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Review

Microbiota of the Tongue and Systemic Connections: The Examination of the Tongue as an Integrated Approach in Oral Medicine

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Abstract: The tongue is able to quickly reflect the state of health or disease of the human body. Tongue inspection is an important diagnostic approach. It is a unique method that allows to explore the pathogenesis of diseases based on the guiding principles of the holistic concept that involves the observation of changes in the lining of the tongue in order to understand the physiological functions and pathological changes of the body. It is a potential method of screening and early detection of cancer. However, the subjective inspection of the tongue has a low reliability index, and therefore computerized systems of acquisition of diagnostic bioinformation have been developed to analyze the lining of the tongue. Next-generation sequencing technology is used to determine the *V2–V4* hypervariable regions of *16S rRNA* to study the microbiota. A lot of neoplasms are identified only at an advanced phase, while in the early stages, many subjects remain in an asymptomatic form. On the contrary, the early diagnosis is able to increase the prognosis of cancer and improve the survival rates of subjects. Evidently, it is necessary to develop new strategies in oral medicine for the early diagnosis of diseases, and the diagnosis of the tongue as a minimally invasive method is certainly one of them.

Keywords: tongue microbiota; host–microbiota symbiosis; tongue diagnosis; systems correlations; minimally invasive; hygiene

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1. Introduction

For thousands of years, doctors have diagnosed a patient's health by inspecting the tongue, especially the back of the tongue. Inspection of the tongue as a method of clinical diagnosis is a unique feature of Traditional Chinese Medicine (TCM).

The tongue inspection is a procedure that has been reported from the Shang Dynasty (from 1600 BC to 1000 BC) that should be performed by the clinical observation the tongue body to evaluate its morphological and color homogeneity. In accordance to the Traditional Chinese Medicine, the tongue is able to absolve a key role of a biosensor, while the different tongue regions are able to provide information to check the diagnosis the organ systems health [1,2].

In accordance to Traditional Chinese Medicine, "A pathology arises associated to a tongue surface coating appearance. The surface coating represents the exterior manifestation, which corresponds to the sustaining disease, and is the primary indication for the diagnosis making". In this way, the appropriate knowledge of the molecular aspects

of tongue response is a key factor to comprehend this semeiotic medical practice [1,3,4]. Several studies on the microbiology of the healthy human tongue, through the sequencing of *16S rRNA*, which is applied for the detection, categorization, and evaluation of the microbic charge within the biological mixtures, have shown that the most abundant phyla are *Fusobacteria*, *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *and Bacteroidetes* [1,4,5]. The variation in the microbiota of the tongue lining recorded by metagenomic sequencing identifies potential biomarkers for several diseases, including the precancerous cascade [1,6,7]. The thin white or reddish tongue coating with 0/1 patina is a symbol of good health (Figures 1 and 2), while the "white-fat" and "yellow-dense" coating (Figures 3 and 4) may be an indication of inflammation, infection, and stress, as well as of immune and endocrine disorders (Figure 5) [8,9]. Thick coat and tongue moisture are increased in tumors. The relationships between tongue diagnosis and diseases, including rheumatoid arthritis, gastritis and precancerous lesions, liver, pancreatic, and lung cancer, were explored [1,10–15]. Tongue-lining microbiota biomarkers could provide a complement for the diagnosis of several diseases from the noninvasive, individualized, and long-term monitoring aspects [1,16]. Tongue diagnosis is a simple, noninvasive method of assessing physiological conditions by observing the thickness and color of the coating [6]. The tongue coating is categorized into three different classes in accordance to their color typologies: white coating (W), yellow coating (Y), and black and gray coating (Table 1). A yellow coat is considered an indication of water retention and heat [3]. The most common syndromes are the following four: external cold syndrome, internal cold syndrome, external heat syndrome, and internal heat syndrome, and are associated with thin white, thick white, thin yellow, and thick yellow tongue coatings [1]. Cold and hot syndromes are two contrary but internally related conditions [8]. According to the TCM theory, the tongue is an external extension of the spleen and stomach [17,18]. Western medicine refers to the indexes of the thickness of the coat on the back of the tongue, classifying it in four degrees [19] (Table 2).

