

# SALIVARY BIOMARKERS FOR THE DIAGNOSIS OF GASTRIC CANCER: A SYSTEMATIC REVIEW

BIOMARCADORES SALIVALES PARA EL DIAGNÓSTICO DE CÁNCER GÁSTRICO: UNA REVISIÓN SISTEMÁTICA

**Natalia Zúñiga-Pérez**<sup>1</sup>

zuniga.natalia.1997@gmail.com

**Humberto Muñoz-Navarro**<sup>1</sup>

munozhumberto300@gmail.com

**César Rivera**<sup>1\*</sup>

cerivera@utalca.cl

## ABSTRACT

**Background:** Recent advancements reveal saliva as a crucial source of diagnostic biomarkers for various diseases, notably gastric cancer. This systematic review evaluates these biomarkers, emphasizing their clinical applicability and potential in early detection. **Methods:** An extensive electronic search was conducted across PubMed/MEDLINE, Scopus, and Web of Science to identify relevant studies. Salivary biomarkers were analyzed as independent variables, with gastric cancer as the dependent variable. The study adhered to a protocol registered with PROSPERO (CRD42021259519). **Results:** Our analysis identified a range of biomarkers, highlighting three proteins - cystatin-B (CSTB), triosephosphate isomerase (TPI1), and deleted in malignant brain tumors 1 protein (DMBT1) - as particularly accurate for gastric cancer diagnosis. **Conclusions:** Salivary biomarkers hold substantial promise for the early detection of gastric cancer. Future research should aim to refine study design and validation for enhancing the quality and applicability of these biomarkers.

**Keywords:** saliva, salivary proteins and peptides, biomarkers, tumor biomarkers, stomach neoplasms

## RESUMEN

**Introducción:** Avances recientes revelan la saliva como una fuente crucial de biomarcadores diagnósticos para diversas enfermedades, especialmente el cáncer gástrico. Esta revisión sistemática evalúa estos biomarcadores, con énfasis en su aplicabilidad clínica y potencial para la detección temprana. **Métodos:** Se realizó una extensa búsqueda electrónica en PubMed/MEDLINE, Scopus y Web of Science para identificar estudios relevantes. Los biomarcadores salivales fueron analizados como variables independientes, con el cáncer gástrico como variable dependiente. El estudio siguió un protocolo registrado en PROSPERO (CRD42021259519). **Resultados:** Nuestro análisis identificó una gama de biomarcadores entre los que destacan tres proteínas: cistatina-B (CSTB), triosa fosfato isomerasa (TPI1) y proteína 1 eliminada en tumores cerebrales malignos (DMBT1), como particularmente precisas para el diagnóstico del cáncer gástrico. **Conclusiones:** Los biomarcadores salivales tienen un gran potencial para la detección temprana del cáncer gástrico. La investigación futura debería apuntar a refinar el diseño del estudio y la validación para mejorar la calidad y aplicabilidad de estos biomarcadores.

**Palabras clave:** saliva, proteínas y péptidos salivales, biomarcadores, biomarcadores tumorales, neoplasias gástricas

**Artículo recibido: 10/11/2023**  
**Arbitrado por pares**  
**Artículo aceptado: 01/02/2024**  
**Artículo publicado: 30/03/2024**

\* **Correspondence to:**

César Rivera  
cerivera@utalca.cl



©Los autores, 2024. Publicado por la Universidad Científica del Sur (Lima, Perú)

**Citar como:** Zúñiga-Pérez N, Muñoz-Navarro H, Rivera C. Salivary biomarkers for the diagnosis of gastric cancer: a systematic review. Rev Cient Odontol (Lima). 2024;12(2):e199  
doi: 10.21142/2523-2754-1202-2024-199

<sup>1</sup> Oral and Maxillofacial Histopathology Laboratory, Department of Stomatology, Faculty of Dentistry, Universidad de Talca. Talca, Chile.

## INTRODUCTION

Gastric cancer, predominantly adenocarcinoma, represents a significant health challenge. This malignant neoplasm arises from the stomach's lining (1) and is known for its propensity to metastasize rapidly to nearby organs (2). Accounting for 95% of gastric cancers, adenocarcinoma is classified based on its invasion depth: early stage when confined to the submucosa and advanced stage upon deeper penetration (2,3). The development of this disease is influenced by a variety of factors including genetic predisposition, aging, lifestyle habits such as excessive alcohol intake and smoking, and diets rich in sodium, red meats, and salt-preserved or smoked foods (4,5). A notable contributor, *Helicobacter pylori* infection, is implicated in approximately 75% of gastric cancer cases (6). Globally, gastric cancer ranks as the fifth most prevalent malignancy and the fourth leading cause of cancer mortality (4), according to GLOBOCAN 2020.

