View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Helsingin yliopiston digitaalinen arkisto

Gynecologic Oncology 143 (2016) 571–577

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Characteristics and outcome of recurrence in molecularly defined adult-type ovarian granulosa cell tumors

Saara Bryk MD [a,b,*], Anniina Färkkilä MD, PhD [a,b], Ralf Bützow MD, PhD [c], Arto Leminen MD, PhD [a], Johanna Tapper MD, PhD [a], Markku Heikinheimo MD, PhD [b,d], Leila Unkila-Kallio MD, PhD [a], Annika Riska MD, PhD [a]

[a] Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, Finland
[b] Children's Hospital, University of Helsinki and Helsinki University Hospital, Finland
[c] Pathology and HUSLAB, University of Helsinki and Helsinki University Hospital, Finland
[d] Department of Pediatrics, Washington University School of Medicine, St Louis Children's Hospital, St Louis, MO, United States

HIGHLIGHTS

- Tumor rupture is the strongest predictive factor for AGCT recurrence.
- AGCT requires active follow up for ten to fifteen years after primary diagnosis.
- Recurrences may develop asymptomatically and in multiple anatomical locations.
- Recurrences significantly increase disease-related mortality.

ARTICLE INFO

Article history:
Received 6 August 2016
Received in revised form 28 September 2016
Accepted 1 October 2016
Available online 10 October 2016

Keywords:
Granulosa cell tumor
Recurrence
Prognosis

ABSTRACT

Objective. Adult-type ovarian granulosa cell tumors (AGCTs) have an unpredictable tendency to relapse. In a carefully validated patient cohort, we evaluated the prognostic factors related to AGCT recurrence.

Methods. We identified all patients diagnosed with AGCT during 1956–2014 in Helsinki University Hospital, with a minimum follow-up of one year (n = 240). After a histological review supplemented with *FOXL2* (402C-G) mutation status analysis, we analyzed the clinical data for association with relapse.

Results. The final cohort included 164 (68%) molecularly defined AGCTs (MD-AGCTs). The majority of the women were postmenopausal (63%), and 92% of tumors were stage I. The median follow-up time was 15.5 years. Fifty-two (32%) patients developed tumor recurrence, of whom 55% had successive recurrences. Multiple-site recurrences were common, and nearly half of the recurrences were asymptomatic. The median time to the first relapse was 7.4 years, and 75% of relapses occurred within ten years after primary diagnosis. The median disease-free survival was 11.3 years. Premenopausal status at initial diagnosis, FIGO stage Ic versus Ia, and tumor rupture associated with relapse. However, tumor rupture was the only independent predictive factor. Of the relapsed patients, 48% died of AGCT in a median time of 15.3 years.

Conclusion. Tumor rupture is the strongest predictive factor for recurrence, and these patients might benefit from a more aggressive initial treatment approach. AGCT requires active follow up for 10 to 15 years after primary diagnosis, since recurrences may develop late, asymptomatically and in multiple anatomical locations.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Adult-type granulosa cell tumors (AGCTs) represent a rare form of ovarian cancer characterized by a single somatic point mutation in the transcription factor *FOXL2* [1–3]. Despite the indolent disease course and relatively favorable prognosis, AGCTs exhibit an unpredictable tendency to late relapse [4–9]. Previous studies have shown that up to one-third of patients with AGCT develop tumor recurrence, even in early stage disease, leading to increased mortality [8,10]. The only clinical factor consistently related to prognosis is tumor stage [4,5,10–15]. Tumor size, tumor rupture, presence of residual tumor, incomplete staging, and even body mass index and diabetes have been suggested as clinical prognostic factors for recurrence [9,10,15–19]. Histopathologic and molecular prognosticators have included nuclear atypia, mitotic rate, diffuse growth pattern and expression of transcription factor GATA4 and human epidermal growth factor receptor HER2 [12,13,18,20,21]. Most of these studies

* Corresponding author at: Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, PO Box 140, 00290 Helsinki, Finland.
E-mail address: saara.bryk@hus.fi (S. Bryk).

http://dx.doi.org/10.1016/j.ygyno.2016.10.002
0090-8258/© 2016 Elsevier Inc. All rights reserved.

have, nonetheless, lacked the diagnostic validation of tumors with *FOXL2* mutation status and suffered from either small number of patients or relatively short follow-up periods [4,14,16,19,20].

As disease relapse accounts for the majority of tumor related morbidity and mortality, the most important challenges in the management of AGCT patients lie in the identification and management of patients at increased risk for relapse. Furthermore, specific guidelines as to how these patients should be followed and treated upon relapse have not been established. The routine follow-up time varies from 2 to 5 years or even beyond 5 years as recommended by the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) [22,23]. The aim of the present study was to analyze the clinical characteristics and prognostic factors related to AGCT recurrence, and to identify an optimal follow-up pattern for these patients, using a large, histologically and molecularly validated single-institute, population-based patient cohort.

2. Materials and methods

The ethics committee of Helsinki University Hospital (HUH) and the National Supervisory Authority for Welfare and Health approved the study. All patients diagnosed with GCT during 1956–2014 in HUH were identified, with a minimum follow-up of one year. A histological review supplemented with immunohistochemical staining was performed on all original slides to confirm the diagnosis, and each case underwent *FOXL2* (402C-G) mutation status analysis, resulting in a patient cohort of

