Lysophosphatidic acid: an ovarian cancer marker

I. Sedláková[1], J. Vávrová[2], J. Tošner[1], L. Hanousek[3]

[1]Department of Gynecology and Obstetrics, University Hospital, Hradec Králové,
[2]Department of Clinical Biochemistry, University Hospital, Hradec Králové,
[3]Department of Gynecology and Obstetrics, Hospital Pardubice (Czech Republic)

Summary

Objective: To determine whether lysophosphatidic acid (LPA) can serve as an ovarian cancer marker, we compared plasma LPA levels in ovarian cancer patients, in women with no ovarian pathology, and in women with benign ovarian tumors. We determined the optimal plasma LPA level cutoff value and correlated clinicopathological parameters with plasma LPA levels. *Method:* Capillary electrophoresis with indirect ultraviolet detection was used to analyze the plasma LPA levels of 133 patients (60 patients with ovarian cancer, 43 women without ovarian pathologies and 30 patients with benign ovarian tumors) during a three-year period. *Results:* Patients with ovarian cancer had a significantly higher plasma LPA level (n = 60, median (med) 16.99 μmol/l, range 4.53-43.21 μmol/l) compared with controls with no ovarian pathology (n = 43, med 2.92 μmol/l, range 0.94-22.93 μmol/l) and patients with benign ovarian tumor (n = 30, med 7.73 μmol/l, range 1.12-28.84 μmol/l) ($p < 0.001$). We found that plasma LPA levels were associated with the International Federation of Gynecology and Obstetrics (FIGO) stage and ovarian cancer histological type. Patients with endometrial ovarian cancer had significantly higher plasma LPA levels in comparison with other histological types of epithelial ovarian carcinoma. *Conclusion:* The plasma LPA level can be a useful marker for ovarian cancer, particularly in the early stages of disease.

Key words: Ovarian cancer; Lysophosphatidic acid; Marker.

Introduction

Ovarian cancer has the worst prognosis of all gynecological malignancies. The poor prognosis results from the inability to detect ovarian tumors at an early, a curable stage, as well as from the lack of effective therapies for the disease in advanced stages. Current treatment, which consists of radical surgery and chemotherapy, has resulted in improved patient survival and quality of life; however, there has been no significant increase in the rate of curing ovarian cancer during the last 30 years. Currently, there are no proven biomarkers that can be used for early detection. The most common biomarker, cancer antigen 125 (CA 125), lacks specificity and is elevated in only around 50% of Stage I ovarian cancer cases [1]. Lysophosphatidic acid (LPA, 1-acyl-2hydroxy-sn-glycero-3-phosphate) is a phospholipid that is elevated in the ascites and serum in ovarian cancer patients [2]. It is produced by malignant ovarian epithelium and activates various biological responses by binding and activating G-protein-coupled receptors. The most prominent LPA effects include stimulation of cell proliferation, cell survival and tumor cell invasion [3]. LPA reduces the sensitivity of ovarian cancer cell lines to apoptosis induced by the chemotherapeutic agent cisplatinum and may also play a role in metastatic competence [4]. It increases the expression of several angiogenic factors, such as interleukin-8, vascular endothelial growth factor, and growth regulated oncogene (GRO), which promote angiogenesis and tumor metastasis [2, 5, 6] The expression levels of the LPA_2 and LPA_3 receptors were significantly increased in ovarian cancer tissue, compared with benign tumors and normal ovarian tissue [7]. While ovarian cancer cells produce LPA, normal ovarian epithelial cells do not [8]. The objective of this prospective study was to determine whether LPA could serve as a new ovarian cancer marker. We measured the plasma levels of LPA in ovarian cancer patients, in woman without ovarian pathologies, and in women with benign ovarian tumors and used these data to correlate LPA plasma levels sensitively and specifically with clinicopathological parameters.