The high mortality rate associated with gastric cancer is largely attributed to delayed diagnosis. Often, the disease remains asymptomatic during initial stages, with symptoms like weight loss, dysphagia, dyspepsia, nausea, vomiting, and early satiety emerging only in advanced stages (7). Early detection significantly improves prognosis, with favorable outcomes observed in cases where surgical intervention is possible. In contrast, late-stage diagnosis often results in a survival period as short as six months (8). Currently, the definitive diagnosis of gastric cancer involves upper digestive endoscopy followed by a biopsy (2), a procedure that is invasive, uncomfortable, and necessitates specialized expertise (9).

The pursuit of early gastric cancer detection presents a formidable clinical challenge. Success in this area could dramatically enhance treatment options and patient prognosis. To this end, the investigation of various biomarkers in bodily fluids, including saliva, has gained momentum. Saliva, a complex fluid harboring components like mRNA, microRNAs, and enzymes, reflects both local and systemic health conditions (10,11) and offers a non-invasive, safe, and cost-effective alternative for diagnostic sampling (12).

However, despite extensive research, clinically reliable salivary biomarkers for early gastric cancer detection remain elusive. Addressing this gap, our systematic literature review aimed to identify, evaluate, and compare salivary biomarkers nearing clinical application. We focused on studies in their validation phase reporting diagnostic accuracy. This approach led us to identify biomarkers across a spectrum of molecular types, with CSTB, TPI1, and DMBT1 proteins demonstrating remarkable diagnostic precision in independent validation settings.

## METHODS

In this study, we conducted a systematic review. Salivary biomarkers, serving as the independent variables, while the presence of gastric cancer was established as the dependent variable. A protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) and assigned the registration code CRD42021259519.

**Diagnostic biomarker:** We defined a diagnostic biomarker as a molecule obtained from studies involving binary logistic regression analysis or Cox proportional hazards modeling, showing a statistically significant association between the biomarker and the outcome (gastric cancer). The calculated risk (odds ratio, OR, or hazard ratio, HR) should have been reported as the risk of a specific outcome in the biomarker group compared to the reference group, with OR/HR > 1 indicating higher risk and OR/HR < 1 indicating reduced risk (13).

**Search strategy:** We conducted a systematic search of all English and Spanish literature in MEDLINE, Scopus, and Web of Science, up to September 20, 2023. Our search strategy resulted from the combination of the following keywords (Table 1).

Table 1. Databases and searches used in this research.

Database	Query
Pubmed/MEDLINE	((("Biomarkers"(Mesh) OR "Biomarkers, Tumor/metabolism"(Mesh) OR "Biomarkers, Tumor"(Mesh)) AND ("Saliva"(Mesh)) AND ("Stomach Neoplasms"(Mesh) OR Gastric cancer (all fields)))
Scopus	((("Biomarkers" OR "Biomarkers, Tumor/metabolism" OR "Biomarkers, Tumor") AND ("Saliva")) AND ("Stomach Neoplasms" OR "Gastric cancer"))
Web of Science	TS="Biomarker*" AND TS="Saliva" AND TS=("Stomach Neoplasms" OR "Gastric cancer") AND TS="Diagnosis"

**Data extraction:** Two review authors independently extracted the data in duplicate. Disagreements were resolved with the intervention of a third investigator, who made the final decision. The following data were extracted from each study: complete reference, study characteristics, country, ethnic origin, sample size (cancer vs. non-cancer), cancer type, type of saliva (stimulated or unstimulated), saliva sample (whole saliva, supernatant, or sediment), and diagnostic capacity values.

**Inclusion and exclusion criteria:** We included observational studies (prospective and retrospective cohort studies, case-control studies, cross-sectional studies) that reported diagnostic accuracy values of salivary biomarkers in gastric cancer. We excluded preliminary studies, conference abstracts, and literature reviews presented in languages other than English or Spanish. After full-text analysis, we excluded studies that did not present an independent validation phase.