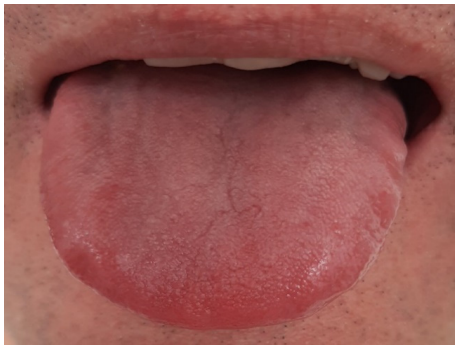

Figure 1. Strawberry tongue health symbol with thin coat (TCM), grade 0/1 according to Miyazaki.

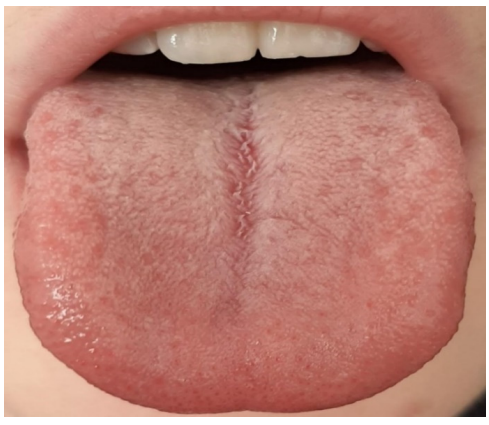

Figure 2. Thin white tongue (TCM), grade 2 according to Miyazaki.

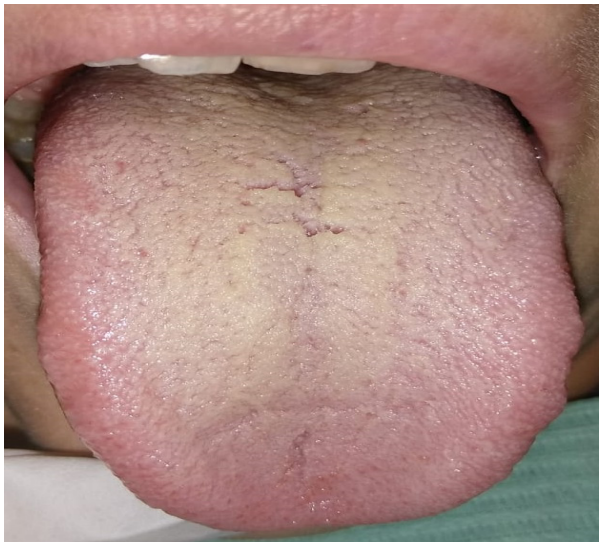

Figure 3. Thick white and yellow tongue (TCM), grade 3 according to Miyazaki.

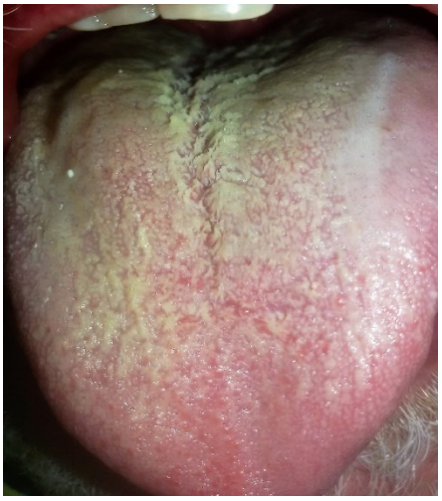

Figure 4. Yellow fat tongue (TCM), grade 2 according to Miyazaki.

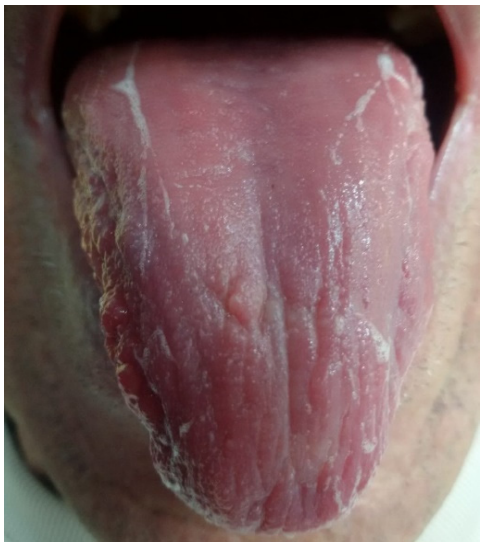

Figure 5. Desquamated coat of tongue related to severe hypovitaminosis.