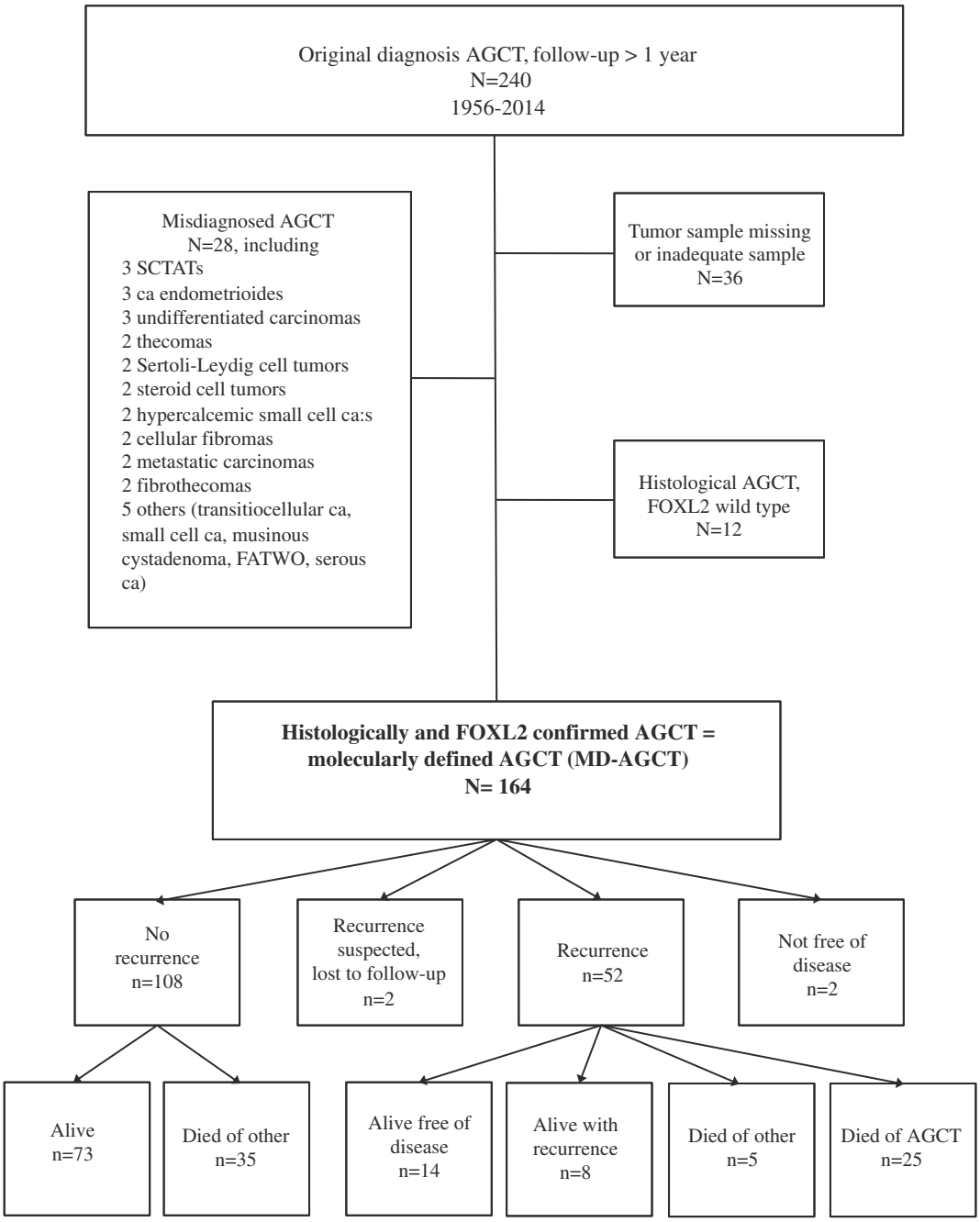

Fig. 1. A histological review supplemented with FOXL2 (402C-G) mutation status analysis was performed on all patients initially diagnosed with adult-type granulosa cell tumors (AGCTs), resulting in a patient cohort of molecularly defined AGCTs (MD-AGCTs, n = 164). Altogether, 76 patients were excluded from the study. Fifty-two patients (32%) relapsed, and 25 (48%) of the relapsed patients died of AGCT. Two patients were lost to follow-up during the study period, each over 15 years after primary diagnosis. SCAT = sex cord tumor with annular tubules, ca = carcinoma, FATWO = female adnexal tumor of probable Wolffian origin.

molecularly defined adult-type granulosa cell tumors (MD-AGCTs). Details of the *FOXL2* analysis have been described in a recent publication [3]. Patients who lacked either histological sample or *FOXL2* mutation status were excluded from the analysis (Fig. 1).

Clinical characteristics were obtained from the patient medical records and included age, menopausal status, parity, use of hormonal treatment and initial symptoms, tumor size, stage, treatment and follow-up regarding both primary and recurrent tumors. Tumor stage was based on the International Federation of Gynecology and Obstetrics (FIGO) criteria [24]. The follow-up information included details on follow-up visits performed at HUH or other units, recurrences, and survival. Survival data were obtained from the Finnish Population Register on Sep 14th 2015, and from death certificates including diagnoses of the cause of death from the Statistics Finland. The clinical data were collected into a database using FileMaker Pro 11.0v4 (FileMaker, Inc., Santa Clara, CA, USA).

The clinical characteristics were evaluated for association with relapse. Disease-free survival was defined as the time period from the date of primary diagnosis to the first confirmed recurrence or last follow-up. Recurrence was recorded only in patients proven tumor-free after initial treatment, i.e. having no residual tumor after surgery or chemotherapy, the latter dependent upon a clean CT scan or second-look operation. This principle was also applied when defining successive recurrences in a single patient. Recurrence sites were grouped in anatomical regions, and recurrence was considered multiple when two or more tumors were simultaneously detected in more than one anatomical region. The year of diagnosis was grouped in two categories (1956–1983 and 1984–2014), mainly based on major developments in imaging (CT-scan and pelvic ultrasound), surgery and introduction of platinum-based chemotherapy [11].

Statistical analysis was performed using JMP Pro 10.0.2 (SAS Institute Inc., Cary, NC, USA). Pearson's Chi-Square Test and Fisher's exact test were used to test associations between groups. Continuous variables were analyzed for normal distribution with the Shapiro-Wilks test and compared with non-parametric test (Mann-Whitney). The disease-free survival curves were obtained using the Kaplan-Meier method and were compared using the log-rank test. Univariate and multivariate analyses were conducted using Cox's regression model. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Primary tumor characteristics and follow-up

After histological and molecular evaluation, 164 of the 240 women (68%) were included in the final analysis (Fig. 1). Mean follow-up time was 16.8 years (median 15.5 years; range 1.0–51.3 years). Patient and tumor characteristics at the time of primary diagnosis are described in detail in Table 1 and Table 2. The mean time in completed hospital-based

Table 1
Clinical characteristics of molecularly defined adult granulosa cell tumor (MD-AGCT) patients according to tumor recurrence (at primary diagnosis).

