Elevated Glutaminase in Epithelial Ovarian Cancer Patients Increased the Suboptimal Cytoreduction

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Citation

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Abstract

Background: Ovarian cancer still ranks second in gynecological cancer after cervical cancer in Indonesia. Most women diagnosed at an advanced stage, the standard treatment for advanced epithelial ovarian cancer (EOC) is cytoreduction. The use of GLS as a predictor of cytoreduction results has never been done before. This study was aimed to compare and combine glutaminase (GLS) and CA-125 as predictor for successful of cytoreduction in advanced epithelial ovarian surgery.

Methods: This cross-sectional study was an observational analytic and conducted in a prospective design. Subjects were patients with EOC stage II to IV who had undergone cytoreduction surgery. GLS and CA-125 were examined by ELISA kit after epithelial histopathology was confirmed. Cut off point (COP) was obtained using receiver operating characteristic (ROC) curve analysis.

Results: There were 53 advanced EOC patients met inclusion criteria. GLS and CA-125 serum level were significantly higher in suboptimal cytoreduction group than optimal cytoreduction group (GLS; 25.78±2.459 vs. 21.68±4.507, p=0.001), (CA-125; 964.22±1722.5 vs. 264.98±251.8, p=0.002). GLS was more specific and accurate than CA-125. COP of GLS was 24.75, CA-125 was 222.5. COP of the combination of GLS and CA-125 was 28.75 with highest score of; 87.5% sensitivity, 71.4% specificity, 81% accuracy, and ROC value of 83.9% (p=0,0001).

Conclusion: Elevated of GLS increased the suboptimal cytoreduction in advanced EOC surgery. Cut off point of the combination of GLS and CA-125 was better to use than COP of GLS, and CA-125 parameter separately.

INTRODUCTION

Ovarian cancer is the cause of death more often than other reproductive system cancers. There are 125,000 women in the world died from ovarian cancer.(1) Based on Indonesia's national cancer registration data in 2012, ovarian cancer still ranks second in gynecological cancer (23.43%) after cervical cancer (63.39%). Most women diagnosed at an advanced stage, more than 50% of all ovarian cancer patients in RSUP Dr. Hasan Sadikin is at an advanced stage.(2) Epithelial ovarian cances is divided into 2 types. Type I tumors include low-grade serous, mucinous, endometroid, and clear cell carcinoma. This type I tumors are slow growing, and genetically stable. In contrast, type 2 is high grade serous carcinoma, this type of tumor is rapid growing, highly aggressive, and genetically instable.(3)

Standard treatment for epithelial ovarian cancer is cytoreduction surgery at an advanced stage [stage 2 and above] followed by adjuvant first-line chemotherapy platinum-taksan [at stage 1C and above]. Tumor residue less than 1 cm is optimal cytoreduction, and tumor residue of more than 1 cm is said to be suboptimal cytoreduction.(4) Primary cytoreduction has benefits include complete surgical staging, sensitivity to chemotherapy or radiotherapy can be increased, and the risk of mutation will be reduced.(5) CA-125 levels are relatively weak predictors associated with cytoreduction surgery results. The application of serum CA-125 levels should be reconsideration and not recommended as a primary predictor of cytoreduction surgery.(6),(7) Cancer cells need glutamine (an amino acid) to supports cancer cells to grow, produce energy, and also biosynthesis.

Glutamine plays a role in the formation of ATP, and maintaining redox homeostasis.(8) In the process of glutaminolysis, the glutaminase enzyme (GLS) works to

change glutamine into glutamate, and then it will be converted into -ketoglutaric (-KG) by glutamate dehydrogenase (GDHS) in order to join the Krebs cycle.(9) An increase in the GLS enzyme indicates the high need for amino acids in plasma. Glutaminase (GLS) expression has increased four times in ovarian cancer with high invasion compared to low invasion.(10) The use of GLS levels in serum (which is an essential component in cell metabolism) as a predictor of cytoreduction results has never been done before. CA-125 examination is often used for cytoreduction prediction, but for the combination with GLS, it has not been investigated. This study was determined to compare and make combination of the two tumor markers to predict cytoreduction results.

METHODS

This cross sectional study was an observational analytic and conducted in a prospective design. The study was conducted in the Obstetrics and Gynecology Department, Oncology Gyencology Division of Faculty of Medicine, Padjadjaran University/Dr. Hasan Sadikin General Hospital, and Laboratory of Molecular Genetics in Padjadjaran University from July 2017 to March 2019. We collected blood sample from patient with suspected ovarian cancer who will undergo cytoreduction. The samples used in this study were patients with a diagnosis of primary epithelial ovarian cancer (EOC) stage II to IV who had undergone cytoreduction surgery. The blood sample was examined for GLS and CA-125 after epithelial histopathology was confirmed. If any patient with a diagnosis of cancer other than ovarian cancer was identified or the ovarian cancer was not epithelial type, histopathological preparations damaged or cannot be assessed, or the operation cannot be continued, the patient was excluded from the sample.

