Original Research

The Role of Akt2 and CA-125 Serum Levels as Predictors for Successful Cytoreduction in Epithelial Ovarian Cancer Surgery

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Summary

Ovarian cancer is one of the common causes of cancer deaths in gynecological malignancies in Indonesia, and indeed the world. Of all classifications of ovarian cancer, the epithelial subtype shows the highest incidence. Cytoreductive surgery is a determinant of prognosis for patients with advanced epithelial ovarian cancer. One possible way to predict the results of cytoreductive surgery is to measure preoperative tumor marker levels. This study aimed to assess the validity of serum levels of Protein kinase B (Akt2) and cancer antigen-125 (CA-125) as outcome predictors for cytoreduction in epithelial ovarian cancer surgery. This was an observational, analytical, cross-sectional study. Akt2 and CA-125 serum levels were examined by enzyme-linked immunosorbent assay (ELISA) and immunoassay technique, respectively. Mean levels of Akt2 and CA-125 were significantly different between optimal and suboptimal cytoreduction groups \((p < 0.05)\). The cut-off point (COP) value obtained for Akt2 was 1.20 U/mL with area under curve (AUC) value of 68.9%, while the COP for CA-125 was 222.50 U/mL with AUC value of 75.3 % \((p < 0.05)\). If the preoperative Akt2 serum level was greater than 1.20 U/mL, and preoperative CA-125 level was greater than 222.5 U/mL, the probability of suboptimal cytoreduction was increased. Thus, our study highlights the possibility for Akt2 and CA-125 levels to be used as serum biomarkers in predicting the success of cytoreductive surgery.

Key words: Epithelial ovarian cancer; cytoreduction; Akt2; CA-125.

Introduction

Ovarian cancer is one of the leading causes of death from cancer in women worldwide. In 2012, there were almost 240,000 new cases of ovarian cancer, ranking it the seventh highest of all cancers in women. By 2018, this figure had risen to almost 300,000, making it the fourth most common cancer in women. Ovarian cancer was the cause of death in 151,917 cases and 184,799 cases worldwide in 2012 and 2018, respectively [1, 2].

Classification of malignant ovarian cancer consists of epithelial-, stromal sex-cordal and germ cell-type; 90% of malignant ovarian cancers are of the epithelial type [3, 4]. The standard management of epithelial ovarian cancer is surgical staging in early-stage (stage 1) cases and cytoreductive surgery in cases of advanced stage (stages 2, 3 and 4), followed by platinum-taxane based chemotherapy [5].

Cytoreductive surgery is performed on advanced stage epithelial ovarian cancer to remove as much of the tumor bulk as possible. Intraoperatively by gross examination, surgery is considered as optimal cytoreduction if residual tumor < 1 cm at greatest dimension, while cytoreduction is suboptimal if the residual tumor is > 1 cm [3, 5, 6]. Saitou et al. reported that patient prognosis only improved when residual tumor nodules were < 1 cm [7].

Measurement of tumor marker levels is among the outcome predictors of cytoreductive surgery. Cancer antigen-125 (CA125) serum level > 420 U/ml was a strong predictor of suboptimal cytoreduction surgery in patients with epithelial ovarian cancer [8]. The combination of CA-125 levels with other tumor markers may help determine management [9]. The increase in CA-125 causes an increase in Protein kinase B (AKT) expression [10]. AKT is a key signaling molecule that regulates cellular processes such as proliferation, cell growth and metabolism as well as serving as a tumor marker [11]. Of the three AKT isoforms, AKT2 expression is found elevated in ovarian cancer [12]. Overexpression of AKT2 leads to increased expression of pyruvate kinase (PKM2) in ovarian cancer cells in nude mice [13]. Elevation of AKT2 expression induced cell migration and invasion in vitro, as well as lung metastasis in vivo, while suppression of AKT2 blocked these effects. STAT3 expression was elevated and NF-κB p65 nuclear translocation was activated both in vitro and in vivo when AKT2 was overexpressed; these effects were inhibited when AKT2 expression was suppressed. AKT2 increases the migration, progression and invasion of ovarian cancer cells in vitro and in nude mice in vivo through PKM2-mediated elevation of STAT3 expression and NF-κB activation [13].
Preoperative combination of AKT2 and CA-125 levels may predict the outcome of cytoreductive surgery, which in turn may help clinicians determine the subsequent management of patients with advanced ovarian cancer.

