DOI: 10.7860/JCDR/2018/30902.11350

Review Article

Oncology Section

Role of Thrombopoietin in the Diagnosis of Ovarian Cancer: A Brief Review

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ABSTRACT

Ovarian Cancer (OC) is the most fatal condition among all gynaecologic malignancies. The survival rate of early and advance stage of OC is 80-90% and 15-20% respectively. This data constitutes the need of a novel biomarker for early diagnosis, which may distinguish malignant tumours from benign ovarian cysts. Presently in practice there are three screening techniques: bimanual pelvic examination, serum Cancer Antigen (CA) 125 and transvaginal ultrasound. Bimanual pelvic examination can detect only 1 in 10,000 ovarian cancers in women presenting to the clinic. CA 125 is raised in 80% of cases of ovarian carcinoma; however, if the carcinoma is limited to ovary, the raised value is seen only in 50% of women. It is mainly useful in postmenopausal women. Ultrasonography also lacks specificity and sensitivity, apart from being costly as a screening test. Studies are going on for search of a biomarker, which may complement CA 125. Most common among these are Human Epididymis Protein 4 (HE4), Thrombopoietin (TPO), CA19-9, human kallikrein 10, human kallikrein 6, osteopontin, claudin 3, DF3 (murine monoclonal antibody), vascular endothelial growth factor, MUC1, mesothelin etc. In 2008, FDA approved the use of HE4 assay for monitoring of progressive disease in patients with epithelial ovarian cancer. Similarly TPO also holds the promise of being a biomarker to complement CA125 and many studies are indeed available to support the association of TPO and OC. In this review article we have summarised and analysed such studies.

Keywords: Cancer antigen 125, Ovarian tumour, Platelets

OVARIAN TUMOURS

WHO has classified ovarian tumours into three major categories: surface epithelial-stromal tumour, sex cord-stromal tumour and germ cell tumour. Surface epithelial-stromal tumour designated as OC in this manuscript, constitute approximately 60% of all ovarian tumours and approximately 90% of all malignant ovarian tumours [1]. OC is the sixth most common gynaecologic malignancy. Its incidence increases with age. So the post menopausal women are the maximum sufferers [2]. The fatality rate of OCs are highest among all gynaecologic malignancies. Most of the cases of OC are diagnosed in advanced stage. This results in long-term survival only in 30% of cases [3]. The survival rate for OC in early and advanced stage is 80-90% and 15-20% respectively. Early stage has been taken till the cancer is limited to ovary (Stage 1A and 1B of FIGO Staging system). Rest all the stages (1C, II, III and IV) have been taken as advanced stage.

This data indicates the need of a novel biomarker, which may distinguish malignant tumours from benign one at early stage [4]. Due to lack of specific symptoms and a potent biomarker, the diagnosis of OC is usually delayed. Survival rate for OC for localised disease, regional metastasis and distant metastasis is approximately 89%, 36% and 17% respectively. The overall five-year survival rate stands around 44%. So the early diagnosis seems to be crucial in increasing the survival rate [5]. If OC is diagnosed in Stage I, cure rate is up to 90%. At present we can diagnose less than 25% of OC in Stage I by combined approach of CA 125 and transvaginal ultrasonography. However, 20% of cases of OC lack CA 125 expression. If we recognise a marker that can detect the OC lacking CA 125 expression, it will improve the diagnostic accuracy significantly [6].

