REFERÊNCIAS BIBLIOGRÁFICAS

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AL-HATAMLEH, M. A. I. et al. Synergistic Effects of Nanomedicine Targeting TNFR2 and DNA Demethylation Inhibitor-An Opportunity for Cancer Treatment. Cells, v. 9, n. 1, p. E33, 20 dez. 2019.

ALI-Fehmi, R., SEMAAN, A., SETHI, S., ARABI, H., BANDYOPADHYAY, S., HUSSEIN, YR, Diamond MP, Saed G, Morris RT, MunkarahAR. Molecular typing of epithelial ovarian carcinomas using inflammatory markers.Cancer. 2011 Jan 15;117(2):301-9.

AMERICAN CANCER SOCIETY. Cancer Facts & Figures 2022. Atlanta, GA, 2022. Disponível em < https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2022.html >. Acesso em: 18 de agosto de 2022.

AMERICAN JOINT COMMITTEE ON CANCER (AJCC). Cancer Staging Manual, ed. 8. New York, Springer, 2017.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). Disponível em http://www.abnt.org.br/. Acesso em 20/09/2021.

BONFITTO, P. et al. Platelet activity is negatively modulated by tumor necrosis factor alpha through reductions of cytosolic calcium levels and integrin alphaIIbbeta3 phosphorylation. Thrombosis Research. Volume 172, P. 44-50. Dezembro, 2018.

CHARLES, K. A. et al. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. The Journal of Clinical Investigation, v. 119, n. 10, p. 3011–3023, out. 2009.

CHEN, X.; OPPENHEIM, J. J. Targeting TNFR2, an immune checkpoint stimulator and oncoprotein, is a promising treatment for cancer. Science Signaling, v. 10, n. 462, p. eaal2328, 17 jan. 2017.

DOBRZYCKA, B. et al. Tumor necrosis factor-alpha and its receptors in epithelial ovarian cancer. Folia Histochemica Et Cytobiologica, v. 47, n. 4, p. 609–613, 2009.

FAHMI, M. N. et al. Cytokines as Prognostic Biomarkers of Epithelial Ovarian Cancer (EOC): A Systematic Review and Meta-Analysis. Asian Pacific journal of cancer prevention: APJCP, v. 22, n. 2, p. 315–323, 1 fev. 2021.

FELDMAN, G. B., KNAPP, R. C. Lymphatic drainage of the peritoneal cavity and its significance in ovarian cancer. American Journal of Obstetrics and Gynecology, 1974. Volume 119, Issue 7, Pages 991-994. ISSN 0002-9378. https://doi.org/10.1016/0002-9378(74)90021-0.

FOGG, K. C. et al. Ovarian cancer cells direct monocyte differentiation through a non-canonical pathway. BMC Cancer. 2020 Oct 17;20(1):1008. doi: 10.1186/s12885-020-07513-w. PMID: 33069212; PMCID: PMC7568422.

GEORGIANNOS, S. N. et al. The immunophenotype and activation status of the lymphocytic infiltrate in human breast cancers, the role of the major histocompatibility complex in cell-mediated immune mechanisms, and their association with prognostic indicators. Surgery. Volume 134, Issue 5, Pages 827-834, 2003. ISSN 0039-6060, https://doi.org/10.1016/S0039-6060(03)00292-7.

GIESELER, F. et al. Heterogeneity of microvesicles from cancer cell lines under inflammatory stimulation with TNF-α. Cell Biology International, v. 42, n. 11, p. 1533–1544, nov. 2018.

GILKS CB, Ionescu D, Kalloger SE, et al. Tumor cell type can reproducibly diagnosed and is of independent prognostic significance in patients with maximally debulked ovarian carcinoma. Hum Pathol. 2008;39:1239–51.

GLOBAL CANCER OBSERVATORY. Disponível em < https://gco.iarc.fr/ >. Acesso em: 18 de agosto de 2022.

GOVINDARAJ, C. et al. Impaired Th1 immunity in ovarian cancer patients is mediated by TNFR2+ Tregs within the tumor microenvironment. Clinical Immunology (Orlando, Fla.), v. 149, n. 1, p. 97–110, out. 2013.

GUO, Y. et al. Potentially functional genetic variants in the TNF/TNFR signaling pathway genes predict survival of patients with non-small cell lung cancer in the PLCO cancer screening trial. Molecular Carcinogenesis, v. 58, n. 7, p. 1094–1104, jul. 2019.

HANAHAN D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.

HASSAN, M. I. et al. Ovarian cancer-induced immunosuppression: relationship to tumor necrosis factor-alpha (TNF-alpha) release from ovarian tissue. Anticancer Research, v. 19, n. 6C, p. 5657–5662, dez. 1999.

HOLDBROOKS, A. T.; BRITAIN, C. M.; BELLIS, S. L. ST6Gal-I sialyltransferase promotes tumor necrosis factor (TNF)-mediated cancer cell survival via sialylation of

the TNF receptor 1 (TNFR1) death receptor. The Journal of Biological Chemistry, v. 293, n. 5, p. 1610–1622, 2 fev. 2018. Immunohistochemical visualization of pro-inflammatory cytokines and enzymes in ovarian tumors - PubMed. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/25007180/>. Acesso em: 17 ago. 2022.

INSTITUTO NACIONAL DO CANCER JOSÉ ALENCAR GOMES DA SILVA (INCA), 2022. Disponível em https://www.gov.br/inca/pt-br . Acesso em 27 de Julho de 2022.

JAMMAL, M. P. et al. Cytokines and Prognostic Factors in Epithelial Ovarian Cancer. Clinical Medicine Insights. Oncology, v. 10, p. 71–76, 2016.

JAMMAL, M. P. et al. Immunohistochemical staining of tumor necrosis factor-α and interleukin-10 in benign and malignant ovarian neoplasms. Oncology Letters, v. 9, n. 2, p. 979–983, fev. 2015.

JIANG, C. et al. Association between the HMGB1/TLR4 signaling pathway and the clinicopathological features of ovarian cancer. Molecular Medicine Reports, v. 18, n. 3, p. 3093–3098, set. 2018.

JO, E. et al. Cordyceps militaris induces apoptosis in ovarian cancer cells through TNF-α/TNFR1-mediated inhibition of NF-κB phosphorylation. BMC complementary medicine and therapies, v. 20, n. 1, p. 1, 13 jan. 2020.

KAAKS, R. et al. Nutrition, hormones, and breast cancer: Is insulin the missing link?. Cancer Causes Control 7, 605–625 (1996). https://doi.org/10.1007/BF00051703

KAMPAN, N. C. et al. Interleukin 6 Present in Inflammatory Ascites from Advanced Epithelial Ovarian Cancer Patients Promotes Tumor Necrosis Factor Receptor 2-Expressing Regulatory T Cells. Frontiers in Immunology, v. 8, p. 1482, 2017.

KARIN, M., LIN, A. NF-κB at the crossroads of life and death. Nat Immunol 3, 221–227 (2002). https://doi.org/10.1038/ni0302-221

KROCKENBERGER M, Dombrowski Y, Weidler C, et al. Macrophage Migration Inhibitory Factor (MIF) contributes to the immune escape of ovarian cancer by downregulating NKG2D1. J Immunol. 2008;180:7338–48

KURMAN, R.J., SHIH, IeM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory.Am J SurgPathol. 2010 Mar;34(3):433-43.

KURMAN. RJ, SHIH, I e M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. Hum Pathol. 2011 Jul;42(7):918-31.

KUROKI, L.; GUNTUPALLI, S. R. Treatment of epithelial ovarian cancer. BMJ (Clinical research ed.), v. 371, p. m3773, 9 nov. 2020.

LI, H. et al. NF-κB/Twist axis is involved in chysin inhibition of ovarian cancer stem cell features induced by co-treatment of TNF-α and TGF-β. International Journal of Clinical and Experimental Pathology, v. 12, n. 1, p. 101–112, 2019.

MARTINS FILHO, A. et al. Correlation of cytokines and inducible nitric oxide synthase expression with prognostic factors in ovarian cancer. Immunology Letters, v. 158, n. 1–2, p. 195–199, abr. 2014.

MEIRA, K. C. et al. Efeitos da idade-período e coorte na mortalidade por câncer do ovário no Brasil e suas grandes regiões. Cad. Saúde Pública 2019; 35(3):e00087018

MOCELLIN S, Rossi CR, Pilati P and Nitti D: Tumor necrosis factor, cancer and anticancer therapy. Cytokine Growth Factor Rev 16: 35-53, 2005.

MOH et al. Cytokines as Prognostic Biomarkers of Epithelial Ovarian Cancer (EOC): A Systematic Review and Meta-Analysis. Asian Pacific Journal of Cancer Prevention, Vol 22; 315-323, 2021

MURTA EFC, NOMELINI RS. Early diagnosis and predictors of malignancy in the evaluation of adnexal mass. CurrOpinObstetGynecol 2006; 18:14-19.

MURTA EFC, SILVA CS, GOMES RAS, TAVARES-MURTA BM, MELO AL. Ultrasonographic criteria and tumor marker assay are good procedures for the diagnosis of ovarian neoplasia in preselected outpatients. Eur J GynaecolOncol 2004; 25:707-712.

NEGUS, R. P. et al. Hypoxia down-regulates MCP-1 expression: implications for macrophage distribution in tumors. Journal of Leukocyte Biology, v. 63, n. 6, p. 758–765, jun. 1998.

NOMELINI, R. S. et al. TNFR2 in tumor microenvironment as prognostic factor in epithelial ovarian cancer. Clinical and Experimental Medicine, v. 18, n. 4, p. 547–554, nov. 2018.

OPALA, T. et al. Evaluation of soluble tumour necrosis factor alpha receptors p55 and p75 in ovarian cancer patients. European Journal of Gynaecological Oncology, v. 26, n. 1, p. 43–46, 2005.

PAWLIK, W. et al. The Clinical Importance of IL-6, IL-8, and TNF-α in Patients with Ovarian Carcinoma and Benign Cystic Lesions. Diagnostics (Basel, Switzerland), v. 11, n. 9, p. 1625, 6 set. 2021.

PENG, C. et al. TNFR1 Regulates Ovarian Cancer Cell Tumorigenicity Through PIK3CB-p110Beta. Current Molecular Medicine, v. 15, n. 5, p. 487–496, 2015.

PIGUET, P. F. et al. Activation of platelet caspases by TNF and its consequences for kinetics. Cytokine, 2002 May 21;18(4):222-30. doi: 10.1006/cyto.2002.0889. PMID: 12126645.

PIURA, B. et al. Distinct expression and localization of TNF system in ovarian carcinoma tissues: possible involvement of TNF-α in morphological changes of ovarian cancerous cells. Anticancer Research, v. 34, n. 2, p. 745–752, fev. 2014.

RABINOVICH A, Medina L, Piura B and Huleihel M: Expression of IL-10 in human normal and cancerous ovarian tissues and cells. Eur Cytokine Netw 21: 122-128, 2010.

RABINOVICH A, Medina L, Piura B, Segal S and Huleihel M: Regulation of ovarian carcinoma SKOV-3 cell proliferation and secretion of MMPs by autocrine IL-6. Anticancer Res 27: 267272, 2007.

SILVA RAJU, J. et al. Prognostic Value of TNFR2 and STAT3 among High-Grade Serous Ovarian Cancer Survivors According to Platinum Sensitivity. Diagnostics (Basel, Switzerland), v. 11, n. 3, p. 526, 16 mar. 2021.

SPROWL, J. A. et al. Alterations in tumor necrosis factor signaling pathways are associated with cytotoxicity and resistance to taxanes: a study in isogenic resistant tumor cells. Breast cancer research: BCR, v. 14, n. 1, p. R2, 6 jan. 2012.

SWANN JB, Vesely MD, Silva A, et al. Demonstration of inflammation- induced câncer and câncer immunoediting during primary tumorigenesis. Proc Natl Acad Sci USA. 2008;105:652-656.

