D-Dimer as a Tumor Marker in Pre-Operative Assessment of Adnexal Masses

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Objective: *To determine the sensitivity, specificity, and predictive value of serum D-dimer testing in preoperative assessment of adnexal masses.*
Material and Method: *D-dimer levels were measured pre-operatively in 200 women diagnosed with adnexal masses who underwent surgical treatment at Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital between June 2007 and May 2008. The CA-125 level was also recorded.*
Results: *When using cut-off value at 500 ng/ml, D-dimer has 91.8% sensitivity, 71.9% specificity, 58.9% PPV, and 95.2% NPV of the tests in differentiating benign from malignant adnexal masses, compared with CA-125, which had 75.4%, 73.0%, 59.7%, and 84.8% respectively (cut-off 65 U/ml). Furthermore, the likelihood ratio to be negative of D-dimer test is high at 0.11. In patients with epithelial ovarian cancer, D-dimer is increased in 83% of early stage (stage 1) ovarian cancer while only 39% of early stage patients have CA-125 level above cut-off value.*
Conclusion: *D-dimer could be a useful test in pre-operative assessment of adnexal masses. In this study D-dimer seems to be better than CA-125 in differentiating benign from malignant adnexal tumors.*

Keywords: *D-dimer, Tumor marker, Adnexal masses, Ovarian tumor, Ovarian cancer*

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Adnexal or ovarian masses encounter a common gynecologic problem and are the major indication for laparotomy. In our institute, about 170 patients with ovarian or adnexal mass undergo surgical exploration and treatment each year and the rate of malignancy in these patients is about 27%[1].

In current practice, we accept transvaginal gray scale ultrasonography and CA-125 levels in evaluating the risk of malignant ovarian tumor. A cut-off 30 U/ml of CA-125 gives 81% sensitivity and 75% specificity[2], which can be compared to the gray scale ultrasound when using morphological index (89% sensitivity and 73% specificity)[3]. Other investigations include magnetic resonance imaging, computed tomography scan, positron emission tomography scan, and Doppler ultrasonography. Those are not routinely used due to cost-effectiveness and experience requirement.

The Royal College of Obstetricians and Gynecologists (RCOG) recommends the use of "Risk Of Malignancy Index" (RMI) that combines age or menopausal status, ultrasound findings, and serum CA-125 levels in pre-operative assessment of ovarian tumors, to determine the risk of malignancy. RMI can increase test specificity to 90% with 70% sensitivity[2,4-8]. Currently, the authors do not have any investigations that have a 100% sensitivity and specificity to discriminate malignant from benign ovarian tumors. Ultrasound frequently fails to differentiate between benign and malignant ovarian tumors. It is important to note that serum CA-125 level was raised (>65 U/ml) in only 33 to 50% of stage I epithelial ovarian cancer cases[2,9]. In addition, levels can be raised in many other malignancies and in benign conditions especially endometriosis.

In patients with malignant ovarian neoplasms, we commonly find abnormality in coagulation and fibrinolytic pathways. The incidence of venous thromboembolism (VTE), (deep vein thrombosis (DVT) and pulmonary embolism) in these patients are seven to 16%[10,11]. It is important to note that these conditions are among the leading causes of morbidity and mortality in ovarian cancer. Neoplastic cells stimulate clotting system via direct thrombin pathway and indirectly by co-stimulating mononuclear cells to

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produce pro-coagulant substances[12]. From present studies, tissue factor is the key pro-coagulant that is produced by cancer cells leading to intravenous thrombosis. These tissue factors and other pro-coagulant substances from cancer and enclose vascular endothelial cells are stimulating the clotting system by various pathways. Cross-linked fibrin networks from pathway are necessary for generating tumor stroma, as the matrix for neo-angiogenesis and preventing these tumor cells from host-defense mechanism[9,13-19].

Blood coagulation and fibrinolytic pathway activated by ovarian cancer cells can be proven by measurement of fibrin degradation product such as serum D-dimer. D-dimer is a stable product of cross-linked fibrin degradation by plasminogen activity. The authors used in diagnosis of acute thromboembolism e.g., DVT and pulmonary embolism and can be measured by enzyme-linked immunosorbent sandwich assay using monoclonal antibodies[20,21]. If ovarian cancer cells make D-dimer rise in human serum, therefore serum D-dimer can be qualified as a tumor marker. The aim of this study is to determine the sensitivity, specificity, and predictive value of serum D-dimer testing in preoperative assessment of adnexal masses to differentiate benign from malignancy.