Materials and Methods

Instrumentation

A Beckman P/ACE 5510 System with System Gold software (Beckman Ins., USA) was used for capillary electrophoresis (CE) separation. On-line indirect UV detection was performed, and the separation temperature was maintained at 25°C. Samples were introduced into the capillary by pressure injection at 3.45 kPa for 10 sec. Separations were carried out under an applied potential of 25 kV at the normal polarity with the cathode placed at the capillary outlet. Between runs, the capillary was rinsed with the separation buffer for 2 min. Each new capillary was conditioned sequentially with 0.1 M NaOH for 1 hr, deionized water for 20 min, methanol for 20 min, and separation buffer for 30 min before use. Each day, the capillary was rinsed with 0.1 M NaOH for 5 min, deionized water for 2 min, and separation buffer for 15 min.

Chemicals and solutions

Lysophosphatidic acid (synthetic, purity > 99%), was purchased from Avanti Polar Lipids, Inc. (USA). Adenosine 5´-monophosphate monohydrate (AMP, 99%), sodium hydroxide (> 99.99%), acetonitrile (> 99.9%) and 8-anilino-1-naphthalenesulfonic acid were obtained from Sigma-Aldrich (Germany). HPLC grade methanol (> 99.9%) and boric acid were purchased from

Revised manuscript accepted for publication February 11, 2008

Lachema (Czech Republic). Thin layer chromatography (TLC POLY SIL) was purchased from Macherey-Nagel (Germany). Aqueous solutions were prepared with deionized water from Millipore-milli-Q plus (France). Stock buffer solutions of 50 mM AMP in various concentrations of boric acid were prepared by dissolving the appropriate amounts of AMP and boric acid in deionized water. The solutions were then adjusted to the desired pH values with 1 M NaOH. Prior to CE analysis, standard working solutions of lysophospholipids were freshly prepared by serial dilution of the standard stock solutions in a solvent of 5% separation buffer in methanol-water (9:1 v/v).

Serum samples preparation

Extraction of serum lipids was performed at 0-4°C to minimize damage to ester bonds. The solution (serum and LPA) was acidified by adding 0.2 ml of 12 mol/l HCl, and mixed with 4 ml of methanol-chloroform (2:1 v/v) for 10 min. After adding 1 ml of chloroform and 1.25 ml of water, the mixture was centrifuged at 1000 g for 10 min. The methanol-chloroform phase (lower phase), which contained the lipids, was removed by pipetting. The solution was evaporated under N2 until approximately 50 µl remained and was then loaded onto a TLC plate. The spot containing LPAs was separated from other lipids by TLC using the solvent system of chloroform-methanol-ammonium hydroxide (65:35:5.5 v/v/v); 0.1% 8-anilino-1-naphthalenesulfonic acid in water was used as spray on the standard LPA. During the first experiment, lipid extraction after TLC separation was used to confirm the position of the LPA spot on the TLC plate. The LPA spot was visualized in a dark room under UV light. During subsequent separations, the LPA spot was detected by water spray to prevent contamination, and then scraped and extracted from the plate. The extract was evaporated under N2 until dry. The dry powder was reconstituted in 1 ml of 5% separation buffer in methanol–water (9:1 v/v), and the solution was subjected to CE analysis.

Patients

We evaluated a sample of patients from the Department of Gynecology and Obstetrics at the University Hospital in Hradec Králové and at the Department of Gynecology and Obstetrics at the Pardubice Hospital over a three-year period (June 2003 to December 2005). Whole blood specimens were obtained from a total of 133 patients pre-operatively, including 60 patients with epithelial ovarian cancer. This included nine patients with Stage I (median (med) age 50.6 years), seven patients with Stage II (med age 56.6 years), 39 patients with Stage III (med age 56.9 years) and five patients with Stage IV (med age 72.4 years) ovarian cancer. Blood specimens were obtained from 43 women with no ovarian pathology (med age 51.2 years) and from 30 women with benign ovarian tumors (med age 43.2 years). Patients who had unclear preoperative ovarian diagnoses, who had CA 125 elevation, were included in the sample of patients with benign ovarian tumors. The clinical cancer stages were determined according to the International Federation of Gynecologists and Obstetricians (FIGO) criteria [9], and the histological subtypes were evaluated according to the World Health Organization (WHO) classification [10].