SZLOSAREK H, Chakravarty P, Leinster DA, Charles KA, Kwong J, Thompson RG, Coward JI, Schioppa T, Robinson SC, Gallagher WM, Galletta L; Australian Ovarian Cancer Study Group, Salako MA, Smyth JF, Hagemann T, Brennan DJ, Bowtell DD and Balkwill FR: A dynamic inflammatory cytokine network in the human ovarian cancer microenvironment. Cancer Res 72: 66-75, 2012.

SZLOSAREK PW and Balkwill FR: Tumour necrosis factor alpha: A potential target for the therapy of solid tumours. Lancet Oncol 4: 565-573, 2003.

TACCHINI-COTTIER et al. Role of TNFR1 and TNFR2 in TNF-induced platelet consumption in mice. J Immunol. 1998 Jun 15;160(12):6182-6. PMID: 9637537.

TORREY, H. et al. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated Tregs. Science Signaling, v. 10, n. 462, p. eaaf8608, 17 jan. 2017.

US TASK FORCE. Recommendation Statement of ovary Cancer in Adults: Screening. US: TaskForce Preventive Services, 2018. Disponível em: https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/ovarian-cancer-screening Acesso em: 09 agosto de 2021.

WANG, T. et al. Elevated Th22 cells and related cytokines in patients with epithelial ovarian cancer. Medicine, v. 96, n. 43, p. e8359, out. 2017.

WIESER, V. et al. Tumor necrosis factor receptor modulator spermatogenesis-associated protein 2 is a novel predictor of outcome in ovarian cancer. Cancer Science, v. 110, n. 3, p. 1117–1126, mar. 2019.

YANG, D. et al. Progranulin promotes colorectal cancer proliferation and angiogenesis through TNFR2/Akt and ERK signaling pathways. American Journal of Cancer Research, v. 5, n. 10, p. 3085–3097, 2015.

ZEPPERNICK F, MEINHOLD-HEERLEIN, I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal câncer. Arch Gynecol Obstet 2014; 290: 839–842.

ZHANG, H.; ZHANG, Y. Olaparib and paclitaxel in combination with carboplatin in treatment of ovarian cancer: influence on disease control. American Journal of Translational Research, v. 14, n. 1, p. 468–475, 2022.

ANEXOS

ANEXO A - TERMO DE CONSENTIMENTO LIVRE E

ESCLARECIDO

TERMO DE CONSENTIMENTO LIVRE, APÓS ESCLARECIMENTO.

TÍTULO DO PROJETO: *"Avaliação de parâmetros da resposta inflamatória em neoplasias ovarianas"*

Eu,

....

........, Registro Hospitalar nº ...., li e/ou ouvi o esclarecimento acima e compreendi para que serve o estudo e qual procedimento a que serei submetido. A explicação que recebi esclarece os riscos e benefícios do estudo. Eu entendi que sou livre para interromper minha participação a qualquer momento, sem justificar minha decisão e que isso não afetará meu tratamento. Sei que meu nome não será divulgado, que não terei despesas e não receberei dinheiro por participar do estudo. Eu concordo em participar do estudo e autorizo a publicação em forma de artigo científico sobre minha doença.

Uberaba, .../ ..../...............

_____   _____

Assinatura do voluntário ou seu responsável legal   Documento de identidade

_____   _____

Assinatura do pesquisador responsável   Assinatura do pesquisador orientador

Telefone de contato da paciente: _____

Telefone de contato dos pesquisadores: 34-3318-5326

ANEXO B – APROVAÇÃO PELO CEP

(PRÓXIMA PÁGINA)

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PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do estroma em neoplasia epitelial de ovário e sua relação com fatores prognósticos

Pesquisador: Rosekeila Simões Nomelini

Área Temática:

Versão: 2

CAAE: 34770014.4.0000.5154

Instituição Proponente: Universidade Federal do Triangulo Mineiro

Patrocinador Principal: Financiamento Próprio
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE MINAS GERAIS

DADOS DO PARECER

Número do Parecer: 877.759

Data da Relatoria: 30/10/2014

Apresentação do Projeto:

O câncer de ovário

O câncer de ovário é uma causa comum de morte entre as neoplasias malignas ginecológicas. Cerca de três quartos dos tumores malignos de ovário apresenta-se em estadiamentos avançados no momento do diagnóstico inicial. É o câncer ginecológico de maior letalidade [1,2].

A maioria das pacientes se encontra em estadiamentos III e IV (FIGO) no momento do diagnóstico [3]. Nesses casos, a principal estratégia terapêutica é a cirurgia de citorredução, seguida de quimioterapia [4]. Apesar dos esquemas quimioterápicos derivados do platinum e, mais recentemente, dos taxanos, os resultados do tratamento não têm obtido melhora importante nas últimas décadas [5]. nos estadiamentos I e II, a sobrevida em 5 anos varia de 80 a 95%, enquanto que nos estadiamentos III e IV, essa percentagem é de apenas 5 a 15% [5-7].

Os marcadores tumorais são substâncias relacionadas à presença ou à progressão de um tumor. O CA – 125 (cancer antigen 125) é um biomarcador não específico para o câncer de ovário, podendo estar elevado no primeiro trimestre da gestação, endometriose, infecções pélvicas e outros tipos de câncer [8]. Mas se associado a outros como o CA - 15.3, CA - 72.4 e CA - 19.9, pode ter aplicação no manejo de massas anexiais [9]. O CA - 19.9 pode estar elevado no subtipo mucinoso, o beta-hCG pode estar aumentado nos tumores de origem germinativa e também no

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coriorcarcinoma [10]. Murta et al. (2004) demonstraram a validade da associação de ultrassonografia e marcadores tumorais na identificação de neoplasias ovarianas, melhorando a sensibilidade e a especificidade como fator preditor de malignidade e conduzindo o cirurgião ao melhor tratamento [11]. Assim, a utilização de marcadores tumorais tem validade na diferenciação de massas ovarianas benignas e malignas, além de sugerir o subtipo histológico [12].

Rossi et al. (2004) estudaram o CA 125 como fator prognóstico (estudo retrospectivo de 82 pacientes), e demonstraram que esse marcador correlacionou com o estadiamento da FIGO, mas não com a idade, o grau, a doença residual após cirurgia, e intervalo livre de doença [13]. Santillan et al. (2005) avaliaram o risco de recidiva de câncer epitelial de ovário em pacientes com níveis séricos de CA125 menor que 35m U/ml, e demonstraram que um aumento progressivo de seus valores, mesmo ainda em níveis normais, poderia ser indicativo de recorrência da doença [14]. Paramasivam et al. (2005) mostraram que o estadiamento cirúrgico completo, o grau histopatológico e os níveis pré-operatórios de CA125 são fatores prognósticos independentes e poderiam ser incluídos como fatores de decisão da realização de quimioterapia [15]. Muramatsu et al. (2005) demonstraram que na avaliação de CA 125 e CA 19.9 séricos e do diâmetro tumoral em pacientes com estádio IA e IC, houve diferenças significativas entre os estádios [16].

Uma outra alternativa em estudo é a avaliação do microambiente tumoral intracístico. CA - 15.3, CA - 125, CA - 19.9 e CEA apresentam alta positividade tanto no soro quanto no fluido intracístico de pacientes com tumores epiteliais malignos de ovário [17,18].

Estudos mostram que outros marcadores podem ser usados nos diagnósticos das neoplasias ovarianas: HE4, GDF-15, Ca 72.4, Octamer-4, Nectin-4, progranulina. O GDF-15 atua como biomarcador de prognóstico potencialmente útil no carcinoma do ovário. O GDF-15 é induzido por citocinas inflamatórias, tais como interleucina-1 e fator de necrose tumoral. Seu aumento está associado a situações patológicas relacionadas à inflamação, lesão tecidual aguda, e malignidade [19]. O GDF-15 é membro da família TGF-beta, também chamada de citocina-1 inibidora de macrófagos (MIC-1), sendo originalmente identificado em linhagens celulares ativados por macrófagos. O GDF-15 regula uma grande variedade de procesos fisiológicos, como indução de apoptose e invasividade tumoral [20]. Concentrações elevadas também têm sido associadas a com um aumento do risco de eventos cardiovasculares em mulheres de idade avançada [21].

A progranulina é encontrada no cromossomo 17q, que pode promover a angiogênese e invasão tumoral. Pode existir uma relação entre os níveis séricos de progranulina e a sobrevida global e livre de doença em câncer epitelial de ovário [22]. Progranulina é um fator de crescimento que pode mediar a progressão do ciclo celular e a motilidade celular. Ela regula a inflamação; pode ter

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ação anti-inflamatória, inibindo algumas ações do fator de necrose tumoral [23].

Citocinas

As citocinas são proteínas expressas pelo sistema imunológico determinantes na regulação da função, crescimento e diferenciação deste, apresentando funções-chave na defesa do hospedeiro. Interleucinas compõem um grande grupo das citocinas produzidas principalmente por células T, embora algumas sejam sintetizadas também por macrófagos e células teciduais. Possuem grande variedade de funções, mas a maioria delas está envolvida na indução da divisão de outras células. As interleucinas são citocinas importantes no estudo da interação tumor-hospedeiro, possuindo propriedades pró ou antitumorais [24]. A inflamação crônica é associada a várias etapas da tumorigênese, incluindo a transformação, proliferação e invasão celular, angiogênese e metástase [25]. As citocinas podem estimular o crescimento celular e contribuir para a metástase. Se permanentemente sintetizadas, estas substâncias podem ser utilizadas como marcadores de ativação do sistema imune. A participação das citocinas na oncogênese revela suas atuações (isolada ou em conjunto com outras citocinas) em atividades imunomoduladoras da resposta imunológica contra neoplasias e, consequentemente, na sinalização entre células inflamatórias e o tecido neoplásico. Essa sinalização poderia inferir ou não em vantagens seletivas ao crescimento das células malignas [26,27].

Além da atividade tradicional da IL-2 na promoção do crescimento dos linfócitos T, ela também participa da ativação, crescimento e estímulo da função tumoricida das células NK [28]. Altos níveis de IL-2 significam aumento dos componentes do sistema imune no ataque contra as células cancerosas. Assim, a IL-2 é utilizada no tratamento de alguns cânceres [29,30]. A IL-6 influencia a função ovariana e no processo de ovulação, fertilização e implantação [31]. Pode estar associada à progressão tumoral através da apoptose de células malignas [32]. É relatada como fator prognóstico do câncer ovariano [33]. Produzidas por macrófagos, monócitos e linfócitos, as interleucinas 8 (IL-8) e 10 (IL-10) podem exercer vários efeitos sobre o sistema imune e estão relacionadas a angiogênese, crescimento e proliferação das células cancerosas [34].

A IL-8 é uma citocina pró-inflamatória, originalmente identificada como quimioatrativa de neutrófilos, esta citocina é produzida por células epiteliais, fibroblastos e tumorais, as quais também possuem receptores para esta interleucina [35]. Sua expressão em células do melanoma humano e do câncer ovariano está correlacionada ao potencial metastático do tumor [24,37,38].

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A IL-10 é uma citocina multifuncional, produzidas pelos linfócitos Th2, pode inibir a resposta imune do tipo celular e as funções das células Th1 (CD4+) imunocompetentes, pelo bloqueio da função de apresentação de antígenos por estas células [39], as quais são capazes de produzir IL-8, acarretando a progressão da malignidade [34,40]. Estudo realizado por Llanes-Fernandez et al. (2009) evidenciou associação inversa entre a IL-10 e a p53, o que reflete o efeito supressor da IL-10 no microambiente do tumor, e sua associação inversa com um marcador de apoptose foi sugerido como indicativo do aumento da agressividade do tumor. Uma possível explicação para o resultado encontrado é o bloqueio da expressão de algumas citocinas pela p53, todavia a regulação negativa destas moléculas pode ser perdida quando há uma deficiência da p53 ocasionada, por exemplo, por mutações [38].