Methods

This study was registered and approved by the Research Ethics Committee, Faculty of Medicine Padjadjaran University/Dr. Hasan Sadikin Hospital, Bandung, Indonesia, under trial registration number 36/UN6.KEP/EC/2018 on April 13th, 2018.

Study subjects

This study was a prospective, observational analysis with a cross-sectional design. The population included patients with a diagnosis of suspected advanced ovarian cancer who would undergo cytoreductive surgery at Dr. Hasan Sadikin General Hospital, Bandung. The inclusion criteria were new patients with suspected epithelial ovarian cancer undergoing cytoreductive surgery who were willing to give informed consent, and who were not suffering from chronic diseases or other tumors. Consecutive patients from the 2018-2019 period attending Dr. Hasan Sadikin General Hospital, Bandung were included.

Before surgery, blood samples were taken from the entire study population for the assessment of serum biomarker levels. Patients who were confirmed histopathologically to not suffer from epithelial ovarian cancer, or who only had stage I disease, were excluded from the study. Patients were also excluded if their histopathological preparations were damaged or could not be assessed or if they were not willing to provide informed consent.

Materials

AKT2 serum examination was performed by ELISA (Enzyme-Linked Immunosorbent Assay. Cloud-Clone Corp., USA). CA-125 examination was done by immunoassay technique analyzed by ADVIA Centaur® XP system (Siemens Healthcare Diagnostics Inc., USA). All techniques were done according to the manufacturer’s instructions.

Statistical Analysis

All data were assessed statistically using SPSS® (24.0.0). For normally distributed data, a paired t-test analysis was used, while a Mann Whitney analysis was used for not normally distributed data. As for categorical data, p-values were analyzed using the Chi-Square test; if the Chi-Square requirements were not met, then Kolmogorov Smirnov and Exact Fisher tests were used. The cut-off point (COP) was made using receiver operating characteristic (ROC) curve 23 analysis.

Results

Fifty-three patients were included in this study, divided into suboptimal and optimal cytoreductive groups. Characteristics of the subjects in the two groups were analyzed. There were no significant differences (p > 0.05) between the two groups regarding patients’ age, parity, height, weight, body mass index, ascites, bleeding, staging, and histopathology (Table 1). Analysis of blood samples taken before surgery revealed the levels of serum AKT2 and CA-125 had a significant difference (p < 0.05) between that of suboptimal and optimal cytoreductive groups (Table 2).

The COP of AKT2 was 1.20 U/mL, with a significant p value (p < 0.05) between COP and results of cytoreduction (Table 3). The AUC value of AKT2 levels with ROC method was 68.9%, implying that 68.9% of patients with AKT2 serum levels greater than 1.20 U/mL would undergo suboptimal cytoreduction with 76.2% specificity, 65.6% sensitivity, and 69.8% accuracy (Figure 1, p = 0.021).

The COP of CA-125 obtained from this study was 222.5 U/mL, with a significant p value (p < 0.05) between COP and results of cytoreduction (Table 4). Based on the ROC method, the AUC value received was 77.5%, suggesting that 77.5% of patients with serum CA-125 levels exceeded 222.5 U/ml. Suboptimal cytoreduction was predicted with 61.9% specificity, 75% sensitivity and 69.8% accuracy (Figure 2, p = 0.002).