Material and Method

This diagnostic test study included all women diagnosed with adnexal masses or ovarian neoplasms submitted to surgical exploration at Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand between June 2007 and May 2008. These patients did not have significant underlying medical conditions such as cardiovascular disease, cerebrovascular disease, coagulation defects, previous malignancy, or previous episodes of thromboembolism. Patients who currently used medications that altered coagulation system e.g., warfarin, heparin, low molecular weight heparin and estrogen therapy, or contraceptive pills were excluded. Informed consent was obtained in all cases. The present study received approval from the institutional review board (IRB).

The day before the operation, peripheral blood samples were drawn from all patients by venipuncture from the antecubital vein for the measurement of D-dimer and CA-125. Blood samples collected with double syringes technique for minimal clots contamination. Plasma was sent to the central laboratory unit, centrifuged and the supernatant was stored at -70°C until assayed. D-dimer was measured by an enzyme-linked immunosorbent sandwich assay or ELISA using monoclonal antibodies (Automated ELISA; VIDAS D-dimer test, bioMerieux's).

For analyzing CA-125 levels, another 5 ml venous blood from each patient was taken in another tube preoperatively. Analysis of serum CA-125 was performed by a CA-125 II kit that utilized the ELISA technique (Automated ELISA; CA-125 II, Cobas).

The results were expressed as mean ± standard deviation (SD). Statistical analysis of the study data was performed by Mann-Whitney U test which assigned $p<0.05$ as statistical significant. All results of serum D-dimer and CA-125 were plotted in a receiver-operator characteristic curve (ROC curve) to find out the best cut-off values. Then two by two tables were used to calculate sensitivity, specificity, and predictive values of both tests. The authors used SPSS software version 13.0 for windows (SPSS Inc., Chicago, US).

Results

Two hundred patients who were diagnosed with adnexal or ovarian tumor were used in the present study. Table 1 compares mean age, body mass index (BMI), pre-operative serum D-dimer and CA-125 levels between benign ovarian tumor (n = 139) and

Table 1. Pre-operative D-dimer and CA-125 levels in benign and malignant adnexal masses

	Benign (n = 139) mean ± SD	Malignant (n = 61) mean ± SD	p-value*
Age	41.02 ± 11.77	51.18 ± 13.34	<0.001
BMI	23.65 ± 4.74	23.73 ± 4.17	0.649
CA-125 (U/ml)	79.30 ± 221.14	691.87 ± 937.09	<0.001
D-dimer (ng/ml)	633.54 ± 1,108.27	2,692.56 ± 1,611.06	<0.001

* Mann-Whitney U test
BMI = body mass index

malignant ovarian tumor (n = 61). Both serum D-dimer and CA-125 are statistically significant higher in the malignancy group ($p<0.001$).

Histopathology of adnexal masses in the present study is shown in Table 2. Endometriotic cysts represented the most common adnexal mass, followed by mature cystic teratoma and serous cystadenoma of the ovary. The incidence of malignant ovarian tumor was 30.5% including metastatic tumors to the ovaries (one was Krukenberg tumor firstly diagnosed from ovarian tissue and the other is a metastatic adenocarcinoma of the Rectum). The word "others" in the table means other benign conditions, eight were subserous and broad ligament leiomyoma, seven were tubo-ovarian abscess including one pelvic tuberculosis, one could not be identified histopathology due to a twisted ovarian cyst and the other was a partubal cysts and pseudocysts.

Table 2. Histopathology of adnexal masses

	Frequency	Percent
Endometriotic cysts	65	32.5
Mature cystic teratoma	18	9.0
Serous cystadenoma	15	7.5
Mucinous cystadenoma	12	6.0
Physiologic cysts	5	2.5
Fibroma-thecoma	3	1.5
Non-mucinous carcinoma	47	23.5
Mucinous carcinoma	9	4.5
Malignant germ cell	2	1.0
Granulosa cell tumor	1	0.5
Metastatic tumors	2	1.0
Others	21	10.5
Total	200	100.0

Table 3. Cut off values, sensitivity and false positive rate (1-specificity) in each cut-off values of D-dimer and CA-125

Test result variable(s)	Positive if greater than or equal to	Sensitivity	1-specificity
D-dimer (ng/ml)	416.0	0.95	0.40
	500.0	0.92	0.31
	560.0	0.92	0.27
	600.0	0.89	0.24
CA-125 (U/ml)	35.0	0.87	0.52
	50.0	0.82	0.35
	65.0	0.75	0.27
	80.0	0.75	0.23

In the present study, 56 patients had epithelial ovarian cancer, the most common histologic subtype being serous (15 persons including 1 borderline serous) followed by clear cell and endometrioid (11 persons), mucinous (9 persons including 1 borderline mucinous), undifferentiated (5 patients), mixed epithelial-stromal (2 patients) and others (transitional cell = 1, small cell = 1).