Table 1. Classification of the lingual patina according to the TCM standard.

Description
Thin white, thick white, and fat white tongue coating
Tongue coating thin yellow, thick yellow, and fat yellow
Thin black and gray tongue coating and thick gray black

Table 2. Classification of the lingual patina according to the Miyazaki standard.

Value	Description
0	No visible patina on the back of the tongue
1	Patina present only on the posterior III of the tongue
2	Patina that completely covers the dorsal surface of the tongue but does not mask the underlying mucosa
3	Very thick patina covering the entire dorsal surface of the tongue

Several studies have shown that *Lautropia* was significantly increased in patients with gingivitis, oral lichen planus, and chronic periodontitis [20–24]. The level of *Capnocytophaga* was higher in the saliva of patients with oral cancer [25,26]. These results indicated that the tongue thin coating (W) is an important reference of the oral microbiota. The salivary *Megasphaera* was observed more abundant in lung cancer patients, associated to an increased rate of white coated tongue [5], fecal *Selenomonas* was increased in colon cancer patients, and the data suggested that (W) thick coat may be correlated with the risk of intestinal tumors [1,27,28]. *Prevotella* has been implicated with periodontal infection [29]. Peri-implant *prevotella maculosa* was increased in patients with smoking mucositis but decreased in patients with nonsmoking mucositis, indicating that thick coating (W) is associated with periodontal disease [28,29]. As for the yellow tongue coating, few potential microbiota have been observed, particularly opportunistic bacteria [20]. Researchers have shown that the oral microbiota is associated with many diseases, such as pancreatic disease, pediatric inflammatory bowel disease, obesity, coronary heart disease, rheumatoid arthritis, gastrointestinal cancer, liver cirrhosis, cardiovascular disease, and pneumonia. The relationship between the tongue lining microbiome and diagnosis was also reported to be relevant for the differentiation of these syndromes [3,9,10,16,17,30]. The mouth environment represents one of the widest microbial reservoirs, and a large quantity of bacterial species are able to provide a complex and stable community. *Mutans streptococci* and red complex bacterial group, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, represent species strongly correlated to a high clinical risk for tooth decay and periodontal pathologies [7,31,32]. In addition, the existence of specific oral bacterial species related to systemic diseases has been reported in recent years [33]. For this reason, oral dysbiosis has attracted attention as an etiology of oral and systemic diseases [34].

After the birth, the oral environment is exposed to a wide variety of microbes, while the human microbiome develops with the organism growth. In this phase, the facultative anaerobic bacterial species such as *streptococcus* and *actinomycetes* are considered the pioneering colonizers of the mouth environment, while the *Streptococcus salivarius* is considered the predominant colonizer species [35]. Although the Firmicutes phylum predominates in both the oral microbiota and the gut, the genus *Streptococcus* within the Firmicutes phylum is rarely detected in the gut microbiota [36]. Initially, a *Streptococcus* species predominance is primarily associated to the first phases of the consolidation of the mouth microbiome. It has also been reported that the formation of the oral indigenous microbiota begins within the first 6 weeks of life, and *Streptococcus* rapidly dominates the oral cavity during this phase [37]. The microbiome quality of the tongue tissues during the transition phase, between 80 and 120 weeks, is more similar to the adults' microbiota composition. Colonies corresponding to *Streptococcus peroris* and *Streptococcus lactarius* decrease exponentially immediately after 30–49 weeks, while *Granulicatella Adjacens*, *Actinomyces odontolyticus*, and

Fusobacterium periodonticum increase over the same period [38]. A drastic compositional change of the language microbiota occurs before the age of 1 year, so diversity and overall bacterial composition reach levels comparable to those of adults by age 2 [7].