Characteristic	Total (%)* N = 164	No recurrence (%)* N = 108	Recurrence (%)* N = 52	p-Value
Year of diagnosis				ns
1956–1983	59 (36.0%)	35 (32.4%)	22 (42.3%)	
1984–2014	105 (64.0%)	73 (67.6%)	30 (57.7%)	
Age				
Median (range), years	54.4 (26–81)	55.6 (27–81)	49.9 (26–80)	0.046
<50	58 (35.4%)	31 (28.7%)	26 (50.0%)	0.008
>50	106 (64.6%)	77 (71.3%)	26 (50.0%)	
Menopause				0.004
No	59 (36.6%)	31 (29.2%)	27 (52.9%)	
Yes	102 (63.4%)	75 (70.8%)	24 (47.1%)	
Parity				ns
Nulliparous	56 (34.6%)	38 (35.5%)	16 (31.4%)	
Primiparous	37 (22.8%)	20 (18.7%)	17 (33.3%)	
Multiparous	69 (42.6%)	49 (45.8%)	18 (35.3%)	
Infertility				ns
Yes	27 (17.3%)	20 (19.4%)	7 (14.3%)	
No	129 (82.7%)	83 (80.6%)	42 (85.7%)	
Oral contraceptives[1]				ns
Yes	54 (43.9%)	40 (47.1%)	14 (40.0%)	
No	69 (56.1%)	45 (52.9%)	21 (60.0%)	
Initial symptoms				ns
Abnormal bleeding	76 (46.6%)	52 (48.2%)	22 (43.1%)	
Abdominal pain	41 (25.2%)	25 (23.1%)	14 (27.5%)	
Abdominal distension	21 (12.9%)	12 (11.1%)	9 (17.6%)	
General symptoms	1 (0.6%)	1 (0.9%)	0 (0.0%)	
Asymptomatic	24 (14.7%)	18 (16.7%)	6 (11.8%)	
Preoperative HRT				ns
Yes	24 (20.0%)	16 (18.6%)	8 (25.8%)	
No	96 (80.0%)	70 (81.4%)	23 (74.2%)	
Postoperative HRT				ns
Yes	47 (49.0%)	34 (47.9%)	13 (52.0%)	
No	49 (51.0%)	37 (52.1%)	12 (48.0%)	

*Of those documented (lost to follow-up n = 2, not free of disease n = 2), ns = not significant, [1]before and after diagnosis, HRT = hormone replacement therapy.

Table 2
Primary tumor and treatment characteristics of molecularly defined adult granulosa cell tumor (MD-AGCT) patients according to tumor recurrence (at primary diagnosis).

Characteristic	Total (%)[a] n = 164	No recurrence (%)[a] n = 108	Recurrence (%)[a] n = 52	p-Value
Tumor size				
Mean (range), cm	10.9 (0.5–30)	10.4	11.7	ns
<10 cm	74 (47.4%)	53 (51.0%)	21 (43.8%)	ns
≥10 cm	82 (52.6%)	51 (49.0%)	27 (56.2%)	
Tumor rupture[b]				0.005
Yes	56 (35.7%)	29 (28.2%)	26 (51.0%)	
No	101 (64.3%)	74 (71.8%)	25 (49.0%)	
Ascites				ns
Yes	33 (22.9%)	21 (21.4%)	12 (27.9%)	
No	111 (77.1%)	77 (78.6%)	31 (72.1%)	
FIGO stage				ns
I	148 (91.9%)	100 (93.5%)	46 (90.2%)	
II	10 (6.2%)	6 (5.6%)	3 (5.9%)	
III	3 (1.9%)	1 (0.9%)	2 (3.9%)	
Surgical approach				
Laparoscopy	31 (18.9%)	21 (19.4%)	10 (19.2%)	ns
Laparotomy	133 (81.1%)	87 (80.6%)	42 (80.8%)	
Surgery				ns
Complete[c]	128 (78.5%)	90 (83.3%)	37 (71.2%)	
Incomplete	35 (21.5%)	18 (16.7%)	15 (28.8%)	
Staging[d]				0.002
Yes	48 (29.8%)	41 (38.3%)	7 (13.7%)	
No	113 (70.2%)	66 (61.7%)	44 (86.3%)	
Lymphadenectomy				0.047
Yes	37 (23.0%)	30 (28.0%)	7 (13.7. %)	
No	124 (77.0%)	77 (72.0%)	44 (86.3%)	
Adjuvant chemotherapy				ns
Yes	28 (17.2%)	20 (18.5%)	8 (15.7%)	
No	135 (82.8%)	88 (81.5%)	43 (84.3%)	
Tumor histology				ns
Follicular/trabeculoid	53 (37.9%)	39 (40.6%)	12 (30.0%)	
Diffuse/sarcomatoid	87 (62.1%)	57 (59.4%)	28 (70.0%)	
Endometrial pathology				ns
Normal	52 (38.0%)	37 (37.8%)	13 (37.1%)	
Polyps	18 (13.1%)	12 (12.2%)	6 (17.1%)	
Hyperplasia	57 (41.6%)	39 (39.8%)	16 (45.7%)	
Carcinoma	10 (7.3%)	10 (10.2%)	0 (0.0%)	

[a] Of those documented (lost to follow-up n = 2, not free of disease n = 2), ns = not significant.
[b] Stage IC1, n = 32, stage IC2, n = 18, stage II n = 5, stage III n = 1), FIGO = International Federation of Gynecology and Obstetrics.
[c] No gynecological organs left.
[d] Peritoneal biopsies and omental biopsy or omentectomy.

routine follow-up was 5.5 years for all patients after primary treatment, and 6.2 and 5.4 years for patients with and without tumor recurrence, respectively ($p = 0.3$). The median number of hospital visits was 11. Transabdominal or transvaginal pelvic ultrasound was performed at least once in routine follow-up on 97 patients (68% of those with data available) and a CT-scan on 41 patients (28%). PET/CT was used nine times, most often unsuccessfully. Serum markers were measured in 101 (71%) patients, and consisted of e.g. estradiol (E2), carcinoembryonic antigen (CEA), CA-125, Inhibin B, and anti-Müllerian hormone (AMH). Routine monitoring for Inhibin B and AMH levels began in 1998 and 2014, respectively, and in primary follow-up either one was measured in altogether 70 patients.