The authors used receiver operator curve (ROC) to find out the best cut-off values of D-dimer and CA-125 (Table 3).

Considering D-dimer, level 500 ng/ml is the threshold level of VIDAS D-dimer test to rule out venous thromboembolism (VTE) in our institute. CA-125 cut-off value at 65 U/ml has reasonably good sensitivity and specificity. Some authors used this cut-off value due to more specificity than the usual 35 U/ml[9].

When using both tests with cut-off points mentioned above, the sensitivity, specificity, and predictive values are compared in Table 4.

The authors categorized malignant ovarian cancer patients into early (stage I) and advanced (stage II-IV) stage of disease to find how many D-dimer and CA-125 were rising in each group (Table 5). In stage I of malignancy disease, 83.3% and 39% of D-dimer and CA-125 were rising respectively.

The authors also observed the incidence of VTE in our study and found two patients with DVT and two patients with DVT and pulmonary embolism (PE) accounting for 2% of DVT and 1% of PE complication in malignancy group. All of these VTE occurred before surgical exploration. No VTE complication was found in benign adnexal tumor.

Table 4. Comparison values between D-dimer and CA-125 in all patients

	SE	SP	PPV	NPV	DA	PLR	NLR
CA-125 (>65 U/ml)	75.4%	73.0%	59.7%	84.8%	73.9%	2.79	0.34
D-dimer (>500 ng/ml)	91.8%	71.9%	58.9%	95.2%	78.0%	3.26	0.11

SE = sensitivity; SP = specificity; PPV = positive predictive value; NPV = negative predictive value; DA = diagnostic accuracy; PLR = positive likelihood ratio (sensitivity/1-specificity); NLR = negative likelihood ratio (1-sensitivity/specificity)

Table 5. Relationship between FIGO stage with levels of D-dimer and CA-125 in patients with EOC & incidence of elevated pre-operative levels of D-dimer and CA-125

FIGO stage	n*	D-dimer (ng/ml)			CA-125 (U/ml)		
		Median	Range	>500 ng/ml, n (%)	Median	Range	>65 U/ml, n (%)
I	18	1,003.0	92-4,973	15 (83.3)	55.24	11-924	7 (38.9)
II-IV	38	3,230.0	500-6,376	37 (97.4)	592.35	28-4,741	36 (94.7)
	56		p<0.001			p<0.001	

* Included only epithelial ovarian cancer, excluded 5 patients with malignant germ cell tumor (n = 2), metastatic tumor (n = 2) and granulosa cell tumor (n = 1)
EOC = epithelial ovarian cancer

Discussion

The incidence of venous thromboembolism in gynecologic malignancy is higher than in other benign conditions; and can occur in 40% of patients[22]. Pelvic surgery that can disrupt circulation predisposing to emboli, also there is systemic activation of coagulation factors in ovarian malignancy by producing several pro-coagulant substances[9,13-19]. Hence, clot production is one cancer characteristic. A degradation product as D-dimer may represent malignant activity and serve as a tumor marker.

Some investigators have demonstrated that serum D-dimer is increased significantly higher in malignant ovarian tumors compared to benign conditions[9,23-28]. One investigator reported that the combined use of D-dimer and CA-125 had 100% specificity to exclude malignant ovarian tumor if both test results were below cut-off value[2,9].

An effective tumor marker should detect ovarian cancer in early stage (high sensitivity), and correctly identify no malignancy if below cut-off screening test result, having a high negative predictive value. In the present study, D-dimer has a high 91.8% sensitivity and high negative 95.2% predictive value. This appears better than CA-125 in differentiating benign from malignant.

Likelihood ratios, the ratio of the probability of the specific test result in patients who do and do not have the disease, has more clinical use. Positive likelihood ratios above 10 and negative likelihood ratios below 0.1 are considered to provide strong evidence to rule in or rule out diagnoses respectively[29]. The 0.11 negative likelihood ratio of serum D-dimer testing in our study confirms the power of test to rule out ovarian malignancy.

