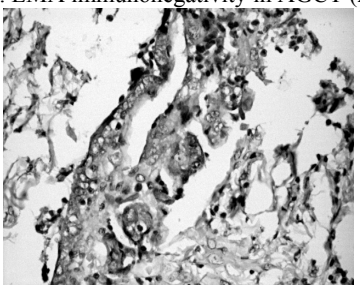

Fig. 10. Vimentin immunopositivity in AGCT (x 10).

CONCLUSIONS

Although considered as benign tumors, only 5-25% of them being malignant, with an indolent course and only local recurrences, in the reported cases the tumors were mainly malignant (66.66%), two of them being highly aggressive, as vascular invasion was noted, and one of them was also advanced, with histologically confirmed implants of the abdominal peritoneal surfaces.

Vimentin, Cytokeratin MNF 116, EGFR and Ki-67 are useful in positive diagnose and in assessment of proliferative activity of AGCTs.

Although relatively ”pure”, one tumor (11.11%) associated an endometrioid ovarian carcinoma, raising the issue of simultaneity of both tumors or of derivation of one tumor from the other, through tumor progression.

REFERENCES

Cronje HS, Niemand I, Bam RH, 1998. Granulosa and theca cell tumors in children: a report of 17 cases and literature review, *Obstet Gynecol Surv,* 53(4):240-247.
Evans AT, Gaffey TA, Malkasian GD Jr, 1980. Clinicopathologic review of 118 granulosa and 82 theca cell tumors, *Obstet Gynecol,* 55(2):231-238.
Gershenson DM, Hartmann LC, Young RH, 2005. Ovarian Sex Cord-Stromal Tumors. In: Hoskins WJ et al, eds. Principles and Practice of Gynecologic Oncology. 4th ed. Baltimore, Md: Lippincott Williams & Wilkins, 1011-1034.
Ko SF, Wan YL, Ng SH, 1999. Adult ovarian granulosa cell tumors: spectrum of sonographic and CT findings with pathologic correlation, *AJR Am J Roentgenol*, 172(5):1227-1233.
Lack EE, Perez-Atayde AR, Murthy AS, 1981. Granulosa theca cell tumors in premenarchal girls: a clinical and pathologic study of ten cases, *Cancer,* 48(8):1846-1854.
Lee WL, Yuan CC, Lai CR, 1999. Hemoperitoneum is an initial presentation of recurrent granulosa cell tumors of the ovary. *Jpn J Clin Oncol,* 29(10):509-512.
Long WQ, Ranchin V, Pautier P, 2000. Detection of minimal levels of serum anti-Mullerian hormone during follow-up of patients with ovarian granulosa cell tumor by means of a highly sensitive enzyme-linked immunosorbent assay, *J Clin Endocrinol Metab,* 85(2):540-544.
Miller BE, Barron BA, Wan JY, 1997, Prognostic factors in adult granulosa cell tumor of the ovary, *Cancer,* 79(10):1951-1955.
Mom CH, Engelen MJ, Willemse PH, Gietema JA, ten Hoor KA, de Vries EG, 2007. Granulosa cell tumors of the ovary: the clinical value of serum inhibin A and B levels in a large single center cohort, *Gynecol Oncol,* 105(2):365-372.
Page R, 2000. Chemotherapeutic agents. In: Pazdur R, Hoskins WH, Wagman L (eds.) Cancer Management: A Multidisciplinary Approach.4th ed. Mellville, NY: PRR Incorporated, 971-980.
Robertson DM, Stephenson T, Pruysers E, 2002. Characterization of inhibin forms and their measurement by an inhibin alpha-subunit ELISA in serum from postmenopausal women with ovarian cancer, *J Clin Endocrinol Metab,* 87(2):816-824.
Robinson JB, Im DD, Logan L, 1999. Extraovarian granulosa cell tumor, *Gynecol Oncol,* 74(1):123-127.
Rubin SC, Sabbatini P, Randall ME, 2000. Ovarian Cancer. In: Pazdur R, Hoskins WH, Wagman L (eds.) Cancer Management: A Multidisciplinary Approach. 4th ed. Mellville, NY: PRR Incorporated, 409-428.
Segal R, DePetrillo AD, Thomas G, 1995. Clinical review of adult granulosa cell tumors of the ovary, *Gynecol Oncol,* 56(3):338-44.
Sehouli J, Drescher FS, Mustea A, 2004. Granulosa cell tumor of the ovary: 10 years follow-up data of 65 patients, *Anticancer Res,* 24(2C):1223-1229.
Spencer HW, Mullings AM, Char G, 1999. Granulosa-theca cell tumour of the ovaries. A late metastasizing tumour, *West Indian Med J,* 48(1):33-35.
Uygun K, Aydiner A, Saip P, 2003. Granulosa cell tumor of the ovary: retrospective analysis of 45 cases, *Am J Clin Oncol,* 26(5):517-521.
Villella J, Herrmann FR, Kaul S, Lele S, Marchetti D, Natiella J, 2007. Clinical and pathological predictive factors in women with adult-type granulosa cell tumor of the ovary, *Int J Gynecol Pathol,* 26(2):154-159.
Young RH, Dickersin GR, Scully RE, 1984. Juvenile granulosa cell tumor of the ovary. A clinicopathological analysis of 125 cases, *Am J Surg Pathol,* 8(8):575-596.
Young RH, Scully RE, 1994. Sex cord-stromal, steroid cell, and other ovarian tumors with endocrine, paraendocrine, and paraneoplastic manifestations. In: Kurman RJ (ed). *Blaustein's Pathology of the Female Genital Tract*. 4th ed. New York, NY: Springer-Verlag; 783-847.

1 I[st] Clinic of Obstetrics and Gynecology Iasi
2 ”Gr. T. Popa” University of Medicine and Pharmacy Iasi, Romania
3 ”Alexandru Ioan Cuza” University, Iasi, Romania
* ioanabuda@yahoo.com