Enquanto algumas citocinas estimulam a proliferação e a invasão do câncer, outras, como os interferons, inibem este processo. O interferon gama (IFN-) é produzido principalmente pelas células Th1 CD4+, CD8+ e NK. Seu efeito antiproliferativo provavelmente é devido ao aumento da morte celular por estimular a atividade de algumas enzimas caspases e exercer atividade antiangiogênica [40] e antitumoral [33]. O TNF- além de ser um dos principais mediadores da inflamação, também é produzido por tumores. Seu papel na tumorigênese inclui transformação e proliferação celular na invasão, angiogênese e metástase [41,42].

Estroma em carcinoma epitelial de ovário

Um grande progresso tem ocorrido no entendimento do papel do sistema imune na progressão tumoral nos últimos anos. A presença de células mononucleares tumor-infiltrantes consiste em linfócitos T auxiliares e citotóxicas, células natural - killer, linfócitos B e macrófagos, demonstrando uma resposta imune ativa possivelmente direcionada contra os antígenos tumorais. A maioria das células T infiltrantes são linfócitos T CD8+ que poderiam mediar uma citotoxicicidade específica contra células tumorais. Por outro lado, os macrófagos (células CD68) são importantes no recrutamento e ativação de linfócitos na presença desses antígenos. Porém, há uma heterogeneidade de macrófagos, podendo resultar em funções antagonistas, podendo inibir ou estimular a proliferação de células tumorais [43,44]. A presença de linfócitos-T infiltrantes (TIL) pode se correlacionar com melhor prognóstico em vários tumores, mas há resultados conflitantes da significância prognóstica de TIL em neoplasia maligna epitelial de ovário [45,46].

Na literatura, tem sido demonstrado que grande número de células T CD3+ são indicativas de uma

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Continuação do Parecer: 877.759

melhor sobrevida em neoplasia maligna de ovário [46,47], e que os linfócitos T CD-8+ são responsáveis por esse efeito [45,48,49]. Por outro lado, a presença de linfócitos T CD4+ regulatórios parecem reduzir a imunidade específica contra tumores, resultando em pobre sobrevida [50]. A localização de TILs nos tumores tem-se mostrado por ser importante fator prognóstico em câncer de ovário [45,51].

A infiltração de células imunes peri e intratumorais podem ser fatores preditores de resposta à quimioterapia [47,49]. Bösmüller et al. (2011) mostraram que a densidade de células T CD3+ e CD8+ no estroma tumoral pode ser um fator preditor da resposta à terapia baseada em platinum [52].

Linfócitos T CD4+ regulatórios (Treg) podem induzir a uma tolerância e suprimir a resposta imune, o que é feito através da secreção de TGF- e interleucina 10 (IL-10), ou por contato direto célula-célula [50]. Os estudos demonstram uma propensão a localização dessas células na região peritumoral [53,54].

É bem estudado que o microambiente tumoral desempenha um papel importante no comportamento do tumor. O estroma pode controlar o crescimento de tumores e invasão. Este compartimento tem uma grande influência relacionada com a resposta imune. A infiltração de células imunes em tumores pode até mesmo determinar a evolução e o prognóstico da doença, e a interação entre as células neoplásicas e o estroma é um fator crítico para o crescimento do tumor [55]. Por isso, o compartimento que será avaliado nesse estudo na imuno-histoquímica para TILs será o estromal.

Recentemente, estudos demonstraram que fibroblastos associados a carcinoma (CAFs) podem promover diretamente tumorigênese através de múltiplos mecanismos, incluindo a angiogênese, proliferação, invasão e supressão imune [56, 57]. Esses efeitos são mediados através da expressão e secreção de vários fatores de crescimento, como o TGF-, VEGF e interleucina-8 (IL8) [58]. Esses efeitos são também estabelecidos pela modulação e status de difeenciação de células inflamatórias mo microambiente tumoral [59]. Além disso, CAFs poderiam afetar a sensibilidade das células do tumor à quimioterapia e à radioterapia [60]. FAP e SMA são considerados marcadores para CAFs em vários tipos de tumores. A eliminação de CAFs in vivo através de uma vacina de DNA cujo alvo é a proteína alfa de ativação dos fibroblastos (FAP) resultou em uma mudança do microambiente imune do padrão Th2 para Th1, melhorando os efeitos antimetastáticos da quimioterapia com doxorrubicina em um modelo murino de câncer de mama [61]. FAP exercem um papel importante na predição da agressividade tumoral em pacientes com carcinoma epitelial de ovário após terapia neoadjuvante. CAFs podem exercer um importante papel

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também na progressão do câncer e metástases, podendo ser alvo para novas estratégias terapêuticas [62, 63].

Dessa forma, parece existir um estreita relação entre CAFs e resposta imune tumoral, o que justifica o estudo dessa relação em neoplasias malignas epiteliais de ovário.

Objetivo da Pesquisa:

1.Investigar a expressão imunohistoquímica de dois marcadores de fibroblastos associados a carcinoma (CAFs), a alfa actina de músculo liso (SMA) e a proteína alfa de ativação dos fibroblastos (FAP), no compartimento estromal de neoplasias benignas e malignas epiteliais de ovário.

2.Investigar a expressão imunohistoquímica citocinas (IL-2, IL-6, IL-10 e TNF-alfa) e de linfócitos tumor-infiltrantes - TILs (CD3, CD4 e CD8) no estroma de neoplasias benignas e malignas epiteliais de ovário.

3.Relacionar a expressão de SMA e FAP com a expressão de citocinas e TILs no estroma de neoplasias malignas de ovário e com a dosagem dessas mesmas citocinas no soro e líquido intracístico.

4.Relacionar a expressão de SMA e FAP, e a expressão de citocinas e TILs no compartimento estromal com fatores prognósticos em carcinoma epitelial de ovário.

Avaliação dos Riscos e Benefícios:

Não há riscos inerentes à pesquisa, já que os protocolos clínicos e cirúrgicos serão mantidos, o lavado peritoneal já faz parte da rotina do procedimento cirúrgico, e a punção do cisto de ovário será realizada após a exérese do mesmo, não prolongando em nada o tempo cirúrgico e nem interferindo no procedimento. O único desconforto devido à pesquisa poderá ser causado pela coleta de sangue, o que será minimizado por ser realizado por profissional habilitado e com as explicações pertinentes oferecidas às pacientes. O risco da perda de confidencialidade será prevenido pela utilização de números e/ou letras para identificação dos casos.

Os benefícios são consequência da melhor compreensão da fisiopatologia da doença e identificação de fatores relacionados ao prognóstico.Considerando que a avaliação clínica e os elementos utilizados para o estudo são parte do diagnóstico e condutas terapêuticas de rotina,

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não há riscos adicionais às pacientes referentes à pesquisa. Os benefícios são consequência da melhor compreensão da fisiopatologia da doença e identificação de fatores relacionados ao prognóstico.

Comentários e Considerações sobre a Pesquisa:
pendências atendidas

Considerações sobre os Termos de apresentação obrigatória:
pendências atendidas

Recomendações:
pendências atendidas

Conclusões ou Pendências e Lista de Inadequações:
pendências atendidas

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Considerações Finais a critério do CEP:

UBERABA, 19 de Novembro de 2014

_____

Assinado por:
ANA PALMIRA SOARES DOS SANTOS
(Coordenador)

UNIVERSIDADE FEDERAL DO
TRIÂNGULO MINEIRO - MG

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do estroma em neoplasia epitelial de ovário e sua relação com fatores prognósticos

Pesquisador: Rosekeila Simões Nomelini

Área Temática:

Versão: 4

CAAE: 34770014.4.0000.5154

Instituição Proponente: Universidade Federal do Triangulo Mineiro

Patrocinador Principal: Financiamento Próprio
FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE MINAS GERAIS

DADOS DO PARECER

Número do Parecer: 1.256.065

Apresentação do Projeto:

pesquisador solicita emenda ao projeto comas seguintes justificativas:

1. No projeto inicial, a proposta era avaliar as citocinas no soro e no líquido intracístico de pacientes com tumores de ovário. Estamos propondo estudar também as citocinas no lavado peritoneal dessas pacientes, ampliando, dessa forma, a avaliação da produção local de citocinas em neoplasias de ovário. Reforçamos que, no termo de

consentimento, já era informado o ato da coleta do líquido peritoneal, então este não

precisará ser modificado.

2. No projeto inicial, a proposta era avaliar as seguintes citocinas no tecido, no soro e no líquido intracístico: IL-2, IL-6, IL-10 e TNF-alfa. Estamos propondo ampliar as citocinas avaliadas nesses locais e também no líquido peritoneal, acrescentando IL-5, IL-8, IL-12, IL-17 e receptores I e II de TNF. Isso se justifica pela importância das mesmas na resposta imune das neoplasias de ovário, e avaliando-se um painel maior de citocinas, pode-se traçar um melhor perfil de fatores associados ao prognóstico em câncer de ovário.

3. Reforço, ainda, que os procedimentos para a coleta do material não serão

significativamente alterados, e que no termo de consentimento inicial já estava previsto a coleta do lavado peritoneal, que é um procedimento já padronizado para qualquer cirurgia de tumor de

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UF: MG   **Município:** UBERABA
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ovário, não aumentando nem o risco e nem o tempo cirúrgico para a paciente.

Objetivo da Pesquisa:

acrescentado:

avaliação das citocinas no lavado peritoneal e avaliação da enzima óxido nítrico sintase reduzida.

Avaliação dos Riscos e Benefícios:

os mesmos apresentados no projeto original

Comentários e Considerações sobre a Pesquisa:

os mesmos apresentados no projeto original

Considerações sobre os Termos de apresentação obrigatória:

apresentados

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

De acordo com a Resolução CNS nº 466 de 2012 e a Norma Operacional nº 001 de 2013 do CNS, o colegiado do CEP em reunião de 02/10/2015 aprova a emenda do projeto.

Conforme orientações da CONEP, após a aprovação do projeto pelo CEP, o pesquisador deve notificar na plataforma Brasil o inicio da pesquisa, bem como apresentar relatórios parciais (semestrais) e final

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Outros	Justificativa.pdf	30/09/2015 09:17:08	Maria José Ferreira de Sousa Covre	Aceito
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_600237_E2.pdf	29/09/2015 14:51:58		Aceito
Outros	Emenda.pdf	03/07/2015 12:24:20		Aceito
Outros	GEP autorizaçao_proj_de_pesquisa.pdf	25/10/2014 17:19:23		Aceito
Projeto Detalhado / Brochura Investigador	Projeto CEP.doc	25/10/2014 17:18:42		Aceito

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Continuação do Parecer: 1.256.065

Outros	patologia especial linha ovário.pdf	25/10/2014 17:12:02		Aceito
Outros	dra+Rosekeial+protocolo+Pj+Pesq+assin+15out.pdf	25/10/2014 17:09:56		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO DE CONSENTIMENTO.doc	25/10/2014 17:07:24		Aceito
Folha de Rosto	folha de rosto escaneada.pdf	13/08/2014 09:36:25		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

UBERABA, 02 de Outubro de 2015

_____

Assinado por:
Marly Aparecida Spadotto Balarin
(Coordenador)

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ANEXO C

DESCRIÇÃO DOS ANTICORPOS UTILIZADOS NA

IMUNOHISTOQUÍMICA DO ESTUDO

Anticorpo	Especificação	Diluição	Tampão/Ph	Controle positivo
TNF	Santa Cruz Biotechnology, Inc. TNFα (52B83): sc-52746 Antibody Lote B0509 Rabbit policlonal IgG 100µg/ml	1:400	Tris-EDTA/ pH 9.0	Tecido de câncer humano
TNFR1	Santa Cruz Biotechnology, Inc. p-TNFR1 (ser 274) Antibody Lote B0509 sc-130220 Rabbit policlonal IgG 100µg/ml	1:100	Tris-EDTA/ pH 9.0	Tecido de câncer humano

TNFR2	Santa Cruz Biotechnology, Inc. p-TNFR2 (D-2) Antibody sc-8041 Rabbit policlonal IgG 100µg/ml	1:50	Tris-EDTA/ pH 9.0	Tecido de câncer humano

Fonte: O Autor.

