

Gastric Juice Biomarkers in Gastric Cancer: New Trends?

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ABSTRACT

As gastric cancer represents the fifth most common cancer diagnosis and the third leading cause of cancer death worldwide, it remains a current social and health issue. A variety of genetic, epigenetic and environmental factors contribute to the development of gastric cancer, although the etiology remains unclear. Timely diagnosis and appropriate treatment play the most crucial role not only in the containment of morbidity and mortality but also in the prognosis. The establishment of novel non-invasive biomarkers into patient management protocols represents a very promising and challenging approach. In this article, we focus on gastric cancer biomarkers with a special interest in gastric juice that may represent a novel, non-invasive, cost-effective 'liquid biopsy'.

Keywords: gastric cancer, gastric juice, biomarkers, guidelines, surgery, surgical oncology.

INTRODUCTION

As gastric cancer (GC) represents the fifth most common cancer diagnosis and the third leading cause of cancer death worldwide, it remains a current social and health issue (1). The incidence of GC is estimated up to 950000 new cases per year and more than 700000 deaths due to malignancy are attributed to this type of cancer annually (2). A predominance in male gender has been observed, with Japan and Korea being particular high endemic areas (3, 4). It is widely known that adenocarcinoma represents more than 90% of gastric malignancies (5).

A variety of genetic, epigenetic and environmental factors contribute to the development of GC, although the etiology of the disease remains unclear. Distal GC is mainly observed in developing countries. *Helicobacter pylori* (HP) infection and dietary habits are major risk factors. In contrast, proximal GC including gastro-esophageal junction (GEJ) lesions usually occur in developed countries, Caucasian populations and higher socio-economic stratum. Gastro-esophageal reflux disease (GERD) and obesity play the main role in etiology (3, 4, 6).

As a rule, gastric malignancy is asymptomatic or has non-specific clinical manifestations in the early stages. Approximately 60% of patients have

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extensive disease that cannot be surgically resected at the time of diagnosis. Endoscopic examination followed by histological analysis (6-8 biopsies) is considered, and will continue to be, the gold standard diagnostic method for GC (7).

Despite new therapeutic advances, gastric malignancies are still associated with a poor long-term survival and dramatic recurrence rates. A five-year survival rate of up to 60% is reported in Asian countries, whereas it does not exceed 25% in the USA and Europe (4). We have to keep in mind that timely diagnosis and appropriate treatment play the most crucial role not only in the containment of this disease's morbidity and mortality, but also in the optimization of prognosis.

The establishment of novel non-invasive biomarkers into patient management protocols is a very promising and challenging approach for distinguishing benign from malignant tumors, predicting the occurrence of GC, determining the stage of the disease when diagnosed, assessing the response to treatment and preventing the recurrence, which represents the main problem for GC patients who have undergone a "therapeutic" resection (8). In this article, we mainly focus on GC biomarkers, with a special interest in gastric juice that may represent a non-invasive cost-effective 'liquid biopsy' and may potentially offer a new 'pathway' in GC management.

Need for novel biomarkers: what is the role of gastric juice?

Timely diagnosis through population-based screening programs represents a great promise to optimize the outcome of GC patients. Is the evolution of novel preventive, diagnostic and prognostic non-invasive biomarkers the key issue?

A biomarker is defined as a quantitatively and objectively measurable index of a biological event. The properties of an 'ideal' biomarker include tissue specificity, a unique nucleotide sequence, resistance to disruptive molecules, minimally invasive, cost-effective, generally available sampling and last but not least, relative stability in extreme temperature conditions and pH values (8). The National Cancer Institute definition of "liquid biopsy" is as follows: "A test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for

pieces of DNA from tumor cells that are in the blood" (9).