This study had passed the ethical review from Research Ethics Committee of the Faculty of Medicine of Universitas Padjadjaran. The patients signed a letter of consent after receiving an explanation of the research procedure. Glutaminase and CA-125 serum level examination was measured with Enzyme-Linked Immunosorbent Assay (ELISA) Kit for GLS and CA-125. The groups was divided into suboptimal cytoreduction and optimal cytoreduction.

Numerical data were tested by unpaired T-test if data were normally distributed with an alternative Mann Whitney test. Categorical data were tested by Chi-Square test with an alternative Kolmogorov Smirnov or Exact Fisher test if the terms of Chi-Square were not met. The cut-off point (COP) was created using receiver operating characteristic (ROC) curve analysis. The statistical analyses were performed using SPSS™ (20.0.0)

RESULTS

There were 53 patients with EOC that met the inclusion criteria on consecutive sampling. There were no significant differences characteristic between suboptimal and optimal cytoreduction groups, therefore the value of GLS, CA-125 preoperative serum, and cytoreduction surgery result did not affected by the subject and tumor characteristics, so it is feasible to carry out further statistical test. Table 2 shows there was significant difference of mean serum level of GLS (ng/mL) and CA-125 (U/mL) between suboptimal and optimal groups, and it was significantly higher in suboptimal cytoreduction group than optimal cytoreduction group (GLS; 25.78±2.459 vs.21.68±4.507, p=0.001), (CA-125; 964.22±1722.5 vs. 264.98±251.8, p=0.002). The quality of the variable's cut off point shown in table 3. Sensitivity of GLS and CA-125 was still in same category as middle sensitivity.

In contrast, CA-125 was low in specificity and accuracy, while GLS was middle in specificity and accuracy (table 3). The value of ROC of GLS was 76.1% which means patients with a GLS value > 24.75 was associated and more likely to have suboptimal cytoreduction surgery to 76.1% (figure 1). The value of ROC of CA-125 was 75.3% which means patients with a CA-125 value > 222.5 was more likely to have suboptimal cytoreduction surgery to 75.3% (figure 2). The highest ROC score was 83.9% obtained from a combination of GLS and CA-125 (figure 3).

Table 1

Background Characteristics of the Study Population

Table 1. Background Characteristics of the Study Population

Variable	Groups		p value
	Suboptimal cytoreduction	Optimal cytoreduction	
	N=32	N=21	
Age (years)			0.131
Mean±SD	50.09±9.686	45.23±13.374	
Median	49.5	51	
Range (min-max)	26.00-64.00	22.00-71.00	
Parity			0.979
0	7(21.9%)	2(9.5%)	
1	4(12.5%)	8(38.1%)	
2	8(25.0%)	1(4.8%)	
>3	13(40.6%)	10(47.6%)	
BMI			0.175
Mean±SD	21.44±4.259	19.90±3.501	
Median	21.2	20.2	
Range (min-max)	10.40-34.70	10.40-26.20	
Ascites			0.195
Mean±SD	1892.50±3126.565	2281.90±5117.13	
Median	575	300	
Range (min-max)	10.00-15000.00	20.00-18000.00	
Stage			0.149
II	5(15.6%)	10(47.6%)	
III	20(62.5%)	11(52.4%)	
IV	7(21.9%)	0(0.0%)	
Histopathology			0.873
Serosa	13(40.6%)	6(28.6%)	
Musinosum	7(21.9%)	10(47.6%)	
Endometrioid	7(21.9%)	2(9.5%)	
Clear Cell	5(15.6%)	3(14.3%)	

SD, standard deviation, p-value only compares mean and proportion (%)

Table 2

GLS, and CA-125 levels

Variable	Groups		p value
	Suboptimal cytoreduction	Optimal cytoreduction	
	N=32	N=21	
GLS (ng/mL)			0.001
Mean±SD	25.78±2.459	21.68±4.507	
Median	25.28	22.47	
Range (min-max)	20.30-30.48	12.82-28.13	
CA-125 (U/mL)			0.002
Mean±SD	964.22±1722.532	264.98±251.883	
Median	600	132.7	
Range (min-max)	4.29–9934.00	5.10–701.00	

p value only compares mean

Table 3

Sensitivity and specificity of predictor cytoreduction scoring of epithelial ovarian cancer

Variable	Cut-off point	Sensitivity	Specificity	AC	LR+	LR-	p value
GLS	24.75	71.90%	71.40%	71.70%	0.79	0.62	0.002
CA-125	222.5	75%	61.90%	69.80%	0.75	0.61	0.007
Combination of GLS and CA-125	28.72	87.50%	71.40%	81.10%	0.82	0.78	0.0001

AC=accuracy classification, LR+= positive likehood ratio, LR-=negative likehood ratio

Figure 1

GLS with Cytoreduction. AUC value obtained from the ROC method was 76.1% CI 62.3%-85.3% (p = 0.001), implicating that GLS can predict cytoreduction correctly in 41 patients out of a total of 53 patients.