Screening Tests for Ovarian Cancer

Presently in practice there are three screening techniques: bimanual pelvic examination, CA 125 and transvaginal ultrasound. Bimanual pelvic examination detects 1 in 10000 OC cases indicating its poor sensitivity and sensitivity. CA 125 is elevated in 80% of OC cases; however, if the cancer is limited only to the ovary, the raised CA 125 is found only in 50% of cases. Raised CA 125 values have also been noted in non malignant gynaecological diseases such as pelvic inflammatory disease, endometriosis and adenomyosis. It is raised in pregnancy, uterine fibroids (benign tumours), pancreatitis, normal menstruation, and liver disease. In healthy female CA 125 is produced by ovary, endometrium and peritoneum. Transvaginal ultrasonography can detect abnormality in ovarian morphology, but is not reliable in differentiating benign from malignant tumours. For screening test, it is sensitive, but it has low positive predictive value. It is also an expensive tool for screening purpose [7]. The statistical data may be better understood by the study of Van Nagell JR Jr et al., which showed following variables of transvaginal ultrasound in screening of OC: sensitivity 81%, specificity 98.9%, Positive Predictive Value (PPV) 9.4% and Negative Predictive Value (NPV) 99.97% [8]. Lastly the definitive diagnostic tool for OC is still surgery. Surgery also has measurable shortcomings. As per the report of Buys SS et al., only 3.5% (20/570) malignant cases can be found after diagnostic surgical excision for OC [9]. Recently serum HE4 assay is gaining popularity, which has been approved by the FDA in 2008 for monitoring of recurrent or progressive disease in patients with OC (Rosen DG et al., 2005) [6]. Thus, to find a novel marker is the need of recent time which may be used in screening, diagnosis and monitoring of therapy in OC.

Serum CA 125 was discovered by Bast R et al., in 1983 [10]. It is also known as MUC 16. It is a member of mucin glycoprotein, contains 22,000 amino acids. CA 125 is the most commonly used screening test for epithelial OC; however, it is more useful in postmenopausal women. It cannot be a reliable marker for early stage disease. Raised CA 125 is seen in 80% cases of advanced cancer and in only 50% cases in early stage (FIGO stage 1A and 1B). Most of the cases of OC with advanced stage are seen in postmenopausal

stage [10]. Malkasian GD Jr et al., conducted study on pre-versus postmenopausal women (sample size 158 women) to look the role of CA 125 in the diagnosis of ovarian tumour. They found that CA 125 was a better marker for the postmenopausal women. Probably this was due to OC being more diagnosed in postmenopausal women [11]. It is also raised in many benign conditions as already mentioned. Report from American College of Obstetricians and Gynecologists, Practice Bulletin; 2007, shows the PPV of CA 125 in women with an adnexal mass ranges from 35% to 91% and the NPV ranges from 67% to 90%. The sensitivity of CA 125 in distinguishing benign and malignant mass is 61% to 90% and specificity is 35% and 91%. This broad range of values appears to be due to different inclusion criteria for premenopausal women in different studies [12].