Statistical Analysis

Comparisons of patients groups were performed using the Kolmogorov-Smirnov and Mann-Whitney tests; p values less than 0.001 were considered statistically significant. To evaluate the diagnostic accuracy of LPA as a marker for ovarian cancer, receiver operating characteristic (ROC) analysis was used.

Results

The ages of the patients, the ovarian cancer stages, grades, and histological types are shown in Table 1. Patients with ovarian cancer had a significantly higher LPA plasma level (med 16.99 µmol/l, range 4.53-43.20 µmol/l) compared with the healthy control group with no ovarian pathologies (med 2.92 µmol/l, range 0.94-22.93 µmol/l) and patients with benign ovarian tumors (med 7.73 µmol/l, range 1.12-28.84 µmol/l) (Table 2, Figure 1). We detected plasma LPA elevation in early stages of ovarian cancer (Table 3). Plasma LPA levels were associated with the FIGO stage and ovarian carcinoma histology type. Patients with endometrial ovarian cancer had a significantly higher plasma LPA level (n = 14, med 22.89 µmol/l, range 4.50-42.58 µmol/l) compared with other histological types of epithelial ovarian carcinoma (serous ovarian cancer n = 26, med. 15.59 µmol/l, range 5.13-42.62 µmol/l, mucinous ovarian cancer n = 9, med 12.52 µmol/l, range 6.83-27.12 µmol/l, clear cell ovarian cancer n = 3, med. 5.07 µmol/l, range 4.53-8.30 µmol/l). The histological grade did not influence the plasma LPA level in this study. We used a 10.4 µmol/l cutoff value, which had 85% sensitivity and 83.7% specificity using the ROC curve (Figure 2).

Table 1. — *Characteristics.*

	Stage I+II (n = 16)	Stage III+IV (n = 44)
Age median (range)	53 (20-62)	64 (40-82)
Stage		
I	9	0
II	7	0
III	0	39
IV	0	5
Grades		
G1	4	5
G2	6	17
G3	1	17
Histological types		
Serous Adenocarcinoma	8	18
Endometroid Ovarian Cancer	4	12
Mucinous Ovarian Cancer	2	6
Clear cell Cancer	2	1
Not determined	2	7

Table 2. — *LPA levels in plasma.*

	Ovarian cancer n = 60	No ovarian pathology n = 43	Benign ovarian tumor n = 30	µmol/l
LPA				
Med	16.99*	2.92*	7.73*	p < 0.001
Range	4.53-43.20	0.94-22.93	1.12-28.84	p < 0.001

* p < 0.001.

Table 3. — *LPA levels in ovarian cancer stages (FIGO).*

Stage	Median (µmol/l)	Range (µmol/l)
I	12.23	4.53 - 43.2
II	16.46	5.13 - 42.58
III	18.59	4.5 - 42.53
IV	35.03	15.05 - 36.05

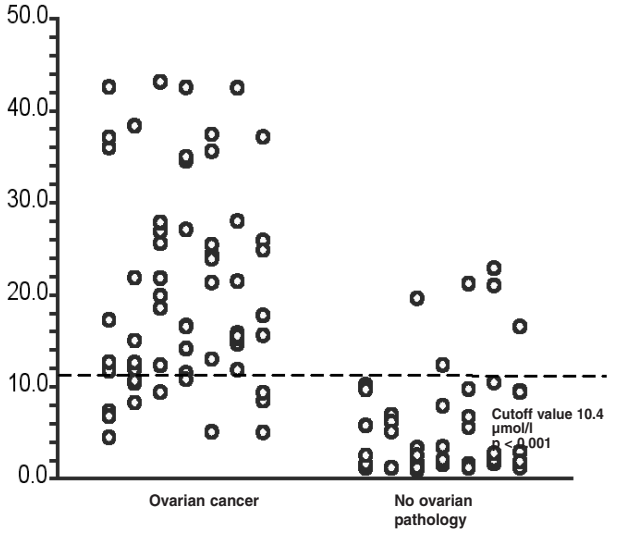

Figure 1. — Total plasma lysophosphatidic acid levels of patients with ovarian cancer and healthy female controls.