Multivariate analysis was obtained by logistic regression test to determine whether AKT2 or CA-125 was more closely related to the cytoreduction result. As Table 5 shows, AKT2 had a comparably smaller p value than CA-125 (p = 0.003 vs. p = 0.005, respectively), and higher odds ratio (OR = 12.404 vs. OR = 10.379, respectively). These data indicate that AKT2 measurement may be more likely...
Table 1. — Comparison of Characteristics of Patients in Optimal and Suboptimal Cytoreduction groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Suboptimal cytoreduction n = 32</th>
<th>Optimal cytoreduction n = 21</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td></td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td>50.09 ± 9.686</td>
<td>45.23 ± 13.374</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td></td>
<td>0.979</td>
</tr>
<tr>
<td></td>
<td>7 (21.9%)</td>
<td>2 (9.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4 (12.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8 (25.0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 3</td>
<td>13 (40.6%)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Mean ± SD</td>
<td></td>
<td>0.164</td>
</tr>
<tr>
<td></td>
<td>153.09 ± 5.348</td>
<td>150.80 ± 4.285</td>
<td></td>
</tr>
<tr>
<td>Weight Preop (kg)</td>
<td>Mean ± SD</td>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>56.25 ± 8.925</td>
<td>51.19 ± 9.825</td>
<td></td>
</tr>
<tr>
<td>Weight Postop</td>
<td>Mean ± SD</td>
<td></td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>50.12 ± 9.317</td>
<td>45.26 ± 8.169</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Mean ± SD</td>
<td></td>
<td>0.175</td>
</tr>
<tr>
<td></td>
<td>21.44 ± 4.259</td>
<td>19.90 ± 3.501</td>
<td></td>
</tr>
<tr>
<td>Ascites</td>
<td>Mean ± SD</td>
<td></td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>1892.50 ± 3126.565</td>
<td>2281.90 ± 5117.13</td>
<td></td>
</tr>
<tr>
<td>Bleeding Volume</td>
<td>Mean ± SD</td>
<td></td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>1312.10 ± 920.868</td>
<td>1357.14 ± 1826.08</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>II</td>
<td></td>
<td>0.149</td>
</tr>
<tr>
<td></td>
<td>5 (15.6%)</td>
<td>10 (47.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>20 (62.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>7 (21.9%)</td>
<td></td>
</tr>
<tr>
<td>Histopathology</td>
<td>Serous</td>
<td></td>
<td>0.873</td>
</tr>
<tr>
<td></td>
<td>13 (40.6%)</td>
<td>6 (28.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucinous</td>
<td>7 (21.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Endometrioid</td>
<td>7 (21.9%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Clear Cell</td>
<td>5 (15.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Age, Weight PostOp (preoperative), and BMI were analyzed by Independent t-Test. Height, Weight PreOp (preoperative), Ascites, and Bleeding Volume were analyzed by Mann Whitney test. Parity, Stage, and Histopathology were analyzed by Kolmogorov Smirnov test.

Table 2. — Comparison of Akt2 and CA-125 in the Suboptimal and Optimal Cytoreduction groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Suboptimal cytoreduction n = 32</th>
<th>Optimal cytoreduction n = 21</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt2</td>
<td>Mean ± SD</td>
<td></td>
<td>0.021*</td>
</tr>
<tr>
<td></td>
<td>1.41 ± 0.555</td>
<td>1.12 ± 0.286</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.65 - 3.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CA-125</td>
<td></td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>964.22 ± 1722.532</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>600.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>4.29 - 9934.00</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 (Mann Whitney Test)

Both AKT2 and CA-125 had p-value < 0.05 according to multivariate analysis, (table 6). The new cut-off point was generated by re-coding AKT2 value based on the original AKT2 COP 1.20. AKT2 values < 1.20 were re-coded to “1” and values > 1.20 were re-coded to “2”. The re-coded values were multiplied by corresponding CA-125 to produce the combined variable. An ROC curve was plotted to accurately predict optimal tumor resection probability.
Table 3. — Comparison between Cut-Off Points of Akt2 in Suboptimal and Optimal Cytoreduction Groups.

<table>
<thead>
<tr>
<th>Cytoreduction</th>
<th>Suboptimal n = 32</th>
<th>Optimal n = 21</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt2 &gt; 1.20</td>
<td>21 (65.6%)</td>
<td>5 (23.8%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Akt2 ≤ 1.20</td>
<td>11 (34.4%)</td>
<td>16 (76.2%)</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 (chi-square test).