THROMBOPOIETIN

Thrombopoietin is also known as myeloproliferative leukaemia virus ligand (*c-mpl*). It is a hematopoietic growth factor belonging to the (Erythropoietin) EPO/TPO family [13]. V-mpl, an oncogen was first discovered from murine myeloproliferative leukaemia virus in 1990. It had capacity to immortalise bone marrow hematopoietic cells from different lineages. Its human homologue, *c-mpl*, was cloned in 1992. TPO, the ligand for *c-mpl*, was cloned in 1994. This is the regulator of megakaryopoiesis. Encoded protein for *c-mpl* gene is CD110. This is composed of 635 amino acids (14). TPO is produced in bone marrow, liver, kidney and spleen. It binds *c-mpl* and gets cleared from circulation [15,16]. Human TPO gene is located on chromosome 3q27. It comprises six exons and five introns. Eight variants of TPO mRNA have been identified yet. TPO-1 has been identified in its full length of mRNA and it has shown proliferative activity [17,18]. Human TPO is a 60-70 kDa, 332 amino acid residue glycosylated polypeptide that plays key role in the growth and development of megakaryocytes and platelets [18]. Conversely platelets have regulatory role on concentration of TPO in plasma [19]. Preoperative thrombocytosis has been described for cervical, endometrial, ovarian and vulvar lesions [20-24]. TPO has shown a regulatory role in proliferation and secretion in porcine ovarian follicular cells [20]. TPO genes have been shown to be expressed by several cancer cell lines e.g., lung, stomach, liver and thyroid [21]. TPO level has also been shown to be increased by inflammatory mediator IL-6 in hepatoma cell lines. IL-6 is produced by macrophages and monocytes [24]. TPO may work as a tumour marker for diagnosis of carcinoma in ovary. As per literature available in past decade, TPO has been shown to be expressed by three malignant tumours: OC, hepatoblastoma and hepatocellular carcinoma. Bottsford-Miller J studied that platelets may work as a biomarker for recurrence of OC. He did a retrospective analysis, in which platelet count was measured twice; one at the time of initial diagnosis and second at the time of recurrence. He noticed that at the time of recurrence, platelet count was increased by 49%. This high value was especially seen when the value of CA125 was normal [25]. Tsukishiro S et al., studied the values of TPO at the time of diagnosis of ovarian tumour. They noticed that histopathologically proven cases of OC had statistically significant values of TPO with respect to benign tumours (cysts) of ovary. They also noticed that there was no significant correlation with platelet count in benign and malignant cases. When the assessment of TPO was combined with CA 125, it showed specificity of 92% in prediction of malignancy, in contrast to 76% for CA 125 alone [26,27]. This discussion justifies that there may be an existence of relationship between OC and either TPO, which requires further study. Similar to this study, Mermer T et al., also identified TPO as a potential tumour marker for the diagnosis of OC. They also noticed high TPO values at the time of diagnosis in cancer cases with respect to benign one [27]. For statistical analysis of serum TPO, they took cut off level 90 pg/mL for diagnosis of malignancy. They noticed sensitivity 52%, specificity 84%, PPV 76% and NPV 63%.

On taking CA 125 alone as a diagnostic marker with cut-off value 30 U/mL, statistical analysis showed sensitivity 92%, specificity 76%, PPV 79% and NPV 84%. When CA 125 was combined with TPO, PPV reached up to 85%. Post therapy serum TPO level in malignant group was significantly low in comparison to preoperative value (p=0.002). In addition, Mermer T et al., also noticed that the high values of TPO were significantly decreased on successful completion of therapy. TPO alone was more cost effective (9.75$) than the combined use of CA 125 (6.5$) and transvaginal ultrasonography (15$). They concluded that TPO can play an additive role for prediction of OC, but it was not superior to CA-125 [27]. So to establish TPO as a diagnostic marker in OC more studies are required. High TPO values in OC may be attributed to direct secretion from tumour tissue or some other growth factors i.e., inflammatory cytokines, which stimulate the target tissue for TPO secretion [27]. Furuhashi M et al., reported a case of ovarian carcinoma expressing thrombopoietin on tissue by immunohistochemistry [28]. TPO expression may be detected in many ways like ELISA, flow cytometry, immunohistochemistry, western blot and molecular techniques. We can study the expression of TPO on all the surgical specimens of ovarian tumour by immunohistochemistry, which is not a very uncommon technique. Sirotkin AV et al., studied that TPO may be a regulator of ovarian function e.g; proliferation, apoptosis, secretion of hormones, steroids, growth factors etc. Furthermore, they also demonstrated that TPO works with protein kinase A to achieve these functions. [29]. Dabrow MB et al., studied that Platelet Derived Growth Factor (PDGF) promoted the growth of ovarian surface epithelial cells in vitro. This may have a role in ovarian carcinogenesis [30].

Erickson-Miller CL et al., studied that Eltrombopag has an inhibitory effect on proliferation of solid tumour cell lines of ovary, lung and breast. Eltrombopag (Promacta®) is an oral Thrombopoietin Receptor (TpoR) agonist. It stimulates megakaryocyte to produce platelets and is helpful in thrombocytopenic patients [31]. Kaszubaska W et al., prepared recombinant human TPO (rhTPO) from Chinese Hamster Ovary (CHO) cell. It showed in vitro megakaryopoiesis in mice [32].