**Quality assessment:** The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) checklist was used (14). Four key domains were analyzed: (1) patient selection, (2) index test, (3) reference standard, and (4) flow and timing. Each domain was assessed in terms of its risk of bias, and the first three domains were also assessed in terms of concerns regarding applicability. The risk of bias and concern about applicability for each domain was considered "low," "high," or "unclear." When the answer to questions about risk of bias and applicability was "low" risk or "low" concern, one point was awarded for each item. Articles were grouped into categories of high quality (6 and 7 points), moderate

quality (4 and 5 points), and low quality (0-3 points). Additionally, we performed a critical appraisal of the articles guided by the Critical Appraisal Skills Program (CASP) tools (<https://casp-uk.net/casp-tools-checklists/>). We assessed the study's validity, its results, and their applicability.

## RESULTS

**Article selection.** The systematic search provided 74 articles, of which we excluded 22 duplicates and 42 for not meeting the eligibility criteria (Figure 1). We obtained 9 articles for full-text review. Among these, 6 articles were excluded as they lacked validation. Finally, we selected 3 articles that met the inclusion criteria.

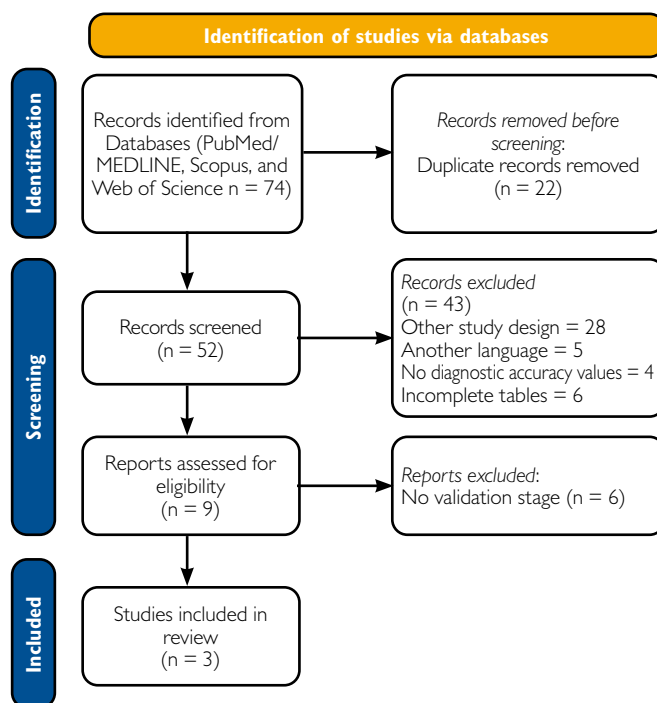


Figure 1. PRISMA Flowchart. A diagram representing the systematic literature search for salivary biomarkers for the diagnosis of gastric cancer patients.

**Study characteristics.** The characteristics of the studies are presented in Table 2. These articles, conducted in Korea and China, were published between 2016 and 2018. They used unstimulated whole saliva as the data source. We identified three different diagnostic methods:

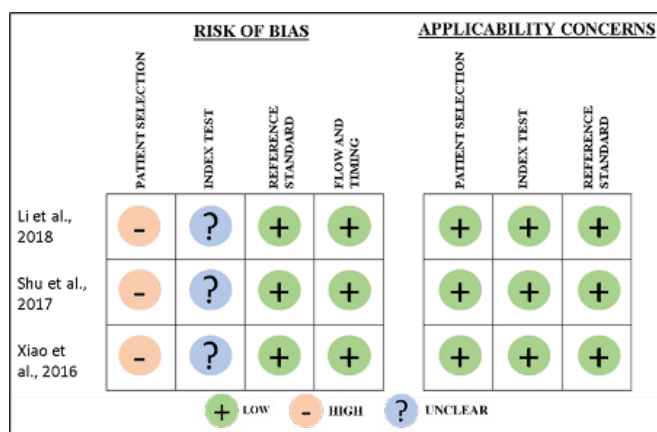
enzyme-linked immunosorbent assay (ELISA), saliva microarrays, lectin transfer analysis, and quantitative reverse transcription polymerase chain reaction (RT-qPCR). Sample sizes for gastric cancer and non-cancer subjects ranged from 20 to 100 participants. One of the studies also reported a sample of patients with atrophic gastritis (a potentially malignant lesion).