3.2. Relapse

Fifty-two of the 164 patients relapsed, resulting in a recurrence rate of 32.1% (Fig. 1). One relapse was observed in 23 patients, two relapses in 17 patients, three in five patients, four in four patients, five in two patients, and six in one patient. The median time to the first relapse was 7.4 years (range 1.1–26.6 years), and the median time between the first and second relapse was 2.9 years (range 0.7–29.6), and the second and third 1.4 years (range 0.7–4.4 years). The majority of first relapses (75%) occurred within ten years, and 87% within 15 years after primary diagnosis (Supplementary Fig. S1).

When evaluating the primary clinical characteristics of patients according to relapse, we found a significantly higher rate of relapses among premenopausal patients (47% vs. 24%, $p = 0.004$). Age distribution differed between the relapsed and non-relapsed groups both as a categorical (⩾ 50 years) and continuous variable, where younger age associated with increased rate of relapses. Tumor rupture, including both perioperative (stage IC1, $n = 32$, stage II $n = 3$), and spontaneous (stage IC2, $n = 18$, stage II $n = 2$, stage III $n = 1$) ruptures, was more common in the recurrent group (51% vs. 28%, $p = 0.005$), as was the lack of staging surgery and lymphadenectomy ($p = 0.002$ and $p = 0.047$, respectively) (Table 2). In routine follow-up, serum markers were monitored and ultrasound performed more often among non-recurrent patients. However, the year of diagnosis as such had no significant impact on recurrence rate (Table 1). In a subanalysis including only stage IC tumors ($n = 53$), we found that the lack of staging surgery and lymphadenectomy were significantly more common among patients with recurrence (Supplementary Table S1).

In Kaplan-Meier and Cox's univariate analyses, the risk of relapse was increased in premenopausal patients, FIGO stage IC versus IA tumors, and in patients with tumor rupture (Fig. 2). The risk was also significantly increased in both stage IC1 and stage 1C2 subgroups alone, when compared with stage 1 A tumors. Premenopausal status was significant by 15 years ($p = 0.04$) and 20 years ($p = 0.02$) of follow-up, whereas stage IC and tumor rupture were significant predictors already by five years of follow-up ($p = 0.02$ and 0.03, respectively). In multivariate analysis, tumor rupture was the only significant risk factor for AGCT recurrence. In stage IC patients, lack of staging surgery and adjuvant chemotherapy were associated with recurrence in univariate analysis. However, in multivariate analysis neither of these was an independent risk factor. When further evaluating the risk for successive relapses after the first recurrence, tumor rupture at the time of recurrence was the only significant clinical risk factor for second relapse.

3.3. Recurrent tumor characteristics

The characteristics of recurrent tumors are presented in Table 3. Recurrences were asymptomatic in 38% of first events of relapse, and in the majority (57–62%) of the successive relapses. Forty-five percent of all asymptomatic tumors were local recurrences in the pelvis. When symptomatic, all recurrences presented most often with abdominal pain. Other, significantly less common symptoms included menstrual cycle disturbances, a palpable mass and abdominal bloating. Thirty-