ANEXO D

ARTIGOS REFERENTES À TESE

Taylor & Francis
Taylor & Francis Group

Dear Rosekeila Nomelini,

Thank you for your submission.

Submission ID	**225418825**
Manuscript Title	**Stromal immunostaining of tumor necrosis factor alpha and its receptors in ovarian neoplasms**
Journal	**Immunological Investigations**

You can check the progress of your submission, and make any requested revisions, on the Author Portal.

Thank you for submitting your work to our journal.
If you have any queries, please get in touch with IIMM-peerreview@journals.tandf.co.uk.

Kind Regards,
Immunological Investigations Editorial Office

Stromal immunostaining of tumor necrosis factor alpha and its receptors in ovarian neoplasms

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Eliângela de Castro Côbo[2]; Renata Margarida Etchebehere[2];

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Word count: 3,194 words.

Abstract

Background: The aim of the study was to evaluate stromal immunostaining of malignant ovarian neoplasms, comparing it with benign ovarian neoplasms and non-neoplastic ovarian lesions.

Methods: Patients treated at the Pelvic Mass Outpatient Clinic were surgically treated according to pre-established criteria. Patients with benign (n=37) or malignant (n=43) ovarian epithelial neoplasia, and non-neoplastic ovarian lesion (n=15) were included in the study. Immunohistochemical study was performed to evaluate stromal TNF-alpha, TNFR1 and TNFR2. The comparison between the groups was performed by the Chi-Square test, with a significance level lower than 0.05.

Results: TNF-alpha stromal immunostaining was more intense (2/3) comparing benign (p<0.0001) and malignant (p<0.0001) ovarian neoplasms with non-neoplastic tumors. TNFR1 immunostaining was stronger (2/3) in the stroma of malignant neoplasms compared with benign neoplasms (p<0.0001), and stronger (2/3) when comparing benign neoplasms with non-neoplastic ovarian lesions (p=0.0002). For TNFR2, stromal immunostaining was stronger (2/3) in malignant neoplasms compared to benign neoplasms (p=0.0091), and stronger in malignant neoplasms compared to non-neoplastic lesions (p=0.0004).

Conclusion: A stronger immunostaining for TNF-alpha e its receptors was found in ovarian cancer, suggesting that they may be targets of further studies to verify their role in carcinogenesis and the progression of ovarian neoplasms.

Key words: TNF-alpha, TNFR1, TNFR2, ovarian tumors.

Main Text Introduction

Ovarian epithelial cancer has a high lethality among gynecological malignancies (Henley et al., 2020). Cytoreductive surgery followed by chemotherapy is still the main treatment (Randall & Rubin, 2021). The American Cancer Society estimates that about 19,880 women will receive a new diagnosis of ovarian cancer for ovarian cancer and 12,810 women will die from ovarian cancer in the United States for 2022 (American Cancer Society, 2022). In Brazil, there were 3,921 deaths from ovarian cancer in 2020 (INCA, 2022).

Tumor stroma plays an important role in ovarian cancer (Davidson et al., 2014; Silva et al., 2020). Ovarian cancer has, in the peritumoral stroma, multiple cell types besides cancer cells, which coordinate tumor survival, growth, invasion and progression (Silva et al., 2020). The tumor microenvironment has molecules that can be potential targets for new cancer therapies. In ovarian cancer, the stroma contains myofibroblasts, endothelial cells, and leukocytes, which may contribute to disease progression. A network consisting of angiogenic factors, proteases, growth factors, immune response-modulating proteins, anti-apoptotic proteins, and signaling molecules to promote tumor cell invasion and metastasis (Davidson et al., 2014).

The chronic production of TNF-alpha in the tumor microenvironment may increase myeloid cell recruitment in an IL-17-dependent manner. This can lead to the tumor-promoting action of this cytokine (Charles et al., 2009). Ovarian cancer has immune-suppression capabilities, and regulatory T cells (Tregs) may contribute to this immune-suppression. Patients with ovarian cancer may have high levels of TNF and Tregs expressing TNFR2, which is associated with suppressive capacity (Kampan et al., 2017). There is increasingly evidence that TNFR2 expression in cancer microenvironment has significant implications in cancer progression, metastasis and immune evasion (Al-Hatamleh et al., 2019). TNFR2+ Tregs were evaluated in patients with ovarian cancer, and TNFR2+ Tregs from tumor-associated ascites were the most potent suppressor T cell fraction. They were more suppressive than peripheral blood TNFR2+ Tregs (Govindaraj et al., 2013).

The aim of the study was to evaluate stromal immunostaining of malignant ovarian neoplasms, comparing it with benign ovarian neoplasms and non-neoplastic ovarian lesions.

Materials and Methods

Patients treated at the Pelvic Mass Outpatient Clinic of the Department of Gynecology and Obstetrics / Oncology Research Institute (IPON) of the Federal University of Triângulo Mineiro – UFTM who were surgically treated according to pre-established criteria (Murta et al., 2004; Murta & Nomelini, 2006). After confirmation of the histopathological diagnosis, patients with benign or malignant ovarian epithelial neoplasia, and non-neoplastic ovarian lesion were included in the study. Exclusion

criteria were torsion of the adnexal pedicle, secondary malignant ovarian neoplasm (metastasis), previous antineoplastic treatment; immunosuppressive diseases and relapse. Borderline ovarian tumors were included in the group of malignant neoplasms.

The following data from the medical records were recorded in a specific database for the study: age, parity, hormonal status, histological type, histological grade, staging (FIGO), immunohistochemistry results for TNF-alpha, TNFR1 and TNFR2.

Informed consent was obtained from all patients included in the study. The study was approved by the UFTM Research Ethics Committee.

Anatomopathological study

It was performed by the Surgical Pathology Service of the UFTM in paraffin sections, and the cases will be reviewed by an observer from the Surgical Pathology Service, to choose the best sections for the immunohistochemical study. The anatomopathological evaluation and staging of cases was performed according to the criteria of the International Federation of Gynecology and Obstetrics – FIGO (Zeppernick et al., 2014).

Immunohistochemistry Study

Specimens obtained by surgical resection were processed in paraffin and reviewed by an experienced pathologist. The selected cases were submitted to new cuts (4 µm) on silanized slides (ATPS - Silane, Sigma® A3648), using the streptoavidin-biotin-peroxidase technique, according to the manufacturer's recommendations. The

specific primary anti-TNF-alpha, anti-TNFR1, anti-TNFR2 antibodies were used in the study.

Positive and negative controls were used. Two observers evaluated the slides, and the interobserver agreement was calculated by kappa. The intensity of immunostaining in the peritumoral stroma was subjectively assessed using 0 to 3: 0 (no staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining). In the immunohistochemical study, the agreement between the two observers was performed using the kappa: κ <0.4: weak agreement; $0.4 \leq \kappa$ <0.8: moderate agreement; $0.8 \leq \kappa$ <1.0: strong agreement; κ =1.0: perfect agreement. All discordant cases were re-evaluated and the result was defined by consensus (figure 1).

Statistical analysis

Data were analyzed by GraphPad Prism software. The comparison between non-neoplastic tumors, benign and malignant neoplasms was performed by the Fisher exact test, with a significance level lower than 0.05.

Results

The study included 95 patients divided into 3 groups (37 benign neoplasms, 43 malignant neoplasms and 15 non-neoplastic lesions). In the malignant neoplasm group, the median age was 56 years (17-81 years), the median parity was 2 deliveries (0-12 deliveries), the median age at menarche was 13 years (10-16 years), the median age at menopause was 49 years (33-57 years).

In the benign neoplasm group, the median age was 48 years (18-69 years), the median parity was 2 deliveries (0-7 deliveries), the median age at menarche was 13 years (11-17 years), the median age at menopause was 49 years (29-55 years)

In the non-neoplastic lesion group, median age was 46 years (35-82 years), median parity was 2 deliveries (0-5 deliveries), median age at menarche was 13 years (11-15 years) , the median age at menopause was 47 years (38-50 years).

According to the International Federation of Gynecology and Obstetrics (FIGO), the stages of malignant neoplasms were: 20 (46.5%) patients IA, 1 (2.3%) patient IB, 3 (7.0%) patients IC2, 1 (2.3%) patient IC3, 1 (2.3%) patient IIB, 2 (4.7%) patients IIIA1(i), I1 (2.3%) patient IIA2, 3 (7.0%) patients IIIB, 9 (27.9%) IIIC patients and 2 (4.7%) IVB patients. Regarding the degree of histological differentiation of malignant tumors, 14 patients (32.6%) had grade 1, 14 patients (32.6%) had grade 2 and 15 (34.9%) patients had grade 3.

TNF-alpha stromal immunostaining was more intense (2/3) comparing benign (p=0.0016) and malignant (p<0.0001) ovarian neoplasms with non-neoplastic tumors. On the other hand, there was no significant difference comparing benign and malignant neoplasms (p=0.2969).

Regarding the TNFR1 immunostaining, it was stronger (2/3) in the stroma of malignant neoplasms compared with benign neoplasms (p<0.0001), and stronger (2/3) when comparing benign neoplasms with non-neoplastic ovarian lesions (p=0.0002). There was no difference comparing stromal TNFR1 between ovarian cancer and non-neoplastic lesions (p=0.231).

For TNFR2, stromal immunostaining was stronger (2/3) in malignant neoplasms compared to benign neoplasms (p=0.0091), and stronger in malignant neoplasms

compared to non-neoplastic lesions (p=0.0004). However, there was no difference comparing stromal immunostaining of benign neoplasms and non-neoplastic lesions (p=0.0933).

Table 1 shows the results of immunostaining between the groups (non-neoplastic lesions, benign neoplasms and malignant ovarian neoplasms).

Discussion

Studies suggest the role of TNF-α and its receptors (TNFR1 and TNFR2) in biology of ovarian cancer and in tumor pathogenesis (Hassan et al., 1999; Piura et al., 2014), and relationship with prognostic factors (Martins-Filho et al., 2014).

TNF-alpha levels were measured in serum and cytosolic fractions of ovarian cancer patients and control patients, demonstrating increased TNF-alpha levels in the cancer patient group. TNF-alpha immunostaining was positive in malignant lesions and negative for normal ovarian tissue (Hassan et al., 1999). One study evaluated by immunohistochemistry the expression of IL-1, IL-6, TGF-β, TNF-α, COX-2, iNOS, and NF-kB in serous and mucinous ovarian cancers, and demonstrated that the expression of IL-1 , TNF-α and COX-2 increased with the stage of the disease in serous and mucinous tumors (Plewka et al. 2014). Serum levels of IL-6, IL-8, and TNF-α were assessed by ELISA; serum IL-8 and TNF-α levels were higher in patients with ovarian cancer compared with benign ovarian cystic lesions. The cutoff level of IL-8 and TNF-α was 4.09 ng/mL and 2.63 ng/mL, respectively (sensitivity and specificity of 70% and 96% for IL-8 and 85.7% and 79.3% for TNF-α) (Pawlik et al. 2021). Another study evaluated

the involvement of T-helper cells and regulatory T cells in epithelial ovarian cancer, and examined the percentages of Th22, Th17, Th1, and regulatory T cells in the peripheral blood of epithelial ovarian cancer, benign ovarian epithelial neoplasm, and healthy control by flow cytometry. The plasma concentrations of IL-22 and TNF-α were significantly elevated in epithelial ovarian cancer patients compared with the other 2 groups. In addition, in patients with ovarian cancer, there was an increased trend of Th22, IL-22, and TNF-α in stage III-IV patients compared with stage I-II patients, and a positive correlation between Th22, Th17, and Th1 cells (Wang et al., 2017). In our study, TNF-alpha stromal immunostaining was more intense comparing benign and malignant ovarian neoplasms with non-neoplastic tumors. On the other hand, there was no significant difference comparing benign and malignant neoplasms.

