Androgen Mediation of Thrombocytosis in Epithelial Ovarian Cancer Biology

Andrew John Li and Beth Young Karlan

Abstract

Purpose: Preoperative thrombocytosis (platelet count $> 400 \times 10^9$/L) at initial exploration for epithelial ovarian carcinoma is associated with decreased surgical cytoreducibility and poor survival. Platelets express androgen receptor (AR), which contains a polymorphic CAG trinucleotide repeat sequence of which the length inversely correlates with AR transactivation function. We hypothesized that androgen-mediated thrombocytosis promotes aggressive ovarian cancer biology.

Experimental Design: Sixty-three patients with epithelial ovarian carcinoma underwent geno-type analysis of the CAG repeat polymorphism in AR. Medical records were reviewed to assess preoperative thrombocytosis, surgical findings, and survival. Data were examined using the Fisher's exact, logistic regression, and Kaplan-Meier analyses.

Results: AR CAG repeat lengths ranged from 8 to 27, with a median of 23. Fifteen of 63 patients (23.8%) showed preoperative thrombocytosis. Short AR allelotype ($\leq 20$ CAG repeats) was associated with a higher incidence of thrombocytosis ($P = 0.04$). The combination of short AR allelotype and thrombocytosis was the only significant factor that predicted inability to achieve optimal surgical cytoreduction ($P = 0.02$). Women with short AR allelotype and thrombocytosis showed statistically decreased progression-free survival (13 versus 37 months, $P = 0.01$) and overall survival (37 versus 65 months, $P = 0.02$) when compared with women with long AR allelotype and normal platelet counts. On multivariate analyses, suboptimal cytoreduction was the only significant factor predictive of disease-specific overall survival ($P = 0.0002$) but the combination of short AR allelotype and thrombocytosis approached statistical significance ($P = 0.08$).

Conclusions: Androgen modulation of thrombocytosis may promote aggressive epithelial ovarian cancer biology.

Thrombocytosis (platelet count $> 400 \times 10^9$/L) has been reported for a variety of solid tumors, including lung, renal, gastric, breast, pancreatic, and colon malignancies (1–6). In gynecologic cancers, preoperative elevations in platelet counts have also been described, with data suggesting that thrombocytosis may function as an independent poor prognostic factor in locally metastatic cervical and advanced-stage ovarian carcinomas (7, 8). Clinical and molecular evidence suggests that sex hormones, specifically androgens, mediate human platelet count and function. In vitro, human platelet aggregation induced by arachidonic acid is enhanced by androgens, and androgen therapy raises platelet counts in patients with myelodysplasia and thrombocytopenia (9, 10). In mice, castration decreases thrombopoiesis and testosterone restores platelet production (11).

Androgens signal through a specific androgen receptor (AR), encoded by a gene on exon 1 of the X chromosome. Length of a polymorphic trinucleotide CAG repeat, which codes for a variable polyglutamine sequence, has been shown to inversely correlate with AR transactivation function; longer AR polyglutamine lengths, encoded by more CAG repeats (ranging between 8 and 31), are associated with lower transcriptional activity and vice versa (12, 13). In epithelial ovarian carcinomas, we have previously shown that a short AR allelotype (with CAG lengths of 19 repeats) is associated with decreased surgical cytoreducibility and poor overall survival (14).

We have hypothesized that androgen-mediated thrombocytosis promotes aggressive epithelial ovarian cancer biology. In this study, we characterized AR CAG repeat lengths in a hospital-based cohort of women with epithelial ovarian carcinoma and attempted to evaluate associations between
preoperative thrombocytosis and short AR allelotype with prognostic clinicopathologic factors.

Materials and Methods

Under an Institutional Review Board–approved protocol, the Gynecologic Oncology Laboratory at Cedars-Sinai Medical Center routinely collects malignant and benign tissue specimens from consenting women undergoing surgical exploration. We queried our database for consecutive patients with stage II to IV epithelial ovarian carcinoma who had available banked serum from their initial cytoreductive surgery between 1995 and 2000. Patients with tumors of low malignant potential and histories of myelosuppressive disorders, acute inflammatory diseases, or splenectomies were excluded from this study. All patients had undergone primary surgical staging by one of four gynecologic oncologists with the intent of optimal tumor cytoreduction; patients who received neoadjuvant chemotherapy were excluded from the review. Following surgical staging, all patients underwent platinum-based chemotherapy. A preoperative automated complete blood count within 14 days of surgery was available for all patients. Thrombocytosis was considered as platelet count > 400 × 10^9/L, consistent with published criteria (1, 2, 6, 7).