### Diagnostic capacity measures in the validation phase.

We found salivary biomarkers of extracellular RNA, glycoproteins, and proteins. In the case of glycoproteins, they were used both individually and in combination. Overall, all biomarkers exhibited high diagnostic capacity measures. However, the combination of CSTB, TPI1, and DMBT1 proteins demonstrated the highest AUC value, indicating superior discriminative capacity between gastric cancer patients and healthy individuals. Regarding sensitivity and specificity, values ranged from 70 to 92 and 72 to 91, respectively. Diagnostic capacity measures are detailed in Table 3.

**Quality assessment.** The QUADAS-2 tool was employed to assess the risk of bias in the studies, covering four key domains: patient selection, index test, reference

standard, and flow and timing. The symbol (-) indicates a high risk of bias, the symbol (+) a low risk of bias, and the symbol (?) suggests an unclear risk of bias. In the risk of bias assessment, the most concerning aspect was the lack of information concerning patient selection and unclear details regarding the index test. Consequently, the assessed articles presented moderate quality (Figure 2).



**Figure 2.** Quality assessment summary (QUADAS-2) of all articles. Quality assessment of comparative diagnostic accuracy studies was used for Xiao et al., 2016 (15), Shu et al., 2017 (16) and Li et al., 2018 (17).

**Table 2.** Key characteristics of included studies.

Study	Country	Ethnicity	Saliva specimen	Method	Sample size		Atrophic gastritis (AG)
					Gastric cancer (GC)	No-cancer (controls)	
Xiao et al., 2016 (15)	Korea	Asian	Unstimulated whole saliva	ELISA	20	20	No reported
Shu et al., 2017 (16)	China	No reported	Unstimulated whole saliva	Saliva microarrays and lectin blotting analysis	23	30	24
Li et al., 2018 (17)	Korea	Asian	Unstimulated whole saliva	RT-qPCR	100	100	No reported

**Table 3.** Diagnostic capacity measures in the validation phase.

Study	Type of biomarker	Biomarker name	Unique combined or Accuracy (ROC curve)	Sensitivity	Specificity	P value
Xiao et al., 2016 (15)	Protein	CSTB, TPII, DMBT1	Combined 93	85	80	<0,05
Shu et al., 2017 (16)	Lectin	VVA and SBA	Combined 89 (80-99)	96	80	<0,001
Shu et al., 2017 (16)	Lectin	VVA	Unique 81 (71-91)	70	91	<0,001
Shu et al., 2017 (16)	Lectin	LEL and DSA*	Combined 83 (71-94)	92	72	<0,001
Li et al., 2018 (17)	Extracellular RNA	3 mRNAs (SPINK7, PPL, and SEMA4B) and 2 miRNAs (miR140-5p and miR301a)	Combined 81 (72-89)	75	83	0,093

Lectins are carbohydrate-binding proteins that discriminate between glycopatterns of glycans based on subtle differences in structure. Several lectins, including VVA, LEL and SBA, are generally used to study altered glycans structures in gastric cancer. \*Combined biomarkers for an atrophic gastritis diagnostic model. Serine peptidase inhibitor Kazal type 7 (SPINK7), perioplakin (PPL), and semaphorin 4B (SEMA4B).

## DISCUSSION

In recent years, various biomarkers in fluids like human saliva have been investigated. This is of significant interest because these biomolecules could contribute to the early diagnosis of diseases such as gastric cancer, which has an unfavorable prognosis due to late diagnosis.

In this study, we identified three types of salivary biomarkers: extracellular RNA (SPINK7, PPL, SEMA4B, miR140-5p, miR301a), carbohydrate-binding proteins (VVA, SBA, LEL, and DSA), and proteins (CSTB, TPI1, and DMBT1). These salivary biomarkers, whether used individually or in combination, exhibited high diagnostic accuracy, represented by their AUC values, making them potentially useful for the detection of gastric cancer and atrophic gastritis.

All the biomarkers were validated in independent cohorts. Initially, they were identified in a discovery phase. Subsequently, the candidate markers advanced to a validation phase, where external samples were used to evaluate diagnostic accuracy. It is noteworthy that during the validation stage, the biomarker's measurement performance characteristics are assessed, determining whether it provides reproducible and accurate data<sup>(18)</sup>. According to Ziegler et al., the development of a biomarker involves several phases before implementation in clinical practice, including initial discovery, external validation, clinical utility, and quantification of cost-effectiveness<sup>(19)</sup>. The aim of exclusively including studies with a validation phase was to focus our review on salivary markers that are nearing clinical use.