In addition, D-dimer has more potential to detect early stage (FIGO stage I) epithelial ovarian cancer than CA-125 (83% vs. 39% rising above cut-off value) our study confirms the low sensitivity of CA-125 to diagnose stage I ovarian cancer as reported in previous studies[2,9].

However, the authors observed lower test specificity of both D-dimer and CA-125 than in the reports[9]. This is probably due to the high incidence of endometrioma patients (33% population). D-dimer testing still has false positive results in some benign conditions particularly those that have inflammatory process.

In conclusion, D-dimer could be a useful marker in pre-operative assessment of adnexal masses. D-dimer testing has a high sensitivity to detect ovarian cancer even in the early stage of disease. Clinical utility includes triaging patients with adnexal or ovarian tumors to appropriate centers and specialty physicians. With the ability to rule out the malignant ovarian tumor, it can aid physician's decision to manage conservatively (non-surgery) in patients who have low risk for malignancy and high surgical risk. Further studies for investigating the combination of D-dimer with other tests similar to the risk of malignancy index (RMI) are being studied.

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What is already known on this topic?

D-dimer is a hematologic marker for thromboembolism. However, it has been used as a tumor marker for ovarian cancer in some reports regarding, its thromboembolism property.

At least five publications[19,24-27] showed that D-dimer level is abnormal in ovarian cancer. Some of them reported the potential of D-dimer as a tumor marker for ovarian cancer[25,26]. Only one study is among Asian population[25].

This study design is a diagnostic test. It showed the sensitivity, specificity PPV, and NPV of D-dimer as a pre-operative marker for adnexal masses. The result is promising if being used as a tumor marker.

What this study adds?

Race is one of the major factor of thromboembolism study. The population of this study is King Chulalongkorn Memorial Hospital, which is the second Asian study, rather than the Japanese[25], to prove that D-dimer could be a tumor marker for ovarian cancer.

Moreover, in this study, D-dimer is superior to CA-125 in diagnostic "early ovarian cancer". The sensitivity of D-dimer compared to CA-125 in this group is 83.3% vs. 38.9%.

Potential conflicts of interest

None.

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การใช้ D-dimer เป็น tumor marker ในการประเมิน adnexal masses ก่อนการผ่าตัด

พงษ์เกษม วรเศรษฐสิน, อมรินทร์ นาควิเชียร

วัตถุประสงค์: *เพื่อศึกษาความไว ความจำเพาะ และ predictive value ของการตรวจ D-dimer ในการประเมิน adnexal masses ก่อนการผ่าตัด การศึกษาเชิงพรรณนาแบบ diagnostic test*

วัสดุและวิธีการ: *ตรวจระดับของ D-dimer และ CA-125 ในผู้ป่วย 200 ราย ที่ได้รับการวินิจฉัยเป็น adnexal masses ก่อนการผ่าตัดที่ภาควิชาสูติศาสตร์-นรีเวชวิทยา โรงพยาบาลจุฬาลงกรณ์ ในระหว่างเดือนมิถุนายน พ.ศ. 2550 ถึง เดือนพฤษภาคม พ.ศ. 2551*

ผลการศึกษา: *เมื่อใช้ระดับ D-dimer ที่ 500 ng/ml จะมีความไว ร้อยละ 91.8 ความจำเพาะ ร้อยละ 71.9 positive predictive value ร้อยละ 58.9 และ negative predictive value ร้อยละ 95.2 ในการแยก adnexal masses ที่ไม่ใช่มะเร็งออกจากชนิดที่เป็นมะเร็ง เมื่อเปรียบเทียบกับการใช้ CA-125 ที่ 65 U/ml จะมีระดับความไว ความจำเพาะ positive predictive value และ negative predictive value ร้อยละ 75.4, 73.0, 59.7 และ 84.8 ตามลำดับ นอกจากนี้ likelihood ratio to be negative ของ D-dimer เท่ากับ 0.11 ในกลุ่มผู้ป่วย epithelial ovarian cancer ระยะที่ 1 มีการเพิ่มขึ้นของ D-dimer ถึงร้อยละ 83 เมื่อเทียบกับ CA-125 ที่เพิ่มเพียงร้อยละ 39*

สรุป: *อาจใช้ D-dimer ในการตรวจประเมิน adnexal masses ก่อนการผ่าตัด ในการศึกษานี้ D-dimer อาจดีกว่า CA-125 ในการแยก adnexal masses ที่ไม่ใช่มะเร็งออกจากชนิดที่เป็นมะเร็ง*