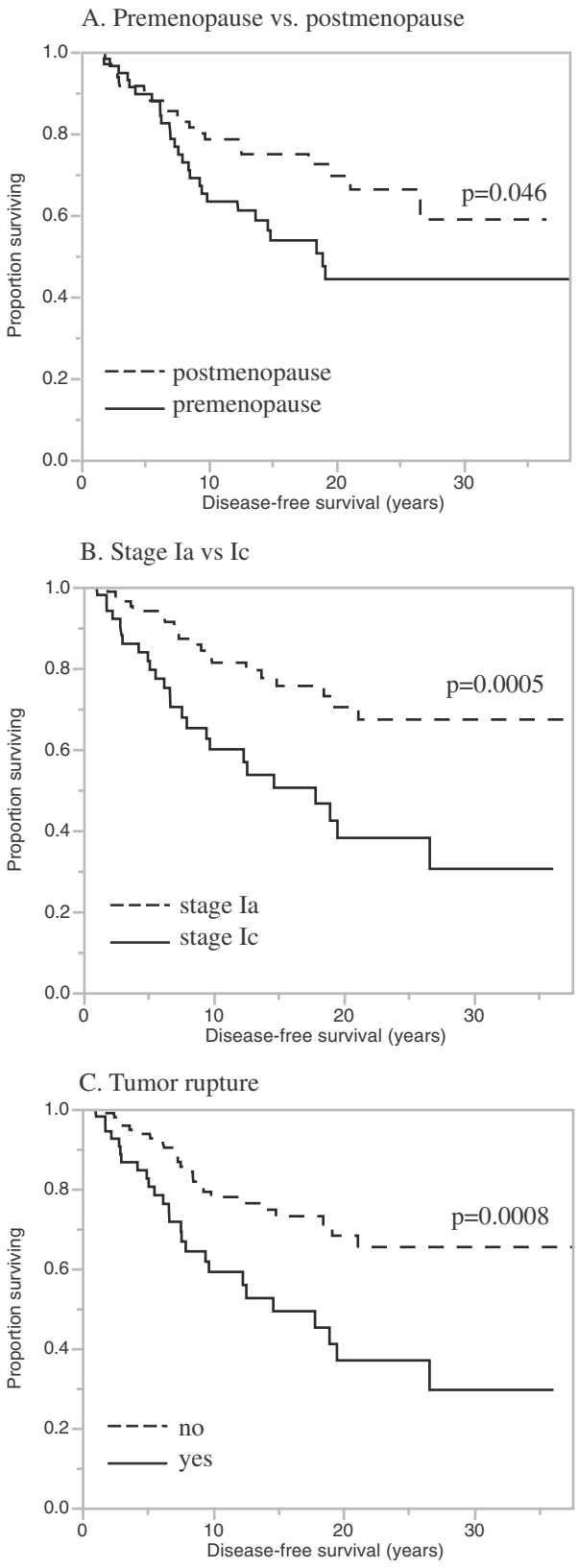

Fig. 2. Kaplan-Meier curves of disease-free survival according to A) menopausal status B) stage Ia versus Ic, and C) tumor rupture.

eight percent of first relapses were discovered by clinical examination alone or in combination with vaginal ultrasound and/or serum markers. Serum marker levels were measured from 64% patients at first relapse, and from 73% of patients at all relapses combined. When measured, CA-

Table 3
Characteristics of molecularly defined adult granulosa cell tumor patients (n = 164), according to order of recurrence.

Characteristic	I recurrence n = 52	II recurrence n = 29	III–VI recurrence n = 23[a]
Year of recurrence			
1956–1983	15 (28.8%)	4 (13.8%)	1 (4.3%)
1984–2014	37 (71.2%)	25 (86.2%)	22 (95.7%)
Age			
<50	15 (28.8%)	8 (27.6%)	4 (17.4%)
>50	37 (71.2%)	21 (72.4%)	19 (82.6%)
Menopause			
No	10 (19.2%)	1 (3.4%)	0 (0.0%)
Yes	42 (80.8%)	28 (96.6%)	23 (100.0%)
Gynecological organs left[b]			
Yes	15 (28.8%)	3 (10.3%)	0 (0.0%)
No	37 (71.2%)	26 (89.7%)	23 (100.0%)
Symptoms			
Yes	30 (62.5%)	11 (37.9%)	9 (42.9%)
No	18 (37.5%)	18 (62.1%)	12 (57.1%)
Ca12-5			
Elevated	8 (33.3%)	5 (27.8%)	3 (42.9%)
Normal	16 (66.7%)	13 (72.2%)	4 (57.1%)
Inhibin-B			
Elevated	16 (88.9%)	17 (94.4%)	12 (100.0%)
Normal	2 (11.1%)	1 (5.6%)	0 (0.0%)
Tumor size (mass)			
<10 cm	22 (45.8%)	18 (62.1%)	10 (45.5%)
≥10 cm	26 (54.2%)	11 (37.9%)	12 (54.5%)
Ascites			
Yes	10 (25.0%)	4 (14.3%)	5 (26.3%)
No	30 (75.0%)	24 (85.7%)	14 (73.7%)
Tumor rupture[c]			
Yes	16 (39.0%)	4 (14.8%)	6 (28.6%)
No	25 (61.0%)	23 (85.2%)	15 (71.4%)
Treatment			
Radical surgery	19 (36.5%)	11 (37.9%)	6 (26.1%)
Surgery + adjuvant[d]	24 (46.2%)	6 (20.7%)	7 (30.4%)
Palliative surgery	2 (3.8%)	1 (3.5%)	1 (4.4%)
CT	3 (5.8%)	3 (10.3%)	4 (17.4%)
CT + other[e]	1 (1.9%)	3 (10.3%)	0 (0.0%)
CT + radiotherapy	0 (0.0%)	1 (3.5%)	0 (0.0%)
Radiotherapy	0 (0.0%)	1 (3.5%)	0 (0.0%)
Aromatase inhibitor	0 (0.0%)	0 (0.0%)	2 (8.7%)
No treatment	3 (5.8%)	3 (10.3%)	3 (13.0%)

[a] III n = 5, IV n = 4, V n = 2, VI n = 1.
[b] Uterus or ovary.
[c] Spontaneus or intraoperative.
[d] Chemo- or radiotherapy, CT = chemotherapy.
[e] Aromatase inhibitor n = 4, bevasizumab n = 2.

125 was elevated in 33% of relapses (in 28% of pelvic, 36% of multiple and 40% of abdominal tumors), and Inhibin-B in 94% of relapses. The clinical use of AMH had only recently been established, and was measured in three patients, showing elevated levels prior to relapse in all cases.

Anatomical sites of recurrence are presented in Fig. 3. Majority of the single recurrences occurred in the pelvis. The second most common single-tumor site was abdominal cavity excluding pelvis, both for first and successive relapses. However, multiple-site recurrence was observed in nearly half of all events of relapse, the proportion remaining similar from 1st to 6th relapses (39–44%). Of all sites, the pelvis was most often involved also in multiple-site recurrence through successive relapses, and the proportion was highest at first relapse (45%). Other sites included the retroperitoneum, liver, inguinal, mediastinal or para-aortic lymph nodes, abdominal wall, lung, and bone (iliac bone, pelvic and thoracic spine). Recurrences in the abdominal wall were seen after both laparotomy (n = 6) and laparoscopy (n = 3).

The majority (70%) of recurrences were treated by surgery with or without adjuvant chemotherapy or radiotherapy (Table 3). Radical surgery with no macroscopic evidence of disease was achieved in 37% of first and 26% of 3rd to 6th recurrences. Ten cases received chemotherapy alone, four cases chemotherapy combined with aromatase inhibitor (letrozole) and/or angiogenesis inhibitor (bevacizumab) and in one case, chemotherapy was used in combination with radiation. Radiotherapy alone was given to one patient with a single recurrent tumor, and hormonal treatment (letrozole) alone to two cases. Four cases underwent palliative surgery, and nine tumors were left untreated due to patient will, old age or poor medical condition.