Genomic DNA was isolated from banked serum using standard procedures (15). Sequences encompassing the polyglutamine CAG repeat region in exon 1 of AR were amplified by hot-start PCR using primers flanking the CAG sequence (5'-TCCAGAATCTGTCTCA-GAGCGTGCC-3' and 5'-GCGTGGAAGGTTCTTGTCCTCTCAT-3') as described by Giovannucci et al. (16). Primers were labeled with fluorescein aminothio to determine sequence length using laser-activated fluorescent dye technology (ABI 377 PRISM and associated software; Applied Biosystems, San Mateo, CA). Representative PCR products were independently sequenced to confirm number of CAG repeat lengths and product identity. Patient data were abstracted from medical records and included preoperative platelet count, surgical and pathologic findings, and time to recurrence and death.

For statistical considerations, a short AR allele length was defined as ≤20 CAG repeats, consistent with published criteria implicating AR allelotype length in breast cancer risk (17). Sample size and power calculations were based on the comparison of overall survival for one patient in the short AR allelotype/thrombocytosis group to each of the six in the long AR allelotype/normal platelets group. Using the log-rank test with a two-sided significance level of 5% and a calculated power of 93%, 60 patients were needed to distinguish a 22-month difference in survival assuming that patients were accrued over a 10-year period. Data were examined using the Fisher’s exact, logistic regression, Cox proportional hazards, and Kaplan-Meier survival analyses. P < 0.05 was considered to be statistically significant.

Results

Sixty-three patients were studied in this cohort. The majority of patients had advanced-stage and high-grade disease at the time of surgery: 58 (92%) had stage III or IV disease and 57 (90%) had grade 3 histology. Fifty-five (87%) patients underwent optimal surgical cytoreductive surgery at initial exploration to residual disease < 1 cm. In this cohort, 15 (24%) women showed preoperative thrombocytosis. In these 15 patients, the median platelet count was 486 × 10^9/L (range, 400 × 10^9/L-756 × 10^9/L). In the 48 women without thrombocytosis, the median platelet count was 287 × 10^9/L (range, 138 × 10^9/L-398 × 10^9/L).

Genotype analysis of all 63 women revealed a median CAG repeat length of 23 (range, 8-29). The range of allele lengths and the frequency of occurrence of each CAG repeat length are shown in Fig. 1. Clinicopathologic characteristics of women harboring a short (≤20 CAG repeats) AR allele versus those harboring a long (>20) AR allele are summarized in Table 1. No differences in age at diagnosis were identified (61.5 versus 60.5 years) between patients within each allelotype group. There were differences in stage at presentation, proportion of patients with suboptimal cytoreduction, and thrombocytosis between patients with short and long AR alleles.

Table 1. Clinicopathologic characteristics of patients with short AR allelotype (≤20 CAG repeats) and long AR allelotype (>20 CAG repeats)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>AR allelotype ≤20, n = 12</th>
<th>AR allelotype &gt;20, n = 51</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>60.5</td>
<td>61.5</td>
<td>NS</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>12 (100%)</td>
<td>47 (92%)</td>
<td>NS</td>
</tr>
<tr>
<td>Grade 3</td>
<td>10 (83%)</td>
<td>48 (94%)</td>
<td>NS</td>
</tr>
<tr>
<td>Suboptimal cytoreduction</td>
<td>3 (25%)</td>
<td>5 (10%)</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytosis</td>
<td>6 (50%)</td>
<td>9 (17%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

NOTE: NS, not significant.
*Suboptimal cytoreduction defined as residual disease > 1 cm.
†Thrombocytosis defined as platelet count > 400 × 10^9/L.

Fig. 1. Distribution of AR allelotype frequencies by the number of CAG repeat sequences in 63 women with epithelial ovarian carcinoma. The number of alleles with a given CAG repeat length is indicated. The median number of CAG repeat sequences was 23 and ranged between 8 and 29 CAG repeats.

Fig. 2. Effect of thrombocytosis and short AR allelotype (≤20 CAG repeats) on progression-free survival. The combination of these factors predicted shorter time to recurrence than in patients with normal platelet counts and long AR allelotype (13.0 versus 37.0 months, P = 0.01).
years, \( P = \text{NS} \)). The distribution of advanced-stage and high-grade disease was evenly distributed between the two groups; although there was a higher incidence of suboptimal cytoreduction in patients harboring a short AR allele (25\% versus 10\%), this difference was not statistically significant. A significant association between short AR allele and preoperative thrombocytosis, however, was identified; 6 of 12 (50\%) patients with a short AR allelotype showed preoperative platelet counts of \( >400 \times 10^9/L \) compared with 9 of 51 (17\%) patients with a long AR allelotype (\( P = 0.04 \)).

Platelets express functionally active AR, which may mediate release of angiogenic growth factors important in cancer biology (18). To examine the effect of thrombocytosis and short AR allelotype on disease progression, we examined Kaplan-Meier survival analyses to identify potential differences in disease-free and overall survival. Patients with both a short AR allelotype (\( \leq 20 \text{ CAG repeats} \)) and thrombocytosis showed a statistically shorter time to recurrence when compared with patients with a long AR allelotype and normal preoperative platelet counts (median survival, 13.0 versus 37.0 months, \( P = 0.01 \); Fig. 2). A short AR allelotype and thrombocytosis also predicted worse overall survival (median survival, 37.0 versus 65.0 months, \( P = 0.02 \); Fig. 3).

