Current scenario of biomarkers in cervical cancer and oncogenesis by HPV

Cenário atual dos biomarcadores no câncer cervical e na oncogênese pelo HPV

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Cervical cancer (CC) is related to HPV infection and represents the third cause of cancer in women. Annually, more than 500,000 new cases are reported worldwide, with significant death rates. It develops due to genetic and epigenetic alterations that control cell growth and differentiation, and may cause death. These alterations induce uncontrolled cell division and invasion of cervical tissue have severe consequences to women's health[1]. CC incidence and mortality drop considerably since the implementation of screening tests and vaccination strategies. Nevertheless, CC continues to have a high incidence, mainly in low-income countries, where these programs do not cover territorial frontiers and there is lack of resources to implement vaccination or screening tests. Oncogenic HPV types reached 25% of cases in Brazil over the last years[2], and there was no modification on HPV types after four years of the vaccination program, according to Tota et al.[3]. Usually, screening tests in Brazil cover women from 25 to 64 years old. According to Teixeira et al.[4], rates of CC under the age of 25 tend to increase, and women over 64 achieved roughly 20% of CC on research of Brazilian women from two high-income cities.

HPV 16 and 18 are the predominant genotypes linked to progression to invasive cancer. HPV uses evasion mechanisms of the immune system in early and late stages of the infection, causing its persistence in cutaneous and mucosal tissues. Its continuous expression of viral proteins E6 and E7 contribute to disease progression[5]. Starting lesions are well defined and classified into cervical intraepithelial neoplasm 1, 2, and 3, with grade 3 being the highest level of evolution. Factors such as age, smoking, sexually transmitted disease, long-term oral contraceptive use, and parity are associated to a higher risk of cervical cancer development[6,7]. Many CIN 1, 2, and some CIN 3 lesions spontaneously revert. Even though, treatment is still needed, because they have high chances to progress[8]. Treatment just after diagnosis can include from large loop excision of the transformation zone (LLETZ), loop electrosurgical excision procedure (LEEP) to cold-knife conization (CKC), and sometimes other treatment modalities, as laser or cryosurgery. Incidence among young women has expressive rates, and treatment can relate to severe consequences in the reproductive function, such as fetal consequences of low birth weight, premature birth, and miscarriage[9,10].

Molecular markers appear to be of fundamental importance, not only to make the diagnostic, but also to help the treatment establishment, working as a tool to assess the individual patient's risk of having cancer. The best biomarker would be 100% sensibility and specificity, which is not a reality yet[1]. Biomarkers specific to cervical cancer would be of great value, making it possible to identify which CC precursor lesions would progress, influencing clinical decision-making. Scientific research focus on finding biomarkers from:

- identification of wrong protein expression due to oncogenes;
- detection of methylation alterations on cell genes, predicting neoplasm process;
- identification of chromosomal or genetic modifications due to viral integration;
- expression of genic polymorphism in association with a better prognostic[1,10,11].

The present editorial was written to provide readers with the recent scenario regarding molecular markers and its impregnability on diagnosis, treatment, and follow up of cervical precancer and cancer patients. According to current literature, research articles report many possible biomarkers to help with cervical cancer diagnosis, treatment, and prognosis. Immunologic markers are of great interest. Programmed Death 1 (PD-1) and its ligand (PD-L1) can blockade T lymphocytes function and allow tumoral growth[12]. Allelic variations of Human Leukocyte Antigen (HLA) genes are associated to genetic susceptibility in cervical cancer development in the presence of high-risk DNA-HPV11. The overexpression of HLA-E seems to trigger signalization pathways to engage Natural Killer (NK) activating receptors, such as NKG2D, contributing to NK activation against viral infection[5]. The allelic *variants* MICA*008:01/04 and MICA*018:01 are associated to the risk of CC development, and MICA-129 *Val* is related to the risk of tumoral development[7].

Squamous cell carcinoma antigen (SCC-Ag) is related to tumoral size, stromal invasion, and disease relapse[1]; Carcinoma Embryonic Antigen (CEA) is linked to cervical squamous neoplasia when it has higher levels and impact on prognostic on cervical adenocarcinoma[1]. There was no association of Mannose-binding lectin (MBL) with lesions progression or with cervical ongenesis[13]. However, products

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of MBL — MASP-1, MASP-2, and MAp-19, showed higher levels related to tumoral progression and a worse prognostic in cervical neoplasia patients[14].

Proteins coded by virus oncogenes E6 and E7 interfere in apoptosis and cell regulation. For that reason, tumor suppressor proteins, such as p53, $p16^{INK4a}$, and Ki-67 were investigated as potential targets. Virus-infected cells express p16 to control irregular cell cycle, which is not present in healthy cervical cells, but is one hundred percent present in high-grade lesions cells[15,16]. Protein 53 (p53) encoded by the gene TP53, seems to be higher in patients with cancer stage II, III and IV, according to the International Federation of Gynecology and Obstetrics (FIGO), older than 48, and with tumor size >= 4 mm and. Absence of p53 is significantly associated with tumors <4 cm, adenocarcinoma and deep invasion.[10]. Histological distribution of Ki-67 is modified, with HPV infection being associated to high-risk HPV infection[16].