Table 4. — Comparison between Cut-off Points of CA-125 in Suboptimal and Optimal.

<table>
<thead>
<tr>
<th>Cytoreduction</th>
<th>Suboptimal n = 32</th>
<th>Optimal n = 21</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-125 &gt; 222.50</td>
<td>24 [75.0%]</td>
<td>8 [38.1%]</td>
<td>0.007*</td>
</tr>
<tr>
<td>Ca-125 ≤ 222.50</td>
<td>8 [25.0%]</td>
<td>13 [61.9%]</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 (chi-square test).

from the combination variable, which resulted in the combination COP was 410.45 U/mL. The new COP had a 75% sensitivity, 61.9% specificity and 69.8% accuracy for predicting optimal cytoreduction (Figure 3, p = 0.001).

Figure 3. — The combination of Akt2 and CA-125 with Cytoreduction. The AUC value obtained from the ROC method was 78.1% (p = 0.001), meaning that combination of Akt2 and CA-125 can predict cytoreduction correctly in 41 patients out of 53 patients.

Discussion

Cytoreductive surgery in epithelial ovarian cancer patients determines prognosis and subsequent therapy. Studies have shown that patients with optimal cytoreduction have a better prognosis. Optimal cytoreduction with a residual mass < 1 cm increases the survival rate by 22 months [14].

The AKT signaling pathway is key in mediating the survival of tumor cells and avoiding apoptosis [13]. A 2014 meta-analysis conducted by Cai et al. that reviewed 11 articles stated that a high expression of AKT was related to poor survival in patients with epithelial ovarian cancer. The authors concluded that only high expression of AKT was significantly related to a poor prognosis, reflecting its oncogenic nature [15].

In this study, the average serum level of AKT2 was statistically significantly different between the optimal and suboptimal cytoreductive group. The COP of AKT2 was 1.20 U/mL, with a significant p value between COP and results of cytoreduction. The AUC value of AKT2 levels with ROC method was 68.9%, implying that 68.9% of patients with AKT2 serum levels more than 1.20 U/mL would undergo suboptimal cytoreduction with 76.2% specificity, 65.6% sensitivity. The results of this study indicate that an increase in AKT expression will make it more difficult to achieve optimal tumor cytoreduction. This is consistent with the theory that high AKT expression is associated with a worse prognosis since oncogenic activity in the Akt sig-
The naling pathway is known to have an essential role in tumori
genesis by influencing the absorption of glucose, amino
acids, and lipids [11]. Thus, AKT2 serum levels can be used to
predict whether results of cytoreduction will be optimal
or suboptimal.

CA-125 is one of the significant predictors for outcome
cytoreduction [16]. Other studies have suggested that the
COP of CA 125 > 100 U/ml increases the occurrence of
suboptimal cytoreduction [17]. Feng et al. stated that CA-
125 could predict optimal or suboptimal cytoreduction. A
pre-operative COP of CA-125 > 500 U/ml had a sensitivity
of 78% and specificity of 73% for predicting suboptimal
cytoreduction [18]. Another study stated that 912 U/ml was
an optimal cut-off value of CA-125 in predicting optimal
and suboptimal cytoreduction [19]. The COP of serum CA-
125 levels still varies.

In our study, the COP of CA-125 was 222.5 U/mL; based
on the ROC method, the AUC value received was 77.5%,
suggesting that 77.5% of patients with serum CA-125 lev-
els exceeded 222.5 U/ml. This was predictive of suboptimal
cytoreduction with 61.9% specificity, 75% sensitivity,
and 69.8% accuracy. The specificity value of AKT2
was higher than that of CA-125, matching the current the-
ory that pre-operative CA-125 serum level is not a specific
marker to diagnose ovarian cancer [3]. CA-125 also
has a low specificity for malignant ovarian cancer because it
often increases in patients with benign endometriosis [15].