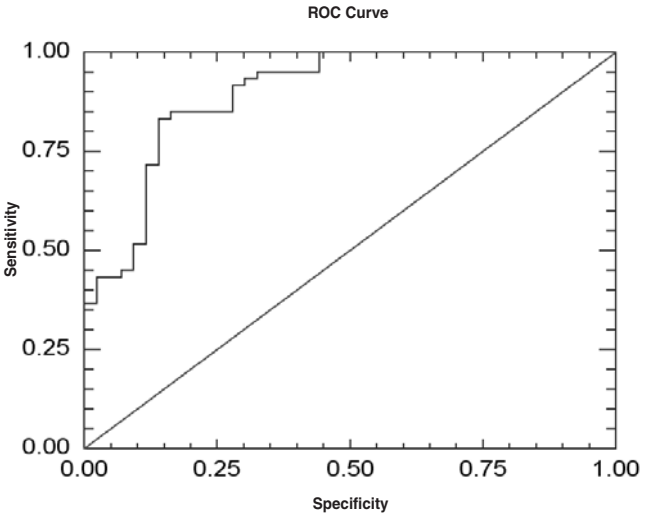

Figure 2. — ROC curve.

Discussion

Higher plasma LPA levels have been reported in ovarian cancer patients than in healthy women [11-14]. A study, which included 48 healthy controls and 48 women with ovarian cancer, showed that plasma LPA levels were elevated in patients with ovarian cancer. Importantly, elevated LPA levels were detected in early-stage ovarian cancer cases compared with the controls [13]. However, another study that measured the LPA plasma levels in 32 patients with ovarian cancer and 32 healthy controls using a liquid chromatography/mass spectroscopy assay found no significant elevation in plasma LPA in cancer patients [15]. The reason for the discrepancy between the findings is unclear, since there were many methodological differences between the two studies, including differences in sample collection, processing, and lipid analyses [11]. The plasma LPA assay offers the possibility of earlier diagnosis of ovarian cancer, a disease that has a poor prognosis, mainly because it is rarely detected in early stages. Identification of effective biomarkers for early cancer detection can improve survival rates [11]. Elevated plasma LPA levels were detected in 90% of patients with Stage I ovarian cancer [13]. Here, we identified that plasma LPA levels were slightly higher in patients with benign ovarian tumors compared with women with no ovarian pathology (Table 2). These results may be influenced by our inclusion of patients suspected of having ovarian cancer, but without histological diagnoses, in the group of women with benign ovarian tumors. Ovarian cancer cells produce LPA but normal ovarian epithelial cells do not [8]. LPA increases the expression of vascular endothelial growth factor (VEGF) in established ovarian cancer cell lines by binding to the LPA2 receptor, which is highly expressed in ovarian cancer cells but not in normal ovarian epithelium [16]. The expression levels of the LPA_2 and LPA_3 receptors were significantly increased in ovarian cancer samples (92.6%) compared with benign tumors (45.5%) and normal ovarian tissue (43.8%) [7]. This study demonstrates plasma LPA elevation in ovarian cancer patients. At present, it is necessary to determine the optimal cutoff value for plasma LPA levels.

Conclusion

Compared to healthy controls and to patients with benign ovarian tumors, elevated plasma LPA levels were detected in ovarian cancer patients. Our results suggest that plasma LPA levels can act as a biomarker for ovarian cancer, particularly in the early carcinoma stages.

Acknowledgement

This project was supported by the Ministry of Health Grant Agency, Nos. 7666-3 and 8768-3.