Buller HR et al., have demonstrated that platelets along with other hemostatic constituents like coagulation and fibrinolytic proteins have an established role in progression of cancer. It works through increasing tumour cell survival [33].

Mechanism of Action of TPO in Ovarian Cancer

Mareacucci R et al., showed that TPO is the major regulator of proliferation and secretion of procrine ovarian follicular cells. Zeimet AG et al., showed that TPO level is increased by an inflammatory process mediated by Interleukin-6 (IL-6), which is produced by macrophages and monocytes. Besbes S et al., studied that normal ovarian tissue as well as ovarian cancer cell expresses TPO. They also showed that TPO is produced by OC cells. Sarkar M et al., demonstrated that the expression of TPO and its receptor *c-mpl* in bovine ovarian follicles [34]. They showed the production of the both (TPO and *c-mpl*) in corpus luteum. Its production varies on different stages of corpus luteum [34]. Besbes S et al., studied the sequence analysis of TPO genetic materials in cell lines of OC and leukemic cell. They showed that the both cells lines expressed the three out of eight variants of TPO, TPO-1 (full length TPO), TPO-2 (12 bp deletion) and TPO-3 (116 bp deletion) variants. On the basis of this data they concluded that TPO could be used as a biomarker for the detection and progression of ovarian pathology.

Secretion of Thrombopoietin in other Medical Conditions

Tsukishiro S et al., showed that serum TPO level was higher in patients with OC than with benign cysts at the time of diagnosis. However, TPO is not specifically secreted in OC. Raised TPO level has been noticed in diverse diseases. Zakynthnos SG et al., studied that value of TPO was raised in sepsis, and also its value correlated well with the severity of sepsis [35]. If a healthy volunteer is infused with endotoxin, it will also show raised TPO value. High TPO value has also been noticed in neonates, children and adult patients with sepsis [36]. Frank Heits et al., studied the association of TPO with Inflammatory Bowel Disease (IBD). The value of TPO

was high in active phase of IBD; however, the mechanism could not be found out [37]. Lupia E et al., have studied that TPO has a role in cardiovascular damage [38]. Mermer T et al., studied on OC and attempted to assess the relationship between serum TPO level and therapeutic response following surgery and chemotherapy. Although, TPO level decreased significantly following treatment, there was no relationship between the patients who responded and who did not respond. This finding limited the relevance of serum TPO in the evaluation of response to treatment. Mermer T et al., also showed that TPO had no relationship with the majority of prognostic factors (age, stage, grade, histology etc.) and their significance in the evaluation of response to treatment [27].

CONCLUSION

Limited studies exist concerning the search of a gold standard marker for OC, which may help in screening, diagnosis and therapeutic monitoring. TPO seems to be a potential diagnostic marker for OC. If proved satisfactory, this will be a major revolution in the field of gynaecological malignancies. Quantitative measurement of TPO is possible by simple techniques like ELISA. This may also be done by immunohistochemistry on ovarian tissue and by molecular technique using real time polymerase chain reaction. This appears to be a potential area for research. Early diagnosis may save numerous human lives. Young researchers need to be promoted to work in this field.