3.4. Survival and outcomes

In the whole cohort, the five- and ten-year overall survival rates were 96.3% and 88.4%, and disease-specific survival rates 98.7% and 93.5%, respectively. The median overall survival among non-recurrent patients was 30.6 years and among recurrent patients 26.5 years (p = 0.03).

Outcomes are presented in Fig. 1. Twenty-seven patients died of AGCT during follow-up. Median disease-free survival (DFS) was 11.3 years. After the first recurrence, median DFS was 3.5 years, and overall survival 8.0 years. A total of six patients have remained disease-free after the first recurrence with a median follow-up of 7.7 years. Nearly half (48%) of the relapsed patients died of AGCT, in a median time of 15.3 years. The proportion of disease-related death was 52% among patients with one recurrence, 31% among those with two, 67% among those with three, 75% among those with four, and 100% among those with five to six recurrences. There was no significant difference in median disease-free survival between patients with one or several successive relapses (7.6 vs. 7.0 years, respectively).

4. Discussion

We have herein studied thus far the largest cohort of *FOXL2* (402C-G) mutation validated granulosa cell tumor patients treated in a single, population-based institute during a six-decade-long period in order to identify reliable risk factors and the clinical picture of tumor recurrence, and guidelines for clinical follow-up. To our knowledge, this is the most comprehensive study to date in analyzing the clinical characteristics of AGCT recurrence. Given the rarity and diagnostic challenges of AGCTs together with the tendency to late recurrences, studies on its clinical course require standardized diagnostics accompanied with decades of follow-up. The molecular diagnostics has been proven crucial in differentiating AGCTs from other, usually more malignant ovarian tumors with significantly worse prognosis [3]. Therefore, the determination of *FOXL2* mutation status is recommended in the primary diagnostics of AGCT [3,25]. Prior studies focusing on AGCT recurrence have, however, lacked the diagnostic accuracy achieved with molecular validation. The 32% recurrence rate with a median time-to-relapse of 7.4 years in this study represents a slightly higher rate and longer median time than observed in most prior works, where recurrence rates vary significantly from 5% to 64% [18,20], although most studies with large patients series, extensive follow-up and tertiary-center treatment have documented a recurrence rate of 20–30% [9,17,26].

Upon relapse, AGCT showed an aggressive course: more than half of the relapsed patients had more than one event of disease recurrence, and nearly half of the relapsed patients eventually died of AGCT. In patients with more than one relapse, the pattern of recurrence resembles that of epithelial ovarian cancer, where the median time to the following relapse shortens by half along with each new recurrence. However, the median survival times of over 25 years among both non-recurrent and recurrent patients reflect the slow and indolent course of AGCT.

We found tumor rupture and stage IC, including both IC1 and IC2, as significant risk factors for recurrence in the patient cohort with mostly stage I tumors, similarly to a recent study [9]. In our cohort, the vast majority of these patients were primarily treated by surgery alone, which is also the current treatment protocol for all stage I AGCTs at HUH. In a subanalysis of stage IC patients significantly more recurrences occurred in patients lacking staging surgery or adjuvant chemotherapy. Additionally, tumor rupture at first recurrence predicted successive relapses. This suggests that patients with tumor rupture might benefit from complete staging including lymphadenectomy, and adjuvant chemotherapy,

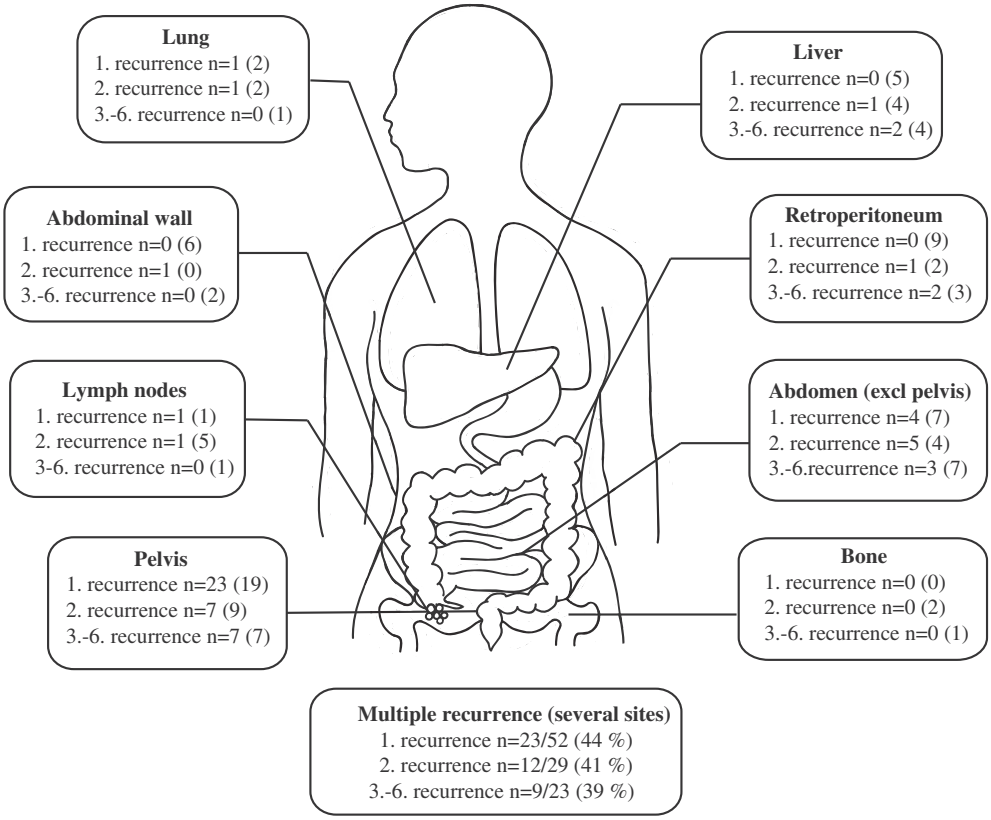

Fig. 3. Sites of recurrent, molecularly defined adult granulosa cell tumors (MD-AGCTs) in 52 patients and 104 events of relapse, by anatomical region and order of recurrence. Multiple tumors simultaneously evident in several anatomical regions were present in 44 cases of relapse, and the sites involved are presented in parentheses. The site of recurrence was unknown in one case.

although these findings were only evident in univariate analysis. Possible chemotherapeutic regimens include BEP, which is the recommended first-line chemotherapy for stages II–IV granulosa cell tumors [22,23]. Alternatively, a combination of paclitaxel-carboplatin may be used [22,23,27]. Staging surgery was only performed on one-third of patients in our whole series, reflecting both the wide treatment era and the unpredictable diagnosis of malignant AGCT. This again highlights the need for accurate diagnostics at presentation.