Piura et al. (2014) extracted total RNA from normal and malignant ovarian tissues and mRNA was analyzed with semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Immunohistochemical study was performed for TNFR1 and TNFR2. TNF-α mRNA and TNFR2 mRNA levels were higher in carcinomas compared with normal ovarian tissues, and TNFR1 mRNA levels were similar. TNFR1 and TNFR2 were mainly localized in the epithelial neoplastic cells of the tumor (Piura et al., 2014). Another study showed that TNFR1 was over-expressed in ovarian cancer, playing an important role in ovarian cancer, with the potential to be a prognostic molecule in ovarian cancer (Peng et al., 2015). In the present study, TNFR1 immunostaining was stronger in the stroma of malignant neoplasms compared to benign neoplasms, and stronger when comparing benign neoplasms with non-neoplastic ovarian lesions. There was no difference comparing TNF R1 stromal between ovarian cancer and non-neoplastic lesions.

Serum TNFR2 levels may have an association with prognostic factors in ovarian cancer (Nomelini et al., 2018). In the evaluation of ascites in patients with ovarian cancer, high levels of immunosuppressive (sTNFR2, IL-10, and TGF-β) and pro-inflammatory cytokines (IL-6 and TNF) were found in this fluid. TNFR2 expression on all T cell subsets was higher on CD4+CD25hiFoxP3+ Tregs (Kampan et al., 2017). On the other hand, a meta-analysis was performed evaluating the associations between circulating levels of C-reactive protein, IL-6, TNF-alpha, and soluble TNFα receptor 2 (TNFR2), and the risk of ovarian cancer, and demonstrated that that elevated levels of C-reactive protein, but not circulating IL6, TNF-alpha, or soluble TNFR2, are associated with an increased risk of ovarian cancer (Zeng et al., 2016). Our data demonstrated that TNFR2 stromal immunostaining was stronger in malignant neoplasms compared to benign neoplasms, and stronger in malignant neoplasms compared to non-neoplastic lesions. But there was no difference comparing stromal immunostaining of benign neoplasms and non-neoplastic lesions.

The main limitation of the study is the heterogeneity of the histological types of ovarian lesions and neoplasms. But this work has many strengths. To our knowledge, it is the first study in the literature that evaluated stromal TNF-alpha and its receptors in 3 groups of ovarian tumors: non-neoplastic lesions, benign neoplasms and malignant neoplasms. And the results demonstrate a stronger immunostaining in ovarian cancer, suggesting that TNF-alpha and its receptors may be targets of further studies to verify their role in carcinogenesis and the progression of ovarian neoplasms.

In conclusion, TNF-alpha stromal immunostaining was more intense comparing benign and malignant ovarian neoplasms with non-neoplastic tumors. TNFR1

immunostaining was stronger in the stroma of malignant neoplasms compared to benign neoplasms, and stronger when comparing benign neoplasms with non-neoplastic ovarian lesions. TNFR2 stromal immunostaining was stronger in malignant neoplasms compared to benign neoplasms, and stronger in malignant neoplasms compared to non-neoplastic lesions.

Acknowledgements

Declaration of interest statement

The authors report no conflicts of interest.

References

Al-Hatamleh MAI, E A R ENS, Boer JC, Ferji K, Six JL, Chen X, Elkord E, Plebanski M, Mohamud R. Synergistic Effects of Nanomedicine Targeting TNFR2 and DNA Demethylation Inhibitor-An Opportunity for Cancer Treatment. Cells. 2019;9(1):33.

American Cancer Society. Cancer facts & figures 2022. https://www.cancer.org/. Accessed 24 July 2022.

Charles KA, Kulbe H, Soper R, Escorcio-Correia M, Lawrence T, Schultheis A, Chakravarty P, Thompson RG, Kollias G, Smyth JF, Balkwill FR, Hagemann T. The tumor-promoting actions of TNF-alpha involve TNFR1 and IL-17 in ovarian cancer in mice and humans. J Clin Invest. 2009;119(10):3011-23.

Davidson B, Trope CG, Reich R. The role of the tumor stroma in ovarian cancer. Front Oncol. 2014 13;4:104.

Govindaraj C, Scalzo-Inguanti K, Madondo M, Hallo J, Flanagan K, Quinn M, Plebanski M. Impaired Th1 immunity in ovarian cancer patients is mediated by TNFR2+ Tregs within the tumor microenvironment. Clin Immunol. 2013;149(1):97-110.

Hassan MI, Kassim SK, Saeda L, Laban M, Khalifa A. Ovarian cancer-induced immunosuppression: relationship to tumor necrosis factor-alpha (TNF-alpha) release from ovarian tissue. Anticancer Res. 1999;19(6C):5657-62.

Henley SJ, Thomas CC, Lewis DR, Ward EM, Islami F, Wu M, Weir HK, Scott S, Sherman RL, Ma J, Kohler BA, Cronin K, Jemal A, Benard VB, Richardson LC. Annual report to the nation on the status of cancer, part II: Progress toward Healthy People 2020 objectives for 4 common cancers. Cancer. 2020 ;126(10):2250-2266.

INCA, 2022. Câncer de ovário. https://www.inca.gov.br/tipos-de-cancer/cancer-de-ovario. Accessed 24 July 2022.

Kampan NC, Madondo MT, McNally OM, Stephens AN, Quinn MA, Plebanski M. Interleukin 6 Present in Inflammatory Ascites from Advanced Epithelial Ovarian Cancer Patients Promotes Tumor Necrosis Factor Receptor 2-Expressing Regulatory T Cells. Front Immunol. 2017 Nov 6;8:1482.

Martins Filho A, Jammal MP, Côbo Ede C, Silveira TP, Adad SJ, Murta EF, Nomelini RS. Correlation of cytokines and inducible nitric oxide synthase expression with prognostic factors in ovarian cancer. Immunol Lett. 2014;158(1-2):195-9.

Murta EFC, da Silva CS, Gomes RA, Tavares-Murta BM, Melo AL. Ultrasonographic criteria and tumor marker assay are good procedures for the diagnosis of ovarian neoplasia in preselected outpatients. Eur J Gynaecol Oncol 2004; 25:707-712.

Murta EFC, Nomelini RS. Early diagnosis and predictors of malignancy in the evaluation of adnexal mass. Curr Opin Obstet Gynecol 2006; 18(1):14-9.

Nomelini RS, Borges Júnior LE, de Lima CA, Chiovato AFC, Micheli DC, Tavares-Murta BM, Murta EFC. TNF-R2 in tumor microenvironment as prognostic factor in epithelial ovarian cancer. Clin Exp Med. 2018;18(4):547-554.

Pawlik W, Pawlik J, Kozłowski M, Łuczkowska K, Kwiatkowski S, Kwiatkowska E, Machaliński B, Cymbaluk-Płoska A. The Clinical Importance of IL-6, IL-8, and TNF-α in Patients with Ovarian Carcinoma and Benign Cystic Lesions. Diagnostics (Basel). 2021;11(9):1625.

Piura B, Medina L, Rabinovich A, Dyomin V, Levy RS, Huleihel M. Distinct expression and localization of TNF system in ovarian carcinoma tissues: possible involvement of TNF-α in morphological changes of ovarian cancerous cells. Anticancer Res. 2014;34(2):745-52.

Plewka D, Kowalczyk AE, Jakubiec-Bartnik B, Morek M, Bogunia E, Kmiec A, Wierzbicki PM, Plewka A. Immunohistochemical visualization of pro-inflammatory cytokines and enzymes in ovarian tumors. Folia Histochem Cytobiol. 2014;52(2):124-37.

Randall TC, Rubin SC. Cytoreductive surgery for ovarian cancer. Surg Clin North Am. 2001 Aug;81(4):871-83.

Silva AC, Jammal MP, Crispim PCA, Murta EFC, Nomelini RS. The Role of Stroma in Ovarian Cancer. Immunol Invest. 2020 May;49(4):406-424.

Wang T, Zhang Z, Xing H, Wang L, Zhang G, Yu N, Wang J, Guo W, Jiang J. Elevated Th22 cells and related cytokines in patients with epithelial ovarian cancer. Medicine (Baltimore). 2017; 96(43):e8359.

Zeng F, Wei H, Yeoh E, Zhang Z, Ren ZF, Colditz GA, Tworoger SS, Su X. Inflammatory Markers of CRP, IL6, TNFα, and Soluble TNFR2 and the Risk of Ovarian Cancer: A Meta-analysis of Prospective Studies. Cancer Epidemiol Biomarkers Prev. 2016 Aug;25(8):1231-9.

Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet 2014; 290(5):839-42.

Legend of figure

Figure 1 - Immunohistochemical staining. Histological sections of ovarian lesions.

A: Stromal immunostaining (2/3) of TNF-α in Mucinous Adenocarcinoma (100x).

B: Stromal immunostaining (0/1) of TNF-α in Non-neoplastic tumor (100x).

C: Stromal immunostaining (2/3) of TNFR1 in Adenocarcinoma (100x).

D: Stromal immunostaining (0/1) of TNFR1 in Serous Cystadenoma (100x).

E: Stromal immunostaining (2/3) of TNFR2 in Serous Adenocarcinoma (100x).

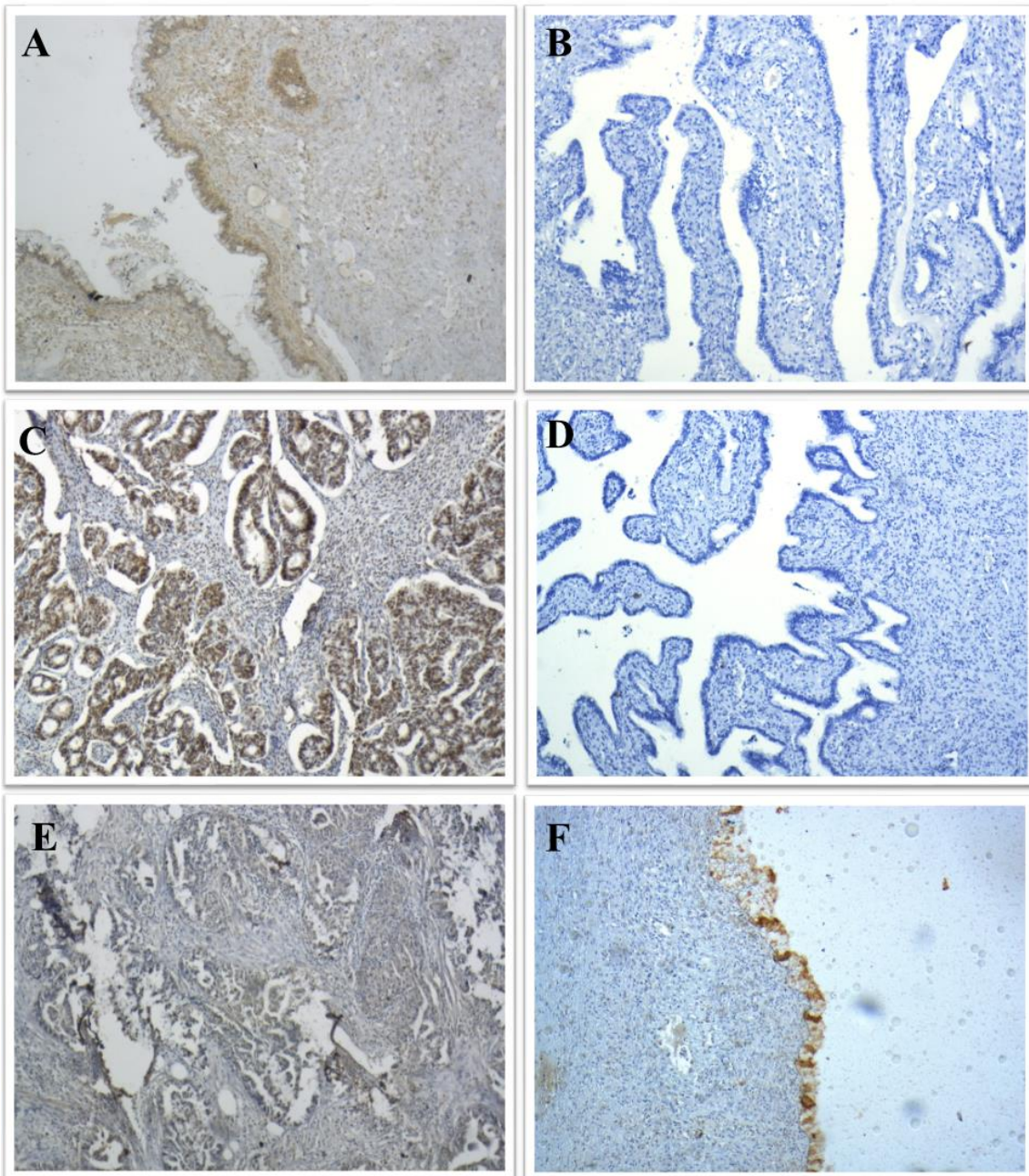F: Stromal immunostaining (0/1) of TNFR2 in Mucinous Cystadenoma (100x).