Some genes are involved in neoplastic processes. Gene cyclin-dependent kinase inhibitor 3 (CDKN3) is higher in cervical cancer patients, related to it in 31.85% of cases with five years of survival. In those patients that died, survival was of 14 months. *In vitro*, its inactivation showed lower tumoral proliferation, which can be used in the future to develop new strategies to treat neoplasm[17]. The methylation process is of importance in the HPV oncogenic pathway, and Fiano et al.[18] showed that DNA methylation in HPV positive women is associated to high-risk CIN. DNA methylation in host genes and HPV genome is associated to cervical oncogenesis[16,18]. Some molecules, such as GHSR, SST, and ZIC1, from 3q chromosome, *in vitro*, are associated to a higher risk of progression from precursor lesions detected by citology[8]. Gene STK31, after aberrant methylation by E7-HPV16, is considered an oncogenic precursor and risk marker to invasive neoplasia[19].

Genotyping HPV is being incorporated in population screening, and it is used to identify tumoral aggressiveness. Whereas HPV 16 is a marker of high-risk lesion, a better disease-free survival was observed in HPV16 cases, more sensitive to treatment, with a lower rate of growth, and better immune response to virus[9]. Besides genotyping, viral load, viral physical status, and circulating fraction of DNA-HPV are biomarkers that need to be better understood[1,16,20,21].

Genomic and immunologic techniques are the oncology vanguard, whose applications include cervical cancer patients. Limitations to the use of these techniques are costs and population size, which contribute to insufficient scientific evidence to change protocols in use.

From an overall reading of the available literature on biomarkers, we can conclude:

- PD-1/PD-L1 showed promising results in other neoplasms, indicating disease progression and specific immunotherapy;
- the methylation status of target genes, such as STK31, is considered an oncogenic precursor and a risk marker to invasive neoplasia, even if it is not a real value for a biomarker;
- DNA-HPV already incorporated as a secondary screening test, which is a substantial tool to help clinic management for papillomavirus patients.

Risk evaluation is of critical significance to choose a therapeutic strategy for patients that face HPV infection. The aim is to bring the best result to a specific patient, according to each clinical situation. Cervical cancer has a significant impact on women's lives, with many deaths related to this disease even with screening tests. Immunological knowledge must be deepened to bring efficient vigilance, a treatment with less morbidity and better survival.

Participation of each author

Newton Sergio de Carvalho: publication theme, review for sending the paper.

Maria da Graça Bicalho: publication theme, review for sending the paper.

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Conflict of interests

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REFERENCES

1. Dasari S, Wudayagiri R, Valluru L. Cervical cancer: Biomarkers for diagnosis and treatment. Clin Chim Acta. 2015;445:7-11. https://doi.org/10.1016/j.cca.2015.03.005
2. Rotelli-Martins CM, Carvalho NS, Naud P, Teixeira J, Borba P, Derchain S, et al. Prevalence of Human Papillomavirus Infection and Associated Risk Factors in Young Women in Brazil, Canada, and the United States: A Multicenter Cross-sectional Study. Int J Gynecol Pathol. 2011;30(2):173-84. https://doi.org/10.1097/pgp.0b013e3181f38dfe
3. Tota JE, Struyf F, Merikukka M, Gonzalez P, Kreimer AR, Bi D, et al. Evaluation of Type Replacement Following HPV16/18 Vaccination: Pooled Analysis of Two Randomized Trials. J Natl Cancer Inst. 2017;109(7):djw300. https://doi.org/10.1093/jnci/djw300
4. Teixeira JC, Maestri CA, Machado HDC, Zeferino LC, De Carvalho NS. Cervical Cancer Registered in Two Developed Regions from Brazil: Upper Limit of Reachable Results from Opportunistic Screening. Rev Bras Ginecol Obstet. 2018;40(6):347-53. https://doi.org/10.1055/s-0038-1660841
5. Franciosi JR, Gelmini GF, Roxo VS, Carvalho NS, Bicalho MDG. Is there a role played by HLA-E, if any, in HPV immune evasion? Scan J Immunol. 2020;91(3):e12850. https://doi.org/10.1111/sji.12850
6. El-Zein M, Ramanakumar AV, Naud P, Roteli-Martins CM, Carvalho NS, Borba PC, et al. Determinants of acquisition and clearance of human papillomavirus infection in previously unexposed young women. Sex Transm Dis. 2019;46(10):663-9. https://dx.doi.org/10.1097%2FOLQ.0000000000001053
7. Von Lisingen R, De França PP, De Carvalho NS, Bicalho MDG. MICA and KLRK1 genes and their impact in cervical intraepithelial neoplasia development in the southern Brazilian population. Human Immunol. 2020;81(5):249-53. https://doi.org/10.1016/j.humimm.2020.02.007
8. Verlaat W, Snijders PJF, Novianti PW, Wilting SM, De Strooper LMA, Trooskens G, et al. Genome-wide DNA Methylation Profiling Reveals Methylation Markers Associated with 3q Gain for Detection of Cervical Precancer and Cancer. Clin Cancer Res. 2017;23(14):3813-22. https://doi.org/10.1158/1078-0432.ccr-16-2641