REFERENCES

[1] Chen VW, Ruiz B, Killeen JL, Coté TR, Wu XC, Correa CN. Pathology and classification of ovarian tumours. Cancer. 2003;97(10 Suppl):2631-42.
[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008, cancer incidence and mortality worldwide: International Agency for Research on Cancer. Lyon, France, 2010. http:// globocan.iarc.fr (accessed May 2012).
[3] Edwards BK, Brown ML, Wingo PA, Howe HL, Ward E, Ries LA, et al. Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. J Natl Cancer Inst. 2005;97:1407-27.
[4] Fountain J, Trimble E, Birrer MJ. Summary and discussion of session recommendations. Gynecol Oncol 2006;103:S23-25.
[5] Ries LA, Harkins D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, et al. SEER cancer statistics review, 1975-2003. Bethesda, MD: National Cancer Institute; 2005.
[6] Rosen DG, Wang L, Atkinson JN, Yu Y, Lu KH, Diamandis EP, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol. 2005;99(2):267-77.
[7] Jelovac D, Armstrong DK. Recent progress in the diagnosis and treatment of ovarian cancer. CA Cancer J Clin. 2011;61(3):183-203.
[8] Van Nagell JR Jr, DePriest PD, Reedy MB, Gallion HH, Ueland FR, Pavlik EJ, et al. The efficacy of transvaginal sonographic screening in asymptomatic women at risk for ovarian cancer. Gynecol. Oncol. 2000;77(3):350-56.
[9] Buys SS, Partridge E, Greene MH, Prorok PC, Reding D, Riley TL, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. Am J Obstet Gynecol. 2005;193(5):1630.
[10] Bast RC Jr, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, et al. A radioummunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med. 1983;309(15):883-87.
[11] Malkasian GD Jr, Knapp RC, Lavin PT, Zurawski VR Jr, Podratz KC, Stanhope CR et al. Preoperative evaluation of serum CA 125 levels in premenopausal and post-menopausal patients with pelvic masses: discrimination of benign from malignant disease. Am J Obstet Gynecol. 1988; 159(2):341-346.
[12] American College of Obstetricians and Gynecologists. Practice Bulletin no. 83: Management of Adnexal Masses. Washington, DC: American College of Obstetricians and Gynecologists; 2007.
[13] Souyri M., Vigon I., Penciolelli J. F., Heard J. M., Tambourin P., Wendling F.A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell. 1990 ;63 (6) :1137-1147
[14] Available from: http://www.genenames.org/cgi-bin/gene_symbol_report?hgnc_id=HGNC:7217.
[15] Bartley TD, Bogenberger J, Hunt P, Li YS, Lu HS, Martin F, et al. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. Cell. 1994;77(7):1117-24.
[16] De Sauvage FJ, Hass PE, Spencer SD et al.Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. Nature. 1994;369 (6481):533-38.
[17] Marcucci R, Romano M, "Thrombopoietin and its splicing variants: structure and functions in thrombopoiesis and beyond," Biochimica et Biophysica Acta-Molecular Basis of Disease, vol. 1782, no. 7-8, pp. 427-432, 2008
[18] Besbes S, Shah S, Al-dybiat I, Mirshahi S, Helfer H, Najah H et al; Thrombopoietin Secretion by Human Ovarian Cancer Cells; Int. J. of Cell Biol; Volume 2017 (2017).
[19] Kaushansky. Thrombopoietin. N Engl J Med. 1998;339(11):746-54.
[20] Hernandez E, Donohue KA, Anderson LL, Heller PB, Stehman FB. The significance of thrombocytosis in patients with locally advanced cervical carcinoma: a Gynaecologic Oncology Group study. Gynecol Oncol. 2000;78:137-42.
[21] Scholz HE, Petru E, Gucer F, Haas J, Tamussino K, Winter R. Preoperative thrombocytosis is an independent prognostic factor in stage III and IV endometrial cancer. Anticancer Res. 2000;20;3983-85.