Table 1: Differences in stromal immunostaining of TNF-alpha, TNFR1 and TNFR2 between malignant, benign and non-neoplastic ovarian tumors.

		0/1	2/3	*p*
TNF (n=37/43)	Benign Neoplasms	11/37 (29.7%)	26/37 (70.3%)	0.2969
	Malignant Neoplasms	8/43 (18.6%)	35/43 (81.4%)	
TNFR1 (n=37/43)	Benign Neoplasms	37/37 (100.0%)	0/37 (0%)	< 0.0001
	Malignant Neoplasms	17/43 (39.5%)	26/43 (60.5%)	
TNFR2 (n=37/43)	Benign Neoplasms	30/37 (81.1%)	7/37 (18.9%)	0.0091
	Malignant Neoplasms	22/43 (51.2%)	21/43 (48.8%)	
TNF (n=37/15)	Benign Neoplasms	11/37 (29.7%)	26/37 (70.3%)	0.0016
	Non-Neoplastic Lesions	12/15 (80.0%)	3/15 (20.0%)	
TNFR1 (n=37/15)	Benign Neoplasms	37/37 (100.0%)	0/37 (0%)	0.0002
	Non-Neoplastic Lesions	9/15 (60.0%)	6/15 (40.0%)	
TNFR2 (n=37/15)	Benign Neoplasms	30/37 (81.1%)	7/37 (18.9%)	0.0933
	Non-Neoplastic Lesions	15/15 (100.0%)	0/15 (0%)	
TNF (n=43/15)	Malignant Neoplasms	8/43 (18.6%)	35/43 (81.4%)	< 0.0001
	Non-Neoplastic Lesions	12/15 (80.0%)	3/15 (97.3%)	
TNFR1 (n=43/15)	Malignant Neoplasms	17/43 (39.5%)	26/43 (60.5%)	0.231
	Non-Neoplastic Lesions	9/15 (60.0%)	6/15 (40.0%)	

TNFR2 (n=43/15)	Malignant Neoplasms Non-Neoplastic Lesions	22/43 (51.2%) 15/15 (100.0%)	21/43 (48.8%) 0/15 (0%)	0.0004

* Fisher's exact test. with a significance level of p<0.05

05-Sep-2022

Dear Dr. Nomelini:

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Stromal expression of TNF and its receptors TNFR1 and TNFR2:

association with blood count parameters

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Abstract

Objective: The objective of this study was to evaluate the relationship of immunostaining of TNF and its receptors (TNFR1 and TNFR2) in the stroma of malignant ovarian neoplasm with hemogram parameters and tumor markers.

Methods: Women with a confirmed diagnosis of malignant or borderline ovarian neoplasm were included in the study. The following data from the medical records were recorded in a specific database for the study: age, hormonal status, histological type, histological grade, staging (FIGO), type of carcinogenesis (type I and type II), blood count parameters and immunohistochemistry results for TNF-alpha, TNFR1 and TNFR2. For the immunohistochemical study, it was utilized streptoavidin-biotin-peroxidase technique, and TNF-alpha and its receptors was evaluated in peritumoral stroma. The values of tumor markers and blood count parameters were compared by Mann-Whitney test. For parameters that were statistically significant, the receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) and to determine the best cutoff values between the weak (0/1) and strong (2/3) immunostaining groups. 3). For data with statistical significance, multivariate analysis was performed. The level of significance was less than 0.05.

Results: For TNF-alpha, analyzing the ROC curves, there was statistical significance for basophils (cut-off value > 0) and neutrophils (cut-off value ≤ 3900) between strong (2/3) and weak (0/1) immunostaining. In relation to TNFR1, a cut-off value of monocytes > 312/mm3 was found. Regarding TNFR2, the cut-off value of CA-125 was ≤ 95.16U/ml. Still in relation to TNFR2, the cut-off value for the absolute number of platelets was ≤ 298000/mm3. There was no statistical significance with the other parameters of the blood count and tumor markers evaluated. After establishing the cut-off values by the ROC curves, a multivariate analysis was performed, which showed that an absolute monocyte count > 312/mm3 is associated with strong stromal immunostaining (2/3) of TNFR1, and a platelet value ≤ 298,000/mm3 is associated with strong (2/3) stromal immunostaining of TNFR2.

Conclusion: Absolute monocyte count > 312/mm^3 is associated with strong (2/3) stromal immunostaining of TNFR1, and platelet count ≤ 298,000/mm^3 is associated with strong (2/3) stromal immunostaining of TNFR2.

Key-words: TNF-alpha, TNFR1, TNFR2, monocytes, platelets, blood count parameters, tumor markers.

Introduction

Ovarian malignant neoplasm comprises several subtypes, but all are still treated as a single disease. This heterogeneity can be a cause of treatment failure (Kossai et al., 2017). Abdominal and pelvic symptoms are non-specific, diagnosis is basically based on transvaginal ultrasound and tumor markers such as CA-125, and treatment is basically surgery followed by chemotherapy (Murta & Nomelini,2006; Roett MA & Evans, 2009).

Ovarian cancer has an unknown pathogenesis, and cytokines may play an important role in the etiology and as prognostic factors. Tumor necrosis factor (TNF) alpha is a cytokine released by monocytes and macrophages and is involved in biological processes such as immunoregulation, growth modulation and cell differentiation. TNF-alpha expression plays a role in ovarian tumor pathogenesis, increasing with disease stage in serous and mucinous tumors (Plewka D. et al., 2014). Soluble TNF-alpha receptor 2 (TNFR2) levels were significantly higher in ovarian carcinoma tissues, while soluble TNF-alpha receptor 1 (TNFR1) mRNA levels were similar in tumor and normal ovarian tissues (Piura B. et al., 2014). TNFR2 stimulates the activation and proliferation of Tregs, which promote cancer cell survival and ensure tumor growth. Tregs that express TNFR2 are associated with maximal immunosuppression capacity (Govindaraj C. et al.), thus TNFR2 levels may represent a higher risk of tumor progression and a poor prognosis factor in ovarian cancer.

Several studies have shown association of TNF-alpha and its receptors with prognosis in ovarian cancer may be targets for new treatment strategies. TNFR2 expression in cancer microenvironment is associated with cancer progression, metastasis and immune evasion. Thus, new therapeutic strategies targeting TNFR2 can improve the management of patients with ovarian cancer (Torrey et al., 2017; Al-

Hatamleh et al., 2019). Furthermore, tumor microenvironment levels of sTNF-R2 (soluble TNF receptor II) had an association with tumor differentiation grades 2 and 3 in ovarian cancer (Nomelini et al., 2018).

Studies show that blood count parameters, such as lymphocyte-to-monocyte ratio, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, platelet count and tumor marker levels are also associated with prognosis in ovarian cancer (Nomelini et al., 2017; Gong et al., 2019; Nomelini et al., 2019; Jammal et al., 2020; Soibi-Harry et al., 2021; Tang et al., 2021). In healthy patients, monocytes are divided into three populations: classical, intermediate, and non-classical, and intermediate monocytes have great expansion in ovarian cancer, with a positive correlation between the proportion of monocytes and peritoneal tumor burden (Mélissa Prat, et al.). The role of systemic inflammation markers has also been demonstrated as biomarkers of poor prognosis in ovarian cancer. A meta-analysis showed that low levels of the pretreatment lymphocyte-monocyte ratio were correlated with high histological grade and advanced stage III and IV tumors (FIGO), being a potential prognostic marker in patients with ovarian cancer (Jun Gong et al. al.).

The objective of this study was to evaluate the relationship of immunostaining of TNF and its receptors (TNFR1 and TNFR2) in the stroma of malignant ovarian neoplasm with hemogram parameters and tumor markers.

Materials and Methods

Women with a confirmed diagnosis of malignant or borderline ovarian neoplasm from the Department of Gynecology and Obstetrics of the Federal University of Triângulo Mineiro – UFTM were included in the study. Exclusion criteria were torsion of the adnexal pedicle, secondary malignant ovarian neoplasm (metastasis), previous antineoplastic treatment; immunosuppressive diseases, infections and relapses.

The following data from the medical records were recorded in a specific database for the study: age, hormonal status, histological type, histological grade,

staging (FIGO), type of carcinogenesis (type I and type II), blood count parameters and immunohistochemistry results for TNF-alpha, TNFR1 and TNFR2.

The blood count and tumor markers were collected in the preoperative period. The hemogram parameters evaluated were hemoglobin, absolute value of neutrophils, band neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes and platelets. RDW, neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) values were also evaluated. NLR was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes. PLR was calculated by dividing the absolute number of platelets by the absolute number of lymphocytes. The tumor markers evaluated were CA-125, CA 15.3 and CA19.9.

A single experienced pathologist reviewed all anatomopathological results and chose the best sections, with sufficient sampling of tumor epithelium and stroma, for the immunohistochemical study. Staging was revised according to the criteria of the International Federation of Gynecology and Obstetrics – FIGO (Zeppernick et al., 2014).

For the immunohistochemical study, the best sections chosen by an experienced pathologist were subjected to the streptoavidin-biotin-peroxidase technique, according to the manufacturer's recommendations. The study used primary antibodies anti-TNF-alpha, anti-TNFR1, anti-TNFR2, with positive and negative controls. Two observers read the blades. The intensity of immunostaining was evaluated in the peritumoral stroma. The score used was: 0 (no staining), 1 (weak staining), 2 (moderate staining), 3 (strong staining). The interobserver agreement was calculated by kappa (κ <0.4: weak agreement; $0.4 \leq \kappa$ <0.8: moderate agreement; $0.8 \leq \kappa$ <1.0: strong agreement; κ =1.0: perfect agreement). Discordant cases were defined by the most experienced researcher.

Informed consent was obtained from all patients included in the study. The study was approved by the UFTM Research Ethics Committee.

Data were analyzed by GraphPad Prism software 6, MedCalc 19.0.4, and IBM SPSS Statistics 20. The values of tumor markers and blood count parameters were compared between the weak (0/1) and strong (2/3) immunostaining groups by Mann-Whitney test. For parameters that were statistically significant, the receiver operating

characteristic (ROC) curve was used to obtain the area under the curve (AUC) and to determine the best cutoff values between the weak (0/1) and strong (2/3) immunostaining groups. 3). For data with statistical significance, multivariate analysis was performed. The level of significance was less than 0.05.

Results

The study included 43 patients with ovarian malignancy. Median age was 56 years (17-81 years). According to the International Federation of Gynecology and Obstetrics (FIGO), the staging of malignant neoplasms was: 20 (46.5%) IA, 1 (2.3%) IB, 3 (7.0%) patients IC2, 1 (2.3%) IC3, 1 (2.3%) IIB, 2 (4.7%) IIIA1(i), 1 (2.3%), 3 (7.0%) IIIB, 9 (27.9%) IIIC, and 2 (4.7%) IVB. Regarding the grade of histological differentiation of malignant tumors, 14 patients (32.6%) had grade 1, 14 patients (32.6%) had grade 2 and 15 (34.9%) patients had grade 3. The molecular subtypes of malignant neoplasms were: type I 19 patients (44.2%) and type II 24 (55.8%) patients.