To determine the effect of androgen-mediated thrombocytosis on overall survival in the context of established prognostic factors, multivariate analysis was done using the Cox regression hazards model considering short AR allele length and presence of thrombocytosis as separate factors and in combination (Table 2). Age, stage, and grade were not significant prognostic factors in this cohort. However, optimal cytoreduction retained strong prognostic significance as an independent positive predictor of overall survival (risk ratio, 5.0; \( P = 0.0002 \)). The combination of a short AR allelotype and thrombocytosis approached statistical significance as a negative prognostic indicator (risk ratio, 0.44; \( P = 0.08 \)).

To further define the role of androgen-mediated thrombocytosis in epithelial ovarian tumor biology, established clinicopathologic factors were examined in relation to AR allele length and platelet count as predictors of optimal tumor cytoreduction at exploratory surgery (Table 3). After controlling for age, stage, and grade, logistic regression analyses identified the combination of a short AR allelotype and thrombocytosis as the only independent negative predictor of tumor resection to residual disease <1 cm (odds ratio, 0.46; \( P = 0.02 \)).

**Discussion**

We have hypothesized that androgen-mediated thrombocytosis promotes aggressive epithelial ovarian cancer biology. To test this hypothesis, we studied AR transactivation function indirectly through genotype analysis in a hospital-based cohort of women with epithelial ovarian carcinomas and examined clinical and pathologic findings in the context of preoperative platelet counts. Platelet expression of polymorphic ARs that correlate with transactivation function may differentially mediate release of angiogenic factors that function in ovarian cancer biology. We identified a statistically significant relationship between a short AR allelotype and preoperative thrombocytosis and found that the combination of short AR allelotype and thrombocytosis predicted decreased progression-free and overall survival. This combination was the sole independent predictor for suboptimal cytoreduction at initial exploratory surgery and approached statistical independence in multivariate analysis as a predictor for overall survival.

We have previously described multivariate analyses identifying preoperative thrombocytosis as an independent poor prognostic factor in a cohort of women with advanced-stage epithelial ovarian carcinomas, suggesting that its function in tumor biology exceeds that of a marker simply reflective of tumor burden (8). To date, this present study is the first exploring a potential relationship between AR activity and platelet function in ovarian cancer. Androgens may mediate platelet function through a receptor-specific mechanism. Megakaryocytes and platelets express AR mRNA and protein.
which is up-regulated by testosterone; furthermore, androgens increase platelet thromboxane receptor density and enhance platelet aggregation response (19, 18). Platelets are known to contain several angiogenic growth factors that are released on activation, including vascular endothelial growth factor, platelet-derived endothelial cell growth factor, transforming growth factor-β, hepatocyte growth factor, and thrombospondin; vascular endothelial growth factor in particular has been shown to mediate platelet involvement in tumor-induced angiogenesis (20, 21). Taken together, these data suggest that platelets express functional AR that modulates release of platelet-specific angiogenic factors important in tumor biology.

Standard therapy in the initial management of epithelial ovarian cancers consists of primary surgical cytoreduction followed by platinum-based chemotherapy. A large meta-analysis of cytoreductive surgery in patients with advanced-stage disease has indicated that maximal tumor resection remains one of the most powerful determinants of survival, yet studies are somewhat limited due to the wide variation in surgical skill (22). Furthermore, skeptics of aggressive surgery argue that the “ability” to achieve minimal residual disease after cytoreduction is more dependent on inherent cancer biology rather than on the skill and commitment of the surgeon (23). In this cohort, we identified that the combination of a short AR allelotype plus the presence of thrombocytosis preoperatively was the only independent predictor for suboptimal resection at exploratory surgery. Despite this finding, optimal surgical resection was done in four of the eight women with both short AR allelotype and thrombocytosis; this may be related to the shared philosophy of aggressive surgical cytoreduction shared by all gynecologic oncologists at our institution. All patients with suspected advanced-stage epithelial ovarian cancers should still be evaluated for surgical cytoreduction as 50% of those with a short AR allelotype and thrombocytosis still underwent optimal tumor resection and thereby had an improved chance for prolonged survival. In this cohort, AR allelotype and preoperative platelet count strongly predicted aggressive tumor biology and may be more useful in the identification of patients for novel up-front or consolidative chemotherapy strategies.

This study is limited in part by the retrospective nature of the review and does not confirm a causal relationship between AR allelotype and platelet function. However, our data suggest a significant association between short AR allelotype and thrombocytosis and survival, and identify this combination as a novel independent predictor for suboptimal surgical cytoreduction. Studies are under way to examine cell cultures harboring ARs of variable CAG repeat lengths to further characterize potential molecular mechanisms responsible for these observed clinical findings and support the potential use of antiandrogen therapy in the management of ovarian cancer.

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