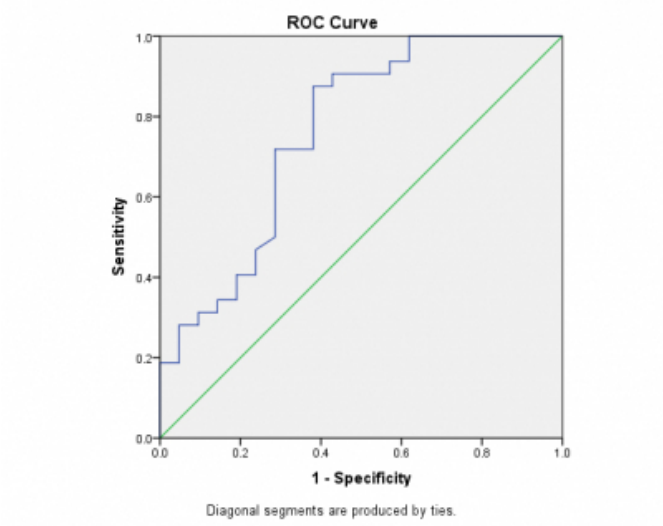


Figure 2

CA-125 with Cytoreduction. The CA-125 AUC value obtained from the ROC method was 75.3% CI 62.5%-88.1% (p = 0.002), implicating that CA-125 can predict cytoreduction correctly in 40 patients out of a total of 53 patients.

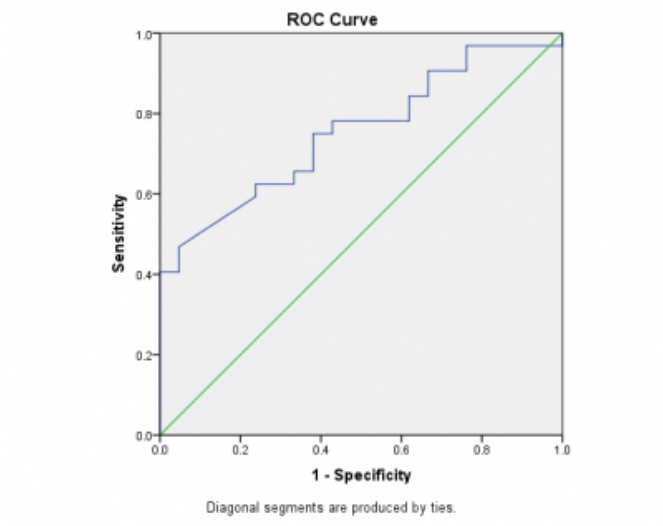
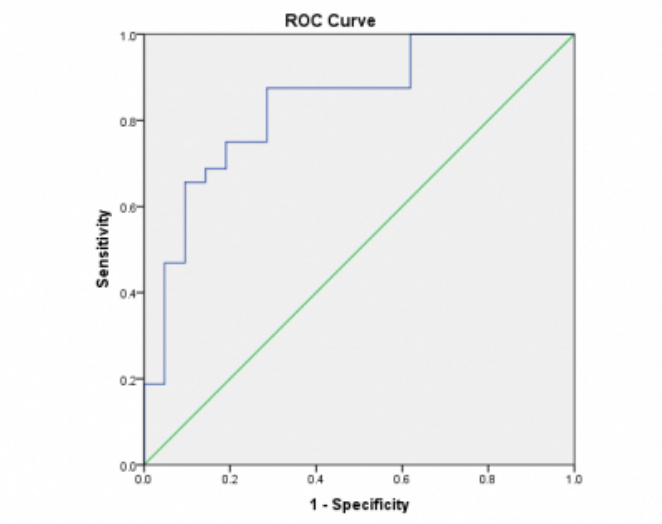


Figure 3

Combination of GLS and CA-125 with Cytoreduction. AUC value obtained from the ROC method was 83.9% CI 72.9%-95% (p = 0.000), implicating that combination of GLS and CA-125 can predict cytoreduction result correctly in 44 patients out of a total of 53 patients