The posterior part of the tongue has a large surface with papillary structures [39], which can retain numerous aerobic and anaerobic microorganisms [40], fungi, metabolites in the blood, saliva, and exfoliated keratinized epithelium. Filiform papillae are a specific structure that involves the formation of the lining of the tongue. This structure causes many cracks and folds that increase the surface of the tongue, and the warm, moist, and nourishing environment provides a suitable medium for colonization, growth, and proliferation of microorganisms [36,41–47]. The mucous membrane of the tongue accumulates more microbes than other parts of the oral cavity do, boasting a relatively complete and independent microecosystem that can be easily harvested from saliva [48,49]. These features imply that more attention should be paid to the tongue microbiota in older adults susceptible to swallowing problems [50]. An increase in tongue lining in edentulous older adults has been associated with aspiration pneumonia and fever [51,52]. Usually, the detached microbes ingested with the salivary fluids move through the esophagus tract to the stomach region, where they go to be inactivated by the action of the gastric acid and proteolytic enzymes. However, alterations in swallowing with aging allow for aspiration into the lower respiratory tract and subsequent lung infection [53]. Older adults with fewer teeth, poor oral hygiene, and more dental caries consistently experience dysbiosis, with a greater relative abundance of *Prevotella*, *Veillonella,* and *Streptococcus* species and a greater number of fungal species. Particular attention should be paid to the state of the tongue by implementing correct oral hygiene habits with particular attention to the back of the tongue. Specifically, mechanical oral hygiene to lower the oral microbial load is recognized as an effective approach to reduce the death rate from aspiration pneumonia, so much so that in some countries, the figure of the oral health professional is guaranteed as standard assistance for the treatment of the oral cavity hygiene in frail elderly in hospitals and nursing homes [54]. Although aspirated saliva contains microorganisms that colonize various oral sites, the bacterial composition indicates that the dominant source is the tongue microbiota [36,41,55].

Some studies have shown that bacteria implicated in periodontal disease, such as *porphyromonas gingivalis*, *tannerella forsythia*, and *treponema denticola,* are a risk factor for atherosclerotic vascular disease, type 2 diabetes, and nonalcoholic fatty liver disease (Figure 6) [56–58]. Furthermore, a repeated oral ingestion of *Porphyromonas gingivalis* promotes systemic inflammation [59].

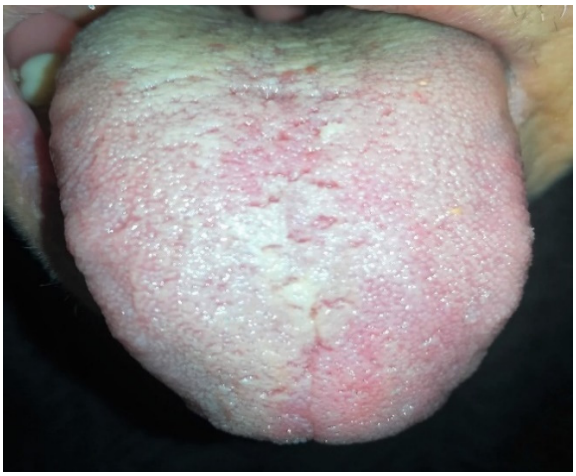

Figure 6. Thick white fatty coat of tongue related to type 2 diabetes, hypertension, and nonalcoholic fatty liver disease.

Since we ingest approximately 600 mL of saliva per day containing up to 10^9 bacteria/10 mL, it is reasonable to suspect that some oral bacteria induce intestinal microbiota disturbances [60].

A growing body of evidence has shown that the microbiome could influence the proliferation of cancer cells [61]. Ten specific microbes have been designated as carcinogenic pathogens by the International Agency for Research on Cancer (IACR). Among these, *Helicobacter pylori* is the most famous organism considered to be the strong inducer of gastric cancer [62,63]. In patients with gastric cancer, the gastric microbiota was predominated by *Veillonella*, *Hemophilus* along with *streptococci, Lactobacillus*, *Prevotella*, and *Neisseria*. It has been noted that some bacteria (*Veillonella*, *Streptococci*, and *Lactobacillus*) have also been observed in the microbial community of the tongue [64]. This finding indicated that the microbiome that lines the tongue is also closely related to the diagnosis of gastric cancer [65].

The purpose of this literature review was to explore the tongue lining microbiota known in the literature and investigate the study methods for screening and early diagnosis of particular systemic diseases such as anemia, amyloidosis, and cancer.

2. Materials and Methods

2.1. General Characteristics

The database search was conducted on PubMed, Google Scholar, and Scopus according to a Boolean search performed by two independent expert reviewers on 27 May 2021. The keyword indicators were (Tongue microb* OR Tongue microbiota) AND systemic disease. A manual search was conducted to improve the article pool.

After the preliminary screening and duplicates removal, the papers abstract and titles were evaluated in order to include the articles for the eligibility assessment.

The articles selected were finally included for the qualitative analysis.