It is reported that human biological fluids (whole blood, serum/plasma, urine, saliva, gastric juice, cerebrospinal fluid) can be a good source of cancer biomarkers with positive predictive value. Gastric juice, seems to be a very suitable source of non-invasive GC biomarkers. It can be easily collected during oesophageal-gastro-duodenoscopy by using a suction device (3) or even on the patient's bed by a nasogastric tube. This biofluid is usually thrown away during an upper gastrointestinal endoscopy. However, it can provide valuable information for the lesion's microenvironment, as it is in direct contact with the tumor (10). Directly secreted by cancer cells products can be collected without being eliminated by the liver. In addition, gastric juice is easier and safer to obtain, compared to a tissue biopsy from the suspicious area (8). After collection, the biofluid can be directly processed or stored for future testing (-80°C) (3).

Upper gastrointestinal (GI) endoscopy combined with tissue biopsy remains the method of choice for GC detection. Sensitivity and specificity values correspond to 69% and 96%, respectively. However, it remains an invasive and expensive method. In Western countries, where GC incidence remains low, endoscopy cannot be considered a cost-effective screening tool for the general population. Also, the effectiveness of this time-consuming method is inextricably linked to the operator's experience (11).

Based on what is known so far, GI endoscopy occupies the third place in the production of waste in healthcare facilities with a significant contribution to greenhouse gas emissions and carbon footprint (12). Patient transport, medical and non-medical equipment, energy consumption, waste and consumables, freight and medical gases are only a few of the causative factors (13). The concept of "green endoscopy" is recently launched in order to raise awareness and reduce the environmental impact of the procedure. Recyclable materials and minimal use of electricity can contribute but are not enough (12). The reduction of inappropriate examinations (followed or not by histological confirmation) and unnecessary endoscopic follow-ups can play a crucial role, as the amount of inappropriate endoscopic procedures reach up to 52% (14). Last but not least, several novel

non-invasive biomarkers, such as gastric juice, can limit the conduction of unnecessary endoscopies for prognostic, diagnostic or reassess purpose (12).

Despite its high diagnostic accuracy, upper GI endoscopy could miss some early-stage lesions, especially in younger patients (<55 years old), female sex, excessive gastric atrophy, adenoma or ulcer, or when the number of fragments taken is insufficient, with a missing rate ranging between 7.5% and 16.6% (15). Linitis plastica is a typical example in which the diffuse infiltrating pattern originating from the submucosal layer (usually leaving the mucosal layer intact), as well as the reactive fibrosis that thickens gastric folds result in false-negative biopsy results in approximately 30-55% of cases. Many diagnostic biomarkers have already been reported concerning linitis plastica detection, such as trypsinogen, long non-coding RNAs, mi-RNAs, mRNAs, proteins and metabolites (16). Although lavage fluid has been studied as a biomarker source and can contribute to decision making, the data regarding gastric juice are quite seldom and future research is deemed necessary.

The potential use of gastric juice as an alternative 'liquid biopsy' has no intent to substitute endoscopic procedure as the gold standard for GC detection, but to improve the accuracy of endoscopic screening in patients with no macroscopically detected lesion during gastroscopy.

Current evidence

Gastric juice was firstly described as a tool for GC primary detection in 1980, when Satake *et al* correlated gastric intestinal metaplasia with elevated gastric juice CEA levels indicating the potential of neoplasia development (17). Although many studies followed, the adoption of tumor markers as GC biomarkers in clinical practice is considered not feasible, due to low diagnostic accuracy.

During the last decade, several novel biomarkers for GC diagnosis have been reported, through the gastric lavage analysis, such as non-coding RNAs, protein-based biomarkers and DNA methylation markers (3). Non-coding RNAs refer to RNAs that do not code into proteins and according to their size they are distinguished in microRNAs, long non-coding RNAs and circular RNAs. They are present in the tumor cell cytoplasm or secreted out of the cell mem-

brane by extracellular vesicles which protect them from degradation. As a result, they are highly stable in biological fluids, such as gastric juice, even in extreme temperature or acidic conditions and can be measured in an important way, using real-time PCR, RNA-sequencing methods or DNA microarrays (8).