DISCUSSION

Cytoreduction surgery for ovarian cancer that leaves no residue or complete resection has a better prognosis. Various studies have proven the effect of optimal cytoreduction on better prognosis and survival 5-year rate time.(5),(11) Cytoreduction surgery is often just able to achieve suboptimal or even only biopsy. The high failure of optimal cytoreduction surgery is because it has not been predicted optimally. Prediction of cytoreduction result assessed in several ways, one of which is by preoperative assessment such as through clinical assessment, and measurement of tumor marker levels. Predictors of cytoreduction results can use clinical examination in patients by assessing the size and movement of the tumor, the presence of ascites, pleural effusion, and the presence of metastases in other organs. (12) There were no significant differences in the distributions of age, parity, BMI, ascites, and staging between the suboptimal and optimal cytoreduction of EOC patients (p>0.05) (Table 1), it means the participants were homogeneous, and bias of cytoreduction due to those factors could be removed and the two groups deserved to be compared.

From table 2 we can see that the mean level of GLS was significantly difference between suboptimal and optimal cytoreduction group. Suboptimal cytoreduction group had higher value of GLS than optimal cytoreduction (p=0.001). It is related to the existing theory that glucose and glutamine

are the primary sources of nutrients that provide bioenergy and also as an intermedia synthesis of macromolecules in proliferating cancer cells. The more cancer cells need glutamine, indicates that the more cancer cells grow and proliferate and surgery will be more difficult. (13)

In table 3 explains the quality of each cut off point. In this study, we compared the cut-off point of GLS with CA-125 to cytoreduction results. The COP of GLS is 24.75, and the COP of CA-125 is 222.5. GLS has a specificity, accuracy, positive likehood ratio, p-value, and AUC value higher to predict cytoreduction than the CA-125. Sensitivity of GLS and This result proves that CA-125 is not a specific tumor marker to diagnose ovarian cancer, and CA-125 was a weak positive and negative predictor of optimal cytoreductive surgery in patients with advanced epithelial ovarian cancer.(14) Glutamate serum levels correlate with tumor aggressiveness.(13) Glutamine has a more critical role in the growth of high invasion ovarian cancer than glucose, and it is also related to the patient's survival rate. Another study has found that the level of cancer cell dependence on glutamine was highly correlated with the rate of cancer invasion. High invasion of ovarian cancer is very dependent on the availability of glutamine.(10)

However, sensitivity of CA-125 was same as GLS, but still higher of 0.3% than GLS. The combination of GLS and CA-125 was made to find the best predictor. The formula is GLS level times with category of CA-125 (1;≤222.5, 2;>222.5), and new cut off point was obtained 28.75. Cut off point of the combination of GLS and CA-125 had sensitivity of 87.5%, specificity of 71.4%, accuracy of 81.8%, positive likehood ratio of 0.82, and negative likehood ratio of 0.78 (p=0.0001) (table 3). It turns out that the combination of GLS and CA-125 has better sensitivity, specificity, accuracy, than GLS and CA-125 separately. The highest value of ROC value (AUC) was also obtained from the combination of GLS and CA-125 than the two tumor marker separately, if the combination score is more than 28.75 was more likely to have suboptimal cytoreduction surgery to 83.9% (figure 1, 2, 3).

There was association between glutamine levels with cell proliferation. The more glutamine was available, the more cells will proliferate. Glutamine deficiency causes cessation of the cell cycle in the G1 phase while an increase in glutamine concentration causes the S phase to gradually increase in ovarian cancer cells. Loss of glutamine in cancer cells could cause significant apoptosis. Glutamine deficiency in ovarian cancer cells can inhibit proliferation through increased the Annexin-V expression. This shows that glutamine can supported cell growth by inducing changes in the cell cycle.(13)

Ovarian cancer cells with high invasive levels depend on glutamine, while low invasive cancer cells do not depend on glutamine. Worse survival rates were related to increased genes that involved in glutaminolysis such as glutaminase (GLS), glutamate dehydrogenase (GDHS), and glutamate oxaloacetate transaminase (GOT1, GOT2).(10) The results of this study were consistent with the existing theory, and previous studies have shown that increased glutaminase expression signifies a high proliferation of invasive cancer cells, thereby increasing the incidence of suboptimal cytoreduction surgery. Elevated glutaminase and CA-125 increased the suboptimal cytoreduction in advanced epithelial ovarian cancer surgery with COP of GLS was 24.75, COP of CA-125 was 222.5, and the best predictor was the COP of the combination of GLS and CA-125 28.75. Hopefully this research could help oncologists to predict the successful of cytoreduction surgery in patient with advanced epithelial ovarian cancer.

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