Younger age as well as premenopausal status at the time of primary diagnosis increased the risk for recurrence. This raises the question whether these premenopausal patients, often treated with sparing surgery, should eventually undergo radical surgery when pregnancy is no longer desired, or latest at menopause. On the other hand, fertility-sparing or incomplete surgery as such was not a risk factor for recurrence, nor for AGCT-related death in a previous study based on virtually the same patient cohort [11]. Moreover, laparoscopy as a primary procedure did not increase the risk for relapse.

The median time to relapse of over seven years calls for long-lasting follow-up - at least ten to fifteen years from primary diagnosis, including regular testing for Inhibin B and/or AMH [28]. Since a significant proportion of patients were asymptomatic at relapse, a clinical follow-up based on symptoms only cannot be recommended. AGCT relapses show variety in both recurrence sites and symptoms, although local recurrences in the pelvis are most common, also at multiple-site recurrence. Hence, pelvic ultrasound remains a standard follow-up tool but a CT-scan is recommended in case of suspected relapse – especially after the first recurrence, when the proportion of asymptomatic and distant tumors increases. AGCTs have a low 18-F-fluorodeoxyglucose (FDG) avidity, and PET/CT is not indicated in the diagnostics and routine follow-up of these tumors, which was also evident in our data [29,30].

Interestingly, hormone replacement therapy after primary diagnosis was given to nearly half of the patients. It had no deteriorating effect on prognosis, instead there was a trend for risk reduction in univariate analysis (data not shown). This is a useful message especially for premenopausal patients, who might suffer from menopausal symptoms after radical surgery. Based on this evidence, there is no need to limit postmenopausal hormone therapy use among patients with a treated AGCT.

The long follow-up period of nearly 60 years is both a strength and a weakness of this study. The median follow-up of 15.5 years (186 months) is the longest reported, and provides reliable data on late-recurring AGCTs. However, this also results in inconsistency in diagnostic and treatment modalities, depending on the treatment era, although the year of diagnosis as such had no significant impact on observed recurrence rates. Nevertheless all cases underwent molecular and histological validation, and thus represent accurately diagnosed AGCTs.

In conclusion, we found that tumor rupture was the strongest predictive factor for recurrence in a large patient cohort with molecularly validated AGCTs. In case of suspected recurrence, CT scan is recommended since multiple and distant recurrences may occur. Premenopausal patients run an increased risk for relapse, and might benefit from eventual radical surgery. The role of adjuvant therapy for stage Ic patients remains to be established, but in the light of the present study, a more aggressive approach might be needed. The relatively high and late recurrence rate confirms that AGCT requires active follow-up of ten to fifteen years after primary diagnosis, complemented with regular monitoring with specific serum markers.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2016.10.002.

Acknowledgements

We thank Dr. Melissa McConechy, Dr. David Huntsman and Dr. Blake Gilks at University of British Columbia and Vancouver General Hospital, BC, Canada for collaboration in the FOXL2 validation of the study cohort

and Ms. Teija Karkkulainen for technical assistance. This study received funding from the Finnish State Funding for University-Level Health Research, The Ida Montini Foundation and University of Helsinki Research Funds.

The authors declare no conflict of interest.

References

[1] S. Jamieson, R. Butzow, N. Andersson, M. Alexiadis, L. Unkila-Kallio, M. Heikinheimo, et al., The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary, Mod. Pathol. 23 (11) (2010) 1477–1485.

[2] S. Jamieson, P.J. Fuller, Molecular pathogenesis of granulosa cell tumors of the ovary, Endocr. Rev. 33 (1) (2012) 109–144.

[3] M.F.A. McConechy, H. Horlings, Molecularly defined adult granulosa cell tumor of the ovary: the clinical phenotype, J. Natl. Cancer Inst. (2016) (in press).

[4] A. Ayhan, M.C. Salman, M. Velipasaoglu, M. Sakinci, K. Yuce, Prognostic factors in adult granulosa cell tumors of the ovary: a retrospective analysis of 80 cases, J. Gynecol. Oncol. 20 (3) (2009) 158–163.

[5] S.T. Schumer, S.A. Cannistra, Granulosa cell tumor of the ovary, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 21 (6) (2003) 1180–1189.

[6] S. Bryk, E. Pukkala, J.I. Martinsen, L. Unkila-Kallio, L. Tryggvadottir, P. Sparén, K. Kjaerheim, E. Weiderpass, A. Riska, Incidence and occupational variation of ovarian granulosa cell tumours in Finland, Iceland, Norway, and Sweden during 1953–2012: a longitudinal cohort study, International Journal of Obstetrics and Gynaecology (2016) (in press).

[7] N. East, A. Alobaid, F. Goffin, K. Ouallouche, P. Gauthier, Granulosa cell tumour: a recurrence 40 years after initial diagnosis. Journal of obstetrics and gynaecology Canada, JOGC = Journal d'obstetrique et gynecologie du Canada: JOGC 27 (4) (2005) 363–364.

[8] A.T. Evans 3rd, T.A. Gaffey, G.D. Malkasian Jr., J.F. Annegers, Clinicopathologic review of 118 granulosa and 82 theca cell tumors, Obstet. Gynecol. 55 (2) (1980) 231–238.