The most promising diagnostic biomarkers were three proteins used in combination. CSTB, a cysteine protein whose expression is reduced in gastric cancer cells, may contribute to the development of this condition by promoting cell proliferation, enhancing metastasis, and inhibiting apoptosis<sup>(20)</sup>. TPI1, an enzyme involved in the glycolytic pathway, is overexpressed in cancers such as esophageal cancer due to increased glycolysis<sup>(21)</sup>, which provides energy for tumor cell growth<sup>(22)</sup>. DMBT1, a complex molecule involved in innate

immune defense and epithelial cell differentiation, is overexpressed in gastric cancer, as inflammation is a key factor in metaplastic progression in the stomach<sup>(23)</sup>. These three biomarkers were validated using ELISA, a widely adopted method known for its ease and speed<sup>(24)</sup>, as in the case of the BioMérieux VIDAS® HIV Panel, a kit for rapid and precise HIV detection. One possible reason for identifying these biomarkers is the presence of exosomes, defined as extracellular vesicles that play a significant role in intercellular communication<sup>(25)</sup>, originating from various cell types, including tumor cells. These exosomes contain diverse molecular content, such as proteins or RNA<sup>(26)</sup>, and are transported over long distances to be eventually found in bodily fluids like saliva<sup>(27)</sup>. The potential of these proteins is so significant that they have been included in a panel of biomarkers patented by David T. Wong in 2018 (US20180188256A1). According to the author, his invention can be used for diagnosing, identifying, or screening individuals with, without, or at risk of having the disease, differentially diagnosing disease states, assessing disease severity or changes in severity, among other potential uses.

The evidence from which these results emerge is of moderate quality. The primary issues in the study designs mainly concern patient selection and the index test. In the selected studies, patients were neither consecutively included nor randomly selected. Additionally, these studies did not avoid a case-control design, which, according to historical arguments, can lead to an overestimation of diagnostic accuracy because the test is evaluated in a group of patients already known to have the disease and in a group of healthy patients<sup>(28)</sup>. Furthermore, the studies did not make it clear whether blinding was employed when interpreting the results of the index test, meaning the results of the reference test were not known. This can increase agreement between both tests, resulting in an overestimation of diagnostic capacity measures such as sensitivity and specificity<sup>(29)</sup>. Future research should address and rectify these identified deficiencies, especially considering that despite the promising findings, saliva is not yet routinely used as a biological fluid for the detection of gastric cancer.

## CONCLUSION

This systematic review underscores the burgeoning potential of salivary biomarkers in the early detection of gastric cancer, a malignancy characterized by its aggressive nature and poor prognosis when diagnosed at advanced stages. The findings of our review, which focused on studies in their validation phase, reveal significant advancements in the identification and validation of salivary biomarkers, particularly the proteins CSTB, TPI1, DMBT1. These biomarkers exhibited remarkable accuracy in distinguishing gastric cancer patients from healthy individuals, as evidenced by high sensitivity and specificity values.

The application of non-invasive diagnostic methods like saliva testing offers a transformative approach to cancer diagnosis, aligning with the growing demand for patient-friendly, cost-effective, and accessible healthcare solutions. The ability to detect gastric cancer through salivary biomarkers not only paves the way for early intervention but also minimizes the reliance on more invasive, uncomfortable, and resource-intensive diagnostic methods like endoscopy. The progression of these biomarkers from laboratory research to clinical application will require collaborative efforts across disciplines, underscoring the importance of interdisciplinary research in advancing cancer diagnostics.

## DECLARATIONS

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and material:** This article contains all supplemental materials. All further information that supports the study's findings is available upon reasonable request from the corresponding author.

**Competing interests:** The authors declare that they have no competing interests.

**Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Authors' contributions:** C.R., N.Z-P., and HM-N. contributed to data acquisition, interpretation and critically revised the manuscript. C.R. contributed to conception, design, project supervision, performed all final analyses, and interpretations. C.R., N.Z-P., and HM-N. drafted the manuscript. All authors gave their final approval and agreed to be accountable for all aspects of the work.

**Acknowledgments:** We dedicate this text to all those who have succumbed to digestive cancers and their families. We hope that in the future, dentistry can contribute to the diagnostic process of these diseases. This work has no sources of funding.