In this regard, Cui *et al* observed significant downregulation of miR-21 and miR-106a, measured by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR), in the GJ of patients diagnosed with GC compared to those with benign diseases (18). Another GJ biomarker, described by Shao *et al* some years ago, was miR-133a, the levels of which were also found significantly reduced in GJ of GC patients (19). Other circulating miRNAs that have been reported are miR-129-1-3p, miR-129-2p, and miR-421 (20, 21). In addition, Skryabin *et al* emphasized the value of extracellular vesicles as promising microRNA source, referring that exosomal miR-135b-3p, miR-199a-3p and miR-451a levels can reflect the potential for malignancy (22).

What is more, H19 firstly described by Li *et al*, is a long-coding RNA upregulated in the GJ of GC patients and is correlated with GC expansion, lymphatic invasion, clinical stage and survival (23). Recently, Wang *et al* demonstrated that LINC00152, which is already correlated with GC progression and metastasis (24), can play a tumor suppressor role by targeting miR-138/SIRT2 axis (25). The statistical significance was improved when the results were combined with classic serum biomarkers (3).

Only a few GJ circRNAs have been studied so far as GC biomarkers. Among them, has_circ_0014717 is increased in GC pathologies and can constitute a useful biomarker for GC screening (26), when has_circ_000780 is significantly downregulated. No correlation with the tumor stage has been reported (27).

Moreover, A1-antitrypsin and its precursor are the protein-based GC biomarkers described by Hsu *et al*, who compared GJ samples of GC patients compared with healthy individuals using proteomic analysis (28). Last but not least, it is widely accepted that hypermethylation of CpG islands can result in oncogenes and tumor suppressor genes silencing, playing an important role in carcinogenesis (3). Analyzing six genes (MINT25, RORA, GDNF, ADAM23, PRDM5,

MLF1) methylation in gastric lavages, Watanabe *et al* concluded that MINT25 was the most specific biomarker for primary tumor detection in GJ (29). More recently, Yamamoto *et al* have shown that hypermethylation of BARHL2 gene not only was related with early-stage GC when detected in GJ samples, but also could intimate residual cancer after a minimally invasive endoscopic resection (30).

The Cytosponge-trefoil factor 3 (TFF3) is a non-endoscopic examination for detecting Barrett's esophagus, representing a triage test for endoscopy. A non-endoscopic cell collection device coupled with an *in-vitro* immunohistochemical specific biomarker identifies intestinal metaplasia. During the procedure, a capsule containing the sponge is swallowed and dissolves in the stomach. Thus, the sponge expands and while being pulled up by an attached string, cells are collected from the whole length of the esophagus (31). In the SUGAR study, Xu *et al* aimed to evaluate the feasibility of combined assessment of PG1, PG2, TFF3, G17 and anti-*H. pylori* antibodies in the serum and in Cytosponge samples. They concluded that combined PG1 computation can identify additional patients with reflux symptoms in need for endoscopy, emphasizing that accessory tests are required, as the Cytosponge does not sample gastric body and antrum (32). Recently, Hadjinicolaou *et al* reported that Cytosponge-TFF3 testing can

track premalignant conditions in the stomach, too. Although, the data are very few so far in order to include the examination in screening programs (33). □

CONCLUSIONS AND FUTURE PERSPECTIVES

Gastric cancer is usually diagnosed in advanced stages, when surgical treatment is not indicated. Promising preventive, diagnostic and prognostic biomarkers can potentially solve this problem. What if they were non-invasive too? Gastric juice has barely been explored as a biomarker source, but with no robust and established evidence so far. Devices analyzing gastric juice in real-time gradually integrate into clinical practice (34), while artificial intelligence (AI) has already proved its utility in dysplasia detection [computer-aided detection (CAdE)], invasion depth estimation, tumor classification-characterization [computer-aided diagnosis (CAdx)] and differential diagnosis between cancers and benign lesions, by using handcrafted algorithms for image analysis (35, 36). Future studies are required to fill this knowledge gap, as the capability of diagnosing GC in early stages, undoubtedly will constitute a promising strategy to optimize patient outcomes in the future. □

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