2.2. Inclusion and Exclusion Criteria

Scientific articles were available on the main medical databases—for which inclusion and exclusion criteria were outlined. More specifically, the inclusion criteria included the following:

- Scientific articles published without time limit;
- International articles in English;
- Experimental studies with no age limits;
- In vivo experimental studies and in vitro experimental studies;
- Expert review;
- Literature reviews;
- Narrative reviews;
- Historical reviews and milestone papers;

The exclusion criteria included the following:

- Scientific articles that dealt more generally with the microbiota of the oral cavity;
- Short communications;
- Opinion papers;
- Book chapter/congress proceedings.

3. Results

3.1. Summary of the Search Output

A total of 267 papers were retrieved from the manual and electronic databases. A total of 250 articles were examined, and a total of 96 were considered for the full text assessment. The eligibility procedure excluded a total of 17 papers, and 79 were considered for the qualitative review (Figure 7).

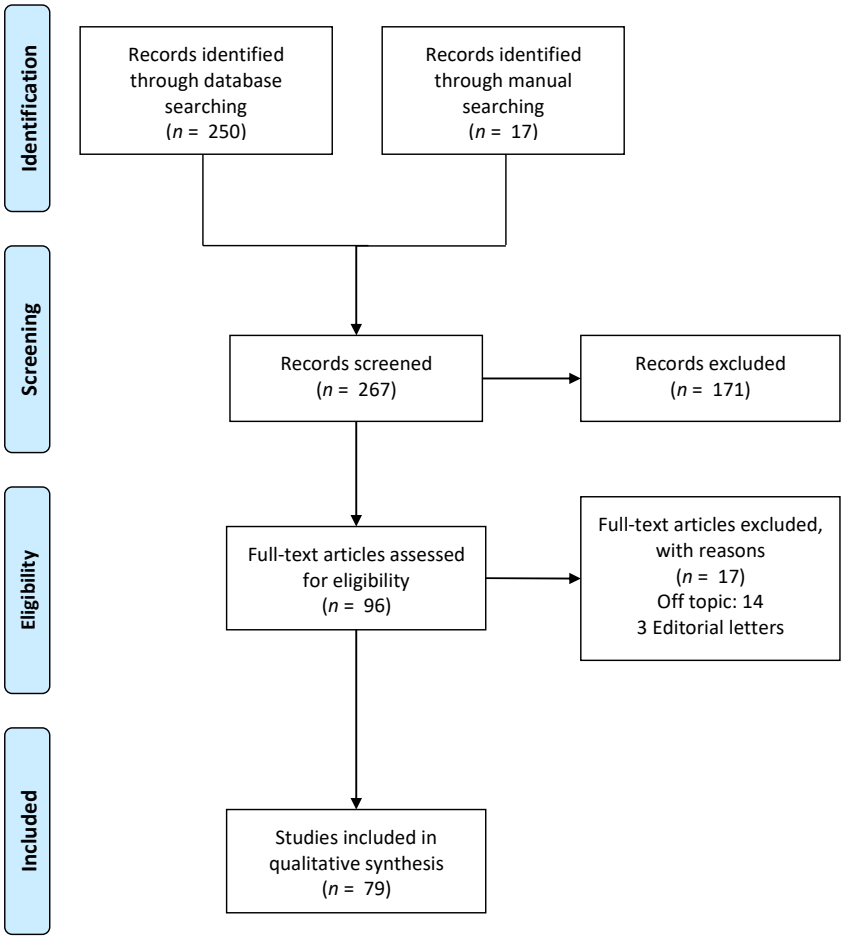

Figure 7. Prisma flowchart assessment of the included study.

3.2. Characteristics of the Included Studies

The most abundant phyla in the oral cavity and the tongue surface were Fusobacteria, Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes [1,4,5].

Streptococcus salivarius was the predominant pioneer colonizer in the newborn, while *S. Mutans* was present exclusively in the oral cavity [34–36]. In literature, it was reported that virulent strains of *Streptococcus salivarius* on the tongue have been reported to have fewer streptococcus throat infections [34–36].