For TNF-alpha, analyzing the ROC curves, there was statistical significance for basophils (cut-off value > 0; sensitivity = 14.3%; specificity = 100%; AUC = 0.571; p = 0.017) and neutrophils (cut-off value ≤ 3900; sensitivity = 54.3; specificity = 100%; AUC = 0.663; p = 0.04) between strong (2/3) and weak (0/1) immunostaining. In relation to TNFR1, using the ROC curve, a cut-off value of monocytes > 312/mm3 was found between strong (2/3) and weak (0/1) immunostaining, with a sensitivity of 92.3% and specificity of 47 .1% and AUC = 0.708 (p=0.013). Regarding TNFR2, the cut-off value of CA-125 was ≤ 95.16U/ml between strong (2/3) and weak (0/1) immunostaining, with a sensitivity of 89.5% and specificity of 52.4 % and AUC = 0.704 (p=0.017). Still in relation to TNFR2, the cut-off value for the absolute number of platelets was ≤ 298000/mm3 between strong and weak immunostaining, with sensitivity of 81% and specificity of 54.5% and AUC = 0.672 (p=0.04) (figure 1). There was no statistical significance with the other parameters of the blood count and tumor markers evaluated.

After establishing the cut-off values by the ROC curves, a multivariate analysis was performed, which showed that an absolute monocyte count > 312/mm3 is

associated with strong stromal immunostaining (2/3) of TNFR1, and a platelet value $\leq$ 298,000/mm3 is associated with strong (2/3) stromal immunostaining of TNFR2 (Tables 1 and 2). Figure 2 shows the differences in the medians of the absolute monocyte count in the weak and strong immunostaining TNFR1 groups, and the differences in the medians of the absolute platelet count in the weak and strong immunostaining groups of TNFR2.

Discussion

The inflammatory response is involved in almost all stages of tumor development. TNF-α is a key mediator in inflammation, is expressed in the ovarian cancer microenvironment, and appears to promote tumor progression by inducing cytokines (Hanahan et al, 2011; Krockenberger et al 2008). Its expression may vary according to the subtype of ovarian neoplasm, which contributes to diagnostic and prognostic factors (Köbel et al, 2008; Gilks et al, 2008). Immune function is compromised by mediators of the systemic inflammatory response, which increase leukocytes, neutrophils, platelets, C-reactive protein, and fibrinogen and decrease lymphocyte concentrations (Zhang et al, 2015). Therefore, carcinogenesis may result from a failure of the immune response.

Several studies show the relationship of TNF-alpha and its receptors with prognostic factors in ovarian cancer (Martins-Filho et al., 2014; Silva et al., 2021) cancer progression (Dobrzycka et al., 2009). Stronger TNF-alpha immunohistochemical satining was associated with histological grade 1 and early stages in ovarian cancer in a study that performed immunohistochemistry for IL2, IL5, IL6, IL8, IL10, TNF-alpha and iNOS (Martins-Filho et al., 2014). One study evaluated the protein expression level of TNFR2 and signal transducer and activator of transcription 3 (STAT3) in high-grade serous ovarian cancer tissues in relation to the platinum-based chemotherapy response and the prognosis outcome. Patients with strong TNFR2 and STAT3 expression had significantly longer progression-free survival interval in the platinum-sensitive group (Silva et al., 2021). The gene and protein expression of HMGB1, TLR4, NF κB and TNF - alfa was significantly increased in the advanced tumor stage and poorly

differentiated group in malignant epithelial ovarian cancer (Jiang et al., 2018). In another study, in patients with ovarian cancer, there was an increased trend of Th22, IL-22, and TNF-α in stage III-IV patients compared with stage I-II patients, evaluated by flow cytometry. (Wang et al., 2017). On the other hand, no differences were observed comparing TNF-alpha in serum and cytosolic fractions in ovarian cancer patients in relation to the histological types (serous, mucinous, and endometrioid carcinomas) and the disease stage (Hassan et al., 1999).

The levels of TNF alpha and its soluble type I (sTNF-R1) and type II (sTNF-R2) receptors were studied in intracystic liquid and serum from benign and malignant ovarian neoplasms. Concentration of sTNF-R2 in the intracystic samples from ovarian cancer was higher than that of the benign neoplasms, and higher intracystic levels of sTNF-R2 associated with grades 2 and 3 tumors (Nomelini et al., 2018). TNFR2 is an important protein target for further studies on the treatment of ovarian cancer because it is present in immunosuppressive Tregs and in human tumors (Chen et al., 2013; Govindaraj et al., 2013). One study suggests that antagonistic TNFR2 antibodies may be a new treatment for TNFR-positive ovarian cancer, by targeting tumor cells and immunosuppressive tumor-associated Tregs (Torrey et al., 2017).

Lymphocytes have relevance in the cellular and humoral antitumor immune response, since their activation and proliferation play a role in cytotoxic cell death and inhibit tumor cells (Zhao et al, 2020). In the same line of reasoning, macrophages originate mainly from monocytes and seem to promote proliferation, invasion and metastasis of tumor cells, by stimulating angiogenesis and inhibiting the antitumor immune response mediated by T cells. A high monocyte count is therefore indirectly associated to tumor progression (Pollard, 2004).

Monocytes are circulating mononuclear phagocytes, ready to extravasate to sites of inflammation and differentiate into monocyte-derived macrophages and dendritic cells. (Geissmann et al., 2003). TNF has pleiotropic actions and can induce both cell death and apoptosis resistance (Karin and Lin, 2002). Tumor necrosis factor (TNF) and its receptors are upregulated during monopoiesis and expressed by circulating monocytes as well as effector monocytes that infiltrate certain sites of inflammation.

Autonomic monocyte TNF may be critical to the function of these cells (Wolf et al., 2017).

Macrophages and endothelial cells infiltrate the peritoneum near epithelial ovarian cancer tumor implants. One study investigated whether the interaction of ovarian cancer cells and tumor-associated macrophages could promote endothelial cell involvement in angiogenesis. The results suggested that the interaction of ovarian cancer cells and tumor-associated macrophages enhances the ability of endothelial cells to promote ovarian cancer progression (Wang et al., 2013). Another study linked the level of blood intermediate monocytes with immunosuppression and tumor burden in the peritoneum, demonstrating that blood intermediate monocytes could be a potential predictive signature of the immune status of ascites and a biomarker of ovarian and ovarian cancer development. response to treatment (Prat et al., 2020). Hopkins et al. (2021) used a murine model to investigate changes in tumor ascites that occur after administration of platinum chemotherapy. Treatment with cisplatin resulted in a significant increase in monocytes in the ascites of tumor-bearing mice. This finding suggests that treatment with this chemotherapeutic agent leads to an increase in antitumor activity in ascites related to changes in ascitic monocytes (Hopkins et al., 2021). Another study demonstrated that the production of TGFα in high-grade serous ovarian carcinoma leads monocytes to differentiate into macrophages. This suggests an important role in the mechanism by which alternatively activated macrophages are generated in the tumor microenvironment (Fogg et al., 2020). Three subpopulations of monocytes can be distinguished in human blood: classical (CD14++CD16-), intermediate (CD14++CD16+), and nonclassical (CD14+CD16++).Monocytes can show the highest expression level of TNFR1 among monocyte subpopulations and nonclassical monocytes can have the highest expression level of TNFR2 (Hijdra et al., 2012). Our study demonstrated that an absolute monocyte count > 312/mm3 is associated with strong stromal immunostaining (2/3) of TNFR1.

Platelets have been recognized as inflammatory cells, but the effects of TNF-alpha and its receptors on the platelet activity is not yet well understood. Tumor necrosis factor-alpha plays an important role in the inflammatory response and acts through two receptors, TNFR1 and TNFR2. One study demonstrated that TNF-α

negatively modulates ADP-induced platelet aggregation via TNFR1/TNFR2 receptors by reducing cytosolic Ca++ levels and by inhibiting c-Src and fibrinogen receptor activation, which take place through cAMP- and cGMP-independent (Bonfitto et al., 2018). Tacchini-Cottier et al. (1998) demonstrated that TNF can induce platelet consumption by acting not on platelets directly but on the TNFR1 of other cells, probably by increasing the release of factors with agonist activity for platelets (Tacchini-Cottier et al., 1998). TNF can induce thrombocytopenia due to reduced platelet lifespan. TNF activates platelet caspases via TNFR1, which results in platelet fragmentation and thrombocytopenia (Piguet et al., 2002). Our study demonstrated an association of lower platelet levels with TNFR2, which was confirmed after multivariate analysis.

The main limitation of our study was the use of a heterogeneous sample of histological types of ovarian cancer. On the other hand, to our knowledge, there are no studies in the literature comparing TNF-alpha and its receptors in the stroma of ovarian cancer with the systemic inflammatory response, represented here by the blood count parameters. Thus, our study is important so that further research on the subject can establish more clearly about the immunological and inflammatory mechanisms that govern the systemic response from the tissue response. The study of these interactions between local and systemic tumor response will also be important in the discovery of new prognostic factors and new targets for the treatment of ovarian cancer, which today is basically performed with surgery and chemotherapy.

Conclusion

Absolute monocyte count $> 312/mm^3$ is associated with strong (2/3) stromal immunostaining of TNFR1, and platelet count $\leq 298,000/mm^3$ is associated with strong (2/3) stromal immunostaining of TNFR2.

Acknowledgements

Declaration of interest

The authors report no conflicts of interest.

References

1. Al-Hatamleh MAI, E A R ENS, Boer JC, Ferji K, Six JL, Chen X, Elkord E, Plebanski M, Mohamud R. Synergistic Effects of Nanomedicine Targeting TNFR2 and DNA Demethylation Inhibitor-An Opportunity for Cancer Treatment. Cells. 2019 Dec 20;9(1):33. doi: 10.3390/cells9010033.

2. Bonfitto PHL, Naime ACA, Lopes-Pires ME, Goulart G, Mendes-Silverio CB, Bueno PI, Castilho RF, Antunes E, Marcondes S. Platelet activity is negatively modulated by tumor necrosis factor alpha through reductions of cytosolic calcium levels and integrin alphaIIbbeta3 phosphorylation. Thromb Res. 2018 Dec;172:44-50. doi: 10.1016/j.thromres.2018.10.008. Epub 2018 Oct 9. PMID: 30359790.

3. Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T. cell phenotype in the inflammatory environment. J Immunol. 2013 Feb 1;190(3):1076-84. doi: 10.4049/jimmunol.1202659.

4. Dobrzycka B, Terlikowski SJ, Garbowicz M, Niklińska W, Bernaczyk PS, Nikliński J, Kinalski M, Chyczewski L. Tumor necrosis factor-alpha and its receptors in epithelial ovarian cancer. Folia Histochem Cytobiol. 2009;47(4):609-13. doi: 10.2478/v10042-008-0117-1.

5. Fogg KC, Miller AE, Li Y, Flanigan W, Walker A, O'Shea A, Kendziorski C, Kreeger PK. Ovarian cancer cells direct monocyte differentiation through a non-canonical pathway. BMC Cancer. 2020 Oct 17;20(1):1008. doi: 10.1186/s12885-020-07513-w. PMID: 33069212; PMCID: PMC7568422.

6. Geissmann, F., S. Jung, and D.R. Littman. 2003. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 19:71–82. http://dx.doi.org/10.1016/S1074-7613(03)00174-2

7. Gong J, Jiang H, Shu C, Hu MQ, Huang Y, Liu Q, Li RF. Prognostic value of lymphocyte-to-monocyte ratio in ovarian cancer: a meta-analysis. J Ovarian Res. 2019 May 31;12(1):51. doi: 10.1186/s13048-019-0527-z. PMID: 31151469; PMCID: PMC6544921.