**Authors' Information:** Natalia Zúñiga-Pérez, Humberto Muñoz-Navarro contributed equally to this work. César Rivera, with extensive expertise in oral medicine, functions as a scientist, educator, and clinician. His professional affiliations include the Chilean Society of Oral and Maxillofacial Pathology, the Chilean Society of Bioinformatics, and the Chilean Association of Immunology.

## Abbreviations

**CSTB:** cystatin-B

**TPI1:** triosephosphate isomerase

**DMBT1:** deleted in malignant brain tumors 1 protein

**OR:** odds ratio

**HR:** hazard ratio

**QUADAS-2:** The Quality Assessment of Diagnostic Accuracy Studies 2

**VVA, LEL and SBA:** carbohydrate-binding proteins

**SPINK7:** serine peptidase inhibitor Kazal type 7

**PPL:** periplakin

**SEMA4B:** semaphorin 4B

**miR:** microRNA

## REFERENCES

- Kanda M, Kodera Y. Recent advances in the molecular diagnostics of gastric cancer. *World J Gastroenterol*. 2015 Sep 14;21(34):9838-52. Available from: <http://dx.doi.org/10.3748/wjg.v21.i34.9838>
- Thrumurthy SG, Chaudry MA, Hochhauser D, Mughal M. The diagnosis and management of gastric cancer. *BMJ*. 2013 Nov 4;347:f6367. Available from: <http://dx.doi.org/10.1136/bmj.f6367>
- Young JJ, Pahwa A, Patel M, Jude CM, Nguyen M, Deshmukh M, et al. Ligaments and Lymphatic Pathways in Gastric Adenocarcinoma. *Radiographics*. 2019 Apr 5;39(3):668-89. Available from: <http://dx.doi.org/10.1148/rg.2019180113>
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021 May;71(3):209-49. Available from: <http://dx.doi.org/10.3322/caac.21660>
- Sasako M, Inoue M, Lin JT, Khor C, Yang HK, Ohtsu A. Gastric Cancer Working Group report. *Jpn J Clin Oncol*. 2010 Sep;40 Suppl 1:i28-37. Available from: <http://dx.doi.org/10.1093/jjco/hyq124>
- Mentis AFA, Boziki M, Grigoriadis N, Papavassiliou AG. Helicobacter pylori infection and gastric cancer biology: tempering a double-edged sword. *Cell Mol Life Sci*. 2019 Jul;76(13):2477-86. Available from: <http://dx.doi.org/10.1007/s00018-019-03044-1>
- Maconi G, Manes G, Porro GB. Role of symptoms in diagnosis and outcome of gastric cancer. *World J Gastroenterol*. 2008 Feb 28;14(8):1149-55. Available from: <http://dx.doi.org/10.3748/wjg.14.1149>
- Rojas Duarte M, Marinkovic Gómez B. Cirugía en medicina general: manual de enfermedades quirúrgicas. Santiago de Chile: Universidad de Chile; 2020.
- White JR, Banks M. Identifying the pre-malignant stomach: from guidelines to practice. *Transl Gastroenterol Hepatol*. 2022 Jan 25;7:8. Available from: <http://dx.doi.org/10.21037/tgh.2020.03.03>
- Zhang CZ, Cheng XQ, Li JY, Zhang P, Yi P, Xu X, et al. Saliva in the diagnosis of diseases. *Int J Oral Sci*. 2016 Sep 29;8(3):133-7. Available from: <http://dx.doi.org/10.1038/ijos.2016.38>
- Setti G, Pezzi ME, Viani MV, Pertinhez TA, Cassi D, Magnoni C, et al. Salivary MicroRNA for Diagnosis of Cancer and Systemic Diseases: A Systematic Review. *Int J Mol Sci*. 2020 Jan 30;21(3). Available from: <http://dx.doi.org/10.3390/ijms21030907>
- Kaczor-Urbanowicz KE, Wei F, Rao SL, Kim J, Shin H, Cheng J, et al. Clinical validity of saliva and novel technology for cancer detection. *Biochim Biophys Acta Rev Cancer*. 2019 Aug;1872(1):49-59. Available from: <http://dx.doi.org/10.1016/j.bbcan.2019.05.007>
- Rivera C, Gallegos R, Figueroa C. Biomarkers of progression to oral cancer in patients with dysplasia: A systematic review. *Mol Clin Oncol*. 2020 Nov;13(5):42. Available from: <http://dx.doi.org/10.3892/mco.2020.2112>
- Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011 Oct 18;155(8):529-36. Available from: <http://dx.doi.org/10.7326/0003-4819-155-8-201110180-00009>
- Xiao H, Zhang Y, Kim Y, Kim S, Kim JJ, Kim KM, et al. Differential Proteomic Analysis of Human Saliva using Tandem Mass Tags Quantification for Gastric Cancer Detection. *Sci Rep*. 2016 Feb 25;6:22165. Available from: <http://dx.doi.org/10.1038/srep22165>
- Shu J, Yu H, Li X, Zhang D, Liu X, Du H, et al. Salivary glycoproteins as potential biomarkers for diagnosis of gastric cancer. *Oncotarget*. 2017 May 30;8(22):35718-27. Available from: <http://dx.doi.org/10.18632/oncotarget.16082>
- Li F, Yoshizawa JM, Kim KM, Kanjanapangka J, Grogan TR, Wang X, et al. Discovery and Validation of Salivary Extracellular RNA Biomarkers for Noninvasive Detection of Gastric Cancer. *Clin Chem*. 2018 Oct;64(10):1513-21. Available from: <http://dx.doi.org/10.1373/clinchem.2018.290569>
- Hunter DJ, Losina E, Guermazi A, Burstein D, Lasserre MN, Kraus V. A pathway and approach to biomarker validation and qualification for osteoarthritis clinical trials. *Curr Drug Targets*. 2010 May;11(5):536-45. Available from: <http://dx.doi.org/10.2174/138945010791011947>
- Ziegler A, Koch A, Krockenberger K, Grosshennig A. Personalized medicine using DNA biomarkers: a review. *Hum Genet*. 2012 Oct;131(10):1627-38. Available from: <http://dx.doi.org/10.1007/s00439-012-1188-9>
- Zhang J, Shi Z, Huang J, Zou X. CSTB Downregulation Promotes Cell Proliferation and Migration and Suppresses Apoptosis in Gastric Cancer SGC-7901 Cell Line. *Oncol Res*. 2016 Oct 27;24(6):487-94. Available from: <http://dx.doi.org/10.3727/096504016X14685034103752>
- Qi YJ, He QY, Ma YF, Du YW, Liu GC, Li YJ, et al. Proteomic identification of malignant transformation-related proteins in esophageal squamous cell carcinoma. *J Cell Biochem*. 2008 Aug 1;104(5):1625-35. Available from: <http://dx.doi.org/10.1002/jcb.21727>
- Akram M. Mini-review on glycolysis and cancer. *J Cancer Educ*. 2013 Sep;28(3):454-7. Available from: <http://dx.doi.org/10.1007/s13187-013-0486-9>
- Sousa JF, Ham AJL, Whitwell C, Nam KT, Lee HJ, Yang HK, et al. Proteomic profiling of paraffin-embedded samples identifies metaplasia-specific and early-stage gastric cancer biomarkers. *Am J Pathol*. 2012 Nov;181(5):1560-72. Available from: <http://dx.doi.org/10.1016/j.ajpath.2012.07.027>
- Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides*. 2015 Oct;72:4-15. Available from: <http://dx.doi.org/10.1016/j.peptides.2015.04.012>
- Meldolesi J. Exosomes and Ectosomes in Intercellular Communication. *Curr Biol*. 2018 Apr 23;28(8):R435-44. Available from: <http://dx.doi.org/10.1016/j.cub.2018.01.059>

26. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol.* 2008 Dec;10(12):1470-6. Available from: <http://dx.doi.org/10.1038/ncb1800>
27. Keller S, Ridinger J, Rupp AK, Janssen JWG, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. *J Transl Med.* 2011 Jun 8;9:86. Available from: <http://dx.doi.org/10.1186/1479-5876-9-86>
28. Lijmer JG, Mol BW, Heisterkamp S, Bossel GJ, Prins MH, van der Meulen JH, et al. Empirical evidence of design-related bias in studies of diagnostic tests. *JAMA.* 1999 Sep 15;282(11):1061-6. Available from: <http://dx.doi.org/10.1001/jama.282.11.1061>
29. Whiting P, Rutjes AWS, Reitsma JB, Glas AS, Bossuyt PMM, Kleijnen J. Sources of variation and bias in studies of diagnostic accuracy: a systematic review. *Ann Intern Med.* 2004 Feb 3;140(3):189-202. Available from: <http://dx.doi.org/10.7326/0003-4819-140-3-200402030-00010>