References

[1] Schwartz P.E., Taylor K.J.: "Is early detection of ovarian cancer possible?". *An. Med.,* 1995, *27*, 519.
[2] Fishman D.A., Bozorgi K.: "The scientific basis of early detection of epithelial ovarian cancer: The National Ovarian Cancer Early Detection Program (NOCEDP)". *Cancer Treat Res.,* 2002, *107*, 3.
[3] So J., Navari J., Wang F.O., Fishman D.A.: "Lysophosphatidic acid enhances epithelial ovarian carcinoma invasion through the increased expression of interleukin-8". *Gynecol. Oncol.,* 2004, *95,* 314.
[4] Xiao Y.J., Schwarz B., Washington M., Kennedy A., Webster K., Belinson J., Xu Y.: "Electrospray ionization spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant versus nonmalignant ascitic fluids". *Anal. Biochem.,* 2001, *290*, 302.
[5] Hu Y.L., Tee M.K., Goetzl E.J., Auersperg N., Mills G.B., Ferrara N. *et al.*: "Lysophosphatidic acid induction of vascular endothelial growth factor expression in human ovarian cancer cells". *J. Natl. Cancer Inst.,* 2001, *93,* 762.
[6] Lee Z., Swaby F.R., Liang Y., Yu S., Liu S., Lu K.H. *et al.*: "Lysophophatidic Acid Is a Major Regulator of Growth-Regulated Oncogene in Ovarian Cancer". *Cancer Research,* 2006, *66,* 2740.

[7] Wang P., Wu X., Chen W., Liu J., Wang X.: "The lysophosphatidic acid (LPA) receptors their expression and significance in epithelial ovarian neoplasms". *Gynecol. Oncol.,* 2007, *104,* 714.
[8] Eder A.M., Sasagawa T., Mao M., Aoki J., Mills G.B.: "Constitutive and lysophosphatidic acid (LPA)-induced LPA production: role of phospholipase D and phospholipase A2". *Clin. Cancer Res*., 2000, *6,* 2482.
[9] Ozols R.F., Rubin S.C., Thomas G., Robboy S.: "Epithelial ovarian cancer". In: Hoskins W.J., Perez C.A., Zouny R.C. (eds.). Principles and Practice of Gynecologic Oncology. Philadelphia: Lippincott-Raven Publishers. 1997, 958.
[10] WHO. WHO Handbook for reporting results of cancer treatment. Geneva (Switzerland), WHO, 1979.
[11] Sutphen R., Xu Y., Wilbanks D., Fiorica J., Grendys E.C. Jr., La Polla J.P. *et al.*: "Lysophospholipids are potential biomarkers of ovarian cancer". *Cancer Epidem.*, 2004, *13*, 1185.
[12] Okita M., Gaudette D.C., Mills G.B., Holub B.J.: "Elevated levels and altered fatty acid composition of plasma lysophosphatidylicholine in ovarian cancer patients". *Int. J. Cancer,* 1997, *71,* 31.
[13] Xu Y., Shen Z., Wiper D.W., Wu M., Morton R.E., Elson P. *et al.*: "Lysophosphatidic acid as a potential biomarker for ovarian and other Gynecologic Cancers". *JAMA,* 1998, *280,* 719.
[14] Sedláková I., Vávrová J., Tošner J., Hanousek L.: "Lysophosphatidic acid in ovarian cancer patients". *Česká Gynekologie*, 2006, *71,* 312.
[15] Baker D.L., Kortison P., Miller B., Riely C.A., Tolley B., Westermann A.M. *et al.*: "Plasma lysophosphatidic acid concentration and ovarian cancer". *JAMA,* 2002, *287,* 3081.
[16] So J., Wang F., Navari J., Fishman D.A.: "LPA-induced epithelial ovarian cancer (EOC) in vitro invasion and migration are mediated by VEGF receptor-2 (VEGF-R2)". *Gynecol. Oncol.,* 2005, *97,* 870.

Address reprint requests to:
I. SEDLAKOVA, M.D., Ph.D.
Department of Gynecology and Obstetrics
University Hospital Hradec Kralové
500-05 Czech republic
e-mail. sedlakiva@seznam.cz