[22] Gucer F, Moser F, Tamussino K, Reich O, Haas J, Arikan G, et al. Thrombocytosis as a prognostic factor in endometrial carcinoma. Gynecol Oncol. 1998;70:210-14.
[23] Lavie O, Comerci G, Daras V, Bolger BS, Lopes A, Monaghan JM. Thrombocytosis in women with vulvar carcinoma. Gynecol Oncol. 1999;72:82-86.
[24] Zeimet AG, Marth C, Muller-Holzner E, Daxenbichler G, Dapunt O. Significance of thrombocytosis in patients with epithelial ovarian cancer. Am J Obstet Gynecol. 1994;170:549-54.
[25] Bottsford-Miller J. True blood: platelets as a biomarker of ovarian cancer recurrence. Gynecol Oncol. 2011;120(suppl 1):S57-58.
[26] Tsukishiro S, Suzmori N, Nishikawa H, Arakawa A, Suzumori K. Preoperative serum thrombopoietin levels are higher in patients with ovarian cancer than with benign cysts. Eur J Obstet Gynecol Reprod Biol. 2008;140(1):67-70.
[27] Mermer T, Terek MC, Zeybek B, Ergenoglu AM, Yeniel AO, Ozsaran A, et al. Thrombopoietin: a novel candidate tumour marker for the diagnosis of ovarian cancer. J Gynecol Oncol. 2012;23(2):86-90.
[28] Furuhashi M, Miyabe Y, Oda H. A case of thrombopoietin producing ovarian carcinoma confirmed by immunohistochemistry. Gynecol Oncol. 1999;74:278-81.
[29] Sirotkin AV, Sanislo P, Schaeffer HJ, Florkovicova I, Kotwica J, Bulla J, Hetenyi L. Thrombopoietin regulates proliferation, apoptosis, secretory activity and intracellular messengers in porcine ovarian follicular cells: involvement of protein kinase A. J Endocrinol. 2004;183(3):595-604.
[30] Dabrow MB, Francesco MR, McBrearty FX, Caradonna S. The effects of platelet-derivedgrowthfactor and receptor on normal and neoplastic human ovarian surface epithelium. Gynecol Oncol. 1998;71(1):29-37.
[31] Erickson-Miller CL, Kirchner J, Pillarisetti K, Ottesen L, Kamel YM, Liu Y, et al. Eltrombopag decreases proliferation of ovarian, lung and breast tumour cell lines. Blood. 2009;114:2409.
[32] Kaszubaska W, Zhang H, Patterson RL, Suhar TS, Uchic ME, Dickinson RW, et al. Expression, purification, and characterization of human recombinant thrombopoietin in Chinese hamster ovary cells. Protein Expr Purif. 2000;18(2):213-20.
[33] Buller HR, Van Doormaal FF, Van Sluis GL, Kamphuisen PW. Cancer and thrombosis: from molecular mechanisms to clinical presentations. Journal Thromb Haemost. 2007;5(Suppl. 1):246-25.
[34] Sarkar M, Schilffarth S, Schams D, Meyer HHD, Berisha B. The expression of thrombopoietin and its receptor during different physiological stages in the bovine ovary. Reproduction in Domestic Animals. 2011;46(5):757-62.
[35] Zakynthinos SG, Papanikolaou S, Theodoridis T, Zakynthinos EG, Christopoulou-Kokkinou V, Katsaris G, et al. Sepsis severity is the major determinant of circulating thrombopoietin levels in septic patients. Crit Care Med. 2004;32(4):1004-10.
[36] Lupia E, Bosco O, Mariano F, Dondi AE, Goffi A, Spatola T, et al. Elevated thrombopoietin in plasma of burned patients without and with sepsis enhances platelet activation. J Thromb Haemost. 2009;7(6):1000-08.
[37] Heits F, Stahl M, Ludwig D, Stange EF, Jelkmann W. Elevated serum thrombopoietin and interleukin-6 concentrations in thrombocytosis associated with inflammatory bowel disease. Journal Interferon Cytokine Res. 1999;19(7):757-60.
[38] Lupia E, Goffi A, Bosco O, Montrucchio G. Thrombopoietin as Biomarker and Mediator of Cardiovascular Damage in Critical Disease. Mediators Inflamm. 2012;2012:39089.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 14, 2017**
Date of Peer Review: **Oct 13, 2017**
Date of Acceptance: **Dec 13, 2017**
Date of Publishing: **Apr 01, 2018**