[9] M.K. Wilson, P. Fong, S. Mesnage, K. Chrystal, A. Shelling, K. Payne, et al., Stage I granulosa cell tumours: a management conundrum? Results of long-term follow up, Gynecol. Oncol. 138 (2) (2015) 285–291.

[10] G. Mangili, J. Ottolina, A. Gadducci, G. Giorda, E. Breda, A. Savarese, et al., Long-term follow-up is crucial after treatment for granulosa cell tumours of the ovary, Br. J. Cancer 109 (1) (2013) 29–34.

[11] S. Bryk, A. Farkkila, R. Butzow, A. Leminen, M. Heikinheimo, M. Anttonen, et al., Clinical characteristics and survival of patients with an adult-type ovarian granulosa cell tumor: a 56-year single-center experience, Int. J. Gynecol. Cancer 25 (1) (2015) 33–41.

[12] J.T. Stenwig, J.T. Hazekamp, J.B. Beecham, Granulosa cell tumors of the ovary. A clinicopathological study of 118 cases with long-term follow-up, Gynecol. Oncol. 7 (2) (1979) 136–152.

[13] E. Björkholm, C. Silfverswärd, Prognostic factors in granulosa-cell tumors, Gynecol. Oncol. 11 (3) (1981) 261–274.

[14] I.H. Lee, C.H. Choi, D.G. Hong, J.Y. Song, Y.J. Kim, K.T. Kim, et al., Clinicopathologic characteristics of granulosa cell tumors of the ovary: a multicenter retrospective study, J. Gynecol. Oncol. 22 (3) (2011) 188–195.

[15] I.E. Ertas, K. Gungorduk, S. Taskin, L. Akman, A. Ozdemir, R. Goklu, et al., Prognostic predictors and spread patterns in adult ovarian granulosa cell tumors: a multicenter long-term follow-up study of 108 patients, Int. J. Clin. Oncol. 19 (5) (2014) 912–920.

[16] A. Suri, E.B. Carter, N. Horowitz, S. Denslow, P.A. Gehrig, Factors associated with an increased risk of recurrence in women with ovarian granulosa cell tumors, Gynecol. Oncol. 131 (2) (2013) 321–324.

[17] H.D. Sun, H. Lin, M.S. Jao, K.L. Wang, W.S. Liou, Y.C. Hung, et al., A long-term follow-up study of 176 cases with adult-type ovarian granulosa cell tumors, Gynecol. Oncol. 124 (2) (2012) 244–249.

[18] H.S. van Meurs, E. Schuit, H.M. Horlings, J. van der Velden, W.J. van Driel, B.W. Mol, et al., Development and internal validation of a prognostic model to predict recurrence free survival in patients with adult granulosa cell tumors of the ovary, Gynecol. Oncol. 134 (3) (2014) 498–504.

[19] S.H. Shim, S.J. Lee, D.Y. Kim, J. Kim, S.N. Kim, S.B. Kang, et al., A long-term follow-up study of 91 cases with ovarian granulosa cell tumors, Anticancer Res. 34 (2) (2014) 1001–1010.

[20] N. Ud Din, N. Kayani, Recurrence of adult granulosa cell tumor of the ovary: experience at a tertiary care center, Ann. Diagn. Pathol. 18 (3) (2014) 125–128.

[21] A. Färkkilä, N. Andersson, R. Bützow, A. Leminen, M. Heikinheimo, M. Anttonen, et al., HER2 and GATA4 are new prognostic factors for early-stage ovarian granulosa cell tumor-a long-term follow-up study, Cancer Med. 3 (3) (2014) 526–536.

[22] N. Colombo, M. Peiretti, A. Garbi, S. Carinelli, C. Marini, C. Sessa, et al., Non-epithelial ovarian cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up, Annals of Oncology: Official Journal of the European Society for Medical Oncology/ESMO 23 (Suppl. 7) (2012), vii20-6.

[23] NCCN Clinical Practice Guidelines in Oncology — Ovarian Cancer. Version 2.2015, 06/22/15. Available at nccn.org [Internet]. 2015.

[24] Figo Committee on Gynecologic Oncology T, Current FIGO staging for cancer of the vagina, fallopian tube, ovary, and gestational trophoblastic neoplasia, International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics 105 (1) (2009) 3–4.

[25] S. Kommoss, M.S. Anglesio, R. Mackenzie, W. Yang, J. Senz, J. Ho, et al., FOXL2 molecular testing in ovarian neoplasms: diagnostic approach and procedural guidelines, Mod. Pathol. 26 (6) (2013) 860–867.

[26] G. Mangili, C. Sigismondi, L. Frigerio, M. Candiani, A. Savarese, G. Giorda, et al., Recurrent granulosa cell tumors (GCTs) of the ovary: a MITO-9 retrospective study, Gynecol. Oncol. 130 (1) (2013) 38–42.

[27] J. Brown, H.S. Shvartsman, M.T. Deavers, L.M. Ramondetta, T.W. Burke, M.F. Munsell, et al., The activity of taxanes compared with bleomycin, etoposide, and cisplatin in the treatment of sex cord-stromal ovarian tumors, Gynecol. Oncol. 97 (2) (2005) 489–496.

[28] A. Färkkilä, S. Koskela, S. Bryk, H. Alfthan, R. Bützow, A. Leminen, et al., The clinical utility of serum anti-Müllerian hormone in the follow-up of ovarian adult-type granulosa cell tumors—a comparative study with inhibin B, Int. J. Cancer (2015).

[29] G. Raj, A. Proietto, K. Jaaback, Positron emission tomography and granulosa cell tumor recurrence: a report of 2 cases, Int. J. Gynecol. Cancer 19 (9) (2009) 1542–1544.

[30] Y.T. Huang, J.C. Lee, A.S. Kumar, Variable F-18 fluorodeoxyglucose avidity of metastatic recurrent adult granulosa cell tumor, Clin. Nucl. Med. 34 (10) (2009) 710–712.