9. Chong GO, Lee YH, Han HS, Lee HJ, Park JY, Hong DG, et al. Prognostic value of pre-treatment human papilloma virus DNA status in cervical cancer. Gynecol Oncol. 2017;148(1):97-102. https://doi.org/10.1016/j.ygyno.2017.11.003
10. Halle MK, Ojesina AI, Engerud H, Woie K, Tangen IL, Holst F, et al. Clinicopathologic and molecular markers in cervical carcinoma: a prospective cohort study. Am J Obstet Gynecol. 2017;217(4):432.E1-432.E17. https://doi.org/10.1016/j.ajog.2017.05.068
11. Termini L, Villa LL. Biomarcadores na Triagem do Câncer de Colo Uterino. DST – J Bras Doenças Sex Transm. 2008;20(2):125-31.
12. Liu C, Lu J, Tian H, Du W, Zhao L, Feng J, et al. Increased Expression of PD-L1 by Human Papillomavirus 16 E7 Oncoprotein Inhibits Anticancer Immunity. Mol Med Rep. 2017;15(3):1063-70. https://doi.org/10.3892/mmr.2017.6102
13. Maestri CA, Nisihara R, Ramos GP, Mendes HW, Messias-Reason I, De Carvalho NS. Mannose-binding lectin does not act as a biomarker for the progression of preinvasive lesions of invasive cervical cancer. Med Princ Pract. 2018;26(6):530-4. https://doi.org/10.1159/000485037
14. Maestri CA, Nisihara R, Mendes HW, Jensenius J, Thiel S, Messias-Reason I, et al. MASP-1 and MASP-2 serum levels are associated with worse prognostic in cervical cancer progression. Front Immunol. 2018;9:2742. https://doi.org/10.3389/fimmu.2018.02742
15. Fonseca FV, Tomasich FD, Jung JE, Maestri CA, Carvalho NS. The role of P16ink4a and P53 immunostaining in predicting recurrence of HG-CIN after conization treatment. Rev Col Bras Cir. 2016;43(1):35-41. https://dx.doi.org/10.1590/0100-69912016001008
16. Valenti G, Vitale SG, Tropea A, Biondi A, Laganà AS. Tumor markers of uterine cervical cancer: a new scenario to guide surgical practice? Updates Surg. 2017;69:441-9. https://doi.org/10.1007/s13304-017-0491-3
17. Barron EV, Roman-Bassaure E, Sánchez-Sandoval AL, Espinosa AM, Guardado-Estrada M, Medina I, et al. CDKN3 mRNA as a Biomarker for Survival and Therapeutic Target in Cervical Cancer. PLoS One. 2015;10(9):e0137397. https://doi.org/10.1371/journal.pone.0137397
18. Fiano V, Trevisan M, Fasanelli F, Grasso C, Marabese F, Bicalho MDG, et al. Methylation in host and viral genes as marker of aggressiveness in cervical lesions: Analysis in 543 unscreened women. Gynecol Oncol. 2018;151(2):319-26. https://dx.doi.org/10.1016/j.ygyno.2018.08.031
19. Yin F, Wang N, Bi XN, Yu X, Xu X-H, Wang Y-L, et al. Serine/threonine kinases 31(STK31) may be a novel cellular target gene for the HPV16 oncogene E7 with potential as a DNA hypomethylation biomarker in cervical cancer. Virology J. 2016;13:60. https://doi.org/10.1186/s12985-016-0515-5
20. Das P, Thomas A, Kannan S, Deodhar K, Shrivastava SK, Mahantshetty U, et al. Human papillomavirus (HPV) genome status & cervical cancer outcome – A retrospective study. Indian J Med Res. 2015;142(5):525-32. https://doi.org/10.4103/0971-5916.171276
21. Huang Y, He Q, Xu K, Zhou J, Yin J, Li F, et al. A new marker based on risk stratification of human papillomavirus DNA and tumor size to predict survival of locally advanced cervical cancer. Int J Gynecol Cancer. 2019;29:459-65. https://doi.org/10.1136/ijgc-2018-000095

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