The quantity of *Capnocytophaga* was significantly higher in the salivary fluids of subjects affected by oral cancer [24,25]. Moreover, salivary *Megasphaera* was more represented in subjects with lung cancer, fecal *selenomonas* was significantly increased in colon cancer patients, and *Prevotella* was implicated in periodontal infection. Peri-implant *prevotella maculosa* was increased in smoking patients but decreased in nonsmoking patients. *Prevotella, Haemophilus, and Streptococcus* were dominant in the lining of the tongue in patients with colorectal cancer. *Porphyromonas gingivalis* was involved in the initiation and progression of rheumatoid arthritis [66–69]. Periodontal disease, caused by *porphyromonas gingivalis, tannerella forsythya*, and *treponema denticola*, was a risk factor for a variety of diseases, such as atherosclerotic vascular disease, type 2 diabetes, and nonalcoholic fatty liver disease [55–57]. There was a predominance of *S. salivarius* and two competitive cohabitation groups among adults aged 70 to 80 years. A group assembled from Group I diners, including *N. flavescens* and *P. pasteri*, was observed in a lower proportion in the microbiota and tongue ecosystem of individuals with fewer teeth, poor oral hygiene, and dental caries. The other group consisted of group II commensals, including *P. histicola, V. atypica, S. salivarius*, and *S. parasanguinis*, which were specifically predominant in the microbiota on the surfaces of the oral mucosa, including the back of the tongue, and constituted only

components less than the microbiota associated with the teeth. An increase in Candida and fungal species was recorded in patients with removable prostheses [36,52–54].

The use of chlorhexidine twice a day was associated with a significant increase in systolic blood pressure after 1 week of use in healthy and normotensive subjects, the frequency of tongue cleansing was a predictor [70].

4. Discussion

The oral cavity represents a key ecosystem for microbial proliferation, while in healthy subjects, the mouth could present over ten billion bacteria. Bacteria are localized in tongue crypts and they could invade into the vascular system through the rich vascularized tissue components of this region [18].

Several studies have suggested a link between the development of the human microbiome and rheumatoid arthritis (RA). *Porphyromonas gingivalis* appears to be involved in the initiation and progression of RA, supported by the high presence of periodontitis [66,67]. In a case-control study, Ceccarelli F., Orrù G., and Pilloni A. et al. analyzed the presence and quantification of *P. gingivalis* in a large healthy cohort, and there was a significant association between the percentage of *P. gingivalis* on the total tongue biofilm and disease activity in patients with RA, suggesting that the microbiological status of the oral cavity could play a role in the mechanisms of inflammation. In this study, the percentage of *P. gingivalis* on the total language biofilm was analyzed for the first time; using this new measurement, an association was identified between this value and disease activity, new information on the bacterium's influence on RA. The authors hypothesized that the presence of *P. gingivalis* can chronically stimulate the immune system, regardless of the presence of periodontitis, leading to a state of chronic systemic inflammation [68].

Next-generation sequencing technology was used to determine V2–V4 hypervariable regions of the 16S rRNA gene to study the tongue lining microbiome in colorectal cancer patients and healthy controls. *Prevotella*, *Haemophilus*, and *Streptococcus* were dominant in the samples from Han et al. In comparison with healthy people, thick tongue lining and wetness were increased in patients with tumors. The dominant color of the tongue in healthy people was reddish, while it was purple in patients with tumors. The relative abundance of *Neisseria*, *Haemophilus*, *Fusobacterium*, and *Porphyromonas* in healthy people was higher than that in patients with tumors. The study suggested that tongue diagnosis may provide a potential screening and early detection method for cancer [69,71].

During the inspection of the tongue, the shape, size, color, and texture of the body and coat should be examined [72]. According to MCT, cold syndrome is associated with oily white tongue, preference for hot food and drink, abdominal tension, abundant clear urine, cold sensation in the lower limbs, loose stools, hypersensitivity to cold, and a preference for heat. Heat syndrome, on the other hand, is associated with halitosis and a thick yellow tongue, a sensation of heat, a preference for cold foods and drinks, a burning sensation in the stomach, constipation, yellow urine, an aversion to heat, and a preference for cold [8,73]. The mouth microbiome is also characterized by the capability to sustain a determinant symbiotic role in blood pressure, maintaining by the release control of nitric oxide (NO), which is a fundamental cardiovascular molecule [74]. Moreover, the administered nitric oxide (NO) through inhalation is able to produce systemic responses and absolve a protective action against the myocardial ischemic reperfusion damage [75]. The nitric oxide (NO) is also produced in the human body by converting arginine to NO [76], while the oral microbial communities supplement host NO production by reducing dietary nitrate to nitrite via bacterial nitrate reductase. Unreduced dietary nitrate is delivered to the oral cavity in saliva, a physiological process called enterosalivary circulation of nitrate [75].