When CA-125 and AKT2 were combined, the new COP
was 410.45 U/mL with a specificity value of 61.9%, sensi-
tivity of 75%, and the same accuracy value of 69.8%. The
AUC value obtained from the ROC method was 78.1%,
meaning that as many as 78.1% of patients with a combina-
tion of CA-125 and AKT2 value more than 410.45 U/mL
may experience suboptimal cytoreduction. Based on the
confidence interval, the combination of AKT2 and CA-125
value ranged from 65.9% to 90.3%. Whether separate or
in combination, AKT2 and CA-125 had the same accuracy
level.

Currently, there is insufficient evidence to recommend
the use of AKT levels in clinical practice as a predictor of
epithelial ovarian cancer prognosis [15]. Further research
will be necessary to substantiate our present findings. How-
ever, as the combination of AKT2 and CA-125 has a higher
AUC value, it can be concluded that the combination of
both tumor markers is beneficial than either used alone.

Finally, the success of cytoreductive surgery still de-
ends on the experience of the surgeon as well as other fac-
tors such as FIGO cancer stage, histopathological type, and
body mass index [19-21]. Findings from this research may
prove useful in helping to predict outcomes following cy-
toreduction and patient prognosis.

The major limitation of our study was insufficient patient
cohort. Therefore, future studies should involve a larger
number of patients to increase the power of the study and
the validity of our findings.

Conclusions

AKT2 and CA-125 serum levels are significantly differ-
ent between the patients in suboptimal and optimal cyto-
reduction groups. AKT2 or CA-125 can be used as single tu-
mor markers to predict cytoreduction, wherein the COP of
AKT is 1.20 U/mL and the COP of CA-125 is 222.5 U/mL.
As both tumor markers are correlated, AKT2 and CA-125
can be used in combination to achieve a new COP of 410.45
U/mL, providing a better AUC value.

Ethics Approval and Consent to Participate

This study was approved by the Research Ethics Com-
mittee, Faculty of Medicine Padjadjaran University/Dr.
Hasan Sadikin Hospital, Bandung, Indonesia, under trial
registration number 36/UN6.KEP/EC/2018 on April 13th,
2018. Suspected ovarian cancer patients were invited to

Table 5. — Multivariate COP of Akt2 and CA-125 Analysis.

<table>
<thead>
<tr>
<th>B</th>
<th>S.E.</th>
<th>p value</th>
<th>OR</th>
<th>95% C.I. for OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Cutoff Akt2</td>
<td>2.518</td>
<td>0.846</td>
<td>0.003*</td>
<td>12.404</td>
</tr>
<tr>
<td>Cutoff CA-125</td>
<td>2.340</td>
<td>0.837</td>
<td>0.005*</td>
<td>10.379</td>
</tr>
</tbody>
</table>

Cytoreduction Groups

Note: *p < 0.05 (logistic regression test)

Table 6. — Comparison between Cut-off Points of CA-125 in Suboptimal and Optimal.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Suboptimal n = 32</th>
<th>Optimal n = 21</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combinations CA-125 dan Akt2</td>
<td>&gt; 410.45</td>
<td>23 (71.9%)</td>
<td>7 (33.3%)</td>
</tr>
<tr>
<td>&lt; 410.45</td>
<td>9 (28.1%)</td>
<td>14 (66.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: *p < 0.05 (chi-square test)
participate in this study. Patients who were willing to become participants in this study were asked to fill and sign an informed consent form.

Funding

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Authors’ Contributions

YMH, GNAW, and SRK designed the research study. YMH, GNAW, AK performed the research. MDLT, KIM, SRK provided help and advice on the experiments. YMH, MDLT, KIM analyzed the data. YMH, KIM, AK, SRK wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Availability of data and materials section

The authors declare that the data will not be shared due to issues of patient confidentiality.

Consent to Publish

All authors declare that consent was given for publication of this study and accompanying images to be published.

Acknowledgments

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Conflict of interest

Authors have declared that no competing interests exist.

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References


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