8. Govindaraj C, Scalzo-Inguanti K, Madondo M, Hallo J, Flanagan K, Quinn M, Plebanski M. Impaired Th1 immunity in ovarian cancer patients is mediated by TNFR2+ Tregs within the tumor microenvironment. Clin Immunol. 2013 Oct;149(1):97-110. doi: 10.1016/j.clim.2013.07.003.

9. Hassan MI, Kassim SK, Saeda L, Laban M, Khalifa A. Ovarian cancer-induced immunosuppression: relationship to tumor necrosis factor-alpha (TNF-alpha) release from ovarian tissue. Anticancer Res. 1999 Nov-Dec;19(6C):5657-62.

10. Hijdra D, Vorselaars AD, Grutters JC, Claessen AM, Rijkers GT. Differential expression of TNFR1 (CD120a) and TNFR2 (CD120b) on subpopulations of human monocytes. J Inflamm (Lond). 2012 Oct 5;9(1):38. doi: 10.1186/1476-9255-9-38. PMID: 23039818; PMCID: PMC3542013.

11. Jammal MP, Martins Filho A, Bandeira GH, Murta BMT, Murta EFC, Nomelini RS. Laboratory predictors of survival in ovarian cancer. Rev Assoc Med Bras (1992). 2020 Feb 27;66(1):61-66. doi: 10.1590/1806-9282.66.1.61.

12. Jiang C, Qu X, Ke H, Gong W, Chen R, Yang W, Cheng Z. Association between the HMGB1/TLR4 signaling pathway and the clinicopathological features of ovarian cancer. Mol Med Rep. 2018 Sep;18(3):3093-3098. doi: 10.3892/mmr.2018.9271.

13. Kossaï M, Leary A, Scoazec JY, Genestie C. Ovarian Cancer: A Heterogeneous Disease. Pathobiology. 2018;85(1-2):41-49. doi: 10.1159/000479006. Epub 2017 Oct 12. PMID: 29020678.

14. Martins Filho A, Jammal MP, Côbo Ede C, Silveira TP, Adad SJ, Murta EF, Nomelini RS. Correlation of cytokines and inducible nitric oxide synthase expression with prognostic factors in ovarian cancer. Immunol Lett. 2014 Mar-Apr;158(1-2):195-9. doi: 10.1016/j.imlet.2014.01.005.

15. Murta EF, Nomelini RS. Early diagnosis and predictors of malignancy of adnexal masses. Curr Opin Obstet Gynecol. 2006 Feb;18(1):14-9. doi: 10.1097/01.gco.0000192967.67567.e9. PMID: 16493254.

16. Nomelini RS, Borges Júnior LE, de Lima CA, Chiovato AFC, Micheli DC, Tavares-Murta BM, Murta EFC. TNF-R2 in tumor microenvironment as prognostic factor in epithelial ovarian cancer. Clin Exp Med. 2018 Nov;18(4):547-554. doi: 10.1007/s10238-018-0508-3.

17. Nomelini RS, Borges Júnior LE, de Lima CA, Chiovato AFC, Micheli DC, Tavares-Murta BM, Murta EFC. TNF-R2 in tumor microenvironment as prognostic factor in epithelial ovarian cancer. Clin Exp Med. 2018;18(4):547-554. doi: 10.1007/s10238-018-0508-3.

18. Nomelini RS, Carrijo Chiovato AF, Abdulmassih FBF, da Silva RC, Tavares-Murta BM, Murta EFC. Neutrophil-to-lymphocyte ratio and platelet count as prognostic factors in ovarian malignancies. J Cancer Res Ther. 2019 Oct-Dec;15(6):1226-1230. doi: 10.4103/jcrt.JCRT_304_17.

19. Nomelini RS, de Carvalho Oliveira LJ, Tavares-Murta BM, Murta EFC. Parameters of blood count and tumor markers: a retrospective analysis and relation to prognostic factors in ovarian cancer. Eur J Gynaecol Oncol. 2017;38(3):364-367.

20. Piguet PF, Vesin C, Da Kan C. Activation of platelet caspases by TNF and its consequences for kinetics. Cytokine. 2002 May 21;18(4):222-30. doi: 10.1006/cyto.2002.0889. PMID: 12126645.

21. Roett MA, Evans P. Ovarian cancer: an overview. Am Fam Physician. 2009 Sep 15;80(6):609-16. PMID: 19817326.

22. Silva Raju J, Abd Aziz NH, Atallah GA, Teik CK, Shafiee MN, Mohd Saleh MF, Jeganathan R, Md Zin RR, Kampan NC. Prognostic Value of TNFR2 and STAT3 among High-Grade Serous Ovarian Cancer Survivors According to Platinum Sensitivity. Diagnostics (Basel). 2021 Mar 16;11(3):526. doi: 10.3390/diagnostics11030526. PMID: 33809542; PMCID: PMC8000880.

23. Soibi-Harry AP, Amaeshi LC, Garba SR, Anorlu RI. The relationship between pre-operative lymphocyte to monocyte ratio and serum cancer antigen-125 among women with epithelial ovarian cancer in Lagos, Nigeria. Ecancermedicalscience. 2021 Sep 14;15:1288. doi: 10.3332/ecancer.2021.1288.

24. Tacchini-Cottier F, Vesin C, Redard M, Buurman W, Piguet PF. Role of TNFR1 and TNFR2 in TNF-induced platelet consumption in mice. J Immunol. 1998 Jun 15;160(12):6182-6. PMID: 9637537.

25. Tang Y, Hu HQ, Tang YL, Tang FX, Zheng XM, Deng LH, Yang MT, Yin S, Li J, Xu F. Preoperative LMR and Serum CA125 Level as Risk Factors for Advanced Stage of Ovarian Cancer. J Cancer. 2021 Aug 9;12(19):5923-5928. doi: 10.7150/jca.62090.

26. Torrey H, Butterworth J, Mera T, Okubo Y, Wang L, Baum D, Defusco A, Plager S, Warden S, Huang D, Vanamee E, Foster R, Faustman DL. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated Tregs. Sci Signal. 2017 Jan 17;10(462):eaaf8608. doi: 10.1126/scisignal.aaf8608. PMID: 28096513.

27. Wang T, Zhang Z, Xing H, Wang L, Zhang G, Yu N, Wang J, Guo W, Jiang J. Elevated Th22 cells and related cytokines in patients with epithelial ovarian cancer. Medicine (Baltimore). 2017; 96(43):e8359. doi: 10.1097/MD.0000000000008359.

28. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet 2014; 290(5):839-42.

Figure legend:

Figure 1: ROC curves relating strong (2/3) and weak (0/1) stromal immunostaining of TNF-alpha with absolute basophil and neutrophil count, TNFR1 with absolute monocyte count and CA 125 value, and TNFR2 with absolute platelet count.

Figure 2: Differences in the medians of absolute monocyte counts in the weak and strong immunostaining groups of TNFR1, and the differences of the medians of the absolute platelet counts in the groups of weak (0/1) and strong (2/3) immunostaining of TNFR2 (p= 0.0266 and p=0.0321, respectively).
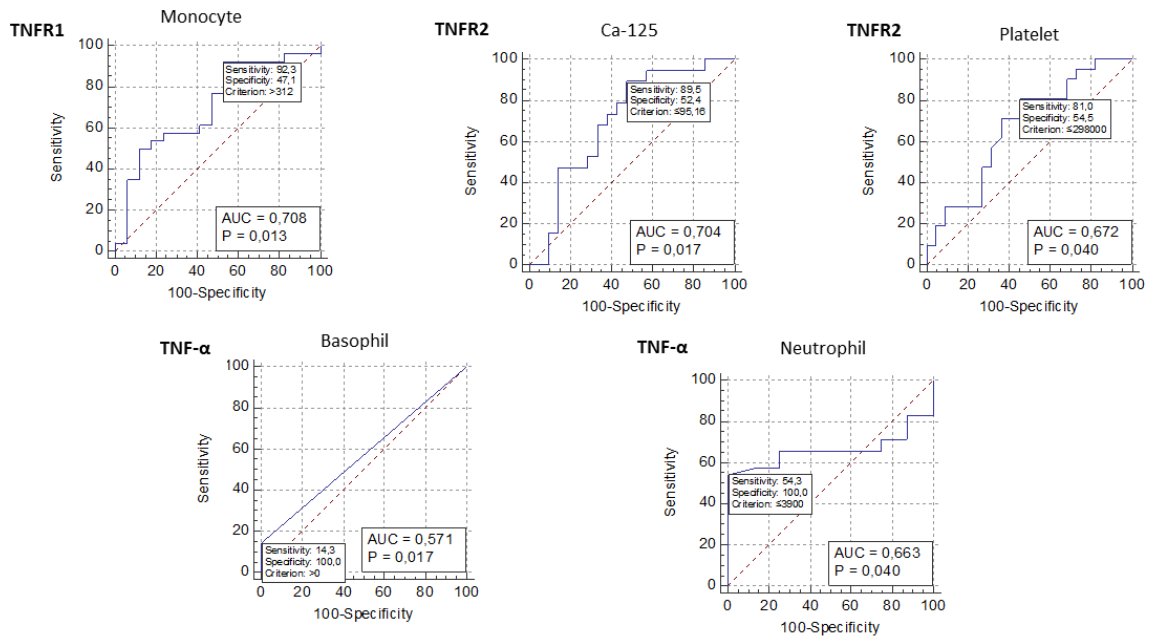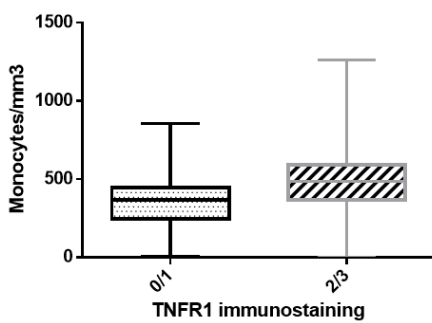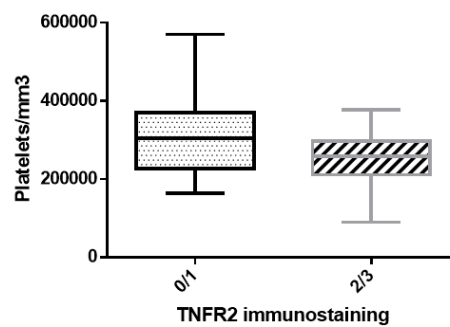
Figure 1

Figure 2

Table 1: Multivariate analysis of the variables age, hormonal status, histological grade, ovarian cancer type, staging and absolute monocyte count considering stromal TNFR1 immunostaining.

Variable	Multivariate analysis	
	OR (95% CI)	p-value
Age (> 50y vs ≤ 50y)	0.318 (0.028-3.591)	0.354
Hormonal status (menopausa vs menacme)	3.904 (0.302-50.5)	0.297
Histological grade (2/3 vs 1)	0.376 (0.025-5.623)	0.479
Type (II vs I)	4.581 (0.326-64.363)	0.259
Staging (III/IV vs I/II)	0.357 (0.052-2.438)	0.294
Monocytes count (> 312/mm^3 vs ≤ 312/mm^3)	11.365 (1.485-86.956)	0.019

Table 2: Multivariate analysis of the variables age, hormonal statuts, histological grade, ovarian cancer type, staging, serum Ca-125 and absolute platelets count considering stromal TNFR2 immunostaining.

Variable	Multivariate analysis	
	OR (95% CI)	p-value
Age (> 50y vs ≤ 50y)	5.9 (0.584-59.643)	0.133
Hormonal status (menopausa vs menacme)	0.153 (0.014-1.664)	0.123
Histological grade (2/3 vs 1)	2.117 (0.184-24.324)	0.547
Type (II vs I)	0.354 (0.031-4.034)	0.403
Staging (III/IV vs I/II)	0.703 (0.131-3.780)	0.681
Ca-125 (≤ 65.16U/mL vs > 95.16 U/mL)	2.457 (0.552-10.948)	0.238
Platelets count (≤ 298,000/mm^3 vs > 298.000/mm^3)	6.739 (1.398-32.483)	0.017