Mitsui and Harasawa reported that the interruption of enterosalivary circulation by the daily administration of oral antiseptics is able to produce a significantly higher systolic blood pressure in humans. With the use of 16S rRNA gene sequencing and analysis, Tribble et al. reported that the application of chlorhexidine as antiseptic mouthwash for 1 week produced significant changes in tongue bacterial communities and resting systolic

blood pressure in healthy, normotensive individuals with documented hygienic behaviors and free from oral pathologies [70]. The frequency of tongue cleaning was a predictor of chlorhexidine-induced changes in systolic pressure and in the composition of the tongue microbiome [70]. Moreover, the chlorhexidine mouthwash administrated twice daily was associated to a significantly higher systolic blood pressure after a 1 week of treatment [77]. This further supports the symbiotic relationship connected with the oral microbiome, which is able to influence the human health status through the nitrate–nitrite–NO entero-salivary pathway. These findings suggest that managing the tongue microbiome by regular cleaning together with adequate dietary nitrate intake provides an opportunity to improve the successful stabilization of the systolic blood pressure [70,78].

Manipulation the human microbiome as a therapeutic target for disease management is a future goal for medicine. Tongue microbiota screening of resistant hypertensive patients may provide new insights into the etiology of their hypertension. The oral cavity is suitable for probiotic and/or prebiotic therapy to promote a balanced microbiota. Rebalancing the oral flora as a means of promoting NO production is a completely new paradigm for biochemistry and physiology, as well as for cardiovascular medicine and dentistry. These studies are new insights into the symbiotic relationship between the host–oral microbiome [79].

Recently, the human microbiota has been investigated deeply due to the rapid development of innovative sequencing approaches, and studies have reported that the oral microbiome is involved with many different metabolic processes, including the digestion of plant derivates, production energetic metabolites such as short-chain fatty acids, inducing the immune homeostasis response, and a protection against different pathogenic agents [79,80]. In general, cancer was considered a disease induced by environmental and genetic factors. A growing body of evidence has shown that the microbiota could influence the proliferation of cancer cells and is responsible for spontaneous cancers in various organs, including skin, colon, liver, breast, and lungs [81].

The salivary microbiota has been reported as a wide reservoir of respiratory and digestive microorganisms and a potential trigger for different pathologies in these systems, while the microbiota localized at the level of the tongue surface represents a consistent source for the salivary microbes [13]. The mouth flora represents the second-widest microbiota after the gut, and more than 700 bacterial species, including fungi, viruses, and protozoa, are localized there. This region is provided by a wide quantity of bio-niches, which show the wide complexity of the oral cavity environment, where the microbial vector is able to colonize different habitats according their intrinsic and metabolic characteristics. Recent studies reported that the oral microbiome is absolved of an important role in oral and systemic health protection and maintenance, while the discovery of 16S rRNA gene next-generation sequencing (NGS) produced a strong contribution for a better comprehension of the interaction complexity of its bacterial component [82].

Recently, it has been reported that diet, lifestyle, smoking, and possibly socioeconomic status can all influence the bacterial profile in the oral cavity. Furthermore, oral hygiene habits can affect the oral microbiota in terms of both the number and diversity of microorganisms [13]. The microbiota on the lining of the tongue is at the forefront of the human digestive system. The biomarkers of the tongue lining microbiome could provide a complement for the diagnosis of several diseases from the noninvasive, individualized, and long-term monitoring aspects and could be a promising integrated contribution to preventive oral medicine [1,16,19]. These aspects could represent a future orientation in the field of the individualized medicine, assembling novel diseases risks categories due to the oral environmental determinants and the microbiota ecosystem.

5. Conclusions

A small organ such as the tongue can be a very effective visual detector of apparently nonvisible pathologies and can be considered as a potential method of rapid cancer screening and diagnosis.

Many cancers are diagnosed at an advanced stage, mainly because most patients are asymptomatic in the early stage. Diagnose in the early stage could increase the number of curable cancers and improve survival. Evidently, it is necessary to develop new strategies in oral medicine for the early diagnosis of diseases, and the diagnosis of the tongue as a minimally invasive method is certainly one of them.

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